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

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LIST OF PUBLICATIONS RELATED TO THE SUBJECT OF THE THESIS

Full papers

1. Palásthy Z., Kaszaki J., Nagy S., Balogh Á., Boros M.: Dual effects of nitric oxide in acute colon obstruction. *Magyar Sebészet 2005; 58: 47-55.*

2. Palásthy Z., Kaszaki J., Lázár G., Nagy S., Boros M.: Intestinal nitric oxide synthase activity changes during experimental colon obstruction. *Scandinavian Journal of Gastroenterology, 2006; 41(8): 910-918.* **(IF: 1.869)**

3. Palásthy Z., Kaszaki J., Érces D., Rácz A., Torday C., Varga G., Vécsei L., Boros M.: Kynurenic acid inhibits intestinal hypermotility and xanthine oxidase activity during experimental colon obstruction in dogs. *Neurogastroenterology and Motility 2008; 20(1): 53-62.* **(IF** 2006: **3.338)**

Abstracts

1. Palásthy Z., Baradnay G., Kaszaki J., Lázár G. Jr., Balogh Á., Boros M.: Nitrogénmonoxid szerepe kísérletes acut vastagbél elzáródásban. *Magyar Sebészet* 1999. 52: 216.

2. Baradnay G., Palásthy Z., Kaszaki J., Lázár G., Balogh Á., Boros M.: The role of nitric oxide in acute motility changes during experimental colonic obstruction. *European Surgical Research 2000. 32 (S1): 12.*

3. Palásthy Z., Kaszaki J., Szalay L., Baradnay G., Balogh Á., Boros M.: The role of mast cells and nitric oxide in leukocyte accumulation during acute experimental colonic obstruction. *European Surgical Research 2001. 33: 135.*

4. Palásthy Z., Kaszaki J., Szalay L., Balogh Á., Boros M.: The effects of selective neuronal NOS inhibition during acute experimental colonic obstruction. *European Surgical Research 2002. 34 (S1): 58.*

5. Palásthy Z., Kaszaki J., Szalay L., Balogh Á., Boros M.: Szelektív neuronalis NOS gátlás hatása kísérletes akut vastagbél elzáródásban. *Magyar Sebészet 2002.* 55: 135.

6. Palásthy Z., Kaszaki J., Lázár G., Nagy S., Boros M.: Dual effects of intestinal nitric oxide synthesis on motility changes during acute colon obstruction. *European Surgical Research 2006. 38 (S1): 8.*

7. Kaszaki J., Palásthy Z., Rácz A., Érces D., Boros M.: Effects of glutamate receptor antagonism on experimental colon obstruction. *Shock 2006. 26 (S1): 26.*

8. Kaszaki J., Érces D., Rácz A., Palásthy Z., Vécsei L., Boros M.: Neuroprotective feature of glutamate receptor antagonism after acute colon obstruction. *European Surgical Research 2007. (S1): 38.*

9. Rácz A., Érces D., Palásthy Z., Kaszaki J., Boros M: A hízósejtek szerepe kísérletes akut vastagbél elzáródásban. *Érbetegségek 2007. S1: 20.*

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

XO: xanthine oxidase

XDH: xanthine dehydrogenase

XOR: xanthine oxidoreductase

1. SUMMARY

Irrespective of the aetiology or the type of the surgical intervention, gastrointestinal (GI) motility disorders are prevailing characteristics in the postoperative period after abdominal surgery. The therapeutic possibilities for dysmotility are rather limited, mainly due to the still unexplored pathophysiology. Intestinal peristalsis is controlled by a complex autonomic neuronal regulation, which is predominantly cholinergic in nature. However, several recent reports have suggested that alternative pathways may significantly modulate the cholinergic GI motility regulation. The main purpose of our studies was to examine the roles of nitrergic and glutaminergic modulation in the colon, in correlation with obstruction-induced motility alterations. A large animal model of colon obstruction was designed, and we performed two series of experiments to investigate the role of nitric oxide (NO) (Study I) and glutamate (Study II). Accordingly, in the first series of experiments we compared the consequences of selective neuronal and non-selective NO synthase (NOS) inhibition on the colonic motility changes during acute experimental ileus. Secondly, we hypothesized that glutamate, a major excitatory neurotransmitter in the central nervous system (CNS), is likely to play a role as an excitatory neurotransmitter in the enteric nervous system (ENS). Consequently, we hypothesized that the inhibition of enteric glutamate receptors by kynurenic acid (KYNA) may influence the motility in the GI tract.

Experiments were performed on inbred mongrel dogs under general anaesthesia. Mechanical occlusion of the mid-transverse colon was maintained for 7 h. In Study I, we observed the haemodynamic and motility parameters, measured the plasma nitrite/nitrate (NO_X) levels, and the NOS activities. Large bowel motility indices were determined by calculating the area under the motility curve as a function of time by a computerized data-acquisition system. Constitutive NOS (cNOS) and inducible NOS (iNOS) activities were determined in tissue biopsies; plasma NO_X levels were measured in the portal blood. Following completion of the baseline studies, the animals were treated with either 7-nitroindazole (7-NI; a selective neuronal NOS (nNOS) inhibitor), or N-nitro-L-arginine (NNA; a non-selective NOS inhibitor). In Study II, the aims were to characterize the motility and associated inflammatory changes during colon obstruction, and to define the consequences of KYNA treatment in this condition. Haemodynamics and motility changes were monitored, and the activities of xanthine oxidoreductase (XOR) and myeloperoxidase (MPO; a marker of leukocyte accumulation) were determined from tissue biopsies.

In the sham-operated group, the cNOS activities differed significantly in the oral and aboral tissue samples (oral: 102.9; *vs* aboral: 62.1 fmol (mg protein)$^{-1}$ min^{-1}). The obstruction

induced a hyperdynamic circulatory reaction, which was accompanied by significant increases in the plasma NO_X level, the tissue iNOS activity, the colon XOR activity and leukocyte accumulation, and a rise in the motility index. NNA treatment decreased the motility index in both intestinal segments for 60 min, but 120 min later the motility index was significantly elevated (a 2.5-fold increase in the oral part, and a 1.8-fold enhancement in the aboral segment). 7-NI decreased the cNOS activity in the oral and aboral parts by approximately 40% and 70%, respectively, and suppressed the motility increase in the aboral colon segment. The administration of KYNA prevented the obstruction-caused decrease in total peripheral vascular resistance (TPR) and increased the tone of the colonic smooth muscles, but permanently decreased the motility index of the characteristic, giant contractions of the colon. The KYNA treatment significantly inhibited the obstruction-induced increases in colon XOR activity and leukocyte accumulation.

NO of neuronal origin is a transmitter that stimulates the peristaltic activity; but an increased iNOS/nNOS ratio significantly modifies the obstruction-induced motility increase. Our results indicate the decisive modulatory role of the glutamate receptors in early colonic motility alterations. KYNA treatment could have a cytoprotective effect based on an indirect inhibition of the superoxide radical and the consequent leukocyte activation.

2. INTRODUCTION

I started my surgical career as a novice medical doctor at the Department of Surgery at the University of Szeged, an institution with long traditions of experimental research on bowel paralysis. Professor Gábor Petri had started to study this syndrome almost four decades ago, and in the 1960s he and his co-workers published several groundbreaking results on the "*pathogenesis and a new therapy of paralytic ileus*" in leading international journals (Petri *et al.* 1967, 1968, 1971). These important experimental findings were later successfully applied at the bedside throughout Hungary[*]. These studies motivated me to focus my attention on this field, and especially on the observation of motility changes in the large bowel.

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 (Bauer *et al.* 2002, Madl *et al.* 2003). Moreover, irrespective of the aetiology or the type of the abdominal surgical intervention, 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.

2.1. Regulation of bowel motility

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.

Intestinal peristalsis is controlled by a complex, autonomic neuronal regulation. 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.

[*] In: Petri Gábor: A "paralytikus" ileus kórtana és sympatholytikus kezelése (Doctoral thesis, Szeged, 1972)

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.* (1988*)* 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*".

The ENS has a relative independence as compared with the rest of the ANS. Nevertheless, the ENS, similarly to the CNS, has sensory receptors which generate stimuli to the network of interneurons and finally to the effector cells (Furness *et al.* 1980, Gershon *et al.* 1981, Lundgren *et al.* 1989). Earlier morphological studies identified varicose swellings along the lengths of autonomic motor nerves, including fibres within the ENS (Gabella *et al*. 1979), and it is generally accepted that these are the sites of release for most neurotransmitters. Many neurophysiologists who study the ENS envisage the release of neurotransmitters as an *en passage* process, occurring as action potentials conducted down nerve fibres into nerve varicosities, functional innervation being defined as the volume through which a neurotransmitter can diffuse from the varicosity and reach postjunctional receptors in sufficient concentrations to produce a physiological response in the neuroeffector cell (Burnstock *et al.* 1981).

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 (Ward *et al.* 2006). In the colon, ICCs located along the submucosal surface of the circular muscle layer (ICCs-SM) also provide a pacemaker function in this organ (Smith *et al*. 1987). A special population of ICCs is distributed over the surface of muscle bundles and within septae that separate muscle bundles and are termed ICCs-SEP (Lee *et al.* 2007). The investigation by Horiguchi *et al.* (2001) demonstrated that these cells may behave much like Purkinje fibres in the heart, conveying and coordinating the spread of pacemaker activity deep into and between muscle bundles and may also be involved in enteric

motor neurotransmission. Functional neurotransmission cannot occur in the absence of these cells (Burns *et al.* 1996, Ward *et al.* 2000). Surgical manipulations of the GI tract, including intestinal resection and anastomosis, lead to dysmotility, which is associated with the disruption of ICC networks (Yanagida *et al.* 2004).

The ICCs possess a variety of receptors for neurotransmitters. Classical excitatory and inhibitory neurotransmitters are concentrated and released from neurovesicles located in enteric nerve terminals or varicose regions of motor nerves.

The motility regulation is predominantly cholinergic in nature (Salzman *et al.* 1995). However, several data suggest that alternative pathways may significantly modulate the cholinergic GI motility regulation. Ward *et al.* (2006) have demonstrated that the ICCs-IM may play a critical role in the reception and transduction of cholinergic and nitrergic neurotransmission. Thus, the local production of NO messenger molecules could be of importance in the regulation of motility and the pathophysiology of dysmotility.

2.2. Nitric oxide

Biological activity for NO was first proposed in 1987 (Monaca *et al.* 1991). NO is a soluble gas and can maintain the connection between cell membranes without synapses. It is a short-lived mediator, formed by the sequential oxidation of the substrate L-arginine by the NOS family of enzymes, leading to the formation of L-citrulline and NO.

There are two main types of NOS: cNOS, which is Ca^{2+}/calmodulin dependent, and iNOS which is Ca^{2+}-independent. cNOS is responsible for the production of NO in a physiological context. In contrast, iNOS produces NO under pathophysiological circumstances. It has been clarified that cNOS has two subtypes: nNOS and endothelial NOS (eNOS). nNOS was first described in the neurons of the CNS and peripheral nervous system, while eNOS is generally found in the endothelium of blood vessels, where it is responsible for vasodilatation. iNOS is mainly located in the cytosol of cells in the immune system.

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 (Qu *et al.* 1999). Moreover, previous studies have shown that NO produced by the iNOS isoform during inflammatory cascade reactions directly inhibits the intestinal smooth muscle contractility (Qu *et al.* 1999, Kalff *et al.* 2000, Türler *et al.* 2002).

