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ON SUBSTANCE-GROUP SPECIFICITY OF ANTIARRHYTHMIC TEST METHODS

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This thesis is dedicated

to

my mother

Marianne Bremer

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I. INTRODUCTION

The search for new drugs able to prevent or to correct cardiac arrhythmias still remains difficult and largely empirical. So far there is no generally accepted procedure for the quantitative assay of these compounds. Although nowadays modern techniques as programmed stimulation are helping to identify the origin, and the precise mechanism of the underlying electrophysiological disturbance in some types of arrhythmias, the development and evaluation of antiarrhythmic compounds is made difficult firstly because the underlying mechanisms of arrhythmias are not fully understood, secondly because animal models of arrhythmias do not necessarily mimic those seen in man.

The importance of producing model arrhythmias in experimental animals, possibly similar to the pathologic phenomena observed in patients, lies in the following facts:

1. It may give a deeper insight into the basic phenomena underlying the pathomechanism of the type of arrhythmia in question by analyzing the method producing the heart rhythm disturbance; furthermore the experimental approach in animals is much less limited than in the patient suffering from arrhythmia.

2. Experimental arrhythmias may serve as a basis for the quantitative assay of antiarrhythmic drugs and may represent a useful tool in the search for effective new agents capable of preventing or suppressing heart rhythm disturbances. It must be born in mind that the collective term arrhythmia includes a number of heart rhythm disturbances of various types which differ considerably from each other in etiology as well in the pathomechanism. Accordingly for each type of arrhythmia occurring in patients, elaboration of a corresponding experimental arrhythmia seems justified. However, this demand is by no means fulfilled at present, and we must be aware of the fact that the majority of the experimental arrhythmias hitherto produced do not correspond to the pathological phenomena observed in the patient. These models were developed under the assumption that if one or more symptoms, objectively measurable parameters of the human heart rhythm disturbance in question, could be reproduced, the mode and site of action of the measures evoking these disturbances could be related to the pathologic changes which underlie the arrhythmia occurring in man. This is, of course, not valid for all cases, but as a working hypothesis it proved quite useful, especially in the search for new antiarrhythmic drugs.

Numerous methods are available for the induction of arrhythmias and many of these have already been extensively reviewed /Szekeres, 1981; Szekeres and Papp, 1971; Caillar and Louis, 1980/. There is another problem representing a major concern of those involved in preclinical drug research, namely the predictability of clinical efficacy of drugs from animal experiments. The answer is often not easy - one could rather speak of a higher or lower probability of agreement between preclinical effects - however in case of the cardiovascular and especially of the antiarrhythmic drugs the situation is more favourable. On the basis of theoretical considerations as well as of purely empirical data the results of animal experiments with antiarrhythmic drugs can be fairly reliably extended to the treatment of patients with heart rhythm disturbances. This is mainly due to the fact that as far as the rules governing cardiac activity are concerned, there is no fundamental difference between man and the other mammalian organisms. If we consider that drugs influencing the more complex and partly inadequately known higher nervous activity - e.g. the psychopharmaca which could be introduced to therapy on the basis of inadequate and indirect evidence from animal experiments, the above assertion concerning

antiarrhythmic drugs seems to be even more firmly established.

In view of the above discussed problems practice has shown that reliability of results concerning validity of antiarrhythmic action and predictive value of experimental models for clinical efficacy can be augmented by using several model arrhythmias for the assay of drugs.

II. OBJECTIVES AND SCOPE OF THE PRESENT EXPERIMENTS

Aim of the present study was to investigate the responsiveness of the most commonly applied antiarrhythmic test methods to different types /and classes/ of antidysrhythmic agents, represented by drugs widely used in the clinical therapy of rhythm disturbances.

It is well known that a host of different tests are needed to assure an adequate evaluation of antidysrhythmic activity of a given compound and a satisfactory predictability of the clinical usefulness from results of animal experiments. A selection of the test methods just adequate for a given class of compounds could reduce the number of idle experiments and thereby save time and money. Elucidation of this differentiated

sensitivity of test methods to different types of antiarrhythmic agents could contribute to the elaboration of a new, more economic strategy for the assay of antidysrhythmic substances. In addition in the present dissertation the general principles of antiarrhythmic assay methods, furthermore of the different techniques for production of experimental arrhythmias as well as the various procedures for evaluating antiarrhythmic activity will be presented and discussed.

III. BASIC PRINCIPLES UNDERLYING THE APPLICATION OF ANTIARRHYTHMIC ASSAY METHODS

As is well known, the cardiac dysrhythmias arise mainly on the basis of some changes in the fundamental electrical properties of the heart. On the other hand, the antiarrhythmic action of drugs results mainly from their ability to correct these electrophysiological changes. Therefore, knowledge of the electrical events in the heart is indispensable for a better understanding of the pathomechanism and therapy of heart rhythm disturbances. However, there is a considerable gap between discoveries in the basic electrophysiology of the heart and their application in antiarrhythmic

therapy in patients.

The fundamental electrical events in the heart and their drug-induced changes can be best studied in the isolated "in vitro" cardiac preparation. One advantage of this latter is that it is relatively simple to prepare and to keep under constant conditions; furthermore, the heart rate, the O_2 supply, the temperature, and the ionic composition of the nutrient solution can be changed separately, according to need. Moreover, it allows a deeper insight into the mode of action of antiarrhythmic drugs, including estimation of membrane transport of ions and of membrane currents. Because of these undeniable advantages, there is a tendency to rely more on "in vitro" experiments than justified.

The main question for the pathophysiologist and the pharmacologist is how far the cardiac electrophysiological changes in vitro and their drug-induced modifications are relevant to prediction of "in vivo" pathological conditions as well as the efficacy of antiarrhythmic drugs "in vivo". Because of the widespread use of in vitro preparations and because of the too far-reaching conclusions drawn from "in vitro" experiments, it should be emphasized that the correlation is by no means as close as generally believed.

In the following, some factors responsible for this discrepancy between results of "in vitro" and "in vivo" experiments will be discussed.

Arrhythmias generally do not arise from a change of a single fundamental electrophysiological parameter /e.g. shortening of repolarization of increased rate of depolarization/ but usually appear as a result of the arrhythmogenic interactions of appropriate changes in several parameters. Thus the antiarrhythmic action of a given drug depends on the net result of its effects on these parameters /Szekeres and Papp, 1971; Szekeres and Papp, 1975/. For instance, one of the principal mechanisms of dysrhythmic activity - circular reentry of impulses around an anatomic obstacle - depends on the relationship between the length of the pathway and the product of conduction velocity and refractoriness, i.e., the length of the impulse. If the length of the impulse exceeds the length of the pathway, the impulse will die off, and no reentry will occur. This may happen either by prolongation of the refractory periods, or by an increase in the conduction velocity, or by both. However, some membrane-stabilizing antiarrhythmic agents not only prolong the refractory period but at the same time depress conduction velocity. Thus, they evoke an antiarrhythmic change in

the first and an arrhythmogenic change in the second fundamental electrophysiologic parameter. It is clear that the drug may interrupt the circus movement of impulses only if the first action is much more pronounced than the second /Cranefield, 1975/.

Arrhythmias originate from pathological changes within the myocardium, or from the influence of extracardiac factors, or from both. The assay of antiarrhythmic drugs is, however, mostly performed on preparations made from normal myocardium. Pathophysiological changes in the myocardium such as hypoxia, ischemia, or ionic shifts may consideraly modify the effect of drugs on the electrophysiological events in the heart.

Even if pathophysiological changes are produced in the experiments in vitro, they reflect only one aspect of the complicated system underlying the mechanism of the observed arrhythmia in vivo /Szekeres and Papp, 1971; Szekeres, 1973/. This is because of the absence of extracardiac factors in experiments in vitro, especially those of hormonal and autonomic nervous regulation. Extracardiac factors, especially together with arrhythmogenic changes of cardiac origin, play a much more important role than generally assumed, not only in the genesis of arrhythmias but in the effectiveness or ineffectiveness of antiarrhythmic drugs. To give an example,

in patients with increased sympathetic tone, high--grade ventricular arrhythmia common during the first hour of infarction may be refractory to lidocaine /Adgey et al. 1971/. This drug was found to depress ectopic activity in only one-third of the patients /Pantridge et al. 1974/. In such cases administration of beta-blockers in graded doses may abolish the arrhythmia /Lemberg et al. 1970; Ribner et al. 1979/.

