# Colon obstruction-induced motility changes - the roles of glutamate and nitric oxide

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***Abbreviations***

ANS: autonomic nervous system

cNOS: constitutive nitric oxide synthase

CNS: central nervous system

CO: cardiac output

eNOS: endothelial nitric oxide synthase

ENS: enteric nervous system

GI: gastrointestinal

GMCs: giant migrating contractions

ICCs: interstitial cells of Cajal

ICCs-IM: interstitial cells of Cajal, intramuscular

ICCs-SEP: interstitial cells of Cajal, distributed over the surface of muscle bundles

ICCs-SM: interstitial cells of Cajal, submucosal

iNOS: inducible nitric oxide synthase

KYNA: kynurenic acid

MAP: mean arterial pressure

MPO: myeloperoxidase

7-NI: 7-nitroindazole

NANC: non-adrenergic non-cholinergic

NMDA: N-methyl-D-aspartate

NNA: N-ω-nitro-L-arginine

nNOS: neuronal nitric oxide synthase

NO: nitric oxide

NOS: nitric oxide synthase

$NO_X$: plasma nitrite/nitrate

SMA: superior mesenteric artery

TPR: total peripheral vascular resistance

XDH: xanthine dehydrogenase

XOR: xanthine oxidoreductase

XO: xanthine oxidase

## 1. INTRODUCTION

In everyday surgical practice, the problems with large bowel motility anomalies are frequent and usually very severe. Different types of mechanical intestinal obstructions are commonly diagnosed during consultations or emergency surgical situations, and the morbidity and mortality rates of these syndromes are still very high. Moreover, irrespective of the etiology or the type of the abdominal surgical intervention, gastrointestinal (GI) motility disorders are prevailing characteristics in the postoperative period. In general, the essential successful treatment of these clinical entities involves normalization of the GI motility. However, the therapeutic possibilities of dysmotility are still rather limited, mainly due to the incompletely explored pathophysiology.

### *1.1. Regulation of bowel motility*

Neurogenic control and coordination of the GI system is based on a reciprocal connection between the GI tract and the CNS through the autonomic nervous system (ANS). Further, local reflexes act in the ENS in an intrinsic manner. In fact, the ENS is part of the ANS, together with the sympathetic and parasympathetic nervous systems, and it has high priority in the regulation and integration of the functions of the GI tract. The ENS consists of interconnected networks of neurons and ganglia which entwine the entire GI tract from the oesophagus to the anal sphincter. The exhaustive works of Jabbour *et al.* showed that the number of neurons in the ENS reaches $10^7$-$10^8$ on average in several species, similar to the number in the spinal cord. Hence, this complex network of enteral autonomic neurons is rightly coined the "*intestinal brain*".

It was subsequently recognized that the motility of the GI tract is automated by the "*pacemaker*" cells of the ENS. Specialized cells known as interstitial cells of Cajal (ICCs) are distributed in specific locations within the tunica muscularis of the GI tract. ICCs serve as electrical pacemakers, providing pathways for the active propagation of slow waves, and are mediators of enteric motor neurotransmission and play a role in afferent neural signalling. Ultrastructural studies have demonstrated that, within the GI tract, the neuroeffector junctions are much more complicated than enteric nerve terminals lying closely apposed to smooth muscle cells. They rather involve specialized synapses that exist between enteric nerve terminals and intramuscular ICCs or ICCs-IM. The ICCs-IM are coupled to smooth muscle cells via gap junctions, and postjunctional responses elicited in the ICCs-IM are conducted to neighbouring smooth muscle cells. In the colon, ICCs located along the submucosal surface of the circular muscle layer (ICCs-SM) also provide a pacemaker function in this organ.

Functional neurotransmission cannot occur in the absence of these cells. Surgical manipulations of the GI tract, including intestinal resection and anastomosis, lead to dysmotility, which is associated with the disruption of ICC networks.

The ICCs possess a variety of receptors for neurotransmitters. The motility regulation is predominantly cholinergic in nature. However, several data suggest that alternative pathways may significantly modulate the cholinergic GI motility regulation.

