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Neurotransmitters of the non-adrenergic non-cholinergic relaxation of proximal stomach

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Abstract. – The proximal third of the stomach (fundus plus oral corpus) relaxes during swallowing so that it can hold large amounts of food with limited increases in intraluminal pressure. This mechanism has been called "receptive relaxation" and is mediated by a vago-vagal reflex. When the food bolus reaches the stomach, gastric relaxation is maintained by another reflex starting from mechanoreceptors in the gastric wall. This second mechanism has been named "adaptive relaxation" or "gastric accommodation" and involves both intramural and vagal reflex pathways, whose inhibitory neurons are always intramural. There was initially a great deal of controversy about the identity of the neurotransmitter/s released by inhibitory neurons, but at present nitric oxide (NO) and vasoactive intestinal polypeptide (VIP) are considered to be the most likely candidates. Several lines of evidence indicate that adenosine triphosphate (ATP) might be implicated too. It seems that these neurotransmitters are co-released from the inhibitory motor neurons and are responsible for the different features of the NANC relaxation induced by low- or high-frequency neuronal firing. NO (and perhaps ATP) would be responsible for the rapid beginning and the initial rapid development of the relaxation evoked by neuronal firing at low- or high-frequency and VIP for the long duration of the relaxation evoked by high-frequency neuronal activation. This review will deal mainly with the physiological characteristics and pharmacological features of the NANC relaxation of the proximal stomach and the evidences favoring or excluding a role as inhibitory neurotransmitters of ATP, NO and VIP in different species.

Key Words:

Non-adrenergic non-cholinergic (NANC), Relaxation, Nitric oxide (NO), Vasoactive intestinal polypeptide (VIP), Peptide histidine isoleucine (PHI), Adenosine triphosphate (ATP), Apamin.

Introduction

The first demonstration that the gastric smooth muscle relaxes in response to vagal stimulation under non-cholinergic experimental conditions (in the presence of atropine) was obtained *in vivo* in the cat and the rabbit by Langley et al.[1]. The reversal by atropine of the effects of vagal stimulation and nicotine was first observed in isolated nerve-muscle preparations of the cat stomach by McSwiney & Robson[2] and Ambache[3], respectively. The explanation given to these findings was that the vagus contained both excitatory (cholinergic) and inhibitory (adrenergic) fibers and that two distinct sets of postganglionic parasympathetic neurons existed, one cholinergic and the other adrenergic[3]. An inhibitory motor response of the stomach was also shown following transmural stimulation in the presence of atropine[4]. At the beginning of the 1960s, the concept of non-adrenergic non-cholinergic (NANC) neurotransmission was proposed for the first time by Burnstock et al.[5,6], who demonstrated inhibitory junction potentials (IJPs) resistant to bretylium, an adrenergic neuron blocker, in the guinea-pig intestinal smooth muscle under non-cholinergic conditions. During the same years it was shown that the relaxation of the stomach induced by vagal stimulation in cats treated with atropine was not affected by adrenergic neuron blockers[7]. Even though the inhibitory motor response was not affected by reserpine, Paton and Vane[4] attributed the relaxation of the guinea-pig stomach caused by transmural stimulation to the release of noradrenaline from adrenergic myenteric neurons. Since then, however, it became clear that a non-adrenergic neurotransmitter was responsible for this kind of response. Consequently, the concept of a NANC inhibitory motor neurotransmission became soon firmly estab-

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lished. Since the end of 1960s, NANC inhibitory motor responses have been demonstrated not only in the gastrointestinal tract of many species, included the human, but also in the genitourinary, respiratory and cardiovascular systems[8-10].

Following the first demonstration of the NANC inhibitory motor responses, a long standing debate on the nature of the NANC neurotransmitters ensued. Adenosine triphosphate (ATP) and vasoactive intestinal polypeptide (VIP) have for a long time been the most accredited candidates. Until the late 1980s, a strong conflict developed between advocates of the "purinergic" or the "vipergic" hypothesis. At that time, nitric oxide (NO) came up as the most certain neurotransmitter released by the inhibitory motor neurons in most systems, including the gastrointestinal tract. At present, there is general agreement that various neurotransmitters are co-released by the inhibitory motor neurons, with NO[11], VIP[12,13] and ATP[14,15] being the most important. In particular, it seems that they have different roles in the stomach, with NO (and, perhaps, ATP) mediating the rapid, short-lived relaxation and VIP being responsible for the late, sustained relaxant responses[16].

