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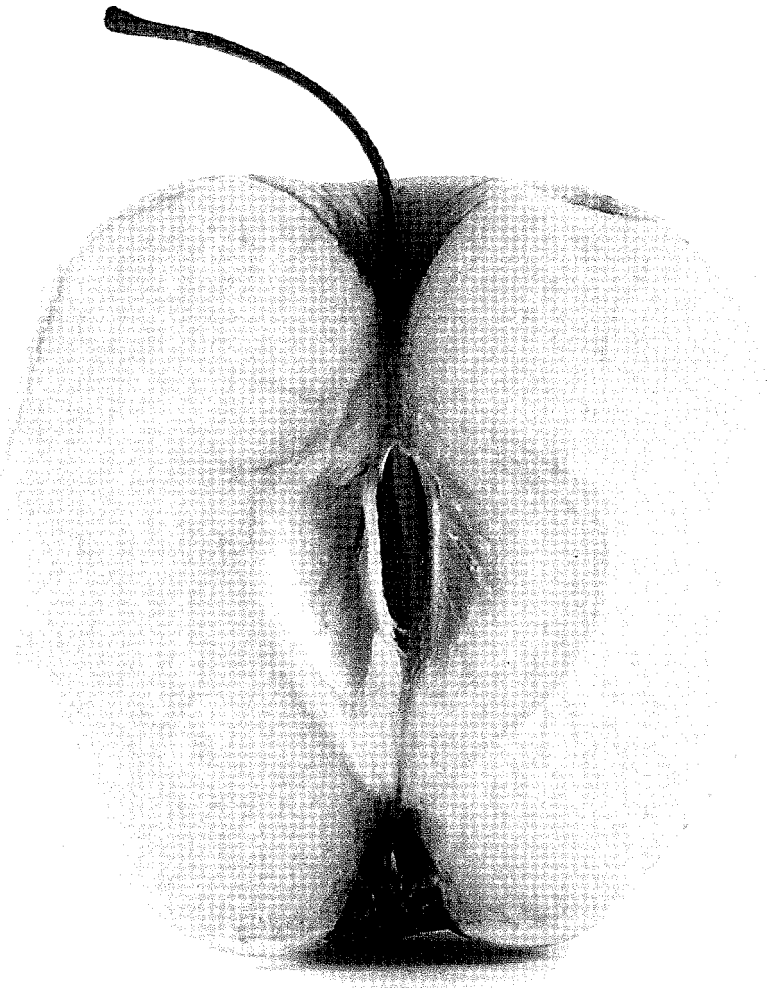
FUNCTIONAL AND MOTILITY DISORDERS OF THE GASTROINTESTINAL TRACT



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N-METHYL-D-ASPARTATE RECEPTOR ANTAGONIST THERAPY IN EXPERIMENTAL COLITIS

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Colitis, idiopathic bowel diseases (IBDs)

Patients with chronic IBDs commonly present with intestinal motility complications. When the gut is inflamed, breakdown of the intestinal barrier function, abnormal secretion and changes in the patterns of motility and visceral sensation all contribute to symptom generation (Lakhan 2010). Inflammation affects not only the mucosa, but extends to the deeper layers of the intestinal wall, causing alterations in nitrergic (Tomita 1998), adrenergic and glutamatergic neurotransmission (Zhou 2006, Kaszaki 2011) and in smooth muscle contractility. The experimental data suggest that inflammation, even if mild, could lead to persistent changes in gastrointestinal (GI) function, resulting in colonic dysmotility and hypersensitivity, even when the preceding infection is restricted to the proximal small intestine. The roles of gaseous and humoral mediators, including nitric oxide (NO), tumor necrosis factor-alpha (TNF-α) and other cytokines have been widely investigated in this condition (Neurath 1997; Tomita 1998; Lindsay 2002). Moreover, it has been revealed that inflammatory alterations in gut function are accompanied by significant changes in activity of the glutamate receptors, which have important roles in the intrinsic neuronal control of GI motility (Kirchgessner 2001).

Glutamate in the enteral nervous system (ENS)

Glutamate immunoreactivity was first detected in subsets of submucosal and myenteric neurones in the guinea-pig ileum (Moroni 1986). It was subsequently shown that glutamate is selectively concentrated in terminal axonal vesicles and can be released after an appropriate stimulus, and functional studies demonstrated the significance of glutamate in the modulation of motor and secretory functions in the gut (Wiley 1991). Glutamate neurotoxicity (necrosis and apoptosis) was later observed in enteric neurons in both intact bowel preparations and cultured myenteric ganglia

(Kirchgessner 2001). It has therefore been suggested that excitotoxicity and overactivation of the enteric glutamate receptors may contribute to inflammation-induced intestinal damage.

