



Evidence for nitric oxide synthase activation by substance P through a mechanism not involving "classical" neurokinin receptors in guinea pig ileum

Rafael Garcia Villar, Christelle Dupuis, Jean Fioramonti, Lionel Bueno

► To cite this version:

Rafael Garcia Villar, Christelle Dupuis, Jean Fioramonti, Lionel Bueno. Evidence for nitric oxide synthase activation by substance P through a mechanism not involving "classical" neurokinin receptors in guinea pig ileum. Digestive Disease Week / 96th Annual AGA meeting, May 1995, San Diego, California, United States. 108 (4), 1995. hal-01600587

HAL Id: hal-01600587

<https://hal.science/hal-01600587>

Submitted on 4 Jun 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - ShareAlike 4.0 International License

April 1995 • Volume 108 • Number 4

Supplement to

Gastroenterology

FORAMOUNT

**Digestive Disease Week
and the
95th Annual Meeting of the American
Gastroenterological Association**

May 14-17, 1995, San Diego, California

**Program of the Annual Meeting of the American
Gastroenterological Association, the American
Association for the Study of Liver Diseases, the
Gastroenterology Research Group, the Society for
Surgery of the Alimentary Tract, and the American
Society for Gastrointestinal Endoscopy**

**Abstracts of Papers Submitted to the American
Gastroenterological Association**

**Abstracts of Papers Submitted to the American
Association for the Study of Liver Diseases**

**Abstracts of Papers Submitted to the Society for
Surgery of the Alimentary Tract**

ABSTRACT FORM FOR: Meetings of the AGA, AASLD at Digestive Disease Week**May 14-17, 1995****San Diego, California**

Type name, address and telephone numbers of author who should receive correspondence in Box G and complete all other parts of the form. **ABSTRACTS MUST BE RECEIVED BY DECEMBER 15, 1994.**

EVIDENCE FOR NITRIC OXIDE SYNTHASE ACTIVATION BY SUBSTANCE P THROUGH A MECHANISM NOT INVOLVING "CLASSICAL" NEUROKININ RECEPTORS IN GUINEA PIG ILEUM.

R. Garcia-Villar, Ch. Dupuy, J. Fioramonti & L. Buéno. INRA, Department of Pharmacology, BP.3, 31931, Toulouse, France.

Both substance P (SP), a tachykinin neuropeptide which contracts, and nitric oxide (NO), a labile transmitter that relaxes intestinal smooth muscle, have been proposed as active components of the non-adrenergic non-cholinergic (NANC) control of gut motility. To address the functional relationships between these two NANC mediators, we tested the hypothesis that NO-synthase (NOS) could be activated after SP-induced contractions in guinea pig ileum, and further, whether the neurokinin (NK) receptor subtypes described so far (i.e. NK1, 2, 3) could be involved in this NOS activation.

Methods: Longitudinal strips of guinea pig ileum (8 x 2 mm) were superfused (Krebs, 37°C, 2 ml/min) with tachykinin agonists (1 pM to 10 µM; SP, GR 73,632 [NK1], GR 64,349 [NK2], senktide [NK3]), and antagonists (1 µM; CP 99,941 [NK1], SR 48,968 [NK2]), as well as KCl (60 mM) and carbachol (CCh; 0.1 µM). The NO-related agents (1 µM) used were: L-NAME, L-NMMA, aminoguanidine (AG), L-arginine (L-ARG) and D-arginine (D-ARG). All drugs were superfused for 3 min. Motility was recorded (isometric system) and quantified (integrated area of activity).

Results: Both SP and NK-receptor agonists (1 pM to 10 µM) produced concentration-dependent contractions, with maximal effects at 0.1-1 µM. The response to 0.1 µM SP was 81±9% (n=16) of that to KCl (60 mM), very close to that elicited by CCh (0.1 µM; 87±5%, n=16), GR 73,632 (0.1 µM; 74±11%, n=12) and senktide (0.1 µM, 76±13%, n=12), but higher than that induced by GR 64,349 (0.1 µM, 52±7%, n=12, p<0.05). The NOS inhibitors L-NAME, L-NMMA, AG (1 µM) superfused before any contractile agent, failed to contract the muscle strips. In contrast, all three provoked a transient contraction (40-70 s) of high amplitude (65±12% compared to KCl, n=24) when superfused 10 min after SP. This contractile response was time-(after SP) and SP-concentration-dependent, and was never obtained when the strip was stimulated by any selective NK-agonist (up to 10 µM), by CCh (0.1 µM) or KCl (60 mM). Blockade of NK1 and NK2 receptors by CP 99,941 and SR 48,968 (1 µM) reduced the response to SP (0.1 µM), but did not prevent the contraction induced by L-NAME, L-NMMA and AG (1 µM) after SP. The response was prevented by L-ARG but not by D-ARG (1 µM).