This review will deal with the most recent aspects concerning the inhibitory motor neurotransmission to the smooth muscle of the stomach of different species, so as to provide the reader with a comprehensive picture of the neurotransmitters responsible for the NANC relaxation of the proximal stomach.

Neurotransmitter/s released by the inhibitory motor neurons of the proximal stomach

Adenosine triphosphate (ATP)

ATP has been the first molecule hypothesized to be the neurotransmitter responsible for the nerve-mediated NANC relaxation of the gastrointestinal smooth muscle[17]. ATP induces monophasic (relaxant) or biphasic motor responses (initial rapid relaxation followed by a sustained contraction) in the proximal stomach of different species, depending on ATP concentrations and, in experiments on strip preparations, the substance used to pre-contract gastric smooth muscle[16]. ATP induces inhibitory motor responses or IJPs in the smooth muscle of rat[18-26], cat[23], guinea-pig[14,27-30] mouse[31-34] and rabbit[35] stomach. Although ATP induces gastric relaxation *in vivo* in the dog[36], ATP-induced contractions are usually observed in strips of canine corpus *in vitro*[37]. ATP has usually a low efficacy in inducing a relaxant effect. This finding has been put in relationship to the fact that ATP also stimulates the production of contracting prostaglandins by the tissues. The latter would then counteract the relaxation induced by ATP and be responsible for the secondary contraction of the biphasic response[21,38]. However, the ATP-induced relaxant response is moderate even when cycloxygenase inhibitors abolish prostaglandin synthesis[21].

Pharmacological tools used to reduce or block ATP-produced relaxations of the proximal stomach were not always able to antagonize the relaxant responses induced by activation of the intramural inhibitory motor neurons. 2-2' Pyridylisatogen tosylate, a substance reported to be a specific ATP receptor antagonist[19,39], tachyphylaxis to ATP[20] or apamin, a blocker of small conductance Ca^{2+}-activated K^+ channels[23], inhibited ATP-produced relaxations, but did not reduce the relaxation caused by electrical field stimulation (EFS) of rat proximal stomach strips. On the contrary, other researchers reported that desensitization to α,β-methylene ATP[22,25] and apamin[25,40,41]were able to antagonize EFS-evoked relaxant responses of rat gastric fundus strips. The desensitizing effect produced by α,β-methylene ATP, however, turned out to be non-specific in at least one study[25].

Conflicting results have been published regarding the role of ATP in the inhibitory motor responses of the proximal stomach in other species. In the guinea-pig, the first studies date back to a few years after the suggestion by Burnstock et al.[18] that ATP or a related substance could be the neurotransmitter responsible for the NANC inhibition of gastrointestinal smooth muscle. Okwuasaba et al.[42] showed that theophylline caused effective antagonism of both ATP-induced and nerve mediated relaxations of isolated strips from the guinea-pig gastric fundus. However, two years later, Baer & Frew[43] reported opposite conclusions by using the same pharmacological tools. Huizinga & Den Hertog[27] definitively demonstrated that theophylline does not antagonize ATP-induced inhibitory responses of the guinea-pig proximal stomach, thus confirming the findings published by Baer & Frew[43]. The P1 purinoceptor antagonists 8-phenyl theophylline and 8-(p-bromophenyl) theophylline reduced the relaxation

of the guinea-pig proximal stomach produced by ATP, suggesting that ATP could be hydrolysed to adenosine, with subsequent activation of P1 purinoceptors. However, the P1 purinoceptor antagonists failed to antagonize the EFS-evoked NANC relaxation[29]. Costa et al.[44] showed that apamin was able to reduce the relaxation of the gastric fundus induced by α,β-methylene ATP, but not that induced by stimulation of enteric inhibitory neurons. On the contrary, the NANC IJP of the circular muscle of the guinea-pig gastric fundus was reduced by apamin or suramin, but whereas apamin inhibited the membrane hyperpolarization produced by ATP, suramin did not, making unlikely the involvement of ATP in this kind of response[45]. In the cat gastric fundus, ATP induces relaxations at high concentrations and its relaxant effect is not antagonized by apamin[23]. In the mouse isolated stomach, apamin and adenosine 5'-*O*-2-thiodiphosphate, which desensitizes P2Y purinoceptors, abolish and reduce the ATP-induced relaxation, respectively, and inhibit the slow component of the relaxation evoked by low-frequency EFS, suggesting that ATP is involved in this response[33]. The increases in gastric volume induced by vagal stimulation *in vivo* in the rabbit are antagonized by reactive blue 2 at doses able to reduce the relaxant responses induced by ATP[35]. These findings do not agree with those of Andrews & Lawes[46], who showed that high doses of α,β-methylene ATP are unable to block the vagally induced fall in gastric corpus pressure in the ferret.

