ELSEVIER

European Journal of Pharmacology 276 (1995) 41–47

ejp

Relaxant influence of phosphodiesterase inhibitors in the cat gastric fundus

Ann J. Barbier, Romain A. Lefebvre *

Heymans Institute of Pharmacology, University of Gent Medical School, De Pintelaan 185, B-9000 Gent, Belgium

Received 8 September 1994; revised 28 December 1994; accepted 30 December 1994

Abstract

The breakdown of the relaxation-inducing second messengers cAMP and cGMP is mediated by phosphodiesterases. Inhibitors of functionally present phosphodiesterases can be expected to induce relaxation by increasing the basic amount of cAMP and/or cGMP. In the cat gastric fundus, vinpocetine, which has some selectivity for phosphodiesterase type I, only induced contractions, but the inhibitors of type III [5-(4-acetimidophenyl)pyrazin-(1*H*)-one; SKF 94120], type IV (rolipram) and type V (zaprinast) phosphodiesterase all caused concentration-dependent relaxation, as did the non-specific phosphodiesterase inhibitor 3-isobutyl-1-methylxanthine (IBMX). The most potent relaxant agent was rolipram (EC_{50} $9 \pm 5 \times 10^{-7}$ M and $3 \pm 1 \times 10^{-7}$ M in longitudinal and circular smooth muscle strips, respectively). These results suggest that type III, IV and V phosphodiesterases are functionally present in the cat gastric fundus and are involved in the regulation of tone. The possible influence of the phosphodiesterase inhibitors on non-adrenergic non-cholinergic (NANC) relaxation induced by nitric oxide (NO), vasoactive intestinal polypeptide (VIP) and train and sustained electrical field stimulation was then tested. Rolipram (3×10^{-8} M), SKF 94120 (10^{-5} M) and IBMX (10^{-6} M) did not potentiate any of the relaxant stimuli studied. Zaprinast (10^{-5} M), the cGMP specific type V phosphodiesterase inhibitor, caused a significant increase of the relaxation induced by exogenous NO and by train electrical field stimulation. These stimuli are thought to induce relaxation via an increase of intracellular cGMP. The type V phosphodiesterase might thus play a role in the metabolism of cGMP, synthesized in the smooth muscle cells by NO, released from NANC nerves.

Keywords: Gastric fundus; NANC (non-adrenergic, non-cholinergic); Cyclic nucleotide; Phosphodiesterase

1. Introduction

The presence of an inhibitory non-adrenergic non-cholinergic (NANC) innervation in the cat gastric fundus is well known (Martinson and Muren, 1963; Martinson, 1965). Both vasoactive intestinal polypeptide (VIP, D'Amato et al., 1988) and nitric oxide (NO, Barbier and Lefebvre, 1993) have been shown to be involved in the relaxation of the cat gastric fundus induced by stimulation of these NANC nerves. It was shown that NO is mainly involved in the short-lasting relaxation elicited by a short train of electrical field stimulation of the NANC nerves, particularly at lower frequencies of stimulation, whereas both NO and VIP contribute to sustained relaxation evoked by long-lasting electrical field stimulation.

The inhibitory neurotransmitters, VIP and NO, exert their relaxant effect via two separate signal transduction mechanisms. The interaction of VIP with its receptor leads to activation of adenylate cyclase, resulting in an increased intracellular content of cyclic adenosine 3′-5′ monophosphate (cAMP; Gozes and Brenneman, 1989). NO promotes the accumulation of cyclic guanosine 3′-5′ monophosphate (cGMP) by activation of soluble guanylate cyclase (Waldman and Murad, 1987). The cyclic nucleotides initiate a cascade of events leading to relaxation.

Cyclic nucleotides are degraded by cyclic nucleotide phosphodiesterases, five main types of which have been described (Beavo and Reifsnyder, 1990). The number of phosphodiesterase types present in a particular cell can vary from three (Nicholson et al., 1991) to five

* Corresponding author. Tel. 32/9/2403373, fax 32/9/2404988.

SSDI 0014-2999(95)00009-7

(Shahid et al., 1991). The phosphodiesterases control the basic amount of cyclic nucleotides in the cell and might influence the cyclic nucleotide response to VIP and NO released from NANC nerves. Selective inhibitors are available for types I, III, IV and V phosphodiesterase (Nicholson et al., 1991). Inhibitors of the phosphodiesterase types present in the smooth muscle cells of the cat gastric fundus can be expected to induce relaxation by increasing the basic amounts of cAMP and/or cGMP (Beavo and Reifsnyder, 1990). Furthermore, the relaxant response to NANC stimulation might be potentiated (Gibson and Mirzazadeh, 1989; Rattan and Moummi, 1989; Rhoden and Barnes, 1990). In this work, the effect per se and the influence on relaxation induced by NO, VIP, and short- and long-lasting electrical field stimulation was studied for the following phosphodiesterase inhibitors: vinpocetine (type I), SKF 94120 (type III), rolipram (type IV), zaprinast (type V) and the non-selective inhibitor 3-isobutyl-1-methylxanthine (IBMX). A preliminary account of parts of these results was given (Barbier and Lefebvre, 1994).

