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Dependence of γ-aminobutyric acid modulation of cholinergic transmission on nitric oxide and purines in cat terminal ileum

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Abstract

The possible involvement of purines and/or nitric oxide (NO) in the γ-aminobutyric acid $(GABA)_A$ receptor-mediated effects on the spontaneous activity of isolated preparations from longitudinal and circular muscles of cat terminal ileum was investigated. GABA had biphasic effects, which were neurogenic and muscarinic. ATP and adenosine dose dependently inhibited the activity of the muscles. A contractile response evoked by the nucleotide only was also observed. The effects of the purines were equipotent and resistant to N^{Ω}-nitro-L-arginine (L-NNA), tetrodotoxin and to desensitization by α,β-methylene adenosine 5′-triphosphate (α,β-meATP), except for the contractile effect of ATP, which was abolished by α,β-meATP. Pretreatment of the preparations with ATP or adenosine produced: (i) desensitization to the effects of the respective purinoceptor agonist only; and (ii) suppression of the GABA-induced responses of longitudinal and circular muscles. Hemoglobin and L-NNA greatly reduced or completely blocked the $GABA_A$-induced relaxation and decreased the $GABA_A$-induced contraction. Our results indicate that purines and NO, to a different extent, mediate the relaxant phase of the GABA effects in both layers. Interactions between muscarinic cholinoceptors and GABA-nitrergic pathway and a concomitant activation of postjunctional P_1 and P_{2y} purinoceptors are suggested to explain the prejunctional biphasic effects of GABA. © 1997 Elsevier Science B.V.

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1. Introduction

Increasing attention has been paid to the functional importance of γ-aminobutyric acid (GABA) and GABAergic mechanisms outside the brain (for review see: Erdö and Wolff (1990)). It is now known that the enteric nervous system of vertebrates contains GABAergic neurons and the performance of GABA in various intestinal regions suggests its physiological role as autonomic neurotransmitter. Recently we have presented evidence that in cat terminal ileum the $GABA_A$ receptor-mediated relaxant and contractile effects, in both longitudinal and circular layers, could be attributed to a modulatory action of $GABA_A$ receptors on cholinergic transmission (Radomirov and Pencheva, 1995). However, we failed to define the target neurons which mediate the biphasic $GABA_A$-induced changes. It is generally accepted that stimulation of the so-called non-adrenergic non-cholinergic (NANC) nerves results in inhibition or excitation of the gut (for review see: Lundberg (1996)). In this connection evidence has been presented that, in various intestinal regions from different species, the canine terminal ileum included, nitric oxide (NO) is among the transmitters for NANC inhibitory nerves related in the action of GABA (Manzini et al., 1986; Boeckxstaens et al., 1991; Kaputlu and Sadan, 1996). Moreover, colocalization of $GABA_A$ receptor immunoreactivity and NO synthase in myenteric neurons has been demonstrated (Krantis et al., 1995). On the other hand the role of purinoceptors and especially of adenosine receptors in GABA release in the central nervous system has recently attracted attention (Kirk and Richardson, 1994), but the data concerning the periphery are scarce and contradictory (Boeckxstaens et al., 1990; Serio et al., 1990; Katsuragi et al., 1993). Several lines of evidence suggest that: (i) ATP, one of the neurotransmitters of the inhibitory NANC nerves in the intestine (Manzini et al., 1985, 1986; Smits and Lefebvre, 1996), might be the endogenous substance released by GABA (Maggi et al., 1984); and (ii)

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purines participate in the modulation of cholinergic transmission pre- and postjunctionally in the gut (Wiklund et al., 1985; Baccari et al., 1994). However, in cat terminal ileum longitudinal and circular muscles, purinoceptors have not been pharmacologically characterized. All this poses questions about: (i) the participation of NO and purines, thought to be NANC transmitters, in neurally mediated responses induced by GABA in longitudinal and circular layers; and (ii) the nature of the purinoceptor subtypes involved.

The present study was therefore designed to study the effects of the purinoceptor agonists, ATP and adenosine, in the longitudinal and circular layers of cat terminal ileum and to understand whether purinergic and nitrergic components participate in the transmission of GABA effects on spontaneous mechanical activity of both longitudinal and circular muscles.

