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Comparison of γ-aminobutyric acid effects in different parts of the cat ileum

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Abstract

The effects of γ-aminobutyric acid (GABA) and those of a $GABA_A$ (muscimol) and a $GABA_B$ (baclofen) receptor agonists were determined on the spontaneous activity of longitudinally or circularly oriented preparations (segments) isolated from terminal, proximal and distal parts of the cat ileum. GABA applied at 1 μM to 2 mM caused dose-dependent biphasic changes (relaxation and contraction) in spontaneous activity of the longitudinal and circular layers in the terminal and distal parts of the cat ileum and monophasic changes (contraction) in the proximal part. The potency of GABA to elicit relaxant and/or contractile effects in different parts of the ileum showed a proximal-to-terminal increasing pattern. In the longitudinal layer of the distal and terminal ileum, muscimol (100 μM) mimicked the relaxation phase of the GABA effect, while baclofen (100 μM) simulated the contractile phase. Bicuculline, atropine and tetrodotoxin abolished GABA- and muscimol-induced relaxation and suppressed, but failed to prevent GABA- and baclofen-induced contractions. In addition, 2-hydroxysaclofen antagonized the baclofen-induced contractile effect, reduced the GABA-induced contractile phase but failed to prevent GABA- and muscimol-induced relaxation. In the circular layer of the same regions, muscimol mimicked the biphasic GABA effects, while baclofen was without effect. Bicuculline, atropine and tetrodotoxin completely prevented the GABA- and muscimol effects, while 2-hydroxysaclofen failed to antagonize them. In the longitudinal and circular layers of the proximal ileum, muscimol (100 μM) exerted a 'GABA-like' transient contractile effect, while baclofen (100 μM) did not elicit any response. Bicuculline, atropine and tetrodotoxin antagonized the GABA- and muscimol-induced contractile responses of longitudinal and circular layers, while 2-hydroxysaclofen was ineffective. The results suggested that the inhibitory and/or excitatory action of GABA on cholinergic transmission in different regions of cat ileum varies along an increasing gradient towards the terminal ileum and is mediated by $GABA_A$ and $GABA_B$ receptors in the terminal and distal ileum and by $GABA_A$ receptors in the proximal ileum.

Keywords: GABA (γ-aminobutyric acid), enteric; $GABA_A$ receptor; $GABA_B$ receptor; Ileum

1. Introduction

The studies of Florey (1953) and Hobbiger (1958) on the effects of γ-aminobutyric acid (GABA) on the mammalian intestine generated great interest in the GABAergic mechanisms outside the brain (for review, see Erdo and Wolff, 1990). An extensive enteric distribution of GABAergic neurons and nerve fibers has been identified in a number of species, including the human (Furness et al., 1989; Krantis et al., 1995, 1998; Williamson et al., 1996). GABA has been demonstrated in more than thirty peripheral tissues but much of the data show it to be an autonomic neurotransmitter only in the enteric nervous system (Jessen et al., 1987). Thus, much attention has been paid to the physiological function of GABA as transmitter in the intestine. Modulation of intestinal motility through activation of GABA receptors could be related to peristalsis and its control (Ong and Kerr, 1983; Taniyama et al., 1987; Fargeas et al., 1988; Grider and Makhlouf, 1992). This prompted us to investigate the effects of GABA and GABAergic drugs on the spontaneous mechanical activity of the longitudinal and circular layers in three different regions of the cat ileum, which is the longest organ of the gastrointestinal tract. In order to retain the integrity of the myenteric plexus of the preparations, the experiments were carried out on isolated segments. The pharmacological effects of some antagonists on the changes induced by

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GABA and GABA receptor agonists were also followed. Since our previous data on the terminal part of cat ileum had shown that cholinergic transmission could be the main target for GABA modulation (Pencheva et al., 1991; Pencheva and Radomirov, 1993; Radomirov and Pencheva, 1995), we compared the histochemical distribution of acetylcholinesterase in all ileal tissues examined.