*1.2. Nitric oxide*

The link between constitutive NO production and the GI nervous system is now well established, as the bulk of the NO is synthesized by nNOS in the submucous and myenteric plexus of the intestinal wall. Moreover, previous studies have shown that NO produced by the iNOS isoform during inflammatory cascade reactions directly inhibits the intestinal smooth muscle contractility. Although this line of reasoning suggests that an altered NO production may lead to dysmotility or more serious GI complications, the exact role of NO in the pathomechanism of obstruction-induced motility changes is still unclear. NO relaxes the smooth muscles directly, but it may also act as a cotransmitter of non-adrenergic non-cholinergic (NANC) inhibitory and descending interneurons. NO may also contribute to intestinal propulsion by inducing neurogenic contractions. *In vitro* observations suggest that non-selective NOS inhibitors enhance the intestinal motility, which indicates the inhibitory neurotransmitter character of NO. In contrast with these observations, Heinemann *et al.* have demonstrated the suppressed contractile activity of the intestinal musculature after the selective inhibition of nNOS.

*1.3. Glutamate*

In the last decade, glutamate was one of the most studied excitatory amino acids in the CNS, and it may be widely presumed that the glutaminergic neuorotransmission plays a role in the ENS too. The kynurenine pathway is the major route of the tryptophan metabolism. It may be activated by free radicals and cytokines which modulate the activity of the enzymes converting tryptophan to kynurenine. The components of the kynurenine pathway have marked effects on the neurons in the CNS. One of the main end-products is quinolinic acid, an agonist of the NMDA-sensitive glutamate receptors. Another arm of the pathway leads to the production of KYNA, which is an antagonist of the strychnine-insensitive glycine allosteric site of the NMDA glutamate receptor subtypes on neurons. Consequently, quinolinic acid can act as a neurotoxin, while KYNA is neuroprotective in the CNS. Several recent studies have suggested that glutamate-mediated facilitatory pathways may modulate the cholinergic transmission in the ENS as an excitatory neurotransmitter. Indeed, glutamate

immunoreactivity has been detected in subsets of submucosal and myenteric neurons in the guinea-pig ileum. At this level, glutamate is selectively concentrated in terminal axonal vesicles and can be released after application of an appropriate stimulus. Moreover, ionotropic NMDA-sensitive glutamate receptors are present and abundantly expressed on enteric cholinergic neurons.

### *1.4. Inflammation*

Inflammation is also an important component of the pathophysiology of bowel obstruction, characterized by an altered permeability of the gut mucosa and the activation of inflammatory cells. It has been shown that the local production of purine and kynurenine metabolites may be involved in the regulation of neuronal activity in inflammatory intestinal disorders as well.

### *1.5. Aims of the dissertation*

The main purpose of our studies was to investigate and clarify the roles of NO and glutamate in the colon obstruction-induced early-phase motility changes. Our experimental series were designed to follow the pathophysiological changes over a period of 420 min in a large animal model of acute mechanical ileus. The aims of Study I were to determine the *in vivo* role of NO in the development of motility changes, and to identify the mechanism by which NO might be produced. Accordingly, we compared the effects of selective and non-selective nNOS inhibition on the colonic motility, and investigated the changes in NOS isoenzyme activity in relation to the occlusion-induced haemodynamic patterns. Our results indicated the decisive role of nNOS in early colonic motility alterations, and the significant modifying potential of the late release of NO derived from the inflammatory iNOS isoform. The ensuing Study II was designed to determine the *in vivo* role of KYNA in the development of motility changes, and to identify the mechanism by which KYNA might influence the accompanying inflammatory process. Accordingly, we compared the consequences of exogenous activation of all subtypes of ionotropic glutamate receptors by KYNA on the colonic motility under physiological (normal) and pathophysiological (obstruction) circumstances.

## 2. MATERIALS AND METHODS

### *2.1. Animals*

The experiments were performed on healthy, inbred dogs of both sexes (body weight range: 12-18 kg) in adherence to the NIH guidelines for the use of experimental animals. The

study was approved by the Ethical Committee for the Protection of Animals in Scientific Research at the University of Szeged.

### *2.2. Surgical procedures*

Surgery was performed under sodium pentobarbital (30 mg $kg^{-1}$ iv) anaesthesia. After a midline abdominal incision, the portal vein was catheterized through the splenic vein for blood sampling. The root of the superior mesenteric artery (SMA) was dissected free and an ultrasonic flow-probe (Transonic Systems Inc., Ithaca, NY, U.S.A.) was placed around the exposed SMA to measure the mesenteric blood flow.