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. The peristalsis of the colon is controlled

by a complex autonomic neuronal regulation in which sensory neurons, interneurons and ascending excitatory and descending inhibitory (motor) neurons take part (Sarna *et al.* 1991). In this process, NO relaxes the smooth muscles directly, but it may also act as a cotransmitter of non-adrenergic non-cholinergic (NANC) inhibitory and descending interneurons (Bult *et al.* 1990, Dalziel *et al.* 1991, Ward *et al.* 1992, Boeckstaens *et al.* 1993, Shuttleworth *et al.* 1993). NO may also contribute to intestinal propulsion by inducing neurogenic contractions (Bartho *et al.* 1995, Holzer *et al.* 1997). *In vitro* observations suggest that non-selective NOS inhibitors enhance the intestinal motility, which indicates the inhibitory neurotransmitter character of NO (Dalziel *et al.* 1991, Ward *et al.* 1992, Boeckstaens *et al.* 1993, Shuttleworth *et al.* 1993). In contrast with these observations, Heinemann *et al.* have demonstrated the suppressed contractile activity of the intestinal musculature after the selective inhibition of nNOS (Heinemann *et al.* 1999).

2.3. Glutamate

Several studies in the 1940s suggested that the oral administration of glutamate could have a beneficial effect on both normal and retarded intelligence. Later, the neurotoxic nature of glutamate emerged in excitotoxic lesions (neuronal death), and it is thought to underlie the pathophysiology of several neurological diseases, including Huntington's disease, status epilepticus, Alzheimer's dementia and olivopontocerebellar atrophy. In 1959, Curtis *et al.* showed that microiontophoretically-applied glutamate could excite spinal neurons. During the subsequent years, this result was confirmed and extended. By the early 1980s, many agreed that some glutamate-like chemical must act as neurotransmitter. A key advance was the introduction of selective antibodies with which to study the immunocytochemical distribution of glutamate.

In the last decade, glutamate was one of the most studied excitatory amino acids in the CNS (Weinberg *et al.* 1999), and it may be widely presumed that the glutaminergic neuorotransmission plays a role in the ENS too. Glutamate is synthesized from gamma-aminobutyric acid. Two types of receptors for glutamate have been identified: ionotropic and metabotropic. The former includes three different types, one of which is the N-methyl-D-aspartate (NMDA)-sensitive receptor, which is coupled to a Na^+ and Ca^{2+} channel.

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 (Mackay *et al.* 2006). The components of the kynurenine pathway have marked effects on the neurons in the CNS (Stone *et al.* 2003). One of the main

end-products is quinolinic acid, an agonist of the NMDA-sensitive glutamate receptors. A second kynurenine metabolite, 3-hydroxykynurenine, can generate free radicals and also exacerbate or contribute to neuronal damage. However, 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 (Perkins *et al.* 1982, Stone *et al.* 2001, Klivényi *et al.* 2004). Consequently, quinolinic acid can act as a neurotoxin, while KYNA is neuroprotective in the CNS (Vécsei *et al.* 1992, Kiss *et al.* 2005).

Far fewer data are available on the role of kynurenine metabolites in the ENS. Several recent studies have suggested that glutamate-mediated facilitatory pathways may modulate the cholinergic transmission in the ENS (Liu *et al.* 1997, Kirchgessner *et al.* 2001). Glutamate is a major excitatory neurotransmitter in the CNS, and thus it is likely to play a role as an excitatory neurotransmitter in the ENS too. 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 (Wiley *et al.* 1986, Liu *et al.* 1997). Moreover, ionotropic NMDA-sensitive glutamate receptors are present and abundantly expressed on enteric cholinergic neurons (Moroni *et al.* 1986, Liu *et al.* 1997).

Inflammation is also an important component of the pathophysiology of bowel obstruction (Madl *et al.* 2003, Törnblom *et al.* 2005), characterized by an altered permeability of the gut mucosa and the activation of inflammatory cells (Törnblom *et al.* 2005). The local production of purine and kynurenine metabolites may be involved in the regulation of neuronal activity in inflammatory intestinal disorders (Forrest *et al.* 2002, 2003).

2.4. 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. Changes in the inflammatory parameters, the local leukocyte accumulation and the activity of XOR the predominant source of superoxide radical production, were also investigated in relation to occlusion-induced haemodynamic patterns. The results indicated that the glutamate receptors decisively modulate the early colonic motility alterations, and demonstrate a significant potential for KYNA to decrease the facilitatory pathways of colonic motility disorders.

3. MATERIALS AND METHODS

3.1. Animals

The experiments were performed on healthy, inbred mongrel dogs of both sexes (body weight range: 12-18 kg) from the Animal House of the University of Szeged in adherence to the NIH guidelines for the use of experimental animals ("Principles of laboratory animal care" NIH publication No. 86-23, revised 1985). The study was approved by the Ethical Committee for the Protection of Animals in Scientific Research at the University of Szeged.

3.2. Surgical procedures

Surgery was performed under sodium pentobarbital (30 mg kg^{-1} iv) anaesthesia. Small supplementary doses of pentobarbital were administered when necessary. During the experiment, the animals were ventilated with room air through an endotracheal tube, using a Harvard respirator. The left femoral artery and vein were cannulated for the recording of mean arterial pressure (MAP) and for fluid and drug administration, respectively. The animals were placed in a supine position on a heating pad for maintenance of the body temperature between 36 and 37 ^{o}C, and received an infusion of Ringer's lactate at a rate of 10 ml kg^{-1} h^{-1} during the experiments. A Swan-Ganz thermodilution catheter (Corodyn TD-E-N, 5011-110-7Fr; Braun Melsungen AG, Melsungen, Germany) was positioned into the pulmonary artery via the right femoral vein to measure the cardiac output (CO).

After a midline abdominal incision, the portal vein was catheterized through the splenic vein for blood sampling. 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,*** *s*train gauge transducers (Experimetria Ltd., Budapest, Hungary) were sutured with an atraumatic technique 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 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.

3.3. Measurements

3.3.1. Haemodynamic 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., Budapest, Hungary). The TPR was calculated via the standard formula.

3.3.2. Colonic motility measurements

The motility index was calculated to estimate the neurogenic function of the intestine (Cowles *et al.* 1978). Briefly, two strain gauge transducers (FSG-02 type; size: 6x15 mm; Experimetria Ltd, Budapest, Hungary) were sutured with 5/0 silk (Braun-Dexon, Melsungen, Germany) onto the appropriate part of the colon. The transducers were connected to an SG-M bridge amplifier and the signals were continuously recorded by a computerized data-acquisition system (HAEMOSYS 1.17; Experimetria Ltd, Budapest, Hungary). The sampling time was 10 min each, with a sampling frequency of 500 Hz; the signal analysis was performed off-line. Large bowel motility indices were determined by calculating the area under the motility curve as a function of time (Huge *et al.* 1998). 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.

3.3.3. Plasma nitrite/nitrate level measurements

Plasma NO_x levels were measured in the portal blood via the Griess reaction (Green *et al.* 1982). 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 (Moshage *et al.* 1995).

3.3.4. 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 *et al* (1993). Briefly, large bowel biopsies kept on ice were homogenized in phosphate buffer (pH 7.4) containing 50 mM Tris-HCl (Reanal, Budapest, Hungary), 0.1 mM EDTA (Serva Feinbiochemica GmbH, Heidelberg, Germany), 0.5 mM dithiotreitol, 1 mM phenylmethylsulfonyl fluoride, 10 μg ml^{-1} soybean trypsin inhibitor and 10 μg ml^{-1} leupeptin. The homogenate was centrifuged at 4 ^{o}C for 20 min at 24 000g and the supernatant was loaded into centrifugal concentrator tubes (Amicon Centricon-100; 100 000 MW cut-off ultrafilter). The tubes were centrifuged at 900g for 150 min and the concentrated supernatant was washed out from the ultrafilter with 250 μl homogenizing buffer. The samples were incubated with a cation-exchange resin (Dowex AG 50W-X8, Na^+ form) for 5 min to deplete endogenous L-arginine. The resin was separated by centrifugation (1500g for 10 min) and the supernatant containing the enzyme was assayed for NOS activity.

For the Ca^{2+}-dependent eNOS activity, 50 μl enzyme extract and 100 μl reaction mixture (pH 7.4, containing 50 mM Tris-HCl buffer, 1 mM NADPH, 10 μM tetrahydrobiopterin, 1.5 mM $CaCl_2$, 100 U ml^{-1} calmodulin and 0.5 μCi [^{3}H]L-arginine (Amersham U.K., specific activity 63 Ci mmol^{-1})) were incubated together for 60 min at 37 ^{o}C. The reaction was stopped by the addition of 1 ml ice-cold HEPES buffer (pH 5.5) containing 2 mM EGTA and 2 mM EDTA. Measurements were performed with the non-selective NOS inhibitor NNA, (Sigma Chem. USA, 3.2 mM) to determine the extent of [^{3}H]L-citrulline formation independent of the NOS activity. iNOS was measured without Ca^{2+}-calmodulin and with EGTA (8 mM).

1 ml reaction mixture was applied to Dowex cation-exchange resin (AG 50W-X8, Na^+ form) and eluted with 2 ml distilled water. The eluted [^{3}H]L-citrulline activity was measured with a scintillation counter (Tri-Carb Liquid Scintillation Analyzer 2100TR/2300TR, Packard

Instrument Co, Meriden, CT, U.S.A.). Protein contents of samples were determined by the Lowry method.

2.3.5. Xanthine oxidase activity

Colon biopsies kept on ice were homogenized in phosphate buffer (pH 7.4) containing 50 mM Tris-HCl (Reanal, Budapest, Hungary), 0.1 mM EDTA (Serva Feinbiochemica GmbH, Heidelberg, Germany), 0.5 mM dithiotreitol, 1 mM phenylmethylsulfonyl fluoride, 10 μg ml^{-1} soybean trypsin inhibitor and 10 μg ml^{-1} leupeptin. The homogenate was centrifuged at 4 ^{o}C for 20 min at 24 000g and the supernatant was loaded into centrifugal concentrator tubes (Amicon Centricon-100; 100 000 MW cut-off ultrafilter). The tubes were centrifuged at 1000g for 90 min and the concentrated supernatant was washed out from the ultrafilter with 250 μl homogenizing buffer. 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 (Beckman *et al.* 1989).

2.3.6. Tissue MPO activity

The activity of MPO, a marker of tissue leukocyte infiltration, was measured in the colon biopsies (Kuebler *et al*. 1995). Briefly, the tissue was homogenized with Tris-HCl buffer (0.1 M, pH 7.4) containing 0.1 mM polymethylsulfonyl fluoride to block tissue proteases, and then centrifuged at 4 ^{o}C for 20 min at 24000g. 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

Our experiments were performed in two series. The numbers of animals in the individual groups and the administered agents are shown in Table I.

Table I. Summary of studies, groups, treatments and numbers of animals.

Study	***Group***	***Treatment***	***n***
Study I	Group 1	Sham-operated	6
Study I	Group 2	Obstruction	8
Study I	Group 3	Obstruction + NNA	6
Study I	Group 4	Obstruction + 7-NI	6
Study II	Group 1	Sham-operated	5
Study II	Group 2	Sham-operated + KYNA	5
Study II	Group 3	Obstruction	6
Study II	Group 4	Obstruction + KYNA	5

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. 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=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} intravenous infusion for 30 min in 20 ml 0.1 M NaOH with the pH adjusted to 7.2-7.4) at 180 min. Dose-response effects were investigated in pilot rat studies. 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. The animals were observed for 420 min, the beginning of obstruction denoting 0 min. 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 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). Non-parametric methods were used. 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. In the Figures, median values and 75th and 25th percentiles are given. p values <0.05 were considered significant.

4. RESULTS

4.1. Haemodynamics

The baseline values of MAP and other macrohaemodynamic variables were not significantly different in the various groups. 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 (data not shown).

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 (data not shown).

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 (Figure 1).