The ultimate object of the search for new antiarrhythmic compounds is to produce clinically useful drugs. So the program for screening effective compounds should involve, besides evaluating antiarrhythmic activity, the detection of possible undesirable side effects. Since experimental arrhythmias in animals are different from those occurring in patients, it is rather difficult to predict clinical usefulness from the results of pharmacologic results. In view of this, the first condition to be considered in selecting antiarrhythmic compounds is a satisfactory agreement between results of pharmacologic and clinical evaluation. Furthermore the method used should be reproducible, simple, and inexpensive.

Antiarrhythmic assay methods may be studied on

1/ isolated parts of the heart /isolated left or right atria, or isolated papillary muscle/,

2/ isolated heart /Langendorff heart preparation or heart-lung preparation/,

3/ more or less intact animal /anesthetized or conscious/.

As regards species mammals are the most used animals in testing and can be divided in two groups. The first group are animals with a relatively small heart and therefore capable of spontaneously restoring ventricular fibrillation to normal sinus rhythm. These include the rat, the guinea pig and the rabbit. Animals of the second group are those with relatively larger hearts such as the dog, pig or monkey and they are not inclined to spontaneous defibrillation.

The rat is particularly suitable for assays where a large number of experimental animals representing a homogeneous population is required for purposes of statistical evaluation. Besides the rat guinea pig and rabbit hearts are good subjects for isolated preparations, and the intact animal of these species is also suitable for assay methods.

In contrast to these animals, the dog is highly inclined to develop lasting ventricular fibrillation upon external stimuli. Its susceptibility exceeds even that of man and due to its relatively large heart size, spontaneous defibrillation is very rare. Accor-

dingly, like the pig it seems to be less suitable for screening of antiarrhythmic compounds than the aforementioned smaller and cheaper animals. On the other hand the relatively large heart size of dogs and pigs makes it possible to perform a detailed analysis of "in situ" electrophysiological changes of preselected compounds with marked antiarrhythmic activity. Here it is advisable to study their electrophysiological and hemodynamic actions on the highly sensitive dog or pig before starting clinical tests.

Pharmacologic testing in healthy volunteers and even more in arrhythmic patients seems to be the most satisfactory procedure, but in view of the multiplicity of different disturbances of rhythm that occur in man, it is important to apply it in a very carefully selected population of patients.

As already mentioned, there are numerous procedures for evaluation of antiarrhythmic activity, employed mainly on an empirical basis without appropriate theoretical foundation. Nevertheless all assay methods used so far, are based on the following three fundamental principles /Szekeres, 1964/:

1. Some type of experimental arrhythmia is produced, and the dose of the antiarrhythmic drug determined which is able to:

a./ abolish persisting arrhythmia /suspending dose/ or

b./ prevent the production of arrhythmia /preventive dose/.

2. Increase due to the antiarrhythmic agent in the intensity of the threshold stimulus just eliciting arrhythmia is determined.
3. Changes induced by antiarrhythmic drugs in the fundamental properties of the heart, as conductivity, excitability, refractoriness, and automaticity, assumed to play a role in the development or perpetuation of arrhythmias, are compared.

All three principles are represented in the assay methods applied by us and described in detail in the chapter of Methods.

IV. MATERIALS AND METHODS

a./ Techniques used for production of experimental arrhythmias

The techniques for production of experimental arrhythmias in animals which may yield useful information can be divided into four groups according to the nature of stimuli eliciting dysrhythmias. Thus these

can be evoked by

1. electrical stimulation of the heart
2. mechanically or thermally produced local conduction disturbances and ectopic foci
3. chemical stimuli /including overdose of drugs/, applied systemically or topically
4. direct excitation of the central nervous system.

The first 3 types of arrhythmogenic stimuli as well as their different combinations proved to be very useful in pharmacological assay of antiarrhythmic agents. The CNS stimulation is mainly of pathophysiological interest.

1/ Induction of arrhythmias by <u>electrical stimulation</u> of the heart has been known for over a century and many variations in technique have been described. Atrial or ventricular fibrillation can be produced by a single electric shock, serial shocks or by progressively increasing the stimulation rate. All electrical methods used to induce fibrillation depend upon the concept that following normal excitation not all cardiac fibers /even those in close proximity/ recover their excitability simultaneously /Mines, 1913; Moe et al. 1964/. This results in a vulnerable period during the cardiac cycle at the end of systole at which time

some fibers have recovered their excitability while others are still refractory. An extra stimulus of sufficiently strong intensity applied during the vulnerable period will precipitate fibrillation. Allessie et al. /1973, 1976/ have mapped the spread of activation of a single premature stimulus and measured refractory periods at multiple sites in the rabbit atrium. These studies have provided the first direct evidence to suggest that the naturally existing non-uniform recovery of excitability is of major importance for the occurrence of re-entrant tachycardias. Presumably if the intensity of the extra stimulus is further increased, the spread of excitation becomes sufficiently disorganized to precipitate fibrillation.

Antiarrhythmic drugs are expected to increase the intensity of the current necessary to evoke fibrillation.

2/ The mechanical /local stretch/ or thermal /focal cooling or rewarming/ interventions alter the fundamental properties of the heart within a circumscribed area of the myocardium, giving rise to local blocks and to spontaneously firing ectopic foci. These in turn may cause asynchronous propagation of impulses leading to irregular cardiac activity.

Local ischemia of the heart brought about by li-

gation of the coronary arteries is one of the most adequate and popular forms of application of the foregoing principle. Arrhythmias so produced are etiologically similar to the arrhythmias occurring in man in association with local ischemia brought about by heart infarction.

After coronary ligation, myocardial excitability increases in the ischemic area as a consequence of hypoxic instability of the membrane /Brooks et al. 1960/. Moreover the action potential is shortened. All these factors favour the appearance of extrasystoles. In prolonged ischemia the resting membrane potential is reduced. Ligation of the left descending coronary artery in dogs produces arrhythmias and to various extent ventricular fibrillation depending on the collateral blood supply of the ischemic myocardium. Therefore Harris introduced the 2 stage ligation method, inserting at least a 30-minute interval between partial and total ligation of the coronary artery. Then the chest is closed and spontaneous respiration restored. Arrhythmia appears in the form of a lasting ventricular tachycardia five to seven hours following complete occlusion, but maximal ectopic activity suitable for assay of drugs appears only on the second day after operation /Winbury and Hemmer, 1955/.

3/ A large number of chemical agents is capable of inducing arrhythmias. The most commonly used are chloroform, aconitine, acetylcholine and ouabain. They are used in various models and are either applied locally, i.e. directly to the myocardium, or injected into the vessels. Ouabain arrhythmias are of great clinical inportance in view of the frequent overdose of cardiac glycosides followed by typical rhythm disturbances.

Nahum and Hoff in 1940 demonstrated that topically applied acetyl-beta-methylcholine induces flutter or fibrillation in the dog and cat heart. Scherf et al. /1950/ subsequently reported a similar action for acetylcholine. The method usually employed is that of Schalleck /1952/ or a modification of it /Hagemann and Pruss; 1979/. Scherf et al. /1947/ introduced the method of applying aconitine on the atrial surface of the dog heart, either by using a cotton pledget immersed into aconitine solution, or by applying aconitine crystals, or by subepicardial injection of the drug. As a consequence, auricular tachycardia, flutter, and occasionally fibrillation appeared, depending upon the concentration used.

Chloroform may sensitize the heart to the action of endogenous catecholamines /Levy, 1914/, which ex-

plains the mechanism of arrhythmias induced by chloroform alone.

b/ Selection of antiarrhythmic compounds

1/ Quinidine

Quinidine, a quinine derivative, is the prototype of class Ia antiarrhythmic agents /Vaughan Williams, 1970/. It decreases the rate of rise of the action potential by interfering with the fast sodium channel. Action potential duration as well as refractory period are prolonged by reducing membrane permeability to passive potassium efflux during phase 3 repolarization /Szekeres and Vaughan Williams, 1962/. Quinidine decreases transmembrane permeability to passive influx of sodium ions during the spike action potential. Electrical V_{max} is reduced by quinidine to a greater extent in ectopic pacemaker tissue than in the sinoatrial node and other normally automatic fibres.

2/ Lidocaine and Mexiletine

Lidocaine is a local anesthetic with class Ib antiarrhythmic action /Bigger and Mandel, 1970/. Mexiletine is structurally related to lidocaine. Both compounds have similar electrophysiologic properties.

