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DOCTORAL DISSERTATION

THE EFFECT OF CEREBRAL DRUGS ON THE ENERGETIC STATE OF NORMAL AND ISCHAEMIC BRAIN

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CONTENTS

I. INTRODUCTION

The multidisciplinary nature of research on neurology and neuropharmacological agents, coupled with the rapid rate of growth of research in this topic, has prompted so many researchers to make available so many reviews on this aspect. It is really very difficult to cope with all, or even the major part of, what is written about brain pathophysiology and pharmacology; especially in regard to cerebral blood flow and brain metabolism in normal and abnormal conditions. Ischaemia is one major factor affecting brain flow, metabolism and function.

This dissertation, is generally aimed to be a single response to the call of so many workers for further investigations and to add some new data to the research in neurosciences and cerebral pharmacology.

Although too much work had been done on cerebral circulation and on the normal and pathological metabolism of the brain, yet there is still a great controversion on the mechanisms of such pathophysiological conditions as hypoxia, anoxia and ischaemia, regarding their etiology, sequences, consequences

and treatment. For this reason much of the work which was done, is not yet confirmed and still awaiting emphasis, proof and clinical application. For instance the pathomechanism of diseases due to cerebral insufficiency or infarction are not yet completely known. Accordingly the mode of action of drugs used to treat those insufficiencies must also be clarified. Many of the today-used cerebrovascular drugs are very effective and beneficial in treatment, although their exact mechanism of action is still not yet proved.

For assaying cerebral drugs, a pathological state must first of all be created and such a state or model must be mimiting, to a great extent, what clinically takes place in such a pathological disease of the patient i.e. the experimental parameters must be more or less, similar to the actual clinical state in the patient.

The different multiple aims of this study are as follows:

1./ To study the pharmacological problems of cerebrovascular diseases with application of physiological or/and anatomical techniques, and to design ischaemic models on which the effects of certain physiological and/or pathological conditions could be

investigated as anaesthesia, hypoxia and ischaemia.

2./ To elucidate the biochemical and pathophysiological mechanisms using ischaemia primarily as a method of modifying cerebral circulation and metabolism, and to integrate the data obtained by both approaches so as to trace the pattern of events, especially in the high energetic state of the brain, occuring during and after the different periods of ischaemia. As well as to study the inherent tolerance of brain cells to ischaemia, and the effect of pH /acidosis due to increased lactate production and the differences in CO_2 production due to hypoxia or ischaemia/ on the viability of brain tissue.

3./ To study the protective effect on ischaemic brain of anaesthesia and some pharmacological agents that affect the rate of brain metabolism or modify its circulation.

4./ To study and try to elucidate the metabolic mechanisms of action on the ischaemic brain of some cerebral oxygenators and cerebrovascular vasodilators namely Papaverin, Cavinton, CH-102, Nitroglycerin and Inosine. Papaverin and Cavinton are well known in cerebral insufficiencies, while CH-102, Nitroglycerin and Inosine are only known in cardiovascular insufficiencies - mainly in angina pectoris - as effective

coronary vasodilators in myocardial ischaemia. Inosine is physiologically known as active myocardial and cerebral metabolite originating from the well known ischaemic metabolite and mediator Adenosine.

The above multidisciplinary goals led my enthusiasm and interest to do my experimental work mainly in cerebral ischaemia with certain preliminary trends or trials to investigate its pathophysiology and pharmacology.

Due to the great interrelationships existing between the heart and brain in the normal and pathological physiology, most of the recent research work done on the heart and brain tissues proved that the two organs are similar in so many physiological, metabolic and haemodynamic properties as could be seen from the common factors regulating both the cerebral and coronary blood flow.

It was found that so many drugs that act on the heart, may similarly and beneficially act on the brain as well as on cerebral circulation.

Although the brain has its main cerebral vascular control namely autoregulation, yet the cerebral flow has a great dependence on the main arterial blood pressure and the arterio-venous blood changes, as well as on the vascular resistance. Those factors may also

reflect and prove the interrelationships between the heart, being the main organ of circulation, and the brain tissue with its cerebral circulation.

There are some physiological and pathological similarities between the heart and brain. For instance, the haemodynamic, metabolic and electrophysiological properties of both tissues are more or less similar in so many normal and pathological aspects. For example hypoxia, anoxia and ischaemia may exist in both tissues with similar causes, mechanisms and consequences. Angina pectoris is considered as a typical myocardial ischaemia, being a state in which the blood supply to the heart through the coronaries is insufficient and thus the oxygen demand/supply ratio is affected. Similar is the state in brain ischaemia, of any etiology, where the oxygen and glucose carried by the blood reach the brain in an insufficient or diminished amounts, accordingly metabolic disorders follows, leading to other haemodynamic and electrophysiological changes as well as to the release of certain metabolites or transmitters.

From those above mentioned similarities and interrelationships between the heart and brain arose the aim and the idea of studying the metabolic effects of some cardiovascular drugs on the brain tissue and

cerebral circulation as nitroglycerin, papaverin and the new product of CHINOIN CH-102.

Inosine and Cavinton are still under investigations. The latter was recently marketed after proving its benefit in cerebral insufficiencies, although its effects on brain metabolic and energetic parameters were still not yet completely investigated. Our study is a trial to fill this empty space of incomplete investigations.

I.2. Terminology: Definitions of some key words

1./ Cerebral hypoxia: is defined as an inbalance between O_2 need and O_2 utilization, such O_2 imbalance induces a decrease in the rate of oxidative phosphorylation and consequently an imbalance between production and utilization of high energy compounds, which in turn, exert feedback mechanism control over the rate of glycolysis.

2./ Ischemia /iske = stop, mia = blood/ is the local and temporary defficiency of blood due to a contraction, ligation or transsection of a blood vessel or due to retention or/and suppression of blood supply to a certain area i.e. defficient blood supply.

Practically it is the disturbance or decrement of the O_2 demand over O_2 supply ratio of the tissue.

3./ Infarction: An area of coagulation or necrosis in a tissue due to anoxia, local anaemia or ischaemia resulting from obstruction of circulation to the area, or from a sudden arrest of circulation in a vessel /anaemic infarction/.

Generally, it is the death of, or affect of, a tissue, mostly section of heart, kidney or brain, due to block or occlusion resulting in cutting off to the blood supply.

4./ Anoxia: Oxygen defficiency or lack. Clinically it is a condition in which the cells of the body do not have or can not utilise sufficient oxygen to perform normal function.

Anaemic anoxia: diminution in the O_2 capacity of the blood due to decrease in the amount of functioning haemoglobin.

Anoxic anoxia: a condition characterized by normal O_2 capacity but diminished O_2 tension in the arterial blood.

5./ Anoxemia: blood anoxia due to defficient aeriation /O_2 content/ of the blood.

6./ Hypoxemia: the state in which there is diminished amount or reduced saturation of O_2 in the arterial blood.

7./ Stroke = Apoplexy: is the sudden uncoscious-

ness, usually causing hemiplegia, caused by a vascular accident in the brain or by haemorrhage, cerebral embolism or thrombosis.

8./ Embolism: is the obstruction of a blood vessel by a solid body refered to as emboli /i.e. plugs/ as, thrombi, fat or air-globules or tumour--cells.

9./ Exsanguination: is the removal of body-blood by means of inducing haemorrhage or cutting of main arteries.

10./ Decapitation: - is the killing of animal by removal of the head.

11./ Ischaemic resolution /or restitution/ - is the subsidence of ischaemic symptoms or the earliest indications of returning to normal i.e. the restoration of viability or functioning to the ischaemic tissue.

12./ Glycolysis: bestly defined by Mac Millan and Siesjö /1971-72/ as the alterations in the energy charge potential /ECP/ which reflects the balance between the energy-using and energy generating processes in the cell. It is a breakdown of glucose in presence of oxygen /aerobic glycolysis/ or in its absence /anaerobic/ to produce energy.

I.3. Numerical expressions of energy states

Different terminologies have been used theoratically and experimentally deriviated to give an exact definition to the state of energy in the brain, muscles or heart or other tissues where energy is stored in the form of high energy phosphate compounds. From those terminologies, the Bioenergetic Index /B.I./ is the most complex indicator to the state of energy.

According to Fedelesova and Ziegelhöffer /1976/

$$B.I. = \frac{/ATP + CP/ \; 10 \; \frac{ATP}{ADN} + \frac{ADP}{ATP}}{\frac{ATP}{CP}}$$

Where B.I. = bioenergetic index

ATP + CP = the available reserves of energy represented by high energy phosphates

ADN = ATP + ADP + AMP /i.e. = Total adenine nucleotides/

ATP/ADN = The degree of degradation and loss of adenine nucleotides

ADP/ATP = The degree of degradation and/or resynthesis

ATP/CP = The relationship between the amount of energy represented by ATP and the energy transfer system in the cell.

This index is determined and calculated in all our experiments and used to express the cerebral state of energy under different conditions.

Energy charge potential /ECP/: energy metabolism was evaluated by oxidative phosphorylation in isolated mitochondria and by quantitating in vivo changes of the cortical energy charge of the Adenylate pool. Atkinson /1968/ found that

$$ECP = \frac{/ATP + 0.5\ ADP/}{ATP+ADP+AMP} = \frac{ATP + 0.5\ ADP}{ADN}$$

and that the energy charge may be an important regulator of metabolic activity since it is defined in terms of actual concentrations of the adenine nucleotides.

Yatsu et al. /1974/ and Lewis et al. /1973/ confirmed the same fact and used this parameter in their experimental studies of ischaemic brain models.

Total Adenine Nucleotides /ADN/: is defined or calculated as ADN = ATP + ADP + AMP. This is also an important parameter for the energy state of a tissue and it is one of the cerebral energy parameters determined beside the bioenergetic index in this dissertation.

II. REVIEW OF LITERATURE

II.1. Energy metabolism of the C.N.S.

Normal brain function requires a continuous supply of neutrients from the blood stream, of these, the most critical are glucose and oxygen. The endogenous stores of glycogen and high energy phosphate compounds are so low that they can support brain function only for minutes. The normal rates of glucose and O_2 consumption by the human brain, and generally the brain of mammals account for 20 to 25 % of the total body consumption although the brain is only 3 % of the total adult body weight /Himwich, 1951; Kety, 1957/. The interest in this part of brain metabolism comes from the apparent lack of obvious work carried out by the brain, in comparison to the mechanical and the biosynthetic work of exporting organs such as liver and Pancreas or the work of the heart and muscles and osmotic work of the kidney. It is to the physiological function of the brain that we must turn in terms of the electrical energy produced by the functioning brain; perhaps as much as 40 % of the energy produced from the oxida-

tive metabolism in the brain /Batchelard, 1975/ is used to support the active transport of Na^+ and K^+ ions. Indeed the brain is unique not only in its electrical excitability, but also in the other quantitative aspects of its intermediary metabolism. It is characterised by having a respiratory quotient, i.e. the ratio of CO_2 produced to the O_2 consumed, of 0.99 /Gibbs et al., 1942/.

The brain is sensitive to any interference in its energy metabolism and the effects of vitamin defficiency in causing convulsions, mental abnormality or retardation are a reflection of this. The metabolic pathways, in which the vitamins function as coenzymes, are common to all organs of the body, and the peculiar disorder of cerebral function is quantitative rather than qualitative i.e. depending highly on the defficient quantity of the specific vitamin.

Interest in energy metabolism of the brain has recieved much carefulness and desire from the observation that: the basal metabolic rates can be increased rapidly, transiently and reversibly by techniques which cause generalized membrane depolarization by means of ions exchange. Thus, in vivo electric shock has been shown to cause 10-20 fold increases in the

rate of carbohydrate metabolism which may last only seconds, untill endogenous supplies of neutrients are exhausted.

Batchelard /1975/ reported that: the rates of energy metabolism in adults were not significantly different during deep anaesthesia, in insulin coma or in diabetic coma.

According to the relationship between concentration of metabolites, the rate of consumption of glucose and O_2 can be quantitatively accounted for by the rates of production of CO_2, Lactate and pyruvate in the normal brain. In laboratory small animals as rats and mice, determinations of rates of CBF and metabolism are more difficult due to the small size of blood vessels and in such animals the results indicate higher rates of metabolism than in man /Batchelard, 1975/. In man the rate of O_2 consumption is about 0.28 μ mol/g/min; and of glucose consumption about 0.33 μ mol/g/min. In young children, the rates of CBF and metabolism may be double those observed in adults, and this is why young children seem to be more resistant to hypoxia or ischaemia.

II. 2. Normal brain metabolism

II. 2.1. Routes of carbohydrate metabolism in the brain

Glucose is utilized in the normal brain almost entirely via the direct routes of glycolysis and the tricarboxylic acid cycle /T.C.A.C./. The individual stages of these pathways occur generally in mammalian organs but with different qualitative and quantitative aspects characteristic of the brain. The brain metabolises glucose very rapidly, and has small reserves of energy but depends on the continuous supply of glucose and O_2 from the circulation. Glucose metabolism in adults is operated by the hexose monophosphate shunt /pentose monophosphate shunt/ glycogenesis, glycolysis and glyconeogenesis.

II. 2.1.1. Glycolysis

The brain uses glucose in different rates according to different species. The normal rate of brain production of lactic acid can be increased by a factor of 100 in convulsions, and in hypoxic or ischaemic conditions for brief periods untill the endogenous sources of energy are depleted.

Glucose is transported to the brain from the circulation through the capillary walls by facilitated diffusion. This initial stage of transport in the utilization of glucose is very important. It is a limiting-factor in regulating the rate of glucose consumption. Glycogen occurs normally in the brain in concentrations of 1.5-2 µ mol/g and disappears rapidly during post mortem autolysis. There is little detectable change in the first minute untill the stores of glucose become depleted. Glycogen is then rapidly lost /Lowry et al., 1964/. Glycogen is also rapidly broken down in hypoglycaemia, anoxia or ischaemia and as a result of convulsions or treatment with stimulants such as the amphetamines /Batchelard, 1975/.

Glycolysis is controlled by glucose transport and the three kinases which are specific cerebral hexokinases: phosphofruktokinase /PFK/ phosphoglyceratekinase /PGK/ and pyruvate kinase /PK/ which are responsible for phosphorylation and maintenance of ATP/ADP ratios in and outside the mitochondria.

II. 2.1.2. Lactate production

The rate of lactate production is used as the main criterion for measuring glycolytic rates in the

brain. Lactate formation is catalysed by lactate dehydrogenase /LDH/, a reversible enzyme in which the equilibrium is in favour of lactate production.

When anaerobic glycolysis is stimulated as in hypoxia, ischaemia or in convulsed states, little pyruvate accumulates because lactate dehydrogenase has a high activity in the brain.

The substrates used by the brain as alternatives to glucose are the other sugars as mannose and maltose which are converted to glucose before reaching the brain.

Under certain unusual conditions, as long starvation, the human and the rat brains only can utilize ketone bodies /Batchelard, 1975/ from the cerebral circulation by oxidation into Acetyl CoA which enters the tricarboxylic acid cycle to play a role in cerebral oxidation.

II. 2.1.3. Control of cerebral glycolysis

Cerebral regulatory mechanisms of glycolysis is mainly involved through regulatory enzymes. There is no clear evidence for any direct hormonal action in the different stages of cerebral glycolysis /Batchelard, 1974/. The rate of cerebral glycolysis under excited states, as during convulsions, may be highly

accelerated.

A regulatory enzyme is defined as an enzyme whose properties are affected by factors other than the availability of its substrates. It is capable of exerting a considerable amount of control over the rate of metabolism through the whole pathway in which it occur.

Lowry et al. /1964/ observed that short periods of ischaemia activated glycolysis where a decrease of glucose, Glucose-6-P, and Fructose-6-P concentrations was observed; while other glycolytic intermediates were increased. Oppositely, glycolysis is inhibited by phenobarbitone /Batchelard, 1975/ and this may give some interpretation to the protective effect of barbiturate anaesthesia.

Studies based on the activating conditions of ischaemia and inhibiting conditions of barbiturates clearly indicated that the control points and the regulatory enzymes of glycolysis are at the stages of: glucose transport, hexokinase, phosphofructokinase and pyruvate kinase.

Other regulatory mechanisms of glycolysis are done by substrates or co-factors concentrations i.e. the limited availability of substrates eg. inorganic phosphate /IP/ which is a substrate for oxidative

phosphorylation as well as for other enzymatic stages needed for glycolysis. According to Batchelard /1974/ the rate of glycolysis is measured as rates of lactate formation. The endogenous concentration of IP is a limiting factor for the anaerobic glycolytic rates. ADP is another substrate needed for glycolysis as well as for phosphoglycrate kinase and pyruvate kinase. /Mg^{2+}/ as a co-substrate is also required as Mg-ADP or Mg-ATP /Batchelard, 1975/. The cytoplasmic ratios of NAD^{+}/NADH are also limiting factors needed for some glycolytic enzymes.

Glycolysis has a great role in cerebral ischaemia or hypoxia. Under such excited states that cause membrane depolarization as ischaemia or hypoxia, glycolytic rates are highly increased, maximum enzymatic activity is reached, there is great expenditure of energy with loss of ATP and CP. Concentrations of ADP and IP are increased and thus their relative enzymes are activated. Also changes in the relative concentrations of ATP, ADP, AMP and IP will affect the hexokinase-phosphofructokinase system.

II. 2.1.4. Gluconeogenesis

Is the endogenous formation of free glucose formed either by glycogen catabolism or reversal of

glycolysis from pyruvate. This process does not occur in the brain due to the low activity of the brain glucose-phosphatases which form glucose from glycogen breakdown.

Glycogenesis: is the formation of cerebral glycogen in the brain from glucose. There is no evidence that glycogen in the brain can be formed endogenously from pyruvate.

The other biochemical pathways involved in the brain are: the hexose - monophosphate shunt /HMP--shunt/ in which the brain metabolises the glucose carbon to form NADPH and pentoses which are required for biosynthesis of lipids and neucleic acids, respectively.

II. 2.2. Oxidation of pyruvate

Whereas the conversion of glucose to pyruvate, then to lactate, through glycolysis or HMP-shunt, is cytoplasmic anaerobic process, the subsequent reactions are mitochondrial and are involved in two pathways.

a./ The Krebs Cycle /Tricarboxylic acid cycle = = T.C.A./: is the cycle in which pyruvate enters through formation of acetyl CoA or oxaloacetate and of which the net result is the passage of the carbon

atom of pyruvate, being originated from glucose, to produce NADH by reducing the oxidized form NAD^+.

b./ The electron transport chain: - involving the reoxidation of NADH and the flavoprotein $FADH_2$ back to NAD^+ and FAD respectively with consequent phosphorylation of ADP to ATP.

II. 2.3. Cerebral transamination reactions

Are carried out by the brain to form amino acids such as glutamate, glutamine and aspartate. The transamination between alfa-oxoglutarate and glutamate is the major reaction in the first stage of the GABA shunt /Batchelard, 1975/.

GABA shunt is the metabolic pathway of gama--aminobutyric acid. Glutamate is very important substrate being involved in a number of important enzymatic reactions in the brain, mainly transamination and glutamine synthesis, which are the main two mechanisms involved in ammonia fixation in the brain and which are catalysed by glutamine synthetase, an enzyme which is inhibited by the convulsant drug Methionine Sulphoximine. Glutamine also acts as a substrate for glutamate decarboxylase which catalyses the removal of CO_2 from glutamate to form GABA which is an important inhibitory neurotransmitter.

II. 2.4. Energy balance and role of cerebral high energy

According to Batchelard /1975/ oxidative metabolism is regulated by the concentration or availability of substrates as ATP, citrate and by NAD^+/ /NADH ratio, ADP/ATP ratio and by some metabolic pathways as glycolysis, oxidative phosphorylation, electron transport chain and the rate of respiration. The ratio ADP/ATP is very important in glycolysis as well as in citric acid /T.C.A./ cycle and in oxidative phosphorylation.

The major use of energy, as ATP, in the brain is used in maintaining cation transport: about 40 % of brain ATP is used to maintain the intracellular concentration of K^+ and extracellular concentration of Na^+, against concentration gradients, by Na^+-K^+-activated ATP-ase of cellular membranes.

Role of energy buffer: cerebral cells are protected from large changes in the ATP/ADP ratio by consumption of creatine phosphate which acts as an 'energy-buffer' to maintain concentration of ATP in the following reaction:

$$\text{ADP + creatine - phosphate} \underset{\text{CK /mitochondr./}}{\overset{\text{CPK /cytoplasmic}}{\rightleftharpoons}} \text{ATP + creatine}$$

Creatine kinase in the brain is either mitochondrial, at the sites of ATP, and thus catalyse the formation of creatine phosphate at the expense of ATP or cytoplasmic /CPK/ at sites of ADP utilization which may act to regenerate ATP /Swanson, 1967/.

Role of cerebral ATP: ATP as a representative of high energy phosphates also share in the brain and nerve action in general and particularly in maintenance of cerebral cationic gradients, ammonia fixation and glutamine synthesis, synthesis of acetylcholine by acetylation of choline, acetylation of other amines and synthesis of sugar phosphates. ATP also plays a great role in other parts of the body than the brain, mainly in muscle contraction /West & Todd, 1955/.

II. 2.5. Metabolism of ATP

From a functional point of view ATP is the high energy phosphate concerned directly with metabolic processes in brain and other tissues. Other high energy phosphates are also important, largely because they react with ADP to keep up the concentration of ATP.

For example:

1,3 - diphosphoglyceric acid + ADP ⟷ ATP ---------- +3 phosphoglyceric acid

2 - phosphoenol pyruvic acid + ADP ⟷ ATP + pyruvic acid

creatine phosphate ---------- creatine

then:

+ ADP ⟷ ATP +

ATP ⟶ ADP + inorganic phosphate+ energy /for metabolic processes/

When ATP is used up and broken down to ADP, the latter takes high energy phosphate groups from diphosphoglyceric and phosphopyruvic acids formed in the anaerobic breakdown of carbohydrate, and from CP to reform ATP. Although some ATP is formed in the anaerobic breakdown of carbohydrate through the process indicated above, the larger quantity arises from the oxidation of carbohydrate, fats and amino acids /West and Todd, 1955/. In this process the excess ATP formed, above immediate needs reacts with creatine to form CP and ADP which is reconverted to ATP by oxidative energy, and the process repeated untill, in the well nourished resting tissue, there are high concentrations of both ATP and CP. When the tissue becomes active, ATP breaks down and is regenerated by the reactions shown above. When the tissue is practically exhausted, all the ATP and CP has been decomposed. CP thus serves as a reservoir of high energy phosphate groups which can be immediately made available as ATP when needed.

II. 3. Energy metabolism in abnormal conditions

Richter and Dawson /1948/ reported that evidence has been obtained that the metabolic activity of the brain is increased in convulsions, trauma, anoxia and

shock. This is indicated by the rise in lactic acid and decrease in high energy phosphates found in the cerebral tissues of animals killed under those conditions. In animals killed under anaesthesia, the reverse takes place, where there is a fall in the cerebral lactate and increase in the labile high energy phosphates.

On their study about brain metabolism in emotional excitement and in sleep the above workers also observed that the lactic acid content of the rat brain was reduced in sleep and increased in emotional excitement. The effect of ischaemia, electrical shock and electrical stimulation in increasing the metabolic rates and fluxes of high energy substrates had already been mentioned in the energy metabolism of the CNS.

II. 3.1. Hypoglycaemia

Is the decrease of blood glucose concentration below the normal level. Glucose is very important to the brain and hypoglycaemic symptoms result from its insufficiency. The symptoms are not due to energy failure because they occur, even if hypoglycaemia is produced by using insulin or deoxyglucose, before any change in cerebral high energy metabolites can be detected. It is thought that consumption of the endo-

genous substrates alternative go glucose e.g. glutamata, alanine, and GABA might result in changing the concentrations of brain excitatory and inhibitory compounds, such changes affect electrical activity and behaviour /Lewis et al., 1974/. Another mechanism is the possibility of a sensitivity of glucoreceptors in the brain to low glucose level.

II. 3.2. Hypoxia and ischaemia

It is well known that the oxygen tension of the air we breathe can be halved without obvious detriment to brain function or behaviour. The brain still receives the O_2 it needs, the CBF rate is raised and the brain can extract O_2 from the blood when the haemoglobin is less than 100 % saturated with O_2. However when O_2 falls to 7 % or 35 mmHg, there is increased utilization of glucose and increased production and no changes in the levels of the high-energy intermediates such as ATP or CP are observed /Cohen et al., 1973/. Glycolysis therefore is increased without change in subsequent oxidative metabolism. The increase in glycolysis is due to activation of hexokinase and pyruvate kinase /Duffy et al., 1972/. Hypoxia may also

affect the synthesis of amine transmitters such as dopamine, noradrenaline and serotonine because molecular O_2 is required for hydroxylation reactions involved in their formation, and recent studies suggest that these reactions are very sensitive to lowered O_2 tension.

II. 4. Pharmacological and physiological aspects of cerebral anoxia and ischaemia

II. 4.1. Energy in the normal and ischaemic brain

Cohen /1975/ reported that in the brain normally over 90 % of cerebral energy is formed through mechanisms utilizing O_2. The remaining energy is derived from degradation of glucose to lactate and pyruvate. In anoxia only O_2 supply is diminished. In anoxia aerobic metabolism stops and glycolysis is stimulated. The earliest biochemical reflection of O_2 defficiency in the brain and the CBF is the increased concentration of the end product of anaerobic glycolysis lactic acid.

In ischaemia, where glucose supply is diminished also, the stores of glucose and glycogen, which is a glucose polymer, are quickly exhausted and metabolism decreases or stops. Unlike other tissues the brain is

unable to employ compensatory mechanisms for maintenance of function and structure when O_2 supply fails. According to Cohen /1975/, clinically, humans can not tolerate more than 4-8 minutes of complete ischaemia, i.e. complete deprivation of blood from all the regions of the brain, without death or irreversible cerebral damage. While in the rats recovery of electrical activity, preservation of structure, and normality of some biochemical parameters were observed after 1 hour of ischaemia. The above worker concluded that normal nerve cells could withstand 60 min of complete ischaemia but that survival might be hindered by extraneural circulatory factors.

II. 4.2. Control of cerebral circulation

The normal arterial blood contains 20 volume % of O_2, all of which is combined with haemoglobin. Although the brain is completely dependent upon cardiovascular activity, there are intrinsic mechanism regulating delivery and utilization of O_2 for cerebral function. This is the so-called autoregulation which maintains O_2 utilization within a physiological range about 3.3 ml/100 g/min /Macilwain and Batchelard, 1971/. The cerebral flow highly depends on the pressure head i.e.

the mean arterial blood pressure of the total body at the level of the head, which is obtained by haemostatic mechanism including central control of the peripheral vascular tone and carotid sinus reflexes. The cerebral autoregulation is usually effective as long as the mean arterial pressure remains above 60-80 mmHg.