Conclusion: Substance P, by a mechanism not involving "classical" NK-receptors (i.e. NK1, 2, 3), enables NOS inhibitors to contract guinea pig ileum in vitro. This effect is likely to result from NOS activation by SP. Thus, L-NAME, L-NMMA, AG by blocking a putative SP-induced NO production, unveil an underlying contractile state whose origin remains to be elucidated.

G. AUTHOR'S NAME, ADDRESS, PHONEName Rafaël GARCIA-VILLARAddress INRA - Department of Pharmacology-Toxicology
180 Chemin de Tournefeuille - BP3
31931 TOULOUSE CEDEX - FRANCETelephone (Office) 33.61.28.51.51 (Fax) 33.61.28.53.10**A. ABSTRACT DESCRIPTOR**

A numbered list of abstract descriptors appears on the reverse of this form. Please enter below the number of the descriptor you have circled that best fits the subject of your abstract. Select one descriptor only.

Descriptor number 42**B. POSTER ONLY**

Check here ☐ if you want your abstract presented only as a poster.

C. PRIZES AND AWARDS

Check the appropriate box if you are applying for an AGA Foundation Student Abstract Prize or AGA Foundation International Travel Award.

- ☐ Student Abstract Prize
☐ International Travel Award

D. DISCLOSURE OF INTEREST/FUNDING

Was this research funded by a pharmaceutical company?

☐ Yes ☒ No

If so, do you have a financial relationship with this company?

☐ Yes ☐ No

E. DDW INFORMATION

Check here ☒ if you are not a member of AGA or AASLD and would like to receive meeting registration information.

F. SUBMISSION FEE

(separate payment must accompany each abstract)

Check Payment \$50.00 # _____

☒ Check must be made payable to W.B. Saunders Company, payable in US dollars, and drawn on a US bank.

Credit Card Payment \$50.00

☐ MasterCard ☐ Visa ☐ American Express
card # _____
exp. date _____
type name _____
signature _____

Purchase Order \$60.00

☐

SUBMIT ABSTRACT PLUS THREE PHOTOCOPIES TO:

AGA
c/o PRISM Productions
5040 Pine Creek Drive
Westerville, OH 43081-4821
Phone: 614/895-1484

I (senior author) affirm that this abstract meets all of the requirements stated in this booklet, that the authors named are familiar with the presented data, and that box D has been completed. On behalf of all of the authors, I hereby transfer copyright in the above abstract to the American Gastroenterological Association.

Signed _____

06 DEC, 1994

- **LOCALISATION OF THE CORTICAL CENTRES FOR HUMAN OESOPHAGEAL SENSATION USING MAGNETOENCEPHALOGRAPHY.** P.L. Furlong, *O. Aziz, K. Singh, Ian Holliday, G. Barnes, G.F.A. Harding & *D.G. Thompson. Depts Vision Sciences & *Medicine, Aston & *Manchester Universities, U.K.

Background: Although mapping of Cortical Evoked Potentials can provide some information about the topography of oesophageal afferents, the precise anatomical representation of oesophageal sensation on the cerebral cortex remains unknown. **Aims:** 1) To identify the anatomical representation of afferents arising from the oesophagus on the cortex. 2) To determine evidence for viscerotopic representation of proximal and the distal oesophageal afferents. **Methods:** 1. **Oesophageal stimulation:** In 3 healthy volunteers, two series of 50 stimuli were applied at a frequency of 0.2 Hz by rapidly inflating a 2 cm silicone balloon to volumes which were clearly perceived but not painful, either 3 cm below the upper oesophageal sphincter or 5 cm above the lower oesophageal sphincter at a frequency of 0.2 Hz. A series of 50 sham inflations were also performed at each site. 2. **Signal Detection:** A 19 channel magnetometer was used to identify the magnetic field waveforms generated by the cerebral cortex during oesophageal stimulation. Recording was made over the parieto-temporal and the frontal cortices bilaterally. Each subject was studied on at least two occasions to obtain evidence for reproducibility. Global field power analysis of the averaged magnetic field waveforms was performed to identify strong dipolar field patterns. Inverse Solution Algorithms were then applied to determine the three dimensional representation of the calculated dipole sources. This technique has been shown to identify cortical sources accurately with an error margin of 2-5 mm. 3. **Coregistration with Magnetic Resonance (MR) Scans:** Three fiducial points (nasion and two preauriculars) were digitised using a magnetic positioning system (Polhemus Navigation System), allowing the location of the head to be accurately determined with respect to the magnetometer. The same fiducial points were marked on MR scans using radio-opaque markers to allow co-registration of magnetometry and MR data in each subject. **Results:** Stimulation of either the proximal or the distal oesophagus evoked reproducible early and late magnetic field wave forms from both hemispheres at a latency of 150 msec to 223 msec and 178 msec to 311 msec respectively. Sham stimulation failed to evoke cortical magnetic fields. Co-registration of calculated dipoles with the MR scans indicated that the early waveforms from the proximal and the distal oesophagus were evoked by two distinct neural sources located in the primary somatosensory cortex close to the vertex, the source for the proximal oesophagus being more medial and superficial than that of the distal oesophagus. In contrast, the late wave forms for both oesophageal regions were localised to a similar area of the insular cortex. **Conclusions:** The centre for human oesophageal sensation is viscerotopically located in both primary somatosensory cortices with secondary representation in the insular cortex.

