● **INHIBITION OF NANC NERVE-INDUCED RELAXATIONS BY NO DONORS IN THE RAT GASTRIC FUNDUS.** G.E. Boeckxstaens, J.G. De Man, B.Y. De Winter, T.G. Moreels, A.G. Herman, P.A. Pelckmans. Divisions of Gastroentrology and Pharmacology, Faculty of Medicine, University of Antwerp (UIA), B-2610 Antwerpen-Wilrijk, Belgium.

Feedback inhibition is an important autoregulatory mechanism of neurotransmitter release. In the present study, we investigated whether the release of nitric oxide (NO) from nonadrenergic noncholinergic (NANC) nerves is also subject to feedback inhibition by studying the effect of the NO donors SIN-1 and nitroglycerin (GTN) on electrically-induced NANC relaxations of the rat gastric fundus. Longitudinal muscle strips of the rat gastric fundus were mounted in organ baths filled with aerated Krebs solution at 37° C. Experiments were performed in the presence of atropine (1 μM) and guanethidine (3 μM), and on a 5-hydroxytryptamine (0.1 μM)-induced contraction. Short periods (10 s) of electrical field stimulation (EFS; 0.5-16 Hz, 1 ms) induced transient nerve-mediated relaxations, previously shown to be nitrergic in nature. Pretreatment of the rat gastric fundus with the NO donors SIN-1 (10-100 μM) and GTN (500 μM), significantly inhibited the responses to EFS (table 1) to the same extent as NO biosynthesis inhibition by nitro-L-arginine (100 μM). Prolonged (3 min) electrical stimulation (16 Hz, 1ms) of the rat gastric fundus induced sustained relaxations, previously shown to be mediated by NO and probably vasoactive intestinal polypeptide (VIP). Pretreatment with SIN-1 (100 μM) and GTN (500 μM) slowed down the onset of the relaxation, an effect similar to that of NO biosynthesis inhibition. In contrast, the concentration-response curves to authentic NO (0.01-10 μM) and VIP (0.01-10 nM) were not affected by pretreatment with SIN-1 (100 μM) or GTN (500 μM)(table 1).

Table 1 Effect of NO-donors on relaxations to EFS, NO and VIP

	2 Hz	4 Hz	NO (30 nM)	VIP (0.3 nM)
control	47±7	59±7	29±11	29±6
SIN-1 (100 μM)	23±10*	28±10*	36±14	26±3
GTN (500 μM)	17±8*	34±6*	23±8	29±6

Results are shown as % decrease of a 5-hydroxytryptamine (0.1 μM)-induced contraction and as mean ± S.E.M. for n=4-6 experiments; *P<0.05, significantly different from control, paired Student t test.

From these results we conclude that the EFS-induced NANC relaxations in the rat gastric fundus are prejunctionally inhibited by NO donors. As this inhibitory effect is similar to that of NO biosynthesis inhibition, these results suggest feedback inhibition of the nitrergic innervation by NO itself in the rat gastric fundus.

● NEURAL MEDIATION OF THE MOTILIN EFFECT ON THE HUMAN ANTRUM. M. Boivin, L. Rivero Pinelo, V. Plourde, S. St-Pierre, P. Poitras. Hôpital Saint-Luc, Université de Montréal, Canada.

To elucidate the mode of action of motilin on the stimulation of the human gastrointestinal motility, we compared the motor response elicitated by exogenous motilin administered in the presence of saline (used as a control), of the muscarinic antagonist atropine or of the $5HT_3$ receptor antagonist ondansetron. Methods: Manometric recording of the interdigestive antroduodenal motility profile was carried out in healthy volunteers until the appearance of a spontaneous period of phase III activity initiated at the antrum and migrating to the duodenum. At the end of the duodenal phase III, the tested blocker was administered IV and was followed 30 min. later by a 10 min. infusion of synthetic human motilin (50 ng/kg); the G.I. motility was then recorded for an additional 60 min. Results: Motilin administered on a background of saline induced a premature phase III migrating from the antrum to the duodenum in every tested subjects (n=5). A low dose of atropine (5 μg/kg/hr) inhibited the motilin effect in 2/5 subjects (p=ns), while a high dose of atropine (15 μg/kg given in 30 min.) blocked the motilin-induced premature antral phase III in all occasions (n=5; p<.01). Motilin given with ondansetron at low dose (8 mg bolus followed by 1 mg/hr) or with ondansetron at high dose (32 mg given in 30 min.) was without effect in 3/7 (p=ns) or in 2/5 (p=ns) subjects respectively. During the administration of atropine 15 μg/kg where motilin always failed to induce a premature antral phase III, a phase III type activity initiated from the duodenum was seen in 4/5 subjects. Conclusions: 1) The induction by motilin of phase III activity in the human antrum is dependant upon muscarinic mediation, suggesting that motilin receptors are located on cholinergic nerves; 2) the participation of serotoninergic mechanisms could not be substantiated; 3) the motor effect of motilin on the human duodenum was still documented despite atropine blockade, suggesting that the effect on the duodenum was not neurally mediated and that motilin receptors are present on duodenal muscles cells; 4) these results are compatible with the existence in human of multiple motilin receptor subtypes, N or M, located respectively on nerves or on muscle cells of the GI tract.