2. Materials and methods

2.1. Preparations

Adult male cats, weighing 3–4 kg, were deprived of food but allowed free access to water for 12 h before the experiments. The animals were killed after anaesthesia with chloralose (80–100 mg/kg i.p.). A 7–8 cm length of the terminal part of the ileum (2–3 cm above the ileocecal sphincter) was quickly removed, rinsed of intraluminal contents and placed in aerated (95% O_2 and 5% CO_2) Krebs solution, containing (mM): NaCl 120, KCl 5.9, $NaHCO_3$ 14.4, NaH_2PO_4 1.2, $MgCl_2$ 1.2, $CaCl_2$ 2.5, glucose 11.5, pH 7.2. The experiments were carried out on segments in order to retain the myenteric plexus. To record the contractile activity of the longitudinal muscle, segments 2 cm long were mounted along the axis of the longitudinal layer in a 20-ml organ bath. Segments approximately 0.8 cm long, were mounted in a 10-ml organ bath along the axis of the circular muscle, using a thread with a large knot situated on the inner part of the gut wall.

2.2. Spontaneous activity

The baths, containing Krebs solution were continuously aerated at 36.5°C. Spontaneous mechanical activity as well as the drug-evoked responses of the longitudinal and circular layers were followed under isometric conditions after standard calibration of a mechanoelectrical transducer (Experimetria, Hungary) connected to a recording device TZ 4620 (Laboratorni Pristroje, Prague) at a tension equivalent to a load of 1 g. There was a 60-min equilibration period with intervening washings before any measurements were made. Acetylcholine (1 nM) added prior to the experiments was used to test the viability of the segments. The tone of the muscle preparations from both layers usually remained unchanged for 120–150 min. Muscle segments which were not spontaneously active or which tended to change their tone after equilibration were discarded.

2.3. Compounds

The compounds used were: GABA (Merck); muscimol (Fluka AG); (±)-baclofen (Research Biochemical); (−)-bicuculline (Sigma); tetrodotoxin (Sankyo); acetylcholine chloride (Germed); atropine sulphate (Merck); pirenzepine dihydrochloride (Sigma); hexamethonium bromide (Serva); guanethidine sulphate (Ciba); naloxone (Sigma); adenosine (Sigma); ATP (Sigma); α,β-methylene adenosine 5′-triphosphate (α,β-meATP; Sigma); N^{Ω}-nitro-L-arginine (L-NNA, Sigma); L-arginine (Sigma); hemoglobin (Sigma). Before the experiments the drugs were dissolved in distilled water and diluted to a final concentration in Krebs solution, except for bicuculline (dissolved in 0.1 N HCl and adjusted to pH 6.5 with 0.1 N NaOH). Drugs were added in volumes not exceeding 0.5–1% of the bath volume.

2.4. Experimental protocol

The effect of GABA, muscimol and baclofen on spontaneous activity were examined at a concentration of 100 μM, which was the EC_{50} value for GABA and GABAergic drugs (Pencheva et al., 1990; Pencheva and Radomirov, 1993). Mechanical responses to GABA were determined in untreated segments and in the presence of antagonists. Intervals of 20–25 min with repeated renewals of the bathing solution were allowed to elapse between each challenge. Drugs remained in contact with the tissue for 2–5 min. The antagonists were used at one concentration only and remained in the organ bath as follows: bicuculline 25 min, tetrodotoxin 10 min; guanethidine 45 min; and hexamethonium 10 min. The effects of atropine, hexamethonium and tetrodotoxin were investigated only once with each preparation.

Concentration–response curves to ATP (0.1 μM–1 mM) or adenosine (0.1 μM–1 mM) for the spontaneous activity of longitudinally or circularly oriented preparations were obtained by cumulative addition of increasing concentrations of the compounds. The same curves were then obtained after desensitization by ATP, adenosine or α,β-meATP. The period of 15–20 min between curves was shown to be sufficient for recovery of the preparations. Concentration–response curves to ATP and to adenosine were made with separate tissues. Desensitization was achieved in separate experiments by applying the agonists at a concentration of 1 μM, with a contact time of 5–7 min and without intervening washings. The curves for ATP or adenosine for the spontaneous activity of segments were also obtained in the presence of tetrodotoxin (0.5 μM; 10 min) and L-NNA (100 μM, 15 min).

