

PHD THESIS

**TRANSMITTERS OF EXTRINSIC AND INTRINSIC NERVES
OF VISCERAL ORGANS
(FUNCTIONAL EVIDENCE)**

Rita Benkó MD

**PhD School: Drug Research
Leader: Prof. Loránd Barthó**

**PhD program: Function and pharmacology of autonomic and
sensory neurons
Leader: Prof. Loránd Barthó**

**Department of Pharmacology and Pharmacotherapy
Faculty of Medicine, University of Pécs**

Pécs

2006

INTRODUCTION

Efferent nerves of the skin, mucous membranes and viscera originate from two sources, i.e., from the autonomic and the somatic nervous systems. These tissues and organs also contain afferent nerves. Nerves within viscera can be classified in different ways. They can be divided into intrinsic neurons (i.e., those with cell bodies and processes within the given organ) and extrinsic ones (that send axons to the given organ).

The present work deals with the functional innervation of the intestinal tract and the urinary bladder. While the gastrointestinal (GI) tract contains a massive intrinsic nervous system of considerable complexity (i.e., the enteric nervous system, ENS), comparatively few and scattered intrinsic nerves can be found in the wall of the bladder. Extrinsic visceral neurons can be either autonomic efferents (parasympathetic praeganglionic, sympathetic postganglionic fibres) or afferents (which, however, may also be able to release transmitters at the periphery).

Autonomic nervous system can be divided into sympathetic, parasympathetic and enteric systems (ENS) (Wood, 1994). The ENS contains approximately as many neurons as the spinal cord (i.e., 10^7 - 10^8 neurons). These intrinsic neurons regulate many GI functions. Extrinsic nerves modulate GI function mainly by influencing intrinsic neurons. Most parts of the intestinal tract are able to function without extrinsic influences.

Intrinsic neurons take place in the ganglionated plexuses of the gut wall (i.e., the myenteric and submucous plexuses). These neurons can influence other neurons and/or smooth muscle cells and other effector tissues (enteric neurons). The most important neurotransmitter of enteric excitatory motoneurons is acetylcholin that acts on muscarinic receptors and thereby contracts circular and/or longitudinal muscles of the gut. When muscarinic receptors are blocked (e.g., by atropine), nerve stimulation is still able to evoke excitatory responses that are mediated by tachykinins (substance P, neurokinin A).

Inhibitory motoneurons of the gut wall release nitric-oxide (NO) as the main transmitter, but this is evidenced for the mediator role of ATP, vasoactive intestinal polypeptide (VIP), pituitary adenylate cyclase-activating polypeptide (PACAP), calcitonin gene related peptide (CGRP) and carbon-monoxide (CO) as well (Goyal and Hirano, 1996).

NO plays a transmitter role in the opening of sphincters, in the peristaltic reflex (descending inhibition), as well as the receptive relaxation of the stomach. NO plays a role in the non-adrenergic, non-cholinergic (NANC) relaxation in response to electrical stimulation, as well as in the regulation of spontaneous tone (see Whittle, 1994). NO is also able to elicit

excitatory responses in the intestine. Some preparations are contracted by NO via a stimulation of intrinsic neurons, while in other preparations NO causes primary contraction on the smooth muscle directly (Barthó and Lefebvre, 1994; Lefebvre and Barthó, 1997).

Extracellular ATP can play a neurotransmitter role („purinergic nerves”; Burnstock, 1972). Moreover, ATP may also play a cotransmitter and modulator role in the nervous system (Burnstock, 1997; Hoyle, 1992). Receptors of ATP have many types, i.e., P_{2X} receptors (currently with 7 subtypes) and P_{2Y} receptors (currently with 8 subtypes; see British J. Pharmacol.: Guide to Receptors and Channels, 2nd edition, 2006). ATP can mediate both excitatory and inhibitory responses; it probably participates in contracting or relaxing vascular smooth muscle, contracting the vas deferens, relaxing GI smooth muscle. Some inhibitory responses of GI preparations are co-mediated by ATP and NO (see Barthó et al., 1998). To date, there has been no evidence for such an interplay in the human GI system.

Sensory neurons of the gut can also be classified as intrinsic and extrinsic. Intrinsic sensory neurons are activated by stretch and other stimuli; they activate interneurons that in turn stimulate enteric motoneurons. These latter mediate the contraction orally and relaxation anally to the stimulus, called „the law of intestine” (Bayliss and Starling, 1899).

Extrinsic sensory neurons originate from the vagus nerve or the spinal cord. A special subgroup of spinal afferents is capsaicin-sensitive neurons (Szolcsányi, 1984). Capsaicin is the pungent substance in paprika (*Capsicum annum*). It stimulates a specific, ion channel-bound receptor (TRPV1). Opening of this channel causes membrane depolarisation in the sensory nerve ending (or varicosity). This, besides afferent activation, causes a release of biologically active substances from these nerve endings, in a manner not dependent on voltage-activated (tetrodotoxin-sensitive) Na⁺-channels or neuronal voltage-dependent Ca²⁺-channels. Biologically active substances released from capsaicin-sensitive afferents include neuropeptides (CGRP, substance P, neurokinin A) and possibly other substances (ATP, NO). All this evokes a variety of tissue responses (e.g., vasodilatation, increase in vascular permeability, smooth muscle contraction or relaxation) (Jancsó et al., 1967, 1968; Szolcsányi, 1984; Barthó et al., 2004).

The urogenital system is also innervated by capsaicin-sensitive afferents. CGRP and tachykinins release in these organs mostly originates from sensory neurones (Maggi, 1995; Barthó et al., 2004). Sensory neuron-mediated responses show inter-species variations and also differ as to different regions of, e.g., the bladder (detrusor muscle, bladder neck). The present work will not deal with capsaicin-sensitive responses of the airways and blood vessels, though this is also a highly interesting and well-studied area.

AIMS

We planned in vitro experiments to get a better insight into the motor responses of visceral organs (GI system, urogenital tract). An important aspect was also the comparison of data obtained on animal preparations with human tissues (obtained from the Department of Surgery). We have been interested in the possible roles of sensory and other NANC transmitters in visceral responses in health and disease, interactions of these substances and possible drug effects upon them (stimulation, antagonism, modulation).

