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## Chronic ingestion of nitrate and nitrite increases in vitro cholinergic contractility and decreases neutrophil contents of the jejunum in rats

Rafael Garcia Villar, Pauline Anton, Stéphane Marty, Vassilia Theodorou, Jean Fioramonti, Lionel Bueno

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Dr R. Garcia-Villan

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**GASTROENTEROLOGICAL ASSOCIATION**

AND

**DIGESTIVE DISEASE WEEK**

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**CHRONIC INGESTION OF NITRATE AND NITRITE INCREASES IN VITRO CHOLINERGIC CONTRACTILITY AND DECREASES NEUTROPHIL CONTENT OF THE JEJUNUM IN RATS.** R. Garcia-Villar, P. Anton, S. Marty, V. Theodorou\*, J. Fioramonti, L. Buéno. INRA, Digestive Pharmacology Unit; \*ESAP, Toulouse, France.

Nitrate and nitrite present in foods and drinking water subserve a controversial human health issue. Thus, consumers blame these compounds and their byproducts (nitrosamines, nitric oxide) for insidious functional and/or structural alterations of the digestive tract that may lead to more severe pathologies.

**Specific aims:** The present study was therefore undertaken to investigate the influence on jejunal contractility, mast cells, and neutrophils, of a two-week oral administration of nitrate or nitrite (sodium and potassium salts) to rats at doses possibly found in food.

**Methods:** Five groups of 8 male Wistar rats (250-300 g) received respectively once daily by intragastric gavage (mg/kg/day; treated groups): NaNO<sub>3</sub> (500), KNO<sub>3</sub> (250), NaNO<sub>2</sub> (8), KNO<sub>2</sub> (5) and distilled water (1ml/day; controls). After 14 days rats were sacrificed; segments of jejunum (20 cm aboral to the LT) were collected for: (i) myeloperoxidase (MPO) spectrophotometric assay for neutrophil infiltration; (ii) mast cell numbers determination (alcian blue/safranin staining); (iii) in vitro contractility studies (construction of concentration-contractile responses curves to carbachol (CCH; a muscarinic cholinergic agonist), serotonin (5-HT), and neurokinin A (NKA); 0.1 nM-1 mM).

**Results:** MPO activity in jejunal tissue was reduced to less than 15% of controls (p<0.05) in rats treated with nitrate but not in those treated with nitrite. No differences in jejunal mast cell numbers were found between the five groups. Maximal contraction induced by CCH was significantly increased reaching 153-159% of controls (p<0.05), while no differences were measured in the responses to 5-HT and NKA, in the four treated groups compared with controls.

**Conclusion:** Ingestion of nitrate or nitrite for two weeks enhanced jejunal smooth muscle responsiveness to cholinergic, but not to serotonergic or tachykinergic stimulation in rats. It can be suggested that alteration of parasympathetic control of GI tract motility and neutropenia resulting from chronic ingestion of nitrate may pave the way for intestinal functional disorders.

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## G4325

**QUANTIFICATION OF CAECOILEAL REFLUX BY GAMMA-SCINTIGRAPHY IN CONSCIOUS PIGS.**

Cuche gaelle, Malbert Charles-Henri, Station de Ress Porcines, Saint-Gilles France

We evidenced recently frequent caecoileal reflux events in pigs (Cuche and Malbert, 1998). However, the method used, i. e. measurement of intraluminal ileal pH, was not quantitatively informative. Thus, this study aimed to quantify the amount of caecal fluids invading terminal ileum using gamma scintigraphy imaging in conscious pigs. Under anaesthesia and aseptic conditions, an infusion silicone tube (ID 5 mm) was inserted in the apex of the caecum and a Y-shaped silicone cannula was located 20 cm proximal to the caecoileal sphincter in 10 pigs (30 ± 2 kg). The animals were allowed to recover for a week after surgery. While the pig was standing in a sling, a guide (ID 10 mm) filled with 40 MBq <sup>67</sup>Ga was introduced into the terminal ileum pointing towards the caecum. This guide will serve to outline the ileal lumen on gamma scans. 200 ml saline labelled with 40 MBq <sup>99m</sup>Tc-DTPA were infused in the caecum at 40 ml.min<sup>-1</sup>. Repetitive one minute scans of the ileocecolonic area were acquired in multi-isotope mode for 30 minutes with high resolution collimator (Apex 500G). Since porcine ileum follows the spiral shape of the colon, the only practicable incidence allowing simultaneous imaging of ileum and caecum was from the rear of the animal. For each frame, the ileum was outlined on the Ga scan and the number of counts (Ic) within a copy of this ROI on the Tc scan was evaluated. Cecoileal reflux was expressed as Ic divided by the total number of counts on the Tc scan. A significant amount of Tc radioactivity was found in the ileal ROI during 4 ± 3 min on each pig. It is uncommon to observe more than one episode during the recording session. None of these refluxes invade the ileum 15 cm proximal to the caecoileal junction and 50 % of them were limited to the first 5 cm of the ileum. For each event, reflux amounted to 13 ± 2 %, a value remarkably constant along frames once the reflux event was detected. In conclusion, this work confirms the existence of cecoileal reflux in pigs. They amount about 13 % of caecal contents. Cuche G., Malbert, C. H. 1998. Relationships between caecoileal reflux and ileal motor patterns in conscious pigs. American Journal of Physiology, 274, G35-G40, 1998.