The biphasic effects of GABA on both longitudinal and circular muscles were further examined in the absence and

in the presence of hemoglobin, L-NNA, L-NNA plus L-arginine and after ATP-, adenosine- or GABA desensitization. The substances were kept in the organ baths before retesting of the effects of GABA as follows: hemoglobin 30 μM, 10 min; L-NNA 100 μM, 15 min; L-arginine (after L-NNA, without washout) 1 μM, 15 min; ATP 1 μM, 5–7 min; adenosine 1 μM, 5–7 min; and GABA 100 μM, 5 min.

2.5. Evaluation of results and statistical analysis

The lowest points of the amplitude of the spontaneous phasic contractions 2–3 min before drug administration were considered the baseline for measurement of the effects of GABA on spontaneous activity in the absence and in the presence of the above-mentioned substances. The relaxant phase of the GABA effect was measured as an area in mm^2, while the contractile phase was expressed as the total sum of the tonic and phasic components in linear units, recalculated as force in mN. The dynamics of the GABA-induced relaxation or contractile phase were evaluated by recalculation of the values as percentages of the corresponding phase of the GABA effects in untreated preparations. The percentage inhibition was finally calculated.

The effects of ATP and adenosine on spontaneous activity in the control preparations, after desensitization by ATP, adenosine or α,β-meATP and in the presence of tetrodotoxin and L-NNA were expressed as percentages of their maximal response. The concentration–effect curves for the purines (in the range of 16 and 84% of the maximum effects), for preparations from 5–8 animals, were subjected to regression analysis. The correlation coefficients of the linear regression lines were between 0.85 and 0.99. The EC_{50} values obtained were expressed as means $\pm$ S.E.M. Significance of differences was assessed by Student's *t*-test for paired and unpaired data at $P < 0.05$. All statistical procedures were done with computer programs (Tallarida and Murray, 1981).

3. Results

In isolated cat terminal ileum the segments with either longitudinal or circular orientation exhibited spontaneous mechanical activity. The activity was characterized by a steady state tone and rhythmic phasic contractions at a rate of 7.8 ± 1.2 to 11.3 ± 1.8 cycles per min. The amplitude of the contractions in the longitudinal muscle layer (12.8 ± 1.8 mN, $n = 20$) was higher than that in the circular (6.8 ± 0.8 mN, $n = 20$) muscle layer.

3.1. GABA effects

As was described earlier (Pencheva et al., 1991; Pencheva and Radomirov, 1993) GABA administered at concentrations of 1 μM to 2 mM induced concentration-dependent biphasic changes in the spontaneous activity of longitudinal and circular muscles. The effect of 100 μM GABA consisted of a first inhibitory phase with a duration between 25–40 s, occurring immediately after GABA administration, and a second stimulatory phase consisting of an increase in tone and in phasic contraction amplitude. The summarized data of Table 1 show that: (i) guanethidine, naloxone or hexamethonium did not change the two phases of GABA effect on the spontaneous activity of longitudinal and circular layers; (ii) bicuculline or tetrodotoxin entirely antagonized the two phases of the GABA effect except for the contractile phase of the GABA-elicited response in the longitudinal layer, which was suppressed but still persisted; (iii) atropine (1–100 nM) concentration dependently inhibited the biphasic response to GABA in both muscle layers and at a concentration of 100 nM it entirely abolished the two phases, except

Table 1
Cat terminal ileum. Influence of antagonists on the relaxant and contractile phases of the GABA effect on the spontaneous mechanical activity of longitudinal and circular layers (summarized results for 6–10 preparations)

Treatment	% Inhibition			
	GABA-induced relaxant phase		GABA-induced contractile phase	
	longitudinal layer	circular layer	longitudinal layer	circular layer
Bicuculline, 30 μM	92.5 ± 4.3	94.8 ± 3.8	28.5 ± 4.3	95.5 ± 3.2
Tetrodotoxin, 0.5 μM	98.4 ± 1.2	97.3 ± 2.1	30.5 ± 3.8	97.5 ± 1.8
Guanethidine, 50 μM	3.5 ± 0.8	3.2 ± 0.4	5.2 ± 0.7	4.3 ± 0.7
Naloxone, 1 μM	7.4 ± 0.9	6.5 ± 0.6	5.8 ± 0.6	6.2 ± 0.8
Hexamethonium, 300 μM	5.2 ± 0.4	4.8 ± 0.5	3.9 ± 0.5	4.3 ± 0.4
Atropine, 1 nM	3.0 ± 0.8	4.2 ± 0.7	15.6 ± 2.3	12.8 ± 1.8
10 nM	52.6 ± 6.3	62.7 ± 7.2	26.5 ± 3.5	48.2 ± 5.3
100 nM	97.4 ± 2.1	95.2 ± 3.1	32.2 ± 4.2	96.5 ± 2.8
Pirenzepine, 1 nM	92.4 ± 3.1	95.2 ± 2.5	34.1 ± 4.2	96.4 ± 2.4
10 nM	94.1 ± 3.1	92.5 ± 3.1	30.7 ± 4.9	97.1 ± 2.3
100 nM	97.2 ± 2.5	94.2 ± 4.6	33.4 ± 4.1	96.3 ± 2.7