2. Materials and methods

2.1. Preparations and spontaneous activity

Adult male cats, weighing 3–4 kg (starved overnight, but allowed free access to water) were subjected to surgical manipulation after anaesthesia with chloralose (80–100 mg kg^{-1} i.p.). A 5–7 cm length of the terminal ileum (2–3 cm above the ileocaecal sphincter), or of the distal ileum (22–25 cm proximal to the ileocaecal sphincter), or of the proximal ileum (65–68 cm proximal to the ileocaecal sphincter), was quickly removed, the intraluminal contents were rinsed out and the tissues were 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 through a thread with a large knot situated on the inner part of the gut wall.

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

2.2. Experimental procedure

After the equilibration period, the mechanical responses to GABA, baclofen or muscimol were determined in untreated segments and in the presence of antagonists in all three ileal tissues. To study the effects of GABA, concentration–response curves within the range of 0.5 μM to 2 mM were obtained in a non-cumulative manner. The effect of a concentration of 100 μM of muscimol and baclofen was evaluated. This concentration proved to be the most effective because dose-dependent responses (1 μM–1 mM) to both agonists were first obtained. The drugs remained in contact with the tissue for no more than 3 min and were then washed out. A 25-min interval with repeated renewal of the bathing solution was allowed to elapse between one challenge and the next. This interval between the single doses of GABA or GABAergic drugs was chosen to prevent any tachyphylaxis. The antagonists were used at one concentration only and remained in the organ baths before retesting of the agonists as follows: bicuculline, 25 min; 2-hydroxysaclofen, 10 min; hexamethonium, 10 min; tetrodotoxin, 10 min; guanethidine, 45 min; naloxone, 10 min; and atropine, 10 min. The effects of atropine, hexamethonium and tetrodotoxin were evaluated only once with each preparation.

2.3. Acetylcholinesterase activity

The experiments were carried out on four adult male cats. Tissue blocks from terminal, distal and proximal parts of the ileum were taken. The samples were fixed by immersion in ice-cold 4% neutral formol calcium for 2 h, and then in the same fixative containing 0.88 M sucrose. Frozen free-floating sections were cut at 20 μm (Reichert-Jung microtome, Austria). The sections were preincubated for 30 min in a solution containing 100 μM tetra-[monoisopropyl]pyrophosphortetramide (*iso*-OMPA) at 4°C and pH 7.0. This inhibitor was included in the same concentration as in the incubation medium of Karnovsky and Roots (1964) in order to inhibit non-specific cholinesterase. Acetylthiocholine iodide was used as a substrate. Incubation was performed for 60 min at 4°C. Control sections were incubated without substrate and gave negative results. After enzyme reaction, the sections were dehydrated, embedded in Entellan and examined by light microscopy (Jenaval, Carl Zeiss, Jena).

2.4. Compounds

GABA (Merck); muscimol (Fluka); (±)-baclofen (Research Biochemical); (−)-bicuculline (Sigma); 2-hydroxysaclofen (ICN Pharmaceutical); tetrodotoxin (Sankyo); acetylcholine chloride (Germed); atropine sulfate (Merck); hexamethonium bromide (Serva); guanethidine sulphate (Ciba); naloxone (Sigma); acetylthiocholine iodide (Boehringer); *iso*-OMPA (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

Table 1
Cat terminal, distal and proximal ileum. Amplitude (mN) of spontaneous phasic contractions and relaxation (EC_{50}, μM; 95% C.L.) and/or contractile (EC_{50}, μM; 95% C.L.) phases of GABA effects on the spontaneous mechanical activity of longitudinal and circular muscle layers

Region	Spontaneous phasic contractions	GABA effects	
	Amplitude (mN)	Relaxation phase [EC_{50}, μM (95% C.L.)]	Contractile phase [EC_{50}, μM (95% C.L.)]
Longitudinal layer			
Terminal ileum	14.5 ± 2.1	62.4 (50.5–74.3)	10.8 (7.7–13.8)
Distal ileum	8.1 ± 1.8[a]	80.5 (72.3–91.0)[a]	43.5 (30.5–58.6)[a]
Proximal ileum	5.5 ± 0.8[a]	–	80.2 (68.3–90.8)[a]
Circular layer			
Terminal ileum	10.8 ± 1.2	94.9 (83.5–109.8)	66.0 (51.2–75.5)
Distal ileum	5.4 ± 1.0[a]	112.5 (95.1–121.3)[a]	75.5 (65.1–94.3)[a]
Proximal ileum	3.2 ± 0.9[a]	–	86.3 (69.5–96.5)[a]

Values represent means ± S.E.M. (8–10 preparations).
[a]Significance of differences vs. the respective values of terminal ileum at $P < 0.05$ (Student's *t*-test for grouped data).

and adjusted to pH 6.5 with 0.1 N NaOH) and *iso*-OMPA and acetylthiocholine iodide (included in incubation medium).