The level of the obstruction was marked by placing a silicone tourniquet catheter around the mid-transverse colon, keeping the neurovascular connections intact. In ***Study I,*** strain gauge transducers (Experimetria Ltd., Budapest, Hungary) were sutured onto the antimesenteric side of the bowel wall to measure the *oral* and *aboral* colonic motility at 10 cm distances from the occlusion point. In ***Study II,*** the transducers were sutured onto the bowel wall, parallel to the circular muscle layer, to measure the colonic motility at a distance of 10 cm *proximally* from the occlusion point. The sampling time was 10 min each, with a sampling frequency of 500 Hz; the signal analysis was performed off-line.

### *2.3. Measurements*

#### *2.3.1. Hemodynamic measurements*

The MAP, portal venous pressure and SMA blood flow were monitored continuously and registered with a computerized data-acquisition system (Haemosys 1.17; Experimetria Ltd., Budapest, Hungary). The CO was determined by thermodilution, using a Cardiostar CO-100 computer (Study I) and a SPEL Advanced Cardiosys 1.4 computer (Study II) (both from Experimetria Ltd.). The TPR was calculated via the standard formula.

#### *2.3.2. Colonic motility measurements*

The *in vivo* colonic motor activity in most species, including humans, dogs and rats, is characterized by three distinct types of contractions: **1)** rhythmic phasic contractions, **2)** giant migrating contractions (GMCs), and **3)** the tone. The GMCs are large-amplitude and long-duration contractions that migrate uninterruptedly over long distances and are associated with mass movements. Large bowel motility indices were determined by calculating the area under the motility curve as a function of time. The amplitude and frequency of the GMCs were calculated, and the tone of the colon was given by the mean value of the minima in the motility curve.

*2.3.3. Plasma nitrite/nitrate level measurements*

Plasma $NO_x$ levels were measured in the portal blood via the Griess reaction. The assay depends on the enzymatic reduction of nitrate to nitrite, which is then converted into a coloured azo compound, detected spectrophotometrically at 540 nm.

*2.3.4. NOS activity measurements*

NO formation in the intestinal tissues was measured via the conversion of [$^3$H]L-citrulline from [$^3$H]L-arginine according to the method of Szabo C *et al.*(1993).

*2.3.5. Xanthine oxidase activity*

The activity of XOR (xanthine oxidase (XO) and xanthine dehydrogenase (XDH), a major source of superoxide radicals in the intestinal tissue, was determined in this ultrafiltered, concentrated supernatant by a fluorometric kinetic assay based on the conversion of pterine to isoxanthopterine in the presence (total XOR) and absence (XO activity) of the electron acceptor methylene blue.

*2.3.6. Tissue MPO activity*

The activity of MPO, a marker of tissue leukocyte infiltration, was measured in the colon biopsies The MPO activities of the samples were measured at 450 nm (UV-1601 spectrophotometer, Shimadzu, Japan), and the data were referred to the protein content.

*2.4. Experimental protocols and groups*

***Study I:*** The animals were randomly allocated to one or other of four groups. Surgery was followed by a recovery period for cardiovascular stabilization, and the baseline variables were then determined during a 30-min control period. Group 1 (n=6) served as sham-operated control, while in groups 2 (n=8), 3 (n=6) and 4 (n=6) complete large bowel obstruction was induced by tightening the tourniquet. The animals in group 3 were treated with NNA (4 mg $kg^{-1}$ intravenously in 20 ml saline) 180 min after the induction of colon obstruction. In group 4, the selective nNOS inhibitor 7-NI (Sigma Chem. USA, 5 mg $kg^{-1}$ in 0.3 ml $min^{-1}$ intravenous infusion for 10 min) was administered 180 min after the onset of obstruction. The animals were observed for 420 min, the beginning of obstruction being taken as 0 min of the experiments. Changes in colonic motility and haemodynamic parameters were registered hourly; blood samples were taken from the portal vein for the measurement of plasma $NO_x$ levels at 0, 60, 180, 300 and 420 min in the postocclusion period. At the end of the experiment, tissue samples were taken from the oral and aboral parts of the large bowel (close to the hepatic and splenic flexures, respectively) for the determination of NOS isoenzyme activities.

