with TTX. Reciprocally, TTX had no effect on GC in the presence of L-NNA or ODQ. GCs were abolished by 10 μM 8-Br-cGMP but unaffected by PKG inhibitor KT5823 (0.2 μM). The frequency and amplitude of GCs were enhanced by 1 μM BAY K 8644 (0.66 ± 0.07/min and 6.7 ± 0.4 g) and abolished by 0.1 μM D-600. Basal cGMP content in strips with myenteric plexus (39 ± 3 pmol/g tissue), was significantly decreased by either 1 μM TTX, 0.1 mM L-NNA, or 1 μM ODQ to 25 ± 3, 19 ± 2, and 22 ± 3 pmol/g tissue, respectively. These treatments had no effect on cGMP content of enzymatically dissociated muscle cells, which was increased 2-fold by 1 μM NO donor SNAP. CONCLUSIONS: 1) Spontaneous contractile activity in rat colon *in vitro* is not generated by cholinergic, serotonergic, or histaminergic input, but is inhibited by tonic NK-3 receptor activation. 2) Constitutive release of NO from enteric neurons modulates spontaneous GC and sustains cGMP level in muscle. 3) PKG activation does not modulate GCs. 4) Calcium influx through L-type channels maintains GC.

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ALTERED NOS EXPRESSION UNDERLIES IMPAIRED MOTILITY CONTROL IN TNBS ILEITIS IN THE GUINEA PIG.

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It is well-known that intestinal inflammation results in altered neural control of g.i. motility. An increase of inducible nitric oxide synthase (iNOS) during intestinal inflammation has been reported in patients with IBD and in animal models. Recently, we showed that after a transient increase on day 1, a significant decrease of NADPH positive neurons was observed on day 3 and 6 after induction of a TNBS ileitis in the guinea pig (Kelles 1999). Aim: To investigate the expression of iNOS and nNOS over time post-inflammation and to correlate this to neural control of motility. Methods: Ileitis was induced with TNBS (60 mg/kg). Expression of iNOS and nNOS mRNA was determined by RT-PCR; GAPDH expression was used as internal standard. In vitro ileal circular muscle responses to electrical field stimulation (EFS; 1, 4, 8, 32 Hz) were evaluated in controls and in inflamed animals before and after administration of L-NAME, a non-selective NOS inhibitor. Data (mean ±SE) were compared by t-test. Results: Histology and a 4-fold increase in MPO confirmed inflammation. RT-PCR showed an increase of iNOS/GAPDH in the LMMP of the guinea pig ileum on day 1 post-inflammation (0.53 ± 0.67 vs 0.13 ± 0.19,$p<0.05$) followed by a decrease in nNOS/GAPDH on respectively day 3 and day 6 post-inflammation (D3: 0.77 ± 0.17 vs 0.13 ± 0.19 and D6: 0.92 ± 0.33 vs 0.42 ± 0.13,$p<0.05$). In control animals, area under the contraction curve was significantly enhanced by L-NAME at all frequencies. In inflamed animals (day 6), L-NAME no longer enhanced contractions (Table). Conclusion: An initial increase of iNOS expression at 24 h is followed by a decreased nNOS expression 3 and 6 days after induction of an TNBS induced ileitis. During inflammation, the nitrergic component to EFS is no longer present, probably reflecting the decrease of nNOS. The decreased nNOS expression may contribute to the altered gut motility as seen during inflammation.

g/cm²	1Hz	8Hz	32Hz
Control-Krebs	1.3±0.3	1.1±0.2	7.4±0.5
Control-L-NAME	1.6±0.4*	2.7±0.5**	9.0±0.6***
Inflamed-Krebs	0.6±0.2	1.3±0.4	8.0±0.7
Inflamed-L-NAME	0.5±0.2	1.7±0.5	8.3±0.7

*=p<0.05;**=p<0.01;***=p<0.001

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DIFFERENTIAL DISTRIBUTION AND TRANSCRIPTIONAL CONTROL OF HUMAN NNOS SPLICE VARIANTS IN THE GASTROINTESTINAL TRACT.