They act like other class I agents on the fast inward current. But in contrast to quinidine both compounds shorten the duration of the action potential. The potassium efflux is increased, while repolarization is shortened. Mexiletine is orally active in contrast to Lidocaine.

3/ Verapamil

Verapamil, a synthetic papaverine derivative, is the prototype of the class IV antiarrhythmic agents /Singh and Vaughan Williams, 1972/, which inhibit membrane transport of calcium and selectively depress nodal tissues. Verapamil has none of the described class I actions. It depresses contractions /negative inotropic effect/ and flattens the plateau of the action potential in atria and ventricles. The slow inward current is restricted.

c/ Methods

1/ Functional refractory period

Guinea pigs of both sexes weighing between 350 and 450 g were sacrified and the left atrium was prepared free in Krebs-Ringer solution at room temperature. After preparation, the left atrium was suspended ver-

tically in an organ bath /Hugo Sachs Elektronik, Hugstetten/ perfused with Krebs-Ringer solution of 32°C temperature and gassed with Carbogen /95 % O_2; 5 % CO_2/. Composition at the nutrient solution was as follows /in g/l/: 7.43 NaCl, 0.35 KCl, 0.28 $MgSO_4$, 0.16 KH_2PO_4, 2.09 $NaHCO_3$, 0.36 $CaCl_2$, 1.5 Glucose and 2 mMol/l Na-pyruvat. The upper end of the atrium was connected to a force-transducer and the lower end to a clasp serving simultaneously as stimulating electrode. 10 min after preparation a force of 1 pond was applied and electrical stimulation /100/min/ for 1 sec of rectangular impulses of 3 msec duration and 1 Hz frequency was started. The contraction amplitude evoked by application of suprathreshold train of impulses was taken as 100 %. The stimulator IR /Hugo Sachs Elektronik, Hugstetten/ delivered after each 8th pacing stimulus a premature extra stimulus. The distance between pacing and extra stimulus was increased stepwise. The minimal coupling time was 50 msec /lying well within the refractory period/ the maximal was 250 msec. After a time of equilibration of 20-30 min, the measurements were repeated 3 times.

The functional refractory period is defined as the minimal time interval between two successive stimuli eliciting an extra beat. This was associated with

a 20 % increase of the contraction amplitude. Drug effects on contractility were estimated at this phase so the basic values represented 120 % of the contraction amplitude measured on applying pacing stimuli only.

2/ Aconitine induced arrhythmias

Rats were anesthetized with sodium pentobarbitone /60 mg/kg given intraperitoneally 15 min prior to aconitine infusion/. A standard lead II ECG was recorded by means of subcutaneous needle electrodes and aconitine nitrate infused at a rate of 1.25 µg/min into a tail vein. Drugs were given intraperitoneally. Control animals were given vehicle only. Premature ventricular beats /PVB-s/, ventricular tachycardia /VT/, ventricular fibrillation /VF/ and finally cardiac arrest followed. This sequence of events is highly consistent in control animals. Experience in this model indicates that in drug treated animals sometimes an abrupt transition from PVB-s to VF may occur. Therefore the time interval until the onset of the first PVB was chosen as an endpoint and the amount of aconitine in /µg/kg/ body weight/ required to induce this type of arrhythmias was estimated. ED_{50} values were defined as the dose of the test drug raising the PVB-inducing arrhyth-

mogenic dose of aconitine by 50 % above that required in control animals.

3/ Local anaesthetic effect

The method of infiltration-anaesthesia was used similar to that described by Bülbring /1945/.

Conscious guinea pigs /Purbright-White/ of both sexes weighing between 400 and 500 g were used. 0.1 ml test solution was administered subcutaneously to the shaved back of the animals. The pain reaction to a mechanical irritation with a needle or its absence was tested before and after application of the test solutions in 5 min intervals. Concentrations of solutions of mexiletine, quinidine and verapamil were: 0.1 %, 0.5 %, 1.0 % and 2.0 %; for lidocaine: 0.1 %, 0.5 % and 1.0 %.

4/ Acetylcholine-induced supraventricular fibrillation

Mongrel dogs of both sexes weighing between 10 and 15 kg were used. Anesthesia was induced and maintained with alpha-chloralose/urethane /75/750 mg/kg/ i.v.. The animals were intubated and artificial respiration maintained by a respirator /Bird, Mark 2/. A femoral artery and a vein were catheterized for

recording of arterial pressure and for i.v. injection, respectively. A left-sided thoracotomy was performed at the 5th intercostal space and the pericardium incised over the left atrium to form a pericardial cradle. The atrial electrogram and conventional lead II ECG were monitored. Atrial flutter or fibrillation was induced by placing 2 drops of a 10 % aqueous solution of acetylcholine directly on the left atrium and then stroking the area gently with a spatula. The duration of atrial fibrillation was determined by noting the return to normal sinus rhythm on the ECG. Supraventricular fibrillation was induced in 20 min intervals. Duration of fibrillation time for all animals without test compounds was between 500 and 600 seconds.

5/ Ventricular electrical fibrillo-flutter threshold

Wistar rats of both sexes weighing between 250-300 g were anesthetized with urethane /1000 mg/kg i.p./. After thoracotomy they were arteficially respirated /Respirator, Hugo Sachs Elektronik, Hugstetten/ and two platinum electrodes were inserted into the left ventricle. Impulses of increasing current intensity of 1 msec duration and 50 Hz fre-

quency were given for 1 sec. Time for determination of thresholds was 1, 3, 5, 10 and 15 min after application of drug. Definition of ventricular electrical fibrillo-flutter threshold: The minimal intensity of current /applied with the above outlined parameters/ which may elicit fibrillo-flutter: a transition of ventricular flutter to fibrillation. The threshold is reached after inducing minimally 3 successive ventricular extrasystoles. The following i.v. doses were used: lidocaine 1.25, 2.5 and 5.0 mg/kg; quinidine: 1.25, and 2.5 mg/kg; mexiletine: 1.25, 2.5 and 5.0 mg/kg; verapamil: 0.15, 0.3 and 0.6 mg/kg.

6/ Electrophysiological parameters in anesthetized, thoracotomized arteficially respirated dogs with intact coronary circulation /"in situ" experiments/

Experimental preparation

Adult mongrel dogs of either sex, weighing 8-15 kg were anesthetized with sodium pentobarbitone /Nembutal, Serva, 30 mg kg^{-1}, i.v./. An endotracheal tube was inserted and the animals were ventillated with room air /respirator, RO-5, SU/ at 10-15 strokes per min. In anesthetized animals the effects mediated by autonomic reflexes are more or less depressed according to the

depth of anesthesia. Therefore, a constant level of anesthesia seemed to be indispensable. To maintain anesthesia at a constant level, additional sodium pentobarbitone was administered by intravenous infusion /0.1 mg kg^{-1} min^{-1}/ continuously. Arterial blood pH and pO_2 were monitored at selected intervals by means of a gas analyser /Astrup, OP-2102/ and pH was maintained at 7.2 $\pm$ 0.2 and pO_2 between 70-90 mmHg, respectively. Body temperature was monitored from a temperature sensing thermopile catheter located in the oesophagus and maintained at 37 $\pm$ 0.5^{o}C by a heating pad.

Mean arterial blood pressure /MABP/ was recorded by means of a catheter inserted into the left femoral artery attached to a pressure transducer /Statham P23Db/ and registered on a Hellige pressure recorder. A canula was inserted into the left femoral vein for drug administration.

Right thoracotomy was performed in the fifth intercostal space. The pericardium was opened and bipolar electrodes /1 mm diameter silver electrode-pairs with 5 mm interelectrode distance embedded in acryl/ were attached on the right atrial appendage near to the sinus node. Another bipolar pair of electrodes /with the same parameters as described above but

embedded in a rubber plate/ was sutured on the surface of the right ventricle. They served as recording electrodes for atrial and ventricular tissue electrograms. In addition further surface bipolar electrodes /inter-electrode distance 3 mm/ were fixed on the right atrium for pacing and on the right ventricle for evoking premature stimuli.

The electrograms and a lead II. electrocardiogram were recorded on a $6NEK_4$ six channel ECG recorder and visualized on an oscilloscope /Tesla, OPD 280 U/. The PQ, QRS and QT intervals were determined by direct measurement of the lead II. electrocardiogram at a paper speed of 200 $mmsec^{-1}$.