2. Materials and methods

2.1. General methodology

Cats of either sex (2.2 – 4.8 kg) were fasted for 24 h before the experiment with free access to water. They were anaesthetized with pentobarbital (30 mg/kg i.p.). The stomach was removed and put in Tyrode's solution. After removal of adhering fat tissue and mesenterium, the stomach was opened along the lesser curvature. The mucosa was removed by sharp dissection. Four longitudinal and circular smooth muscle strips (20×3 mm) were cut from the ventral part. All strips were suspended between parallel platinum electrodes in glass organ baths containing 18 ml of Tyrode's solution, under a preload of 1 g. Strips were used immediately or stored at 4°C in Tyrode's solution for a maximum of 4 h. The equilibration was 60 min for strips which were used immediately after preparation, and at least 30 min for strips which had been stored. The bathing solution was bubbled with 95% O_2 and 5% $C0_2$ and kept at a temperature of 37°C; it was changed every 15 min. The composition of the Tyrode's solution was (mM): NaCl, 136.8; KCl, 2.7; $CaCl_2$, 1.8; $MgCl_2$, 1.6; NaH_2PO_4, 0.4; $NaHC0_3$, 11.9 and glucose, 5.6. The Tyrode's solution contained atropine (10^{-6} M), 5-hydroxytryptamine (3×10^{-6} M) and guanethidine (4×10^{-6} M). Electrical field stimulation (40 V, 1 ms) was applied via a Grass S88 stimulator and relaxations were recorded isotonically (Bioscience T3 Isotonic Transducers) on a Graphtec Linearcorder FWR 3701.

2.2. Experimental protocols

After the equilibration period, the tissues were relaxed by addition of 10^{-5} M sodium nitroprusside as reference for other relaxant responses. The tissues were then rinsed at 5-min intervals until tone had recovered to the level before administration of sodium nitroprusside, or until tone did not further recover after three further rinsing sessions.

In the experiments concerning the relaxant effect of the phosphodiesterase inhibitors, vinpocetine (10^{-6}–10^{-4} M), SKF 94120 (10^{-6}–10^{-4} M), rolipram (10^{-8}–10^{-4} M), zaprinast (10^{-6}–10^{-3} M) and IBMX (10^{-6}–10^{-4} M) were added in a cumulative way to both circular and longitudinal muscle strips, while the solvent was added to parallel control strips. Only one type of phosphodiesterase inhibitor was studied per tissue.

To study the influence of the phosphodiesterase inhibitors on NANC relaxation, the relaxation in response to NO, VIP and electrical field stimulation was studied before and after 30-min incubation with a fixed concentration of phosphodiesterase inhibitor; parallel control tissues received the solvent of the phosphodiesterase inhibitor. Each phosphodiesterase inhibitor was tested only in longitudinal (IBMX, rolipram) or circular muscle strips (SKF 94120, zaprinast). The response to 10^{-5} M NO and to 10^{-7} M VIP was studied in half of the tissues, while the response to electrical field stimulation (4 Hz, 10 s train and 2 Hz, sustained until a plateau response was obtained) was studied in the other half.

2.3. Drugs and materials

5-Hydroxytryptamine monohydrate was obtained from Janssen Chimica (Belgium). Atropine sulphate, IBMX, guanethidine sulphate and sodium nitroprusside were from Sigma Chemical Co. (USA). VIP was from Cambridge Research Biochemicals (UK) and Sigma. SKF 94120 [5-(4-acetimidophenyl)pyrazin-(1*H*)-one], zaprinast, rolipram and vinpocetine were gifts from SmithKline Beecham (UK), Rhône-Poulenc (UK), Schering AG (Germany) and Thieman Arzneimittel (Germany), respectively. IBMX was solved in 50%, and rolipram and vinpocetine in 100% ethanol, zaprinast in 20% ethanolamine and SKF 94120 in 0.1 N NaOH. A saturated NO solution was prepared according to Kelm and Schrader (1990) from NO gas (l'Air Liquide, Belgium).

2.4. Analysis of data

Peak relaxant responses to the different stimuli were recorded and expressed as means ± S.E.M.; n refers to the number of experimental animals unless otherwise indicated. The EC_{50} of the phosphodiesterase in-

hibitors was determined by linear interpolation in each concentration-response curve as the concentration responsible for 50% of the maximal relaxant response. As administration of 10^{-5} M NO to cat gastric fundus smooth muscle strips elicits a multiphasic response consisting of an initial peak relaxation followed by a sustained relaxation (Barbier and Lefebvre, 1993; see also Fig. 2), the peak and sustained relaxation were measured separately. The Wilcoxon signed-ranks test for paired observations was used to compare NANC relaxant responses before and in the presence of a phosphodiesterase inhibitor. The Mann-Whitney U-test was used to compare the EC_{50} in longitudinal and circular smooth muscle strips. A difference was considered statistically significant at $P < 0.05$.