N-Methyl-D-aspartate (NMDA) receptors: motility regulation and inflammation. The receptors that mediate the glutamate signal are divided into two main classes, which can be subdivided according to the pharmacological agents capable of specifically activating the appropriate receptor. The ionotropic receptors, such as the kainate, alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid and NMDA receptors, are ligand-gated ion channels, while the metabotropic receptors are coupled to G-proteins. Ionotropic NMDA-sensitive glutamate receptors are present and abundantly expressed on enteric cholinergic neurons (Moroni 1986; Wiley 1991).The GI motility response basically depends on the outcome of myenteric plexus activation, and the net effect combines contractile and relaxant signals. The participation of peripheral NMDA glutamate receptors in this mechanism is of special interest, but the exact mediatory pathways and the neurotransmitters released concomitantly have not yet been fully elucidated. Recent studies suggest that hyperalgesia in colitis is caused by the enhanced activity of NMDA receptors in the spinal nociceptive pathways and on the peripheral (Zhou 2006) and central ends of primary afferent neurons (Li 2006). More importantly, other *in vivo* data have indicated that the expression of NMDA receptors is enhanced in peripheral inflammatory reactions (Tan 2008) and receptor up-regulation occurs on the neurons of the myenteric plexus in TNBS-induced colitis too (Zhou 2006).

It has been postulated that nitrergic neurotransmission, one of the forms of intrinsic innervation, mediates peristaltic waves, inhibitory responses or relaxing mechanisms of the GI tract (Ekblad 1994, Kohjitani 2005). Our earlier data led to the conclusion that NO may play a complex role in the regulation of the motility of the inflamed colon: NO of neuronal origin is a transmitter that stimulates the peristaltic activity of the colon, since nonselective NO synthase (NOS) inhibition gave rise to a transient inhibition of the motility, whereas the administration of a selective neuronal NOS inhibitor elicited long-lasting motility inhibition (Palásthy 2006). It seems reasonable that activation of the myenteric NMDA receptors, followed by a massive Ca^{2+}

influx through the NMDA receptor-ion complex, may stimulate NO synthesis, which would result in turn in colonic smooth muscle relaxation.

In contrast, several studies have indicated that NMDA induces contraction of the ileal smooth muscle (Moroni 1986). It should be added that the formation of reactive oxygen species (ROS) during the inflammatory process may provide an explanation for this finding. Superoxide production has been demonstrated to be triggered by NMDA activation and the reaction is inhibited by superoxide dismutase (Lafon-Cazal 1993). Xanthine oxidoreductase (XOR)-dependent ROS production is known to occur in the early phase of colon obstruction and also during experimental colitis (Kaszaki 2008; Varga 2010). NO in combination with XOR-derived superoxide radicals leads to the formation of peroxynitrite, a well-known inhibitor of NOS activity and highly injurious to a variety of cells (Beckman 1990). The enhanced production of peroxynitrite and the decreased NOS activity eliminate the *relaxant* effect of endogenous NO. Taken together, the excessive accumulation of glutamate with subsequent activation of the ligand-gated, ion channel NMDA receptors opposes the NO-dependent relaxation (Kohjitani 2005).

NMDA receptor antagonism

Kynurenic acid (KynA), one of the few known endogenous excitatory amino acid receptor blockers, has high affinity for the glycine coagonist site of the NMDA receptors and a broad spectrum of antagonistic properties in supraphysiological concentrations (Sas 2007). It is a product of an alternative tryptophan metabolism, the kynurenine pathway, which is the major route for the conversion of tryptophan to NAD and NADP, leading to the production of a number of biologically active molecules with neuractive properties. KynA is able to reduce the excitotoxic damage to the CNS (Choi 1988) and it is additionally effective against neurodegenerative disorders (Klivényi 2004).