2.5. Statistics

The lowest amplitude of the spontaneous phasic contractions 2–3 min before drug application was considered as baseline for measurement of the effects of GABA on spontaneous activity in the controls and in the presence of antagonists. The relaxation phase was measured and calculated as area (in square millimeter). The contractile phase was expressed as the total of the tonic and phasic components in linear units and recalculated as force in millinewton. The monophasic contractile effect of GABA in the proximal part of the ileum was measured as maximal amplitude of the phasic contraction in linear units, recalculated in millinewton. The responses were expressed as percentages of the maximal response to GABA. The concentration–effect curves for the preparations isolated from six different animals were analyzed by the method of linear regression described by Tallarida and Murray (1981). Student's *t*-test for grouped data at $P < 0.05$ was used to compare the EC_{50} values, with 95% confidence limits (C.L.) and the values for the amplitude and frequency of the phasic contractions.

3. Results

3.1. Spontaneous activity

In either orientation, longitudinal or circular, the segments isolated from terminal, proximal and distal parts of the cat ileum showed spontaneous mechanical activity. The activity was characterized by rhythmic phasic contractions without significant changes in tissue tone. Segments which were not spontaneously active (4%) or tended to change tone (3.5%), were not used. The amplitude of the phasic contractions in both longitudinal and circular layers was significantly lower in the distal and proximal parts of the ileum than in the terminal part; the lowest values were recorded for the proximal ileum (Table 1). A longitudinal-to-circular decreasing pattern of the amplitudes of the contractions was also observed in all the three ileal parts. The frequencies of the phasic contractions did not differ between the terminal (9.5 ± 1.2 cycles min^{-1}) and distal (8.1 ± 1.6 cycles min^{-1}) ileum in the two layers, but were significantly decreased in the preparations from the proximal (6.2 ± 0.7 cycles min^{-1}) ileum.

3.2. GABAergic effects

GABA applied at concentrations of 0.5 μM to 2 mM caused concentration-dependent biphasic changes in the spontaneous mechanical activity of the longitudinal and circular layers in the terminal and distal part of the ileum and monophasic changes in the proximal part (Fig. 1). Like the effects in the terminal ileum described earlier (Pencheva et al., 1991; Pencheva and Radomirov, 1993) the GABA-induced changes in the distal ileum consisted of a first inhibitory phase followed by a second stimulatory

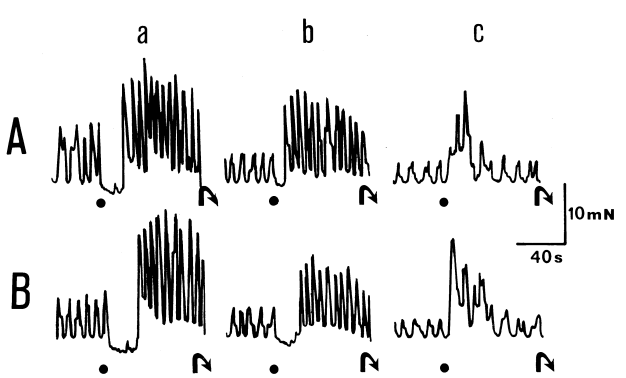

Fig. 1. Cat terminal (a), distal (b) and proximal (c) ileum. Effect of GABA (100 μM) on the spontaneous mechanical activity of the longitudinal (A) and circular (B) layers. (●), application; (downwardly curved right arrow) washing.