3. Results

3.1. Relaxant effects of the phosphodiesterase inhibitors

Vinpocetine (10^{-6}–10^{-4} M) produced only contractile effects and was not investigated further. Fig. 1 shows the relaxant effects of the other phosphodiesterase inhibitors in longitudinal (1A) and circular (1B) muscle strips. For zaprinast, the concentration-response curve reached a plateau. For rolipram, the mean concentration-response curve first tended to reach a plateau but at the higher concentrations tested, the effect again increased; this shape was, however, not present in all strips. The EC_{50} and E_{max} values are given in Table 1. For IBMX and SKF 94120, the concentration-response curve did not tend to a plateau at the highest concentrations tested; the real E_{max} and EC_{50} for these two substances might thus have been somewhat higher than those reported in Table 1. Rolipram was clearly the most potent drug. IBMX was more effective than the other phosphodiesterase inhibitors, especially in the longitudinal smooth muscle strips. The EC_{50}s in longitudinal and circular muscle strips were not significantly different from each other.

In the parallel control strips, the solvent of vinpocetine caused a contraction of $11.4 \pm 6.2\%$ in longitudinal muscle strips, and a relaxation of $29.6 \pm 32.5\%$ in circular muscle strips. The solvent of SKF 94120 had no effect per se except at the concentration corresponding to 10^{-4} M SKF 94120, where it caused a relaxation of $25.9 \pm 27.3\%$ (circular) and $6.4 \pm 4.8\%$ (longitudinal smooth muscle strips). The solvent of rolipram had no consistent relaxant effect in circular smooth muscle strips, while the concentration corresponding to 10^{-4} M rolipram caused a relaxation of $13.0 \pm 5.4\%$ in longitudinal smooth muscle strips. The solvent of zaprinast caused a tone increase at the concentrations corresponding to 3×10^{-4} M ($13.8 \pm 5.3\%$) and 10^{-3} M zaprinast ($33.1 \pm 19.3\%$) in the longitudinal muscle strips. In the circular muscle strips the solvent of zaprinast had no effect. The solvent of IBMX had no influence on tone.

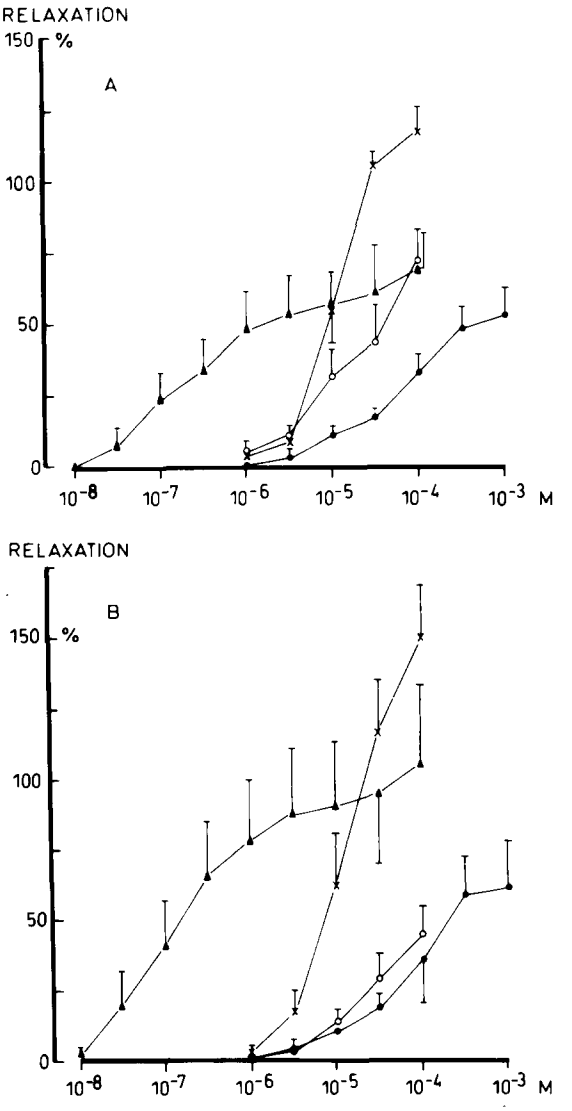

Fig. 1. Concentration-response curves for the relaxant effect of phosphodiesterase inhibitors in cat gastric fundus smooth muscle strips. (A) Longitudinal; (B) circular. (×) IBMX (10^{-6}–10^{-4} M), (●) zaprinast (10^{-6}–10^{-3} M), (▲) rolipram (10^{-8}–10^{-4} M), (○) SKF 94120 (10^{-6}–10^{-4} M). All data are the means ± S.E.M. for six to eight strips from four to six cats.