To elicit motor responses of innervated smooth muscle preparations we used the sensory stimulant capsaicin, as well as non-selective „field” stimulation of intramural nerves. We have taken the „identity of effect” as an important criterion, but the „identity of specific antagonism” as the most decisive argument in the identification of a transmitter. In other words, the effect, but first of all, specific inhibition of the exogenous putative transmitter and the stimulation-evoked responses has to be largely similar. Also a specific inhibition of synthesis (NO) or using of genetically modified animals can be regarded as ways of specific inhibition.

Our work is basic research; it helps to understand the function of viscera in health and disease; on the long term, our results can be useful for clinical diagnosis and therapy. Moreover, some conclusions as to the peripheral neurotransmitter systems may have relevance for the central nervous system as well. First of all, primary sensory neurons most probably release the same substances from their peripheral and central endings. Since transmitter identification is easier in viscera than in the spinal cord, the conclusion drawn from such experiments may have implications for nociception and antinociception as well.

Specific aims of the experiments

1. We tested the effects of capsaicin and their mechanisms in circular muscles of various segments of the human intestine, with special emphasis on the NO-guanylate cyclase system. We also tested different species of laboratory animals for a presence of NO-mediated effects of capsaicin.
2. The action of the endocannabinoid anandamide on the human gut has also been tested, with special attention to its possible interaction with TRPV1 receptor.

3. A possible interaction between the NO and purinoceptor-mediated systems have been examined on the rat ileum longitudinal and the human colon circular strips in the mechanism of electrical field stimulation-evoked relaxation.
4. The hypothesis that, along with NO, other guanylate cyclase-stimulating transmitter (e.g. CO) can be involved in the NANC relaxant responses has been examined on several GI preparations.
5. Functional innervation of the urinary bladder has been examined by pharmacological means; we tried to separate cholinergic, purinergic and (sensory) peptidergic responses by using specific drugs.
6. The effect of experimental diabetes on these responses has been studied and the effect of capsaicin has been pharmacologically characterised.

EXPERIMENTAL

Role of NO in the motor effects of capsaicin in intestinal circular muscle preparations of various species

Introduction

Capsaicin shows multiple motor effects on GI preparations. In the guinea-pig ileum, both the neurally-mediated contraction and a relaxant response have been detected (see among others Barthó and Szolcsányi, 1978; Szolcsányi and Barthó, 1978; Barthó et al., 1994, 1999, 2000). The latter may be mediated by CGRP (Barthó et al., 1987; 1991). Capsaicin evokes relaxation on human gut preparations (Maggi et al., 1988, 1990a,b). CGRP is not involved in this tetrodotoxin-resistant response. A possible role of a VIP-like peptide has been proposed (Maggi et al., 1989, 1990a,b).

The aim of the present study was to examine the effects and mechanisms of action of capsaicin on the circular muscle of the human sigmoid colon, other human GI preparations and on the colon of the mouse and guinea-pig, with special reference to the NO-guanylate-cyclase and the purinergic systems. We also tested if the endocannabinoid anandamide is able to mimic or modify the effect of capsaicin on the human colon, since anandamide has been reported to activate TRPV1 receptors (Smart et al., 2000), besides stimulating cannabinoid receptors (Szolcsányi, 2000).

Methods

Guinea-pig proximal colon strips (mucosa-denuded), mouse distal colon zig-zag preparations, circular (mucosa-denuded) strips of human ileum, appendix, ascending and sigmoid colon were studied in conventional organ bath experiments, using oxygenised Krebs solution. Movements were recorded isotonicly. Human appendix and guinea-pig colon preparations had a high spontaneous tone; the other preparations were moderately pre-contracted. NANC conditions were maintained by atropine and the adrenergic blocking drug guanethidine. Responses are expressed as % of the maximal relaxation due to isoprenaline. Capsaicin was given only once to each preparation.

Results

In the human sigmoid colon, both the NO synthesis inhibitor L-NOARG (100 μ M) and the guanylate cyclase inhibitor ODQ (1 μ M) strongly and specifically inhibited the effect of capsaicin. The inhibitory action of L-NOARG was reversed by L-arginine (5 mM). ODQ prevented the relaxation in response to the NO donor sodium nitroprusside (500 nM-1 μ M). Tetrodotoxin was ineffective against capsaicin.

L-NOARG also inhibited the relaxant effect of capsaicin on the human ileal, appendix and ascending colonic circular muscle, as well as on the mouse colon, but not on the guinea-pig colon. The effect of capsaicin was partly inhibited on the mouse colon, but not on the rest of preparations. The P₂ purinoceptor antagonist PPADS (50 μ M) did not influence the effect of capsaicin. PPADS inhibited relaxation induced by the P₂ purinoceptor agonist α,β -methylene ATP (10-30 μ M). The endogen cannabinoid receptor agonist anandamide (1-300 μ M) failed to relax the human colon. The colon of TRPV1 „knockout” mice (Davis et al.,2000) failed to respond to capsaicin.

Discussion

Our data show for the first time that NO is an important mediator of the capsaicin-induced relaxation in the human ileal, appendix and colonic circular muscle, as well as the mouse, but not the guinea-pig colon. A partial inhibitory action of tetrodotoxin on the capsaicin-induced response in the mouse colon might indicate an involvement of intrinsic neurones and/or a sensory axon reflex mechanism. On the other hand, PPADS-sensitive purinoceptors are unlikely to participate in the effect of capsaicin in the human colon.

Capsaicin-sensitive and endocannabinoid mechanisms may have complex interactions. Anandamide has been shown to stimulate TRPV1 receptors (and upon prolonged exposure can also inhibit these receptors). On the other hand, activation of cannabinoid receptor has an inhibitory action on capsaicin-sensitive afferents (see Smart et al., 2000; Szolcsányi, 2000). In our experiment on the human colon, anandamide failed to show any effect. A possible explanation for this may be that the entry of anandamide into sensory neurons is not sufficiently quick to compensate for its elimination (anandamide acts at the intracellular part of the TRPV1 receptor; De Petrocellis et al., 2001).