NMDA antagonism: experimental results

An important feature of KynA is its ability to reduce inflammatory reactions in the colon (Varga 2010). In our studies, we employed haptene-induced rat colitis, a frequently used IBD model. In animal experiments, 2,4,6-trinitrobenzensulfonic acid (TNBS) is an appropriate substance with which to model the symptoms of human colitis and to test new therapeutic possibilities. A single intracolonal (ic) TNBS enema causes extensive transmural colitis with

ulcerations that last longer than 8 weeks. As compared with the control group, rats with TNBS colitis exhibit a weight loss (Morris 1989), visceral hyperalgesia (Zhou 2008), significant elevations of plasma TNF-α, tissue NOS and XOR activities (Varga 2010) and leukocyte accumulation, as evidenced by increased level of myeloperoxidase (MPO) activity. In our first study series, the animals were anesthetized with sodium pentobarbital (50 mg/kg bw ip), and macrohemodynamic parameters, including mean arterial pressure (MAP), heart rate (HR) and cardiac output (CO; by a thermodilution technique) were recorded with a SPEL Advanced Cardiosys 1.4 computer (Experimetria Ltd., Budapest, Hungary). After a midline abdominal incision, a strain gauge transducer (Experimetria Ltd., Budapest, Hungary) was sutured onto the colon wall 3 cm distal from the cecum for the measurement of colonic motility. At the end of the experiments, blood samples were taken for the determination of TNF-α concentrations, while the XOR and MPO activities and extent of NO production [the level of nitrite/nitrate (NO_x), stable end-products of NO] were measured in colonic tissue biopsies (Varga 2010).

Table 1.

Effects of synthetic KynA derivatives (KADs) on hemodynamic, motility, and biochemical parameters.

Groups (n=5-10)	Mol. weight	Hemodynamic parameters	Motility parameters	Biochemical parameters
SZR-72	295.7	-	⇩	-
KAD-79	337.8	⇩	⇩	⇧
KAD-80	335.8	⇩	⇧	⇧
KAD-81	321.8	-	⇩	⇧
KAD-82	323.8	⇩	⇩	-
KAD-83	351.8	-	⇩	-
KAD-100	356.5	-	-	⇩
KAD-101	382.2	⇩	⇧	⇩
KAD-104	358.2	-	-	⇩
KAD-105	384.2	⇩	⇩	⇩
KAD-106	342.2	-	⇩	-
KAD-107	368.2	-	⇧	⇩
KAD-108	344.2	⇩	⇩	⇩
KAD-761	286.3	-	-	⇧

With this model, we tested a variety of synthetic KynA derivatives (KADs) containing different side-chain modifications (the analogs were synthetized by the Institute of Pharmaceutical Chemistry, University of Szeged) 48 h after colitis induction and the data were compared with the results on the untreated control group (Table 1). Compounds with macrohemodynamic side-effects (unwanted MAP, CO or HR changes) or dysmotility were then excluded, and finally two KynA analogs (SZR-72 and KAD-106) were chosen for further examinations. In these subgroups, the motility inhibitory potential and anti-inflammatory effects of the KADs were analyzed in a subacute TNBS-induced colitis model.

The animals received enemas in a total volume of 0.25 ml, containing only the solvent (25% ethanol) in the case of the sham-operated group, or 34 mg TNBS for the other 3 groups, 17 h before the start of monitoring. The next day, the animals were anesthetized and surgery was performed to monitor the hemodynamics and colonic motility for 6 h. The groups treated with the KADs received an iv infusion containing 10 mg kg^{-1} SZR-72, or 11.5 mg kg^{-1} KAD-106 (equimolar doses) dissolved in 0.9% saline with the pH adjusted to 7.2-7.4 in a total volume of 1 ml. The 30-min treatments started 18 h after the ic instillation of TNBS. At the end of the experiments, full-thickness tissue samples were taken to determine the colonic MPO and XOR activities and level of NO production. The plasma TNF-α concentration was determined in venous blood samples with an ELISA kit (Biomedica Hungaria Kft, Hungary).

As compared with the baseline values there were no significant changes in the hemodynamic parameters in the sham-operated group during the observation period. The CO was significantly higher in the colitis groups than in the sham-operated group, while both KADs reduced the increased CO, though the effect of SZR-72 lasted longer. There were no differences in HR or MAP between the groups.

To estimate the excitatory status of the ENS, the motility index (determined via the area under the motility curve) was calculated as a marker of the condition of neuronal control. At the beginning of the observation period, the motility index was higher in the TNBS-treated groups than in the sham-operated animals and it remained significantly higher throughout the later phase of the experiments. SZR-72 decreased the motility index

significantly, whereas KAD-106 treatment did not cause a significant decrease in motility index, though a decreasing tendency was observed (Figure 1).