● ELASTIC PROPERTIES OF THE NORMAL HUMAN ESOPHAGUS: A BIOCHEMICAL STUDY. L. Bonavina, M. Venturi, * L. Colombo, A. Segalin, * E. Mussini, A. Peracchia. Department of General Surgery and Surgical Oncology, University of Milan. * Mario Negri Institute for Pharmacological Research, Milan. ITALY

The compliance of the esophageal wall has been recently investigated using impedance planimetry. Experimental studies have demonstrated regional variations in collagen content and passive elasticity of the esophagus. The aim of the present study was to evaluate collagen and elastin content in the human esophagus, to determine whether regional differences occur. 128 specimens of the entire wall at 3 levels above and below the carina from cricopharyngeal muscle to esophagogastric junction (EGJ) were obtained from the freshly harvested esophagus of 10 cadavers, median age 66.0 yrs (range 42-80) , with no evidence of esophageal and connective tissue disease. The protocol was approved by the Ethics Committee of the Ospedale Maggiore Policlinico, Milan. 4-hydroxyproline (Hyp) (collagen) and isodesmosine (IDES) and desmosine (DES) (elastin) content were measured with standard methods. The results are shown in the table below (values are expressed as means ±SD) :

	4-Hyp µg/mg	Collagen µg/mg	IDES ng/mg	DES ng/mg	Elastin µg/mg	Collagen Elastin
			a	c	e	g
Upper esophagus (n = 62)	4.43± 2.10	33.49± 15.36	66.09± 30.63 b	61.47± 32.61 d	23.13± 12.86 f	1.45± 1.19 h
Lower esophagus (n= 66)	4.21± 1.37	31.96± 10.07	84.51± 41.64	86.16± 50.41	32.79± 20.78	0.97± 0.48

a vs b, c vs d, e vs f, g vs h p < 0.01 (Mann-Whitney's U test)

IDES,DES and elastin content was significantly greater in the esophagus below the carina, with a peak between 4 and 7 cm from EGJ (IDES 88.2± 39.9, DES 90.7± 49.5, elastin 36.4 ± 23.0); 4-Hyp and collagen content was not significantly greater in the upper esophagus. The ratio of collagen to elastin was therefore markedly lower in the distal esophagus. In conclusion, regional variations in elastin content and in collagen to elastin ratio are present in the human esophagus, with a peak from 4 to 7 cm above the EGJ, which corresponds to the epiphrenic ampulla.

● FEDOTOZINE REVERSES SPINAL AND SUPRA-SPINAL C-FOS EXPRESSION IN RESPONSE TO PERITONITIS-INDUCED DIGESTIVE ILEUS IN RATS. B. Bonaz*, P.J.M. Rivière**, X. Pascaud**, J.L. Junien** and C. Feuerstein*. *Laboratory of Neurophysiology, INSERM U318, CHU, 38043 Grenoble cedex 09 and **Institut de Recherche Jouveinal, 94265 Fresnes cedex, FRANCE.

Fedotozine, a kappa opioid receptor agonist, reverses abdominal surgery- or peritonitis-induced digestive ileus in rats possibly through a peripheral action on sensory afferents (1,2). Abdominal surgery induces c-fos expression, a marker of neuronal activation, in selective brain nuclei controlling digestive motility in rats (3). Purpose: 1) to map spinal and supraspinal pathways activated by peritonitis-induced digestive ileus in rats. 2) to characterize primary afferent fibers involved in this activation. 3) to investigate the effect of fedotozine on peritonitis-induced c-fos expression. Methods: peritonitis was induced by 0.6% acetic acid (AA) ip injected (10ml/kg) in conscious male fasted SD rats either a) *untreated* (n=10), b) pretreated 14 days before with *capsaicin* (125mg/kg sc; n=5), c) preatreated 30 min before with *fedotozine* (15 mg/kg sc; n=5) or d) pretreated 2 h before fedotozine with the kappa antagonist *nor BNI* (30 mg/kg sc; n=3). Control animals (n=15) received the vehicle alone. Sixty min after ip injections of AA, rats were perfused with 4% paraformaldehyde. Thirty micron frozen sections of the brain and spinal cord were processed for Fos immunoreactivity (Fos-IR) using a rabbit antibody against Fos protein (Oncogene Science; 1/500) (3). The effect of fedotozine alone on Fos-IR was also investigated. Results: 1) almost no Fos-IR was observed in controls. 2) peritonitis induced Fos-IR in the spinal cord (laminae I, V, VII, X), nucleus tractus solitarius (NTS; medial part), parabrachial nucleus, supraoptic (SON) and paraventricular nucleus (PVH) of the hypothalamus mainly in the parvocellular part known to contain CRF perikarya. 3) capsaicin pretreatment blocked peritonitis-induced Fos-IR in all structures tested. 4) fedotozine alone induced Fos-IR in the NTS and consistently decreased by 65% peritonitis-induced c-fos expression in the PVH, SON and spinal cord. 5) nor BNI reversed the effect of fedotozine on peritonitis-induced Fos-IR. Conclusions: 1) peritonitis caused by ip injection of AA induces c-fos expression in the spinal cord and selective brain nuclei involved in the control of digestive motility in rats. This effect is conveyed through capsaicin-sensitive afferent fibers. 2) the reversal effect of fedotozine on c-fos expression in the spinal cord and the PVH argues for an action at peripheral afferent sites and a possible effect of the PVH on peritonitis-induced digestive ileus through CRF efferent pathways.

(1) Gastroenterology 104: 724-731, 1993. (2) J. Pharmacol. Exp. Ther. 270: 846-850, 1994. (3) J. Comp. Neurol. 349: 212-222, 1994.