Nitroergic-purinergetic interactions on human and rat intestinal preparations

Introduction

NANC responses due to electrical field stimulation are predominantly mediated by intrinsic neurons situated in the myenteric plexus; these nerves are insensitive to capsaicin. Both extracellular ATP and NO are respected neurotransmitters in the GI tract. NO-mediated mechanisms can be inhibited by a blockade of the NO synthase. For inhibiting purinergetic transmission receptor antagonists, ATP desensitisation and, with some reservations, the K⁺-channel blocker apamin can be used.

The aim of the present experiment was to assess the combined effectiveness of NO synthase inhibition and P₂ purinoceptor antagonism. The effect of guanylate cyclase inhibition has also been tested on the NANC relaxation. The questions to be answered were as follows.

- (a) Do purinergetic nerves participate in the NANC relaxation?
- (b) If yes, what is their functional relation to nitroergic mechanisms? Can a combined inhibition of these mechanisms fully account for the NANC response?
- (c) Do nitroergic nerves act via an activation of the guanylate cyclase also involved in the NANC relaxation?

Methods

Myenteric plexus-longitudinal muscle strips of the rat ileum were prepared with the method of Paton and Vizi (1969). Human colonic tissue was obtained and circular strips were prepared as described above. Organ bath experiments with electrical field stimulation were conducted and movements were recorded isotonicly. Rat preparations were precontracted with

prostaglandin $F_{2\alpha}$ and human strips with histamine. NANC relaxations were tetrodotoxin-sensitive in both preparation.

Results

Rat ileal strip

NANC relaxation to stimulation was reduced by L-NOARG (from 38 to 26 % relaxation, $p < 0,05$). Apamin alone caused a reduction by one third, while L-NOARG plus apamin caused a nearly full inhibition. L-NOARG plus the purinoceptor antagonists PPADS (50 μM) or suramin (100 μM) practically abolished the NANC relaxation. PPADS alone caused a moderate inhibition. Subsequent administration of L-NOARG caused full inhibition.

The inhibition of guanylate cyclase with ODQ (1 μM) failed to add to the reduction of the NANC response by L-NOARG. ODQ strongly inhibited the relaxant effect of nitroglycerin (13 μM). PPADS (50 μM) strongly and specifically inhibited relaxation induced by ATP (1 μM) or α,β -methylene ATP (10 μM).

Human sigmoid colon

NANC relaxation in response to electrical field stimulation (1 or 10 Hz) was reduced to its half by L-NOARG (100 μM). A further strong inhibition was produced by an addition of PPADS (50 μM) plus suramin (100 μM), but either PPADS or suramin alone (in the presence of L-NOARG) was without effect. Likewise, a combination of PPADS and suramin was ineffective in the absence of L-NOARG. The guanylate cyclase inhibitor ODQ (3 μM) reduced the NANC relaxation in the absence, but not in the presence of L-NOARG.

PPADS plus suramin strongly, while PPADS (but not suramin) moderately inhibited the relaxant effect of α,β -methylene ATP (5 μM). PPADS plus suramin failed to influence relaxations evoked by the adrenergic β -receptor agonist isoprenaline or the Ca^{2+} -channel blocker nifedipine.

Discussion

Purinergetic and nitrenergic nerves co-mediate the NANC relaxation in the rat longitudinal and human colonic circular muscle. Our study has been the first to investigate the combined effects of NO synthase inhibition and purinoceptor antagonism. These data show that the two mechanisms together can account for practically the entire NANC relaxation, while Smits and Lefebvre (1996), using ATP desensitisation, concluded that a third transmitter (which is not

CO) is also involved. The apamin-sensitive (L-NOARG-resistant) component of the response may be identical with the purinergic one. Apamin, however, can also inhibit the effects of other relaxants (e.g., neuropeptides).

P₂ purinoceptors seem to be involved in the NANC relaxation of the human colon as well, however, their interaction with the nitrenergic mechanisms seems to be more complex. First, a combination of PPADS and suramin was necessary to inhibit the non-nitrenergic response. We believe that this combination covers a wider scale of purinoceptors than either drug alone. Second, even in combination these drugs failed to reduce the NANC relaxation in the absence of L-NOARG, i.e., a non-additive relationship exists between the two systems.

The guanylate cyclase inhibitor ODQ reduced the NANC response in both the rat (Tanovic et al., 2001) and human preparations, but failed to add to the inhibitory action of the NO synthase inhibitor L-NOARG. This seems to indicate that (a) NO acts via the guanylate cyclase and (b) we do not have to assume a mediating role of any other stimulant of the guanylate cyclase (e.g., CO) in the NANC relaxation.

Effect of experimental diabetes on purinergic, cholinergic and peptidergic motor responses evoked in the rat urinary bladder by electrical field stimulation and capsaicin

Introduction

Purinergic neurotransmission has long been known to play a role in bladder functions in mammals (see Hoyle, 1994; Burnstock, 1997). There are data on other neurotransmitters as well. Capsaicin causes atropine-resistant contraction in the rat bladder; this is inhibited by previous capsaicin treatment or tachykinin antagonists, but not by tetrodotoxin (Maggi et al., 1991). This shows that capsaicin releases, in a tetrodotoxin-insensitive manner, tachykinins from sensory nerve endings and thereby causes contraction of the rat bladder smooth muscle. By applying long-term electrical stimulation Meini and Maggi (1994) demonstrated a capsaicin-sensitive tonic contraction in the bladder detrusor muscle.

Diabetic neuropathy encounters autonomic, somatomotor and sensory neurons. A possible manifestation of neuropathy is bladder dysfunction. Data of the literature are contradictory as to the effects of experimental diabetes on the effect of capsaicin on the

bladder. Both reduced (Kamata et al., 1992; Pinna et al., 1994) and unchanged responses to capsaicin have been described (Santicioli et al., 1987; Dahlstrand et al., 1992).

The aim of the present study was to investigate the effect of experimental diabetes on cholinergic, purinergic and capsaicin-sensitive responses of the rat bladder. Better than any other study, we separated these kinds of responses from each other by pharmacological means. Capsaicin-sensitive responses were evoked by capsaicin administration or by long-term electrical stimulation. Moreover, it was tested whether capsaicin is able to elicit relaxation in precontracted preparation or to inhibit cholinergic or purinergic neurotransmission.

Methods

Streptozotocin treatment

Male Wistar rats were given streptozotocin (50 mg/kg) or its solvent, 8 weeks before the experiments. Animals with blood glucose below 13 mM were excluded.