His-bundle electrogram was recorded by introducing a 6F /USCI, USA/ tripolar catheter via the right femoral artery into the heart. Correct placement of the catheter was determined during overpacing the heart by 200 beat per min and monitored on the oscilloscope and registered on $6NEK_4$ six channel ECG recorder. The His-bundle electrogram was filtered to transmit only between 50-500 Hz. The AH and HV intervals, indicative of the AV nodal and infranodal His-Purkinje conduction times respectively, were determined by direct measurement at 200 $mmsec^{-1}$ paper speed.

Programmed electrical stimuli

Determination of the electrophysiological parameters was carried out completely automatically using a ZX 81 personal computer system. According to the program, the computer controlled both the timing of the pacing stimuli as well as that of the extra stimuli elicited by means of a Grass S44 stimulator. The evoked tissue potential was registered from the heart and directed via amplifier and filter system to the computer. This latter introduced according to the program protocol the pacing and the premature stimuli and measured the tissue responses, as well as their time interval at predetermined times. The parameters were visualized on a TV screen and at the same time recorded on paper by means of a printer.

Determination of the sinus node recovery time /SNRT/

After estimation of the diastolic threshold, the pacing frequency of the sinus node and the duration of the overdrive pacing was set within the limits allowed by the program. The right atrium was paced at a rate of 60 impulses per min above the normal sinus rate with square wave pulses of 2 msec duration and 2 times diastolic threshold strength. After pacing for 2 minutes,

stimulation was stopped by the program and the time interval between the last pacing impulse and the first spontaneously appearing sinus beat /which is the SNRT/, as well as the normal sinus cycle length /SCL/ were automatically calculated and recorded both on the TV screen and by the printer. The computer also automatically calculated the corrected sinus node recovery time /CSNRT/ as a difference of the cycle length of the first escape beat and the normal sinus cycle length /CSNRT = SNRT - SCL/.

Estimation of the atrial effective refractory period /AERP/

The AERP was measured by means of bipolar electrode pairs sutured on the surface of the right atrium. One bipolar electrode served as pacing electrode, whereas the other one for recording the evoked tissue potentials. The pacing frequency and the delay of the first extra stimulus could be set within the limits given by the program. The time interval between the pacing stimulus and the extra stimulus /elicited after 10 normal paced stimuli/ was automatically increased by 2 msec /which is still within the atrial absolute refractory period/ until the first premature atrial stimulus appeared. The time interval between the last pacing sti-

mulus and the first premature beat gives the AERP in msec.

Estimation of AV node refractoriness /AVRP/

Refractory period of the AV node was determined by using atrial extra stimuli. The heart was paced at a rate of 10 stimuli per min over the normal sinus rate. The program automatically increased the pacing frequency until the ventricular impulse disappeared indicating that the AV node became refractory to the last stimulus. The refractory period was defined as the longest pacing interval in msec, failing to result in a conducted ventricular impulse.

Estimation of the ventricular effective refractory period /VERP/

VERP was measured by means of two bipolar electrodes sutured on the surface of the right or left ventricle and was determined as described previously for the atrial myocardium.

Drug administration

According to the experimental protocol 4 groups consisting of six animals each were formed. In every group at first control recordings were made and repeated at least twice. In the first group cumulative doses of

2.0, 4.0 and 6.0 mg/kg lidocaine, in the second group the same doses of mexiletine, in the third group the same doses of quinidine and finally in the fourth group doses of 0.05, 0.1 and 0.2 mg kg^{-1} verapamil were administered by i.v. bolus injection. After each dose the changes in the electrophysiological parameters were followed continuously until they returned to nearly the control level. The following abbreviations for the parameters measured were used: Sinus cycle length = SCL; sinus node recovery time = SNRT; corrected sinus node recovery time = CSNRT; atrial effective refractory period = AERP; ventricular effective refractory period = VERP; A-V refractory period = A-VRP; Atrial-His conduction time = AH; His-ventricular conduction time = HV; PQ-time = PQ; QRS-time = QRS; QT--time = QT.

7/ Statistics

The results were expressed as Mean $\pm$ S.E.M. Significance of the difference from initial value was evaluated by means of Student's unpaired t-test and values were considered significant at level = $p < 0.05$.

V. RESULTS

1/ Effect on functional refractory period and contractile force in isolated guinea pig atria

According to Table 1 mexiletine evoked the most expressed prolongation of the functional refractory period reaching 70 % in 10^{-5} Mol/l concentration. Lidocaine was active at 10 times higher i.e. 10^{-4} Mol/l concentration only. Also quinidine and verapamil proved to be less active in this respect. What concerns the effect on contractility verapamil exerted a strong negative inotropic effect i.e. it reduced contractility force by 55 % at $5x10^{-5}$ Mol/l concentration. The negative inotropic effects of lidocaine and mexiletine were less pronounced. Quinidine did not affect contractility in 10^{-6} and 10^{-5} Mol/l concentration ranges.

2/ Effect in the rat aconitine test

In Table 2 ED_{50} values increasing threshold dose of aconitine for production of PVB-s by 50 % obtained with quinidine, lidocaine, mexiletine and verapamil treatment are compared. Accordingly aconitine induced arrhythmias in rats have shown a selective sensitivity to Class I antiarrhythmic agents. The order of potency

Table 1.

Concentration dependent drug induced changes in functional refractory period and contraction force of the isolated left atrial preparation of guinea pigs

Drug	Concentration Mol/l	% change in: Refractory period	Contractility
Lidocaine	10^{-6}	- 4.1±3.9	- 3.5±5.8
	10^{-5}	9.0±3.2	- 9.3±4.8
	10^{-4}	78.0±6.0	-31.9±6.4
Verapamil	10^{-6}	13.1±3.9	-15.5±9.8
	10^{-5}	54.9±4.0	-25.4±6.1
	$5x10^{-5}$	73.0±5.2	-55.4±9.0
Quinidine	10^{-6}	6.5±4.4	0 ±8.6
	10^{-5}	25.3±3.8	3.5±7.6
Mexiletine	10^{-6}	18.9±3.6	-11.4±8.2
	$3x10^{-6}$	23.2±6.2	-13.3±8.0
	10^{-5}	66.9±8.4	-21.1±5.6

Values are mean ± S.E.M. of 6 preparations for each drug and concentration used

Table 2.

ED_{50} values /mg/kg i.p./ of antiarrhythmic drugs raising the amount of i.v. administered aconitine required to produce PVBs by 50 %. Experiments performed with 8 anesthetized rats for each drug

Drug	ED_{50}	Class
Quinidine	31	Ia
Lidocaine	48	Ib
Mexiletine	61	Ib
Verapamil	inactive	IV

is quinidine > lidocaine > mexiletine. Verapamil proved to be inactive in the rat aconitine test.

3/ Local anaesthetic effect

As shown in Fig. 1., mexiletine exerted the most persistent local anaesthetic effect independently of the concentration used. Nearly 2-hours after application of a 2.0 % solution of this drug no pain reaction was to be induced. Local-anaesthetic effect of lidocaine is also dose-dependent and nearly as much expressed as that of mexiletine but with a shorter time of action. The effects of quinidine in 0.5, 1.0 and 2.0 % solution are similar, but duration of action is shorter than that of lidocaine and especially of mexiletine. Verapamil in all concentrations had only a marginal local anaesthetic effect of very short duration.