Only one paper was published in which ATP concentrations were measured in the incubation medium of gastric preparations. In this study, it has been shown that high-frequency EFS induces ATP release from rat gastric fundus strips. This release was tetrodotoxin (TTX)-sensitive[22]. No data, however, are available in the literature concerning the frequency- and the calcium-dependence of ATP release.

In 2000, a paper was published the title of which stated that ATP is the third inhibitory NANC neurotransmitter in the rat gastric fundus[24]. This conclusion was based on the evidence that PPADS, a P2 receptor antagonist, or apamin greatly reduced the non-nitrergic nonpeptidergic component of the NANC relaxation induced by low-frequency EFS. Non-nitrergic nonpeptidergic conditions were achieved with an inhibitor of nitric oxide synthase (NOS), N^G-nitro-L-arginine methyl ester (L-NAME), plus a peptidase, α-chymotrypsin. However, apamin is a peptide sensitive to the degrading effect of α-chymotrypsin, as shown by the fact that apamin is no longer effective in inhibiting ATP-induced relaxation of the rat gastric fundus in the presence of the peptidase[26]. In addition, PPADS was unable to inhibit the non-nitrergic nonpeptidergic component of the NANC relaxation induced by high-frequency EFS[26]. In this latter study, apamin, but not PPADS, inhibited the non-nitrergic nonpeptidergic component of the NANC relaxation of the rat gastric fundus observed in the presence of L-NAME plus an anti-VIP serum[26]. Thus, it seems that a third inhibitory neurotransmitter exists in the rat gastric fundus, the effect of which is apamin-sensitive; however, it cannot be identified with ATP.

VIP and related peptides

The role of VIP, a 28-amino-acid peptide, as a neurotransmitter was recognized a few years after its isolation by Said & Mutt in 1970[47] from the porcine small intestine[48,49]. It is co-synthesized from the same gene precursor with PHI, a 27-amino-acid peptide (P) with an N-terminal histidine (H) and a C-terminal isoleucine (I) isolated from porcine duodenum by Tatemoto & Mutt in 1981[50]. VIP and PHI co-localize in neurons of various tissues and produce similar biological effects in various systems[51]. Two C-terminal-extended forms of PHI, i.e. peptide histidine glycine (PHI-Gly) and peptide histidine valine [PHV(1-42)], have been isolated and characterized. They co-localize with PHI in a number of rat tissues, including the stomach, where they account for 65% of the detectable PHI-LI[52].

VIP, PHI and related peptides induce concentration-dependent relaxations of fundus strips prepared from the rat stomach[20,53-55]. PHI, PHI-Gly and PHV(1-42) are 2-7 times less potent than VIP in inducing a relaxation[55]. The relaxations produced by these peptides are not immediate (differently from NO- or ATP-induced relaxant responses), begin after a certain time lag and are slowly developing. On the contrary, EFS-evoked relaxations start without any latency and develop rapidly, independently of the frequency used. In addition, peptide-induced relaxations are long-lasting, i.e. the muscle tone existing before peptide administration is recovered gradually when exogenously applied peptides are removed from the bath medium, and the recovery period is concentration-dependent[55]. VIP is significantly more potent and effective than PHI and related peptides

when the muscle tone during the gradual recovery of its basal level is expressed as area under the curve (AUC), a parameter indicating the duration of the relaxation[55]. A similar feature is observed when gastric fundus strips are stimulated by high-frequency EFS[55], suggesting that these peptides could be responsible for the long duration of high-frequency EFS-induced relaxation of the proximal stomach[26,55]. Findings from release experiments support this hypothesis. In fact, VIP-like immunoreactivity (LI) and PHI-LI are released from the rat gastric fundus only by high-frequency EFS in a frequency-dependent, Ca^{2+}-dependent and TTX-sensitive manner[51,55,56].