Isolated bladder

Two detrusor preparations were made up from each animal. Movements were recorded isotonicly. Electrical field stimulation (120 V, 0.1 ms, single pulses or 1 Hz for 30 s, or 10 Hz for 40 s) was applied. PPADS (50 μ M) plus suramin (100 μ M) was administered to inhibit purinergic (thus isolate cholinergic) responses and atropine (1 μ M) for studying purinergic responses. Capsaicin-sensitive responses were studied in the presence of atropine and purinoceptor antagonists. Guanethidine (3 μ M) was present throughout for an inhibition of sympathetic responses.

Results

Electrical stimulation in the presence of purinoceptor antagonists

Preparations from solvent-treated animals exhibited „twitch” response to single shocks and tonic contraction to 1 Hz stimulation (17 and 46 % of the maximal contraction, respectively). Atropine (1 μ M) reduced these responses to less than their half. Quite similar results were obtained on the bladders of diabetic rats.

On solvent-treated animals, capsaicin tachyphylaxis failed to influence the effects of electrical field stimulation, indicating that they were mediated by capsaicin-insensitive nerves. Responses were blocked by tetrodotoxin (1 μ M).

Electrical stimulation in the presence of atropine

Preparations from solvent-treated animals showed twitch responses (approximately 15 and 40 %, with single shocks and 1 Hz stimulation, respectively). Significantly higher responses were obtained in bladders from diabetic rats (26 and 53 %, respectively). PPADS plus suramin caused approximately 50 % inhibition in both the control and diabetic group. Capsaicin tachyphylaxis was ineffective on this type of contraction as well.

Long-term electrical stimulation

In the solvent group and in the presence of atropine and PPADS plus suramin, 10 Hz stimulation for 40 s resulted in an initial, quick, followed by a smaller, sustained contraction. Tetrodotoxin (1 μM) practically abolished both phases of this response. Capsaicin tachyphylaxis (1 μM for 40 min, without rinsing) slightly decreased the quick and strongly inhibited the tonic phase of the response.

In bladders of diabetic animals the tonic component of the response to electrical stimulation was less than half of the controls (while the initial one was slightly enhanced). Capsaicin tachyphylaxis further reduced the tonic component (practically to zero).

Table 1

Effect of capsaicin tachyphylaxis to contractions evoked by sustained electrical stimulation (10 Hz for 40 s) on the rat bladder detrusor preparation. Fast and slower (tonic) phases of the response were distinguished. Mean \pm S.E.M. *-Significant difference from the control response (Wilcoxon's signed rank test); #-significantly different from the solvent-treated group (Mann-Whitney test); n denotes the number of preparations.

	Control 10 Hz 40 s	Capsaicin tachyphylaxis (1 μM)	n
<i>Solvent-treated group</i>			
quick	26.1 \pm 4.0	19.7 \pm 2.7*	12
sustained	25.2 \pm 4.5	4.7 \pm 1.6*	12
<i>Diabetic group</i>			
quick	40.3 \pm 3.2 [#]	31.4 \pm 4.0* [#]	12
sustained	10.4 \pm 2.5 [#]	1.3 \pm 0.9*	12

Capsaicin-induced contraction

30 nM and 1 μM capsaicin was administered in a cumulative fashion, each for 3 min. Atropine (1 μM), PPADS (50 μM) and suramin (100 μM) was present throughout the

experiments. Responses to capsaicin were approximately 20 % smaller in the diabetic than in the solvent treated group. The difference proved statistically significant at 1 μ M of capsaicin. Capsaicin pretreatment abolished the contractile effect of capsaicin (30 nM or 1 μ M), both in the solvent-treated and in the diabetic group.

Pharmacological sensitivity of the capsaicin-induced contraction was studied in a separate group of untreated animals. The excitatory response to capsaicin (300 nM) was not influenced by tetrodotoxin (1 μ M) or a combination by tetrodotoxin (1 μ M) plus ω -conotoxin GVIA (an inhibitor on neuronal Ca^{2+} channels; 500 nM). Neither the tachykinin NK_1 receptor antagonist SR 140 333 (200 nM), nor the tachykinin NK_2 receptor antagonist SR 48 968 (200 nM) had any inhibitory effect on the capsaicin-induced contraction. By contrast, a combination of the two tachykinin antagonists strongly (by approximately 80 %) inhibited the effect of capsaicin.

Capsaicin fails to relax the bladder

We examined the possibility of a relaxant effect of capsaicin that is normally masked by the contractile one. Preparations were treated with tachykinin antagonists plus atropin or purinoceptor antagonists. Capsaicin (1 μ M, 2 min before stimulation) failed to influence responses to single electrical shocks or 1 Hz stimulation. In preparations treated with tachykinin antagonists only and precontracted with acetylcholin (1 μ M) capsaicin (1 μ M) did not evoke any relaxant response.

Effect of purinoceptor antagonists on the contractile action of ATP in the rat bladder

A combination of PPADS (50 μ M) and suramin (100 μ M) strongly inhibited the contractile effect of exogenous ATP (1-100 μ M).

Discussion

As far as it was possible, the cholinergic, purinergic and capsaicin-sensitive neuronal responses of the bladder were separated by pharmacological means. The nature of the „remaining” responses in the presence of atropine and purinoceptor antagonists remains obscure. Cholinergic and purinergic responses (as well as the „remaining” contraction) seem to be relatively insensitive to experimental diabetes (purinergic responses have in fact increased).

Early observations of Maggi et al. (1991) on the tachykininergic mediation of the contractile response of capsaicin on the rat bladder were confirmed in the present experiments with the aid of receptor subtype-specific tachykinin receptor antagonists (SR 140 333 and SR

48 968). Since neither of these substances was effective on its own, a supra-additive collaboration between NK₁ and NK₂ receptors has to be presumed. We did not find evidence for any kind of acute inhibitory effect of capsaicin on the smooth muscle tone or on cholinergic or purinergic responses.

A selective strong inhibition of the tonic capsaicin-sensitive contraction in diabetic rats makes it likely that diabetes damages capsaicin-sensitive neurons rather than smooth muscle cells.