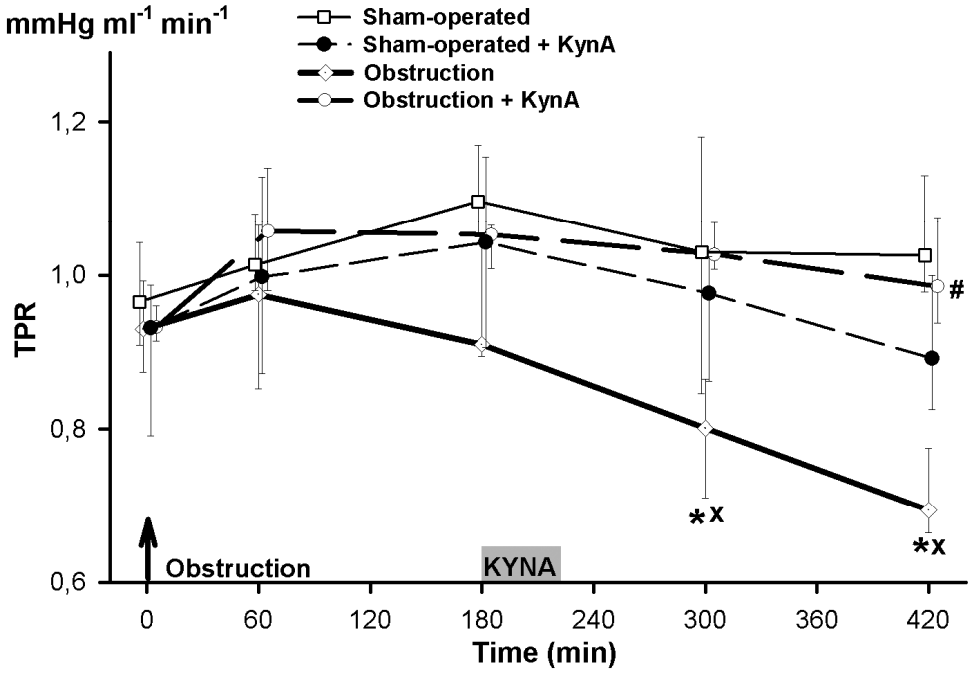

Figure 1. *Changes in TPR in the sham-operated (empty squares), KYNA-treated sham-operated (full circles with dashed line), colon obstruction (empty diamonds), and KYNA-treated obstruction (empty circles with dashed line) groups. The plots demonstrate the median values and the 25th (lower whisker) and 75th (upper whisker) percentiles, * p<0.05 within groups vs baseline values, ^{x} p<0.05 between groups vs sham-operated group values, $^{\#}$ p<0.05 between KYNA-treated group vs obstructed group values.*

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 (Figure 2).

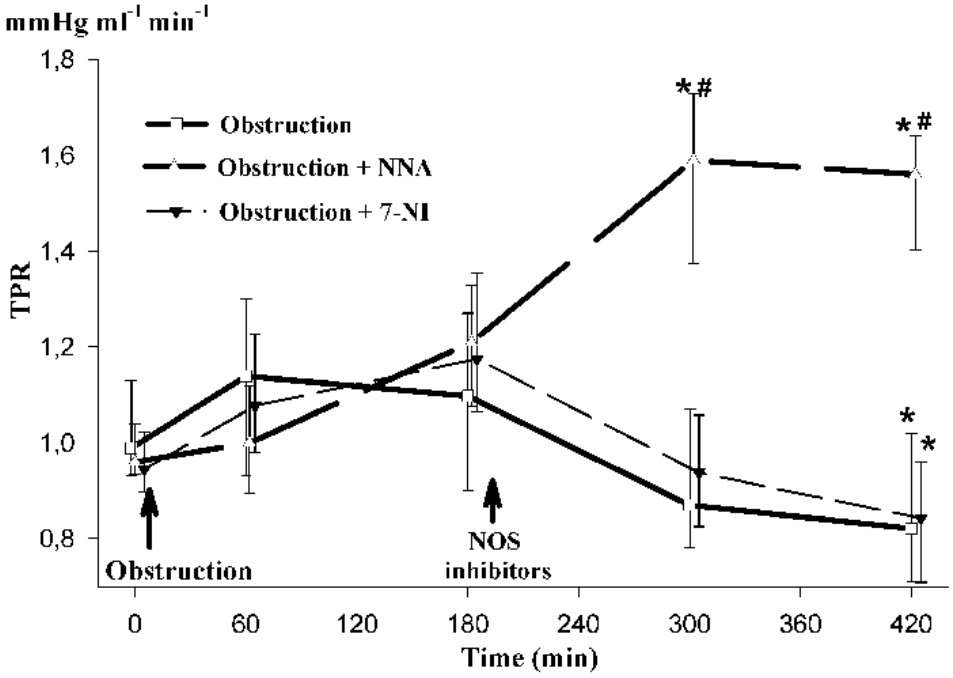

Figure 2. *Changes in TPR in colon obstruction and non-selective NOS inhibitor NNA treatment (empty triangles), or selective nNOS inhibitor 7-NI treatment (full triangles). The plots demonstrate the median values and the 25th (lower whisker) and 75th (upper whisker) percentiles * p<0.05 within groups vs baseline values, ^{x} p<0.05 between groups vs sham-operated group values, $^{\#}$ p<0.05 between NOS inhibitor-treated groups vs obstructed group values.*

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 (Figure 3).

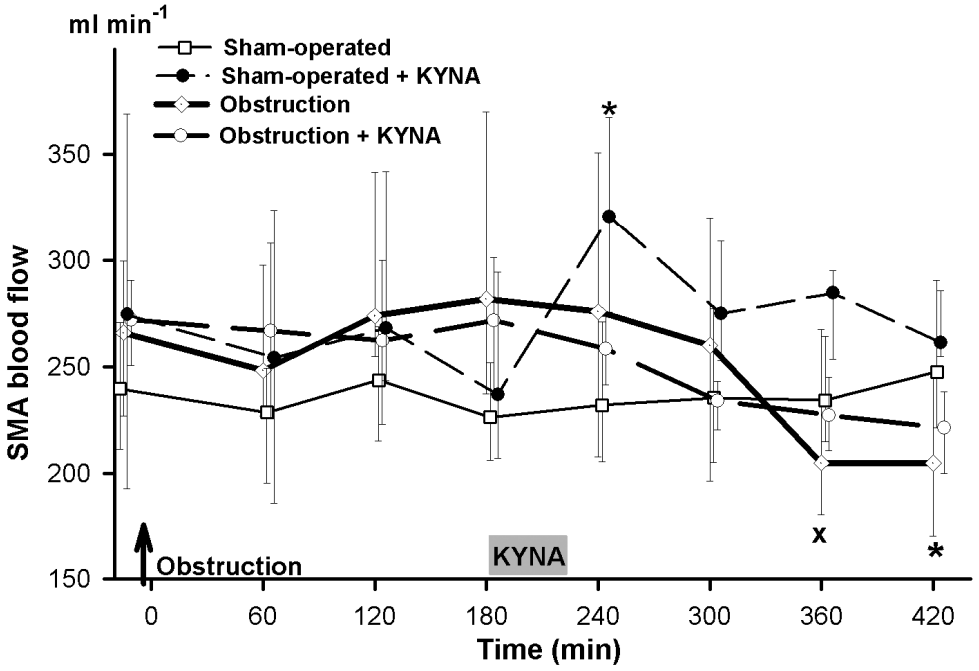

Figure 3. *Changes in SMA blood flow in the sham-operated (empty squares), KYNA-treated sham-operated (full circles with dashed line), colon obstruction (empty diamonds), and KYNA-treated obstruction (empty circles with dashed line) groups. The plots demonstrate the median values and the 25th (lower whisker) and 75th (upper whisker) percentiles, * p<0.05 within groups vs baseline values, ^{x} p<0.05 between groups vs sham-operated group values, $^{\#}$ p<0.05 between KYNA-treated group vs obstructed group values.*

4.2. 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 (Figure 4).

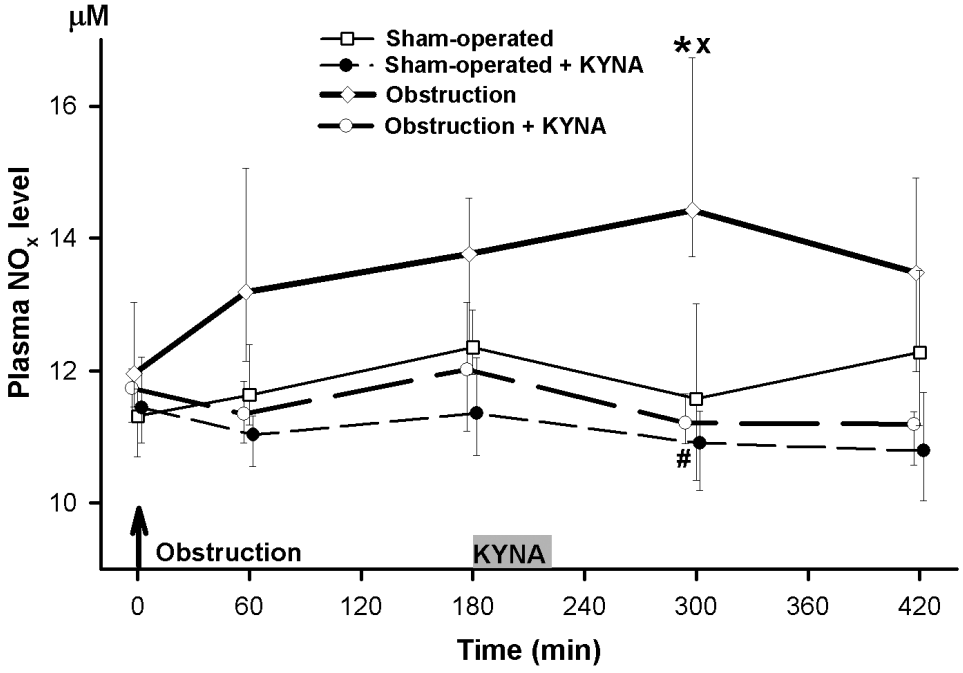

Figure 4. *Changes in plasma NO_X levels in the sham-operated (empty squares), KYNA-treated sham-operated (full circles with dashed line), colon obstruction (empty diamonds), and KYNA-treated obstruction (empty circles with dashed line) groups. The plots demonstrate the median values and the 25th (lower whisker) and 75th (upper whisker) percentiles, * p<0.05 within groups vs baseline values, ^{x} p<0.05 between groups vs sham-operated group values, $^{\#}$ p<0.05 between KYNA-treated group vs obstructed group values.*

Both specific and non-specific NOS inhibitors significantly depressed the increase in plasma NO_X level as compared with the baseline and the obstruction-treated control group (Figure 5).