Values represent means $\pm$ S.E.M. for percentage inhibition of GABA-induced relaxant or contractile phases.

Table 2
Cat terminal ileum. Inhibitory and/or contractile effects (EC_{50}; μM) of ATP and adenosine on the spontaneous activity of longitudinal and circular layers in control preparations, after ATP-, adenosine- or α,β-meATP-desensitization and in the presence of tetrodotoxin (1 μM) and N^{Ω}-nitro-L-arginine (L-NNA, 100 μM)

Agonist	Response	Controls	After desensitization by:			In the presence of:	
			ATP	adenosine	α,β-meATP	Tetrodotoxin	L-NNA
ATP	Longitudinal layer						
	Contr.	217 ± 52	1010 ± 240[a]	180 ± 40	N.E.	165 ± 32	128 ± 36
	Inh.	18 ± 8	1060 ± 128[a]	19 ± 4	21 ± 5	17 ± 6	20 ± 5
	Circular layer						
	Contr.	285 ± 65	1580 ± 280[a]	260 ± 35	N.E.	320 ± 46	211 ± 34
	Inh.	24 ± 9	1700 ± 350[a]	25 ± 7	23 ± 6	29 ± 5	26 ± 6
Adenosine	Longitudinal layer						
	Inh.	12 ± 7	14 ± 6	1580 ± 280[a]	16 ± 7	11 ± 5	14 ± 7
	Circular layer						
	Inh.	21 ± 6	26 ± 5	1150 ± 180[a]	24 ± 8	25 ± 4	20 ± 6

The EC_{50} values are the means ± S.E.M. of at least 6 observations.
[a]Significance of differences versus controls at $P < 0.05$ (Student's t-test for grouped data). Abbreviations: N.E., no effect; Contr., contractile effect; Inh., inhibitory effect.

for the contractile effect on the longitudinal muscle layer; (iv) the bicuculline-sensitive GABA-induced relaxation phase was completely eliminated by pirenzepine (1 nM); GABA failed to contract the circular muscle layer in the presence of pirenzepine, while the contractile phase of the GABA effect in the longitudinal muscle layer was decreased. The $GABA_A$ receptor agonist muscimol (100 μM), entirely mimicked the GABA-induced biphasic effect in the circular layer and the relaxation phase of the GABA effect in the longitudinal layer, while the $GABA_B$ receptor agonist, baclofen (100 μM), mimicked only the contractile phase of the GABA effect in the longitudinal layer but failed to induce any changes in the circular layer (data not shown).

3.2. Purine effects

ATP (0.1 μM–1 mM) or adenosine (0.1 μM–1 mM) concentration dependently and reversibly inhibited the spontaneous activity of the longitudinal and circular layer from the cat terminal ileum. A concomitant contractile response evoked only by the nucleotide at concentrations between 10 μM and 1 mM, was also observed. Adenosine did not induce contractions in the two layers. The values derived from the concentration–response curves for the inhibitory effects of adenosine and for the inhibitory and contractile effects of ATP, are presented in Table 2. According to the EC_{50} values, ATP and adenosine showed equipotent inhibitory effects. The EC_{50} values for the inhibitory effects of ATP were significantly lower than those for the contractile effects of ATP.