4/ Effect on supraventricular arrhythmias induced by acetylcholine and concomitant mechanical stimulation

It is clear from Table 3 that in this model of supraventricular arrhythmia quinidine proved to be the most effective in reducing the duration of the period of atrial fibrillation /AF/ and this effect was dose-dependent. 5.0 mg/kg caused a reduction of fibrilla-

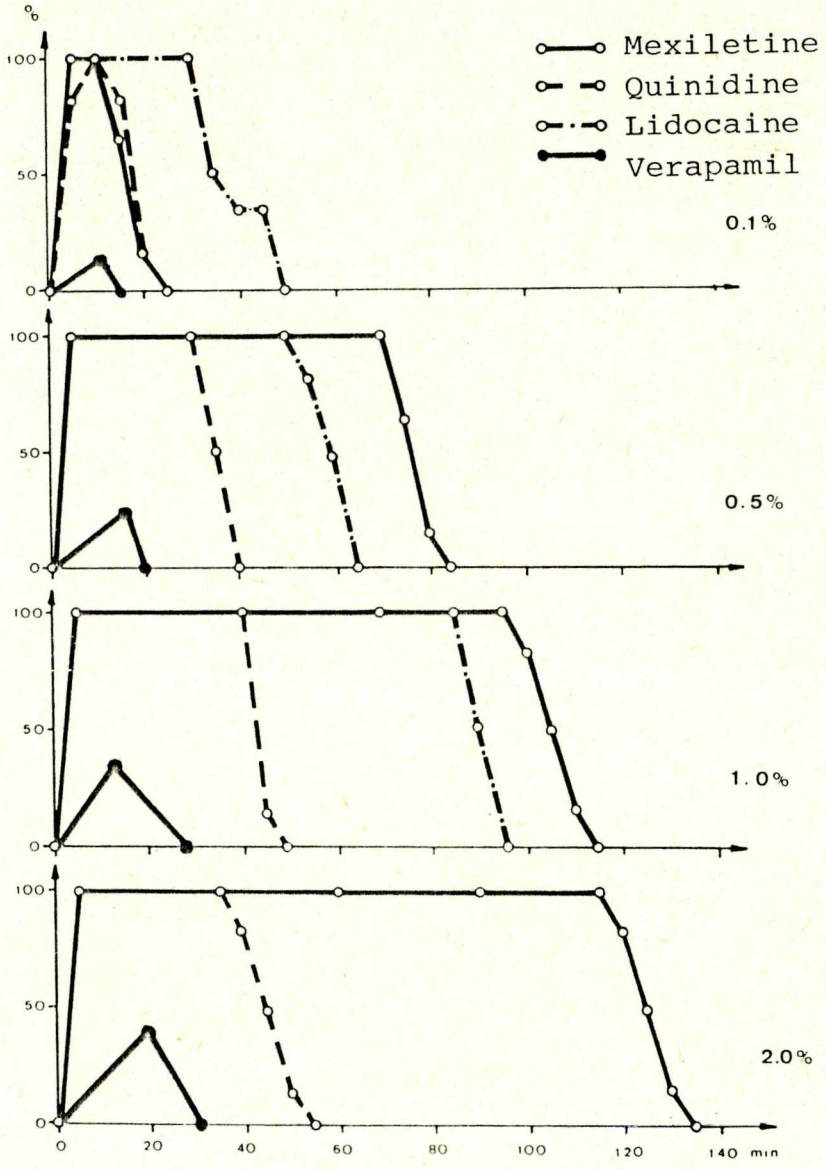

Fig. 1. Time dependent protection from pain produced by local anesthetic effect of different antiarrhythmic drugs.
Ordinate: % protection from pain
Abscissa: time in minutes

tion time of 83 % soon after administration of the drug. At this time 10.0 mg/kg nearly completely abolished AF. With both doses the action was present even after 80 min which is a rather long lasting effect. Lidocaine and mexiletine were rather ineffective in this respect and even application of a 5 mg/kg dose failed to produce an early shortening exceeding 50 % of the fibrillation-time. Verapamil reduced fibrillation-time in a dose-dependent manner. Soon after administration of 0.4 mg/kg 78 % shortening of fibrillation time was observed and a weak protective action was seen even after 80 min.

5/ Effect on the ventricular fibrillo-flutter threshold in rats

Results are presented in Table 4. Accordingly all compounds tested increased the ventricular electrical fibrillo-flutter threshold. The most marked effect in increasing ventricular fibrillo-flutter threshold was produced by mexiletine showing a 136 % increase in the first min after administration of 5.0 mg/kg dose. This effect was of reasonably long duration after i.v. application of the drug and proved to be dose-dependent. An increase similar to that produced by mexiletine was observed after vera-

Table 3.

Drug induced time dependent change in the duration of acetylcholine + mechanical stimulation induced atrial fibrillation periods as % of the control values

Drug and dose mg/kg	Duration of atrial fibrillation msec Control	Change in % of control value Time after application of drug 2′	20′	40′	60′	80′
Quinidine						
2.5 n=7	535.3 ±37.6	-45.3 ±8.9	-24.8 ±4.9	-10.1 ±4.8	- 6.7 ±3.7	
5.0 n=7	559.2 ±49.6	-83.3 ±11.1	-30.3 ±14.3	-19.1 ±9.8	- 8.8 ±6.4	
10.0 n=8	510.1 ±53.4	-92.0 ±8.0	-74.0 ±13.1	-39.8 ±10.1	-21.4 ±9.4	-10.8 ±8.4
Lidocaine						
1.25 n=7	563.8 ±48.9	-18.3 ±8.4	- 9.7 ±4.9	- 6.7 ±3.9		
2.5 n=7	538.4 ±33.7	-24.3 ±11.1	-14.4 ±8.4	- 8.7 ±5.6		
Mexiletine						
2.5 n=7	591.4 ±43.9	-29.4 ±11.4	-21.7 ±9.1	-13.2 ±8.4	- 7.1 ±6.4	
5.0 n=7	577.3 ±39.4	-41.4 ±14.1	-30.1 ±13.8	-18.3 ±9.9	- 8.4 ±7.4	
Verapamil						
0.1 n=7	601.1 ±47.6	-14.7 ±8.4	- 8.4 ±6.7			
0.2 n=7	589.0 ±43.9	-29.4 ±8.4	-19.4 ±9.8	-10.4 ±10.1		
0.4 n=7	581.0 ±61.1	-78.4 ±12.1	-51.3 ±9.8	-39.4 ±11.2	-29.4 ±9.2	-11.1 ±8.7

n = number of animals

Values are mean ± S.E.M.

Table 4.

Drug induced time dependent changes in ventricular electrical fibrilloflutter thresholds /FFT/ as % of control value

Drug and dose	FFT in µA	Change in % of control value Time after application of drug				
mg/kg	Control	1′	3′	5′	10′	15′
Lidocaine						
1.25 n=7	68.3 ±6.4	34.8 ±11.7	8.5 ±5.1	5.3 ±4.5	0.1 ±2.6	0.8 ±2.4
2.5 n=7	69.4 ±7.1	59.5 ±9.8	42.2 ±15.1	44.7 ±9.7	7.3 ±6.3	4.0 ±4.1
5.0 n=6	78.1 ±8.3	77.2 ±15.7	61.9 ±12.1	51.0 ±12.1	35.8 ±16.4	26.4 ±9.1
Quinidine						
1.25 n=7	73.4 ±6.3	21.4 ±12.1	20.1 ±13.4	18.5 ±8.4	13.9 ±7.9	9.8 ±7.1
2.5 n=5	69.3 ±8.1	37.4 ±11.7	30.2 ±12.8	18.7 ±6.1	14.5 ±8.3	9.1 ±6.1
Mexiletine						
1.25 n=6	73.8 ±6.4	89.7 ±18.3	59.8 ±16.4	46.1 ±13.1	31.4 ±10.0	16.3 ±8.9
5.0 n=7	76.4 ±8.3	136.8 ±24.7	93.4 ±22.8	86.5 ±16.4	46.3 ±17.3	27.1 ±16.1
Verapamil						
0.15 n=6	73.9 ±4.8	21.9 ±9.4	17.3 ±11.7	13.3 ±8.4	10.1 ±8.4	8.4 ±7.8
0.3 n=6	78.4 ±9.1	77.4 ±22.4	49.8 ±19.7	36.4 ±16.4	31.1 ±15.4	18.7 ±9.1

n = number of animals

Values are mean ± S.E.M.

pamil and lidocaine /77 % and 77 % respectively/. Duration of this effect was also longer than 15 min as quinidine has only weak effects in increasing fibrillo-flutter threshold.

6/ Electrophysiological parameters in anaesthetized, thoracotomized arteficially respirated dogs with intact coronary circulation

According to Table 5a. and b. cardiac cycle length /SCL/ was reduced by all drugs investigated in a dose dependent manner i.e. all drugs exerted a negative chronotropic action. In this respect verapamil proved to be specially active. The drugs also prolonged the sinus node recovery time corrected for cycle length.

What concerns the atrial effective period the Class I antiarrhythmic agents but also verapamil prolonged it to about the same extent whereas the ventricular effective refractory period was less affected by lidocaine mexiletine and verapamil and not affected by quinidine. The same is valid for the atrioventricular effective refractory period which was not affected by quinidine, only moderately prolonged by lidocaine and mexiletine and very strongly augmented by verapamil. In agreement with these latter findings only verapamil was capable of inducing a significant prolongation of

Table 5a.