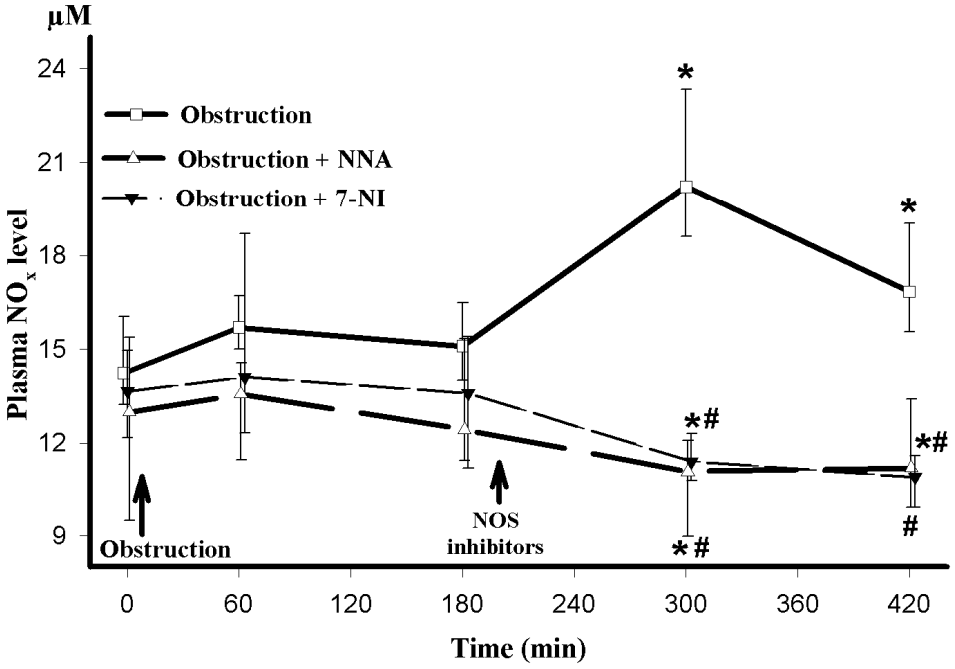

Figure 5. *Changes in plasma NO_X levels in colon obstruction and non-selective NOS inhibitor NNA treatment (empty triangles), or selective nNOS inhibitor 7-NI treatment (full triangles) groups. The plots demonstrate the median values and the 25th (lower whisker) and 75th (upper whisker) percentiles * p<0.05 within groups vs baseline values, ^{x} p<0.05 between groups vs sham-operated group values, $^{\#}$ p<0.05 between NOS inhibitor-treated groups vs obstructed group values.*

4.3. Changes in NOS isoenzyme activity

In the sham-operated group, the cNOS activities differed significantly in the oral and aboral tissue samples (oral cNOS: M=102.9; p75=123.5; p25=69.3; *vs* aboral cNOS: M=62.1; p75=88.2; p25=37.8 fmol (mg protein) $^{-1}$ min^{-1}; p=0.0423). Similarly, the activity of cNOS was significantly higher in the oral bowel segment in the obstructed group (oral cNOS: M=112.6; p75=128; p25=90.4; *vs* aboral cNOS: M=67.1; p75=78.5; p25=62.9 fmol (mg protein) $^{-1}$ min^{-1}; p=0.0143).

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 (Figure 6).

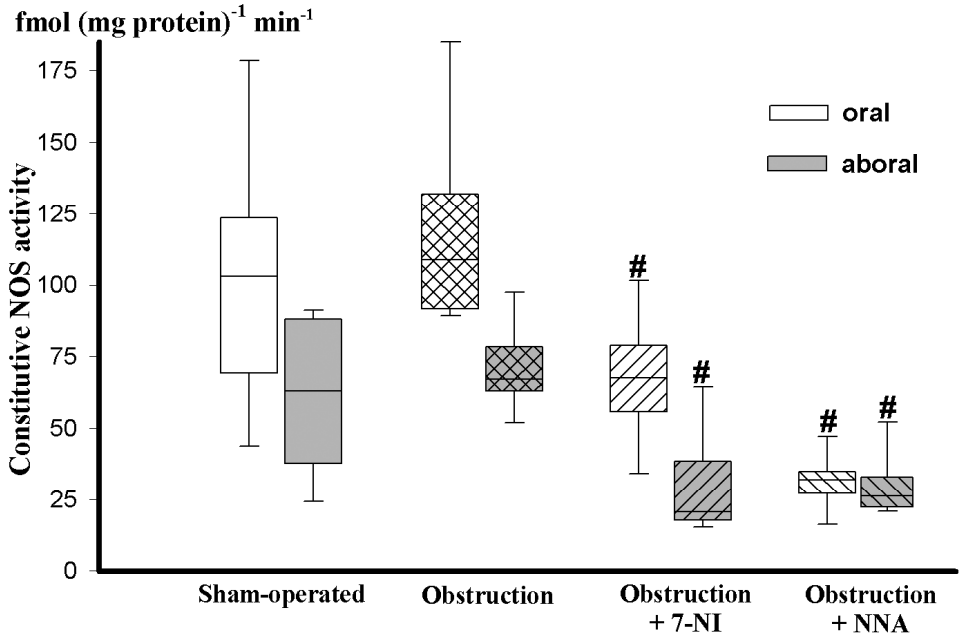

Figure 6. *Changes in cNOS activities orally (white boxes) and aborally (grey boxes) (fmol (mg protein)$^{-1}$ min^{-1}) in colonic tissue from saline-treated sham-operated (empty box), obstruction-treated (checked box), obstruction + 7-NI-treated (left striped box) and obstruction + NNA-treated (right striped box) animals. The plots demonstrate the median (horizontal line in the box) and the 25th (lower whisker), and 75th (upper whisker) percentiles.* $^{\#}$ *p<0.05 between NOS inhibitor-treated groups vs obstructed group values.*

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 (Figure 7).

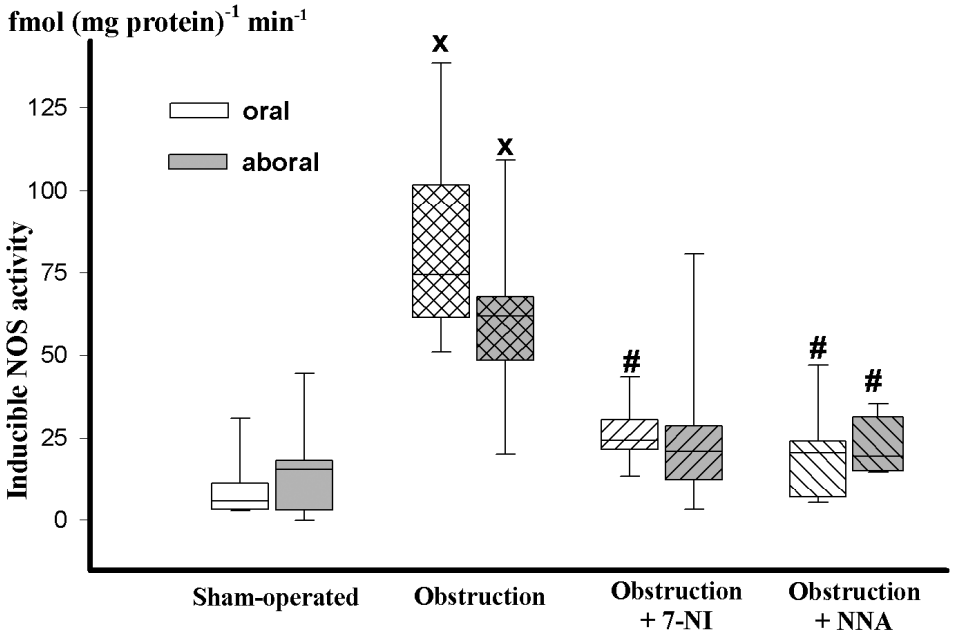

Figure 7. *Changes in iNOS activities orally (white boxes) and aborally (grey boxes) (fmol (mg protein)$^{-1}$ min^{-1}) in colonic tissue from saline-treated sham-operated (empty box), obstruction-treated (checked box), obstruction + 7-NI-treated (left striped box) and obstruction + NNA-treated (right striped box) animals. The plots demonstrate the median (horizontal line in the box) and the 25th (lower whisker), and 75th (upper whisker) percentiles.* ^{x} *$p<0.05$ between groups vs sham-operated group values,* $^{\#}$ *$p<0.05$ between NOS inhibitor-treated groups vs obstructed group values.*

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 (Figure 7).

4.4. 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 (M=3.74; p75=4.612; p25=3.32; *vs* the sham-operated M=0.84; p75=1.22; p25=0.5 µmol (mg protein)$^{-1}$ min^{-1}). The activity of XDH was also elevated

significantly in the obstructed group (M=14.9; p75=19.7; p25=13.5; *vs* the sham-operated M=1.48; p75=6.69; p25=0.95 μmol (mg protein)$^{-1}$ min^{-1}), indirectly indicating an accumulation of hypoxanthine as an end-product of ATP degradation. The nonselective NMDA receptor antagonist treatment therapy significantly inhibited the obstruction-induced increases in the XO and XDH activities (Figure 8).

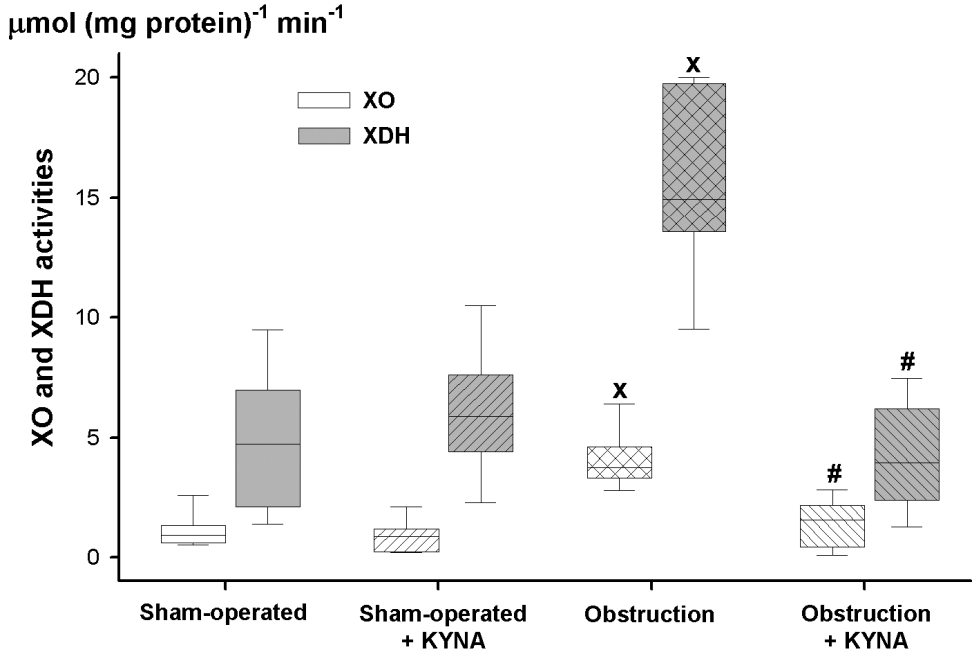

Figure 8. *Changes in activity of XO (white boxes) and XDH (grey boxes) (μmol (mg protein)$^{-1}$ min^{-1}) in colonic tissue from sham-operated (empty box), sham-operated + KYNA-treated (left striped box), obstruction-treated (checked box), and obstruction + KYNA-treated (right striped box) animals. The plots demonstrate the median (horizontal line in the box) and the 25th (lower whisker), and 75th (upper whisker) percentiles.* ^{x} *p<0.05 between groups vs sham-operated group values,* $^{\#}$ *p<0.05 between KYNA-treated groups vs obstructed group values.*

MPO is a marker enzyme of neutrophilic leukocyte accumulation in tissues. Its activity was 373.8 mU (mg protein)$^{-1}$ (p25=255; p75=437) and 426 mU (mg protein)$^{-1}$ (p25=391; p75=502) in the non-treated and KYNA-treated, sham-operated animals, respectively. After obstruction induction, the MPO activity increased significantly in the proximal colon (M=782; p25=615; p75=939). The KYNA treatment induced a significant

decrease in the MPO activity (M=572; p=468; p75=686) of the large bowel as compared with the non-treated obstructed group (Figure 9).