SUMMARY OF THE NEW RESULTS

Our results concerning the mechanisms of visceral motor responses revealed substantial species differences; while in the guinea-pig intestine mainly tachykinin-mediated excitation and CGRP-mediated inhibition is found (see Barthó et al., 2004), an NO-mediated relaxant response has been found in the human intestinal circular muscle and in the mouse colon. The lack of effect of tetrodotoxin on the response to capsaicin in human preparations makes an involvement of intrinsic nitrergic neurons unlikely; the possibility that NO originates from the sensory neurons themselves can be raised but has not been proven yet. To this end, we plan to test inhibitors of neuronal NO synthase.

Co-transmission is an increasingly recognised phenomenon in the peripheral as well as in the central nervous system. In nearly all of our findings co-transmission is likely to play a role; purinergic and nitrergic mechanisms (in an apparently additive manner) in the rat ileal NANC relaxation, the same (but a clearly non-additive manner) in the human colonic NANC relaxation; purinergic and cholinergic mechanisms in autonomic responses of the rat bladder; finally, tachykinin NK1 and NK2 receptor-mediated (hence, probably involving the two preferential endogenous agonist ligands, substance P and neurokinin A) in the response to capsaicin, likewise in the rat bladder.

With a sequential use of the NO synthase inhibitor L-NOARG and the guanylate cyclase inhibitor ODQ we proved a mediating role of this enzyme in the „nitrergic” responses of intestinal preparations, but we did not find any evidence for parallel involvement of another guanylate cyclase stimulating transmitter (e.g., CO).

We have demonstrated a fairly selective damaging effect of experimental diabetes on the capsaicin-sensitive neurons supplying the rat urinary bladder.

ACKNOWLEDGEMENTS

I express my sincere thanks to *Prof. Loránd Barthó*, leader of our laboratory research activity; colleagues and coworkers, *Drs. Zsófia Lázár, Sarolta Undi and Mátyás Wolf; László Lénárd Jr.* for introducing me to some experimental designs; *Prof. Gyula Mózsik and Dr. Ágnes Király* for critically reading the first version of this work. My parents lent me continuous moral support.

REFERENCES

- Barthó L, Benkó R, Patacchini R, Pethő G, Holzer-Petsche U, Holzer P, Lázár Z, Undi S, Illényi L, Antal A, Horváth ÖP, 2004. Effects of capsaicin on visceral smooth muscle: a valuable tool for sensory neurotransmitter identification. *Eur. J. Pharmacol.* 500: 143-157.
- Barthó L, Kóczán G, Holzer P, Maggi CA, Szolcsányi J, 1991. Antagonism of the effects of calcitonin gene-related peptide and of capsaicin on the guinea-pig isolated ileum by human alpha-calcitonin gene-related peptide(8-37). *Neurosci. Lett.* 129: 156-159.
- Barthó L, Lázár Zs, Lénárd LJr, Benkó R, Tóth G, Penke B, Szolcsányi J, Maggi CA, 2000. Evidence for the involvement of ATP, but not of VIP/PACAP or nitric oxide, in the excitatory effect of capsaicin in the small intestine. *Eur. J. Pharmacol.* 392: 183-188.
- Barthó L, Lefebvre R, 1994. Nitric oxide induces acetylcholine-mediated contractions in the guinea-pig small intestine. *Naunyn-Schmiedeberg's Arch. Pharmacol.* 350: 582-584.
- Barthó L, Lénárd LJr, Patacchini R, Halmi V, Wilhelm M, Holzer P, Maggi CA, 1999. Tachykinin receptors are involved in the "local efferent" motor response to capsaicin in the guinea-pig small intestine and oesophagus. *Neuroscience* 90: 221-228.
- Barthó L, Lénárd LJr, Szigeti R, 1998. Nitric oxide and ATP co-mediate the NANC relaxant response in the guinea-pig taenia caeci. *Naunyn-Schmiedeberg's Arch. Pharmacol.* 358: 496-499.
- Barthó L, Pethő G, Antal A, Holzer P, Szolcsányi J, 1987. Two types of relaxation due to capsaicin in the guinea-pig ileum. *Neurosci. Lett.* 81:146-150.
- Barthó L, Szolcsányi J, 1978. The site of action of capsaicin on the guinea-pig isolated ileum. *Naunyn-Schmiedeberg's Arch. Pharmacol.* 305: 75-81.
- Bayliss WM, Starling EH, 1899. The movements and innervation of the small intestine. *J. Physiol.* 24: 99-143.
- Burnstock G, 1972. Purinergic nerves. *Pharmacol Rev.* 24: 509-560.
- Burnstock G, 1997. The past, present and future of purine nucleotides as signalling molecules. *Neuropharmacol.* 36: 1127-1139.
- Caterina MJ, Julius D, 2001. The vanilloid receptor: a molecular gateway to the pain pathway. *Ann. Rev. Neurosci.* 24: 487-517.
- Clapham DE, Montell C, Schultz G, Julius D, 2003. International Union of Pharmacology. XLIII. Compendium of voltage-gated ion channels: transient receptor potential channels. *Pharmacol. Rev.* 55: 591-596.
- Dahlstrand C, Dahlström A, Ahlman H, Jonsson O, Lundstam S, Norlén L, Pettersson S, 1992. Effect of substance P on detrusor muscle in rats with diabetic cystopathy. *Br. J. Urol.* 70: 390-394.
- Davis JB, Gray J, Gunthorpe MJ, Hatcher JP, Davey PT, Overend P, Harries MH, Latcham J, Clapham C, Atkinson K, Hughes SA, Rance K, Grau E, Harper AJ, Pugh PL, Rogers DC, Bingham S, Randall A, Sheardown SA, 2000. Vanilloid receptor-1 is essential for inflammatory thermal hyperalgesia. *Nature* 405: 183-187.
- De Petrocellis L, Bisogno T, Maccarrone M, Davis JB, Finazzi-Agro A, DiMarzo V, 2001. The activity of anandamide at vanilloid VR1 receptors requires facilitated transport across the cell membrane and is limited by intracellular metabolism. *J. Biol. Chem.* 276: 12856-12863.
- Goyal RK, Hirano I, 1996. The enteric nervous system. *New England J. Med.* 334: 1106-1115.
- Hoyle CH, 1992. Transmission: purines. In: *Autonomic Neuroeffector Mechanisms*, ed. Burnstock G, Hoyle CH; Harwood Academic Publishers, Chur, 1992, pp. 367-407.
- Hoyle CH, 1994. Non-adrenergic, non-cholinergic control of the urinary bladder. *World J. Urol.* 12: 233-244.
- Jancsó N, Jancsó-Gábor A, Szolcsányi J, 1967. Direct evidence for neurogenic inflammation and its prevention by denervation and by pretreatment with capsaicin. *Br. J. Pharmacol. Chemother.* 31: 138-151.
- Jancsó N, Jancsó-Gábor A, Szolcsányi J, 1968. The role of sensory nerve endings in neurogenic inflammation induced in human skin and in the eye and paw of the rat. *Br. J. Pharmacol. Chemother.* 33: 32-41.
- Kamata K, Inoue K, Kasuya Y, 1992. Changes in contractile responses of the urinary bladder to substance P in streptozotocin-induced diabetic rats. *Gen. Pharmacol.* 24: 547-553.
- Lecci A, Santicioli P, Maggi CA, 2002. Pharmacology of transmission to gastrointestinal muscle. *Curr. Op. Pharmacol.* 2: 630-641.
- Lefebvre RA, Barthó L, 1997. Mechanism of the nitric oxide-induced contraction in the rat isolated small intestine. *Br. J. Pharmacol.* 120: 975-981.
- Maggi CA, 1995. Tachykinins and calcitonin gene-related peptide (CGRP) as co-transmitters released from peripheral endings of sensory nerves. *Prog. Neurobiol.* 45: 1-98.
- Maggi CA, Giuliani S, Santicioli P, Patacchini R, Said SI, Theodorsson E, Turini D, Barbanti G, Giachetti A, Meli A, 1990a. Direct evidence for the involvement of vasoactive intestinal polypeptide in the motor response of the human isolated ileum to capsaicin. *Eur. J. Pharmacol.* 185: 169-178.