3.2. Influence of phosphodiesterase inhibitors versus relaxant stimuli

As the phosphodiesterase inhibitors had the same relaxant effect in both types of strips (see 3.1.), each phosphodiesterase inhibitor was further investigated in only one type of strip (IBMX and rolipram in longitudinal, and zaprinast and SKF 94120 in circular smooth muscle strips). In these subsequent experiments, the

Table 1
EC_{50} and E_{max} for the relaxant effect of phosphodiesterase inhibitors

	EC_{50}	E_{max}
Longitudinal smooth muscle strips		
SKF 94120	$2.3 \pm 0.8 \times 10^{-5}$ M	72.2 ± 11.9%
Rolipram	$0.09 \pm 0.05 \times 10^{-5}$ M	69.4 ± 14.3%
Zaprinast	$7.4 \pm 1.9 \times 10^{-5}$ M	52.9 ± 8.7%
IBMX	$1.1 \pm 0.3 \times 10^{-5}$ M	117.0 ± 8.6%
Circular smooth muscle strips		
SKF 94120	$2.2 \pm 0.3 \times 10^{-5}$ M	44.5 ± 11.4%
Rolipram	$0.03 \pm 0.01 \times 10^{-5}$ M	106.1 ± 28.0%
Zaprinast	$7.9 \pm 1.6 \times 10^{-5}$ M	60.9 ± 16.2%
IBMX	$1.4 \pm 0.4 \times 10^{-5}$ M	148.5 ± 18.7%

EC_{50} was calculated with the relaxant effect at the highest concentration studied taken as 100%. E_{max} was expressed as percentage of the response to 10^{-5} M sodium nitroprusside. All data are the means ± S.E.M. for six to eight strips from four to six cats.

lowest concentration of the phosphodiesterase inhibitor that consistently induced relaxation was used, except for IBMX. IBMX was first administered in a concentration of 3×10^{-6} M, but this induced a far more pronounced relaxation than when administered during the construction of the concentration-response curve. Therefore, 10^{-6} M IBMX was used, which induced a tone decrease of 8.8 ± 6.3% in one series ($n = 6$; experiments with electrical field stimulation) and a tone increase of 2.9 ± 7.2% in the other ($n = 6$; experiments with NO and VIP). The tone decrease induced by 10^{-5} M zaprinast was 19.3 ± 11.2% ($n = 6$) and 12.4 ± 16.6% ($n = 7$), respectively. SKF 94120 (10^{-5} M) caused a tone decrease of 34.1 ± 13.4% ($n = 8$) and 25.8 ± 8.0% ($n = 7$). For 3×10^{-8} M rolipram the tone decrease was 14.4 ± 9.4% ($n = 6$) and 3.4 ± 12.8% ($n = 7$).

In the presence of 10^{-5} M SKF 94120 the peak relaxant response to 10^{-5} M NO and the effect of sustained stimulation at 2 Hz were decreased (Table 2, $P < 0.05$). The solvent of SKF 94120 did not influence the relaxant responses. Rolipram (3×10^{-8} M) did not potentiate the relaxant response to any of the relaxant stimuli studied. Likewise, its solvent did not influence the relaxations. In the presence of 10^{-5} M zaprinast, the plateau component of the relaxant response to 10^{-5} M NO was considerably potentiated ($P < 0.05$, see Fig. 2 and Table 2); the relaxation induced by train electrical field stimulation at 4 Hz was moderately potentiated ($P < 0.05$). The solvent of zaprinast did not influence the effects of the relaxant agents. IBMX (10^{-6} M) did not influence the relaxation induced by NO, VIP or electrical stimulation. The solvent of IBMX

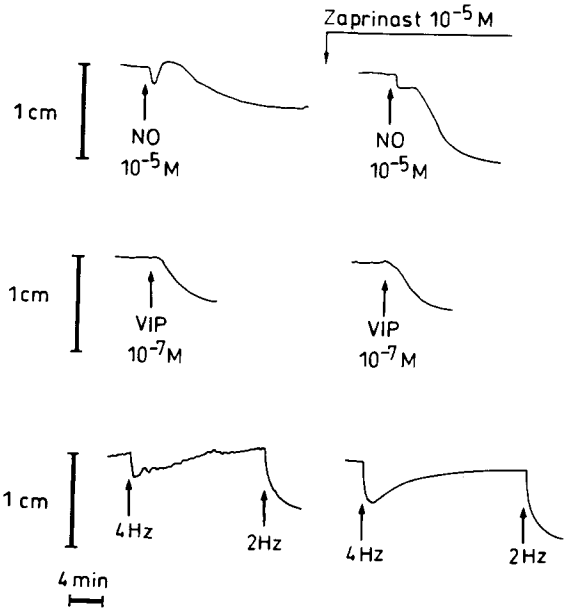

Fig. 2. Traces of the relaxant response to 10^{-5} M NO, 10^{-7} M VIP and electrical field stimulation at 4 Hz (train) and 2 Hz (sustained) in the cat gastric fundus, before and after 30-min incubation with 10^{-5} M zaprinast.