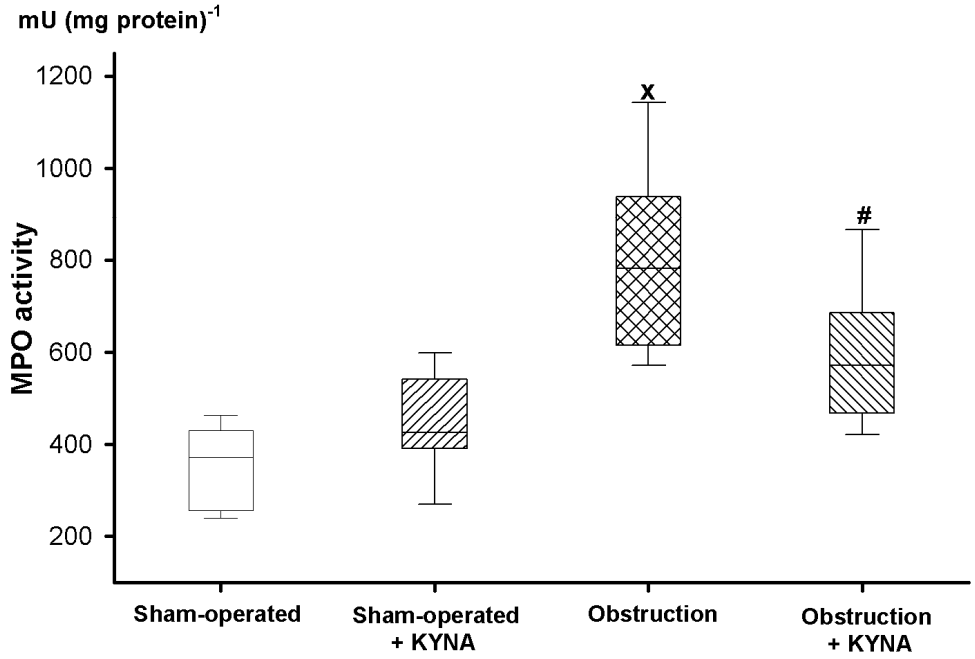

Figure 9. *Changes in activity of MPO (mU (mg protein)$^{-1}$) in colonic tissue from sham-operated (empty box), sham-operated + KYNA-treated (left striped box), obstruction-treated (checked box), and obstruction + KYNA-treated (right striped box) animals. The plots demonstrate the median (horizontal line in the box) and the 25th (lower whisker), and 75th (upper whisker) percentiles.* ^{x} *p<0.05 between groups vs sham-operated group values,* $^{\#}$ *p<0.05 between KYNA-treated groups vs obstructed group values.*

4.5. 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 (Figures 10 and 11).

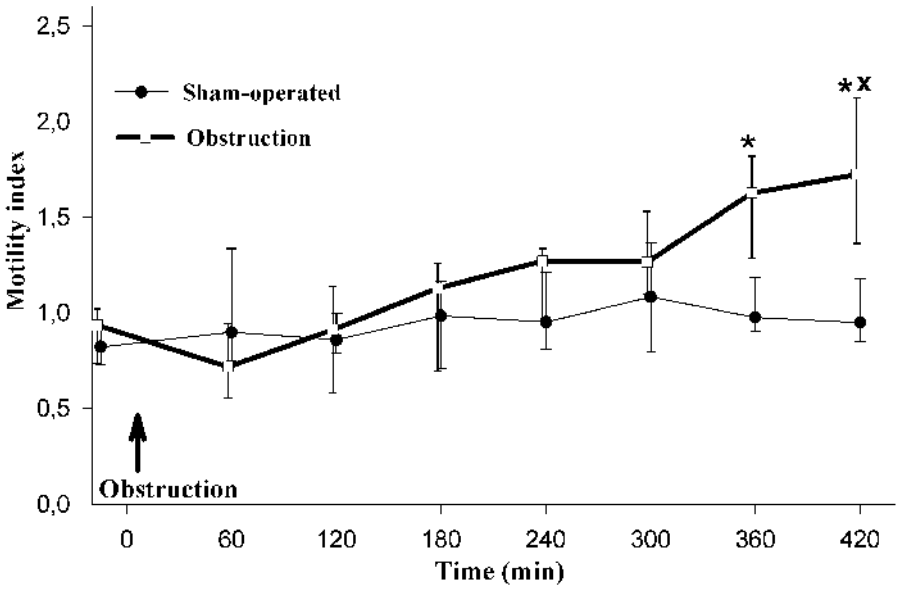

Figure 10. *Changes in motility index of the proximal colon segment, in the sham-operated group (full circles) and during colon obstruction (empty squares). The plots demonstrate the median values and the 25th (lower whisker) and 75th (upper whisker) percentiles * p<0.05 within groups vs baseline values, ^{x} p<0.05 between groups vs sham-operated group values, $^{\#}$ p<0.05 between NOS inhibitor-treated groups vs obstructed group values.*

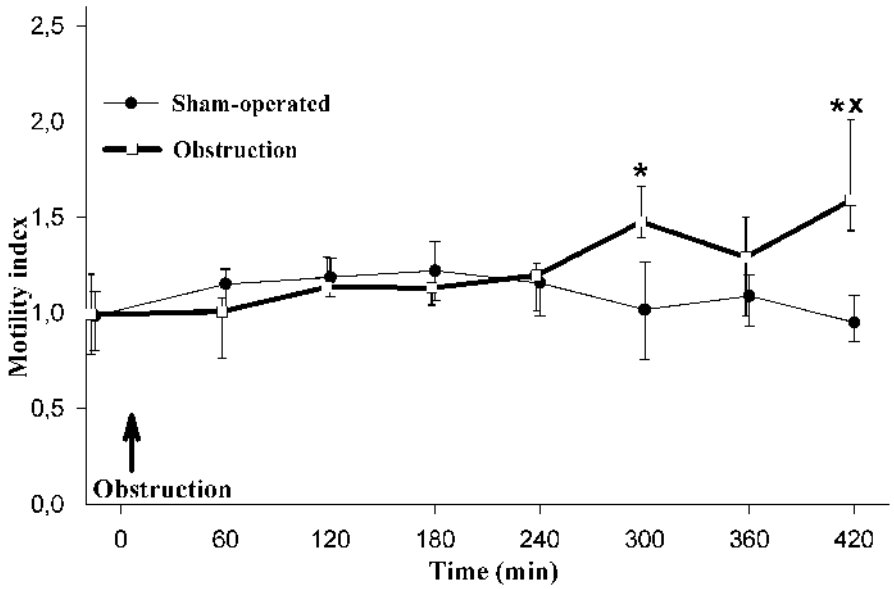

Figure 11. *Changes in motility index of the distal colon segment in the sham-operated group (full circles) and during colon obstruction (empty squares). The plots demonstrate the median values and the 25th (lower whisker) and 75th (upper whisker) percentiles * p<0.05 within groups vs baseline values, ^{x} p<0.05 between groups vs sham-operated group values, $^{\#}$ p<0.05 between NOS inhibitor-treated groups vs obstructed group values.*

This change was significant by the end of the observation period. 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 (Figures 12 and 13).

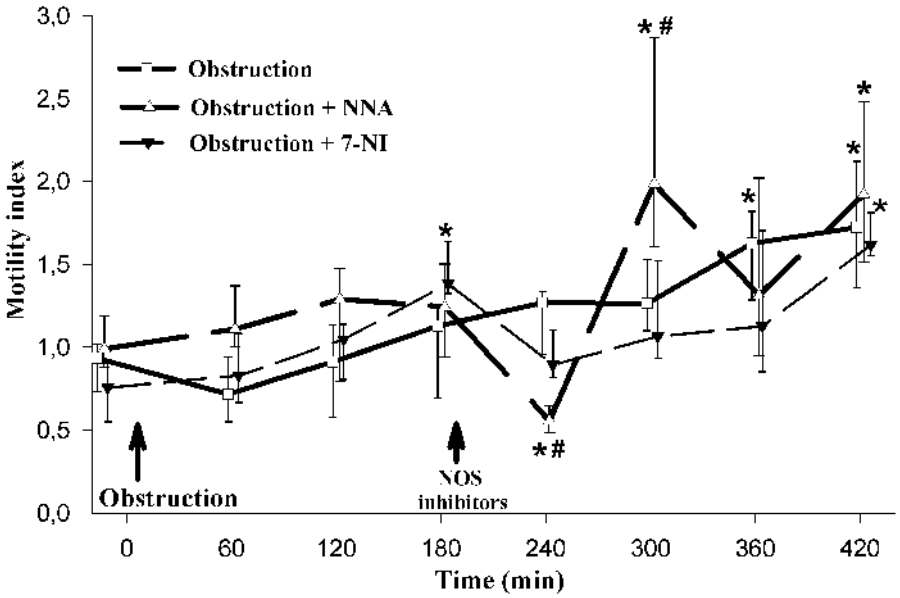

Figure 12. *Changes in motility index of the proximal colon segment, in colon obstruction (empty squares) and non-selective NOS inhibitor NNA treatment (empty triangles), or selective nNOS inhibitor 7-NI treatment (full triangles). The plots demonstrate the median values and the 25th (lower whisker) and 75th (upper whisker) percentiles * p<0.05 within groups vs baseline values, ^{x} p<0.05 between groups vs sham-operated group values, $^{\#}$ p<0.05 between NOS inhibitor-treated groups vs obstructed group values.*

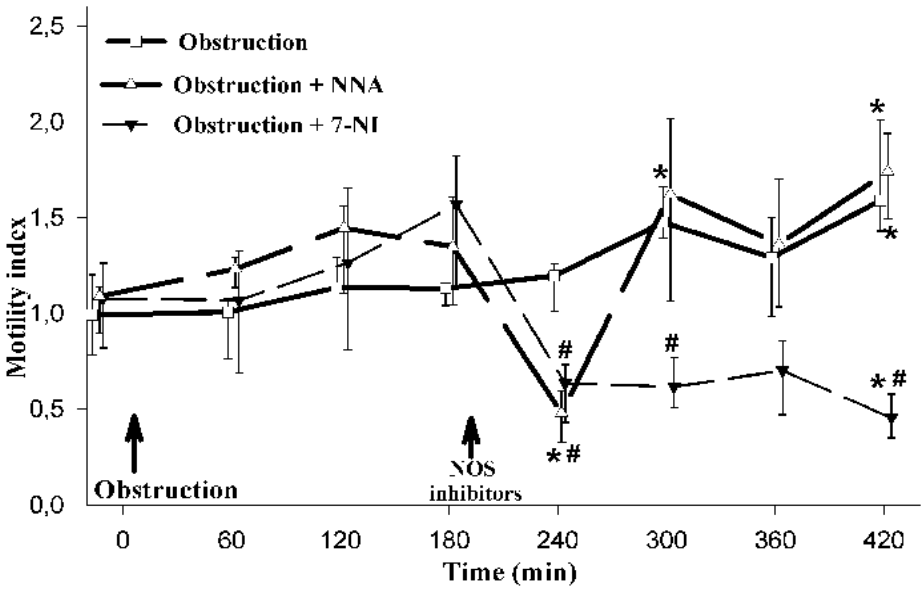

Figure 13. *Changes in motility index of the distal colon segment, in colon obstruction (empty squares), and nonselective NOS inhibitor NNA treatment (empty triangles), or selective nNOS inhibitor 7-NI treatment (full triangles). The plots demonstrate the median values and the 25th (lower whisker) and 75th (upper whisker) percentiles * p<0.05 within groups vs baseline values, ^{x} p<0.05 between groups vs sham-operated group values, $^{\#}$ p<0.05 between NOS inhibitor-treated groups vs obstructed group values.*

The KYNA treatment significantly inhibited the obstruction-induced increase in the motility index and 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 (Figures 14 and 15).