The cerebral flow /CBF/ is regulated by changes in cerebrovascular resistance /CVR/ which is the resultant of all factors tending to force blood flow through the brain, and by the intracranial pressure /ICP/ which also affect the CVR; because when it is increased, it compresses the cerebral vessels and causes their resistance. Blood viscosity also affects CBF, since decreased viscosity as in anaemia causes the CBF to increase. Pathological changes in the cerebral vessels eg. sclerosis also affect the resistance and flow.

The most and single important factor in the autoregulatory mechanism is the cerebrovascular tone /elasticity/ which regulates vasodilation or constriction. Neurogenic effects are of some minor role on the tone, sympathetic stimulation or sympathomimetics tend to decrease CBF /Harper et al., 1972/. Chemical factors have great effects on the vascular tone since

increased CO_2 in the arterial blood increases CBF; and inhalation of 5-7 % CO_2 increases CBF by 75 % and equally is effective 10 % O_2. Hyperventilation decreases CBF.

II. 4.3. Regulation of cerebral blood flow and its relation to the heart blood flow: the role of Adenosine as a common factor in the regulation of blood flow in heart and brain

The brain and heart regulation of blood flow are, to some extent, similar, although there are some differences concerning the relative responsiveness to the specific factors influencing the flow in each tissue.

According to Berne and Rubio /1977/ the factors controlling or influencing the flow in the brain and heart are mainly: neural factors, local factors, tissue pressure, myogenic and metabolic factors consisting of the integrated roles of potassium, osmolarity, CO_2 and O_2 tensions as well as the metabolic role of adenosine.

The local factors are mainly the autoregulation, due to which the brain flow remains constant regardless of alterations of the blood pressure /per-

fusion pressure/. Autoregulation is similar in both organs, heart and brain but is more prominent in the brain.

The tissue pressure, specially in the brain, being in a rigid enclosure and thus constant volume, does not seem to play a great role in the regulation of either cerebral or coronary flow because any increase in the B.P. is normally countered by an increase in the vascular resistance so that the cerebral capillary pressure and blood flow remain relatively constant. The myogenic factor was thought to be the myogenic response to alteration in B.P. namely: a constriction with increased and dilatation with decreased pressure of the pial arteries. This response is of minor importance compared to the metabolic response. It is thought that even this intravascular pressure of the pial vessels might mainly represent a response to metabolites from the brain caused by the alteration of perfusion pressure Berne and Rubio /1977/.

The metabolic factors are the most important ones regulating and affecting the cerebral and myocardial blood flow. Those factors are mainly related to the metabolic activity of the tissue and its oxygen supply. Several mediators have been proposed to

correlate and link parenchymal tissue metabolism to blood flow. The most important mediators are CO_2, oxygen and potassium concentrations, osmolarity and adenosine. Carbon dioxide /CO_2/ is considered the principal agent in the control of cerebral circulation /Lassen, 1974/. Hypercapnia highly increases the CBF and hypocapnia decreases it.

O_2-tension is known to produce dramatic effects on the cerebral and myocardial vascular resistance. A reduction in arterial pO_2 induces vasodilation while an increased pO_2 elicits vasoconstriction in a degree proportional to the changes in pO_2 /Lassen, 1974, and Rubio, 1974/. This effect of pO_2 could be due either to a direct action on the vascular smooth muscle or indirectly by the release of a vasodilator metabolite from the parenchymal tissue when the O_2 - supply becomes inadequate. It appears much more likely that a decrease in parenchymal tissue pO_2 via release of vasodilator metabolites, propably adenosine, might be responsible for changes in cerebral and coronary vascular resistance.

The physiological role of potassium /K^+/ as a mediator for cerebral or coronary blood flow is still in doubt. According to Berne and Rubio /1977/ a small increase in the extracellular K^+-concentration increa-

ses the vascular conductance in several tissues, and that in the heart, infusion of K^+ induces a slight increase /15 %/ in coronary flow and cardiac work. In the brain an increase of potassium in artificial CBF, bathing the pial vessels, induces vasodilation. Hypoxia was thought to cause a very slight change in the extracellular K^+ and yet this change /1 mM/ was able to produce a four — fold increase in CBF.

Osmolarity: In the brain, topically applied hypertonic solutions increased the diameter of the pial arterioles. But neither increased K^+ nor hyperosmolarity are not principal components in the mediation of metabolically induced vasodilation in brain and heart /Berne and Rubio, 1977/.

II. 4.4. Adenosine

Recent studies indicate that cerebral and coronary blood flow may be controlled mainly by the adenosine concentration of the interstitial fluid. It is well known that adenosine is formed in ischaemic cerebral and cardiac tissues. According to Berne et al. /1974/ adenosine is a potent vasodilator that readily crosses most of the cell membranes, it can arise from endogenous ATP under conditions of hypoxia and is ra-

pidly destroyed by enzymatic action or removed by washout, and so this nucleoside appears to be a good metabolic mediator of vasodilation.

Rubio et al. /1974/ observed that there is a striking parallelism between adenosine formation, adenosine release and coronary flow in hypoxic heart. Adenosine levels in the heart were also observed to increase several folds with increased cardiac work /by aortic constriction in rats/ and within a few seconds after the onset of ischaemia or hypoxia. The principal source of adenosine is the parenchymal tissue and is formed by the action of 5'-nucleotidase on AMP, which increases in concentration as a result of ATP degradation when O_2 supply is not sufficient to maintain normal oxidative phosphorylation. Some of the formed adenosine enters the interstitial fluid and reaches the resistance vessels thus inducing vasodilation. But the large fraction of adenosine is rephosphorylated to AMP by adenosine-kinase in heart cells. Some adenosine enters the vessels and is degraded to inosine by adenosine deaminase, and a large fraction of inosine is then split to hypoxanthine and ribose-1-phosphate in the capillary endothelium, pericytes and erythrocytes and is catalyzed by the nucleoside phosphorylase /Berne and Rubio, 1977/. Most of the myocardial nucleo-

tides are recovered via direct phosphorylation of adenosine, the so-called "salvage pathway". While the small amount that escape as degradation products in the venous blood are mainly replaced by "de novo synthesis" from small precursors and probably by synthesis from hypoxanthine in the blood.

The ischaemic brain also produces large amounts of adenosine, a fact which may reflect that the nucleoside might also play a vasodilator role in cerebral circulation.

Berne at al. /1974/ confirmed that adenosine is rapidly /within 2-3 sec/ formed in the brain; escapes into the CBF and is a potent dilator of cerebral arterioles when applied topically to the pial vessels, but not so when administered intraarterially. Intravascular administration of adenosine failed to reach the brain vascular smooth muscles because of the blood-brain barrier /BBB/. The pial vessels show a dose-response relationship to the topically applied adenosine. The intravenously administered adenosine is incorporated into cardiac nucleotides at a rate 20 times faster than into cerebral nucleotides because of the BB-barrier. Electrical stimulation of brain tissue as well as stimulation by seizures causes adenosine formation in brain tissue. Hyperventilation

and the resulting hypocapnia cause an increase in brain adenosine, probably secondary to the reduced CBF. Oppositely hypercapnia, which increases the CBF, causes a reduction in brain adenosine /Berne et al., 1974/. Adenosine and H^+ are thought to work in co-operation because both induce cerebral arteriolar dilation and contribute to the vasodilation observed in asphyxia, ischaemia and increased cerebral metabolism. However, hypercapnia and hypocapnia increases and decreases, respectively, the cerebral adenosine levels because of the CO_2-induced flow changes and alterations in brain O_2 supply to which adenosine production responds. The above observations as well as other recent studies on the heart and brain give an increasing body of evidence that a common factor on the control of blood flow in those two vital tissues is the nucleoside, adenosine. The other factors, mainly CO_2, H^+ /or pH/, K^+ and the myogenic activity contribute to the myocardial and cerebral blood flow, but the relationships between those metabolic mediators is still not yet clarified. Very recently on the mechanism of action of adenosine, certain studies on cultured vascular smooth muscle indicated that adenosine greatly enhances the cyclic AMP levels of those muscles; and that increase in cyclic AMP is usually associated with

relaxation or vasodilation. Another possibility for its action is that adenosine interferes with calcium uptake as shown by the latter workers in guinea pig atrium.

According to Szentmiklósi et al. /1976/ adenosine is released from cardiac and brain cells during hypoxia when the possibility of heart or brain ischaemia is increased, and that this adenosine was observed to have effects similar to those of hypoxia in the heart; and that those effects were significantly diminished by aminophylline, the competitive antagonist of adenosine. It is assumed that in the heart and brain hypoxia or ischaemia, the increased tissue levels of adenosine might contribute to the functional and metabolic impairments of the brain or heart.

II. 5. Complications of anoxia and ischaemia

The primary response to anoxia is an increase in CBF. In anoxia and ischaemia the CBF becomes sufficiently impaired, the brain begins to extract more O_2 per unit volume in an attempt to maintain oxidative metabolism. Thus the arterio venous O_2 difference /AVO_2/ is increased. When cerebral anoxia exceeds 5 min, the phenomenon of "No-reflow" occurs. On resto-

ration of O_2 supply, blood flow in ischaemic regions of the brain remains decreased due to diminished capillary activity in those areas. However, Cohen /1975/ reported that focal cerebral ischaemia in monkeys could exist for 2-24 hours without producing this phenomenon. Local "no reflow" phenomenon seems to be due to vascular damage because continuous generalized or focal anoxia paralyses cerebral autoregulatory mechanisms.

The foetus and newborns brains survive more severe anoxia than the adult brain. This difference has been attributed to a relatively greater role of glycolysis in energy production in the immature organisms /Himwich et al., 1942/, since in the foetus and newborns a main source of energy is obtained through glycolysis and since most of the metabolic enzymes have not been developed and not functioning in the newborns.

II. 5.2. Effect of carotid and bicarotid occlusion in relation to ischaemia & anoxia

Focal stagnant anoxia occurs whenever blood flow through a supplying local artery is unadequate. The carotid arteries provide the major suppliers of

cerebral circulation. Narrowing of the carotid lumen may result without production of any anoxic or ischaemic symptoms. Thus neurological symptoms in early life may not appear even if blood flow is absent in both carotids and vertebral arteries. Here blood is supplied through collaterals from the external carotid circulation. CBF is significantly decreased only when a carotid artery is narrowed over 90 % thus considerable stenosis of the lumen may result without producing any symptoms. Clinically, Cohen /1975/ mentioned that complete clamping of the carotid artery decreased CBF in most patients in whom the collaterals were not functioning well. There is extreme variation in ability to tolerate complete acute occlusion of one carotid artery. Occlusion of one carotid has been reported to result in abolition of auto-regulatory responses, thus preventing hypercapnia from increasing CBF. However increasing systemic blood pressure was effective in increasing CBF.

II. 6. Pharmacological approach to the prevention and treatment of cerebral ischaemia and anoxia

Despite the numerous investigations, satisfactory methods of treatment have not yet been employed.

The principles involved for primary management of cerebral ischaemia and anoxia have been directed towards:

1./ Prevention of further anoxic episodes by preventing further oxygen deficiency, caring to the careful function of the heart, circulation and blood pressure. Anoxic and anaemic anoxia should be treated.

2./ Increasing O_2 availability in defficient areas including attempts to deliver increased amounts of O_2 on a cellular level through: a./ increasing CBF, the inspired O_2 concentration, preventing cerebral vasodilation and decreasing the cerebral CO_2 concentration and by hyperventilation, b./ pharmacologically, through; - vasodilating agents as papaverine and hexobendine, c./ maintenance of cerebral circulation through the use of anticoagulants to a limited extent, to prevent dangers of haemorrhages, and/or the use of inhibitors of platelet adhesiveness such as dipyridamole /Persantin/, acetyl salicylic acid and salphinpyrazone to decrease adhesiveness and so to prevent cerebral occlusive diseases /Kalendovsky et al., 1975/.

3./ Reduction of cerebral edema: cerebral edema is a dangerous complication of anoxia because the swelling may be sufficient to eliminate CBF. Here

dehydrating agents as mannitol and glycerol have been employed. Dextran, steroids and dexamethazone are also beneficial to reduce edema and so increase CBF.

4./ Diminution of cerebral metabolic requirements: by means of hypothermia /body cooling/ which decreases the cerebral needs and energy requirements thus results in higher concentrations of high energy phosphates within the brain and so they have been effective in prolonging the tolerance to anoxia in the experimental animals /Cohen, 1975/.

5./ Increasing the availability of energy supplies: eg. by infusion of glucose with alkali, to decrease the lactate and CO_2 acidity effect /Dawes et al., 1964/. Pentoxifylline: which is a xanthine-derivative, reduces cerebral edema and treats experimental cerebral infarctions. It also increases energy availability through inhibition of break down of cyclic AMP-phosphodiesterase.

6./ Prevention of further tissue damage: - through maintainance of CBF. Acidosis is a significant consequent of anoxia, since increased concentrations of tissue lactic acid are the earliest changes reported. The brain, CSF and the CBF lactate increases with O_2 defficiency. Acidosis has a markedly detrimental effect on cerebral energy production /Cohen

et al., 1973/. Thus alkalosis was employed by Dawes et al. /1964/ to combat the acidosis when administered with glucose as an additional energy source.

Secondary treatment intended to correct pathophysiological phenomena such as coma, impaired respiratory exchange, dehydration, chemical and electrolyte imbalance are also important in this aspect.

II. 7. Experimental cerebral ischaemia

II. 7.1. Studies on the effects of ischaemia

The metabolism of a tissue totally deprived of its blood supply changes immediately from a steady - state condition, in which substrates are continually supplied and some end - products and by - products continually removed, to a "closed system".

Under such conditions of ischaemia, the following consequences occur: 1./ oxidative phosphorylation and metabolism stops or decreases, 2./ metabolism becomes dependent on endogenous substrates, 3./ end products accumulate, specially pyruvate and lactate, 4./ energy reserves decline, 5./ there is a tendency of reactions towards equilibrium and finally failure of function frequently following or followed by major structural disorders or disruption /Maker & Lehrer, 1971/.

The biochemical, pathological and neurophysiological mechanisms involved in ischaemic or anoxic brain have been discussed according to the results obtained by our experiments as well as from the collected results and data obtained by other workers in the same aspect. Although it is very difficult to correlate orderly such great data, obtained from different workers with different methodologies and accordingly different results and interpretations, yet it is the aim of this study to practice some of those experiments as well as to collect & compare the previous data and literature obtained by other workers. The cerebral blood flow /CBF/ is very important being highly related to brain metabolism. It is well known that in normal conditions, there is a precise link between regional metabolism and blood flow in the brain. In pathological states, there may be an uncoupling between CBF and metabolism due to many mechanisms /Maker & Lehrer, 1971/; in particular anaerobic glycolysis, membrane depolarization and altered neurotransmitter release, are relevant for the final damage of the tissue.

II. 7.2. Experimental models of brain ischaemia

Various methods which have been used in studying brain ischaemia were discussed by so many workers and subjected to critical evaluation regarding their advantages, limitations and the aim of using them. This is particularly important because workers in this field have not always considered critical sources of errors and methodological disadvantages which, must affect the validity and interpretation of the data obtained.

Experimental studies on brain ischaemia and infarction were done in the past by using extracranial arterial ligation in dogs and rabbits and recording the degree of neurological impairment and recovery. Today arterial ligation remains a popular and widely used model of human stroke for the production of brain ischaemia and infarction /Yatsu, 1975/. The different in vivo and in vitro experimental models of brain ischaemia could be summarized in the following.

In vivo models:

1./ Decapitation; 2./ vascular ligation; 3./ vascular ligation + hypoxia; 4./ vascular emboli /embolization/; 5./ exanguination /total bleeding/; 6./ isolated-head perfusion; 7./ increased CSF pressure; 8./ hypotension + hypoxia.

In vitro models:

1./ Brain slices; 2./ Subcellular organells: mitochondria, synaptosomes.

Each of those methods has both advantages and limitations. In vitro models of ischaemia such as the use of brain slices allow precise control of the experimental environment and evaluation of mechanisms at cellular or subcellular levels. For example enzymatic reactions and their kinetics, transport mechanism and functional activity as oxidative phosphorylation by isolated mitochondria can be studied precisely. But such slices or models being not intact are of less physiological environment.

On the other side results of in vivo models of cerebral ischaemia have greater application to humans and animals but the large number of pharmacological, physiological and metabolic variables applied upon an intact animal preparation render the interpretation less precise.

Inspite of the different disadvantages of both in vivo and in vitro models of brain ischaemia, each has provided a valuable information on the pathology and pharmacology of ischaemic infarction. These investigations, being searching for the basis of pathophysiology of the disease and on the different therapies

maximizing microcirculatory restoration and on diagnostic metabolic parameters or agents identifying viable ischaemic tissue, will improve the clinical efforts attempting to reduce ischaemic damage. The major experimental models of cerebral ischaemia as mentioned above, were applied and reviewed as follows:

1./ Vascular ligation and embolization: vascular ligation has been a popular and widely used method of creating cerebral ischaemia. Yatsu /1975/ pointed out that this technique most closely mimics clinical stroke. Vascular arterial embolization have been used by Kogure et al. /1974/ to avoid cranial surgery. Both ligation and embolization models have been greatly and successfully used by studies on CBF, cerebral edema, neurophysiological and neurochemical investigations. Levine /1960/ produced cerebral ischaemia by unilateral carotid artery ligation combined with hypoxia in rats. The hemisphere ipsilateral /on the same side/ to the ligation was found ischaemic; while the contralateral hemisphere anoxic. Recently, salford et al. /1971/ have shown that the biochemical and neuropathological alteration in the hemisphere ipsilateral to the carotid ligation were compatible with anoxic damage alone and not indicative of ischaemia.

Their study and their different interpretations shows the complexity of vascular occlusion because the degree of collateral circulation is variable in animals and more abundant than in humans.

2./ Exsanguination and hypotension: - this method involves either haemorrhagic shock or hypotension induced with ganglionic blockers such as Trimethaphan /Arfonad/. Exsanguination has been effectively utilized by Nilson /1971/ for biochemical studies on brain tissues. Limitations of such models are the possibilities of systemic metabolic effects that influence CBF and metabolism, as well as coagulation disturbances intravascularly.

3./ Isolated - head perfusion model: The isolation of cerebral circulation from systemic influences achieves greater control over the brain parameters. Accordingly this technique was used to trace biochemical changes in the brain along with neurophysiological parameters.

For cerebral ischaemia this technique offers no advantages over the existing methods, but remains as a powerful tool to study the in vivo effects of some specific substances on brain metabolism.

4./ Increased cerebrospinal fluid /CSF/ pressure: This technique offers the advantage of global, /li-

mited/ ischaemia while minimizing systemic metabolic effects which may influence cerebral metabolism. To produce global ischaemia limited to brain, exsanguination of the cerebrum has been accomplished by increasing the CSF pressure sufficiently to obstruct the arterial flow. Furthermore the increased pressure evacuates the cerebral venous system of blood.

According to Siesjö and Ljunggren /1973/, this method involves the infusion of artificial CSF, such as Elliot solution, into the subarachnoid space, with simultaneous monitoring of the CSF pressure.

5./ Hypotension plus hypoxia: - for purposes of producing global cerebral ischaemia without circulatory arrest, Yatsu et al. /1974/ reported an experimental model, involving the use of intravenous trimetophan to lower the B.P. in awake artificially-ventilated rabbits to achieve hypotension. Then for hypoxia 96 % N_2 and 4 % O_2 ventilation was introduced, and so ischaemia was observed.

Limitations of this model are the same as the "Exsanguination and hypotension" models. Also as with other in vivo operative techniques adequate anaesthetic, surgical and monitoring equipments are necessary.

6./ Brain slices and subcellular organells: - such organells as mitochondria, microsomes and synapto-

somes offer the opportunity of controlling and regulating specific metabolites and environmental conditions. This is advantageous in studying molecular mechanisms and action of pharmacological agents. But have the disadvantage that by separation of brain cells and their subcellular organells from their complex in vivo environment, they become in vitro model and such in vitro techniques are not physiological and of less possibility to be translated into clinical cerebral ischaemia. Only in vitro retinal tissues have the advantage of maintaining in situ cellular integrity and allowing neurophysiological studies /Webster & Ames, 1965/.

II. 7.3. Recent advances in experimental methods

Recently Villa et al. /1978/ used another new method of total ischaemia by clamping both carotid arteries and vertebral arteries of Beagle dogs. Other models were also produced by clamping the main cerebral arteries on monkeys. On rats and dogs some ischaemic models were produced by occluding or/and cutting the aorta.

Takahashi and Aprison /1964/ have introduced a new method called the near-freezing method by which it

was possible to obtain brain tissue for the determination of acetylcholine /ACH/ content in the specific brain areas. This method involves the immersion of the animals into liquid nitrogen for accurately measured short time intervals then decapitating and analysing the brain contents. For varification of this near--freezing technique, the workers compared the results with two other methods namely freezing completely in liquid nitrogen by immersion for 5 min, and the other was by decapitation with a Harward rat guillotine, removing the brain then freezing and analysing for ACH content. The main disadvantage of this near-freezing technique is the technical difficulty in measuring ACH content in specific areas of the brain rather than in the whole brain.

Many studies have used the method of Levine /1960/ to produce ischaemic lesions in rodent /rat/ brain. This method involved occlusion of one carotid artery followed by a period of anoxia; and is not suitable for measuring acute changes.

Kárpáti et al. /1978/ and Biró et al. /1976/ on studying the effects of Cavinton on cerebral ischaemia, produced ischaemia on conscious immobilized cats by inflating a blood pressure cuff wrapped around the neck after both carotids being occluded.

Unilateral anoxic ischaemic brain lesions were produced in adult rats killed by exsanguination during the phase of anoxia caused by increasing the flow of nitrogen, such method was used by Atkinson and Spector /1964/ in studying metabolism of glucose in anoxic ischaemic brain.

Fieschi et al. /1978/ developed a model of experimental focal transient cerebral ischaemia in rabbits, that closely approaches clinical situations in man. It is an embolic ischaemia produced by infusing ADP solution into the carotid artery.

However, a simple way to cause the complete cessation of blood flow to the brain is by decapitation. It is the most simplest and effective method of producing ischaemia. It has only one disadvantage; most of the studies have found higher rates of change of labile brain metabolites during freezing following decapitation than when the animals are frozen whole /Lowry et al., 1964/ and Thorn et al. /1958/. This finding has been attributed to the wide neuronal-discharge /depolarization/ following decapitation which stimulates metabolism above the normal levels. However the possibility that some circulation to the brain is maintained during freezing of the whole animal without decapitation is also a disadvantage in the determination of the normal levels

of substrates as well as the abnormal levels in ischaemia caused by a method other than decapitation.

Changes of temperature of the surroundings greatly affect the analysis of substrates. Thorn et al. /1958/ reported that a fall in brain temperature during an experimental procedure may slow the rate of metabolic change. Thus the temperature control is very important.

Anaesthesia also is a very important factor to be regarded during experimentation. Generally anaesthesia results in increase in brain glucose and glycogen and slowing of metabolism before and during ischaemia. It may also slow the changes induced by decapitation /Lowry et al., 1964; Gatfield et al. 1966/.

II. 7.4. Evaluation of the in vivo and in vitro models and freezing techniques

In the determination of brain metabolic rates, slices perhaps represent the most nearly physiological preparation for in vitro studies but they constitute only local samples of the brain whereas quantitative in vivo studies are made on the whole brain. Suspensions of whole brain in complete medium, corrected for loss of activity due to homogenization, might give a

better comparison but those suspensions have been studied only from small animals.

The most common method used for small animals is rapidly freezing the whole animal by total immersion in liquid nitrogen. This is based on the original method described by Granholm et al. /1968/, who froze brains of animals by pouring liquid N_2 over their exposed skulls where a plastic funnel was placed directly over the skull of the anaesthetized animal and liquid nitrogen was poured in. The whole--body freezing technique requires no anaesthetic but has the disadvantage that the animal briefly convulses. The funnel freezing technique has the disadvantage that anaesthesia in involved and required.

The in vivo techniques mentioned are more difficult and have some limitations due to the difficulties in varying the immediate environment of the brain cells, and the difficulties in conducting many chemicals or neutrients from the blood stream to the brain, as well as the types of cells of the CNS being heterogenous, it is difficult to distinguish the different aspects of metabolism in each type of them, and accordingly regional brain metabolism would be very difficult to trace.

Because of those limitations the in vitro techni-

ques are mainly developed with the object of overcoming those limitations. But still, inspite of their development, the in vitro techniques themselves have their own inherent limitations namely: 1./ many cerebral functions depend on the architectural and structural relationships between cells and their processes in variqus regions of the brain, some of which are certainly to be disrupted or stopped; and 2./ the tested cells in the tissue of brain analysed is greatly affected by the outside or out- of -body medium in vitro; as well as the concentration of some sensitive metabolites, mainly lactate, is removed quickly from the brain to the venous blood in situ but remains in the immediate environment of the cells in vitro.

However, these in vitro techniques have contributed significantly to our knowledge of cerebral function and to study metabolic and neurophysiological phenomena through studies on the effects of membrane depolarization on the cerebral metabolic rate /Macilwain, 1955/. Those techniques also allowed a good medium to study the central effects of barbiturates, chlorpromazine and other metabolic and psychogenic agents on cerebral metabolism /Basharahil, 1975/.

II. 8. Protection of the ischaemic brain

II. 8.1. Protective means valuable in preventing ischaemic brain damage

Many experimental trials were directed towards the phenomenon of protecting the brain and preserving its biochemical function from ischaemic damage. This phenomenon operates mainly by reducing the brain metabolism through hypothermia and/or anaesthesia. According to Yatsu /1975/ those experimental attempts were directed towards the energy-related processes namely: the increment of energy synthesis via mitochondrial oxidative phosphorylation and the energy charge of the adenylate pool; as well as the decrement of the energy-depending processes.

The experimental and clinical protective anti-ischaemic therapies were classified by Meyer et al. /1972/ according to their ability to 1./ increase the cerebral flow - CBF - to the ischaemic areas, 2./ reduce cerebral and vascular edema; and 3./ reduce blood coagulation and platelet aggregation.