## ● G4326

**CHRONIC INGESTION OF NITRATE AND NITRATE INCREASES IN VITRO CHOLINERGIC CONTRACTILITY AND DECREASES NEUTROPHIL CONTENT OF THE JEJUNDUM IN RATS**

R Garcia-Villal, INRA, Digest Pharmacology Unit, Toulouse Cedex 9 France; P Anton, INRA, Toulouse France; S Marty, INRA, Digest Pharmacology Unit, Toulouse France; V Theodorou, ESAP, Toulouse France; J Fioramonti, L Bueno, INRA, Digest Pharmacology Unit, Toulouse France

Nitrate and nitrite present in foods and drinking water subserve a controversial human health issue. Thus, consumers blame these compounds and their byproducts (nitrosamines, nitric oxide) for insidious functional and/or structural alterations of the digestive tract that may lead to more severe pathologies. **Specific aims:** The present study was therefore undertaken to investigate the influence on jejunal contractility, mast cells, and neutrophils, of a two-week oral administration of nitrate or nitrite (sodium and potassium salts) to rats at doses possibly found in food. **Methods:** Five groups of 8 male Wistar rats (250-300 g) received respectively once daily by intragastric gavage (mg/kg/day; treated groups); NaNO<sub>3</sub> (500), KNO<sub>3</sub> (250), NaNO<sub>2</sub> (8), KNO<sub>2</sub> (5) and distilled water (1 ml/day; controls). After 14 days rats were sacrificed; segments of jejunum (20 cm aboral to the LT) were collected for: (i) myeloperoxidase (MPO) spectrophotometric assay for neutrophil infiltration; (ii) mast cell numbers determination (alcian blue/safranin staining); (iii) in vitro contractility studies (construction of concentration-contractile responses curves to carbachol (CCH; a muscarinic cholinergic agonist), serotonin (5-HT), and neurokinin A (NKA); 0.1 nM-1 mM). **Results:** MPO activity in jejunal tissue was reduced to less than 15% of controls (p<0.05) in rats treated with nitrate but not in those treated with nitrite. No differences in jejunal mast cell numbers were found between the five groups. Maximal contraction induced by CCH was significantly increased reaching 153-159% of controls (p<0.05), while no differences were measured in the responses to 5-HT and NKA, in the four treated groups compared with controls. **Conclusion:** Ingestion of nitrate or nitrite for two weeks enhanced jejunal smooth muscle responsiveness to cholinergic, but not to serotonergic or tachykinergic stimulation in rats. It can be suggested that alteration of parasympathetic control of GI tract motility and neutropenia resulting from chronic ingestion of nitrate may pave the way for intestinal functional disorders.

## G4327

**ROLE OF TACHYKININ NK<sub>1</sub>, NK<sub>2</sub> AND NK<sub>3</sub> RECEPTORS IN MODULATION OF VISCERAL HYPERSENSITIVITY IN THE RAT.**

Genevieve-Anne Gaudreau, Janie McDuff, Victor Plourde, CHUM, Saint-Luc - Univsite de Montreal, Montreal, PQ Canada