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Six distinct 5'splice variants of nNOS in the human GI-tract (GIT) are generated by usage of 3 separate promoters or alternative splicing. They either encode for full length nNOSα or the N-terminally truncated nNOSβ or nNOSγ proteins. The promoter regions of the 3 different first exons show multiple transcription factor binding sites (e.g. AP-1 which can be regulated by phorbol esters). The aim was to characterize the mRNA expression of nNOS splice variants along the human GIT, to express the predicted nNOS proteins (nNOSα, nNOSβ, nNOSγ) in an isolated cell system and to investigate possible differential effects of phorbol esters on the promoter activities. Methods: Expression of nNOS splice variants was analyzed by RT-PCR/ southern blotting in human gastric antrum and corpus, small intestine, colon and recto-sigmoid. Expression vectors for nNOSα, β and γ were transfected into COS cells and recombinant proteins were analyzed by western blotting or ^{3}H-arginine-^{3}H-citrulline assays. nNOS promoter gene constructs of the 5'flanking region of exon $1_{5'1}$, $1_{5'2}$ and $1_{5'3}$ were transfected into HeLa cells. Luciferase and β-galactosidase activities were measured in presence of an active (PMA, 0.1 μM) or an inactive phorbol ester (0.1μM). Results: There was a distinct and site specific expression of nNOS splice variants. nNOSα-$1_{5'1}$ mRNA was expressed in gastric corpus and antrum, colon and recto-sigmoid, but not in small intestine. mRNA of nNOSα-$1_{5'2}$, nNOSβ-$1_{5'2}$ and nNOSγ was found in small intestine, colon and recto-sigmoid, whereas gastric corpus and antrum lack nNOSγ. nNOSα-$1_{5'3}$ and nNOSβ-$1_{5'3}$ variants were present in all regions. Heterologous eukaryotic expression of N-terminal nNOS proteins revealed bands of approximately 160 kDa for nNOSα, 135 and 125 kDa for nNOSβ and 125 kDa for nNOSγ in the Western blot. All forms showed functional NOS activity in the NOS assay. The promoter activities of exon $1_{5'2}$ (+46%)and exon $1_{5'1}$ (+10%) were stimulated by PMA, whereas the promoter of exon $1_{5'3}$ was downregulated (-17%). Conclusions: 5'nNOS splice variants are differentially expressed along the human GIT, indicating a site specific transcriptional control of the nNOS gene. Such a differential regulation of nNOS promoter activities was shown with phorbol esters. Expression of the different 5'splice variants resulted in functionally active proteins (nNOSα, β and γ) with predicted size. The differential distribution of these variants could be implicated in different biological functions. SFB-391-C5

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SWALLOW-INDUCED LOWER ESOPHAGEAL SPHINCTER RELAXATION *IN VIVO* IN NEURONAL NITRIC OXIDE GENE KNOCK OUT MICE.

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Background. Substantial experimental evidence indicates nitric oxide (NO) to be an important inhibitory neurotransmitter involved in lower esophageal sphincter (LES) relaxation. Preparations of LES, treated with chemical blockers of nitric oxide synthase (NOS) or obtained from neuronal NOS gene disrupted (nNOS-/-) mice, do not relax in response to electrical field stimulation. However, it is not known whether swallow-induced LES relaxation is similarly affected in nNOS-/- mice. Aims. 1. Test the feasibility of intraluminal manometry to study swallow-induced LES relaxation in anesthetized mice; 2. To assess the degree of impairment in swallow-induced LES relaxation in nNOS-/- mice. Methods. Micro-sized esophageal perfusion manometry was conducted in pentobarbital-anesthetized adult wild type (WT) and nNOS-/- mice of both sexes, using a custom-designed 3-channel catheter assembly (Dentsleeve, Australia). Each channel had an ID of 0.3 mm and an OD of 0.6 mm and was perfused with water at 5 μl/min. Each port had a typical raise rate of >400 mm Hg/s and a compliance of 1.2 μl/100 mm Hg. Mice were instrumented with intravenous and intrarterial cannulae. Swallows were evoked by injecting 0.03 cc saline into the pharyngeal cavity. The LES was characterized by a basal high pressure zone that relaxed to swallowing. Results. As shown below, L-NAME treated wild type mice and nNOS-/- mice showed marked impairment in LES relaxation. Conclusions. 1. *In vivo* LES manometry is feasible in mice and like other animal species, mouse LES maintains a basal tone that relaxes to swallow. 2. Swallow-induced LES relaxation in nNOS-/- mice is markedly inhibited but not completely abolished as may be expected from *in vitro* data. We conclude that nNOS is responsible for about 70% of swallow-induced LES relaxation. Other neurotransmitters like VIP or ATP may be responsible for the remaining relaxation.

	Basal LES pressure (mm Hg)	Swallow-induced LES relaxation (%)
WT (N=5)	23±4†	83±8
WT+L-NAME (100 mg/kg,iv; N=5)	31±7	28±7*
nNOS-/- (N=4)	48±22	24±7*

†Mean±SEM; *indicates significant difference ($p<0.05$) from corresponding wild type; student t-test