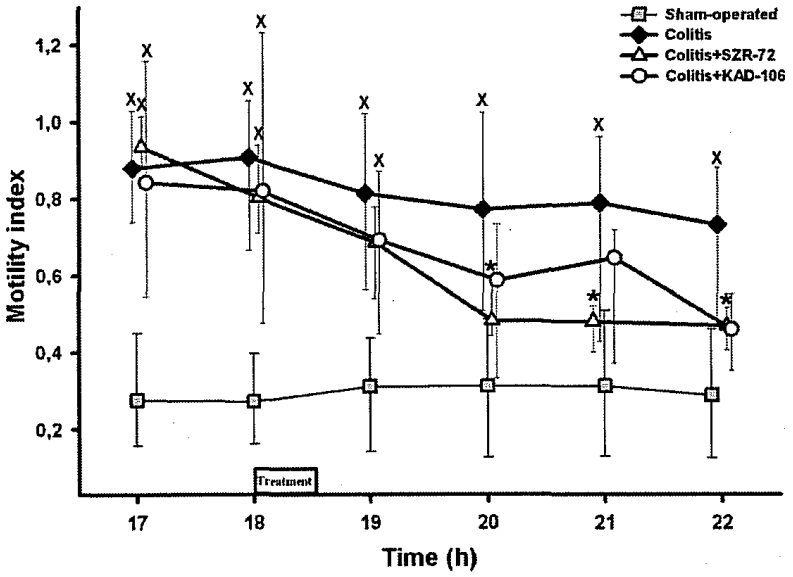

Fig.1. Changes in colon motility index in the sham-operated (shaded squares with a thin continuous line), colitis (black diamonds with a thick line), SZR-72 (empty triangles with a thick line) and KAD-106-treated colitis (empty circles with a thick line) groups. The box indicates the time of treatment with synthetic NMDA antagonist. The plots depict 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.

Data relating to the anti-inflammatory capabilities of the KADs are presented in Table 2. The plasma TNF-α concentration and tissue XOR and MPO activities were increased significantly in the proximal colon as compared with those in the sham-operated group 23 h after colitis induction. Both SZR-72 and KAD-106 induced a significant decrease in the MPO activity of the large bowel relative to the non-treated colitis group, and the SZR-72 treatment significantly decreased the TNF-α level and XOR activity too. Activation of the NMDA receptors and subsequent NO production may be the key in both motility and local inflammation changes. This study revealed that the TNBS-induced mucosal damage was associated with a significant increase in colonic NO_x level. The SZR-72 treatment decreased NO production significantly, while KAD-106 administration did not significantly influence the elevated colonic NO_x level as compared with that in the colitis group.

Table 2.

Effects of colitis and NMDA antagonism on colon myeloperoxidase (MPO) activity [U*(mg*protein^{-1})], colon xanthine oxidoreductase (XOR) activity [(pmol*(min*mg)$^{-1}$)], plasma nitrite/nitrate level (NO_x) [μmol] and plasma TNF-α levels (ng ml^{-1}).

		MPO	XOR	NO_x	TNF-α
Sham-operated	Median	0.725	24.8	15.59	3.48
	25p; 75p	*0.67; 0.883*	*21.75; 27.5*	*14.59; 15.97*	*1.24; 8.76*
Colitis	Median	1.41 x	44.2 x	18.81 x	13.82 x
	25p; 75p	*1.101; 1.651*	*73.6; 48.43*	*17.37; 20.29*	*10.39; 18.06*
Colitis+SZR-72	Median	0.924 #	18.3 #	16.31 #	7.27
	25p; 75p	*0.876; 1.002*	*15.53; 22.18*	*14.24; 16.65*	*4.09; 10.24*
Colitis+KAD-106	Median	0.438 #	34.6 x +	15.94	12.54 x
	25p; 75p	*0.27; 0.589*	*30.43; 39.75*	*13.24; 18.62*	*11.98; 13.99*

x $p < 0.05$ relative to the sham-operated group; # $p < 0.05$ relative to the colitis group;
+ $p < 0.05$ relative to the colitis+SZR-72 group

In summary, our results have demonstrated an important role of NMDA receptor activation in the pathophysiology of TNBS-induced colitis. Neuroprotection is a therapeutic strategy aimed at slowing or halting the progression of primary neuronal loss following acute or chronic diseases. The KynA analog SZR-72 significantly inhibited the colon motility, and also exerted a potentially protective, anti-inflammatory effect due to the inhibition of XOR activity, colonic NO production and concomitant leukocyte accumulation in the inflamed tissues. KAD-106 administration likewise resulted in significant, but moderate anti-inflammatory effects in the TNBS-induced colitis model. It remains to be established whether the findings in this experimental model are applicable to humans. However, together with previous observations, the present data suggest that inhibition of the enteric NMDA receptors may provide a novel neuroprotective option via which to influence intestinal hypermotility and inflammatory processes simultaneously.

Acknowledgments

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