Table 2
Relaxant effect of NO, VIP and electrical field stimulation before and in the presence of phosphodiesterase inhibitors in the cat gastric fundus

	SKF 94120 (10^{-5} M)		Rolipram (3×10^{-8} M)	
	Before	After	Before	After
NO (10^{-5} M, peak)	59.2 ± 16.0	34.0 ± 8.7 [a]	50.9 ± 6.1	56.3 ± 12.7
NO (plateau)	35.2 ± 13.5	24.1 ± 7.4	26.1 ± 11.7	39.2 ± 11.6
VIP (10^{-7} M)	58.0 ± 15.4	50.8 ± 11.6	80.1 ± 14.8	87.2 ± 23.8
4 Hz (train)	55.0 ± 7.1	54.0 ± 7.8	53.4 ± 16.0	42.6 ± 17.0
2 Hz (sustained)	94.5 ± 21.9	79.3 ± 17.1 [a]	98.7 ± 26.2	84.4 ± 24.1
	Zaprinast (10^{-5} M)		IBMX (10^{-6} M)	
	Before	After	Before	After
NO (10^{-5} M, peak)	31.0 ± 5.2	41.1 ± 8.1	53.2 ± 13.9	42.7 ± 16.9
NO (plateau)	37.4 ± 13.2	90.7 ± 30.6 [a]	22.8 ± 12.0	41.3 ± 16.7
VIP (10^{-7} M)	57.5 ± 16.8	70.0 ± 11.1	49.1 ± 19.0	55.7 ± 24.4
4 Hz (train)	41.3 ± 7.0	53.7 ± 8.2 [a]	61.4 ± 1.6	73.0 ± 7.5
2 Hz (sustained)	70.6 ± 9.7	84.9 ± 7.5	99.7 ± 11.7	98.7 ± 8.1

All data are the means ± S.E.M. for six to eight strips. [a] $P < 0.05$.

caused a decrease of the peak relaxant response to 10^{-5} M NO (from 60.3 ± 11.4% to 42.0 ± 10.0%, $P < 0.05$) and an increase of the relaxation elicited by sustained electrical field stimulation at 2 Hz (from 138.4 ± 31.3% to 187.0 ± 5.3%, $P < 0.05$).

4. Discussion

When a particular phosphodiesterase isoenzyme is functionally present in the cat gastric fundus smooth muscle cells, one expects an inhibitor of this enzyme to produce relaxation. Vinpocetine, which shows some selectivity for phosphodiesterase type I and relaxes the rat aorta in the concentration range of 10^{-7} to 10^{-4} M (Souness et al., 1989), only induced contraction, so that the functional presence of phosphodiesterase type I under the experimental conditions seems unlikely. The phosphodiesterase types III, IV and V inhibitors all relaxed the cat gastric fundus in a concentration-dependent way. The type III phosphodiesterase inhibitor, SKF 94120, relaxed the cat gastric fundus in concentrations ranging from 10^{-6} M to 10^{-4} M. This range is higher than that required to induce relaxation in the guinea-pig trachea and the canine lower oesophageal sphincter, but similar to that for the opossum lower oesophageal sphincter (Table 3). The EC_{50} in the cat gastric fundus was about 15 times higher than the K_i versus the type III phosphodiesterase, reported for the cardiac ventricle (Nicholson et al., 1989). This should not mean that the relaxant effect of SKF 94120 in the cat gastric fundus is not due to phosphodiesterase type III inhibition; we have to take in account the species difference and, even in the same tissue, a clear discrepancy can occur between the K_i for phosphodiesterase inhibition and the EC_{50} for relaxation (see e.g. the 100-fold difference for zaprinast in the rat aorta, Souness et al., 1989). The type IV phosphodiesterase inhibitor, rolipram, was the most potent phosphodiesterase inhibitor in the cat gastric fundus, the EC_{50}

Table 3
Relaxant effects of phosphodiesterase inhibitors in respiratory and gastrointestinal tissues