The main factors affecting the recovery of the ischaemic tissue are: microcirculatory restoration, cerebral and vascular edema, viability of tissue plas-

ticity, neuronal regeneration and the neurophysiological and chemical functions.

Hypothermia as a means of reducing ischaemic infarctions did not yet recieved sufficient clinical trials, but it was observed, experimentally, that it prolonged the cerebral function following circulatory arrest, and that the brain metabolism was significantly reduced when halothane anaesthesia was combined with hypothermia /30°C/. Yatsu /1975/ also observed that the rate of survival after circulatory arrest for 5 min, was increased by hypothermia. Kramer et al. /1968/ observed that hypothermia increased the ischaemic cerebral ATP by 3-4 folds.

The mechanism of cerebral protection by hypothermia is not yet clear, but reduced metabolism seems to play a great role. As a reflection of cerebral metabolic rate /$CMRO_2$/, hypothermia reduces the conduction of peripheral nerves, reduces the neuromuscular transmission and depresses the subcortical and spinal responses to peripheral stimulation /Yatsu, 1975/.

II. 8.2. Anaesthetic protection and barbiturate anesthesia in cerebral ischaemia

According to Cohen /1975/ barbiturates are used beside hypothermia as a means of decreasing the cereb-

ral needs and has been experimentally employed with drug therapy. Barbiturates have also been effective in preventing as well as in prolonging the tolerance to anoxia and ischaemia in experimental animals /Cohen, 1973/. Wilhjelm and Arnfold /1965/ demonstrated an increased tolerance of mice and protective effect to hypoxia with different anaesthetics as thiopental, halothane and cyclopropane.

Mechanisms accounting for the anaesthetic protection were not yet investigated, but prevention of seizures was believed to play a role as well as the reduction of the cerebral metabolic demands and diminution of the cerebral energetic requirements, a mechanism which is shared also by hypothermia.

Nilsson /1971/ found protection with barbiturates in rats subjected to cerebral haemorrhagic shock. Yatsu /1975/ and Nilsson /1971/ concluded that the reduced fall of cerebral ATP supports the view that slowed metabolism is a mechanism by which certain anaesthetics, mainly barbiturates, provide cerebral protection. This was the metabolic mechanism of anaesthetic protection.

However, Smith et al. /1974/ thought that the protective action of barbiturates is probably through the reduction of the cerebral flow /CBF/ and intracra-

nial pressure and not on biochemical consequences to ischaemia. But even this view, according to the experimental data of those workers, suggest that the better improvement observed was in part a consequence of reduced cerebral metabolism. Most of the workers give evidence to the metabolic mode of action. Lowry et al. /1964/ & Gatfield et al. /1966/ observed that anaesthesia increases the brain glucose and glycogen and slows the changes induced by decapitation. Batchelard /1975/ reported that phenobarbitone inhibits glycolysis and increases the concentration of glucose, glucose-6-phosphate, and fructose-6-phosphate, and decreases the concentration of fructose diphosphate and the subsequent glycolytic substrates in ischaemic brains.

Anaesthetics at low concentration, inhibit the respiration of brain tissue /Quastel, 1955/ and thus decrease the O_2 consumption of the brain, accordingly cerebral activity, glucose and glutamate oxidation as well as the acetylation processes, mainly of choline, are all disturbed. The main action of anaesthetics is on the oxidative part of respiration and metabolism. In contrast to other tissues, the brain is very sensitive to anaesthetics because the carbohydrate breakdown is the main feature of its metabolism and thus the specific inhibitory effects of anaesthetics, and

narcotics in general, in glucose breakdown, affects all the oxidative mechanisms of carbohydrates.

Brody and Bain /1951/ claimed that barbiturates, at low concentration, cause an uncoupling of phosphorylation from oxidation through inhibited oxidative synthesis of ATP.

Richter and Dawson /1948/ reported that in the brain of anaesthetized animals there is a fall in lactate and increase in the labile high energy phosphates. Nembutal /pentobarbitone/ inhibits the formation of high energy phosphate bonds by brain tissue by interfering with O_2 utilization. Clinically, Yatsu /1974/ designed a clinical use of barbiturates in thrombotic strokes.

A multiple mechanism of action has recently been suggested by /Nemoto, 1977/ for the protective action of barbiturates on the ischaemic brain. This mechanism is thought to be mediated through: 1./ reduction of brain O_2 metabolism, 2./ reduction of the post-ischaemic osmotic gradients within the brain to prevent hypoperfusion and thus to aid the restoration of autoregulation, 3./ prevention of cerebral edema by preventing the post-ischaemic brain hyperosmolality and finally, 4./ by stabilizing the lysosomal membranes to prevent enzyme activation which results in cytolysis and infarction. This multiple mechanism was confirmed, to

some extent, by Smith et al. /1974/ who also evidenced Nemoto general findings which suggested that thiopental may block the oxidation of lactate thus reduce the ischaemic brain O_2 demands and reduce the post-ischaemic development of continued hypoxia and no-reflow phenomenon caused by the hypoperfusion.

II. 9.1. The cerebral effects of some circulatory and metabolic drugs

The drugs investigated in this study were: Pentobarbital /Nembutal/ a well known anaesthetic and cerebral depressant of the barbiturate group which was investigated to see its protective effects to the ischaemic brain, cavinton: a newly introduced to the market and clinically to the medical use by the Hungarian chemical works of Gedeon Richter Ltd. /Budapest/ as a cerebral vasodilator and metabolic oxygenator; Chinoin CH-102 which is a new isoquinoline derivative synthetized by Chinoin Pharmaceutical Works - Budapest; and Papaverin which is a well known vasodilator extensively studied in cardiovascular experiments but yet slightly investigated as a cerebral vasodilator or cerebral metabolic modifier. Nitroglycerin and inosine were also studied to investigate if they can provide any protective effects to the ischaemic brain.

II. 9.2. Development of two new Hungarian cerebrovascular agents: Cavinton and CH-102

II. 9.2.1. Cerebrovascular actions of Cavinton

Cavinton has a main specific dilatory action on the cerebral vessels. It improves the O_2 utilization and brain tolerance to anoxia. It slightly decreases the mean B.P., the cerebral vascular resistance and the total peripheral resistance. According to Cavinton Literature /1979/, cavinton increases remarkably, about 40 %, the cerebral nutritive flow due to its active vasodilatory effects. It also prevent the effect of aggregating agents and so inhibits the thrombocyte aggregation and adhesion to the wall of the vessels.

Biochemically, cavinton increases the tissue concentration of cyclic AMP, serotonin and ATP, which favourably influence brain function. It increases the aerobic and anaerobic glucose metabolism. According to Rosdy et al. /1976/ this compound has a main influence on the biogenic amine levels of the rat brain, where noradrenaline, 5-HT and dopamine were increased after its administration. According to Kárpáti et al. /1978/ and Biró et al. /1976/ cavinton is very active in increasing cerebral flow /CBF/, cardiac output and the heart rate. The circulatory peak effects are reached within one minute after the i.v. injections. Pál

/1979/ reported that, clinically, cavinton improves cerebral blood supply and increases the O_2 consumption of cerebral cells. Due to this effect it produces a favourable circulatory and energetic conditions in cerebrovascular diseases as well as in retinal and auditory circulatory diseases.

The most important pharmacological actions of cavinton are those exerted on the cerebral and systemic circulation. According to Török /1967/ cavinton: a./ elicits dilatation and increases the blood flow of the cerebral vessels in the whole brain, especially in the cortex, thalamus and hypothalamus. Its onset of action was rapid and long-lasting, b./ dilates the meningeal vessels in rats and the capillary area as shown by the increased nutritive flow, c./ exerts a potent cerebral vasodilatation that enhances the cerebral metabolism. According to those properties it was concluded /Remedia Richter, 1977/ that cavinton in animal experiments, generally improves the cerebral perfusion and the O_2 supply of the brain thus aids brain regeneration induced by hypoxia.

Mode of action: The different mechanisms by which cavinton is thought to act could be summarized in the following points: 1./ through vascular effects namely: smooth muscle relaxation and thrombocyte aggregation or adhesion inhibiting effects. According to Bencsath

et al. /1976/ cavinton inhibits adenosine-transport through the membrane of the red-cells, thus adenosine constantly appears in plasma. According to Berne et al. /1974/ adenosine plays an important role in the normal regulation of CBF on one hand and as a thrombocyte-adhesion inhibiting agent on the other hand /Cavinton medical Literature, 1979/.

2./ Through increased O_2 - metabolism of the brain: where cavinton promotes the O_2 utilization of cerebral cells, increases the physiological vasodilatation in hypoxia by improving autoregulation and increasing blood adenosine. It also increases the tolerance of the brain cells to hypoxia.

3./ Through the energy state of the brain: where cavinton increases the tissue concentration of cyclic AMP, by inhibiting phosphodiesterase and stimulating adenyl cyclase, thus favourably influencing the cerebral function and metabolic processes, by increasing the level of carbohydrate substrates and ATP, and increasing the blood flow and the local perfusion pressure, through increasing the brain level of catecholamines /Cavinton Literature, 1979/.

The metabolic effects of cavinton have an anabolic tendency in the CNS, by increasing tissue nutrition and improving the energetic conditions, thus increasing the brain tolerance against harmful circulatory

influences or O_2-insufficiencies. Cyclic AMP-phosphodiesterase /PDE/ is more intensively inhibited by cavinton than by theophyllin. Catecholamines are known to increase the synthesis of cyclic AMP. And cavinton was observed to increase the levels of noradrenaline and dopamine in the brain of rats/Rosdy et al. 1976/. Cavinton increases the ATP level at the expense of AMP, probably by increasing the level of cyclic AMP which is responsible for activating the 6-phosphofructokinase, an enzyme which regulates glucose metabolism. Cavinton has been shown to increase the available carbohydrate substrates, the blood glucose level and the glycogen content of the liver and heart.

The therapeutic indication fields of cavinton were reported by Braun Pál /1979/ to be neuropsychiatric, otological and ophthalmological ones.

II. 9.2.2. Chinoin "CH-102"

CH-102 or /HE-165/ is a new isoquinoline derivative recently synthetized at the Chemical Research Dept. of Chinoin Pharmaceutical Works /Budapest/ and it is still under investigations in our institute for its myocardial haemodynamic and antianginal properties. Some of the pharmacological properties of CH-102 have already been studied at the Department of Pharmacology

of Szeged Medical University.

According to Szekeres /1977/ and Krassói et al. /1977/ oral or intravenous administration of this agent in low doses, proved to be a highly potent antianginal agent with long lasting effect, while with an intravenous high dosage a very marked respiratory stimulation, was observed. CH-102 was found to stimulate respiration in normal animals as well as in animals with respiratory depression. It was found to decrease the vascular resistance, especially of the coronaries and thus exert its favourable antianginal effect, partly at least, through this resistance - lowering and secondly through increasing the venous system capacity. The metabolic effects of this compound on ischaemic heart model were studied by Takáts et al. /1977/. CH-102 was found to increase the myocardial nutritional circulation and so it has beneficial antiischaemic - antianginal effects in experimental angina, but there is no correlation between its coronary flow increase and its protective effect against ischaemic ST-segment changes. Haemodynamic parameters, contractility, B.P. and cardiac output, as well as metabolic changes, O_2 and lactate uptake, reflected its protection against ischaemic alterations. Metabolically; Takáts et al. /1973 and 1977/ observed that on the ischaemic heart model, CH-102 inhibited or prevented the decrease in

ATP and CP and the elevation of lactate induced by ischaemia. Following ischaemia, the lowest ADP level was observed after pretreatment with CH-102 /Takáts I. et al., 1973; and Szekeres, 1977/. According to the above observations it was concluded that CH-102 is a new potential antianginal drug, potent coronary dilator that increases blood flow not only in the large coronary arteries, but also enhances the capillary "nutritional" circulation of the normal and ischaemic myocardium. Under the influence of CH-102 the myocardial O_2 uptake and the heart O_2 supply/O_2 demand ratio was increased with consequent improvement of myocardial oxygenation.

This drug was also reported to exhibit a marked antianginal action in experimental coronary insufficiency. It has a very favourable therapeutic ratio and exerts a long lasting antianginal activity after oral administration /Szekeres et al., 1976/.

CH-102 was proved to exert a dose - dependant long lasting increase in total coronary flow through coronary vasodilatation.

On coronary occlusion, the drug temporarily improved the circulation to the ischaemic area, as well as to the non-infarcted area /Szekeres et al., 1977/. On other vascular regions, the drug slightly reduced

the resistance of the pulmonary, femoral and carotid arteries, accordingly their arterial flow was slightly increased. The decrease of resistance of the carotid arteries may play a great role in increasing the cerebral flow to the brain.

II. 9.3. Vasodilator drugs in cerebrovascular use

Review of literature here will be limited to the nitrites, the group of nitroglycerin, and papaverin. While the nitrites have widespread clinical use, there is relatively little experimental work on their cerebrovascular actions. Papaverine currently has little therapeutic uses but according to Bass and Toole /1975/ it is considered the standard agent against which cerebral vasodilators are compared.

In contrast to hypertension, the therapy of hypoxic or ischaemic flow insufficiencies is directed towards the correction of the inbalance between the tissue requirements and its delivery and removal of various materials by the blood stream, specially O_2--delivery.

II. 9.3.1. The Nitrites

The term "nitrite" refers to a group of therapeutic agents that includes organic and inorganic nit-

rates and nitrites. Only the organic members are used as vasodilators, especially, nitroglycerin /glyceryl trinitrate/.

The mechanism of action of nitrites is principally acting by relaxing smooth muscles, regardless of innervation or location. Bass and Toole /1975/ mentioned that the main characteristics of its cardiovascular actions are the dilatations of all blood vessels, specially capacitance vessels, leading to a fall in venous return, cardiac output and blood pressure. Pharmacologically the basic action and all the complex effects of nitrites in the different organs or systems could be interpretated in terms of an effective, non-specific relaxation of smooth muscles /Goodman and Gilman, 1965/. On the cardiovascular system: - the vascular smooth muscle relaxation is the most important that leads to decreased vascular resistance and mean systemic arterial pressure.

The most important is the effect on coronary vessels of which the relaxation and vasodilatation are the main mechanisms utilized in the treatment of angina pectoris. The coronary dilatation produced by nitroglycerin is less than that which can be induced by Papaverin or aminophylline /Goodman and Gilman, 1965/. On the heart muscle: nitrites might have the possibility of altering some of the myocardial meta-

bolism, and changes in cardiac performance appear to be secondary to actions on vascular smooth muscle.

Recent studies proved that nitroglycerin is a clinically active agent with strong coronary vasodilator effects. It possesses a protective effect against heart ischaemic ST-segment elevation, lactate uptake diminution and is able to increase coronary flow in the ischaemic heart and to reduce the size of the ischaemic foci. Szekeres /1977/ reported that the beneficial effects of nitroglycerin is its ability to induce large coronary arterial dilatation and increased collateral flow and to cause redistribution of myocardial blood flow from epicardial to endocardial regions.

On the cerebral /meningeal/ vessels: nitrites are very effective vasodilators. Some increase in intracranial pressure may accompany the dilatation of cerebral vessels but of insufficient magnitude or duration to be dangerous /Goodman and Gilman, 1965/.

The therapeutic uses of nitrites /Nitroglycerin/ are: 1./ In angina pectoris, where nitroglycerin may improve angina by reducing the demand or by increasing the supply of O_2. The simplest interpretation of the remarkable effect of nitroglycerin is its improvement of the blood flow to ischaemic areas, in the myocardium, by dilating coronary vessels. Accordingly,

the different systemic therapeutic uses of nitroglycerin are angina pectoris, paroxysmal noctural dyspnoe and acute myocardial infarctions /Bass and Toole, 1975/. 2./ Neurologically: nitrites were shown to be potent cerebral vasodilators. Pial arteries, veins and minute vessels were dilated by them. Nitrites transiently increased the flow /CBF/. Local application of nitroglycerin improved the middle cerebral artery spasms /Bass and Toole, 1975/. It is unfortunate that the above neurological observations did not lead to clinical use in cerebrovascular diseases, may be because of the short duration of action and the potential side effect of nitrites in decreasing the cerebral flow due to hypotension. Accordingly the neurological uses of nitrites are those which require elevation of CBF and the reduction of vasospasms.

II. 9.3.2. Papaverin

The main pharmacological effect of papaverine is smooth muscle relaxation. In addition, it has a moderate quinidine-like effect on the heart and coronary vasodilator properties /Goth, 1974/. Papaverine is the classical vasodilator among non-specific smooth muscle relaxants and its effect on vascular smooth muscle are more prominent. Szekeres et al. /1967 and

1976/ have interpretated the disadvantage of papaverine in the cardiovascular system as being possessing a strong coronary dilating effect, thus it causes an increase in the heart rate and contractility leading to increased myocardial O_2 consumption, which is harmful to the ischaemic changes. The same disadvantage might be also exerted in the brain tissue and cerebral circulation and this might be the reason why papaverin is not used or not clinically prefered in cerebrovascular insufficiencies.

However inspite of that, and in addition to its action on coronary vessels, papaverin is capable of relaxing other arteries thus widely used in vasospasms accompanying peripheral arterial embolism, pulmonary embolism and cerebrovascular thrombosis /Goth, 1974/.

The mechanism of most actions of papaverin is thought to be the direct smooth muscle relaxation. A number of studies have dealt with its antagonism of cyclic AMP - phosphodiesterase, because its vasodilation correlates with elevated levels of cAMP. This is a result of impaired degradation of cAMP by this enzyme.

Oishi et al. /1978/ confirmed that papaverin inhibits phosphodiesterase thus leads to increased cyclic - AMP, and that its vasodilating mechanism is different from that of Ca^{++}-antagonists. The cerebral

vasodilation caused by papaverin was thought to be a direct action /Oishi et al., 1978/, not influenced by propranolol, atropine or diphenhydramine, i.e. adrenergic, cholinergic or histaminergic mechanisms were not involved in its action. On the other side, the antianginal action of papaverin, as well as prenylamine, was shown to be partly mediated through adrenergic mechanism via β-receptors thus causing coronary vasodilatation, increased metabolic rate and coronary flow /Szekeres, 1977/. Accordingly selective β-blockers as propranolol and pronethalol can block or diminish the latter effects of papaverine on the heart, but not on the brain. On the heart metabolism papaverin improved the myocardial oxygenation through increasing the O_2 supply/O_2-demand ratio, an effect which was potentiated after β-adrenoceptor blockade /Szekeres et al., 1967/.

The main pharmacological actions of Papaverin are relaxation of the cardiac and smooth muscles specially of the larger arteries. This is direct antispasmodic action irrespective of innervation and includes the coronary, cerebral, peripheral and pulmonary arteries musclature, specially when the vessels are in spasm as in pulmonary or peripheral embolism /Goodman and Gilman, 1965/.

On the CNS and cerebral vessels: - Its direct va-

sodilating action on the cerebral vessels increases CBF, and decreases the cerebrovascular resistance and in some hypoxic conditions it restores the cerebral oxygen utilization to normal. These effects may explain its benefit in cerebrovascular insufficiencies. Neurologically papaverin is used in cerebral infarctions, migraine, experimental vasospasms, where it relaxes smooth muscles and produces vasodilation and increased CBF. It increases the regional CBF in regions of ischaemia, accordingly the oxygen availability to the brain of such patients increases /Bass & Toole, 1975/.

II. 9.4. Inosine

Inosine is a well known metabolite resulting from the breakdown of adenosine by oxidative deamination or from inosine monophosphate by dephosphorylation. Its concentration increases on the heart or brain tissue on cases of hypoxia or ischaemia. It is thought that the body uses this metabolite by successive enzymatic phosphorylations to produce the adenyl high energy phosphates specially ATP.

According to Leprán I. /not published/ and, Leprán and Szekeres /1980/ it was proved to have a good positive inotropic effect on the heart, without deteriorating the O_2 supply/O_2 demand ratio, thus increasing

the myocardial contraction in acute myocardial ischaemia. It also balances the myocardial and other tissues demand and supply of O_2. Inosine is mainly thought to exert a regulatory role in myocardial and cerebral circulations, similar to that of adenosine; as well as a protective effect in hypoxic heart or brain. The primary action mechanism of this protective effect is still controversial. It is not yet clear whether direct coronary vasodilating effect or some other metabolic effect takes place. Berne and Rubio /1977/ have shown that exogenous inosine is able to incorporate into myocardial nucleotides and provides ready substrate for ATP resynthesis. Liang et al. /1980/ reported that inosine increases the collateral perfusion of the ischaemic myocardium following coronary occlusion; and recently those latter workers also have found that inosine antagonizes the cyclic AMP phosphodiesterase in a dose - dependent manner. More recently, Leprán and Szekeres /1980/ in a trial to study the effect of inosine in some other types of O_2 defficiencies, proved that: - a./ in coronary artery ligation in rats, pretreatment with inosine /200 mg/kg i.p./ resulted in a significantly higher survival rate after occlusion; and that on isoprenaline - induced myocardial necrosis, improvement was observed with inosine which produced a 67 % protection against necrosis and necrotic

enzymes. On cerebral ischaemia caused by bilateral carotid occlusion and transsection under ether anaesthesia the above workers observed that severe ischaemia took place and that inosine treatment resulted in increasing the survival rate significantly from 10 % in the control, to 64 % within 20 hours. Those recent investigations demonstrate a prominent protective effect of inosine in several types of tissue anoxia or ischaemia.

In a trial to investigate the mechanism of action of inosine and inosine monophosphate /IMP/ in the brain, Liang and coworkers /1980/ observed that brain cyclic AMP-phosphodiesterase catalyses the hydrolysis of both cyclic AMP and cyclic GMP, and that inosine, IMP and hypoxanthine competitively inhibited the hydrolysis of cAMP and cGMP by the brain cyclic nucleotide phosphodiesterase. The order of potency of their inhibiton was inosine, IMP, then hypoxanthine respectively. Accordingly; inosine, IMP and hypoxanthine all inhibit the hydrolysis of cAMP by a competitive mechanism through one common site of action, thus leading to inhibited binding of cAMP to the enzyme cAMP-phosphodiesterase /Liang et al., 1980/. This mechanism seems to be similar to that suggested by Berne and Rubio /1977/ for the mechanism of action of adenosine in the heart and brain as a vasodilatory agent to the coronary and cerebral vessels.

III. MATERIAL AND METHODS

All the experiments were performed on adult male wistar rats of average weight 200-250 mg, feeded with standard diet and normal tap water. Wherever anaesthesia was necessary pentobarbital /nembutal/ 45 mg/kg i.p. was administered. The freezing techniques and the application of the ischaemic models were described in the appropriate experiments.

III. 1. Experimental procedures

During this study, many experiments have been carried over; the following outlines show the main experiments we adopted:

1./ Determination of the normal values of rat brain substrates and metabolites.

2./ Application and effect of ischaemia on rat-brain caused by decapitation: and a./ effect of 15 sec. ischaemia /before freezing of the head/,
b./ effect of 30 sec. ischaemia /before freezing of the head/ on brain substrates, energy charge /ECP/ and Bioenergetic Index /B.I./.

3./ Application and effects of ischaemia on rat brain caused by bilateral carotid occlusion and transsec-

tion for:

a./ 5 min, 15 min, 60 min and 240 min before freezing, against control experiments with anaesthesia,

b./ 4 hours before freezing in control with the normal values,

c./ 1-2 day before freezing.

4./ Application of ischaemia on rat brains by bilateral carotid occlusion only /no transsection/ for 3 days before freezing.

5./ A study of the effect of 15 min barbiturate anaesthesia on the normal levels of substrates and high energy phosphate-ester levels of rat brain; and utilization of such anaesthesia as a protective mechanism against the onset and consequences of cerebral ischaemia.

6./ The effect of combined cerebral ischaemia for 15 min and anaesthesia on the normal metabolism of the rat brain. The above experiments were the pathophysiological part of the dissertation.

The pharmacological part was concerned with the protective effect of different drugs on the metabolism of the ischaemic brain; and was consisting of the following experiments:

7./ Study of the effect of CH-102, cavinton and papaverin on the high energy phosphates, lactate, bioener-

getic Index /B.I./ and energy charge potential of the ischaemic rat brain.

8./ Comparative study of the protective effect of Nitroglycerin, Inosine and Papaverin on the metabolic parameters of the ischaemic rat brain.

III. 2. Experiment /1/: Determination of the normal values of rat brain substrates

The determination of normal values of brain metabolites, which is an indicator expressing the normal rate of rat brain metabolism, is very important and considered the basis for such research. Because, by these values the other abnormal values, caused by abnormal physiological or pathological mechanisms as in ischaemia, anoxia or hypothermia; as well as the study of cerebral pharmacologically active agents, could be compared. Without such comparison, it is difficult to identify such abnormal effects or mechanisms. The values of our results were compared with those of other workers on the same purpose using rats or mice so as to be sure and satisfied of our own results & interpretations.

III. 3. Experimental procedures and techniques

3.1. Animals used were white male wistar rats of average body weight 250-300 g.

3.2. No anaesthesia was used here since anaesthesia will affect the normal metabolism and thus the normal values.

3.3. Reagents used: are all pure & analytical, obtained from Boehringer Mannheim GMBH diagnostica - Biochemica; except Nicotinamide adenine dinucleotide /NAD/ and LDH crystals which were obtained from Reanal /Budapest-Hungary/. The general method of freezing, extraction and neutralization was used according to the principles of Bergmeyer /1974/ and Hans Adam /1974/.

3.4. Freezing: - Liquid nitrogen has been used as the freezing substance, since it has a temp. of -186 ^{o}C. The head of the rat, or even better the total body of the rat, had been immersed in liquid nitrogen for a sufficient period of time /2-3 min/, so that all enzymatic activity of the brain could be stopped as soon as possible. In case of other experiments where decapitation is required, then only the separated decapitated head has to be directly and quickly submerged in liquid Nitrogen. The separated head was broken with great care to prevent any pieces of bones from mixing with the brain tissue. Sufficient amount of the pure frozen brain tissue was taken /400-500 mg/ in a deep previously-cooled porcelain mortar containing liquid Nitrogen. This frozen tissue had been devided into two parts, each about 200 mg, in a separate mortar contai-

ning liquid nitrogen. The weight of the brain tissue in each mortar had been accurately measured.