- Maggi CA, Giuliani S, Zagorodnyuk V, 1996. Calcitonin gene-related peptide (CGRP) in the circular muscle of guinea-pig colon: role as inhibitory transmitter and mechanisms of relaxation. *Regulatory Peptides* 61: 27-36.
- Maggi CA, Patacchini R, Santicioli P, Giuliani S, 1991. Tachykinin antagonists and capsaicin-induced contraction of the rat isolated urinary bladder: evidence for tachykinin-mediated cotransmission. *Br. J. Pharmacol.* 103: 1535-1541.
- Maggi CA, Santicioli P, Del Bianco E, Geppetti P, Barbanti G, Turini D, Meli A, 1989. Release of VIP- but not CGRP-like immunoreactivity by capsaicin from the human isolated small intestine. *Neurosci. Lett.* 98: 317-320.
- Maggi CA, Theodorsson E, Santicioli P, Patacchini R, Barbanti G, Turini D, Renzi D, Giachetti A, 1990b. Motor response of the human isolated colon to capsaicin and its relationship to release of vasoactive intestinal polypeptide. *Neuroscience* 39: 833-841.
- Meini S, Maggi CA, 1994. Evidence for a capsaicin-sensitive, tachykinin-mediated, component in the NANC contraction of the rat urinary bladder to nerve stimulation. *Br. J. Pharmacol.* 112: 1123-1131.
- Paton WDM, Vizi ES, 1969. The inhibitory action of noradrenaline and adrenaline on acetylcholine output by guinea-pig ileum longitudinal muscle strip. *Br. J. Pharmacol.* 35: 10-20.
- Pinna C, Bolego C, Puglisi L, 1994. Effect of substance P and capsaicin on urinary bladder of diabetic rats and the role of the epithelium. *Eur. J. Pharmacol.* 271: 151-158.
- Santicioli P, Gamse R, Maggi CA, Meli A, 1987. Cystometric changes in the early phase of streptozotocin-induced diabetes in rats: evidence for sensory changes not correlated to diabetic neuropathy. *Naunyn-Schmiedeberg's Arch. Pharmacol.* 335: 580-587.
- Smart D, Gunthorpe MJ, Jerman JC, Nasir S, Gray J, Muir AI, Chambers JK, Randall AD, Davis JB, 2000. The endogenous lipid anandamide is a full agonist at the human vanilloid receptor (hVR1). *Br. J. Pharmacol.* 129: 227-230.
- Smits GJM, Lefebvre RA, 1996. ATP and nitric oxide: inhibitory NANC neurotransmitters in the longitudinal muscle-myenteric plexus preparation of the rat ileum, *Br. J. Pharmacol.* 118: 695-703.
- Szolcsányi J, 1984. Capsaicin-sensitive chemoceptive neural system with dual sensory-efferent function. In: *Antidromic Vasodilatation and Neurogenic Inflammation*, ed. Chahl LA, Szolcsányi J, Lembeck F; Akadémiai Kiadó, Budapest, 1984, pp. 27-56.
- Szolcsányi J, 2000. Anandamide and the question of its functional role for activation of capsaicin receptors. *Trends Pharmacol. Sci.* 21: 203-204.
- Szolcsányi J, Barthó L, 1978. New type of nerve-mediated cholinergic contractions of the guinea-pig small intestine and its selective blockade by capsaicin. *Naunyn-Schmiedeberg's Arch. Pharmacol.* 305: 83-90.
- Tanovic A, Jimenez M, Fernandez E, 2001. Actions of NO donors and endogenous nitric oxide on the longitudinal muscle of rat ileum *in vitro*: mechanisms involved. *Life Sci.* 69: 1143-1154.
- Whittle BJ, 1994. Nitric oxide in gastrointestinal physiology and pathology. In: Johnson, L.R. (Ed.) *Physiology of the Gastrointestinal Tract*, 3rd ed. Raven Press, New York, pp. 267-294.
- Wood JD, 1994. Physiology of the enteric nervous system. In: Johnson, L.R. (Ed.) *Physiology of the Gastrointestinal Tract*, 3rd ed. Raven Press, New York, pp. 423-475.