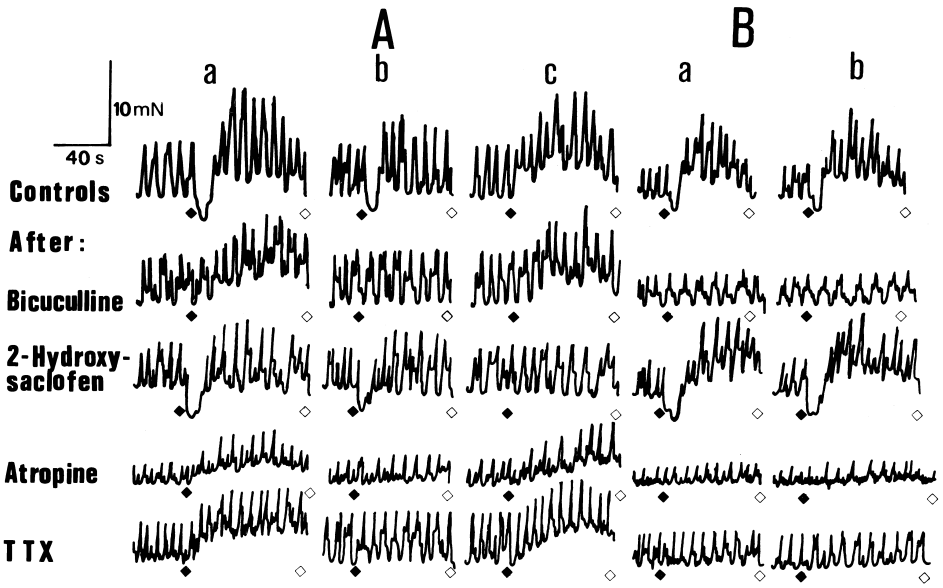

Fig. 2. Cat distal ileum. Effects of GABA (100 μM; a), $GABA_A$ agonist, muscimol (100 μM; b) and $GABA_B$ agonist, baclofen (100 μM; c) on the spontaneous mechanical activity of the longitudinal (A) and circular (B) layers in the absence (Controls) and presence (After:) of bicuculline (30 μM), 2-hydroxysaclofen (300 μM), atropine (30 μM) and tetrodotoxin (TTX; 0.5 μM). (◆) Application; (◇) washing.

phase in both layers. The inhibitory phase was a 5- to 10-s transient relaxation, while the second stimulatory phase consisted of a sustained contraction with a tonic and a phasic component, developing simultaneously for about 60–80 s (Fig. 2A,a,B,a). The duration of the biphasic effect in the distal ileum was slightly reduced as compared to that in the terminal part of the ileum. In the longitudinal layer of the distal ileum, the $GABA_A$ receptor agonist, muscimol (100 μM), mimicked the relaxation phase of the GABA effect and slightly increased the amplitude of the spontaneous contractions (Fig. 2A,b), while the $GABA_B$ receptor agonist, baclofen (100 μM), simulated the stimulatory phase with its tonic and phasic components and sustained character (Fig. 2A,c). In the circular layer, muscimol (100 μM) completely mimicked the biphasic character of the GABA effect, including the tonic component of