Tachykinins are involved in visceral pain but the relative contribution of tachykinin receptors is unclear. In animal models of visceral sensitization, repetitive colorectal distensions (CDs) result in visceral afferent nerve sensitization. **AIMS:** To investigate the effects of NK<sub>1</sub>, NK<sub>2</sub> and NK<sub>3</sub> receptor blockade in visceral hypersensitivity induced by CDs. **METHODS:** Visceral pain thresholds (VPT) were determined in male Sprague Dawley rats before or following a sensitization paradigm consisting of 10 repetitive CDs (duration 1 min followed by 30 sec rest) at 30 or 50mmHg. VPT were evaluated by measuring the pressure threshold (increments of 5mmHg, duration 3 min, 1 min rest) required to evoke the first abdominal muscle contraction (mean of 3 measurements). NK receptors antagonists, RP67580 (6.5 nmol), SR48968 (6.5 nmol) and R820 (6.5 nmol), acting at NK<sub>1</sub>, NK<sub>2</sub> and NK<sub>3</sub> receptors respectively, were administered intrathecally 15 min prior to the assessment of VPT. **RESULTS:** VPT (31.0±11.0mmHg) were significantly lowered following either 30 or 50mmHg CDs. VPT were significantly increased by RP67580 in rats sensitized with 30 (12.9±2.5 to 27.7±3.9 mmHg; p<0.05) or 50mmHg (12.61.7± to 32.1±8.8 mmHg; p<0.05). VPT were also significantly increased by R820 in rats sensitized with 30 (14.0±2.9 to 28.7±8.2mmHg; p<0.05) or 50mmHg (16.3±2.3 to 33.2±10.6 mmHg; p<0.05). SR48968 (≤ 65 nmol) did not affect VPT. **SUMMARY:** Repetitive CDs at 30 or 50mmHg significantly lowered VPT suggesting visceral sensitization and this effect was reversed by NK<sub>1</sub> and NK<sub>3</sub> receptor antagonists but unaffected by NK<sub>2</sub> blockade. **CONCLUSIONS:** Our results suggest that tachykinins with actions at spinal NK<sub>1</sub> and NK<sub>3</sub> receptors are involved in visceral sensitization induced by repetitive CDs.

## G4328

**INVOLVEMENT OF NMDA RECEPTORS IN RAT VISCERAL HYPERALGESIA.**

Genevieve-Anne Gaudreau, Victor Plourde, CHUM, Saint-Luc - Univsite de Montreal, Montreal, PQ Canada

N-methyl-D-aspartate (NMDA) receptors are involved in spinal transmission of nociceptive input. In the rat, repetitive colorectal distensions (CDs) result in visceral afferent nerve sensitization. **AIMS:** To investigate a possible role of NMDA receptors in the development of visceral hypersensitivity. **METHODS:** Visceral pain thresholds were determined in male Sprague Dawley rats before or following a sensitization paradigm consisting of 10 repetitive CDs (duration 1 min followed by 30 sec rest) at 30 or 50mmHg. Visceral pain thresholds were evaluated by measuring the pressure threshold (increments of 5mmHg, duration 3 min, 1 min rest) required to evoke the first abdominal muscle contraction (mean of 3 measurements). To assess the role of NMDA receptors, the NMDA receptor antagonist, MK-801, was administered intraperitoneally (i.p.; 30nmol/kg) or intrathecally (i.t.; 10nmol) under light halothane anesthesia, 15 min before the sensory thresholds were determined in conscious unanesthetized animals. **RESULTS:** Normal visceral pain thresholds (31.0±11.0mmHg) were significantly lowered following either 30 or 50mmHg CDs. MK-801 administered i.p. to rats sensitized by 30mmHg increased the pain threshold from 17.0±3.3 to 30.8±7.5mmHg (p<0.05) and in rats sensitized by 50mmHg from 13.3±2.5 to 22.3±6.9mmHg (p<0.05). Following i.t. MK-801 administration, pain thresholds in rats sensitized with 30mmHg were significantly increased from (13.9±2.0)mmHg to (27.0±9.0mmHg) (p<0.05) and with 50mmHg from (15.0±3.9)mmHg to (25.9±6.9)mmHg; p<0.05). **SUMMARY:** Both 30 and 50mmHg-induced hypersensitivity was abolished by i.p. or i.t. MK-801. **CONCLUSIONS:** Those results support a role for NMDA receptors in visceral hypersensitivity induced by repetitive CDs.

## ● G4329

**NEMATODE-INDUCED HYPERMASTOCYTOSIS MODIFIES THE JEJUNAL MOTOR RESPONSE TO CCK-8 IN RATS: ROLE OF MAST CELLS AND CCK RECEPTORS**

J Gay, J Fioramonti, R Garcia-Villar, L Bueno, INRA, Digest Pharmacology Unit, Toulouse France

Intestinal hypermastocytosis is a common feature of various digestive disturbances such as inflammatory bowel diseases. In rats, jejunal hypermastocytosis accompanied by enteric nerves remodeling can be observed during several weeks after infection with the nematode *Nippostrongylus brasiliensis* (Nb). The aim of this study was, first to evaluate whether jejunal motor responses to CCK-8 were modified by post-infection hypermastocytosis, and second to determine the CCK receptors involved in the motor response. **METHODS:** 8 groups of 8 male Wistar rats were used: 4 control groups were matched with 4 groups infected at day 0 with 3500 third-stage larvae of Nb (in 0.5 ml sterile water; sc). At day 30, 3 groups of NiCr electrodes were implanted 10 cm apart in mid-jejunum. Myoelectrical recordings started 7 days after surgery. The duration of disruption of the migrating myoelectrical complex (MMC) pattern was evaluated in both control and post-infected groups after CCK-8 (50 µg/kg, ip), under baseline