As far as other species are concerned, VIP relaxes strips of the proximal stomach or the *in vitro* or *in vivo* whole stomach of different species: guinea-pig[30,57,58], mouse[33,59,60], cat[23,61-64], dog[65-67], pig[68-70], rabbit[71], ferret[46] and human[72]. In addition, many researchers reported VIP-LI release from the stomach of different species: in response to EFS from rat gastric fundus or antrum strips[22,41], and from guinea-pig gastric fundus strips[30], in the venous effluent from a vascularly perfused isolated rat stomach preparation in response to vagal stimulation[73] and in the gastric vein *in vivo* in response to vagal stimulation in the cat[61] and in the dog[65]. VIP- and PHI-LI co-release in the gastric venous effluent has also been shown in the dog *in vivo* in response to vagal stimulation[66]. In many of these studies, EFS or vagal stimulation has been performed at high-frequency (≥10 Hz), confirming that high-frequency stimulation is necessary to evoke VIP-LI release from gastric neurons.

The role of VIP as an inhibitory motor neurotransmitter in the proximal stomach has been demonstrated in many studies. Unfortunately, useful receptor antagonists are not easily available to evaluate the involvement of VIP in physiological responses. For this reason, in most published studies, peptidases or anti-VIP sera were the pharmacological tools most commonly used. In the rat gastric fundus, the first study showing a role for peptides in the NANC relaxation was published in 1987 by De Beurme & Lefebvre[74]. They showed that α-chymotrypsin and trypsin, at concentrations able to abolish VIP-induced relaxation, did not reduce the maximal amplitude of the NANC relaxation induced by EFS at an intermediate frequency (5 Hz), but inhibited the relaxation measured after EFS cessation. Trypsin was also shown to be able to inhibit the relaxations induced by low-frequency EFS at a pulse train length >30 sec[75]. Trypsin's inhibitory effects on the NANC relaxation were progressively greater with longer train durations[75]. In these initial studies, no direct proof of a VIP involvement in NANC relaxation was obtained. They only demonstrated the existence of a peptidergic component, mainly responsible for the maintenance of the response. They also suggested that the initial rapid phase of the relaxation is due to other neurotransmitters that, in addition, are able to produce maximal relaxation when the peptidergic component is blocked. Other studies confirmed that trypsin or α-chymotrypsin, at concentrations able to block the relaxant response to VIP, reduces the EFS-induced relaxation of the rat gastric fundus[40,73,76,77].

Definitive proof of VIP involvement in the neurally induced relaxation of the rat proximal stomach came from the use of specific antisera. Three studies published in the late 1980s and early 1990s reported that anti-VIP sera, at dilutions that abolished VIP-induced relaxation, inhibited the NANC relaxation evoked by EFS at an intermediate frequency (5 Hz)[40,78,79]. Similarly to what was shown for α-chymotrypsin and trypsin, anti-VIP sera significantly reduced the amplitude of relaxation measured at the end of the stimulation, not the maximal amplitude[78,79]. A clarification of the role of VIP (and PHI) in the NANC relaxation of the gastric fundus came from the evaluation of the effects of peptidases and antisera on the response evoked by high-frequency EFS. α-Chymotrypsin and an anti-VIP serum, at concentrations able to abolish VIP-induced relaxation, greatly attenuated the AUC of high frequency EFS-evoked relaxant responses, but did not affect their maximal amplitude[26,55]. An anti-PHI serum induced a qualitatively similar effect[55]. These studies showed that a peptidergic component accounts for about 70-75% of the duration of the relaxant response evoked by high-frequency EFS. Most of this component is due to the action of VIP. A peptidergic component seems also to be present also in the relaxation induced by low-frequency EFS, but VIP and PHI do probably not contribute to this response. Another conclusion that can be drawn from the studies is that the other neurotransmitters involved in the NANC inhibitory neurotransmission are able to compensate for the inhibition of the peptidergic component as far as the amplitude of the response is concerned. No "rescue" mechanisms are foreseen as far as the duration of the response is concerned.

The picture is more complicated if we look at studies performed on the stomach of other species. Actually, the first study evaluating the effect of an anti-VIP serum on the EFS-induced NANC relaxation of the proximal stomach was performed in the guinea-pig[30]. These authors reported that an anti-VIP serum significantly reduced the relaxation induced by low-frequency EFS. These results were questioned by Lefebvre et al.[80], who showed that a NOS inhibitor abolished low-frequency EFS-evoked NANC relaxations of the guinea-pig gastric fundus. The effect of peptidases or anti-VIP sera on the duration of high-frequency EFS-evoked NANC relaxations has not yet been evaluated in this species. However, it is very probable that a peptidergic component is present also in the NANC relaxation of the guinea-pig proximal stomach, as suggested by Lefebvre et al.[80]. Strong evidence for a role of VIP in the neurally mediated relaxation of the proximal stomach has been put forward in the cat[62,64,81]. In this species, trypsin[62,81], α-chymotrypsin[64] and an anti-VIP serum[62] reduce the NANC inhibitory motor response induced by EFS of gastric strips. Evidence for the involvement of one or more peptides in the NANC relaxation of the proximal stomach is available also for the mouse[33], the pig[69], the ferret[46], the dog[67] and the human[72]. In the human gastric fundus, Tonini et al.[72] showed that the desensitization to the relaxant effect induced by VIP abolished the component resistant to N^G-nitro-L-arginine (L-NOARG, a NOS inhibitor) of the NANC relaxation induced by high-frequency EFS.