Electrophysiological parameters after Lidocaine, Mexiletine, Quinidine and Verapamil in situ experiments in dogs with intact coronary circulatia

Drug	n	Dose (mg/kg^{-1})	SCL (msec)	SNRT (msec)	CSNRT (msec)	AERP (msec)	VERP (msec)	A-V RP (msec)	P - Q (msec)	QRS (msec)	Q - T (msec)	A - H (msec)	H - V (msec)
Lidocaine	6	C	356±26.5	412±18.5	56± 9.5	107± 3.6	130± 3.6	186± 9.4	100± 7.0	60± 6.0	150±10.1	80±10.0	20± 6.0
	6	2	379±30.9 NS	468±32.6 NS	89± 2.4*	115± 4.0 NS	136± 3.8 NS	205± 7.7 NS	100± 6.0 NS	60± 6.0 NS	170±12.0 NS	80± 9.8 NS	20± 5.4 NS
			+ 6%	+ 14%	+ 59%	7%	+ 5%	+10%	0%	0%	+13%	0%	0%
	6	4	435±45.8**	534±50.4**	99±12.1***	127± 5.0*	147± 4.3*	223± 8.2*	110± 8.0 NS	60± 7.2 NS	190±11.8*	80±11.0 NS	23± 7.2*
			+22%	+30%	+ 77%	+19%	+11%	+20%	+10%	0%	+27%	0%	+15%
	6	6	505±39.5***	642±49.6***	137±12.4	157± 7.8**	162± 4.8***	243± 5.4**	110± 8.0 NS	70± 7.9	250±15.4**	110±11.8*	25± 5.4**
			+42%	+56%	+145%	+47%	+25%	+31%	+10%	17%	+67%	38%	+25%
Mexiletine	6	C	330±32	400±40	70± 8.8	99± 6.0	154± 2.7	187±11.9	80± 5.0	50± 5.0	160± 5.0	70± 8.0	20± 4.1
	6	2	332±19 NS	407±33 NS	85± 6.9 NS	105± 5.7 NS	158± 4.7 NS	215±21.2 NS	80± 4.0 NS	50± 4.0 NS	170± 7.1 NS	75± 7.1 NS	20± 3.8 NS
			+ 1%	+ 2%	+ 21%	+ 6%	+ 3%	+15%	0%	0%	+ 6%	+ 7%	0%
	6	4	398±25*	487±46 NS	89±12.4 NS	112± 5.5 NS	170± 7.0*	225±14.9	80± 6.0 NS	50± 5.0 NS	180± 7.2 NS	80± 8.9 NS	25± 6.1*
			+21%	+22%	+ 27%	+13%	+10%	+20%	0%	0%	+13%	+14%	25%
	6	6	482±27**	620±46*	138± 9.7*	147± 7.0***	187± 8.0**	250±15.5**	80± 6.0 NS	50± 5.0 NS	190± 6.4*	90± 7.4**	30± 7.2**
			+46%	+55%	+ 97%	+48%	+21%	+33%	0%	0%	+19%	+29%	+50%
Quinidine	6	C	358±25	420±25	62± 8.7	103± 3.4	162± 8.3	190±10.6	80± 9.0	70± 5.1	215± 9.8	50± 5.1	25± 3.1
	6	2	404±30 NS	460±32 NS	72±15.2 NS	109± 3.1 NS	166± 8.6 NS	198± 7.8 NS	80± 5.3 NS	70± 5.1 NS	220± 8.1 NS	50± 4.8 NS	25± 4.2 NS
			+13%	+ 9%	+ 16%	+ 5%	+ 2%	+0.5%	0%	0%	+ 2%	0%	0%
	6	4	477±33*	560±41*	83± 6.8*	123± 6.1*	170± 7.1 NS	208± 8.5 NS	80± 7.2 NS	70± 4.0 NS	230± 9.1 NS	50± 5.3 NS	30± 6.1*
			+33%	+33%	+ 34%	+19%	+ 4%	+ 9%	0%	0%	+ 7%	0%	+20%
	6	6	582±40**	710±42**	128±12.5***	143± 9.6**	180±10.3 NS	214± 9.7 NS	80± 7.1 NS	70± 4.2 NS	240± 7.0*	50± 6.1 NS	30± 6.1*
			+63%	+69%	+103%	+38%	+11%	+12%	0%	0%	+12%	0%	+20%

continuation in Table 5b.

Table 5b.

Drug	n	Dose (mg/kg^{-1})	SCL (msec)	SNRT (msec)	CSNRT (msec)	AERP (msec)	VERP (msec)	A-V RP (msec)	P - Q (msec)	QRS (msec)	Q - T (msec)	A - H (msec)	H - V (msec)
Verapamil	6	C	318±31.0	380±46.0	49± 8.0	107± 3.1	140± 5.0	188± 3.1	95± 2.1	73± 6.1	226± 5.1	81± 7.1	28± 1.2
	6	0.05	389±49.1*	440±57.1*	51± 8.1*	125± 7.8 NS	152± 4.3 NS	249± 9.8**	113± 1.2**	73± 8.1 NS	233± 6.2 NS	91± 8.1 NS	29± 0.8 NS
			+22%	+15%	+ 4%	+17%	+ 9%	+32%	+16%	0%	+ 3%	+12%	+4%
	6	0.10	482±20.8***	565± 3.8**	63± 7.2***	133± 8.7 NS	169± 8.7*	293± 8.7**	125± 2.1**	75± 8.7 NS	238± 5.0 NS	104± 8.7 NS	32± 0.7 NS
			+51%	+48%	+ 29%	+24%	+21%	+56%	+32%	+3%	+ 5%	+28%	+14%
	6	0.20	587±38.7***	670± 4.2***	86± 9.0***	146±11.7 NS	176± 5.1**	372±11.1**	160± 6.1***	100± 8.1***	263± 6.8**	131±11.2 NS	36± 0.9***
			+84%	+76%	+ 76%	+36%	+26%	+98%	+68%	+37%	+16%	+61%	+25%

Mean ± S.E.M.; % = difference from the control value; * $p < 0.0$; ** $p < 0.01$; *** $p < 0.001$; NS = non significant

Abbreviations: SCL = sinus cicle length; SNRT = sinus node recovery time; CSNRT = corrigated sinus node recovery time; AERP = atrial effective refractory period; VERP = ventricular effective refractory period; A-VRP = A-V refractory period; A-H = atrial-His conduction time; H-V = His-ventricular conduction time; C = control

the PQ distance. This latter drug has also significantly increased the width of the QRS complex and the QT distance while the other three Class I antiarrhythmic agents did not affect these parameters significantly. The verapamil induced prolongation of PQ distance is mainly due to a depression of the His-ventricular conduction /H-V/ because atrial-His bundle /A-H/ interval was not substantially affected.

VI. DISCUSSION

Aim of the present investigations was to assess the value of the different assay methods for a possible specificity to antiarrhythmic drugs with dissimilar pharmacological profile and belonging to different classes of antidysrhythmic agents. To achieve this it seemed to us reasonable to discuss the mechanism and predictive value of the different test methods one by one and their responsiveness to the drugs tested.

The usefulness of i.v. aconitine induced rhythm disturbances for testing antiarrhythmic drugs is well established /Fekete and Borsy, 1964; Vargaftig and Vorignet, 1969; Szekeres and Papp, 1971; Szekeres, 1964/. The advantages of applying this test in rats

can be summarized as following: a/ The method does not fail to produce arrhythmias and all forms of ventricular arrhythmias may occur, including ventricular extrasystoles and ventricular tachycardia. b/ The duration of the arrhythmias is long enough to make beyond any doubt determination of the arrhythmia moderating or abolishing action of the tested drug possible. The duration of antiarrhythmic action can be estimated as well. c/ The method is of good reproducibility, i.e. identical doses of aconitine cause arrhythmias of about the same severity and duration. d/ The different rat strains represent a very homogenous population and since rats are relatively inexpensive, so large number of animals can be used for evaluation. e/ The assay is less time-consuming than other methods because f/ no surgical intervention is required. Another advantage of this method is that since compounds to be tested are given prior to anaesthesia, an indication on possible toxic or side effects can also be obtained.

Thus, a number of additional informations are available when using this simple model.

On the other hand it is well established, that aconitine is a powerful membrane depolarizing agent. Its effect has been ascribed to activation of action

potential Na^+ ionophore /Catterall, 1975/. The antiarrhythmic agents belonging to Class I /Singh and Hauswirth, 1974/ have a pronounced membrane stabilizing action. These and our own results with the aconitine method suggest, that the antiarrhythmic action of Class I antiarrhythmics /mexiletine, quinidine and lidocaine/ is very closely related to their membrane-stabilizing effects, which is also responsible for their protective action against aconitine induced arrhythmias in the rat.