- **CHARACTERIZATION OF P₂ RECEPTOR-MEDIATED CONSTRICTION OF SUBMUCOSAL ARTERIOLES IN GUINEA PIG ILEUM.** J.J. Galligan, A. Herring and T. Harpstead. Department of Pharmacology & Toxicology, Michigan State University, E. Lansing, MI 48824

Sympathetic nerve stimulation causes constriction mediated by ATP acting at P₂ receptors in submucosal arterioles. In the present study, the P₂ receptor subtype mediating this response was characterized. A computer-assisted video monitoring system was used to measure drug-induced changes in arteriolar diameter (40-80 μ m) in small pieces of submucosa *in vitro*. Intracellular microelectrodes were used to record membrane potential of arteriolar smooth muscle. The rank order potency for constriction caused by several ATP analogs was (EC₅₀, μ M): α,β -methylene ATP (0.3) > β,γ -methylene ATP (4.1) = 2-methylthioATP (4.5) > ATP (12) > ADP (150) > AMP (>300). Constrictions caused by α,β -methylene ATP were competitively blocked by suramin (K_B = 3.2 μ M) and noncompetitively blocked by pyridoxal phosphate-6-azophenyl-2',4'-disulfonic acid (PPADS, 0.3-3 μ M). Norepinephrine (NE)-induced constrictions were unaffected by suramin (100 μ M) or PPADS (3 μ M). α,β -methylene ATP (1 μ M) depolarized arteriolar smooth muscle by 35 \pm 2 mV (resting membrane potential, -81 \pm 4 mV, n = 4). Extracellular solutions with 0 added calcium blocked constrictions caused by α,β methylene ATP (0.1-1.0 μ M) but not those caused by NE (3-10 μ M). Nifedipine (0.3-1 μ M) blocked constrictions caused by 40 and 60 mM KCl but not those caused by α,β methylene ATP (0.1-3 μ M). These data indicate that P_{2X} receptors mediate ATP-induced constriction of submucosal arterioles. Although activation of P_{2X} receptors causes smooth muscle depolarization, calcium entry through voltage-operated, nifedipine-sensitive calcium channels does not contribute to P_{2X}-mediated vasoconstriction. P_{2X}-mediated vasoconstriction is caused by calcium entry through ATP-gated channels. (Supported by the American Heart Association, DK40210 and NS01738)

- **INCREASED OESOPHAGEAL SENSITIVITY TO DISTENSION DURING PHYSICAL AND MENTAL STRESSFUL CONDITIONS.** F. Galeazzi, M.G. Lucà, G. Mastropalo and Remo Naccarato. Department of Gastroenterology - University of Padova - ITALY

Patients with oesophageal complaints often refers their symptoms to stressful events, but the oesophageal dysmotility patterns evoked by physical and mental stress are rarely related to symptoms. The aim of this study was to evaluate whether the sensitivity of the oesophagus to distension is altered after mental and physical experimental stress.

METHODS. In 8 patients (4 males, 17 - 51 yrs) referred for oesophageal symptoms (6 heartburn, 1 non cardiac chest pain and 1 dysphagia) with normal upper g.i. endoscopy and oesophageal manometry, a stepwise distension (2 ml every 10 seconds up to 20 ml) of the oesophagus was performed with a latex balloon (4x2x2 cm) 10 cm above LOS. Sensory (first perception of distension) and pain thresholds were assessed in basal conditions and two more times in the same session, in a random order: 1) after physical stress (cycles of 45-second immersion of non dominant hand in iced water followed by 15 seconds at room temperature until patient's tolerance) and, 2) after a mental test (sequence of logical and practical operations for 30 minutes while having a dichotomous listening disturbance). Blood pressure and heart rate were monitored during the tests and 20-minute recovery periods were allowed between the stressors.