In conclusion, there is no doubt that part of the neurally evoked relaxation of the proximal stomach is mediated by one or more peptides. In the NANC relaxation induced by low-frequency neuronal firing, the peptidergic component becomes apparent only when NANC neurons are stimulated for a prolonged period of time. In the NANC relaxation induced by high-frequency neuronal firing, the peptides involved have been identified, at least in the rat, cat and human, to be VIP and PHI, which are responsible for the slow recovery of basal tone after the cessation of neuronal activation.

Nitric oxide

It is by now well established that NO is a neurotransmitter in the central and peripheral nervous system. In particular, its role as a neurotransmitter released from the inhibitory motor neurons has been demonstrated in different tissues such as the gastrointestinal, respiratory, genitourinary and vascular systems (for a recent review concerning the gastrointestinal tract, see Toda & Herman[11]). NO and L-citrulline are produced in equimolar amounts by the enzymatic activity of NOS, which catalyzes the reaction between L-arginine and O_2. NO was first identified as endothelium-derived relaxing factor (EDRF)[82] and subsequently shown to be a peripheral inhibitory neurotransmitter[83]. Three NOS isoforms have been isolated and characterized: endothelial (eNOS), neuronal (nNOS) and inducible (iNOS). The neuronal form is constitutive and Ca^{2+}- and calmodulin-dependent[84].

NO concentration-dependently relaxes the smooth muscle of the proximal stomach of various species: rat[85], guinea-pig[80], mouse[86], cat[81], dog[67] and pig[69]. In all these species, the effects induced by bolus administrations of NO are short-lasting, as NO is a labile molecule with a very short half-life. However, it has been shown that continuous NO infusions produce maintained relaxations[87].

The demonstration that NO is involved in the NANC inhibitory neurotransmission to the smooth muscle came from the use of some L-arginine analogues that inhibit the enzymatic activity of NOS. The first to be used, and consequently the most used along the years, were N^G-monomethyl-L-arginine (L-NMMA), L-NOARG (also called L-NNA or L-NA) and L-NAME. Li & Rand[79], by using L-NMMA, the least potent among the three L-arginine analogues, were the first to demonstrate a role of NO as an inhibitory motor neurotransmitter in the stomach. They showed that L-NMMA, but not its enantiomer D-NMMA, inhibited the EFS-induced NANC relaxation of rat gastric fundus strips[79]. The reduction caused by L-NMMA was greatest for relaxations induced by low-frequency or short-duration EFS. In addition, L-NMMA did not reduce the maximal amplitude of the relaxation induced by long-duration EFS at an intermediate frequency (5 Hz), but slowed the initial phase of relaxation[79]. It was evident from this first study that NO is particularly important in determining the rapid beginning of the relaxation. In addition, these results indicated that other neurotransmitters were able to compensate for the blockade of NO as far as the maximal amplitude of the response is concerned. Many other studies followed these original findings, confirming and widening them. They showed that NOS inhibitors nearly abolish the NANC relaxation induced by low-fre-

quency (≤4 Hz) EFS and significantly reduce that evoked by high-frequency EFS when very short (10 s) stimulations are performed[16,75,76]. On the contrary, when long pulse trains of stimulation are used, NOS inhibitors abolish only the response evoked by very low frequency EFS (0.25-0.5 Hz), significantly reduce the relaxation induced by EFS at low frequencies (1-4 Hz) and do not significantly inhibit that induced by high-frequency EFS[16,75,76]. Thus, high-frequency EFS, independently of the length of pulse trains, induces the release of additional neurotransmitters besides NO, which are responsible for the long duration of the relaxant response. It is very probable that they are identical with VIP and PHI[26]. Neurotransmitters other than NO are also released in response to low-frequency EFS, and their action can be seen only when long pulse trains are used. It seems that one or more peptides are released in response to low-frequency EFS, but the available findings do not support their identification as VIP and related peptides[55,75]. In addition to peptides, a neurotransmitter that acts through mechanisms sensitive to apamin but is different from ATP is also released, at least in response to high-frequency EFS[26].