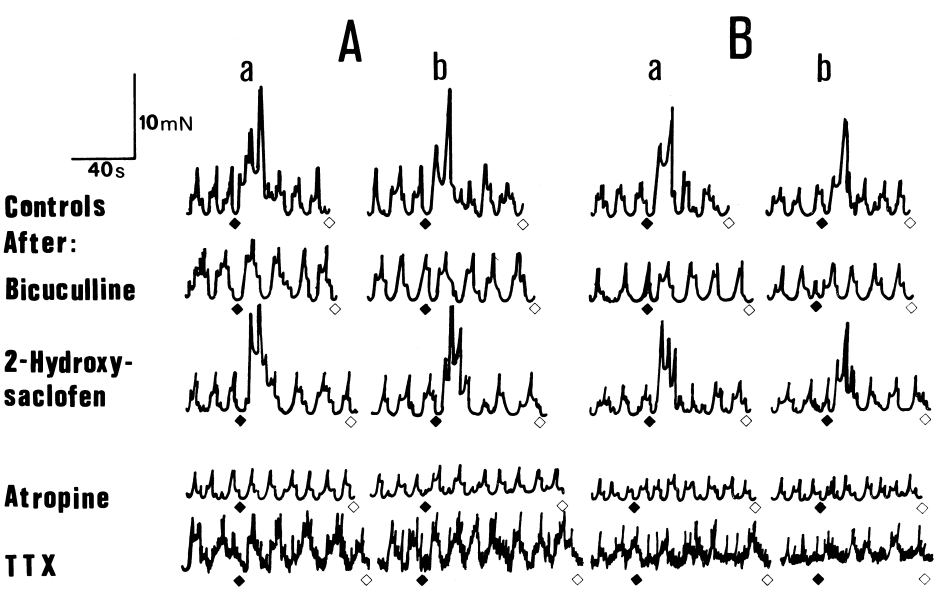

Fig. 3. Cat proximal ileum. Effects of GABA (100 μM; a) and $GABA_A$ agonist muscimol (100 μM; b) on the spontaneous mechanical activity of the longitudinal (A) and circular (B) layers in the absence (Controls) and presence (After:) of bicuculline (30 μM), 2-hydroxysaclofen (300 μM), atropine (30 μM) and tetrodotoxin (TTX; 0.5 μM). (◆) Application, (◇) washing.

the contractile phase (Fig. 2B,b), while baclofen was without any effect (data not shown). On the other hand the action of GABA in the proximal part of cat ileum was a transient contractile effect in both layers (Fig. 3A,a,B,b). This effect developed for no more than 20 s and was manifested as an increase in phasic contraction amplitude, with no changes of tone. In the same region, muscimol (100 μM) exerted a 'GABA-like' contractile effect with a transient pattern, and no increase in tone in either longitudinal or circular layer (Fig. 3A,b,B,b), while baclofen (100 μM) was ineffective (data not shown).

The relaxation or contractile phases of GABA effects on the spontaneous mechanical activity of the longitudinal and circular layers in the different parts of the ileum showed a proximal-to-terminal increasing pattern (Table 1). The EC_{50} values for both phases of the GABA effects in the distal ileum were significantly higher than the EC_{50} values for the terminal ileum preparations. The absolute values for the relaxation or contractile phase measured as areas and amplitudes respectively were more than 50% lower for the preparations from the distal ileum (15.5 ± 3.7 mm^2 and 25.8 ± 4.6 mm^2 as area and 11.2 ± 1.2 mN and 8.1 ± 1.9 mN as amplitude in the longitudinal and circular layer, respectively) as compared to those from the terminal ileum (34.7 ± 4.8 mm^2 and 62.5 ± 10.5 mm^2 as area and 18.4 ± 4.5 mN and 15.0 ± 4.9 mN as amplitude in the longitudinal and circular layer respectively). The EC_{50} values for the contractile effects of GABA in the proximal ileum were highest in the longitudinal or circular layer, while the amplitude of the contractions (9.0 ± 0.9 mN and 8.2 ± 1.5 mN in the longitudinal and circular layer, respectively) was lowest, though similar to that in the distal ileum.

3.3. Effects of GABAergic drugs in the presence of antagonists

Since the pharmacological analysis of GABA effects in the terminal ileum has been described previously, we now followed the responses of the longitudinal and circular layer to GABAergic drugs in the presence of antagonists in the proximal and distal parts of the ileum. In the longitudinal layer of the cat distal ileum the $GABA_A$ receptor-selective antagonist, bicuculline (30 μM), the M-cholinergic blocker, atropine (30 μM), and tetrodotoxin (0.5 μM) antagonized the GABA- and muscimol-induced relaxation and suppressed, but failed to prevent, the GABA- and baclofen-induced contraction (Table 2; Fig. 2A). The $GABA_B$ receptor selective antagonist, 2-hydroxysaclofen (300 μM), antagonized the baclofen-induced contractile effect, reduced the GABA-induced contractile phase (especially its tonic component), but failed to prevent the GABA- and muscimol-induced relaxation and the muscimol-induced increase of the phasic contractions. In the circular layer of the same region, bicuculline, atropine and tetrodotoxin completely prevented the GABA- and muscimol-induced biphasic effect, which was resistant to 2-hydroxysaclofen (Table 2; Fig. 2B). In the longitudinal or circular layer of the distal ileum the *N*-cholinergic blocker, hexamethonium (300 μM), guanethidine (50 μM), which prevented neurotransmitter release from the sympathetic nerve terminals, and the opioid receptor antagonist naloxone, (1 μM), did not antagonize the effects of GABA (Table 2), muscimol or baclofen (data not shown).