3.5. Deproteinization & extraction: - Here the best agent used to carry both processes is perchloric acid, 0.5 normal solution. This solution can extract the acid-soluble components and deproteinize proteins & enzymes which can be easily removed by centrifugation 7000 r.p.m., for 10 min. The supernatant was neutralized by 3M K_2CO_3. a./ To the brain sample in the first mortar 10 times its weight of 0.5 N perchloric acid was added, carefully grinded with pestle, with repeated additions of liquid nitrogen and thoroughly mixed to be homogeneous. This portion was kept for enzymatic determinations.

b./ To the tissue in the other mortar 50 times the weight of 0.5 N perchloric acid was added, well grinded, homogenized and kept for the chemical Non-enzymatic determinations of inorganic phosphate, total phosphate and creatine phosphate.

c./ Centrifugation: the amounts of already pulverized homogenous mixture of the tissue and acid in each mortar, were centrifuged after melting, in Janeczky K24 centrifuge at $-4^{o}C$ with 7000 r.p.m. for 10 minutes. Supernatants were separated or pipetted into clean dry labelled-test tubes for subsequent analysis.

3.6. Neutralization: - Only for the enzymatic determination supernatants have to be neutralized. Non--enzymatic chemical-determinations need no neutralization because their analytical technique is not affected by acids, as well as the reaction medium for the chemical determinations, usually for inorganic and total phosphates needs to be acidis. For the enzymatic analysis the sample must be later neutralized by a solution of K_2CO_3 so as not to affect enzymes.

1 ml portions of the centrifugated perchloric acid extracts were pipetted into clean dry test-tubes, then titrated with 3M K_2CO_3 solution using a 0.2 ml capillary pipette and methyl orange as indicator, with continuous stirring till the end point; /usually the volume of potassium carbonate used or required is about 10 % the volume of supernatant used/. The neutral solution was allowed to stand for 10 min in ice water then decanted; or could be directly centrifuged to remove the precipitated $KClO_4$ /potassium perchlorate/ then the neutral supernatant was immediately analysed /0.1-0.2 ml samples/ for determination of ATP and CP, which are hydrolysed on standing, then for other enzymatic determinations of ADP, AMP and lactate as in the following procedures.

III. 3.7. Non-enzymatic analysis /Wahler & Wollenberger 1958/: Chemical-spectrophotometric determinations of inorganic phosphate /IP/, total phosphate /P_t/ & creatine phosphate /CP/

3.7.1. Total phosphate content /P_t/ determination

1./ From the perchloric acid extract 1 ml was pipetted in a stoppered centrifugation tubes. 3 mls of 0.005 M sodium molybdenate was added to it. The mixture was left for 1/2 hour then 4 mls of Isopropyl acetate was added; shaken for 30 seconds in an ice bath & centrifuged. 2 mls of the upper phase was pipetted into a clean dry test tube. About 150 mg of stannous chloride /$SnCl_2$/ powder was added to the supernatant and dissolved. 1 ml of cellosolve was added, well shaken and poured into a clean glass cuvette for spectrophotometric measurement. Another blank cuvette was prepared containing only 3 mls of cellosolve. Concentrations were read spectrophotometrically after 15 minutes at wavelength 710 nm and filament lamp. The extinction /E_{P_t}/ was read. P_t is refering to the total phosphate.

2./ For calculation: - From the standard curve of phosphate content the concentration of the total phosphate was calculated as: P_t = extinction E_{P_t} x 0.0175 in µ mol/g.

Notes:

1./ In the above procedure for P_t determination, the molybdenate was mixed after half an hour with isopropyl acetate. This time was enough for molybdenate to react with creatine phosphate and thus to split it into creatine and phosphate CP $\longrightarrow$ Cr + P, and so we get the total phosphate P_t = P + IP.

2./ The 1/2 hour was enough for the reaction sodium molybdenate + creatine phosphate $\xrightarrow{\text{acid}}$ creatine + + sodium phosphomolybdenate.

3./ Isopropyl acetate was used to extract the inorganic and free phosphate in the complex form of sodium phosphomolybdenate.

4./ Stannous chloride was used only to give the blue colour for spectrophotometric readings.

III. 3.7.2. Determination of the inorganic phosphate and creatine phosphate

1./ From the perchloric acid extract diluted /x50/ 1 ml was pipetted in a stoppered centrifugation tube previously containing a cold mixture of 3 mls sodium molybdenate and 4 mls isopropyl acetate solutions. The tube was stoppered well and quickly shaken in an ice bath for 1/2 min, then taken out of the bath and centrifuged. From the upper layer, 2 mls were pipetted into a

clean dry test tube. About 150 mg of stannous chloride powder was added and shaken. 1 ml of cellosolve then added, mixed well and poured in a glass cuvette. In a blank, control, cuvette only 3 mls cellosolve was added. 15 minutes later, test cuvettes were measured spectrophotometrically and calculated as in the previous method for total phosphate.

Calculations of IP and CP:

IP = Extinction E_{IP} x 0.0175 in μ mol/g of wet tissue

CP /μ mol/g/ = P_t - IP.

Comments: The above mentioned procedure is used effectively in the determination of IP since no other quicker and more precise method could be used.

However for determination of CP it is not the most suitable method to be used and instead the enzymatic determination method is much more quicker and more accurate. The chemical determination of CP is a long process and thus errors might enter from any point of the chemical, technical or instrumental procedures. Any errors in the determination of total phosphate or IP will reflect itself on the result of CP concentration. Therefore, during all our procedures used in this study only the enzymatic determination for CP was used.

III. 3.8. Enzymatic methods of determination those are used for:

a./ ATP & CP /H.U. Bergmeyer /1974/, Bücher /1977/, Dennemann /1961/,

b./ ADP & AMP /Hans Adam /1965; 1974/ and Jaworek et al. /1974//,

c./ Lactate /H.J. Hohorst, Kreutz & Bücher /1965/ and Hohorst /1974/.

From the above determinations the following parameters were calculated:

d./ the total adenine nucleotides ADN = ATP+AMP+ADP

e./ the bioenergetic index /B.I./ as mentioned previously in the introduction.

III.3.8.1. Determination of ATP and CP

1./ 1 ml of the neutralized extract was pipetted into a small clean, dry labelled test tube previously cooled and submerged in an ice - cooled water bath. This extract was used for all enzymatic determinations.

2./ Into the test-cuvette, the following mixture was pipetted from Boehringer-combination ATP-determination kit:

3 mls solution I /which is the buffer solution of triethanolamine

+ $MgSO_4$ + the substrate Glyceryl -3-P./

+ 0.2 ml sol II /NADH/

+ 0.1 ml of the sample /i.e. neutral extract/ and mixed well.

3./ A blank cuvette was made containing 3.3 ml of diluted picric acid solution with which extinction E_o of the tested sample must be arranged within the range 0.500 - 0.700 using quartz cuvettes of 1 cm width. The zero extinction /E_o/ of the tested solution was measured spectrophotometrically, with the instrument at wave length 340 nm and deuterium lamp.

4./ 0.04 ml or 40 µl of suspension III /GADPH+ +PGK+GDH+TIM enzymes/ was added to the test cuvette, mixed and the extinction /E_1/ was read each five min till a constant reading was obtained /after about 15--20 min/.

5./ 5-10 mls of a fresh solution of ADP containing 1 mg/ml was prepared, 0.1 ml of this solution was added to the test cuvette, mixed and the new extinction E_2 was read each five minutes for 15-20 min. This time is enough to convert the ATP completely into ADP according to the reaction Glycerate 3-P+ATP $\xrightarrow{PGK}$ Glycerate 1,3-di-P+ADP.

6./ 50 µl of a freshly prepared CPK solution /containing 3 mg/0.3 ml redistilled water/ was added to the test cuvette followed again 40 µl of suspension III, mixed and the new extinction /E_3/ was read each five or ten minutes till the reaction stopped or slowed down /after 30-40 min/. Here: CP $\xrightarrow{CPK}$ Cr + P; and ADP + P—— ATP;

and the produced ATP was determined.

7./ Calculations:

$$dE_{ATP} = E_o - E_1$$

$$dE_{CP} = E_2 - E_3$$

$$\text{ATP content} = \frac{/W + V_1/ \times /V_2 + V_3/ \times V_c}{e \times W \times V_2 \times V_t} \times dE_{ATP} = dilK_{ATP} \times dE_{ATP}$$

$$\text{CP content} = \frac{/W + V_1/}{W \times e} \times \frac{/V_2 + V_3/}{V_2} \times \frac{V_c}{V_t} \times dE_{CP} = dilK_{CP} \times dE_{CP}$$

where: - dE = the difference in optical density,

W = weight in gms of brain tissue taken for enzymatic analysis,

V_1 = the volume in mls of perchloric acid used for deproteinization /x10/,

V_2 = the volume of the extract before neutralization /usually 1 ml/,

V_3 = volume in mls of K_2CO_3 used for neutralization /10 % the total volume of extract i.e. usually 0.1 ml/,

V_c = total volume in cuvette /usually 3.14 ml for ATP cuvette and 3.33 for CP cuvette/,

V_t = total volume of sample used /usualla 0.1 ml/,

e = extinction coefficient of NADH,

$dilK$ = dilution constant of ATP or CP.

According to the above equations it was found that:

$ATP = dE_{ATP} \times 32.45$

$CP = dE_{CP} \times 34.29$

/where 32.45 and 34.29 are constant factors for the calculation of ATP and CP respectively./

III. 3.8.2. ADP and AMP determination /Hans Adam, 1965 and 1974/, /Jaworek,Gruber and Bergmeyer, 1974/

Here also the test-sample was the already neutralized extract.

1./ In a clean quartz cuvette, of 1 cm width, the following solutions from Boehringer ADP-AMP determination kit were added: - 2.5 ml Triethanolamine HCl buffer solution /pH 7.5/,

+ 0.2 ml /200 µl/ solution 2 /phosphoenol pyruvate+KCl+$MGSO_4$/

+ 0.2 ml /200 µl/ " 3 /NADH solution/

+ 0.02 ml /20 µl/ " 4 /LDH suspension/

+ 0.2 ml /200 µl/ sample. The Mixture was well mixed in the test cuvette and E_o measured spectrophotometrically against a blank cuvette containing 3 mls of dilute picric acid, at wavelength 340 n.m., U.G. 11 filter and deuterium lamp. /E_o have to be in the range of 0.600-0.500/.

2./ 5 minutes later, 0.02 ml /20 µl/ of suspension 5 /phosphokinase/ was added and left for 15 min, during each five minutes of which the extinction E_1 was read.

3./ 0.02 ml /20 µl/ suspension 6, which is monophosphate kinase, was then added, mixed and left for about 20-35 min, during each five min of which the extinction E_2 was observed.

4./ Calculations:

$$dE_{ADP} = E_o - E_1$$

$$dE_{AMP} = E_1 - E_2$$

$$\text{ADP } \mu \text{ mol/g} = \frac{/W + V_1/}{W \times e_{ADP}} \times \frac{/V_2 + V_3/}{V_2} \times \frac{V_c}{V_t} \times dE_{ADP} = K_{ADP} \times dE_{ADP}$$

$$\text{AMP} = \frac{/W + V_1/ \times /V_2 + V_3/ \times /V_c/}{W \times e_{AMP} \times V_2 \times V_t} \times dE_{AMP} = K_{AMP} \times dE_{AMP}$$

/where e_{ADP} = extinction factor for ADP = 6.22

e_{AMP} = extinction factor for AMP = 12.44/

Similarly as in calculating the final constant factors for ATP and CP determination, the following deriviations were also used:

ADP /in µ mol/g/ = $dE_{ADP} \times 32.45$

AMP /in µ mol/g/ = $dE_{AMP} \times 16.33$

5./ The test principles are: ADP + PEP $\xrightarrow{PK}$ ATP + Pyruvate

Pyruvate + NADH + H^+ $\xrightarrow{LDH}$ Lactate + NAD^+; then AMP+ATP $\xrightarrow{MK}$ 2ADP

III. 3.8.3. Lactate determination /H.J. Hohorst et al., 1965 and Hohorst, 1974/

1./ A buffer enzyme mixture was prepared for each sample from the following: - 1.55 ml Hydrazine glycine buffer /pH 9.8/ + 1.45 mls water + 3 mg NAD + 10 µl LDH and where mixed.

2./ Into a quartz test cuvette 2.9 mls of the above buffer-enzyme mixture was pipetted. And into another blank cuvette 3 mls of the same mixture was pipetted. E_o was measured spectrophotometrically at 340 nm using deuterium lamp.

3./ 0.1 ml /100 µl/ of the neutralized test extract was added into the test cuvette, mixed, and the extinction E_1 was read each 5 min for 20-25 min.

4./ The optical density difference dL was calculated as:

$$dL = E_1 - E_o$$

Lactate content of the sample could be calculated in µ mol/g as:

$$L = \frac{/W + V_1/ \times /V_2 + V_3/ \times /V_c/}{e_L \times W \times V_2 \times V_t} \times dE_L$$

where V_c = total volume in the test cuvette = 3.0 mls /Constant/

e_L = extinction constant for Lactate = 6.29 mls /constant/..

$\therefore$ Lactate concentration = $dE_L \times 57.556$

/where 57.556 is a constant factor for the calculation of Lactate./.

The test theory: - Lactate + NAD^+ + hydrazine $\xrightarrow[pH\ 9.5]{LDH}$ pyruvate-hydrazone + $NADH^+$ + H_3O. High concentration of diphosphopyridine nucleotide and LDH are required to obtain a quantitative fast reaction in which the formation of NADH increases the optical density. Hydrazine was used to form a complex with the resultant pyruvate so as to prevent or decrease the reversility of the reaction and so to shift the reaction towards completion i.e. to the oxidation of lactate completely into pyruvate.

III.3.8.4. Total Adenine Nucleotides /ADN/: is calculated from the formula: ADN = ATP + ADP + AMP using the values of these nucleotides as obtained above from their enzymatic determinations.

III.3.8.5. The Bioenergetic Index /B.I./: is calculated from Fedelesova and Ziegelhöffer /1976/ equation:

$$B.I. = \frac{/ATP + CP/ \quad 10\ \frac{ATP}{ADN} + \frac{ADP}{ATP}}{\frac{ATP}{CP}}$$

The value of B.I. is determined by calculating the different parameters obtained by the enzymatic determinations and the ADN values calculated.

III. 3.8.6. The energy charge potential /ECP/: was sometimes used in the analysis. It was calculated according to Atkinson /1968/ equation and Lewis et al. /1973/ where:

$$ECP = \frac{/ATP/ + 0.5\ /ADP/}{/ATP/ + /ADP/ + /AMP/} = \frac{ATP + 1/2\ ADP}{ADN}$$

III. 4. /Experiment 2./ Application and effect of 15 sec and 30 sec ischaemia on rat brain metabolism caused by decapitation

Animals used: 15 male Wistar rats of average weight 250-300 g were used in two groups. No anaesthesia was used. Ischaemia was applied by decapitating the heads as quickly as possible using a very sharp instrument and a stop watch. In the first group of 9 rats,the heads were frozen in liquid N_2 after 15 sec. While in the other group the heads were frozen after 30 sec. The heads were left in liquid Nitrogen till completely frozen /about 3-5 min/ then analysed for brain metabolites as previously described in the determination of the normal values of brain substrates. The obtained values or results were compared with those of the normal values /as control/.

III. 5. /Experiment 3./ Application and the effect of ischaemia caused by bilateral carotid occlusion and transsection on rat brain

White adult male wistar rats 200-300 grams of body weight were used. The animals were weighed and anaesthetized by nembutal /Pentobarbital/ 45 mg/kg intraperitonial. Nembutal being previously dissolved in 0.9 % NaCl solution.

The animals were left for 10-15 minutes till completely sleeping; the skin of the neck was cut after removing a part of the hair.

The tissues of the neck were removed and cleaned till the carotid artery /left or right/ was identified. The carotid was exposed cleaned and separated from the vagus nerve, taking care not to cut or even to disturb the vagus. The carotid artery was quickly ligated by applying two ligatures with a small distance between them, using polypropylene Ethicon sterile suture and the surgical forceps. The carotid artery was then transsected between the two ligatures. The two ligatures were necessary to prevent excessive haemorrhage which may cause death. The other carotid artery was similarly operated.

The carotids and tissues of the neck were put again in order and the outer skin of the neck was sutured again using surgical sterile thread.

The animals were left in the above conditions for different periods of time /i.e. different periods of ischaemia/ as follows:

The first group for 5 min /before freezing/

" second " " 15 " " "

" third " " 60 " " "

" fourth " "240 " " "

" fifth " " 1-2 day " "

A control experiment with another group of anaesthetized animals was done as above except that the carotids of those controls were neither occluded nor transsected /shame operation/. Those controls were left for 15 min after being operated and before freezing. All the animals "experimental and control" were then totally frozen in liquid nitrogen after the specified periods of ischaemia for the test-animals and of anaesthaesia for the controls.

When completely frozen, the heads were seperated, the skulls were broken and the brain tissues were carefully removed, weighed frozen and analysed for the different ischaemic values of brain metabolites and cerebral parameters as previously described.

It is to be noted that the fifth /group/ of the experiments was aimed to trace the rate of tolerance of the animals to such prolonged period /1-2 days/ of ischaemia and to observe the rate of survival of the rats after

such ischaemia.

III.6. Experiment 4./ Application of ischaemia on rat brains by bilateral carotid occlusion only, without transsection, for 3 days /i.e. 72 hours-ligation/

A group of 6 adult male Wistar rats, each about 250-300 g of body weight were used. Anaesthetized by nembutal, i.p. injected 45 mg/kg body weight, and operated. Both carotid arteries were exposed as described previously, cleaned and occluded withouth being transsected. 3 days later, the operated animals were frozen in liquid nitrogen and analysed for brain metabolites to investigate the effects and severity, if there is, of such occlusion on the normal brain substrates concentration.

III.7.8. /Experiment 5./ A sutdy of the effect of 15 min barbiturate anaesthesia on normal and ischaemic brain metabolism in rats: and, utilization of such anaesthesia as a protective mechanism against cerebral ischaemia

A group of 15 adult male Wistar rats of average weight 200-250 g were treated by Pentobarbitone /nembutal/ 45 mg/kg i.p. The animals were left for 15 min,

during which they fell asleep. They were then frozen quickly in liquid nitrogen. The heads were separated, frozen skulls were broken and samples of the pure brain tissues were analysed separately for each animal. The values of metabolites and substrates of the anaesthetized brain were compared with those of the normal brain of the control awake animals.

The combined effect of anaesthesia and 15 min cerebral ischaemia on the normal rat brain metabolites and cerebral parameters was done using a group of 12 male adult Wistar rats, weighed and anaesthetized with nembutal 45 mg/kg /i.p./. Then operated as previously mentioned in experiment 3. The values obtained were compared with those of the normal values being the control for this experiment.

III. 9. The pharmacological experiments

III. 9.1. Development of our methods for evaluating cerebral antiischaemic action

Elucidation of the pathomechanism underlying cerebral hypoxia and ischaemia as well as development of new effective antiischaemic drugs has been rendered difficult by the lack of an adequate experimental models for the disease.

For the screening of antihypoxic drugs, a modified quantitative assay was used based on the quantitative measurements of metabolic changes occuring in the brains of intact anaesthetized rats, a test which is thought to be more sensitive and selective than other screening methods. The analytical procedures were done on the different metabolites and parameters of the normal rat brain as well as the ischaemic rat brain to evaluate the changes occuring in ischaemia.

Several ischaemic models were tried namely: decapitation, bilateral carotid occlusion and later bilateral carotid occlusion and transsection for different periods of time, to elucidate the sequence of changes occuring during such ischaemia.

The effect of some cerebral drugs was estimated using the latter model whereby the difference in the changes occuring in the brain metabolism, expressed in terms of concentrations of high energy metabolites /in μ mol/g/ and the energy charge potential /ECP/ as well as the bioenergetic index /B.I./ were estimated, calculated and evaluated in comparison with those values obtained for the normal animal or the control ischaemic animals with, and/or without treatment. This difference on concentrations of metabolites before and after treatment gives an account of the action or properties of the drug on the brain of the treated animals.

III. 10. /Experiment 6./ The effect of Papaverine, Cavinton and CH-102 on cerebral energy state during experimental ischaemia in rats

Experiments were performed on male Wistar rats of averge weight 200-250 grams, previously anaesthetized with Nembutal 45 mg/kg /i.p./. When completely anaesthetized, the drugs were injected intravenously in the following doses: papaverin HCl 2 mg/kg, Cavinton 2 mg/kg and CH-102 0.2 mg/kg respectively. Each drug was used for a separate group of animals. 15 min later, cerebral ischaemia was produced by bilateral bicarotid occlusion and transsection. After 15 min, the animals were frozen completely /in toto/ in liquid Nitrogen. The frozen skulls were broken, the brain tissues were carefully removed, weighed and analysed. In another group of animals, the same technique was used as above except that a control solution of the solvent /50 % solution of propylene glycol/ was used instead of the drugs. This latter group was used as a control for determination of the control ischaemic values.

The brain tissue samples were analized as described before.

III. 11. /Experiment 7./ Comparative study of some vasodilators: Papaverin, Nitroglycerin and Inosine on ischaemic cerebral and metabolic parameters of the rat brain

Three groups of animals were used for the assay of the above drugs and a fourth group was used as a control. The animals available here were of white adult male Wistar rats of about 250-300 grams of weight. Each group was formed of six animals. The animals were anaesthetized with nembutal 45 mg/kg /i.p./; 15 minutes later, the drugs were injected in the following order: Papaverin HCl 2 mg/kg /i.v./, Nitroglycerin 0.1 mg/kg /i.v./ and Inosine 200 mg/kg /i.p./ respectively.

The above drugs were used dissolved in normal saline /0.9 % NaCl solution/. Accordingly the control animals were injected with the same physiological solution /i.v./ in a dose of 1 ml/kg body weight.

It may worth mentioning that Nitroglycerin was available as a 1 % alcoholic extract and was serially diluted in an isotonic normal solution /0.9 % NaCl/ which was later used as the control solution. The amount of alcohol contained in the extract was not accounted for in the control since a very small dose /less than 0.3 ml/ of the diluted extract was used for each animal.

Inosine was used in a comparatively higher dose

than the other agents because according to the personal communication of Leprán István, a dose less than that used may give no or unremerkable effect on the large vessels of the cerebrovascular system. And this latter agent was used intraperitonially because being in such high concentration and being dissolved in warm solution of 0.9 % NaCl solution i.e. supersaturated, it has the probability to affect the lumen of the vessels or preciptating on their walls on cooling. Accordingly the i.p. route was prefered.

The treated animals were left for 15 min so that they can attain the physiological stability, as well as for the drugs to be absorbed, distributed and metabolised so that they can exert their action on the blood vessels especially on those of the cerebral circulation and metabolism.

The animals were then carefully operated and the carotids were bilaterally occluded and transsected to produce the cerebral ischaemia. 15 min after transsection the animals were completely submerged in liquid nitrogen. The skulls were separated and broken. Brain tissues were carefully removed, cleaned, weighed and analysed for substrates of both the treated and the control groups. From the values of substrates obtained the total adenine nucleotides /ADN/, the bioenergetic index /B.I./ and the energy charge potential /ECP/ were calculated.

IV. RESULTS AND DISCUSSIONS

All the results obtained were expressed as concentrations of the different brain metabolic substrates, and values of other parameters /ADN, ECP and B.I./. The concentrations were expressed as μ mol/g wet weight of brain tissue. The tissues used were of the total brain in homogenised extracts and thus the values of substrates give informations of the total, normal and abnormal, brain metabolism. Most of the results were tabulated and shown in graphs, after being calculated to get the mean values and the standard errors followed by statistical analysis, using Student-test tables with the aid of the programmed Wang computer to calculate the statistical significance of the results compared to the normal or the control values.

IV. 1. Determination of the normal values

As shown in /Table 1./ the values of the different parameters were obtained from the brain of the conscious animals totally immersed in liquid nitrogen. To be in certainity, these values obtained were compared with those of other workers in the same aspect. The amount$ of ATP found in brain extracts /2.95 μ mol/g/ was in accordance with the values observed by Mac Millan

Table 1.

Normal values of rat brain high-energy phosphate esters and other metabolites as compared to those of other workers

Substrates /μ mol/g/	ATP	ADP	AMP	CP/2/ creatine P /enz.det./	I.P. inorganic PO_4	total PO_4 P_t	L lactate	ADN[x]	ECP	B.I.[x]
Normal values as obtained by other workers in rats /in μ mol/g wet brain tissue weight/	3.07±0.02/ 3/	1.28±0.00/10/	0.67±0.00/10/	2.39±0.40/ 5/	4.79±0.08/ 8/	7.79±0.00/8/	4.5 ±3.8 /10/		0.926±0.002/ 7/	
	2.29±0.2 / 5/	0.296±0.01/3/	0.20±0.04/ 8/	2.17±0.20/ 8/	4.50±0.00/12/	6.40±0.00/9/	3.8 ±0.00/10/		0.934±0.003/11/	
	2.22±0.00/10/	0.28±0.14/ 8/	0.04±0.01/11/	1.59±0.10/ 6/	5.10±0.00/9/		2.10±0.00/12/			
	2.57±0.10/11/	0.38±0.02/11/	0.04±0.02/ 7/	2.56±0.06/ 4/			2.22±0.13/ 4/			
	2.54±0.04/ 4/	0.42±0.03/ 7/		4.05±0.29/11/			1.35±0.28/11/			
	2.85±0.04/ 7/									
Normal values obtained by our experiments /μ mol/ /g/	2.95±0.10 n = 14	0.98±0.07 n = 14	0.80±0.05 n = 8	2.58±0.08 n = 12	3.80±0.22 n = 10	6.07±0.44 n = 9	4.55±0.47 n = 10	4.37±0.21 n = 8	0.71±0.04 n = 8	30.90±2.13 n = 6

Symbols and references: - 1/ The values chosen from other workers were only those for the parameters practically measured and used in this study. There are other important brain metabolites as glucose, oxygen, glycogen, pyruvate and citrate etc. Which have already been measured and extensively studied by so may workers /see the references below No. 3,4,5,6,7,11,12,13,14,15,16,17,18 and 19/

2/ The values of CP which were enzymaticaly determined

x = The values of /ADN/ and /B.I./ are recently introduced and so not used by those workers mentioned above.

n = Number of experiments or number of the rats of which the respective values were considered and calculated to have the mean values expressed above with the calculated standard errors.