Tissue	Phosphodiesterase inhibitor	EC_{50}	Concentration [a]
Opossum lower oesophageal sphincter [1]	SKF 94120	Not given	10^{-6}–10^{-4} M (effective)
Guinea pig trachea [2]	SKF 94120	2.08×10^{-6} M	10^{-9}–10^{-4} M (tested)
Canine lower oesophageal sphincter [3]	SKF 94120	Not given	10^{-8}–3×10^{-4} M (effective)
Bovine trachea [4]	Rolipram	1.9×10^{-8} M	3×10^{-9}–10^{-6} M (effective)
Guinea pig trachea [4]	Rolipram	9.8×10^{-8} M	3×10^{-9}–3×10^{-6} M (effective)
Pig bronchus [4]	Rolipram	5.6×10^{-5} M	10^{-6}–3×10^{-4} M (effective)
Mouse trachea [4]	Rolipram	$> 10^{-5}$ M	3×10^{-8}–10^{-5} M (effective)
Guinea pig trachea [5]	Rolipram	2.1×10^{-7} M	10^{-9}–3×10^{-5} M (effective)
Human bronchus [5]	Rolipram	5.9×10^{-7} M	10^{-9}–10^{-4} M (effective)
Human bronchus [6]	Rolipram	3.4×10^{-8} M [b]	10^{-8}–10^{-5} M (effective)
	Rolipram	5.2×10^{-8} M [c]	10^{-8}–10^{-5} M (effective)
Bovine trachea [7]	Rolipram	1.6×10^{-7} M [b]	10^{-8}–10^{-4} M (effective)
	Rolipram	7.9×10^{-8} M [d]	3×10^{-8}–10^{-4} M (effective)
Opossum lower oesophageal sphincter [1]	Zaprinast	Not given	3×10^{-6}–10^{-4} M (effective)
Guinea pig internal anal sphincter [8]	Zaprinast	Not given	10^{-9}–10^{-5} M (tested)
Canine lower oesophageal sphincter [3]	Zaprinast	Not given	3×10^{-7}–3×10^{-6} M (effective)
Opossum lower oesophageal sphincter [9]	Zaprinast	Not given	3×10^{-7}–10^{-4} M (effective)
Mouse anococcygeus muscle[10]	Zaprinast	Not given	10^{-5}–10^{-4} M (effective)
Rat gastric fundus [11]	Zaprinast	3.3×10^{-4} M	3×10^{-6}–10^{-3} M (effective)
Bovine trachea [7]	Zaprinast	4.0×10^{-5} M [b]	3×10^{-7}–10^{-4} M (effective)
	Zaprinast	6.3×10^{-6} M[d]	3×10^{-7}–10^{-4} M (effective)
Rat gastric fundus [11]	IBMX	2.9×10^{-5} M	3×10^{-6}–3×10^{-4} M (effective)
Guinea pig stomach [12]	IBMX	Not given	3×10^{-5}–3×10^{-4} M (tested)
Guinea pig trachea [2]	IBMX	0.95×10^{-6} M	10^{-9}–10^{-4} M (tested)
Bovine trachea [7]	IBMX	2.5×10^{-6} M [b,d]	3×10^{-7}–10^{-4} M (effective)

[1] Rattan and Moummi (1989); [2] Bryson and Roger (1987); [3] Barnette et al. (1990); [4] Tomkinson et al. (1993); [5] Cortijo et al. (1993); [6] Qian et al. (1993); [7] Shahid et al. (1991); [8] Baird and Muir (1990); [9] Barnette et al. (1989); [10] Gibson and Mirzazadeh (1989); [11] Barbier and Lefebvre (1992); [12] Bitar and Makhlouf (1982). [a] Whenever possible, the concentration range in which the phosphodiesterase inhibitor induced more than 10% relaxation is indicated (effective). If this is not clear from the original article, the entire concentration-range tested is given (tested). [b] Tissue precontracted by metacholine, [c] precontracted by acetylcholine, [d] precontracted by histamine.

being in the same range as found in the human and guinea pig airways (see Table 3). The experiments of Tomkinson et al. (1993), showing a wide range in EC_{50} values in the same tissue between different species, illustrate that the functional significance of a phosphodiesterase type can differ greatly between species. Although it was not found with all strips, the concentration-response curve of rolipram tended to be biphasic. This has also been observed with other tissues and might indicate interaction with a phosphodiesterase type other than phosphodiesterase IV (Shahid et al., 1991; Cortijo et al., 1993); the phosphodiesterase inhibited by the higher concentrations of rolipram is most probably the phosphodiesterase type III (Harris et al., 1989). Zaprinast induced relaxations of the cat gastric fundus in a concentration range similar to that effective in other gastrointestinal and respiratory tissues (Table 3). Poor penetration of whole tissue preparations might contribute to the rather low relaxant potency of zaprinast in many tissues (Chilvers et al., 1991). Our results thus suggest the functional presence of phosphodiesterase types III, IV and V in the cat gastric fundus; the cyclic nucleotide affinity at these phosphodiesterase isoenzymes is cGMP = cAMP, cAMP $\gg$ cGMP and cGMP $\gg$ cAMP, respectively. Inhibition of all these types and of the non-evaluable type II might contribute to the relaxant effect of IBMX, which was the most effective relaxant phosphodiesterase inhibitor. It indeed inhibits the five phosphodiesterase isoenzymes non-selectively (Shahid et al., 1991), although it has been observed to preferentially potentiate cAMP-dependent relaxant responses (Fujimoto and Matsuda, 1990; Barbier and Lefebvre, 1992). In how far phosphodiesterase IV inhibition is the major determinant of the relaxant effect of IBMX in the cat gastric fundus cannot be deduced from our results.