In the proximal part of the cat ileum, bicuculline (30 μM) antagonized the GABA- and muscimol-induced contractile effects in both muscle layers (Table 2; Fig. 3). The bicuculline-sensitive contractile effects of GABA and muscimol were eliminated by atropine (3 μM) and tetrodotoxin (0.5 μM), while 2-hydroxysaclofen (300 μM), hexamethonium (300 μM), guanethidine (50 μM) and naloxone (1 μM) did not antagonize them.

3.4. Distribution of acetylcholinesterase reaction

The myenteric ganglia and connecting nerve bundles in the different parts of the ileum were well outlined by the

Table 2
Cat distal and proximal ileum. Influence of antagonists on the relaxation and/or contractile phases of the GABA (100 μM) effect on the spontaneous mechanical activity of longitudinal and circular layers

Treatment	% Inhibition in distal ileum GABA effects				% Inhibition in proximal ileum GABA effects	
	Relaxation phase		Contractile phase		Contractile phase	
	Longitudinal layer	Circular layer	Longitudinal layer	Circular layer	Longitudinal layer	Circular layer
Bicuculline, 30 μM	93.1 ± 3.9	90.3 ± 7.8	32.6 ± 4.1	94.7 ± 3.6	95.8 ± 2.3	96.4 ± 2.1
2-Hydroxysaclofen, 300 μM	4.8 ± 1.2	3.5 ± 1.2	66.8 ± 8.4	2.8 ± 0.7	1.5 ± 0.5	2.5 ± 0.7
Hexamethonium, 300 μM	6.8 ± 0.9	7.4 ± 0.8	2.4 ± 0.6	3.7 ± 0.5	4.3 ± 0.7	3.2 ± 0.8
Tetrodotoxin, 0.5 μM	96.4 ± 2.7	97.1 ± 2.5	30.8 ± 5.6	91.8 ± 4.1	97.1 ± 2.6	96.8 ± 2.1
Guanethidine, 50 μM	2.5 ± 0.6	3.1 ± 0.8	3.6 ± 0.7	4.3 ± 0.6	3.7 ± 0.6	5.4 ± 0.9
Naloxone, 1 μM	8.9 ± 1.1	8.5 ± 0.9	6.3 ± 0.8	5.2 ± 0.9	6.7 ± 0.5	7.2 ± 0.5
Atropine, 3 μM	97.7 ± 2.0	96.4 ± 2.9	36.3 ± 4.2	96.7 ± 3.1	95.4 ± 3.2	96.8 ± 2.9

Data for at least six effects obtained with tissue from different animals are summarized. Values represent means ± S.E.M. for percentage inhibition of GABA-induced relaxation or contractile phase.

acetylcholinesterase-positive nerve elements. In the ganglia there were acetylcholinesterase-negative and, rarely, acetylcholinesterase-positive nerve cell bodies, but the neuropil of the ganglia and the nerve bundles showed high acetylcholinesterase activity. Nerve bundles branched in the smaller nerve bundles and terminated in single fibres among the smooth muscle cells. The network formed by the nerve fibres was richer in the circular layer than in the longitudinal muscle layer. No differences were seen in the dimension and density of the myenteric ganglia, the length of the interganglionic nerve bundles, and the density of the acetylcholinesterase positive nerve fibres in the circular muscle layer among the terminal, distal and proximal parts of the ileum. However, histochemistry of the samples from terminal ileum in both longitudinal and circular layer showed more intensive staining than did samples from the proximal and distal ileum (data not shown), suggesting higher activity of the acetylcholinesterase positive nerve structures in the terminal ileum.