3/ Mac.Millan and Siesjö /1971/ 4/ Gatfield P.D. et al. /1966/ in mouse brain 5/ Weyne et al. /1977/ 6/ Saunder's Handbook of biological data /congress Library/ U.S.A./ /1967/ 7/ Eklöf et al. /1971/ 8/ Lolley and Sampson /1962/ 9/ Olsen and Stone /1955/ 10/ Spector R.G. /1965/ 11/ Winn et al. /1979/ 12/ Dawson and Richter /1950/.

and Siesjö /1971/ and others as shown in the table. ADP concentration was found to be 0.98 μ mol/g, while AMP was 0.80 μ mol/g which was in accordance with the values of Spector /1965/. CP was found to be 2.58 μ mol/g, which was near to that of Weyne et al. /1977/. The inorganic phosphate /IP/ concentration was, more or less, stable in the normal state /3.80 μ mol/g/. The excessive degradation of adenine nucleotides and CP increase the concentration of IP, as in ischaemia. The total phosphate was about 6.07 μ mol/g, but depends mainly on the concentrations of CP and IP. The lactate concentration was another important factor in brain metabolism, being very sensitive to ischaemia or hypoxia. Our normal value of lactate was 4.55 μ mol/g and was in accordance with the value of Spector /1965/. The total adenine nucleotides ADN was 4.37 μ mol/g, in the normal state. This parameter was recently used and so very few workers calculated its value, mainly in the brain and heart of different experimental animals. The bioenergetic index /B.I./ was also very recently introduced by Fedelesova and Ziegelhöffer /1976/ and it has been used by very few workers in the ischaemic myocardial metabolism, mainly in our institute by Szekeres L. /1978/ and Takáts et al. /1977/. In our experiments, in case of the brain, the normal B.I. was found to be about 30.90. The energy charge potential /ECP/ in the normal state of the rat

brain was 0.71 ± 0.04 as calculated from Atkinson equation.

IV. 1.2. Discussion and comments on the techniques followed for the determination of normal values

All the procedures used for evaluating the brain substrates were generally based on the spectrophotometric studies of many investigators according to the optical test introduced by Otto Warburg in 1936 which was based on the fact that the reduced nicotinamide adenine-dinucleotides /NADH and NADPH/ absorb light with a peak between 338.5 and 340.5 nm, while the oxidized forms NAD and NADP show no absorption between 300 and 400 nm /Mattenheimer, 1976/.

The determination of metabolites with enzymes has the advantage of specificity, while the chemical determination of metabolites very often give too high results because compounds of similar structure might react with the metabolite. During spectrophotometric analysis, the incubation of the samples into the cuvettes was specifically done at room temp. /about 25-28 ^{o}C/.

Neutralization of the acidic extract was important since excessive perchlorate, from perhloric acid extracts, can slow some of the enzymatic reactions by changing the

favourable pH needed for such reactions. In addition it may create some slow side reactions which could disturb the analysis. During the analysis, ATP and CP were both measured on the same sample as quickly as possible. When the reaction with ATP was complete, ADP and the creatine kinase /CK/ were added to estimate CP.

ADP and AMP were also measured on the same sample. For AMP determination, ATP was added to convert AMP, in presence of adenylate kinase which is specific only for 5' - AMP, into ADP. Stability of substrates during storage and analysis was very important. The most sensitive substrates, ATP and CP, were analysed as quickly as possible after neutralization.

There is a general comment regarding the sources of errors: - During the whole experiments, effort was made to control all sources of errors. There are at least five kinds of possible errors: a./ Freezing may not be fast enough to stop enzymatic processes and the chemical change may continue in the frozen state, b./ change may occur during preparation of the tissue for extraction, c./ acidic extracting agents may affect some metabolites, d./ loss may occur, as a result of incomplete extraction or instability during storage of extracts. The weight, size and age of the animals used may probably give rise to possible sources of errors. Because, even the normal values are known to be different for

the young adult animals from those of the small ones. In such errors, the following factors may contribute: 1./ the less satisfactory manner of preparing original extracts, specially from the brain of adult animals, 2./ the longer time required to freez the heads of adults. 3./ The cerebral values of substrates cannot be measured untill the possibility of contamination with bone fragments is controlled. With relatively small or young rats brains, great difficulty was encountered in avoiding minute fragments of bone from the skull.

In this study, great care and attention was delivered to the above sources of errors and how to get rid of them or at least to minimise their effects on our results.

IV. 2.1. Effect of ischaemia of different durations caused by decapitation

Table 2. and the figures 1, 2, 3, 4 and 5 illustrate the brain metabolites concentrations as well as the parameters used to characterize the energy state of the tissue obtained at the end of the 15 sec. and 30 sec. ischaemic periods.

In both groups ATP and CP had been markedly decreased, while lactate, ADP, AMP and IP were markedly

Table 2.

Effect of ischaemia caused by decapitation for 15 sec. and 30 sec. in normal brain substrates and energy parameters in rate

Substrates /in μ mol/g/	ATP	ADP	AMP	CP	IP	P_t	L	ADN	ECP	B.I.
Normal values /control/	2.95±0.10	0.98±0.07	0.80±0.05	2.58±0.08	3.80±0.22	6.07±0.44	4.55±0.47	4.37±0.21	0.71±0.04	30.90±2.13
	n = 14	n = 14	n = 8	n = 12	n = 10	n = 9	n = 10	n = 8	n = 8	n = 6
Isch. 15 sec. after decapitation	1.66±0.13	1.30±0.12	1.62±0.11	1.89±0.14	5.80±0.22	6.49±0.36	8.58±0.58	5.14±0.31	0.43±0.04	13.39±2.63
	n = 7	n = 7	n = 5	n = 6	n = 7	n = 8	n = 7	n = 5	n = 5	n = 3
	xxx	x	xxx	xxx	xxx	N.S.	xxx	x	xxx	xxx
Isch. 30 sec. after decapitation	1.02±0.16	1.13±0.16	3.13±0.39	1.62±0.19	5.91±0.65	7.17±0.80	12.16±1.02	5.12±0.64	0.32±0.03	11.69±3.63
	n = 6	n = 6	n = 6	n = 6	n = 4	n = 4	n = 4	n = 6	n = 6	n = 6
	xxx	N.S.	xxx	xxx	xxx	N.S.	xxx	N.S.	xxx	xxx

Results were evaluated in μ mol/g of tissue wet weight ± Standard errors. i.e. Mean ± S.E.

N.S. = no significance P = significant difference from the normal value.

x = $P < 0.05$ xx = $P < 0.01$ xxx = $P < 0.005$

n = number of animals or experiments used.

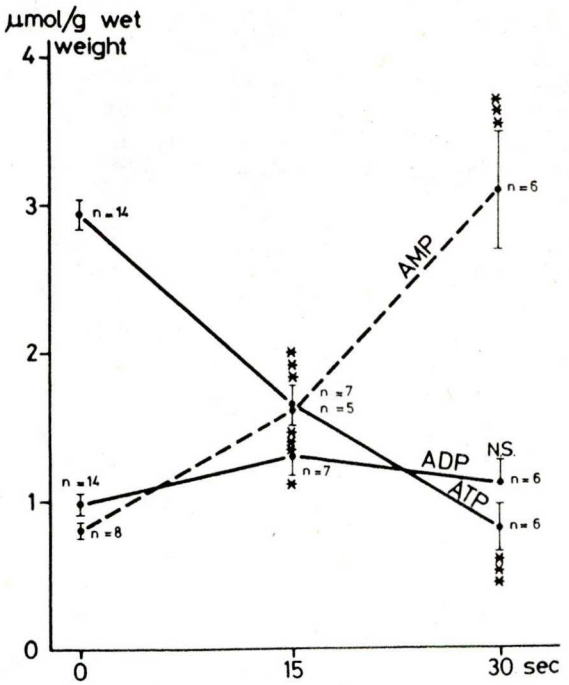

Fig. 1. Effects of 15 and 30 seconds ischaemia caused by decapitation on the normal levels of ATP, ADP and AMP of rat brain.

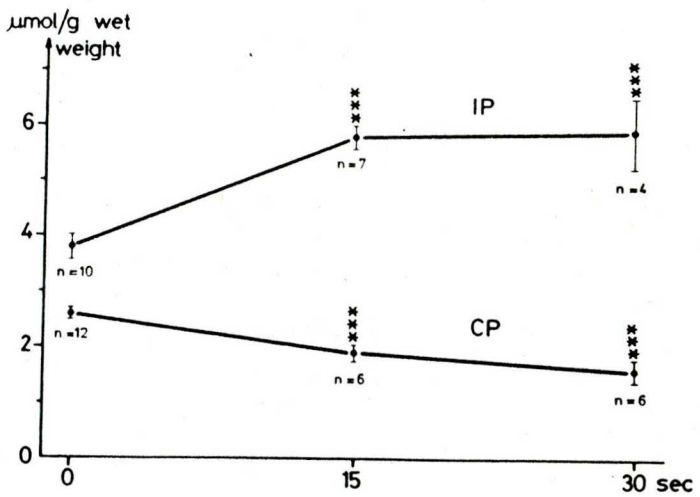

Fig. 2. The time-course effects of decapitation 15 and 30 seconds before freezing on the normal concentrations of creatine phosphate /CP/ and inorganic phosphate /IP/ of rat brain.

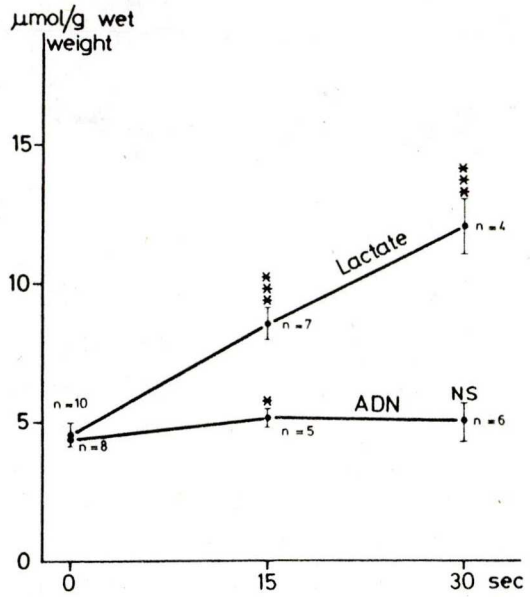

Fig. 3. The effect of 15 and 30 sec ischaemia, after decapitation, on the normal lactate concentration and the total adenine nucleotides /ADN/ of rat brains.

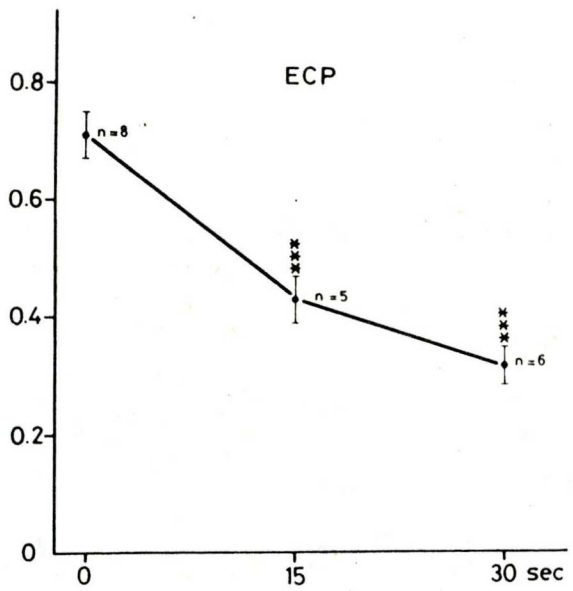

Fig. 4. Changes on the normal energy charge /ECP/ after 15 sec and 30 sec of ischaemia caused by decapitation on rat brain.

increased. Lactate was nearly doubled after 15 sec and was about 3 folds the control value after 30 sec of ischaemia. The rate of change of those parameters was proportional to the period of ischaemia. Higher changes were observed on the 30 sec than the 15 sec after decapitation. Even ADP, which was elevated within 15 sec began to decrease on the 30 sec; a fact which may reflect the breakdown of ADP into AMP as could be shown from the high value of AMP at the end of 30 sec. ATP was decreased to about 55 % of the normal after 15 sec, while 30 sec of ischaemia decreased its value to 1.02 μ mol/g which was about 33 % of the normal.

AMP was double the normal value at the 15 sec and then four times the normal at the 30 sec. CP was below the normal in the 2 groups, being 1,89 after 15 sec and 1.62 μ mol/g after 30 sec. The total adenine nucleotides /ADN/ was slightly increased by decapitation but was more or less constant between the 15 and 30 sec.Lactate content varied between 8.58 μ mol/g which was about double the normal value at the 15 sec, and 12.16 μ mol/g i.e. about 3 folds after 30 sec. The inorganic phosphate /IP/ increased from 3.80 to 5.80 μ mol/g after 15 sec and to 5.91 μ mol/g after 30 sec. The above results may reflect the increased breakdown of CP and other high energy phosphates, due to ischaemia, the consequence of which was the higher inorganic phosphate levels. The total phos-

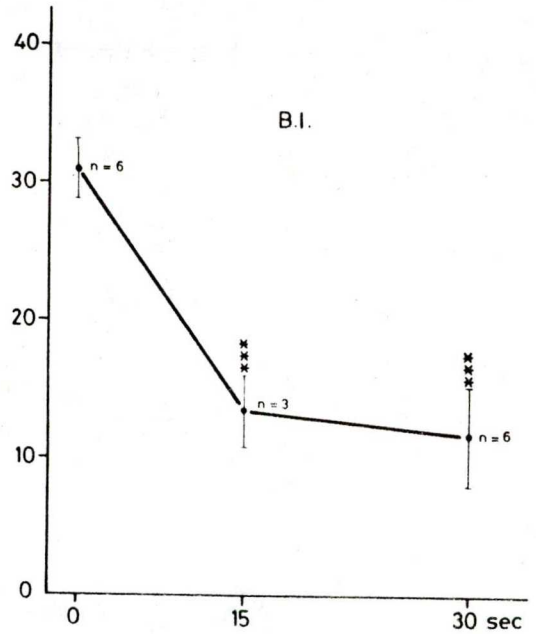

Fig. 5. The bioenergetic index /B.I./ 15 and 30 sec after decapitation and before freezing compared to its normal value in rat brains.

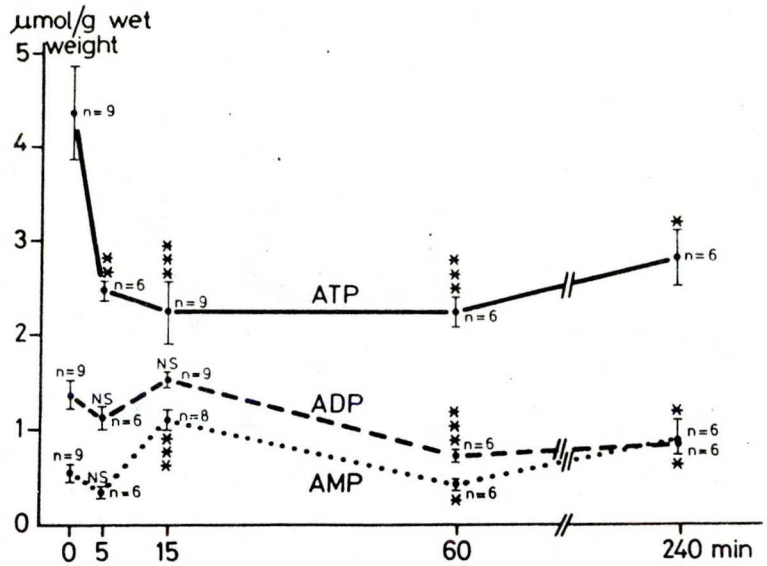

Fig. 6. The time-course effects of bilateral carotid occlusion and transsection for 5, 15, 60 and 240 min before freezing on the substrate concentrations of ATP, ADP and AMP of the anaesthetized rat brain.

phate /P_t/ slightly increased to 6.49 then more elevated to 7.17 μ mol/g, 15 sec and 30 sec respectively after decapitation. The bioenergetic index /B.I./ was markedly decreased to about 43 % of the normal value after 15 sec and more decreased to 38 % after 30 sec of ischaemia. The energy charge potential /ECP/ decreased from 0.71, originally, to 0.43 then to 0.32 after 15 and 30 sec of ischaemia respectively.

The above results obtained were, more or less, in accordance with those obtained, for most of the parameters, by Lowry and Passonnaeu /1964/, Lowry et al. /1964/, Ljunggren et al. /1974/ and Maker et al. /1966/. In both groups the lactate concentration was showing continuous increase above the normal value. This accumulation of lactate may indicate the existence of hypoxia. Although hypoxic changes are mainly catabolic in nature, yet the increased values of the organic phosphates namely AMP, ADP, and ADN, as observed above, after 15 and 30 sec may indicate a considerable degree of restitution of the energy metabolism. Thus the total adenine nucleotides /ADN/, at the end of 30 sec seemed to be restored to values within 15-20 % of the normal. This effect may reflect the fact that the severity of lactic acidosis, being the remarkable signal of ischaemia, as expressed by the high concentration of lactate, did not completely stop the restitution of energy metabolism although it

affected the concentrations of CP and ATP and shifted them towards catabolism or increased consumption. The differences in ATP and CP concentrations as well as those increments of AMP and ADP, may partly have reflected ischaemic values. In our results the changes in the concentrations of CP, were inversely proportional to those of IP and P_t.

IV. 2.2. Discussion of the effect of ischaemia of different durations caused by decapitation

Previous studies on metabolism of the whole brain and nerve indicate that, when the blood supply of the brain is cut off, function may be maintained for a short time through utilization of the energy reserve. This reserve has only four major components ATP, CP, glycogen and glucose. Consequently the rates of change of these substances during brief periods of complete ischaemia could be a good measure of the metabolic rate. In this experimental model, there was a production of complete ischaemia by decapitation.

The substances measured account for all the high energy phosphate compounds available to the brain after its blood supply was cut off. As shown from the results, ischaemia increased the glycolytic rates in both groups of rats. The changes in the concentration of ATP and CP

may reflect that the phosphorylation steps were facilitated to make those increases in the glycolytic rates. As found by Lowry et al. /1964/, the phosphorylation reactions were the steps controling glycolysis in the brain and there is no evidence that other step facilitates glycolysis. Those steps are the phosphorylation of glucose and fructose-6-phosphate and the phosphorolysis of glycogen. After the blood supply has been cut off, the brain is forced to use its emergency sources of energy as long as they are available. Therefore, the analytical results of metabolites could be examined from two points of view: how fast are the energy reserves used and in what order? and what are the mechanisms by which these energy supplies are mobilized. And perhaps may be more to the point of brain protection against ischaemia is the question, what are the mechanism for keeping the energy supplies from being used untill needed?

Without a supply of oxygen the rate of energy use or the metabolic rate, can be calculated, in terms of the high energy phosphate use, from the changes in the above mentioned four substrates of the energy reserve. According to /Lowry et al., 1964/, the rates of use of these compounds in ischaemia differs markedly among the different animals used and in general their order of depletion is CP, glucose, ATP and glycogen, with the

rates of the last two being equal. In our results, the order of depletion of the major energy phosphates, expressed as rate of consumption in both groups of animals was CP then ATP. While for the other substrates analysed, the rate of accumulation was in the order of lactate, Pi, AMP, ADP then P_t respectively after 15 sec. While after 30 sec the order of accumulation was lactate, AMP, Pi, P_t then ADP respectively.

The energetic state of the brain, as calculated in terms of ADN, ĖCP and the B.I., was slightly higher after 15 sec than 30 sec after decapitation. Generally speaking; decapitation highly decreased the total energetic state, in both cases compared to that of the normal value. ATP is not depleted untill the rate of delivery of the high energy phosphate from CP and from conversion of glucose into lactate is less than that needed. Lowry and coworkers observed that glycogen in respect to glucose is similar to ATP when related to CP in the time and conditions of depletion mentioned above, in the case of ischaemia but not necessarily the same in the case of anaesthetized animals. From the rate of consumption of ATP and CP and the accumulation of ADP, AMP, IP, P_t and lactate, it could be assumed that the initial rate of use of ATP and CP seemed to be maintained for the first 15 seconds, as in the first group of ischaemic rats; but in the second group the rate of use decrea-

sed in the later 15 seconds, possibly by that time the more active cerebral cells have exhausted their reserve of ATP, CP and glucose. The rates of ATP and CP consumption in the second group fell to less than half the initial rate and remained nearly constant for CP, and slightly decreasing for ATP in the second 15 sec of ischaemia.

Oppositely were the changes in the concentration of lactate. Lactate in anaerobic glycolysis in known to be a good mirror reflecting the changes in the stores of glucose and glycogen within experimental limits. The degree of production of lactate after 15 and 30 sec of ischaemia may indicate that the rate of depletion of glucose and glycogen during the first 15 sec is approximately equal to that during the later 15 sec.

The changes in ADP and AMP /as shown in table 2./ were consistent with those in ATP, but were assumed to be more accurate measures of ATP breakdown only in the early stages of ischaemia when other metabolic changes were small. The ADN value observed 30 sec after decapitation agreed with those of Lowry et al. /1964/.

Changes in IP values agreed within reasonable limits with the changes calculated from decrease in CP, ATP and ADP, as well as with those values obtained by Lowry and co-workers. In a trial to interpretate the above mentioned data we may find that, shutting off the

blood supply converts the brain into a closed biological system, and at the same time limits the chemical events to those which can occur anaerobically.

However, according to Batchelard /1975/ a kinetic property of P-fructokinase /PFK/, which is a regulatory enzyme in the control of glycolysis, is that it is completely inactivated in presence of high local levels of ATP, but activated in ischaemia where ATP is decreased. Lardy and Parks /1956/ also found that ATP is inhibitory to PFK and that this inhibition of the enzyme may be overcomed by low levels of either ADP, AMP, Pi, cyclic AMP, or fructose diphosphate. Combination of these substances or even very slight changes or increments, in their concentration can cause a large increase in PFK activity and this is one of the most important mechanisms of the ischaemic changes. According to our results the only substances among those deinhibitors, that increased significantly in the ischaemic rat brains at the time of peak glycolysis were IP, ADP and AMP.

However, because of the fall in ATP and CP, there were the observed increases in ADP which were supposed to be due to the degradation of ATP and the equilibrium constant for the reaction catalysed by creatine kinase. In addition, the adenyl kinase /AMP-kinase/ seemed to be activated by decapitation to maintain equilibrium among adenine nucleotides as well as to protect the

ischaemic brain through a vasodilatory mechanism, probably via adenosine release to induce a vasodilatory compensation. Accordingly the observed concentration of AMP was increased, proportionally, much more than the local ADP concentration; because AMP = ADP^2/ATP /at equilibrium/ and ATP is decreasing.

Those considerations seemed to be in agreement with Lowry and Passonneau /1964/ who formulated a theory of glycolytic control which stated that, whenever ATP formation does not keep with ATP use, then ADP and IP must increase, however slightly. Through the activity of adenylate kinase this results in an even greater percentage increase in AMP. The combination of increased IP, ADP and particularly AMP, increased P-fructo-kinase activity in a somewhat autocatalytic way, since the products or the produced substrates: fructose diphosphate and ADP are both deinhibitors. Creatine kinase tends to inhibit the reaction somewhat by removing part of the ADP, but not the IP.

A point of interest to be mentioned here is that there is a great difference in the rates of metabolism of the energy phosphates in the brains of adults and new-born animals. The latter were noticed to have lower rate than adults. This might be due to a lower basal metabolic rate in newborns, since there is a lower proportion of active neurons in the immature brains

/Gringard and Mcilwain, 1955/. Accordingly such a difference in the age, weight or size of the experimental animals must be put in consideration during handling of the animals used in such experiments. Several investigators had studied similar models of decapitation from the point of view considering cerebral circulatory regulation, mainly through evaluation of the adenosine metabolite. From those Berne et al. /1974/ and Rubio et al. /1975/ confirmed that the brain can produce adenosine rapidly in anoxic or ischaemia cases. Winn et al. /1979/ reported that within 10 seconds of ischaemic onset, cerebral adenosine increases by more than 3-fold with the major increase occuring within 5 seconds. However, most of the workers agree in the fact that the high energetic changes taking place in the ischaemic brain and after decapitation are mainly due to the high neuronal changes in the brain.

IV. 3.1. Results and discussion of the metabolic changes in rat brain following cerebral ischaemia caused by bilateral carotid occlusion and transsection for 5, 15, 60 and 240 min and 1-2 days

Since cerebral circulation is mainly affected through both carotid arteries and the vertebral arte-

ries, then any disturbance, specially constriction, occlusion or transsection to all or any of these arteries will lead to cerebral ischaemia. Whether this ischaemia is partial or total depends on which of those arteries are closed or transsected. Occlusion or transsection of all the above arteries supplying the cerebral circulation leads to total or complete ischaemia as in the case of decapitation, which as previously mentioned, is very serious and fatal and difficult to be applied in the assay of cerebral drugs, because ischaemic changes take place very quickly and the animals die soonly.

The partial or incomplete type of ischaemia could be applied by occlusion or/and transsection of only some of the arteries supplying the brain so that a part of the circulation is still going on to supply the brain tissue. Here in our model, this latter type was chosen and was done by bilateral occlusion and transsection of both left and right carotids. This experiment was aimed to study the sequence of events as well as to trace the successive and gradual effects caused by insufficient oxygen and blood supply to the brain tissue for gradually increasing periods of time. It is also aimed to study the degree of tolerance of the brain to such periods of ischaemia, and accordingly the rate of survival of the animals observed during the time courses of ischaemia.

The design of a control experiment to the above experimental groups was slightly puzzling, since within the first hour of ischaemia the effect of the anaesthetic agent was very remarkable and reflected itself in the quantitative values of brain metabolites, while in the 4-hour ischaemia, the effect of anaesthesia was already diminished in most of the animals, and towards it termination in the others. In the 1-2 days ischaemic group, it was supposed that the anaesthetic have already been eliminated. According to this difficulty, and in order to trace equally all the groups, a control of the 15 min anaesthesia was chosen, because most of the groups were analysed while still under the effect of anaesthesia except the last group. But for satisfaction table 4. and figures 11., 12. were aimed to give an account for the 4-hour group if considered out of anaesthesia. A summary of all the results was given in tables 3 and 4 and in the figures 6—12. The time-course of effects could be traced as follows in comparison with the control groups:

1./ Changes in ATP and CP: It was clear in table 3. that 5 min of bilateral carotid occlusion and transection caused a relatively high decrease of ATP, about 45 % from the control. 15 min and 60 min later the rate of decrease was very smaller. Similarly were the changes in CP but with lesser degree of change in the

Table 3.