***Study II:*** The protocol was essentially the same as in Study I; only the administered drugs were different. Group 1 (n=5) served as sham-operated control, while in group 2 (n=5) the animals were treated with the non-specific glutamate receptor antagonist KYNA (Sigma Chem. USA; 50 mg $kg^{-1}$ in 0.7 ml $min^{-1}$ iv infusion for 30 min in 20 ml 0.1 M NaOH with the pH adjusted to 7.2-7.4) at 180 min. In groups 3 (n=6), and 4 (n=5), complete large bowel obstruction was induced by tightening the tourniquet. The animals in groups 1 and 3 were treated with the vehicle for KYNA, while in group 4, KYNA was administered 180 min after the onset of obstruction. At the end of the experiment, tissue samples were taken from the proximal part of the large bowel (close to the hepatic flexure) for the determination of inflammatory enzyme activities.

### *2.5. Statistical analysis*

Data analysis was performed with a statistical software package (SigmaStat for Windows, Jandel Scientific, Erkrath, Germany). Friedman repeated measures analysis of variance on ranks was applied within the groups. Time-dependent differences from the baseline (0 min) for each group were assessed by Bonferroni's method, and differences between groups were analysed with Kruskal-Wallis one-way analysis of variance on ranks, followed by Bonferroni correction for pairwise multiple comparison. $p$ values $<0.05$ were considered significant.

## 3. RESULTS

### *3.1. Hemodynamics*

In the animals with colon obstruction, MAP displayed a slightly decreasing tendency during the observation period. NNA treatment increased MAP significantly during the later stages of the experiments, but MAP did not change significantly in the 7-NI-treated animals as compared with the non-treated group with colon obstruction. The administration of KYNA did not significantly change the values of MAP in either the sham-operated or the colon-obstructed groups.

In parallel, the obstruction caused a significant CO elevation after 300 min. NNA significantly decreased the obstruction-caused CO elevation, whereas 7-NI did not influence this change, and the CO was not significantly different from that in the control group with large bowel obstruction. KYNA treatment caused a significant, slight increase in CO in the sham-operated animals, as compared with the non-treated sham-operated group. However, KYNA treatment did not influence the obstruction-induced CO elevation.

The TPR did not change in the sham-operated group, while it gradually decreased after colon obstruction. KYNA treatment did not cause an alteration in the sham-operated group, but inhibited the obstruction-induced decrease in TPR. The changes 360 min after obstruction were statistically significant.

A continuous TPR increase was observed after non-selective NOS inhibition by NNA; the change was statistically significant 300 min after obstruction. In contrast, the administration of 7-NI did not alter the obstruction-induced TPR decrease.

In the sham-operated animals, KYNA administration caused a transient, significant increase in SMA blood flow. However, there were no significant differences in the SMA blood flow changes in the colon-obstructed animals with or without KYNA treatment.

### *3.2. Plasma $NO_x$ levels*

In the sham-operated groups with or without KYNA treatment, the plasma $NO_x$ level in the portal blood did not change significantly. The obstruction of the colon elicited a gradual, statistically significant increase in plasma $NO_x$ level. KYNA treatment significantly suppressed the increase in plasma $NO_x$ level as compared with the baseline and the obstruction-treated control group. Both specific and non-specific NOS inhibitors significantly depressed the increase in plasma $NO_x$ level as compared with the baseline and obstruction-treated control group.

### *3.3. Changes in NOS isoenzyme activity*

In the sham-operated group, the cNOS activities differed significantly in the oral and aboral tissue samples. Similarly, the activity of cNOS was significantly higher in the oral bowel segment in the obstructed group.

The nNOS inhibitor therapy decreased the cNOS activity in the oral and aboral parts of the large bowel by approximately 40% and 70%, respectively, the difference between the cNOS activities remaining significant ($p$=0.0317). NNA significantly decreased the cNOS activity, by approximately 70%, in both segments of the large bowel.

The iNOS activity was 5.8 fmol (mg protein)$^{-1}$ min$^{-1}$ (p25=3.2; p75=11) in the oral biopsies from the sham-operated animals, and an activity of 15.6 fmol (mg protein)$^{-1}$ min$^{-1}$ (p25=3; p75=18.1) was measured aborally. After obstruction induction, the iNOS activity increased 10-fold in the oral segment and a 4-fold elevation was demonstrated in the aboral segment. The non-selective and the selective NOS inhibitor treatment likewise induced significant decreases in iNOS activity in both parts of the large bowel as compared with the non-treated obstructed group.