PUBLICATIONS INCLUDED IN THIS THESIS

- Barthó L, Benkó R, Lázár Z, Illényi L, Horváth ÖP, 2002, Nitric oxide is involved in the relaxant effect of capsaicin in the human sigmoid colon circular muscle. *Naunyn-Schmiedebergs' Arch. Pharmacol.* 366: 496-500. IF: 2,6
- Benkó R Lázár Z, Pórszász R, Somogyi G, Barthó L, 2003, Effect of experimental diabetes on cholinergic, purinergic and peptidergic motor responses of the isolated rat bladder to electrical field stimulation or capsaicin. *Eur. J. Pharmacol.* 478: 73-80. IF: 2,4
- Benkó R, Lázár Z, Undi S, Illényi L, Antal A, Horváth ÖP, Rumbus Z, Wolf M, Maggi CA, Barthó L, 2004, Inhibition of nitric oxide synthesis blocks the inhibitory response to capsaicin in intestinal circular muscle preparations from different species. *Life Sci.* 76: 2773-2782. IF: 2,2
- Barthó L, Benkó R, Patacchini R, Pethő G, Holzer-Petsche U, Holzer P, Lázár Z, Undi S, Illényi L, Antal A, Horváth ÖP, 2004, Effects of capsaicin on visceral smooth muscle: a valuable tool for sensory neurotransmitter identification. *Eur. J. Pharmacol.* 500: 143-157. IF: 2,4
- Benkó R, Undi S, Wolf M, Magyar K, Tóvölgyi Z, Rumbus Z, Barthó L, 2006, P₂ purinoceptors account for the non-nitregic NANC relaxation in the rat ileum. *Naunyn-Schmiedebergs' Arch. Pharmacol.* 4: 319-324. IF: 2,1
- Under preparation for publication:*
- Benkó R, Undi S, Wolf M, Illényi L, Kassai M, Cseke L, Horváth ÖP, Antal A, Magyar K, Tóvölgyi Z, Barthó L, 2006. P₂ purinoceptor antagonists inhibit the NANC relaxation of the human colon *in vitro*. *Neuroscience*.

OTHER PUBLICATIONS

- Lénárd L Jr, Lázár Z, Benkó R, Szigeti R, Báthori Z, Tóth GK, Penke B, Barthó L, 2000, Inhibitory effect of PACAP(6-38) on relaxations induced by PACAP, VIP and non-adrenergic, non-cholinergic nerve stimulation in the guinea-pig taenia caeci. *Naunyn-Schmiedebergs' Arch. Pharmacol.* 361: 492-497. IF: 2,9
- Barthó L, Lázár Z, Lénárd L Jr, Benkó R, Tóth G, Penke B, Szolcsányi J, Maggi CA, 2000, Evidence for the involvement of ATP, but not of VIP/PACAP or nitric oxide, in the excitatory effect of capsaicin in the small intestine. *Eur. J. Pharmacol.* 392: 183-188. IF: 2,2

- Rózsai B, Lázár Z, Benkó R, Barthó L, 2001, Inhibition of the NANC relaxation of the guinea-pig proximal colon longitudinal muscle by the purinoceptor antagonist PPADS, inhibition of nitric oxide synthase, but not by a PACAP/VIP antagonist. *Pharmacol. Res.* 43: 83-87. IF: 0,9
- Lázár Z, Shahbazian A, Benkó R, Tóth G, Penke B, Barthó L, Holzer P, 2001, PACAP(6-38) inhibits the effects of vasoactive intestinal polypeptide, but not PACAP, on the small intestinal circular muscle. *Eur. J. Pharmacol.* 431: 259-264. IF: 2,2
- Lázár Z, Benkó R, Bölskei K, Rumbus Z, Wolf M, Holzer P, Maggi CA, Barthó L, 2003, Actions of endothelin and corticotropin releasing factor in the guinea-pig ileum: no evidence for an interaction with capsaicin-sensitive neurons. *Neuropeptides* 37: 220-232. IF: 2,2
- Benkó R, Undi S, Wolf M, Barthó L, 2005, Effects of acute administration of and tachyphylaxis to α,β -methylene ATP in the guinea-pig small intestine. *Basic & Clinical Pharmacology & Toxicology* 97: 369-373. IF: 1,5
- Barthó L, Undi S, Benkó R, Wolf M, 2005, Ideg-simaizom transzmitterek a gyomor-bélhuzamban: A motilitászavarok lehetséges háttere. *Praxis* 14 (12): 14-19.
- Barthó L, Undi S, Benkó R, Wolf M, Lázár Z, Lénárd L Jr, Maggi CA, 2006, Multiple motor effects of ATP and their inhibition by PPADS in the small intestine of the guinea-pig. *Basic & Clinical Pharmacology & Toxicology* 98: 488-495. IF: 1,5
- Undi S, Benkó R, Wolf M, Illényi L, Horváth ÖP, Antal A, Csontos Z, Vereczkei A, Barthó L, 2006, Purinergic nerves mediate the non-nitric oxide relaxation of the human ileum in response to electrical field stimulation. *Brain Res. Bulletin* (in press). IF: 2,5

PRESENTATIONS AT MEETINGS

- Lénárd L Jr, Barthó L, Benkó R, Báthori Z, Tóth G, Penke B, PACAP-antagonista gátolja a nem-adrenerg, nem-kolinerg elemnyedést a coecumban. MITT vándorgyűlés, Harkány, 1999. jan. 27-30. [**Abstract:** *Neurobiology* 7: 349-350, 1999]
- Barthó L, Lénárd L Jr, Benkó R, Tóth G, Penke B, PACAP antagonist inhibits non-adrenergic, non-cholinergic (NANC) relaxation in the guinea-pig taenia caeci. *Neuropeptides - Ferrara 1999* (9th Annual Meeting of the European Neuropeptide Club, Ferrara, Italy, 12-15 May, 1999. [**Abstract:** *Regul. Pept.* 80:146, 1999]
- Barthó L, Lénárd L Jr, Szigeti R, Benkó R, Báthori Z, Tóth G, Penke B, Nitric oxide, ATP and pituitary adenylate cyclase activating polypeptide (PACAP) mediate the relaxant response of the guinea-pig taenia caeci. Second European Congress of Pharmacology, July 3-7, 1999, Budapest, Hungary. [**Abstract:** *Fundam. Clin. Pharmacol.* 13/Suppl. 1, 1999, 77s]