Effect of ischaemia caused by bilateral carotid occlusion & transsection for different periods on the brain substrates of rats

Substrates /in μ mol/g/	ATP	ADP	AMP	CP	IP	P_t	L	ADN	ECP	B.I.
Nembutal anaesth. /15 min/ control	4.38±0.49	1.37±0.15	0.54±0.08	3.12±0.16	3.82±0.34	5.32±0.29	5.89±0.80	6.29±0.33	0.80±0.03	37.79±3.37
	n = 9	n = 9	n = 9	n = 9	n = 7	n = 7	n = 9	n = 9	n = 9	n = 9
5 min. Isch. bilateral carotid occlusion & transection	2.48±0.10	1.11±0.11	0.34±0.04	2.43±0.08	5.82±0.36	7.47±0.25	5.80±0.57	3.93±0.18	0.77±0.01	30.98±1.60
	n = 6	n = 6	n = 6	n = 6	n = 6	n = 6	n = 6	n = 6	n = 6	n = 6
	xx	N.S.	N.S.	xx	xxx	xxx	N.S.	xxx	N.S.	N.S.
15 min	2.23±0.32	1.52±0.07	1.07±0.07	2.38±0.25	4.30±0.25	4.72±0.32	6.12±0.35	4.75±0.41	0.62±0.04	27.28±2.41
	n = 9	n = 9	n = 8	n = 11	n = 10	n = 12	n = 6	n = 11	n = 9	n = 9
	xxx	N.S.	xxx	x	N.S.	N.S.	N.S.	xx	xxx	x
60 min /1 hr./	2.25±0.17	0.72±0.03	0.31±0.03	2.26±0.07	4.50±0.84	5.88±0.89	6.01±0.67	3.28±0.35	0.80±0.02	32.51±1.92
	n = 6	n = 6	n = 6	n = 6	n = 6	n = 6	n = 6	n = 6	n = 6	n = 6
	xxx	xxx	x	xxx	N.S.	N.S.	N.S.	xxx	N.S.	N.S.
4 hours. /240 min/	2.84±0.31	0.84±0.10	0.88±0.16	2.77±0.26	7.15±1.63	9.21±1.71	12.32±2.29	4.55±0.28	0.68±0.05	36.07±6.68
	n = 6	n = 6	n = 6	n = 6	n = 4	n = 5	n = 4	n = 6	n = 6	n = 6
	x	x	x	N.S.	x	x	xxx	xxx	x	N.S.

All the symbols used above carry the same designation and meanings as mentioned previously /in table 2./.

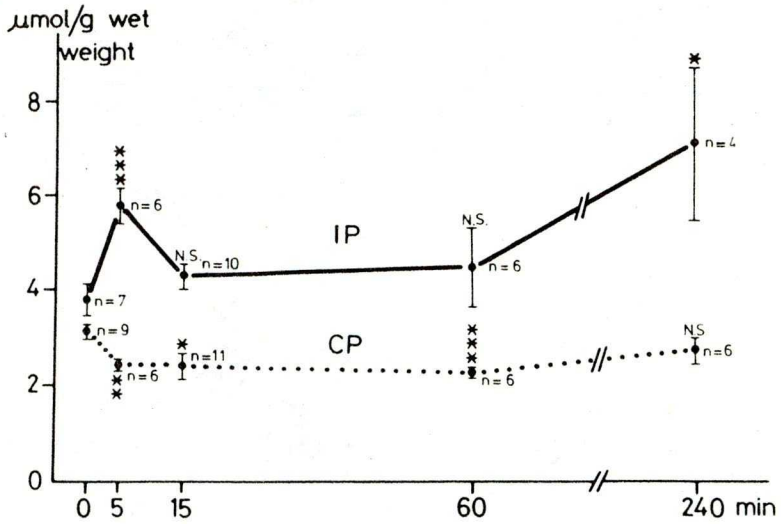

Fig. 7. The time-course effects of ischaemia caused by bilateral carotid occlusion and transsection for the periods 5, 15, 60 and 240 minutes, before freezing the animals, on the values of inorganic phosphate /IP/ and creatine phosphate /CP/ of anaesthetized rat brain.

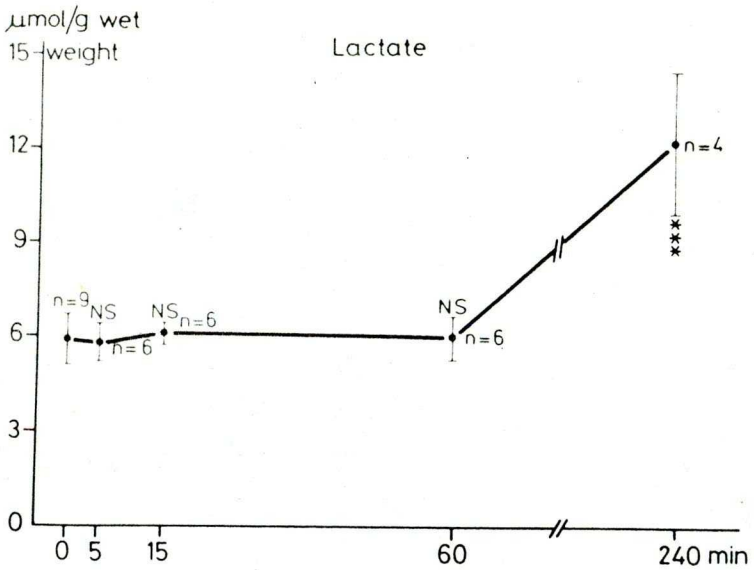

Fig. 8. The time-course changes on the lactate concentration in the ischaemic brain of rats following bilateral carotid occlusion and transsection for 5, 15, 60 and 240 min before freezing of the animals.

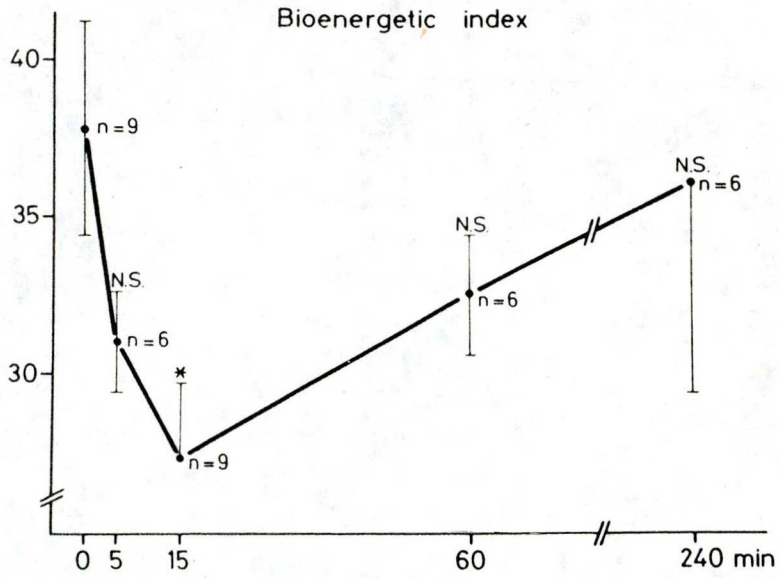

Fig. 9. Changes on the bioenergetic index /B.I./ of the anaesthetized rat brain following ischaemia caused by bilateral carotid occlusion and transsection for several periods 5, 15, 60 and 240 min before freezing .

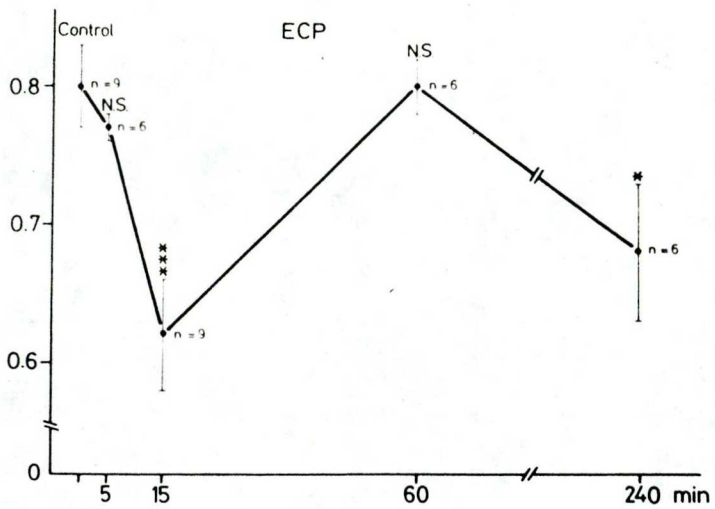

Fig. 10. Changes on the energy charge /ECP/ of the anaesthetized rat brain following ischaemia caused by bilateral carotid occlusion and transsection for 5, 15, 60 and 240 min before freezing the animals .

first 5 min. After 4 hours, both ATP and CP began to increase slightly above the 60 min concentrations. These results may reflect 2 facts; a high metabolic rate was occuring within the first 5 min due to the ischaemic changes; and that restitution of energy anabolism or biosynthesis was also still going on beside the ischaemic catabolic effects on the high energy compounds. This might be a compensatory protective mechanism to the brain caused by the autoregulation and the blood supply which was still reaching the ischaemic brain from the other vertebral and intra-cerebral collateral vessels. The 5 min ischaemia clearly showed that ATP was quickly and highly consumed even than CP in the first 5 min of ischaemia.

2./ ADP and AMP changes: - these parameters are interdependent. The concentration of each depends on the other and on the conditions controlling their metabolism. The results showed a moderate decrease in both metabolites after 5 min of ischaemia. After 15 min the concentrations increased to 1.52 and 1.07 μ mol/g respectively. These increments were resulting from the ischaemic - induced breakdown of ATP into ADP and consequently ADP into AMP. One hour later, again they were catabolized with different degrees and at the end of 240 min, the ADP was below the control value while AMP was above its control value. This may reflect the fact

that ischaemic and hypoxic changes tend to shift brain metabolism towards the breakdown of ADP to AMP more than the reversible reaction. This may also reflect a protective mechanism to the ischaemic brain through increased AMP concentration which is known to yield adenosine, on breakdown, a factor which is recently known to have a great role in brain autoregulation and cerebral vasodilation.

3./ Inorganic phosphate /IP/ and total phosphate /P_t/. IP is a very sensitive factor in ischaemic studies because the breakdown of CP in such conditions yields a part of its phosphate as inorganic. And since the total phosphate is approximately equal to IP + CP then changes of any of the IP or P_t reflect itself on the other. Table 3 shows that bicarotid occlusion and transsection increased the values of both IP and P_t, during all the periods, above their control values. The rate of increase of these metabolites was very high during the first 5 min but was then gradual during the other periods. This may reflect the excessive change of CP during the first 5 min of ischaemia, as well as the metabolic breakdown of AMP to a less degree. Fig. 7. shows the time-course relationship between IP and CP.

4./ Lactate: - The results obtained for lactate were generally in accordance with those obtained by so many workers: Spector /1965/, Dawson and Richter /1950/,

Villa et al. /1978/, Siesjö and Nilson /1974/ and Eklöf et al. /1971/. Table 3. and the figures 8 and 12 show the degree and the time course of those changes. It was observed that non significant increase took place in the first 5 min, 15 min and 60 min respectively, but a very significant increase took place in the period between, 60 min and 4 hours. The concentration of lactate after 4 hours accounts to more than double the control-value. This may reflect the fact that within a certain short periods of hypoxia, the brain tissue has the ability to make use of the increasingly produced lactate in biosynthetizing glucose or for oxidising lactate, in the presence of the little oxygen supplied, to pyruvate and finally to CO_2 + H_2O with the yield of energy. But on chronic hypoxia, the brain tissue loses its ionic stability and pH by the excessive lactic acidosis and thus loses its ability to synthetise glucose or glucose-6-phosphate. In the same time the cerebral circulation also loses its ability to wash-out the increasingly accumulating lactic acid.

5./ The total adenine nucleotides /ADN/: this parameter was used to express the adenine nucleotides pool which is one of the most important sources of energy supplying the brain. Some workers did not prefer to use this parameter because it does not account for

the other sources of energy but only for the adenine metabolites. In our study, the values calculated for ADN were low in the ischaemic animals compared to the control. It was 6.29 μ mol/g initially but abruptly decreased to 3.93 after 5 min of ischaemia due to the decreased values of ATP, ADP and AMP. At the end of the 15 min; a slight restitution occurred where an increase of ADN was observed, which might be due to the increased ADP and AMP values.

6./ The energy charge potential /ECP/: - the control value of ECP was 0.80 and gradually decreased to 0.77 then 0.62 after 5 and 15 min respectively. This was in accordance with changes in ATP. After 4 hours the ECP, due to the comparatively increased values of ADN generally and of ATP, ADP and AMP, was still below the initial value.

7./ The bioenergetic index /B.I./: - this complex factor was advantageous over ADN and ECP parameters in that it accounts for the most important fragments constituting the high energy of the whole brain, mainly adenyl phosphates and CP. The results, in table 3. and figures 9. and 12. showed that there was a gradual decrease in the B.I. from 37.79 initially to 30.98 and 27.28 after 5 and 15 minutes respectively. At the end of 60 min, the value again increased to 32.51 and then to 36.07 after 4 hours. But still this latter value is below the

control. This may reflect a decrease of the total energetic state of the brain within the first 5-15 min, followed by a slight improvement or restitution after 60-240 min towards the control value. This might be due to the already observed improvement of ATP, CP and ADN at 240 min after transsection.

IV. 3.2. The effect of 4-hour bilateral carotid occlusion and transsection on the normal levels of brain metabolites and substrates

This is only a comparative study aimed to give a comparison between the ischaemic changes observed at the end of 4 hours and the normal values of substrates of the awake non-ischaemic animals. Table 4. and the figures 11-12. demonstrate this. Such results may be of some value if there is a question or doubt in the lasting effect of the anaesthetic activity after 4 hours in the anaesthetized ischaemic animals. Table 4. shows that after 240 min of ischaemia, the ATP and ADP values were slightly decreased from their normal values. Similarly AMP was not significantly changed from the normal. It could be concluded that the above ischaemic effects were not remarkably expressed when compared with the normal values, a fact which may mirror the still existing effect of anaesthesia. Also the observed ischaemic value of CP

Table 4.

Effect of 4-hour and 1-2 days cerebral ischaemia caused by bilateral carotid occlusion & transsection on some normal cerebral energetic parameters of rat brain

Substrates /in μ mol/g/	ATP	ADP	AMP	CP	IP	P_t	L	ADN	ECP	B.I.
Normal values /control/	2.95±0.10	0.98±0.07	0.80±0.05	2.58±0.08	3.80±0.22	6.07±0.44	4.55±0.47	4.37±0.21	0.71±0.04	30.90±2.13
	n = 14	n = 14	n = 8	n = 12	n = 10	n = 9	n = 10	n = 8	n = 8	n = 6
4-hour	2.84±0.31	0.84±0.10	0.88±0.16	2.77±0.26	7.15±1.63	9.21±1.71	12.32±2.29	4.55±0.28	0.68±0.05	36.07±6.68
	n = 6	n = 6	n = 6	n = 6	n = 4	n = 5	n = 4	n = 6	n = 6	n = 6
	N.S.	N.S.	N.S.	N.S.	xx	x	xxx	N.S.	xxx	N.S.
1-2 days n = 6	Unfortunately, out of the 6 operated rats, 5 died within the first 24 hours. The sixth died next day, All the animals died after being showing symptoms of blindness, loss of ability to eat and drink, as well as severe disorders in movement, balance and behaviour.									

All the symbols used above carry the same designations or meanings as mentioned before in table 2.

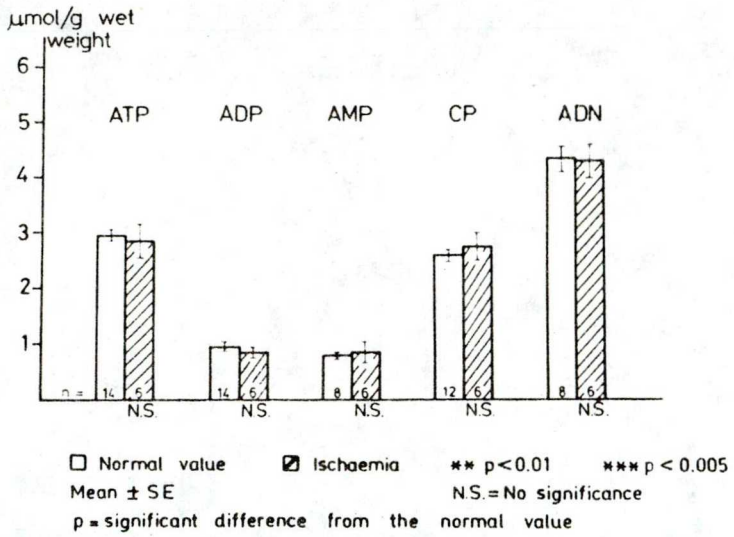

Fig. 11. The effect of ischaemia caused by bilateral carotid occlusion and transsection, for 4 hours on the normal values of ATP, ADP, AMP, CP and ADN of rat brains.

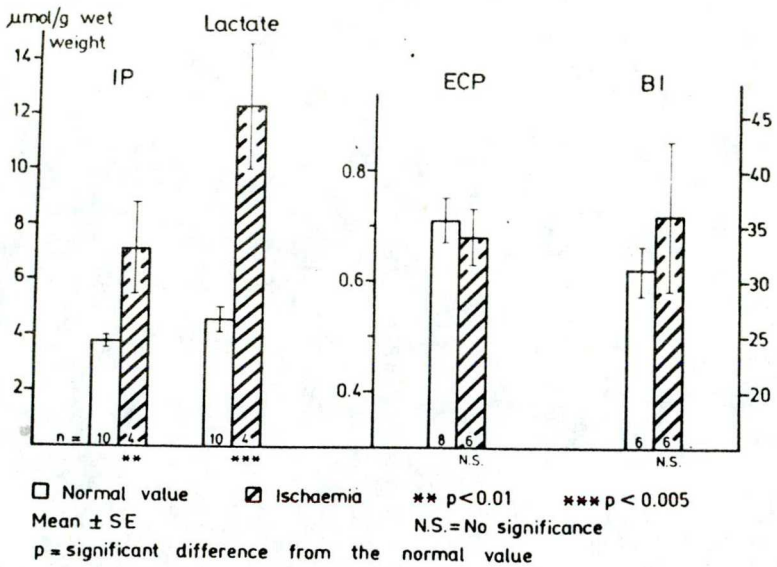

Fig. 12. The changes on the normal values of rat brain of inorganic phosphate /IP/, lactate, energy charge /ECP/ and bioenergetic index /B.I./ induced by bilateral carotid occlusion and transsection for 4 hours before freezing of the animals.

was not expected because it was higher even than the normal value. If this observation was not due no anaesthesia as concluded above then experimental errors might be involved in this case, and further investigations might be required to clarify this. The inorganic and total phosphates /IP, P_t/ were both significantly increased after 4 hours. Similarly, lactate was also very significantly increased by more than 100 %. The latter three parameters IP, P_t and lactate were showing the expected symptoms of hypoxia and ischaemia, and our values for these substrates were in accordance with those of some workers using similar techniques of analysis as Siesjö and Nilson /1974/, Spector /1965/ and Dawson and Richter /1950/.

ADN, was more or less, constant. The energy charge /ECP/ depression was significantly observed after 4 hour ischaemia; while the B.I. was non significantly increased. Although this latter result was also unexpected, yet the still-existing effect of anaesthesia, and the increased values of ADN and CP as well as the probability of energy restitution in the brain may account for this increment of B.I. after the 4 hours of ischaemia. This may confirm our conclusion that the effect of anaesthesia was still existing even after 4 hours. And accordingly the effect of anaesthesia has to be considered and counted for by an anaesthetized control during the analysis of

substrates even after 4 hours from transsection.

IV. 3.3. The effect of bilateral carotid occlusion and transsection for 1-2 days

This experiment, or group of animals, was designed to give the values of brain substrates and ischaemic parameters 1-2 days after bicarotid occlusion and transsection as well as to observe the degree of tolerance of rats to such prolonged ischaemic changes, and accordingly to record the rate of survival after 24 hours.

Unfortunately, out of the six operated animals, 5 rats died within the first 24 hours. The last animal of the group died next day within 30-32 hours after the transsection. All the rats died after being showing symptoms of blindness, loss of ability to eat and drink, as well as severe disorders in movement, balance and behaviour. These effects, specially those observed on the eyes, were mostly due to the ischaemic changes on the brain tissue and cerebral circulation which supplies the cerebral cortex, cerebellum, eye structures and other cerebral regions, in which the disturbance of blood supply seemed to cause those mentioned observations as a consequence of circulatory insufficiency.

The survival rate was 100 % in all the group exposed to ischaemia of periods ranging from 5 min to 240 min;

but was 16.67 % after 24 hours and zero % after 48 hours.

According to the above observations, it could be concluded that in terms of survival, the animals can tolerate a period of 4 hours of bilateral carotid occlusion and transsection but difficult to bear longer period than that, and that 24 hours or more after such ischaemia seems to be very fatal to the life of the whole animal.

IV. 3.4. Comments on the choice of our ischaemic model for the pharmacological experiments

To have a model very similar to that of the clinical condition in the animals, several models were investigated: decapitation was the first of those, but it produced a complete ischaemia which the brain blood supply was completely cut and the brain hardly withstand such changes even for a very short period. Accordingly the metabolic changes were very high and quickly took place after decapitation and probably before freezing, a fact which makes this model unsuitable for measuring the changes in substrates following ischaemia, with or without pretreatment.

Many workers, have used this model only to investigate the metabolic effects or to follow the consequences, or rate of tolerance of the brain tissue, to such type of ischaemia. Most of them did not prefer this type of comp-

lete ischaemia, being fatal and clinically non practical, as well as being experimentally very difficult to trace exactly its effects on brain metabolism or to assay the effects of drugs on such model.

Another model of producing cerebral hypoxic ischaemia was then tried by bilateral carotid occlusion, without transsection. But our results led to the conclusion that such model is not a suitable ischaemic one since the metabolic and energy-state parameters were found more or less normal and different from those characterizing the partial or total ischaemia even after 3 days from occlusion of both carotid arteries. Accordingly this model was excluded from being the suitable sensitive model required for the assay of cerebral drugs.

A third model was investigated namely bilateral bicarotid occlusion and transsection for certain periods before freezing. The different periods of ischaemia applied were 5 min, 15 min, 60 min and 240 min respectively. The effects of ischaemia were investigated in terms of the high energy phosphates, lactate, inorganic phosphate and the other parameters. The results obtained by this last model were, more or less, satisfying and were found to be in accordance with some results obtained by other workers. Accordingly this model was satifactorily selected to be the suitable for the assay of compounds acting on the ischaemic brain. From the different periods of ischae-

mia caused by bilateral carotid occlusion and transsection, the 15 min was specifically chosen for the assay because it was the most suitable period for the ischaemic metabolic symptoms to appear. The 5 min ischaemia was not enough to show all the changes, and in such short period the drug may not show its peak of action and metabolic changes.

Similarly the 1 hour and 4 hour ischaemic models were not selected because the brain tissue showed great sensitivity and irreversible alterations, to ischaemic changes, which could not be protected by the pharmacological agents. On the other hand: some of the anaesthetized animals were sensitive enough to recover from anaesthesia during this 1-4 hours while others remain on deep sleep. Such non--uniformity in the sleeping-time due to anaesthesia favours the choice of the 15 min period. In addition, most of the investigated drugs have their favourable action within a short period of time, specially, after intravenous administration.

In this selected model the different types of pharmacologically active drugs: papaverin, CH-102, cavinton, nitroglycerin and inosine were assayed.

The effect of anaesthesia, as a protective mechanism against cerebral ischaemia, and of anaesthesia combined with ischaemia were also studied in the same model and the observed results showed an improvement in the

ischaemic brain.

IV. 4.1. Effect of 3 day bilateral carotid occlusion on the normal values of metabolites and cerebral parameters of the rat brain

The aim of this experiment was to investigate a new model of cerebral ischaemia which could be easily used for analysis of pharmacological agents. The results were summarized in table 5. and graphically demonstrated in figures 13-15. The results were completely different from those of the previous experiment. Here all the animals tolerated this type of ligature for 3 days without any death and even without serious abnormalities, and the rate of survival of the animals after 3 days was 100 % while when the carotids were transsected the survival rate after 1 day was only about 17 % and after 2 days was zero %. Our results and observations on the above model were in accordance with those observed generally by Johanson et al. /1978/ and Salford et al. /1971/, although the latter occluded only one /unilateral/ carotid artery.

Johanson reported that in rats and rabbits, the ligation of the common carotid artery is often harmless due to the existence of circle of Willis; and that it only causes a slight increase in the main arterial pressure. In table 5. all the parameters analysed were increased

Table 5.

Effect of 3-day bilateral carotid occlusion - with no transection - on normal metabolic high energy phosphates, lactate, B.I. & E.C.P. of rat brain

Substrates /in μ mol/g/	ATP	ADP	AMP	CP	IP	P_t	L	ADN	ECP	B.I.
Normal values /control/	2.95±0.10	0.98±0.07	0.80±0.05	2.58±0.08	3.80±0.22	6.07±0.44	4.55±0.47	4.37±0.21	0.71±0.04	30.90±2.13
	n = 14	n = 14	n = 8	n = 12	n = 10	n = 9	n = 10	n = 8	n = 8	n = 6
3-day bilateral bicarotid occlusion	3.22±0.79	1.12±0.22	1.09±0.10	2.93±0.20	4.71±1.38	6.29±1.25	5.56±0.57	5.55±0.59	0.70±0.05	35.40±4.44
	n = 5	n = 6	n = 6	n = 6	n = 5	n = 5	n = 6	n = 5	n = 6	n = 5
	N.S.	N.S.	xx	N.S.	N.S.	N.S.	N.S.	x	N.S.	N.S.

where: - N.S. = no significant ; n = number of animals used in the experiment

P = significant difference from the normal /control values

x = $P < 0.05$ xx = $P < 0.01$ Mean = average ± S.E.

above the control value except ECP which was slightly decreased. ATP and CP increments were not significant. Only AMP and ADN showed significant alterations. The behaviour of metabolites other than ATP and CP was as could be expected from hypxia or ischaemia. But the elevations of ADN and B.I. were unexpected if the model was ischaemic. The values of lactate and the ECP were the most expected results from an ischaemic model. Lactate was increased. The effect of ligation on lactate concentration was rather similar to that reported by Siesjö and Nilson /1974/ and Salford et al. /1971/. The ECP was not significantly decreased after 3 day ligation from the normal value. This also agreed with Eklöf et al. /1971/ who reported, that there were no changes in the ECP values of the animals ligated only, but progressive changes occured in the energy state when such animals were made hypotensive. Observations on ECP obtained by Salford et al. /1971/ on unilateral carotid occlusion on rats were also similar to those mentioned in our results. Whether this observation may mean that unilateral and bilateral carotid ligation were of similar effects or not, could not by surely concluded now and further investigations might be needed to prove such conclusion. It is to be noted that the results were compared with the normal values although the animals were anaesthetized. But this anaesthetic effect was not counted for in the controls

because after 3 days the effect of anaesthesia was supposed to be nil, and so when the animals frozen, they were completely conscious.