So the results with the aconitine method indicate that this test method responds primarily to drugs having Class I action.

Indeed with this method verapamil failed to exert any action. As it is known, verapamil is a Ca^{2+}--antagonist and so it belongs to Class IV of the antiarrhythmic agents /Vaughan Williams, 1970/. Nevertheless our above results are not sufficient to conclude that verapamil, as well as other Class IV agents would fail to protect against ventricular arrhythmias. However, clinical experience as well as experimental data /Szekeres et al., 1987/ show that concentrations sufficiently high to abolish ventricular arrhythmias cannot be used because of the high sensitivity of the atrioventricular conduction to

verapamil. So the danger of inducing an A-V block furthermore the very expressed negative inotropic action is the limiting factor in using higher doses of this drug /Szekeres et al. 1987/.

Another form of chemically induced arrhythmias is the supraventricular fibrillation induced by concomitant application of acetylcholine and mechanical stimulation. Nahum and Hoff demonstrated in 1940 that topically applied acetyl-β-methylcholine induces atrial flutter or fibrillation in the dog and cat. Scherf et al. /1950/ later reported on a similar action for acetylcholine. The method usually employed is that of Schalleck /1952/: A cotton-wool swab is soaked in a 5 % solution of acetylcholine and placed on the region of the sinus node. After 1 min contact period, atrial fibrillation is induced by pinching the atrium with forceps. The disadvantage of this method is the great variability in duration of the fibrillation period varying between 15 and 20 min. Therefore it was decided to modify Schallecks' method so as to assure the measurement of drug induced activity and to quantify this activity on an intensity and duration basis. Atrial flutter or fibfillation was induced by placing 2 drops of a 10 % aqueous solution of acetylcholine directly to the

atrium and then stroking the area gently with a spatula. No attempt was made to prevent the acetylcholine solution from the mixing with the pericardial fluid. A sufficient amount of the solution remained in contact with the atrium to allow the induction of atrial fibrillation. In control experiments, atrial fibrillation could be induced neither by stroking the atrium nor by contact with acetylcholine alone. Induction of fibrillation was done in 20 min intervals. The good reproducibility is demonstrated by the results obtained as control values: fibrillation time has always between 510 and 600 seconds. The other advantage of this method is that the animal can be used as its own control. At the end of each testing and after finishing the whole experiment the duration of fibrillation period was established again. A deviation of 7-9 % to the control values from the beginning was allowed. This deviation of 7-9 % is in a good accordance with the biological deviation. Thus, this modification allows one to compare both the potency and the duration of antiarrhythmic agents, i.e. shortening of fibrillation time and return of fibrillation to sinus rhythm, respectively.

The mechanism of this special form of supraventricular tachyarrhythmia /mostly atrial fibrillation

and fibrillo-flutter/ is not yet clear. Evidence indicates that the tachyarrhythmia induced by acetylcholine is the result of a hyperpolarisation and a conduction block /Trautwein, 1963/. This block could develop in the area being in direct contact with acetylcholine. The tachyarrhythmia triggered by mechanical stimuli could then be perpetuated by re--entry of impulses facilitated by the delayed conduction and conduction block /Moe and Abildskov, 1959/.

The above described form of atrial tachyarrhythmia should be particularly sensitive to agents such as quinidine, which decrease intraatrial conduction velocity and increase the refractory period.

The results show, that quinidine is really the most powerful agent, if tested with the above mentioned method. It decreases duration of fibrillation time in a dose-dependent manner. The most pronounced effects are seen after 10.0 mg/kg. Initially a nearly total cessation of fibrillation can be observed. Even after 80 minutes a marginal antiarrhythmic action of 10 % reduction in fibrillation time is present. These results are in good agreement with the results presented by Hageman and Pruss, 1979 using a method similar to our modification. Lidocaine, as a Class Ib agent acting mainly in ventricular arrhyth-

mias exerts only a weak antiarrhythmic effect in this model. Even if given in the rather high dose of 2.5 mg/kg, which may already produce CNS side-effects, the maximal reduction of duration of fibrillation time is only 24 %. Similarly weak effects were seen after mexiletine, representing also a Class Ib type of action. These findings indicate, that the model of acetylcholine induced arrhythmia is a good tool in differentiating between Class Ia and Class Ib actions.

On the other hand from the clinical point of view the Class I agents appear to be so different from each other that many physicians are reluctant to accept that they may have a similar mode of antiarrhythmic action. All Class I agents share the capacity of depressing the fast Na^+ inward current but they have additional properties which may distinguish them. These additional properties necessitate their subdivision /Harrison et al. 1980/. Lidocaine and mexiletine shorten monophasic action potentials and Q-T interval in vivo. They do not lengthen QRS in sinus rhythm, but prolong refractory period as measured by programmed stimulation or by isolated heart /left atrial/ preparations. In contrast to this quinidine widens the QRS at high con-

centrations, and lengthens action potential duration. The refractory period is prolonged absolutely and also relative to APD as shown by programmed stimulation on the heart "in situ" and in isolated heart preparations. In sum one can say, that from the pharmacological and the clinical point of view the above mentioned agents can be put into the following subdivisions of the Class I agents:

quinidine: Class Ia; Lidocaine: Class Ib; and

mexiletine: Class Ib.

Thus our results are in accordance with the classification of Vaughan Williams, 1970.

Although on the basis of its electrophysiological actions as well as on its effect on ionic currents verapamil cannot be included into group Ia /"quinidine like"/ this agent proved to be very active against supraventricular model arrhythmias. Marked effects are seen after 0.3 mg/kg evoking an initial shortening of the duration of fibrillation time by 77 % and with a long duration of action, i.e. for more than 80 min. These data obtained from pharmacological investigations in animals are in accordance with clinical data /Rinkenberger et al. 1980/. Verapamil has become the agent of choice for the acute termination of paroxysmal supraventricular tachycardia /PSVT/ or junctional tachy-

cardias both arising mainly of AV nodal reentry. This mechanism is also responsible for the protection of the ventricles in atrial flutter and atrial fibrillation when the number of impulses conducted to the ventricles is reduced to more physiological values /Szekeres et al. 1987/.

Vaughan Williams reported in his review of 1984 about the local anaesthetic action of the Class I antiarrhythmic agents. It is well-known that quinidine exerts a local anaesthetic effects on nerves. Anaesthetic effects were firstly described by Bulbring /1945/ and this method of infiltration-anaesthesia is well-established. Burn /1956/ pointed out that beside other pharmacological activities drugs with antiarrhythmic actions also possess local anaesthetic activity. This activity is associated with the effect of reducing the membrane permeability to sodium. This is the so-called membrane-stabilizing effect /Fleckenstein, 1949; 1969; Vaughan Williams, 1967; 1968/. Although properties of nervous and cardiac membranes and consequently the action potentials are different, the basic phenomena regulating transmembrane transport of sodium and potassium ions are essentially the same and therefore estimation of local anaesthetic activity is a valid procedure in assessing membrane stabilizing

activity. Thus quinidine, lidocaine and mexiletine have rather marked local anaesthetic effects. Verapamil however has shown only marginal effects. These results indicate that generalization of Burn /1956/ is not justified because not all antiarrhythmics possess local anaesthetic effect.

The above results allow us to conclude that the test of local anaesthetic effect may distinguish agents of Class I action as quinidine, lidocaine, mexiletine /irrespective of the fact whether they belong to Class Ia or Class Ib subgroup/ - from all other antiarrhythmic agents including verapamil. It should be noted here that very high /non-specific/ doses of beta-adrenoceptor blocking agents /with no intrinsic sympathomimetic activity/ may also exert some membrane stabilizing action. These results obtained with the local anesthesia test are in agreement with our earlier discussed data obtained by means of the aconitine-method, where the agents possessing membrane-stabilizing effects proved to be highly effective, but not verapamil. Thus both methods seem to be selective for agents with Class I action.