- Lázár Z, Lénárd LJr, Benkó R, Tóth G, Penke B, Szolcsányi J, Maggi CA, Barthó L, Evidence for the participation of P₂ purinoceptors in the excitatory effect of capsaicin in the small intestine. The 6th European Congress of Pharmaceutical Sciences, September 16-19, 2000, Budapest, Hungary. [Abstract: Eur. J. Pharmaceutical Sci. 11/Suppl. 1, 2000, S113]
- Barthó L, Lázár Z, Lénárd LJr, Benkó R, Tóth G, Penke B, Szolcsányi J, Maggi CA, P₂ purinoceptors, but not vasoactive intestinal polypeptide/pituitary adenylate cyclase activating polypeptide or nitric oxide, play a role in the excitatory effect of capsaicin on myenteric neurons. Hung. Physiol. Soc./Br. Physiol. Soc. Meeting, Budapest, 27-29 May, 2000. [Abstract: J. Physiol. Lond. 526 P, 167 P.]
- Lázár Z, Benkó R, Barthó L, Nem nitrerg, nem VIP-erg gátló válasz légsőben. Magyar Idegtudományi Társaság (MITT) VIII. Konferenciája, Szeged, 2001. január 24-27.
- Barthó L, Benkó R, Lázár Z, Illényi L, Horváth ÖP, A kapszaicin mozgató hatása humán vastagbélben. A Magyar Élettani Társaság (MÉT) kongresszusa, Szeged, 2001. június 6-8.
- Barthó L, Benkó R, Lázár Z, Illényi L, Horváth ÖP, Motor effects of capsaicin in the human colon. 7th Meeting of the Austrian Neuroscience Association, Seggau/Leibnitz, Styria, Austria, 2-5 September, 2001.
- Lázár Z, Shahbazian A, Benkó R, Tóth G, Penke B, Barthó L, Holzer P, PACAP(6-38) inhibits the effects of VIP, but not PACAP, on the small intestinal circular muscle. 7th Meeting of the Austrian Neuroscience Association, Seggau/Leibnitz, Styria, Austria, 2-5 September, 2001.
- Benkó R, Lázár Z, Pórszász R, Barthó L, Csökkent zsigeri kapszaicin-érzékeny válaszok streptozotocin-diabéteszes patkányban. A Magyar Diabetes Társaság XVI. Kongresszusa, Debrecen, 2002. május 30-június 2. [Abstract: Diabetologia Hungarica 10, Suppl. 1, 10. old., 2002]
- Barthó L, Benkó R, Lázár Z, Illényi L, Horváth ÖP, Kapszaicin-érzékeny idegek lokális efferens hatásai humán colonon. A Magyar Gasztroenterológiai Társaság 44. Nagygyűlése, Balatonaliga, 2002 június 4-8. (Motilitási Szekció, június 6.)
- Benkó R, Lázár Z, Undi S, Rumbus Z, Wolf M, Barthó L, A nitrogén-monoxid szerepe a kapszaicin hatásában különböző fajok bél körkörös izomzatán. A Magyar Élettani Társaság (MÉT) kongresszusa, Debrecen, 2004. június 7-9.
- Benkó R, Undi S, Wolf M, Illényi L, Kassai M, Cseke L, Barthó L, Nitrerg-purinerg interakciók tengerimalac-, patkány- és humán izolált bélen. A Magyar Élettani Társaság (MÉT) kongresszusa, Budapest, 2005. június 2-4. [Extended abstract: Nitrergic-purinergic interactions in the guinea-pig, rat, and human intestine. Acta Physiol. Hung. 92 (3-4): 244-245.]
- Barthó L, Benkó R, Undi S, Pethő G, Sensory neurotransmitters and modulators: the basis of analgesic research. 1st BBBB Conference on Pharmaceutical Sciences, Siófok, Hungary, September 26-28, 2005.
- Undi S, Benkó R, Wolf M, Magyar K, Barthó L: A guanilat-cikláz gátló ODQ hatása az elektromos téringerléssel kiváltott simaizom-válaszokra és a perisztaltikus reflexre. A Magyar Experimentális Farmakológia Tavasz Szimpóziuma, Budapest, 2005. június 6-7. [Abstract: Inflammopharmacology 13: (5-6) 572-573.]
- Wolf M., Undi S, Benkó R, Magyar K, Tóvölgyi Z, Barthó L, Egy guanilat-cikláz gátló vegyület hatása az elektromos téringerléssel kiváltott simaizom-válaszokra és a perisztaltikus reflexre. A Magyar Élettani Társaság (MÉT) LXX. Vándorgyűlése, Szeged, 2006. június 7-9.

- Undi S, Wolf M, Benkó R, Illényi L, Cseke L, Kassai M, Vereczkei A, Horváth ÖP, Antal A, Barthó L, Purinerg gátló válasz humán ileumon. A Magyar Élettani Társaság (MÉT) LXX. Vándorgyűlése, Szeged, 2006. június 7-9.
- Undi S, Wolf M, Benkó R, Illényi L, Kassai M, Cseke L, Antal A, Horváth ÖP, Barthó L, P₂ purinoceptor antagonisták gátló hatása human colon NANC relaxációjára *in vitro*. A Magyar Experimentális Farmakológia Szimpóziuma, Pécs, 2006. június 3.
- Benkó R, Undi S, Wolf M, Magyar K, Illényi L, Kassai M, Cseke L, Horváth ÖP, Antal A, Barthó L, NO and ATP co-mediate the non-adrenergic, non-cholinergic (NANC) relaxation in the human colon and rat ileum. 15th World Congress of Pharmacology, IUPHAR, Peking, 2006. július 2-7. [**Abstract:** Acta Pharmacologica Sinica, 2006, Suppl. 1: P 110.]
- Undi S, Benkó R, Wolf M, Lázár Z, Lénárd L Jr., Maggi CA, Barthó L, Effects of ATP and alpha, beta-methylene ATP (ABMA) and their inhibition by PPADS in the non-stimulated and field-stimulated guinea-pig ileum. 15th World Congress of Pharmacology, IUPHAR, Peking, 2006. július 2-7. [**Abstract:** Acta Pharmacologica Sinica, 2006, Suppl. 1: P 111.]