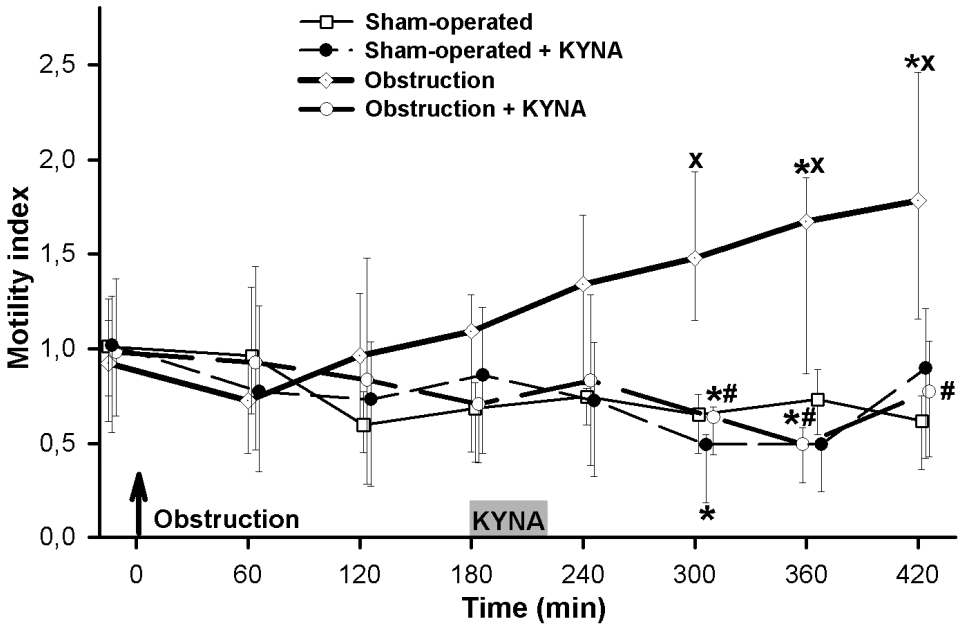

Figure 14. *Changes in motility index of the proximal colon in the sham-operated (empty squares), KYNA-treated sham-operated (full circles with dashed line), colon obstruction (empty diamonds), and KYNA-treated obstruction (empty circles with dashed line) groups. The plots demonstrate the median values and the 25th (lower whisker) and 75th (upper whisker) percentiles, * p<0.05 within groups vs baseline values, ^{x} p<0.05 between groups vs sham-operated group values, $^{\#}$ p<0.05 between KYNA-treated group vs obstructed group values.*

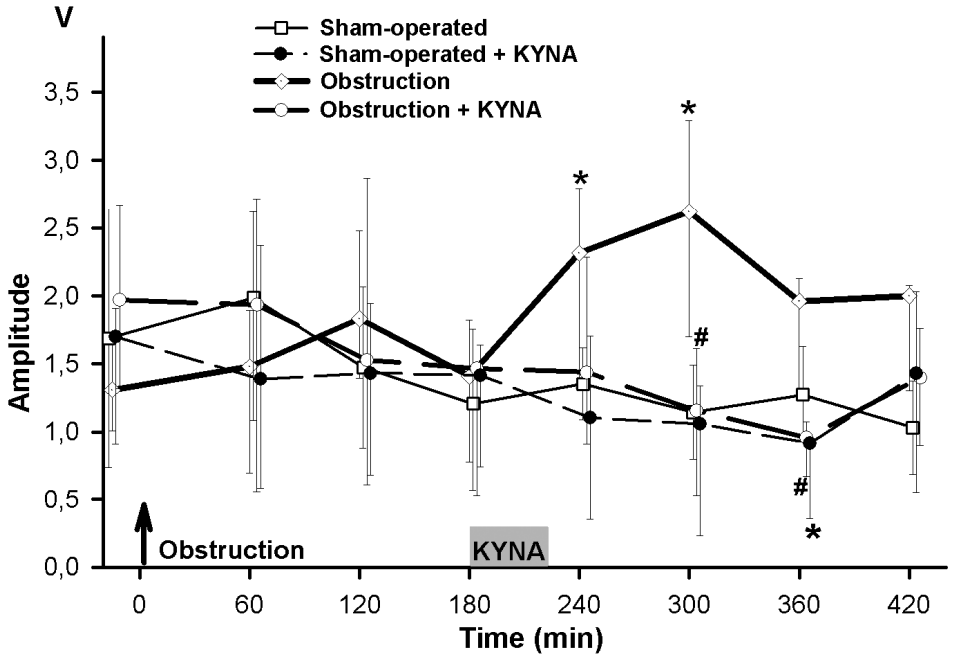

Figure 15. *Changes in amplitude of GMCs of the proximal colon in the sham-operated (empty squares), KYNA-treated sham-operated (full circles with dashed line), colon obstruction (empty diamonds), and KYNA-treated obstruction (empty circles with dashed line) groups. The plots demonstrate the median values and the 25th (lower whisker) and 75th (upper whisker) percentiles, * p<0.05 within groups vs baseline values, ^{x} p<0.05 between groups vs sham-operated group values, $^{\#}$ p<0.05 between KYNA-treated group vs obstructed group values.*

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 (Figure 16).

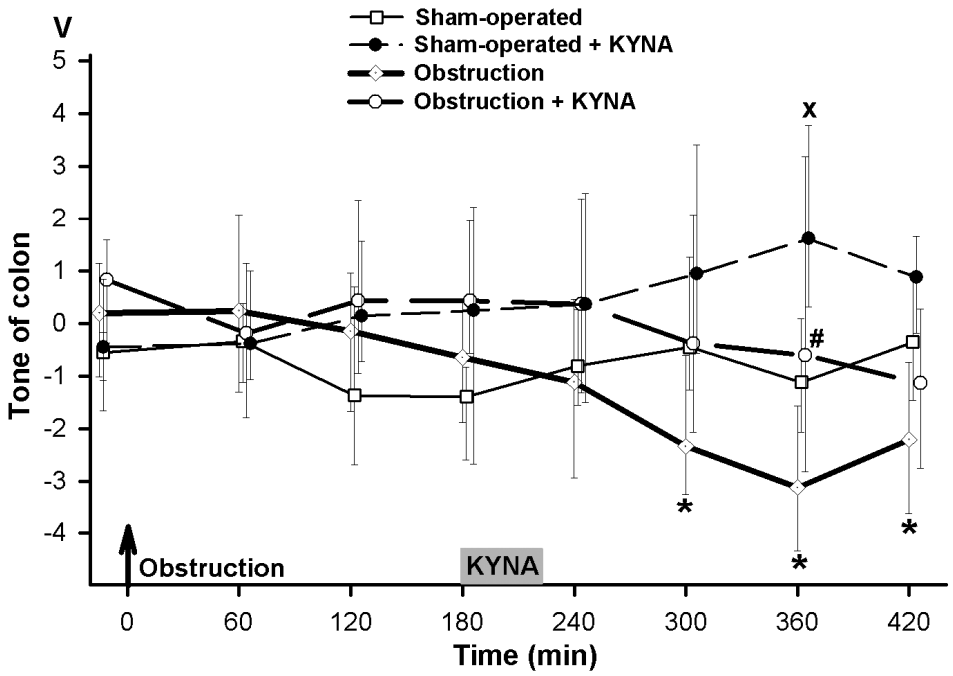

Figure 16. *Changes in tone of the proximal colon in the sham-operated (empty squares), KYNA-treated sham-operated (full circles with dashed line), colon obstruction (empty diamonds), and KYNA-treated obstruction (empty circles with dashed line) groups. The plots demonstrate the median values and the 25th (lower whisker) and 75th (upper whisker) percentiles, * p<0.05 within groups vs baseline values, ^{x} p<0.05 between groups vs sham-operated group values, $^{\#}$ p<0.05 between KYNA-treated group vs obstructed group values.*

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 (Figure 17).

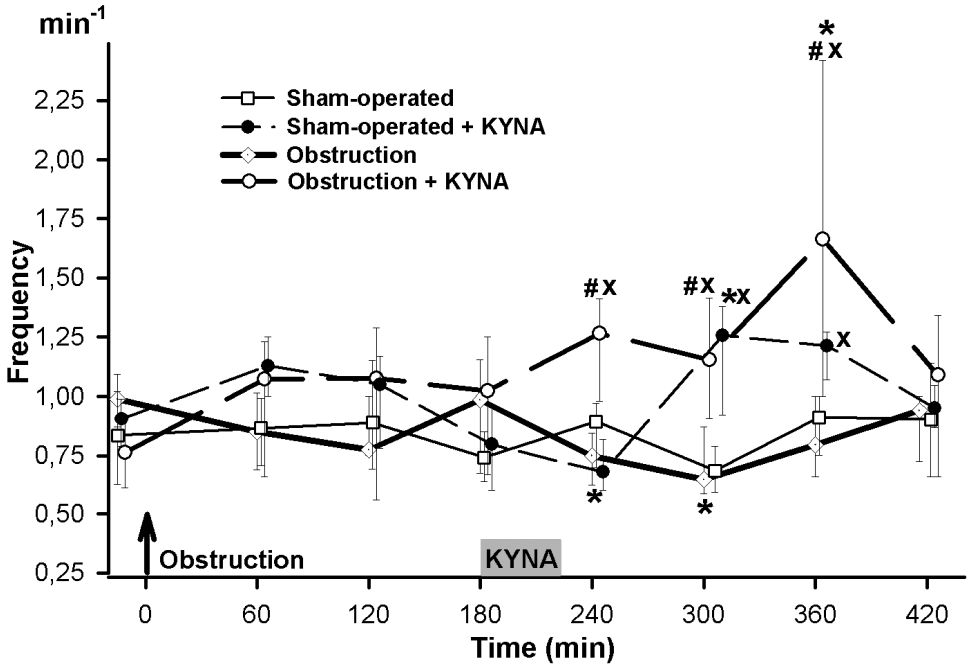

Figure 17. *Changes in frequency of GMCs of the proximal colon in the sham-operated (empty squares), KYNA-treated sham-operated (full circles with dashed line), colon obstruction (empty diamonds), and KYNA-treated obstruction (empty circles with dashed line) groups. The plots demonstrate the median values and the 25th (lower whisker) and 75th (upper whisker) percentiles, * p<0.05 within groups vs baseline values, ^{x} p<0.05 between groups vs sham-operated group values, $^{\#}$ p<0.05 between KYNA-treated group vs obstructed group values.*

5. DISCUSSION

Inflammation can be a significant factor in the development of motility changes in functional bowel disorders (Madl *et al.* 2003, Törnblom *et al.* 2005), but the connection between alterations in intestinal motor function and local inflammatory activation is still unclear. Abdominal surgery causes postoperative GI dysmotility, which can progress to paralytic ileus. Surgery causes inflammatory responses leading to a loss of ICCs, which generate intestinal pacemaker activity (Yanagida *et al.* 2007). In the early phase of bowel obstruction, similarly as mentioned above, an inflammatory process is generated. Our study design allowed us to follow the time course of the obstruction-induced inflammatory and motility changes in the large intestine in the acute phase of mechanical ileus, and to investigate the roles of nitrergic and glutaminergic neurotransmission in this scenario.

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 haemodynamic 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 (Bone *et al.* 1991).

Conflicting data have been reported on inflammation-induced motility alterations. It was recently suggested that, in proinflammatory conditions, the activation of resident macrophages in the tunica muscularis and the upregulation of cytokines may affect the smooth muscle contractility (Won *et al.* 2006). 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 (Kalff *et al.* 2003). 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 (Hellström *et al.* 1997). Indeed, the obstruction-induced motility alterations are time-dependent, characteristically changing in parallel with the development of inflammation. This phenomenon was observed in our earlier study too, when the mechanical intestinal obstruction-induced time-dependent changes in motility patterns were examined in a 36-h period. The motility of the proximal segment increased during the first 8 h, and then gradually decreased in the next 16 h, while the motility of the distal segment increased later. This process was accompanied by a parallel significant increase in cholinergic activation (and an elevated release of acetylcholine) (Kaszaki *et al.* 1987).

In the intact, conscious state, the predominant motor activity of the colon is characterized by the GMCs, which are stimulated by acetylcholine release from cholinergic excitatory neurons (Sethi *et al.* 1991). It has been suggested that the excitatory transmission to the intestinal smooth muscle is predominantly cholinergic in nature (Starke *et al.* 1989), and could be modulated by nonadrenergic noncholinergic (NANC) inhibitory or other facilitatory pathways.