In the second part of our work, the possible modulation of cyclic nucleotide generation during NANC relaxation by the phosphodiesterase isoenzymes was investigated. The relaxant stimuli were chosen to be representative of the cAMP and cGMP pathways activated in NANC neurotransmission. The cGMP pathway was investigated by means of exogenous NO and short train electrical field stimulation, which leads to the release of NO from NANC nerves (Barbier and Lefebvre, 1993). The other NANC neurotransmitter, VIP, was used to probe the cAMP-dependent pathway. Finally, sustained electrical field stimulation, which leads to a joint release of NO and VIP, was also studied. In these experiments a low concentration of phosphodiesterase inhibitors had to be used in order to exclude a too pronounced effect on tone before the relaxant stimuli were administered. This of course implies that the targeted phosphodiesterase is not maximally inhibited, and this may explain some of our negative results such as the non-effect of SKF 94120, rolipram and IBMX on the VIP-induced relaxation. In the rat gastric fundus and in the guinea pig trachea, IBMX likewise did not influence the VIP-induced relaxation although it potentiated the relaxant effect of the β-adrenoceptor agonist, isoprenaline (Shikada et al., 1991; Barbier and Lefebvre, 1992). Only zaprinast was able to potentiate a number of the relaxant stimuli tested: the relaxation elicited by short train electrical field stimulation and the protracted phase of the relaxation to NO. This contrasts with our results in the rat gastric fundus, where 3×10^{-5} M zaprinast did not potentiate the NO-induced relaxation or the relaxation induced by train electrical field stimulation, although it did potentiate the relaxant effect of the NO donor, sodium nitroprusside (Barbier and Lefebvre, 1992). To explain these results we hypothesized that zaprinast might be effective to potentiate a cGMP-mediated effect when this effect is prolonged and accompanied by a sustained accumulation of cGMP. This seems to be corroborated by the present results: both the NO-induced relaxation and the relaxation induced by train electrical field stimulation were clearly more protracted in the cat gastric fundus than in the rat gastric fundus and were indeed potentiated by 10^{-5} M zaprinast. No significant potentiation of the relaxation induced by sustained electrical field stimulation at 2 Hz was observed. VIP contributes to this response (Barbier and Lefebvre, 1993), which is thus probably also related to accumulation of cAMP. This will not be influenced by the cGMP-specific phosphodiesterase inhibitor, zaprinast. A potentiation of the NANC relaxant response to electrical field stimulation by zaprinast was also found in the mouse anococcygeus (Gibson and Mirzazadeh, 1989) and the guinea pig airways (Rhoden and Barnes, 1990). Our data indicate that, in the cat gastric fundus, phosphodiesterase V is functionally involved in the regulation of cGMP produced by NO, both when exogenously administered and when endogenously released from NANC nerves. We have no explanation for the inhibitory effect of SKF 94120 on the peak relaxant effect of NO and the relaxation induced by sustained electrical field stimulation. SKF 94120 per se in a concentration of 10^{-5} M produced the most pronounced relaxation among the phosphodiesterase inhibitors in this type of experiments. When tone is decreased, relaxant responses will tend to become smaller but it is not clear why this would become manifest for NO and sustained electrical field stimulation, but not for VIP and train electrical field stimulation.

The results of this study indicate that type III, IV and V phosphodiesterases are involved in the regulation of tone in the cat gastric fundus. They furthermore provide evidence that type V phosphodiesterase is involved in the regulation of the NO-mediated increase

in intracellular cGMP content after stimulation of NANC nerves.

Acknowledgements

This study was financially supported by a grant of the Investigation Fund of the University of Gent (01107393) and by grant 3.0007.92 from the Belgian Fund for Medical Scientific Research (FGWO).

References

Baird, A.A. and T.C. Muir, 1990, Membrane hyperpolarization, cyclic nucleotide levels and relaxation in the guinea-pig internal anal sphincter, Br. J. Pharmacol. 100, 329.

Barbier, A.J. and R.A. Lefebvre, 1992, Effects of 3-isobutyl-1-methylxanthine and zaprinast on non-adrenergic non-cholinergic relaxation in the rat gastric fundus, Eur. J. Pharmacol. 210, 315.

Barbier, A.J. and R.A. Lefebvre, 1993, Involvement of the L-arginine: nitric oxide pathway in nonadrenergic noncholinergic relaxation of the cat gastric fundus, J. Pharmacol. Exp. Ther. 266, 172.

Barbier, A.J.M. and R.A. Lefebvre, 1994, Functional responses to phosphodiesterase inhibitors in the cat gastric fundus, Abstr. 10th World Congress of Gastroenterology, Los Angeles, October, 2-7th, 2699P.

Barnette, M., T.J. Torphy, M. Grous, C. Fine and H.S. Ormsbee, III, 1989, Cyclic GMP: a potential mediator of neurally- and drug-induced relaxation of opossum lower esophageal sphincter, J. Pharmacol. Exp. Ther. 249, 524.

Barnette, M.S., M. Grous, T.J. Torphy and H.S. Ormsbee, III, 1990, Activation of cyclic AMP-dependent protein kinase during canine lower esophageal sphincter relaxation, J. Pharmacol. Exp. Ther. 252, 1160.

Beavo, J.A. and D.H. Reifsnyder, 1990, Primary sequence of cyclic nucleotide phosphodiesterase isozymes and the design of selective inhibitors, Trends Pharmacol. Sci. 11, 150.

Bitar, K.N. and G.M. Makhlouf, 1982, Relaxation of isolated gastric smooth muscle cells by vasoactive intestinal peptide, Science 216, 531.