The refractory period of the cardiac-muscle is an important determinant of both initiation and termination of arrhythmias. In vitro techniques to determine

the effects of agents on refractoriness are therefore widely used to detect potential antiarrhythmic activity. Most methods are based on direct or indirect determination of the refractory period /Dawes, 1946; Govier, 1965/. The advantage of using these methods is that they give an additional information about contractile force, pointing to the possible negative inotropic effect of the drug tested and all this in a very early screening phase. Lidocaine, mexiletine and verapamil all markedly prolonged the functional refractory period in the isolated guinea pig atria. The prolongation induced by these agents is in the same range: 67 %-78 %. Only quinidine was less effective in this respect /25 %/. This finding suggests that the prominently supraventricular antiarrhythmic action of quinidine is only partly based on prolongation of the functional refractory period. What concerns the contractile force it is conspicuous that while verapamil evokes a prolongation of the refractory period by 76 % the same concentration may reduce the contractile force by more than 55 %. This finding points to the importance of the relationship between prolongation of the refractory period and diminution of the contractile force. This relationship was more favourable for lidocaine and mexiletine. Quinidine had no negative inotropic effects in this test in the

concentrations applied, but prolongation of functional refractory period was as mentioned above 25 % only. Our results with the programmed electrical stimulation in dogs show that a concentration of quinidine higher then used by us "in vitro" can be safely applied. Administered in such concentrations it is obvious that quinidine is as potent as verapamil in prolonging atrial refractory period in situ.

Experimental induction of arrhythmias by electrical stimulation of the heart has been known for a very long time /Mines, 1913; Moe et al. 1954; Szekeres and Papp, 1971; Marshall et al. 1981/. All electrical methods used to induce fibrillation depend upon the concept that following normal excitation not all cardiac fibers /even those in close proximity/ recover their excitability simultaneously. This results in a vulnerable period during the cardiac cycle at which some fibers have recovered their excitability while others are still refractory. An extrastimulus of sufficiently strong intensity applied during the vulnerable period will precipitate fibrillation. Antiarrhythmic drugs are expected to increase the intensity of the current necessary to evoke fibrillation. So, determination of ventricular fibrillation thresholds may yield useful informations and have the following advantages:

a/ the changes produced are reversible,

b/ the changes in susceptibility to arrhythmia can be observed continuously,

c/ the procedure is suitable for investigating all factors responsible for arrhythmia,

d/ very frequent determinations are possible,

e/ the development of arrhythmogenic or antiarrhythmic action of the agent can be investigated from minute to minute,

f/ exact data can be obtained on the maximum effect and on duration of activity of the drug,

g/ the animal can be used as a control of itself.

All agents tested, increase the threshold for electrically induced ventricular fibrillo-flutter, but in different degree. Quinidine, a powerful agent in supraventricular arrhythmias, increased the ventricular threshold only moderately. Mexiletine, very active against ventricular arrhythmias in clinical use, proved to be the most powerful agent in this model. Verapamil comes very close to the activity of mexiletine, but to draw conclusions from these data on ventricular antiarrhythmic activity in man is not easy, because clinical data are doubtful /Schwartz, 1981/. Certainly depression of contractility and of atrioventricular conduction as dose-limiting factors should also be taken into con-

sideration /Szekeres et al. 1987/.

Programmed electrical stimulation facilitated the estimation of the electrophysiological parameters in intact animals. This means that in an experiment "in vivo" all influences on behalf of the autonomic nervous system are becoming effective. On the other hand, the main difficulty of a comparative analysis of different antiarrhythmic drugs lies in the fact that they affect the hemodynamics to a different degree. Therefore the doses commonly used in therapy were applied. It is that nearly all antiarrhythmic agents exert a pronounced negative inotropic effect, which may limit the clinical use of these drugs. All drugs increased sinus cycle length i.e. reduced heart rate, furthermore prolonged SNR and the CSNRT, also AERP and VERP were prolonged to nearly the same extent. A-V refractoriness of was increased most intensively by verapamil. This is very important because as mentioned repeatedly AV-block may represent the limiting factor in the clinical use of verapamil. In accordance with this the A-V conduction /i.e. prolongation of P-Q interval/ was markedly increased by verapamil and only marginally by the three other agents. Looking at the A-H and H-V intervals quinidine has marginal effects only. Mexiletine was the most effective to prolong H-V interval in-

dicating that it may also inhibit A-V conduction but mostly on the H-V level. The prolongation of A-H and H-V intervals by lidocaine are not so pronounced but care must also be taken. So it is concluded that the beneficial antiarrhythmic effects of all compounds tested is at least partly due to their electrophysiological effects observed.

VII. SUMMARY AND CONCLUSIONS

In the present study responsiveness of six commonly applied antiarrhythmic test methods to membrane-active drugs was investigated. Aim of the experiments was to assess the value of the individual tests in differentiating among certain subtypes of antiarrhythmic agents representing different pharmacological profile and classes according to Vaughan Williams classification of antiarrhythmic drugs.

The i.v. aconitine test method in rats as well as the local anesthesia test in guinea pigs responded primarily to agents possessing Class I type /fast Na^+--channel inhibitory action/ irrespective of the fact whether they were Class Ia subtype /as quinidine, depressing conduction and prolonging repolarisation/ or Class Ib subtype /as lidocaine or mexiletine characterized

by some increase of conduction velocity in the partly depolarized fibres and by a shortening of repolarization. Thus by means of the above test methods this group can be clearly distinguished from other membrane active antiarrhythmic agents /as verapamil representing a Class IV action, selectively blocking the Ca^{2+}-channels and suppressing the slow inward Ca^{2+}-current and the slow potentials/.

On the other hand the test methods of acetylcholine and mechanical stimulus induced supraventricular tachyarrhythmia /atrial fibrillo-flutter and fibrillation, AF/ in anesthetized dogs as well as the estimation of the ventricular electrical fibrillo-flutter threshold /FFT/ in anaesthetized rats may distinguish between Class Ia and Class Ib agents. Thus quinidine possessing predominantly supraventricular antiarrhythmic action very effectively protected from acetylcholine induced AF in the dog but was not effective in ventricular FFT test in rats. In contradiction with this mexiletine and lidocaine proved to be ineffective in the acetylcholine induced atrial fibrillation test, but were highly active in the ventricular fibrillo-flutter threshold test.

The simple test of estimation of the refractory period in isolated guinea pig auricles provides an opportunity for a simultaneous determination of changes in

the contractile force. Thus the relationship between the favourable action prolonging the refractory period and the unfavourable negative inotropic effect can be established. In this sense verapamil which effectively prolonged the refractory period also greatly depressed contractile force. This relationship was much more favourable for lidocaine and mexiletine. Quinidine in the doses used did no prolong refractory period but it did not affect contractility either.

Such exploratory type of "in vitro" tests should however be completed with tests based on "in vivo" experiments as the estimation of basic electrophysiological parameters by programmed stimulation in the anaesthetized dog. These experiments have shown that from the electrophysiological point of view there is no counter-indication to using doses of quinidine higher than applied "in vitro". These higher doses proved to be effective in prolonging atrial and ventricular refractory periods as well. On the other hand this type of test also revealed that increase of the dose of verapamil to sufficiently high values protecting from arrhythmias of ventricular origin is not possible because of the very expressed depression of the atrioventricular conduction. Thus threatening A-V block may limit the use of verapamil in ventricular arrhythmias. A-V

conduction was less affected by quinidine, lidocaine and mexiletine. Such type of "in vitro" and "in vivo" analysis of drug induced changes in the main electrophysiological parameters underlying the mechanism of arrhythmias could reveal the relative importance of the individual parameters in the protective action of the different antiarrhythmic drugs.

As a result of the above presented and discussed experiments an additional conclusion of practical value for search of new antiarrhythmic agents can be drawn, namely more expedient and economic strategy for the assay of antidysrhythmic substances is suggested. Detection of antiarrhythmic action by means of the ventricular /and possibly auricular/ electrical fibrillo-flutter method seems to be the first step. This and the acetylcholine induced atrial fibrillation test may give information on supraventricular or ventricular site of action. As a second phase estimation of the refractory period in isolated perfused atria /and papillary muscles/ could give information about one the most important electrophysiologic parameter involved in antiarrhythmic action and also point to possible adverse effects of the drug on contractile force. The aconitine test and the local anaesthetic action do also belong to the assay methods of the second phase. These tests are capable of

distinguishing drugs with Class I action from other antiarrhythmic agents.

The third step of testing could be the estimation of electrophysiological changes on the dog heart "in situ" by programmed stimulation.

The great advantage of this procedure lies in the fact that many informations about the electrophysiological mechanism underlying arrhythmogenic and antiarrhythmic activity can be obtained.

Further steps should include tests related to experimental /ischaemic/ injury of the heart, steps not analysed in the present study.

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