*3.4. Changes in XOR and MPO activities*

In the treated and non-treated sham-operated groups, the XO and XDH activities did not differ significantly. The activity of the superoxide anion-producing XO was significantly increased after the obstruction. The activity of XDH was also elevated significantly in the obstructed group, indirectly indicating an accumulation of hypoxanthine as an end-product of ATP degradation. The non-selective NMDA receptor antagonist treatment therapy significantly inhibited the obstruction-induced increases in XO and XDH activities.

MPO is a marker enzyme of neutrophilic leukocyte accumulation in tissues. After obstruction induction, the MPO activity increased significantly in the proximal colon. The KYNA treatment induced a significant decrease in the MPO activity of the large bowel as compared with the non-treated obstructed group.

*3.5. Colonic motility changes*

The colonic motility index and the amplitude of the GMCs did not change in the sham-operated group during the time course of the experiments. The motility of the colon segments orally and aborally to the obstruction was only slightly elevated until 300 min following obstruction induction; a gradual, approximately 1.5-fold increase was observed in both segments by 420 min.

The NNA treatment caused a transient motility decrease at 60 min after administration, but 120 min later the motility index was significantly elevated. This motility change was greater in the oral part than in the aboral colon segment. Treatment with 7-NI slightly decreased the motility of the colon in the oral segment, while a prolonged, significant motility inhibition was observed in the colon segment aborally to the obstruction.

The KYNA treatment decreased the amplitude of the GMCs as compared with the non-treated obstruction group, while in the sham-operated group the treatment caused significant decreases in the motility index and the amplitude of the GMCs at 300 min and 360 min.

The tone of the proximal colon, defined as the mean value of the minimum points in the motility curve, was significantly decreased after the obstruction, and this change was significantly inhibited by KYNA treatment after 360 min. In the sham-operated animals, the non-selective NMDA receptor antagonist treatment caused a 2-fold increase in the tone of the proximal colon as compared with the baseline and the control value.

The frequency of contractions did not differ in the sham-operated and obstructed groups during the observation period. However, the administration of KYNA caused

significant, 1.4 and 1.6-fold elevations, respectively, in the frequency of the GMCs, which were characterized by a decreased amplitude, irrespectively of the obstruction.

## 4. DISCUSSION

In this canine model, experimental blockade of the intestinal passage increased the large bowel motility, and triggered a hyperdynamic circulatory reaction 5 h after obstruction, accompanied by a significant $NO_x$ level elevation in the plasma, increased iNOS and XO activation and leukocyte accumulation in the proximal colon. The colon obstruction-induced hemodynamic changes were characterized by an increased CO and a reduced TPR, similarly as observed in early human sepsis. This hyperdynamic cardiovascular response may be regarded as a compensatory reaction through which the organism tries to accommodate to the evolving septic metabolic changes.

There is now good evidence that postoperative ileus initiates the activation of transcription factors, upregulates proinflammatory cytokines, and increases the release of kinetically active mediators (inducible NO and prostaglandins), important factors in the recruitment of leukocytes and the suppression of motility. On the other hand, Hellström *et al.* have demonstrated that low doses of endotoxin cause marked changes in myoelectric activity in the small intestine, with repetitive bursts of spike potentials and a simultaneous increase in the transit of the intestinal contents. Indeed, the obstruction-induced motility alterations are time-dependent, characteristically changing in parallel with the development of inflammation.

### *4.1. Role of nitric oxide: Study I*

NO is a universal chemical mediator of GI intercellular communication and its pathogenetic role has been also verified in sepsis and mucosal permeability changes. Further, it has been demonstrated that the overproduction of NO caused by the iNOS isoform contributes significantly to the cardiovascular and intestinal motility failure during this condition. Yanagida *et al.* observed that the activity of ICCs and pacemaking was greatly attenuated in the absence of NO derived from iNOS. Non-selective NOS inhibitors reduce both constitutive and inductive NO production; thus, in parallel with the increased blood pressure, they also lead to a drastic decrease in the CO. Indeed, this haemodynamic pattern evolved in the early phase of bowel obstruction after non-selective NOS inhibition. Selective nNOS inhibition, however, efficiently decreased the obstruction-caused plasma $NO_x$ level elevation, and did not influence the hyperdynamic circulatory response. This indicates that NO produced by both eNOS and iNOS isoforms accounts for the obstruction-induced hemodynamic changes.