Bryson, S.E. and I.W. Rodger, 1987, Effects of phosphodiesterase inhibitors on normal and chemically-skinned isolated airway smooth muscle, Br. J. Pharmacol. 92, 673.

Chilvers, E.R., M.A. Giembycz, R.A.J. Challiss, P.J. Barnes and S.R. Nahorski, 1991, Lack of effect of zaprinast on methacholine-induced contraction and inositol 1,4,5-trisphosphate accumulation in bovine tracheal smooth muscle, Br. J. Pharmacol. 103, 1119.

Cortijo, J., J. Bou, J. Beleta, I. Cardelus, J. Llenas, E. Morcillo and R.W. Gristwood, 1993, Investigation into the role of phosphodiesterase IV in bronchorelaxation, including studies with human bronchus, Br. J. Pharmacol. 108, 562.

D'Amato, M., F.A. De Beurme and R.A. Lefebvre, 1988, Comparison of the effect of vasoactive intestinal polypeptide and non-adrenergic non-cholinergic neurone stimulation in the cat gastric fundus, Eur. J. Pharmacol. 152, 71.

Fujimoto, S. and T. Matsuda, 1990, Effects of pimobendan, a cardiotonic and vasodilating agent with phosphodiesterase inhibiting properties, on isolated arteries and veins of rats, J. Pharmacol. Exp. Ther. 252, 1304.

Gibson, A. and S. Mirzazadeh, 1989, *N*-Methylhydroxylamine inhibits and M&B 22948 potentiates relaxations of the mouse anococcygeus to non-adrenergic, non-cholinergic field stimulation and to nitrovasodilator drugs, Br. J. Pharmacol. 96, 637.

Gozes, I. and D.E. Brenneman, 1989, VIP: molecular biology and neurobiological function, Mol. Neurobiol. 3, 201.

Harris, A.L., M.J. Connell, E.W. Ferguson, A.M. Wallace, R.J. Gordon, E.D. Pagani and P.J. Silver, 1989, Role of low K_m cyclic AMP phosphodiesterase inhibition in tracheal relaxation and bronchodilation in the guinea pig, J. Pharmacol. Exp. Ther. 251, 199.

Kelm, M. and J. Schrader, 1990, Control of coronary vascular tone by nitric oxide, Circ. Res. 66, 1561.

Martinson, J., 1965, The effect of graded vagal stimulation on gastric motility, secretion and blood flow in the cat, Acta Physiol. Scand. 65, 300.

Martinson, J. and A. Muren, 1963, Excitatory and inhibitory effects of vagus stimulation on gastric motility in the cat, Acta Physiol. Scand. 57, 309.

Nicholson, C.D., S.A. Jackman and R. Wilke, 1989, The ability of denbufylline to inhibit cyclic nucleotide phosphodiesterase and its affinity for adenosine receptors and the adenosine re-uptake site, Br. J. Pharmacol. 97, 889.

Nicholson, C.D., R.A.J. Challiss and M. Shahid, 1991, Differential modulation of tissue function and therapeutic potential of selective inhibitors of cyclic nucleotide phosphodiesterase isoenzymes, Trends Pharmacol. Sci. 12, 19.

Rattan, S. and C. Moummi, 1989, Influence of stimulators and inhibitors of cyclic nucleotides on lower esophageal sphincter, J. Pharmacol. Exp. Ther. 248, 703.

Rhoden, K.J. and P.J. Barnes, 1990, Potentiation of nonadrenergic neural relaxation in guinea pig airways by a cyclic AMP phosphodiesterase inhibitor, J. Pharmacol. Exp. Ther. 252, 396.

Shahid, M., R.G.M. Van Amsterdam, J. De Boer, R.E. ten Berge, C.D. Nicholson, and J. Zaagsma, 1991, The presence of five cyclic nucleotide phosphodiesterase isoenzyme activities in bovine tracheal smooth muscle and the functional effects of selective inhibitors, Br. J. Pharmacol. 104, 471.

Shikada, K., A. Yamamoto and S. Tanaka, 1991, Effects of phosphodiesterase inhibitors on vasoactive intestinal peptide-induced relaxation of isolated guinea-pig trachea, Eur. J. Pharmacol. 195, 389.

Souness, J.E., R. Brazdil, B.K. Diocee and R. Jordan, 1989, Role of selective cyclic GMP phosphodiesterase inhibition in the myorelaxant actions of M&B 22,948, MY-5445, vinpocetine and 1-methyl-3-isobutyl-8-(methylamino)xanthine, Br. J. Pharmacol. 98, 725.

Tomkinson, A., J.-A. Karlsson and D. Raeburn, 1993, Comparison of the effects of selective inhibitors of phosphodiesterase types III and IV in airway smooth muscle with differing β-adrenoceptor subtypes, Br. J. Pharmacol. 108, 57.

Waldman, S.A. and F. Murad, 1987, Cyclic GMP synthesis and function, Pharmacol. Rev. 39, 163.