Many other studies have demonstrated the involvement of NO in the NANC relaxation of the rat stomach. *In vitro*, NOS inhibitors have been shown to reduce the relaxation of: 1) fundus strips activated by EFS[88,89], an increase of K^+ ions in the incubation medium[89] or nicotine[90]; 2) corpus strips activated by EFS[91]; 3) the vascularly perfused isolated stomach activated by vagal stimulation or the nicotinic receptor agonist 1,1-dimethyl-4-phenylpiperazinium (DMPP)[73]. *In vivo*, NOS inhibitors inhibited the accommodative reflex of the stomach[92] and reduced the gastric relaxation induced by vagal stimulation[93]. It has also been shown that the EFS-induced NANC IJP of fundus circular smooth muscle cells is reduced by NOS inhibitors[88,89].

Many studies have evaluated the effects of NOS blockade on the proximal stomach of other species. As for the guinea-pig, two important papers were published by Desai et al. in 1991[94,95]. They showed that NOS inhibitors, under NANC conditions, were able to abolish the adaptive relaxation of the whole isolated stomach, measured as rapid increase in its intraluminal volume, induced by stepwise increases in intragastric volume and pressure[94], the nicotinic receptor agonist DMPP[94] or vagal stimulation[95]. These authors excluded the participation of other neurotransmitters in these responses[95]. This concept was confirmed by them in a paper published three years later, in which they presented findings that seemed to exclude an involvement of VIP in the relaxation of the guinea-pig stomach induced by vagal stimulation[57]. On the contrary, as already discussed above, the findings published by Lefebvre et al.[80] do not seem to exclude the involvement of other neurotransmitters besides NO in the EFS-evoked NANC relaxation of guinea-pig gastric fundus strips.

Different groups evaluated the effects of NO blockade on the NANC relaxation of the mouse gastric fundus. NOS inhibitors abolished or greatly reduced the NANC relaxation of longitudinal muscle strips evoked by short-duration EFS (1-8 Hz)[31,96]. Also in this species, NO seems to be responsible for the fast component of the relaxation of longitudinal or circular strips induced by short-duration, high-frequency EFS[59,60]. On the contrary, NOS inhibitors do not significantly reduce the sustained component of the relaxation[59,60] that is mediated by a peptidergic neurotransmitter[59]. Similar results were obtained when EFS-induced decreases in intragastric pressure of the isolated stomach were studied[33].

NOS inhibitors have also been shown to reduce the NANC relaxation of the pig[69], cat[81], dog[67] and human[72] proximal stomach. In all these papers, the authors conclude that two major components in the NANC relaxation of the stomach can be distinguished: a nitrergic one and a peptidergic one, very probably mediated by VIP.

The synthesis of NO has been evaluated in two studies performed on the rat gastric fundus. Boeckxstaens et al.[85] using a superfusion bioassay have shown that EFS of fundus strips causes a TTX-sensitive release of a labile substance that dilatates de-endothelialized rings of rabbit aorta. The released substance was very probably NO, because the relaxation of aorta rings was inhibited by a NOS inhibitor, N^G-nitro-L-arginine. In another study, the synthesis of NO was evaluated by measuring L-citrulline concentrations in the incubation medium of fundus strips[97]. EFS increased the levels of L-citrulline in a Ca^{2+}-dependent and TTX-sensitive manner. A NOS inhibitor blocked the EFS-evoked production of L-citrulline. Surprisingly, the curve concentration-response for the inhibitory effect of the NOS inhibitor on EFS-induced L-citrulline production was to the left of that for the inhibitory effect on the NANC relaxation.

Conclusions

In conclusion, the available data suggest that: 1) NO is responsible for the immediate beginning of the gastric NANC relaxation and for its rapid development; 2) the continuous stimulation of nitrergic nerves can sustain the relaxant response; 3) non-nitrergic components appear only when long-duration stimulations are performed; 4) a peptidergic component can be observed in response to either low- or high-frequency neuronal stimulation; 5) VIP, and perhaps PHI, are released in response to high-frequency neuronal activation and are responsible for the long duration of the relaxation; 6) an apamin-sensitive component can be distinguished in the relaxation, that does not seem to be mediated by ATP; 7) the different components are able, within some limits, to compensate for the blockade of the other ones in determining the amplitude of the response.

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