5.1. Role of nitric oxide: Study I

NO is a universal chemical mediator of GI intercellular communication (Salzman *et al.* 1995) and its pathogenic role has been also verified in sepsis and mucosal permeability changes (Moncada *et al.* 1991, Sun *et al.* 2004). 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 (Hellström *et al.* 1997, Kalff *et al.* 2000). Yanagida *et al.* observed that the activity of ICCs and pacemaking was greatly attenuated in the absence of NO derived from iNOS (Yanagida *et al.* 2007). Non-selective NOS inhibitors (such as arginine analogues) 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 (Klabunde *et al.* 1991, Kilbourn *et al.* 1992). 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 haemodynamic changes.

The relative weight of NOS in the obstruction-induced motility dysfunction is less clear. In our study, there was significant difference between the activities of the cNOS isoenzymes in the different large bowel segments in the sham-operated group. The continuous or constitutive synthesis of NO in the intestinal tract is mainly ensured by nNOS (Qu *et al.* 1999), but both known cNOS isoforms are present in the myenteric neurons of the colon. Determination of their exact activity is limited by the fact that both eNOS and nNOS are Ca^{2+}-dependent, and at present these isoenzymes 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 (Moore *et al*. 1996). 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. Nevertheless, the 7-h colonic obstruction was followed by an enhanced iNOS activity.

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 (Sun *et al.* 2004) and intestinal motility in both the small and large intestines (Mizuta *et al.*1999). On the basis of this observation, the inhibitory role of NO in the

regulation of intestinal motility is anticipated. Indeed, it is now generally accepted that NO is a neurotransmitter which mediates relaxation (Bult *et al.*1990, Dalziel *et al.* 1991, Ward *et al.* 1992, Boeckstaens *et al.* 1993, Shuttleworth *et al.* 1993). 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. We may assume that this event was not triggered by the lack of relaxation-mediating NO only, but also by a mediator predominance that enhanced smooth muscle constriction. Indeed, this phenomenon was earlier described as a side-effect of NOS inhibition (Richard *et al.* 1995). Similarly, when Ohta *et al.* compared the *in vivo* effects of different routes of NNA administration, intravenous NNA infusion resulted in increased peristalsis, while intra-cerebroventricularly administered NOS-inhibitor therapy suppressed the motility of the colon (Ohta *et al.* 1996). 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 (Bartho *et al.* 1995). 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 (Holzer *et al.*1997). Our results confirm that this process mainly involves nNOS-derived NO, as decreased colon motility was demonstrated after selective nNOS inhibition (Heinemann *et al.*1999).

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 (Takahashi *et al.* 1998). Our results confirm this observation, because the cNOS activity was significantly higher orally in the sham-operated group and in the animals with colon obstruction, too. Moreover, the rich oral nitrergic innervation can not be inhibited by a given amount of nNOS inhibitor as effectively as the distal part with its poorer innervation. 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 (Mancinelli *et al.* 2001).

These data suggest that NO may play a rather complex role in the regulation of the motility of the obstructed colon.

- 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.
- 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.
- 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.

5.2. Role of glutamate: Study II

Glutamate or its endogenous receptor agonists/antagonists may participate in the modulation of the enteric cholinergic function, since activation of the NMDA receptors enhances acetylcholine release from the myenteric neurons in the ileum and colon (Wiley *et al.* 1991). Besides being one of the main excitatory transmitters in the CNS, glutamate can act either as a neurotransmitter in the peripheral nervous system or at least as a modulator of classical transmitter systems (Liu *et al.* 1995, Sinsky *et al.* 1998, Kirchgessner *et al.* 2001). In particular, there is now evidence for glutamate release from neurons and the presence of glutamate receptors in the intestines in non-human species (Ren *et al.* 1999), and receptors of the NMDA subtype in the myenteric plexus (Moroni *et al.* 1986). This subtype is preferentially activated by quinolinic acid and blocked by KYNA (Stone *et al.* 1982, 2001, 2003). These data therefore indicated that NMDA subtype receptors play a role in the gut motility, and activation by glutamate could increase the contractile activity. Our results have revealed that glutamatergic facilitation does indeed take part in an obstruction-induced increase in colon motility.

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 (Stone *et al.* 2001, Mackay *et al.* 2003). 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 (Forrest *et al.* 2002, 2003). In pathological conditions (infections, ischaemia or traumatic brain injury), dramatic increases in quinolinic acid concentrations have been demonstrated (Stone *et al.* 2001). 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 (Rios *et al.*1991).

Glutamate neurotoxicity (necrosis and apoptosis) has been observed in a subset of enteric neurons in both intact bowel preparations and cultured myenteric ganglia (Kirchgessner *et al.* 1997). Taken together, 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 (Kiss *et al.* 2005). 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 (Sas *et al.* 2003), which can be blocked by atropine or a NOS inhibitor. The systemic administration of L-kynurenine dose-dependently, but not selectively, elevates the level of KYNA in the brain. 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.

There is a close relationship between amelioration of the capillary blood flow and a decrease in the leukocyte-endothelial interaction in the intestines (Wolfárd *et al.* 2002). The MPO activity is a quantitative marker of the leukocytes accumulated in the tissue (Kuebler *et al.* 1996). A decreased MPO activity was found in the obstructed colon segment following KYNA treatment, and this could be related to the partial elimination of XOR-dependent oxygen radical production.

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 (Tong *et al.* 2003). 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. On this basis, it should be mentioned that an increase in the intestinal wall tension could stimulate acetylcholine release (Tong *et al.* 2003).

- Our results demonstrate an important role for glutamate receptors in the pathophysiology of acute colon obstruction-induced motility changes.
- These findings reveal that KYNA not only significantly inhibits the contraction of the GMCs in the colon, but also exerts a protective, anti-inflammatory effect due to the indirect inhibition of oxygen radical production and leukocyte activation.

To summarize the results of our experiments (Study I and Study II), 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, besides the cholinergic neurotransmission 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 (Vizi *et al. 2001*). The ICCs play a critical role in the reception and transduction of

excitatory and inhibitory neurotransmission (Ward *et al.* 2006). The synthesized NO, as a soluble transmitter of the ICCs easily penetrates biological membranes and conducts or mediates the stimuli to the neighboring 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.

6. REFERENCES

Bartho L, Lefebvre RA. Nitric oxide-mediated contraction in enteric smooth muscle. *Arch Int Pharmacodyn Ther* 1995; 329:53-66.

Bauer AJ, Schwarz NT, Moore BA, Turler A, Kalff JC. Ileus in critical illness: mechanisms and management. *Curr Opin Crit* Care 2002; 8:152-157.

Beckman JS, Parks DA, Pearson JD, Marshall PA, Freeman BA: A sensitive fluorometric assay for measuring xanthine dehydrogenase and oxidase in tissues. *Free Rad Biol Med* 1989; 6: 607-615.

Boeckstaens GE, Pelckmans PA, Herman AG, Maercke YM. Involvement of nitric oxide in the inhibitory innervation of the human isolated colon. *Gastroenterology* 1993; 104:690-697.

Bone RC. The pathogenesis of sepsis. *Ann Intern Med* 1991; 115:457-469.

Bult H, Boeckxstaens GE, Pelckmans PA, Jordaens FH, Van Maercke YM, Herman AG. Nitric oxide as an inhibitory non-adrenergic non-cholinergic neurotransmitter. *Nature* 1990; 345:346-347.

Burns AJ, Lomax AEJ, Torihashi S, Sanders KM, Ward SM. Interstitial cells of Cajal mediate inhibitory neurotransmission in the stomach. *Proc Natl Acad Sci* 1996; 93:12008–12013.

Cortesini C, Cianchi F, Infanto A, Lise M.: Nitric oxidesynthase and VIP distribution in enteric nervous system in idiopathic chronic constipation. *Dig Dis Sci* 1995, 40:2450-2455.

Cowles VE, Condon RE, Schulte WJ, Woods JH, Silin LF. A quarter Wheatstone bridge strain gauge force transducer for recording gut motility. *Am J Dig Dis* 1978; 23:936-942.

Curtis DR, Phyllis JW, Watkins JC, Chemical excitation of spinal neurones. *Nature* 1959; 183:611–612.

Dalziel HH, Thornbury KD, Ward SM, Sanders KM. Involvement of nitric oxide synthetic pathway in inhibitory junction potentials in canine proximal colon. *Am J Physiol* 1991; 260:G789-G792.

Ellis H.: The clinical significance of adhesions: focus on intestinal obstruction. *Eur J Surg* 1997, 577:5-9.

Forrest CM, Youd P, Kennedy A, Gould SR, Darlington LG, Stone TW. Purine, kynurenine, neopterin and lipid peroxidation levels in inflammatory bowel disease. *J Biomed Sci* 2002; 9: 436-442.

Forrest CM, Gould SR, Darlington LG, Stone TW. Levels of purine, kynurenine and lipid peroxidation products in patients with inflammatory bowel disease. *Adv Exp Med Biol* 2003; 527:395-400.

Green LC, Wagner DA., Glogowski J, Skipper PL, Wishnok JS, Tannenbaum SR. Analysis of nitrate, nitrite and [^{15}N]nitrate in biological fluids. *Anal Biochem* 1982; 126:131-138.

Heinemann A, Holzer P. Intestinal motor depression by 7-nitroindazole through an action unrelated to nitric oxide synthase inhibition. *Pharmacology* 1999; 59:310-320.

Hellström PM, Al-Saffar A, Ljung T, Theodorsson E: Endotoxin actions on myoelectric activity, transit, and neuropeptides in the gut. Role of nitric oxide. *Dig Dis Sci* 1997; 42:1640-1651.

Holzer P, Lippe ITH, Tabrizi AL, Lénárd L, Barthó L. Dual excitatory and inhibitory effect of nitric oxide on peristalsis in the guinea pig intestine. *J Pharmacol Exp Ther* 1997; 280:54-161.

Huge A, Kreis ME, Jehle EC, Ehrlein HJ, Starlinger M, Becker HD, Zittel TT. A model to investigate postoperative ileus with strain gauge transducers in awake rats. *J Surg Res* 1998; 74:112-118.

Kalff JC, Schraut WH, Billiar TR, Simmons RL, Bauer AJ. Role of inducible nitric oxide synthase in postoperative intestinal smooth muscle dysfunction in rodents. *Gastroenterology* 2000; 118:316-327.

Kalff JC, Turler A, Schwarz NT, Schraut WH, Lee KK, Tweardy DJ, Billiar TR, Simmons RL, Bauer AJ. Intra-abdominal activation of a local inflammatory response within the human muscularis externa during laparotomy. *Ann Surg* 2003; 237:301-315.

Kaszaki J, Budai D, Őry Z, Nagy S, Petri G. Examination of cholinerg mechanisms in experimental mechanical ileus. *Kísérletes Orvostudomány* 1987; 3:302-310.

Kilbourn RG, Griffith OW. Overproduction of nitric oxide in cytokine-mediated and septic shock. *J Natl Cancer Inst* 1992; 84:827-831.

Kirchgessner AL. Glutamate in the enteric nervous system. *Curr Opin Pharmacol* 2001; 1:591-596.

Kirchgessner AL, Liu MT, Alcantara F. Excitotoxicity in the enteric nervous system. *J Neurosci* 1997; 17:8804-8816.

Kiss C, Vécsei L. Neuroprotection and the kynurenine system. In: Kynurenines in the brain: from experiment to clinics. (ed) Vécsei L; *Nova Sciences Publishers*, New York, 2005; pp.173-191.

Kiss JP, Vizi ES: Nitric oxide: a novel link between synaptic and nonsynaptic transmission. *Trends Neurosci* 2001; 4:211-215.

Klabunde RE, Ritger RC, Helgren MC.: Cardiovascular actions of inhibitors of endothelium-derived relaxing factor (nitric oxide) formation/release in anesthetized dogs. *Eur J Pharmacol* 1991;199:51-59.

Klivényi P, Toldi J, Vécsei L.: Kynurenines in neurodegenerative disorders: therapeutic consideration. *Adv Exp Med Biol* 2004; 541:169-183.