Pretreatment of the preparations with ATP (1 μM) or adenosine (1 μM) produced desensitization to the effects of the respective purinoceptor agonist only. After ATP desensitization the EC_{50} values of the dose–response ATP curves for the preparations from longitudinal and circular layers were greatly increased, while those of the adenosine curves were not significantly changed (Table 2). After pretreatment with adenosine only, the EC_{50} values of the adenosine dose–response curves were also greatly increased. α,β-meATP, added of a single concentration (1–10 μM) resulted in a long-lasting increase (15–25%) of the basal tone of the preparations from both longitudinal and circular layers. After α,β-meATP desensitization (5–10 min preincubation of the preparations with the compound), the dose-dependent inhibitory effects of ATP or

Table 3
Effect of desensitization by ATP, Adenosine or GABA on the relaxant and contractile phases of GABA (100 μM) effects on the spontaneous mechanical activity of longitudinal and circular layers

Desensitizing agent	Concentration (μM)	% Inhibition			
		GABA-induced relaxant phase		GABA-induced contractile phase	
		longitudinal layer	circular layer	longitudinal layer	circular layer
ATP	1	40.5 ± 5.8	40.3 ± 6.2	17.2 ± 4.8	22.1 ± 5.0
Adenosine	1	45.1 ± 5.7	40.8 ± 5.1	18.4 ± 5.3	21.7 ± 6.3
GABA	100	94.5 ± 5.4	95.2 ± 7.3	90.2 ± 9.1	89.8 ± 9.5

Values represent means ± S.E.M. (6–10 preparations).

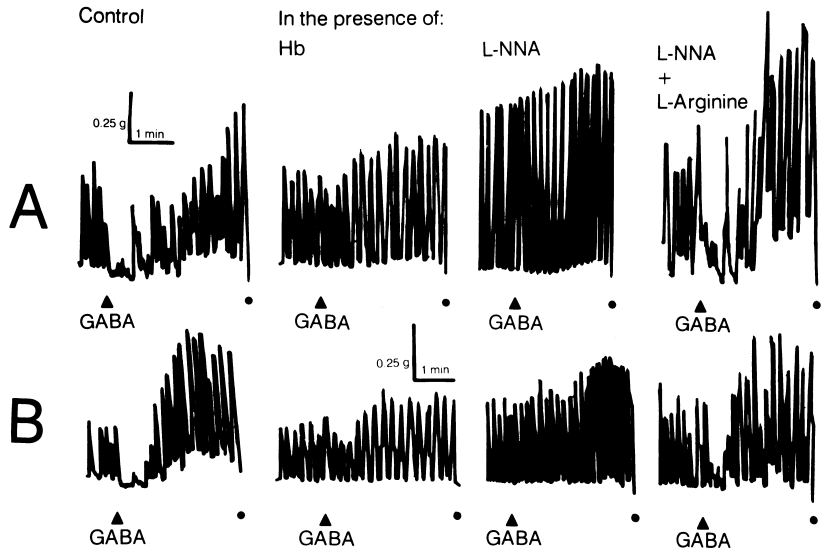

Fig. 1. Cat terminal ileum. Influence of hemoglobin (Hb; 30 μM), N^{Ω}-nitro-L-arginine (L-NNA, 100 μM) and L-NNA (100 μM) plus L-arginine (1 mM) on the biphasic effects of GABA (100 μM; control) on the spontaneous activity of longitudinal (A) and circular (B) layers.

adenosine were not changed, while the contractile effects of ATP were completely abolished in both layers.

Preincubation with tetrodotoxin (0.1 μM; 10 min) or L-NNA (30–100 μM; 15–20 min) resulted in an about 10–20% increase of the amplitude of the spontaneous phasic contractions. In the presence of tetrodotoxin or L-NNA, the ATP- or adenosine dose–response curves were not altered as compared to the controls.

3.3. Purinergic component of GABA effect

After desensitization to ATP or adenosine the relaxation and contractile phases of the GABA-induced responses of longitudinal and circular layers were suppressed (Table 3). The percentage inhibition of the relaxant phase was similar after ATP- or adenosine-induced desensitization and did not exceed 45%. The influence of purinoceptor desensitization on the GABA-induced contractile phase was weaker than that on the GABA-induced relaxation phase. In the presence of GABA (100 μM) a subsequent challenge with GABA (100 μM) greatly reduced or completely blocked the biphasic responses of both longitudinal and circular layers.

3.4. Nitrergic component of GABA effect

Preincubation of the preparations from both layers with haemoglobin (30 μM; 15 min) increased the amplitude of the spontaneous phasic contractions by about 10–20%. In the presence of hemoglobin the relaxant and contractile phases of the GABA effect were significantly reduced in the longitudinal and circular layers as compared with the controls (Fig. 1; Table 4). L-NNA (100 μM; 15 min) greatly reduced or completely blocked the $GABA_A$-induced relaxation and decreased the contractile phase in both muscle layers. In other experiments the segments were treated with L-arginine (1 mM) for 10 min and then L-NNA was added to the bath. In this case the biphasic effects of GABA were not significantly changed.