The continuous or constitutive synthesis of NO in the intestinal tract is mainly ensured by nNOS, but both known cNOS isoforms are present in the myenteric neurons of the colon. eNOS and nNOS can not be differentiated by conventional biochemical means. The *in vivo* specificity of 7-NI towards nNOS is due to a higher neuronal uptake as compared with endothelial cells. The significant decrease in cNOS activity after nNOS inhibition allowed the conclusion that nNOS is responsible for at least 40% of the basal NO production of the canine colon.

Here, we have reported the first observations on the intestinal NOS isoenzyme activity in correlation with obstruction-induced motility alterations. The results revealed that NO is crucially involved in the mechanism of motility alterations through iNOS activation. Under physiological conditions, the inhibition of NO production leads to a significantly increased luminal pressure and intestinal motility in both the small and large intestines. On the basis of this observation it is now generally accepted that NO is a neurotransmitter which mediates relaxation. Our results partially support this notion, since non-selective NOS inhibition transiently decreased the motility index in both intestinal segments for approximately 60 min. However, after this period, the intestinal motility increased dramatically. Indeed, this phenomenon was earlier described as a side-effect of NOS inhibition. When Ohta *et al.* compared the *in vivo* effects of different routes of NNA administration, intravenous NNA infusion resulted in increased peristalsis, while intracerebroventricularly administered NOS-inhibitor therapy suppressed the motility of the colon. These findings are in accord with the report by Bartho and Lefebvre of $Ca^{2+}$-dependent contraction enhancement effects on a longitudinal muscle specimen after NO-agonist administration. The explanation for this apparent contradiction may be that the NO-related regulation of the intestinal motility comprises two different parts, separated in time: an initial excitatory period is followed by an inhibitory relaxation. Our results confirm that this process mainly involves nNOS-derived NO, as decreased colon motility was demonstrated after selective nNOS inhibition.

However, it is noteworthy that the 7-NI-induced inhibition of the motility was less strong in the oral segment than in the aboral part of the colon. The cause of this disparity may be the different NANC innervation of the intestinal segments. It has been shown that the number of nitrergic neurons is significantly higher in the myenteric plexus of the proximal colon than in the distal part of the large intestine. The administration of an equipotent 7-NI dose therefore elicited a higher rate of inhibition, and thus decreased the motility more effectively distally.

In our experiments, the activation of iNOS and the overproduction of NO reached a level characteristic of early sepsis, but these biochemical changes did not correlate with the moderately increasing motility index in the oral and aboral colon segments. Our results indicated that the NO originating from iNOS modifies the excitatory profile of the regulatory process in the examined time frame. This is supported by the finding that selective iNOS inhibition therapy positively influenced the conditions under which motility inhibition had been attained.

In conclusion, these data suggest that NO may play a rather complex role in the regulation of the motility of the obstructed colon:

1) NO of neuronal origin is a transmitter that stimulates the peristaltic activity of the colon, since non-selective NOS inhibition transiently inhibits the motility, while the administration of a selective nNOS inhibitor elicits long-lasting motility inhibition.
2) In parallel, the non-specific inhibition of NO leads in the long run to a significant motility increase. This delayed effect could indicate suppression of the neurotransmission of an inhibitory motor neuron, inhibition of the motility-decreasing effect of iNOS, or the predominance of constrictor mediators that act on the smooth muscle elements of the intestinal wall.
3) As an inherent component of the septic process accompanying acute colon obstruction, significant but different quantities of inductively produced NO are present in the proximal and distal segments of the colon; this could result in a considerably increased iNOS/nNOS ratio, and hence moderate the obstruction-induced motility increase.

***4.2. Role of glutamate: Study II***

The enzymes of the kynurenine pathway are activated by inflammation and immune stimulation, leading to large increases in the generation of the NMDA agonist quinolinic acid and its antagonist, KYNA. The balance between the relative concentrations of these substances during an inflammatory response could therefore have a profound influence on the excitability of the enteric neurons and hence on the motility of the gut. In pathological conditions (infections, ischaemia or traumas), dramatic increases in quinolinic acid concentrations have been demonstrated. Although quinolinic acid is a relatively weak agonist at the NMDA receptors, its *in vivo* excitotoxicity is similar to that of NMDA, and several of its metabolites, including toxic free radicals, can enhance the neurotoxicity. Moreover, quinolinic acid can increase the formation of reactive oxygen species both through a direct

Fenton-like interaction with iron, and through the NMDA receptor-activated increase in intracellular $Ca^{2+}$ level, which results in a higher XOR activity.