The above results did not reflect a state of total or acute ischaemia but were, more or less, expressing those of the normal animals. The survival rate of the animals was 100 % after the 3 days. This may mean that bilateral carotid occlusion, with no transsection, produced a certain harmless degree of ischaemia as shown from the sensitive parameters. This last conclusion was similar to what Salford et al. /1971/ and Johanson et al. /1978/ had already stated. The very slight changes observed in our results for ATP, CP, ADP, ECP, B.I. and even lactate were not significant. A state which may reflect the fact that: bilateral carotid occlusion has no remarkable changes on the metabolic or energetic state of the brain unless it is combined with transsection /Basharahil et al. /1979 and 1980/ hypoxia, anoxia or hypotension /Eklöf et al., 1971/.

IV. 4.2. Discussion

The above results obtained in our experiment could be discussed in the light of the previous workers of similar study. Levine /1960/ was the first who tried to apply anoxic-ischaemia in rats by carotid ligation. And

later Eklöf et al. /1971, Salford et al. /1971/ and Johansson et al. /1978/ have proceeded on similar experimental studies.

Our results, could be concluded in a similar manner to those observations previously mentioned by Levine namely: unilateral, as well as bilateral, carotid ligation alone can not result in sufficient ischaemia to produce lesions. However, carotids of rats when uni- or bilaterally ligated then subjected to anoxic anoxia, they developed lesions. The rate of mortality was zero in our case and negligible in Levine's experiments. But when Levine added anoxia, for certain periods, death of some of the animals was observed. Levine et al. /1959/ observed that the severity of infarctions was proportional to the duration of exposure to, and depth of, anoxia.

Those latter workers observed that it would be desirable to maintain the ischaemia for a period of time after anoxia in order to produce anoxic ischaemic lesions. The above results and observations confirm the agreed fact, of Eklöf et al. /1971/, Salford et al. /1971, Johansson et al. /1978/, Levine /1960/ and Basharahil et al. /1979/ that uni- or even bilateral carotid ligation is not a suitable or effective method to produce cerebral ischaemic circulatory or metabolic changes, unless it is combined with anoxia or hypoxia. This fact was also reflected by, and was in accordance with, our general

results and conclusion.

Regarding the cerebral vascular and metabolic aspect of anoxia and ischaemia: in anoxic anoxia there is only O_2 defficiency while ischaemic anoxia involves deffi-ciency of oxygen, glucose and other materials and is complicated by accumulation of waste products, as lactate. It had been shown that anoxic anoxia produced the same effects as ischaemia and that the vascular damage of ischaemia is mediated by anoxia. The distribution of anoxic or ischaemic infarctions depends both on a "vascular" and a "systemic" factors. In our experiment carotid ligation constituted the vascular factor while the brain tissue succeptability or vulnerability constitutes the systemic /endogenous/ factor.

Our results may reflect the fact that: it is unlikely that unilateral or bilateral carotid ligation causes remarkable impairment in the total blood flow of the brain, as could be shown from the low concentration of lactate /5.56/ in comparison with total ischaemic values obtained by decapitation /8-13 μ mol/g/.

Regarding the sensitivity of the brain: recent biochemical studies demonstrated that the brain is very resistant to pure hypoxemia; and that as long as an adequate blood pressure is upheld to brain, the arterial and cerebral venous oxygen tensions can be reduced remarkably without causing changes in the energetic state/MacMillan

and Siesjö /1971/, Salford et al. /1971/ and Eklöf and Siesjö /1971/.

Their results suggested that a critical degree of ischaemia gives rise to an inhomogenous blood flow with regional anoxia, but this ligation alone at normal blood pressure did not lead to any significant metabolic changes. The CBF was the only parameter observed by Reivich /1964/ to be reduced by a 45-50 % from the normal by such ligation, without any remarkable changes neither in the individual adenine nucleotides nor in the ECP, at normal blood pressure.

To conclude we may mention that our results, in general were in accordance with most of the observations previously mentioned by the above workers i.e. no cerebral metabolic or energetic failure was observed as long as the mean arterial blood pressure was kept constant or normal. The increase of lactate, AMP and IP observed might show the inability of the cerebral circulation to carry on its washout activity and to remove such accumulating metabolites. Such conclusion may confirm the observations of Reivich. The comparatively low increase of lactate compared to other ischaemic models, may contribute a main factor in the preservation of tissue viability and energy metabolism in this model.

IV. 5-6.1. The effect of barbiturate anaesthesia and anaesthesia when combined with ischaemia on the normal levels of substrates and energetic parameters of rat brain: and The possibility of utilizing anaesthesia as a protective mechanism against cerebral ischaemia

This study was aimed to compare the effects of anaesthesia on the brain of normal awake animals and on the ischaemic brain as well. The choice of the normal values as control was to achieve two integrated aims. Firstly, to investigate the effect of anaesthesia on the normal brain metabolism; and secondly to observe the effects of ischaemia on already anaesthetized animals when compared to the normal. Combination of those two aims might, to a great extent, aid in showing the ability of anaesthesia to protect the ischaemic brain.

On this study, the design of a control was slightly puzzling, since to investigate directly the protective effect of anaesthesia on ischaemic brain needs a control of awake ischaemic animals. And this was difficult to attain because application of ischaemia by bilateral carotid occlusion and transsection needs preanaesthesia. Indeed this study gives an account of the effect of ischaemia on anaesthetized brain; rather than of anaesthesia on the ischaemic brain. However since ischaemia without anaest-

hesia had already been investigated, using the decapitation technique; and because the effect of anaesthesia on normal brain can act as a control to the effect of ischaemia on anaesthetized animals, then some comparison of the results obtained in this study could be justified.

Table 6. and figures 13., 14. and 15 are summarizing all the results. As shown ATP and CP were significantly increased, with nembutal anaesthesia, but when combined with ischaemia both ATP and CP decreased below the normal value. ATP increase by anaesthesia, and its decrease by ischaemia were both more significant than those of CP. The increase of ATP and CP by anaesthesia reflects the slow metabolic rate of the brain caused by anaesthesia. ADP was increased by anaesthesia and more significantly elevated when combined with ischaemia. AMP was depressed with anaesthesia but again increased above its normal value after application of ischaemia.

The IP was, more or less, constant in anaesthesia but increased when combined with ischaemia. The total phosphate /P_t/ was slightly decreased with anaesthesia. All the above parameters may contribute to the protective effect of nembutal anaesthesia as reflected by the increased concentrations of ATP, ADP and CP in anaesthesia, and, to some extent by the slight effect of ischaemia on those parameters during the combined case. The effect of anaest-

Table 6.

Effect of anaesthaesia and anaesthaesia combined with ischaemia on the normal rat brain parameters of high energy phosphates, lactate, ECP and B.I.

Substrates /in μ mol/g/	ATP	ADP	AMP	CP	IP	P_t	L	ADN	ECP	B.I.
Normal values /control/	2.95±0.10	0.98±0.07	0.80±0.05	2.58±0.08	3.80±0.22	6.07±0.44	4.55±0.47	4.37±0.21	0.71±0.04	30.90±2.13
	n = 14	n = 14	n = 8	n = 12	n = 10	n = 9	n = 10	n = 8	n = 8	n = 6
Nembutal anaesth. /45 mg/kg/	4.38±0.49	1.37±0.15	0.54±0.08	3.12±0.16	3.82±0.34	5.32±0.29	5.89±0.80	6.29±0.33	0.80±0.03	37.79±3.37
	n = 9	n = 9	n = 9	n = 9	n = 7	n = 7	n = 9	n = 9	n = 9	n = 9
	xxx	x	x	xxx	N.S.	N.S.	N.S.	xxx	N.S.	N.S.
Combined anaesth. + ischaemia /15 min/	2.23±0.32	1.52±0.07	1.07±0.07	2.38±0.25	4.30±0.25	4.72±0.32	6.12±0.35	4.75±0.41	0.62±0.04	27.28±2.41
	n = 9	n = 9	n = 8	n = 11	n = 10	n = 12	n = 6	n = 11	n = 9	n = 9
	x	xxx	xxx	N.S.	N.S.	x	x	N.S.	N.S.	N.S.

All the symbols used above carry the same meanings as mentioned before /in table 2./.

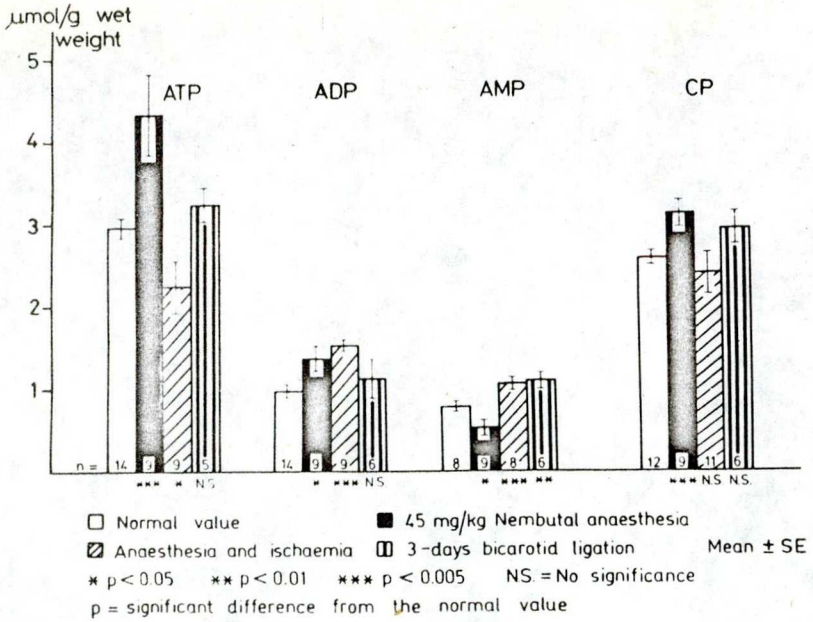

Fig. 13. The effect of nembutal anaesthesia and of anaesthesia when combined with ischaemia, caused by bilateral carotid occlusion and transsection for 15 min, as well as the effect of 3 day bilateral carotid ligation, without transsection, on the normal values of ATP,ADP,AMP and CP of rat brain.

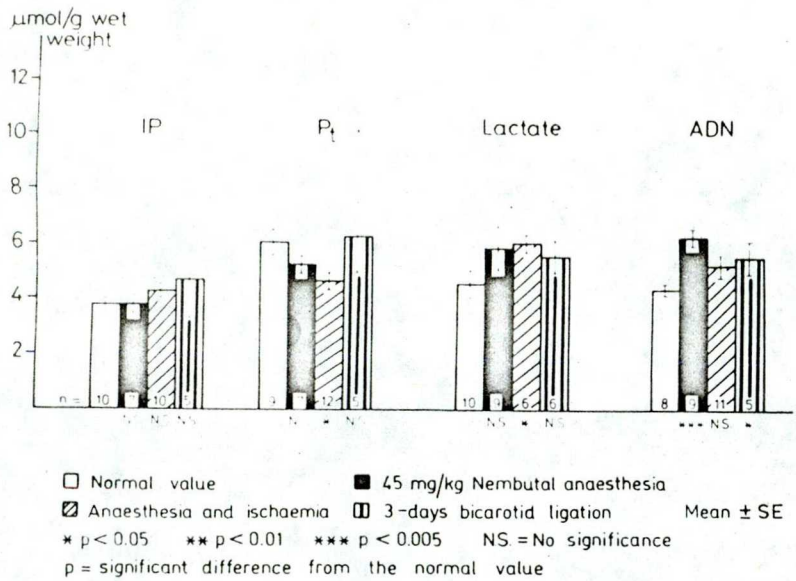

Fig. 14. The changes on the normal rat brain concentrations of inorganic phosphate /IP/, total phosphate /P_t/, lactate and the total adenine nucleotides /ADN/ following anaesthesia /nembutal 45 mg/kg/, anaesthesia combined with 15 min ischaemia and 3 day bilateral carotid ligation.

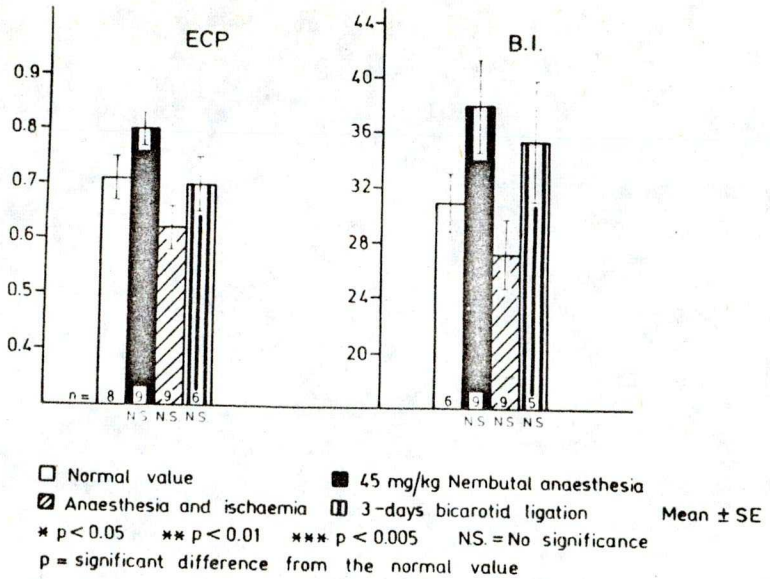

Fig. 15. The changes of the normal cerebral values, in rat brains, of two energetic parameters: the energy charge /ECP/ and the bioenergetic index /B.I./ following nembutal anaesthesia, anaesthesia combined with 15 min ischaemia and 3 day bilateral carotid occlusion, without transsection.

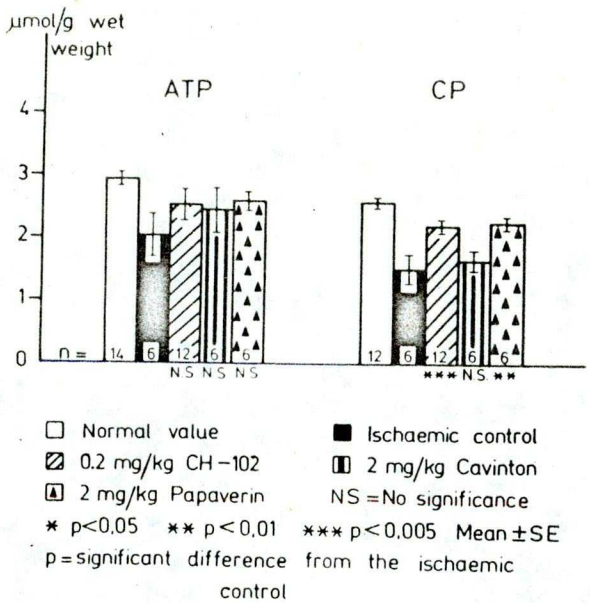

Fig. 16. The effect of papaverine, cavinton and CH-102 on the concentrations of adenosine triphosphate /ATP/ and creatine phosphate /CP/ of anaesthetized ischaemic rat brain.

hesia was still existing in the combined case but was masked by ischaemia. All those observations seemed to account for the protective effect of anaesthesia during ischaemia. Lactate was slightly elevated during anaesthesia then significantly increased when combined with ischaemia. The ADN was significantly increased with anaesthesia, but very slight increase was observed in the combined case. This might be due to the combination of the opposing anaesthetic and ischaemic effects on adenine nucleotides, especially ATP. Those observed improvements on ADN, compared to its normal value can not be interpretated by any reason other than anaesthesia.

The energy charge /ECP/ and the bioenergetic index /B.I./ were both increased on anaesthesia, showing the relatively improved energetic state of the brain. But on combination with ischaemia, their values remarkably declined down to a value even less than the normal. This might have reflected the drastic catabolic effects of ischaemia on the energetic state, as well as the protective effects of anaesthesia mediated through energy-conservation.

The results obtained for ATP, CP and IP, but not lactate, in both cases, of the anaesthesia alone and when combined with ischaemia, were similar to those observed in mouse brain by Gatfield et al. /1966/, who concluded that anaesthesia increases the concentration of glucose,

glycogen, ATP and CP, but decreases that of lactate, and that anaesthesia generally diminishes the rate of consumption of the energy reserves and reduces the rate of use of energy substrates.

All the parameters observed, in our study, during the combined case as well as those values of ATP and ADP in the only anaesthetized animals, were in coincidence with those of Winn et al. /1979/ except their values for lactate which, compared to ours, were very low in all the cases.

Our results were in agreement, to a higher extent, with those of Albaum et al. /1946/ and Dawson and Richter /1950/ who used similar freezing techniques, similar doses of nembutal and animals /rats/ similar to those used in our study.

IV. 5-6. 2. Discussion

Anaesthesia was found to increase the levels of ATP, CP and B.I., as well as the energy charge by decreasing the rate of consumption of the high energy phosphates. Studies of Falbergrova et al. /1970/ and Gatfield et al. /1966/ showed that anaesthesia increased the concentration and decreased the rate of consumption of glucose, glycogen and the high energy phosphates in the brain; and that the metabolic effects of anaesthesia depends on the dose,

depth and duration of anaesthesia. The low value of lactate after ischaemia could show either a decreased production of lactate or an increased consumption of it and both possibilities indicate a protective effect of the anaesthetic against lactacidosis, which is of detrimental effect to the brain viability and to the cerebral enzymatic reactions.

Our results indicated that although anaesthesia had preserved the brain high energetic state and decreased its rate of degradation, yet when ischaemia was applied in the anaesthetized animals, it depleted most of the high energy metabolites and thus decreased the ECP and the B.I. This may indicate two facts namely: the ischaemic catabolic changes were more severe than the protective anabolic changes of nembutal, and that unless the effect of anaesthesia was existing, the situation will be more worsened since anaesthesia reflected a high rate of protection to the ischaemic brain. Recent studies of Nemoto /1977/ indicated a remarkable recovery of functional neurologic damage in monkeys and dogs by administering thiopental 90 mg/kg, at 5 min after 16 min of ischaemia and this recovery was increasing with time after ischaemia i.e. proportional to the postischaemic time; and that complete recovery was attained after 7 days. It was also observed by Smith et al. /1974/ what Nemoto confirmed that: early barbiturate administration 5 min after ischaemia,

was much more better and effective in accelerating the restoration of function and recovery of brain viability than later administration. It is generally known that the longer the duration of ischaemia the less is the chance of recovery. According to Nemoto /1977/ the suggested mechanisms of nembutal protection to the ischaemic brain damage could be elucidated through: a./ reduction of brain oxygen metabolism. Such metabolic reduction decreases the degree of hypoxia and anaerobic glycolysis and aids the recovery of reflow, b./ reduction of osmotic gradients within the brain in order to attain constant pH and to prevent the dangreous effect of lactacidosis, c./ stabilization of lysosomal membranes due to the adjusted osmotic gradients and cellular pH, and d./ increased resistance of brain cells to the development of edema. Nemoto findings also suggested that a barbiturate may block the oxidation of lactate thus reducing brain oxygen demands during the postischaemic state and therefore reduce the development of continued hypoxia. This latter suggestion is in accordance with our observations with nembutal also since the lactate concentration was always higher than the normal value in cases of ischaemia and even in cases of anaesthesia with no ischaemia.

Comparison of those mild changes of ischaemia when combined with anaesthesia with the previous severe changes

of ischaemia alone, after decapitation, or bicarotid occlusion and transsection, shows clearly the beneficial protective effect of anaesthesia in general, and barbiturate, or nembutal, anaesthesia in particular, against the serious autolytic effects of ischaemia on the brain viability and function.

Pharmacology: results and discussions

IV. 7. Effect of Papaverin, Cavinton and CH-102 on cerebral energy state during experimental ischaemia in rats

The assay of drugs acting against cerebral ischaemia is rather difficult, especially if the protective or clinical action of a long term treatment should be studied. The clinical and pharmacological attempts to treat cerebral ischaemia are always related with improvement of cerebral circulation and are using such interventions which may increase this circulation, as vasodilators or brain tissue oxygenators, as well as protective mechanisms leading to the preservation of brain tissue viability. Brain metabolizers or oxygenators tend to improve the cerebral oxygen supply/demand ratio. Thus drugs reducing cerebral ischaemia don't only affect the blood supply and circulatory parameters of the brain, but also change

the level of substrates and metabolites characterizing the energetic state of the brain /Basharahil, Takáts and Szekeres, 1980/.

The aim of this study was to compare the effect, on energy metabolism and its different biochemical parameters in rat brain tissue, of the agents: papaverin, CH-102 and cavinton.

Alteration in the physiological concentrations of the creatine phosphate /CP/, ATP, ADP, AMP, total adenine nucleotides /ADN/, lactate /L/, energy charge /ECP/ and the bioenergetic index were observed. The means of the observed and calculated results were summarized in table 7. and graphically shown in figures 16-19.

In the ischaemic control animals,/15 min bilateral carotid occlusion and transsection/ significant changes were observed in comparison with the normal values. There was a decrease in the concentrations of ATP and CP. ADP and lactate were increased. AMP and ADN were not significantly changed.

The ECP and B.I. were both decreased in the control animals. The normal and the ischaemic control values of ECP were near to those obtained by /Villa et al., 1978/. The above observations were, more or less, in accordance with the previously mentioned results of the 15 min bilateral bicarotid occlusion and transsection, although numerically not exactly the same, which may be due to the

Table 7.

Effect of some cerebral vasodilators and oxygenators /CH-102, Cavinton & Papaverin HCl/ on metabolic and high energy parameters of the ischaemic rat brain

Substrates /in μ mol/g/	ATP	CP	ADP	AMP	ADN	L	B.I.	ECP
Normal values	2.95±0.10	2.58±0.08	0.98±0.07	0.80±0.05	4.37±0.21	4.55±0.47	30.90±2.13	0.71±0.04
	n = 14	n = 12	n = 14	n = 8	n = 8	n = 10	n = 6	n = 8
Ischaemic control /+50 % Propylene Glycol/	2.06±0.34	1.52±0.22	1.37±0.09	0.86±0.15	4.12±0.42	6.25±0.54	14.19±2.58	0.65±0.04
	n = 6	n = 6	n = 6	n = 6	n = 6	n = 5	n = 6	n = 6
CH-102 /0.2 mg/kg/ i.v.	2.56±0.22	2.21±0.08	1.33±0.13	0.56±0.07	4.46±0.22	6.07±0.61	24.51±1.43	0.72±0.02
	n = 12	n = 12	n = 12	n = 12	n = 12	n = 9	n = 12	n = 12
	N.S.	xxx	N.S.	x	N.S.	N.S.	xxx	N.S.
Cavinton /2 mg/kg/ i.v.	2.46±0.35	1.65±0.15	1.13±0.09	0.51±0.04	4.09±0.37	6.93±0.24	17.25±1.89	0.73±0.03
	n = 6	n = 6	n = 6	n = 6	n = 6	n = 6	n = 6	n = 6
	N.S.	N.S.	N.S.	x	N.S.	N.S.	N.S.	N.S.
Papaverin HCl /2 mg/kg/ i.v.	2.60±0.15	2.25±0.10	0.69±0.11	0.70±0.07	3.98±0.15	5.11±0.81	27.66±1.85	0.47±0.01
	n = 6	n = 6	n = 6	n = 6	n = 6	n = 6	n = 6	n = 6
	N.S.	xx	xxx	N.S.	N.S.	N.S.	xxx	x

/All the abreviations and symbols used above carry the same meanings as in table 2.

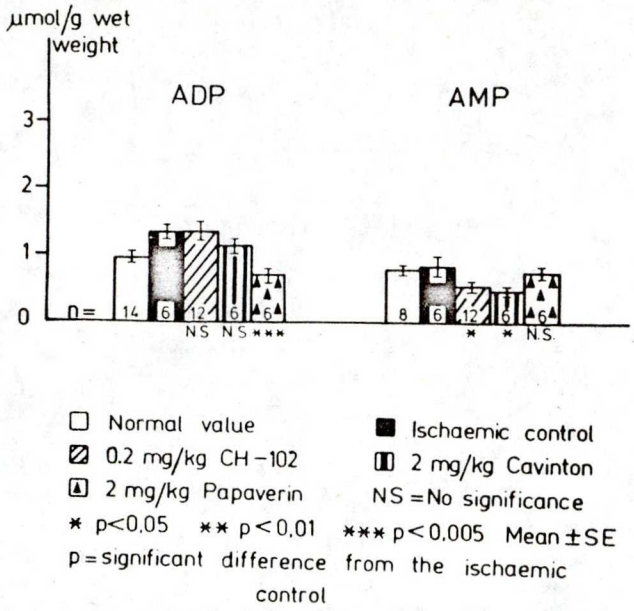

Fig. 17. The effect of papaverine, cavinton and CH-102 on the brain concentrations of adenosine diphosphate /ADP/ and adenosine monophosphate /AMP/ of anaesthetized ischaemic rats.

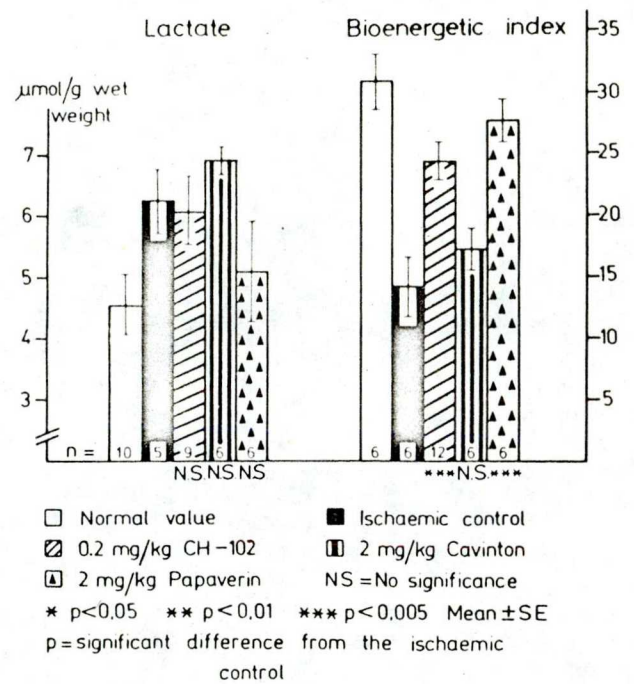

Fig. 18. The metabolic effect of papaverine, cavinton anc CH-102 on the levels of lactate and on the bioenergetic index /B.I./ of the ischaemic rat brain.