RESULTS. Blood pressure and heart rate increased by 17.7% and 11% and by 9.7% and 14.8% during the cold stress the mental stress respectively. The sensory and pain oesophageal thresholds (ml of balloon inflation) significantly decreased with the two stressful events as follows:

	Basal	Cold Stress	Mental Stress
Sensory Threshold	9.0 \pm 2.0	6.2 \pm 2.3 *	6.2 \pm 2.3 *
Pain Threshold	16.6 \pm 4.7	10.9 \pm 4.0 **	14.2 \pm 5.8 **

(M \pm SD) * p < 0.005 vs basal values; ** p < 0.02 vs basal values

CONCLUSIONS. Physical and mental experimentally induced stress reduce the sensory and pain oesophageal thresholds, being the perception of distension is not influenced by the specific type of stress applied. The mental and the physical stresses seem more active on sensory and pain thresholds respectively. It is possible that in stressful situations patients' oesophageal symptoms are not only due to changes in motility patterns but also to an high increase in oesophageal sensitivity.

- **EVIDENCE FOR NITRIC OXIDE SYNTHASE ACTIVATION BY SUBSTANCE P THROUGH A MECHANISM NOT INVOLVING "CLASSICAL" NEUROKININ RECEPTORS IN GUINEA PIG ILEUM.** R. Garcia-Villar, Ch. Dupuy, J. Fioramonti & L. Bueno. INRA, Department of Pharmacology, BP.3, 31931, Toulouse, France.

Both substance P (SP), a tachykinin neuropeptide which contracts, and nitric oxide (NO), a labile transmitter that relaxes intestinal smooth muscle, have been proposed as active components of the non-adrenergic non-cholinergic (NANC) control of gut motility. To address the functional relationships between these two NANC mediators, we tested the hypothesis that NO-synthase (NOS) could be activated after SP-induced contractions in guinea pig ileum, and further, whether the neurokinin (NK) receptor subtypes described so far (i.e. NK1, 2, 3) could be involved in this NOS activation.

Methods: Longitudinal strips of guinea pig ileum (8 x 2 mm) were superfused (Krebs, 37°C, 2 ml/min) with tachykinin agonists (1 pM to 10 μ M; SP, GR 73,632 [NK1], GR 64,349 [NK2], senktide [NK3]), and antagonists (1 μ M; CP 99,941 [NK1], SR 48,968 [NK2]), as well as KCl (60 mM) and carbachol (CCh; 0.1 μ M). The NO-related agents (1 μ M) used were: L-NAME, L-NMMA, aminoguanidine (AG), L-arginine (L-ARG) and D-arginine (D-ARG). All drugs were superfused for 3 min. Motility was recorded (isometric system) and quantified (integrated area of activity).

Results: Both SP and NK-receptor agonists (1 pM to 10 μ M) produced concentration-dependent contractions, with maximal effects at 0.1-1 μ M. The response to 0.1 μ M SP was 81 \pm 9% (n=16) of that to KCl (60 mM), very close to that elicited by CCh (0.1 μ M; 87 \pm 5%, n=16), GR 73,632 (0.1 μ M; 74 \pm 11%, n=12) and senktide (0.1 μ M, 76 \pm 13%, n=12), but higher than that induced by GR 64,349 (0.1 μ M, 52 \pm 7%, n=12, p<0.05). The NOS inhibitors L-NAME, L-NMMA, AG (1 μ M) superfused before any contractile agent, failed to contract the muscle strips. In contrast, all three provoked a transient contraction (40-70 s) of high amplitude (65 \pm 12% compared to KCl, n=24) when superfused 10 min after SP. This contractile response was time- (after SP) and SP-concentration-dependent, and was never obtained when the strip was stimulated by any selective NK-agonist (up to 10 μ M), by CCh (0.1 μ M) or KCl (60 mM). Blockade of NK1 and NK2 receptors by CP 99,941 and SR 48,968 (1 μ M) reduced the response to SP (0.1 μ M), but did not prevent the contraction induced by L-NAME, L-NMMA and AG (1 μ M) after SP. The response was prevented by L-ARG but not by D-ARG (1 μ M).

Conclusion: Substance P, by a mechanism not involving "classical" NK-receptors (i.e. NK1, 2, 3), enables NOS inhibitors to contract guinea pig ileum *in vitro*. This effect is likely to result from NOS activation by SP. Thus, L-NAME, L-NMMA, AG by blocking a putative SP-induced NO production, unveil an underlying contractile state whose origin remains to be elucidated.