Glutamate neurotoxicity (necrosis and apoptosis) has been observed in a subset of enteric neurons in both intact bowel preparations and cultured myenteric ganglia. These data indicate that excitotoxicity may occur in the ENS as well, and overactivation of the enteric glutamate receptors may contribute to the intestinal damage produced by obstruction, anoxia or ischaemia. Since the glutamate receptors are involved in functional bowel disorders, the neuroprotective abilities of KYNA have been tested. KYNA is a broad-spectrum antagonist at all subtypes of ionotropic glutamate receptors, but it is preferentially active at the strychnine-insensitive glycine allosteric site of the NMDA receptor. KYNA itself only poorly penetrates the blood-brain barrier, and thus the protective effects of KYNA are limited for the CNS. It follows that the intravenous administration of KYNA targets only the peripheral nervous system.

The mechanism whereby an elevated KYNA level leads to an increase in SMA blood flow or the inhibition of XOR activity has not been elucidated. However, it has been reported that the administration of L-kynurenine results in a significant immediate increase in corticocerebral blood flow under normal or ischaemic circumstances, which can be blocked by atropine or a NOS inhibitor. This raises the possibility that KYNA may exert its neuroprotective effect not only by inhibiting excitatory neurotransmission, but also by increasing the blood flow. Our results demonstrating decreased XO and MPO activities following KYNA treatment confirm this hypothesis. Another possible explanation would be a substrate analogue non-specific inhibitory effect of KYNA on XOR activity, since there is structural similarity to hypoxanthine/xanthine, the substrate for XOR.

The relative weight of KYNA treatment in the modification of the obstruction-induced motility dysfunction was significant. Our results indicate that glutamate receptors contribute to the excitatory profile of the motility pattern in the examined time frame, since non-selective NMDA receptor antagonism treatment significantly decreased the motility index and amplitude of the GMCs. Our results are consistent with the findings of Tong *et al.*, suggesting that mGluR8 agonists increase the motility by inhibiting nitrergic relaxation and possibly by facilitating cholinergic contractions. However, the increases in colon tone and frequency of contractions with limited amplitude point to the possible role of some other facilitating mechanism. Since KYNA is not only a broad-spectrum antagonist of all subtypes of ionotropic glutamate receptors, but also a non-competitive antagonist at the alpha7 nicotinic receptor, the role of an excitatory cholinergic pathway as concerns the increased tone could

not be excluded. In conclusion, our results demonstrate an important role for glutamate receptors in the pathophysiology of acute colon obstruction-induced motility changes.

1) These findings reveal that KYNA significantly inhibits the contraction of the GMCs in the colon.
2) KYNA exerts a protective, anti-inflammatory effect due to the indirect inhibition of oxygen radical production and leukocyte activation.

*To summarize* the results, the data suggest that, presumably through the co-functioning of the triple unit of the nerve, the ICCs and the smooth muscle cells in the ENS, the nitrergic and glutaminergic mechanisms play supplementary, important roles in the regulation of colonic motility. The function of this triad could probably be that nerves stimulate the NMDA receptors of the ICCs through the release of glutamate. The activation of NMDA receptors induces $Ca^{2+}$ influx, and causes constitutive NO production by a $Ca^{2+}$/calmodulin-dependent process. The ICCs play a critical role in the reception and transduction of excitatory and inhibitory neurotransmission. The synthetised NO, as a soluble transmitter of the ICCs easily penetrates biological membranes and conducts or mediates the stimuli to the neighbouring smooth muscle cells. This possible attachment of the glutaminergic and nitrergic mechanisms seems to be supported by the result of our Study II. KYNA treatment not only significantly inhibited the obstruction-induced increase in the motility index of the colon, but also significantly decreased the plasma $NO_X$ levels.

It remains to be established whether the findings in this experimental model are applicable to humans. However, together with previous observations, these data strongly suggest that medication with an appropriate selective iNOS inhibitor prior to intestinal surgery protects against postsurgical dysmotility and reduces the severity of postoperative ileus. Furthermore, the suppression of the hypermotility function of the NMDA receptors might be beneficial in serving as an incremental tool which can influence the excitotoxicity complications after an acute colon obstruction. We hope that these findings will result in the near future in a more effective approach via which to reduce the morbidity and mortality rates of these still dangerous clinical entities.

## LIST OF PUBLICATIONS

### Full papers

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