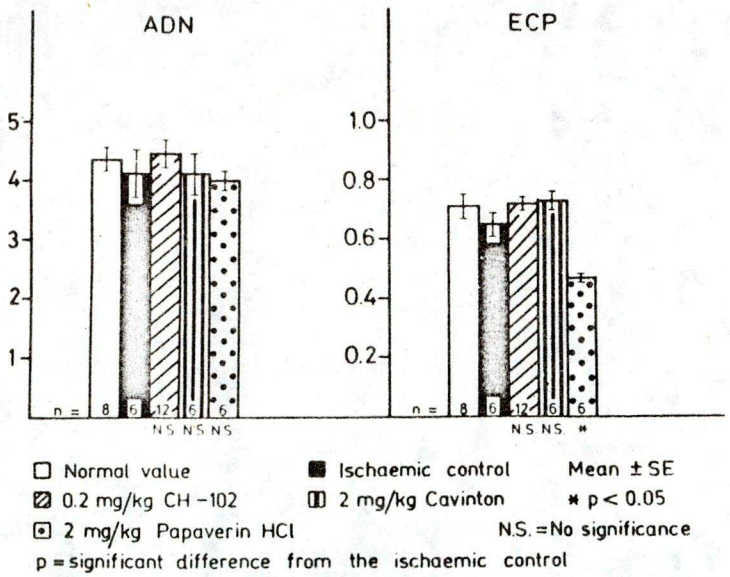

Fig. 19. Changes caused by papaverine, cavinton and CH-102 on the total adenine nucleotides /ADN/ and energy charge /ECP/ of the ischaemic rat brain

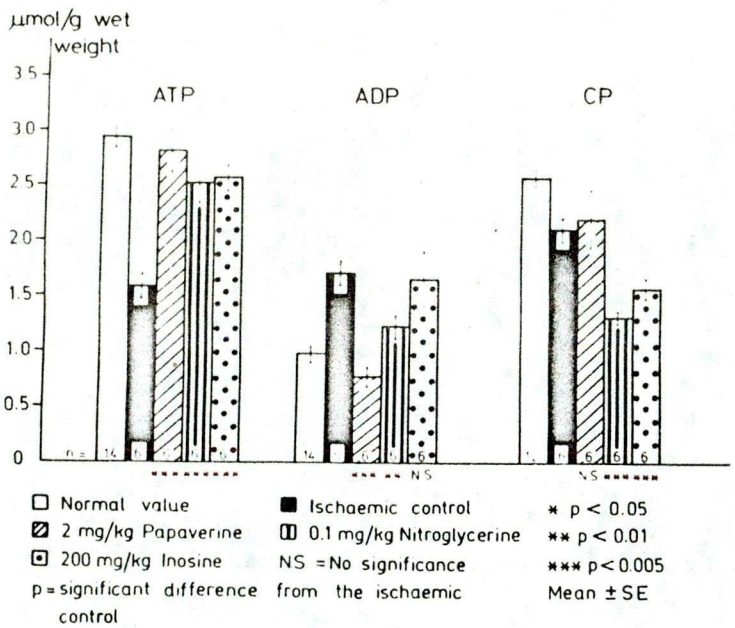

Fig. 20. Comparative effects of papaverine, nitroglycerine and inosine on the concentrations of adenosine triphosphate /ATP/, adenosine diphosphate /ADP/ and creatine phosphate /CP/ on the brains of anaesthetized ischaemic rats .

smaller number of animals used in this group, or the effect of the solvent, propylene glycol, which might have played some role in the isotonicity, pH or the viscosity of the circulating blood.

On the treated animals: - All of the studied drugs increased the concentration of ATP and CP as well as the B.I. by different degrees and decreased the concentrations of ADP and AMP in different proportions in control with the ischaemic values before treatment /Basharahil, Takáts and Szekeres, 1980/.

ATP was not significantly increased, while CP was more significantly increased by papaverin and CH-102 than with cavinton. ADP was only significantly decreased by papaverin but non significant decrease was observed by the others. The decrease of AMP was significant with CH-102 and cavinton. Cavinton in reducing AMP was more effective than both papaverin and CH-102. The results of cavinton obtained in our study for ATP, ADP and ADN were generally in agreement with those reported on rat brain by Rosdy et al. /1976/, although numerically their values were different due to some modifications in their method. The above workers used the parameter $\frac{ATP}{ADP}$, their value was 1.44 for the control and 1.56 with cavinton. This was near to the value of this parameter in our results being 1.50 and 2.34 for the control and cavinton treatment respectively.

To compare our results for papaverin with other workers, unfortunately very few literature were available for the cerebral action of papaverin; but no data was found on the action of CH-102, on the ischaemic rat brain. Our results regarding the effect of papaverin on the concentration of brain metabolites specially ATP, CP, ADP, AMP and the ECP were very similar to those of Villa et al. /1978/.

ADN was non significantly affected being slightly increased by CH-102 but was around the control value with cavinton. Papaverin decreased its value by about 3.4 %. Lactate was decreased by CH-102 and, specially, by papaverin. This may reflect the wash-out capabilities of the latter two agents to remove lactic acidosis from the brain through increasing the rate of the cerebral flow or the brain tissue viability and capability to utilize the accumulated lactate by converting it into pyruvate, which can easily enter the citric acid cycle to supply energy by its oxidation.

The energy charge /ECP/ was elevated by cavinton and CH-102 but significantly decreased by papaverin. The bioenergetic index /B.I./ increase was remarkable with papaverin and CH-102 respectively, and was non significant with cavinton. Generally speaking, although all of the studied drugs increased the concentrations of ATP, CP and B.I. by different degress, yet papaverin seemed

to be the most effective in this respect as well as in decreasing the concentration of lactate.

From the above results, it could be concluded that all the three agents, in the applied doses, produced a protective effect to the energetic state of the ischaemic brain. The increased values of ATP, CP and B.I. indicate the improvement of the total energetic state of the brain tissue. Such sensitive metabolic effects might help or lead to the elucidation of the mechanism of action of such agents.

Among the most important mechanisms protecting the brain against hypoxia or ischaemia are anaesthesia, vasodilatory and metabolic ones. The vasodilatory mechanism is thought to be mediated through vascular effects, namely relaxation of the vascular smooth muscles, leading to vasodilation which might be caused by the accumulation of adenosine, the nucleoside recently found to play an important role in the normal autoregulation of the cerebral blood flow /Berne et al., 1974/. Adenosine on another side, was thought to act as a thrombocyte - adhesion inhibiting agent /Cavinton Literature, 1979/, thus leads to an increased cerebral flow and decreased vascular resistance, actions which the above treated drugs might exert through its release. The metabolic mechanism to such agents, if found, could be mediated through the increased oxygen utilization of the brain, as proven to be caused by ca-

vinton /Cavinton Literature, 1979/, thus leading to increased tolerance to hypoxia and increased biosynthesis of ATP well as increased formation of cyclic AMP.

Papaverin produced the best values of cerebral energetic parameters that, to some extent, approached those of the normal values if the effect of the anaesthetic is put into consideration, being also, a protective agent to ischaemic cerebral tissues.

From the results discussed above, it could be concluded that in the aspect of improving the bioenergetic state of the ischaemic brain and in the doses of the different drugs used: papaverin seemed to be the most effective, followed by CH-102, then cavinton /Basharahil, Takáts and Szekeres, 1980/.

It might be of interest to mention at the end, that from the above three agents tested, only cavinton is offical agent that has clinically important cerebral indications. CH-102 is still under investigations. Although papaverin is known to possess cerebral vasodilating properties, yet cavinton is clinically prefered for its specific cerebral metabolic action and vascular properties as well as its ability to increase the systolic pressure. Cavinton also has the advantage that its duration of action is more prolonged than papaverin /Török, 1979/ and that it is more specific and selective in decreasing the cerebral vascular resistance, without any side effect on

the peripheral vascular system or on the central nervous system. Such properties neither papaverin nor CH-102 have not yet proved to possess.

IV. 8. Comparative study of the effect of nitroglycerin, inosine and papaverin on ischaemic rat brain

All the results were summarised in table 8. and graphically expressed in figures 20., 21. and 22. This experiment is similar to the previous one and both could be considered as one study. Papaverine HCl being the most effective agent in the previous experiment and being considered as the standard agent against which cerebral vasodilators are compared /Bass and Toole, 1975/, it was chosen again here to compare with it or standardize the cerebral metabolic properties of nitroglycerin as well as inosine. All the agents used were dissolved in normal saline /0.9 % NaCl/ to be isotonic for parentral administration. The same solvent, normal solution, was used for the control group. Accordingly a slight differences could be observed in the values of metabolites and parameters of this control and that of the last experiment as well as in the effects of papaverin in both experiments. But the observed differences were not remarkable /compare table 7., 8/. However, our results and conclusions were again compared with those

of Villa et al. /1978/ who used normal solution in the control animals. And whose general conclusions on the effect of ischaemia and the action of papaverine were in agreement with ours.

Changes on the treated animals with:

1./ Papaverin HCl /2 mg/kg i.v./: produced an improvement on the energetic state as reflected by the significant increase in ATP which was 83 % higher than the control. CP was similarly increased by 17 %. ADP decreased by 54 %; while AMP was non significantly changed. Those results were similar to those obtained with papaverin in the previous study and which were already reported /Basharahil et al., 1980/.

The relative differences between the results of the two experiments might be due to the animals being here slightly bigger /250-300 g/ than the ones available in the previous group /200-250 g/. This difference could exert changes in the acceleration of tissue freezing. Other experimental changes might be due to the different solvents used, each solvent being of different physical and rheological properties which might have exerted some effect in the brain cellular permeability, blood viscosity or the absorpability of the animal tissue to the normal solution and the other relatively viscous solution /50 % propylene glycol/ although the latter solvent was made isotonic. This effect of the changes in viscosity as well as the

Table 8.

Effect of Papaverine, Nitroglycerin and Inosine on some cerebral metabolic parameters of the ischaemic rat brain

Substrates /in μ mol/g/	ATP	CP	ADP	AMP	ADN	L	B.I.	ECP
Normal values	2.95±0.10	2.58±0.08	0.98±0.07	0.80±0.05	4.37±0.21	4.55±0.47	30.90±2.13	0.71±0.04
	n = 14	n = 12	n = 14	n = 8	n = 8	n = 10	n = 6	n = 8
Ischaemic control /+0.9 % NaCl Soln/	1.57±0.15	2.11±0.11	1.71±0.11	0.40±0.05	3.70±0.18	5.85±0.32	21.95±1.32	0.66±0.01
	n = 6	n = 6	n = 6	n = 6	n = 6	n = 5	n = 6	n = 6
Papaverin HCl /2 mg/kg/ i.v.	2.87±0.20	2.46±0.26	0.78±0.09	0.43±0.07	4.05±0.21	5.52±0.85	33.20±4.84	0.80±0.03
	n = 6	n = 6	n = 6	n = 6	n = 6	n = 6	n = 6	n = 6
	xxx	N.S.	xxx	N.S.	N.S.	N.S.	x	xxx
Nitroglycerin /0.1 mg/kg/ i.v.	2.53±0.18	1.31±0.05	1.24±0.11	0.40±0.05	4.17±0.11	3.61±0.54	12.24±0.91	0.76±0.02
	n = 6	n = 6	n = 6	n = 6	n = 6	n = 6	n = 6	n = 6
	xxx	xxx	xx	N.S.	x	xx	xxx	xxx
Inosine /200 mg/kg/ i.p.	2.57±0.12	1.58±0.09	1.66±0.25	0.41±0.06	4.64±0.27	3.44±0.37	14.75±1.19	0.74±0.02
	n = 6	n = 6	n = 6	n = 6	n = 6	n = 6	n = 6	n = 6
	xxx	xxx	N.S.	N.S.	x	xxx	xxx	xx

All the symbols and abreviations used above carry the same meanings as in table 2.

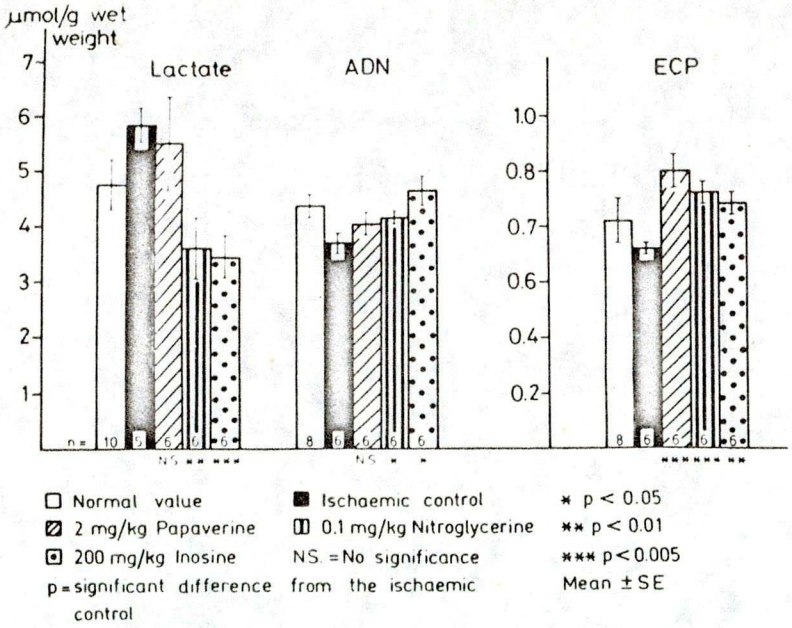

Fig. 21. The comparative metabolic effects of papaverine, nitroglycerine and inosine on the cerebral concentrations of lactate, total adenine nucleotides /ADN/ and the energy charge /ECP/ of the brain of ischaemic rats .

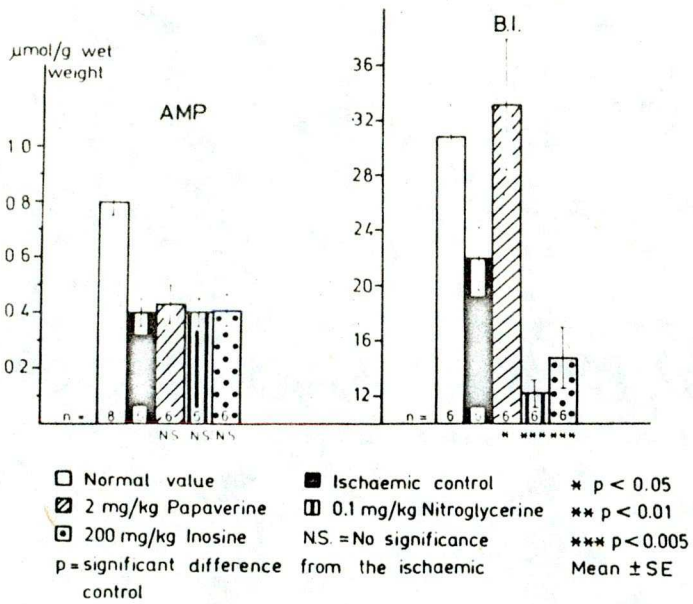

Fig. 22. Protective metabolic effects of papaverine, nitroglycerine and inosine on the cerebral concentration of adenosine monophosphate /AMP/ and the bioenergetic index /B.I./ of the ischaemic rat brain .

effect of 0.9 % NaCl solution in improving the ischaemic cerebral vessels was mentioned and confirmed by Ljunggren et al. /1974/. ADN seemed to be more better in this study with papaverin where an increase of 9 % of the control value was observed thus reflecting an improvement of the adenyl pool and its restoration.

Papaverine led to a decreased value of lactate by 6 % from the control which is less than the decrease obtained /18 %/ when propylene glycol was used as solvent. The energy charge /ECP/ was significantly increased by about 21 %. This value reflected the improvement of the cerebral energetic state which was mainly due to the increase of ATP. Bioenergetic index /B.I./ was significantly increased to about 51 % from the control.

All the above changes with papaverin namely the increased values of ATP, CP, ADN, B.I. ECP and the inhibited concentrations of ADP and Lactate were the main remarkable symptoms of improvement of the cerebral energetic state as well as of the cerebral flow.

2./ Effect of nitroglycerin: - this agent led to a marked increase /61 %/ in ATP. But unfortunately CP was significantly decreased /38 %/ from the control. ADP also decreased by about 28 %, but AMP was not affected by nitroglycerin. The increased values of ATP and ADN reflected an improvement in the energetic state of the ischaemic brain.

Nitroglycerin was more effective than papaverin in the lactate removal circulatory parameter according to which lactate was depressed by 38 % with nitroglycerin while papaverin decreased lactate by only 6 %. This parameter may also reflect the capability of the drugs to increase the cerebral availability and consumption of oxygen and thus the increased ability of the cerebral tissue to oxidise lactate to pyruvate, which on further oxidation yields CO_2 + energy with the subsequent formation of ATP molecules. And this pathway might have played an important role in the improved values of adenyl nucleotides. Accordingly, this factor together with ATP or ADN changes may constitute, at least, a part of a metabolic mechanism of action of nitroglycerin as well as papaverin.

The bioenergetic index /B.I./ with nitroglycerin was, in contrast to papaverin significantly inhibited by 44 %; while the energy charge /ECP/ was increased by 15 % from the control. These two parameters were reflecting the fact that the total energetic state of the brain was shifted towards increased consumption of energy /catabolism/ to a degree, more than what the total anabolic pathway or restitution can provide.

3./ Effect of inosine: - ATP was significantly increased by about 64 % from its control value. But, similar to nitroglycerin, CP was decreased by 25 % with inosine. ADP was decreased 3 % and in the same time AMP increased by

3 % from the control. Although those observations reflected an improvement in the adenine nucleotides, yet they also reflected a worse situation in the total energy state which might be due to the inhibition of CP. ADN was increased by 25 % thus confirming the improvement of the adenyl pool. Lactate concentration was inhibited by 41 % from the control a fact which may show an improvement in the cerebral circulation as a consequence of lactate wash - out and the possibility of improved tissue oxygenation, which might have led to the utilization of the accumulated lactate in the ischaemic model. Such metabolic effect of inosine might be confirmed by the improved ECP, which was increased from its control value by 12 %.

The bioenergetic index /B.I./, being highly affected by the CP depression, was decreased below the control value by about 33 %. This might have reflected the disturbance of the total energetic state and the incapability of inosine, and nitroglycerin as well, to favour the total energetic state of the ischaemic brain towards restitution and improvement. The increased degradation of CP, even in presence of inosine or nitroglycerin seemed to play a great role in this failure.

According to the above results, it could be concluded that papaverin was the most effective due to its ability to increase ATP, CP, ADN as well as the ECP and

B.I.; and to decrease the value of ADP and lactate. All those effects when combined together may certainly indicate improved cerebral circulation and cerebral energetic state.

Nitroglycerin and inosine failed to achieve the improvement of CP and B.I. But they still have beneficial effects on the improvement of ATP, ADN and ECP as well as some circulatory improvement as could be observed from the decrement of lactate. On respect to their percentage improvements on the above mentioned parameters, it could be concluded that: papaverin was the most effective agent, followed by inosine and finally nitroglycerin. The latter two agents were compared with regard to their increase of ATP and ADN, and their lactate removal ability which was 41 % and 38 % respectively for inosine and nitroglycerin.

General conclusion: - On further comparison between the effects of the agents used in the last experiment and those used above, on the metabolic and cerebral parameters of the ischaemic brain, it could be concluded that the order of cerebral antiischaemic effectivity seemed to be as follows: papaverin, CH-102, cavinton, inosine then nitroglycerin respectively.

It might be for the above mentioned reasons or observations that inosine and nitroglycerin were not clinically used in cerebral ischaemic infarctions.

SUMMARY

The aim of this study was 1./ to investigate some models of cerebral ischaemia using different techniques and to chose from those models the most suitable one for investigating the metabolic effects of the pharmacologically active metabolic or cerebrovascular agents, 2./ to study the protective effects of barbiturate anaesthesia in cerebral ischaemia, as well as to study the protective metabolic and antiischaemic effects of different pharmacological agents i.e. papaverin, nitroglycerin and inosine; as well as those of the newly-developed Hungarian agents Cavinton and Chinoin - CH-102 on the brain of anaesthetized ischaemic rats.

The review of literature cited in this study was slightly detailed in the aspects of pathophysiology, biochemistry and pharmacology of cerebral ischaemia as well as the controversion existing among those workers which requires the clarification of their different points of view. The etiology, effects, consequences and pharmacology of cerebral ischaemia was reviewed as well as the different experimental models used by different workers.

The methods used to induce experimental cerebral ischaemia were applied on male Wistar rats, of average weight 200-300 g feeded on normal diet and tap water.

The substrates analysed and investigated were adenosine triphosphate /ATP/, adenosine diphosphate /ADP/, adenosine monophosphate /AMP/, creatine phosphate /CP/, inorganic phosphate /IP/, total phosphate /P_t/ and lactate /L/ as well as the calculated cerebral energetic parameters: total adenine nucleotides /ADN/, energy charge potential /ECP/ and the bioenergetic index /B.I./. The substrate concentration of the above metabolites was evaluated as μ mol/g of wet brain tissue, in addition to their statistical analysis of the standard errors and significance.

The ischaemic models applied in this study were done by:

1./ Decapitation for 15 sec and 30 sec, before freezing the heads, on awake conscious animals. On comparing the concentrations of substrates after the two periods significant changes were observed, already in the early stage of ischaemia after decapitation. Those alterations were even more expressed in the prolonged stage of this model. In both stages, the alterations observed were a decrease in ATP and CP concentrations as well as in ECP and B.I. values while ADP, AMP, IP and lactate were increased. Those changes reflect the main metabolic alterations in the ischaemic brain. Because of the quick severe detrimental changes following decapitation and due to the irreversible changes following this model of total cerebral ischaemia, as shown from

the brain sensitive substrates and parameters, it was concluded that this model is unsuitable for the pharmacological investigations of cerebral drugs.

2./ Bilateral carotid occlusion for 3 days: this method did not produce any significant changes in the energy state of the brain. The substrate analysis of this model proved that such intervention is not enough to produce cerebral ischaemia. In this model neither ischaemic lesions nor damage of cerebral viability or function were observed, as long as the mean arterial blood pressure was kept constant or normal. This method produced no detrimental effects on the substrate concentrations of the metabolic and energetic state of the brain. The sensitive parameters ATP, CP, ADN and B.I. were not affected; and only AMP, IP and lactate were slightly increased. It was concluded that bilateral carotid occlusion, produces no remarkable energetic or metabolic changes in rat brain, unless it is combined with other pathological means such as hypoxia, anoxia, hypotention or transsection of the occluded arteries. Due to the insensitivity of this model to indicate the metabolic changes, it was not prefered as a model of choice for the pharmacological investigations.

3./ Bilateral carotid occlusion and transsection for 5, 15, 60 and 240 minutes respectively before freezing of the total animals. In those models the time-course

changes, observed during ischaemia, were traced and discussed from the metabolic and energetic points of view. During the 4 hours period following, such ischaemia significant changes were observed. ATP, CP and ADP levels were decreased while AMP, IP and lactate were elevated above the control. Those signs were characteristic for the ischaemic alterations.

To study the rate of tolerance of the brain tissue and the degree of survival of the animals following the selected type of ischaemia for more prolonged periods than those stated above, it was observed that 5 animals out of 6 died within the first 24 hours. All the animals died after being showing severe symptoms of cerebral insufficiency, blindness and behavioural disorders. It was concluded that such prolonged periods of ischaemia after transsection of the carotids are very serious and fatal to the brain and to the animal survival as a whole. By comparing the different models from the pharmacological points of view, the model of 15 min ischaemia was chosen for the pharmacological investigations of the studied cerebral protective and antiischaemic drugs.

4./ The effect of anaesthesia on the normal and ischaemic brain was investigated in the above selected model of ischaemia, using Nembutal 45 mg/kg i.p. Normal and ischaemic brains anaesthesia caused an elevation in the values of ATP, CP, ADP, ADN and B.I. The degree of

elevation was more pronounced in the anaesthetized than in the anaesthetized-ischaemic animals. Those parameters reflect the improvement of the total energetic state of the brain due to anaesthesia even in the ischaemic brain. The normal ECP was improved; lactate and IP were both slightly increased on anaesthesia and on combination of anaesthesia and ischaemia, a fact which reflects beneficial protective effect of anaesthesia in general and barbiturate - anaesthesia in particular on the brain viability and function and especially on its metabolic and energetic state.

The pharmacological part of this dissertation was a comparative analytical study of the effects of: papaverin, cavinton and CH-102 as well as nitroglycerin and inosine, in cerebral ischaemia generally, and on the energetic state of the ischaemic brain in particular. Alterations in the concentrations of CP, ATP, ADP, AMP, lactate, ADN, ECP and B.I. were studied on the already selected model of cerebral ischaemia. Papaverine 2 mg/kg, CH-102 0,2 mg/kg and cavinton 2 mg/kg produced a protective effect to the reduced energy state of the ischaemic brain. CP and ATP concentrations were increased, while ADP, AMP and lactate levels were decreased. B.I. was also increased indicating the improvement of the bioenergetic state. CH-102 and papaverine proved to be the most effective in this respect.

On the other part of the pharmacological study papaverin 2 mg/kg nitroglycerin 0.1 mg/kg and inosine 200 mg/kg were found to increase the ischaemic values of ATP, ADN and ECP; and to decrease those of lactate. CP and B.I. values were improved by papaverin only. The other agents decreased the values of those parameters in different degrees.

Accordingly papaverine seems to be the most effective agent among those tested. The other agents still have some beneficial effect on the improvement of the adenyl pool of the high energy phosphates as well as in the cerebral circulation as compared from the lactate--washout - or consumption. In those aspects inosine was comparatively more effective than nitroglycerin. Comparing the activity of these agents with those of the previous study; the order of increasing effectivity seems to be papaverine, CH-102, cavinton, inosine then nitroglycerin respectively. According to these experimental results we may state that vasodilating drugs, reducing cerebral ischaemia, do not only affect the blood supply of the brain but also change the metabolites characterizing the energetic state of the brain tissue.

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Igazgató Ur!

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