Kuebler WM, Abels C, Schuerer L, Goetz AE: Measurement of neutrophil content in brain and lung tissue by a modified myeloperoxidase assay. *Int J Microcirc* 1996; 16: 89-97.

Lee HT, Hennig GW, Fleming NW, Keef KD, Spencer NJ, Ward SM, Sanders KM, Smith TK.: Septal interstitial cells of Cajal conduct pacemaker activity to excite muscle bundles in human jejunum. *Gastroenterology* 2007; 3:907-917.

Liu MT, Rothstein JD, Gershon MD, Kirchgessner A: Glutamatergic enteric neurons. *J Neurosci* 1997; 17:4764-4784.

Mackay GM, Forrest CM, Stoy N, Christofides J, Egerton M, Stone TW, Darlington LG: Tryptophan metabolism and oxidative stress in patients with chronic brain injury. *Eur J Neurol* 2006; 13:30-42.

Madl C, Druml W. Systemic consequences of ileus. *Best Pract Res Clin Gastroenterol* 2003; 17:445-456.

Mancinelli R, Fabrizi A, Vargiu R, Morrone L, Bagetta G, Azzena GB. Functional role of inducible nitric oxide synthase on mouse colonic motility. *Neurosci Lett* 2001; 311:101-104.

29. Mizuta Y, Takahashi T, Owyang C. Nitrergic regulation of colonic transit in rats. *Am J Physiol* 1999; 277:G275-G279.

Marzinzig M, Nussler AK, Stadler J, Marzinzig E, Barthlen W, Nussler NC, Beger HG, Morris M Jr, Brückner UB: Improved methods to measure end products of nitric oxide in biological fluids: nitrite, nitrate, and S-nitrosothiols. *Nitric oxide: Biology and Chemistry 1* 1997, 177-189.

Mitolo-Chieppa D, Mansi G, Rinaldi R, Montagnani M, Potenza MA, Genualdo M, Serio M, Mitolo CI, Rinaldi M, Altomare DF, Memeo V: Cholinerg stimulation and nonadrenerg, noncholinerg relaxation of human colonic circular muscle in idiopathic chronic constipation. *Dig Dis Sci* 1998, 43:2719-2726.

Mizuta Y, Takahashi T, Owyang C: Nitrergic regulation of colonic transit in rats. *Am J Physiol* 1999, 277:G275-G279.

Moncada S, Palmer RMJ, Higgs EA. Nitric oxide: physiology, pathophysiology and pharmacology. *Pharmacol Rev* 1991; 43:109-142.

Moroni F, Luzzi S, Franchi-Micheli S, Ziletti L. The presence of N-methyl-D-aspartate-type receptors for glutamic acid in the guinea pig myenteric plexus. *Neurosci Lett* 1986; 68:57-62.

Moshage H, Kok B, Huizenga JR, Jansen PL. Nitrite and nitrate determinations in plasma: a critical evaluation. *Clin Chem* 1995; 41:892-896.

Moore PK, Bland-Ward PA. 7-Nitroindazole: an inhibitor of nitric oxide synthase. *Methods Enzymol* 1996; 268:393-398.

Németh H, Toldi J, Vécsei L.: Role of kynurenines in the central and peripheral nervous systems. *Curr Neurovasc Res* 2005; 3:249-260. Review.

Ohta D, Sarna SK, Condon RE, Lang IM. Inhibition of nitric oxide synthase in the brain suppresses colonic motor activity. *Am J Physiol* 1996; 70:G717-G724.

Papachristodoulou A, Zografos G, Markopoulos C, Fotiadis C, Gogas J, Sechas M, Skalkeas G: Obstructive colonic cancer *J R Coll Surg Edinb* 1993, 38:296-298.

Perkins MN, Stone TW. An iontophoretic investigation of the action of convulsant kynurenines and their interaction with the endogenous excitant kynurenic acid. *Brain Res* 1982; 247:184-187.

Petri G, Porszasz J. Peristalisis and sympathetic activity. *Lancet* 1967; 2(7531):1420-1

Petri G, Pórszász J, Szenohradszky J. Pathogenesis and a new therapy of "paralytic ileus" *Langenbecks Arch Chir* 1968; 322:441-5.

Petri G, Szenohradszky J, Porszasz-Gibiszer K. Sympatholytic treatment of "paralytic" ileus. *Surgery* 1971; 3:359-67.

Qu XW, Wang H, Rozenfeld RA, Huang W, Hsueh W. Type I nitric oxide synthase (NOS) is the predominant NOS in rat small intestine. Regulation by platelet-activating factor. *Biochim Biophys Acta* 1999; 1451:211-217.

Qu XW, Wang H, De Plaen IG, Rozenfeld RA, Hsueh W: Neuronal nitric oxide synthase (NOS) regulates the expression of inducible NOS in rat small intestine via modulation of nuclear factor kappa B. *FASEB J* 2001, 15:439-446.

Ren J, Hu H-Z, Liu S, Wood JD. Glutamate modulates transmission in the submucosal plexus of guinea-pig small intestine. *Neuroreport* 1999; 10:3045–3048.

Richard V, Hogie M, Clozel M, Löffler B-M, Thuillez C. In vivo evidence of an endothelin-induced vasopressor tone after inhibition of nitric oxide synthesis in rats. *Circulation* 1995; 91:771-775.

Rios C, Santamaria A. Quinolinic acid is a potent lipid peroxidant in rat brain homogenates. *Neurochem Res* 1991; 16:1139-1143.

Richard V, Hogie M, Clozel M, Löffler B-M, Thuillez C: In vivo evidence of an endothelin-induced vasopressor tone after inhibition of nitric oxide synthesis is rats. *Circulation* 1995, 91:771-775.

Salzman AL. Nitric oxide in the gut. *New Horiz* 1995; 3:352-364.

Sarna SK. Physiology and pathophysiology of colonic motor activity (1). *Dig Dis Sci* 1991; 36:827-862.

Sarna SK. Physiology and pathophysiology of colonic motor activity (2). *Dig Dis Sci* 1991; 36:998-1018.

Sas K, Csete K, Vécsei L, Papp JG. Effect of systemic administration of L-kynurenine on corticocerebral blood flow under normal and ischemic conditions of a brain in conscious rabbit. *J Cardiovasc Pharmacol* 2003; 42:403-409.

Sethi AK, Sarna SK. Colonic motor activity in acute colitis in conscious dogs. *Gastroenterology* 1991; 100: 954–963.

Shuttleworth CW, Sanders KM, Keef KD. Inhibition of nitric oxide reveals non-cholinergic excitatory neurotransmission in the canine proximal colon. *Br J Pharmacol* 1993; 109:739-747.

Sinsky M, Donnerer J. Evidence for a neurotransmitter role of glutamate in guinea pig myenteric neurons. *Neurosci Lett* 1998; 258:109-112.

Smith TK, Reed JB, Sanders KM. Origin and propagation of electrical slow waves in circular muscle of canine proximal colon. *Am J Physiol* 1987;252(2 Pt 1):C215-24.

Sun Y, Fihn B-M, Jodal M, Sjövall H: Inhibition of nitric oxide synthesis potentiates the colonic permeability increase triggered by luminal bile acids. *Acta Physiol Scand* 2004; 180:167–175.

Starke K, Göthert M, Kilbinger H: Modulation of neurotransmitter release by presynaptic autoreceptors. *Physiol Rev* 1989; 69:864-989.

Stone TW. Cell-membrane receptors for purines. Review. *Biosci Rep* 1982; 2:77-90.

Stone TW. Kynurenines in the CNS: from endogenous obscurity to therapeutic importance. *Progress in Neurobiology* 2001; 64:185–218.

Stone TW, Mackay GM, Forrest CM, Clark CJ, Darlington LG. Tryptophan metabolites and brain disorders. *Clin Chem Lab Med* 2003; 41:852-859.

Szabo C, Wu CC, Mitchell JA, Gross SS, Thiemermann C, Vane JR: Platelet-activating factor contributes to the induction of nitric oxide synthase by bacterial lipopolysaccharide. *Circ Res* 1993; 73:991-999.

Takahashi T, Owyang C. Regional differences in the nitrergic innervation between the proximal and distal colon in rats. *Gastroenterology* 1998; 115:1504-1512.

Tong Q, Kirchgessner AL. Localization and function of metabotropic glutamate receptor 8 in the enteric nervous system. *Am J Physiol Gastrointest Liver Physiol* 2003; 285:G992-G1003.

Törnblom H, Abrahamsson H, Barbara G, Hellström Pm, Lindberg G, Nyhlin H, Ohlsson B, Simrén M, Sjölund K, Sjövall, H, Schmidt PT, Öhman L: Inflammation as a cause of functional bowel disorders. *Scand J Gastroenterol* 2005; 40:1140-1148.

Türler A, Moore BA, Pezzone MA, Overhaus M, Kalff JC, Bauer AJ. Colonic postoperative inflammatory ileus in the rat. *Ann Surg* 2002; 236:56-66.

Vanderwilden JM, De Laet HM, Schiffmann SN, Mailleux P, Lowenstein CJ, Snyder SH, Vanderhaegen JJ: Nitric oxide synthase distribution in the enteric nervous system of Hirschsprung's disease. *Gastroenterology* 1993; 105:969-973.

Vécsei L, Miller J, Macgarvey U, Beal MF. Effects of kynurenine and probenecid on plasma and brain tissue concentrations of kynurenic acid. *Neurodegeneration* 1992; 1:17–26.

Vécsei L, Schwab F.: Kynurenine and its metabolites in nervous system diseases. *Orv Hetil*. 1992; 133(29):1803-7.

Ward SM, Dalziel HH, Thornbury KD, Westfall DP, Sanders KM. Nonadrenergic, noncholinergic inhibition and rebound excitation in canine colon depend on nitric oxide. *Am J Physiol* 1992; 262:G237-G243.

Ward SM, Beckett EAH, Wang X-Y, Baker F, Khoyi M, Sanders KM. Interstitial cells of Cajal mediate cholinergic neurotransmission from enteric motor neurons. *J Neurosci.* 2000; 20:1393–1403.

Ward SM, Sanders KM.: Involvement of intramuscular interstitial cells of Cajal in neuroeffector transmission in the gastrointestinal tract. *J Physiol* 2006; 576(Pt 3):675-82.

Wiley JW, Lu Y, Owyang C: Evidence for a glutamatergic neural pathway in the myenteric plexus. *Am J Physiol* 1991; 261:G693-G700.

Weinberg RJ: Glutamate: An excitatory neurotransmitter in the mammalian CNS. *Brain Res Bull* 1999; 50(5-6):353-354.

Wolfárd A, Kaszaki J, Szabó C, Szalay L, Nagy S, Boros M. Prevention of early myocardial depression in hyperdynamic endotoxemia in dogs. *Shock* 2000; 13:46-51.

Wolfárd A, Szalay L, Kaszaki J, Sahin-Tóth G, Vangel R, Balogh Á, Boros M. Dynamic in vivo observation of villius microcirculation during small bowel autotransplantation: effects of endothelin-A receptor inhibition. *Transplantation* 2002; 73:1511-1514.

Won KJ, Suzuki T, Hori M, Ozaki H. Motility disorder in experimentally obstructed intestine: relationship between muscularis inflammation and disruption of the ICC network. *Neurogastroenterol Motil* 2006; 18:53-61.

Yanagida H, Sanders KM, Ward SM.: Inactivation of inducible nitric oxide synthase protects intestinal pacemaker cells from postoperative damage. *J Physiol* 2007; 582(Pt 2):755-65.

Yanagida H, Yanase H, Sanders KM, Ward SM.: Intestinal surgical resection disrupts electrical rhythmicity, neural responses, and interstitial cell networks. *Gastroenterology* 2004; 127(6):1748-59.

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7. ANNEX