Table 4
Influence of hemoglobin, N^{Ω}-nitro-L-arginine (L-NNA) and L-NNA plus L-arginine on the relaxant and contractile phase of the GABA (100 μM) effect on the spontaneous activity of longitudinal and circular layers

Treatment	Concentration (μM)	% Inhibition			
		GABA-induced relaxant phase		GABA-induced contractile phase	
		longitudinal layer	circular layer	longitudinal layer	circular layer
Hemoglobin	30	68.5 ± 7.2	65.3 ± 5.8	38.5 ± 6.2	55.6 ± 6.8
L-NNA	100	88.6 ± 9.2	84.3 ± 7.6	37.3 ± 8.7	70.4 ± 9.3
L-NNA + L-arginine	100 + 1	9.8 ± 4.2	12.5 ± 3.8	8.3 ± 2.5	11.3 ± 3.5

Values represent means ± S.E.M. (6–10 preparations).

4. Discussion

Pharmacological analysis of the GABA effect confirmed our previous findings (Pencheva et al., 1991; Pencheva and Radomirov, 1993; Radomirov and Pencheva, 1995) that: (i) the relaxation phase of the GABA effect in both muscle layers and the contractile phase of the GABA effect in the circular layer are mediated by $GABA_A$ receptors; (ii) $GABA_A$ and $GABA_B$ receptors could be involved in the contractile phase in the longitudinal layer; $GABA_B$ receptors are located on the smooth muscle cells (Pencheva et al., 1990); and (iii) the GABA effect in both muscle layers is neurogenic and muscarinic by nature; it was sensitive to tetrodotoxin and to the muscarinic-receptor antagonists, atropine and pirenzepine, but was not changed by hexamethonium. Pirenzepine (1 nM), which has a higher affinity for the M_1 cholinoceptor subtype than for the M_2 subtype (Kilbinger and Nafziger, 1985; Vizi et al., 1989), entirely prevented the bicuculline-sensitive GABA action in both muscle layers, while atropine did not discriminate between them. All these results suggest that GABA provoked the release of acetylcholine from presynaptic cholinergic terminals via bicuculline-sensitive $GABA_A$ receptors. This probably results in activation of the M_1 subtype located on different postsynaptic neurons which release inhibitory or excitatory neurotransmitters. The contractile phase of the GABA effect on the muscle layers of cat terminal ileum could be attributed to the release of acetylcholine from postsynaptic neurons, except for the tetrodotoxin-sensitive part in the longitudinal layers. However, it might be suggested that purines and NO are the neurotransmitters determining the relaxant phase of the GABA effects.

The equipotency of ATP and adenosine established in this study, and the suggestions of some authors about different specific purinoceptors types in the intestine (Abbracchio and Burnstock, 1994; Dalziel and Westfall, 1994; Chang et al., 1995), make the use of putative antagonists for selective blockade unreasonable. The present results prompt suggestions concerning the type of receptors mediating the postjunctional inhibitory and contractile effects of purines. ATP-induced inhibition of the spontaneous activity of the preparations in both layers is probably mediated through stimulation of receptors similar to P_{2y} purinoceptors because: the ATP-induced inhibitory responses were greatly reduced after ATP, but not adenosine desensitization and the ATP inhibition was not altered after α,β-meATP desensitization. Arguments for the ability of P_{2x} postjunctional purinoceptors to mediate ATP-induced contractions are: the blockade of ATP-induced contractions after α,β-meATP desensitization and the inhibition of ATP contractile effects only by ATP desensitization. A per se postjunctional contractile action of adenine nucleotides, but not of nucleosides, has been proved by Wiklund et al. (1985) in the ileum. Therefore, the ATP-induced contractile responses in the present study, could be considered to be P_{2x}-mediated rather than a result of ATP-stimulated prostaglandin biosynthesis (Kasakov and Vlaskovska, 1985). The inhibitory responses to adenosine are probably mediated via receptors which have some of the characteristics of P_1 purinoceptors because the EC_{50} values for adenosine inhibition were not changed after ATP desensitization, while they were greatly increased after adenosine desensitization. Furthermore, desensitization by α,β-meATP did not change the adenosine-elicited inhibitory responses. The same constellation of purinoceptors has recently been demonstrated in cat colon circular muscle (Venkova et al., 1994). In the canine terminal ileum and the ileocecal junction, relaxation induced by ATP was blocked by tetrodotoxin and inhibitors of NO synthase (Boeckxstaens et al., 1990). In the present experiments on cat terminal ileum, as in the rat ileum (Smits and Lefebvre, 1996), the effects induced by purines and adenosine were influenced by neither tetrodotoxin nor L-NNA, suggesting that in the longitudinal and circular muscles ATP and adenosine evoke responses via a direct smooth muscle action which is not mediated by nitric oxide.

The finding that ATP- or adenosine-desensitization of the muscle reduced GABA-induced relaxation shows that the purines play a certain role in the transmission of GABA action in cat terminal ileum. Probably adenosine and ATP act via separate pathways. However, since at present there are no data on concomitant blockade of both pathways, it is difficult to suggest nucleoside–nucleotide interactions which would participate in the GABA action. It seems that GABA-induced acetylcholine release activates M_1 cholinoceptors located on postsynaptic purinergic or NANC nerves, which release ATP and/or adenosine. The released purines participate in the relaxant responses of both muscle layers by activating postjunctional P_1 and P_{2y} purinoceptors. Since the design of the pharmacological analysis of the GABA action yields information about the nature of the components involved rather than about their quantitative correlations, the total percentage inhibition of the GABA-induced relaxation phase exceeded 100. That the GABA-induced contractile phase is also affected by desensitization to ATP or adenosine, though to a small extent, might be considered a result of: (i) modified contractility after treatment with ATP or adenosine; (ii) ATP- or adenosine-influenced cholinergic mechanisms mediating this phase; and (iii) interdependence of the two phases.

The present results provided further evidence that, in the longitudinal and circular muscles of the cat terminal ileum, the $GABA_A$ receptor-mediated responses are much affected by the inhibitor of NO synthase, L-NNA, and by the scavenger of NO, hemoglobin. Furthermore, the substrate for NO synthesis, L-arginine, prevented the inhibitory effects of L-NNA on the $GABA_A$-induced responses of longitudinal and circular layers. Thus the $GABA_A$ receptor-mediated effects are closely related to the NO synthesis in these tissues. These findings are in agreement with the data about co-localization of GABA or

$GABA_A$ receptors and NO synthase in the intestine (Krantis et al., 1995; Williamson et al., 1996). Substantial evidence for NO as a transmitter in the gastrointestinal tract has been presented (for review see: Brookes, 1993). However, so far, it has been suggested that NO is a transmitter of NANC nerves. Acting upon this suggestion many authors presented data about the role of NO in the effects of GABA, ATP and other mainly relaxant substances (Manzini et al., 1985; Li and Rand, 1990; Boeckxstaens et al., 1991; Bogers et al., 1991). The present study showed that the $GABA_A$ receptor-mediated responses in the cat terminal ileum, which we found to be cholinergic and muscarinic, could also be mediated by NO. This conclusion is supported by the recent data on the modulation of cholinergic neurotransmission by NO in intestinal preparations (Baccari et al., 1993, 1994; Rand and Li, 1993; Wiklund et al., 1993; Hryhorenko et al., 1994). Kilbinger and Wolf (1994) proved that endogenous NO inhibits the depolarization-evoked release of acetylcholine and further suggested that NO released within myenteric ganglia acts as a transmitter on postganglionic cholinergic neurons. Thus the coupling between muscarinic (probably of M_1 subtype) cholinoceptors and activation of NO synthase demonstrated in rat cortical cultures (Castoldi et al., 1993) could release simultaneously NO and acetylcholine, resulting in both inhibition and excitation of the muscles. This model of transmitter interactions could explain the prejunctional biphasic actions of GABA effected through a modulatory action on cholinergic transmission mediated via $GABA_A$ receptors.

In conclusion, the $GABA_A$ receptor-mediated effects in the longitudinal and circular muscle layers of the cat terminal ileum might be attributed to: (i) interactions between muscarinic cholinoceptors and GABA-nitrergic pathway; and (ii) concomitant activation of postjunctional P_1 and P_{2y} purinoceptors.

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