4. Discussion

The present study demonstrated the effect of GABA along the cat ileum. The actions of GABA and GABAergic drugs on longitudinal and circular muscle of cat distal ileum appeared to be similar in pattern and pharmacological behaviour to those on the adjacent terminal ileum described elsewhere (Pencheva et al., 1991; Pencheva and Radomirov, 1993). Thus, in both regions GABA induced biphasic changes in the mechanical activity of segments isolated from longitudinal and circular layers. The relaxation phase in both muscle layers is probably mediated by $GABA_A$ receptors because: (i) it was mimicked by muscimol; (ii) it was sensitive to bicuculline, but resistant to 2-hydroxysaclofen; and (iii) it was not induced by baclofen. $GABA_A$ and $GABA_B$ receptors could be involved in the contractility phase of the longitudinal muscle layer because: (i) muscimol increased the spontaneous contractions and this effect was resistant to 2-hydroxysaclofen; (ii) baclofen exerted a contractile effect sensitive to 2-hydroxysaclofen; and (iii) GABA-induced contractions were decreased but not prevented by bicuculline or by 2-hydroxysaclofen. Because part of the GABA- or baclofen-induced contraction in the longitudinal muscle layer in the terminal and distal ileum was insensitive to tetrodotoxin, a direct effect of GABA, mediated by $GABA_B$ receptors located on the smooth muscle could be assumed (Pencheva et al., 1990). In the circular layer of these two regions only $GABA_A$ receptors mediate the GABA-induced contraction because: (i) muscimol mimicked the GABA response; (ii) the GABA and muscimol effects were prevented by bicuculline but resistant to 2-hydroxysaclofen; and (iii) baclofen did not alter the spontaneous activity. However, differences in the potency of GABA action between the terminal and distal part of the cat ileum could be suggested because: (i) the EC_{50} values for the GABA effect in the terminal ileum for both relaxant and contractile phases were significantly lower than those for the effect in the proximal part of the ileum; and (ii) the absolute values for the relaxation and contractility phases were significantly higher in the terminal ileum than for the distal ileum. The present results also suggest that GABA mainly induces transient contractions of the cat proximal ileum longitudinal and circular muscles by activation of $GABA_A$ receptors. Indeed, the GABA-induced contractions were: (i) antagonized by bicuculline, but persisted after 2-hydroxysaclofen; and (ii) mimicked by muscimol, but not by baclofen. These data agree with observations for guinea-pig ileum (Kaplita et al., 1982; Giotti et al., 1983), guinea-pig duodenum (Barbier et al., 1989) and cat colon (Taniyama et al., 1987) where $GABA_A$ receptors elicit only transient cholinergic contractile responses. Despite the qualitative and quantitative differences in the performance of GABA among the three ileal regions examined, some common features of GABA action could be assumed. First, release of neurotransmitters from the sympathetic nerve terminals or activation of opiate receptors is not involved in the GABAergic mechanisms of action in any of the three cat gut tissues examined. Second, the GABA effects were antagonized by atropine, but not affected by hexamethonium. Third, tetrodotoxin blocked the GABA effect, except for the smooth muscle components in the longitudinal layer of the distal and terminal ileum. This suggests a neurogenic and muscarinic nature of the GABA effects in all ileal parts studied. This suggestion is in accordance with much of the data in the literature (Giotti et al., 1985; Fargeas et al., 1988; Schwörer and Kilbinger, 1988), regardless of the differences with respect to pattern, receptor specificity, design of the experiment, species or topographical characteristics, etc. The potency of GABA to induce relaxation or contraction is maximal in the terminal ileum. Thus, the inhibitory and/or excitatory action of GABA on cholinergic transmission in different ileal regions via activation of muscarinic receptors, varies with an increasing gradient towards the terminal ileum. This show GABA to be a member of the group of neuromodulators which affect motility in a different way via a different influence on acetylcholine release (Kleinrok and Kilbinger, 1983), activating muscarinic receptors (Schwörer and Kilbinger, 1988; Fargeas et al., 1988).

The ratio between the excitatory and the inhibitory neurotransmitters of the intrinsic nervous system is of great importance for the motility of different regions along the gut. The present results showed the highest acetylcholinesterase activity in the terminal part of the ileum as compared to the proximal and distal parts. Both amplitude and frequency of the spontaneous contractile activity of the longitudinal and circular layers also showed a proximal-to-distal or a circular-to-longitudinal increasing pattern. These results are consistent with the finding of Atanassova

et al. (1990) that, in canine ileum 25 cm proximal to the ileocolonic sphincter, the amount of acetylcholine released accounts for 58% of that in the terminal ileum, taken to be 100%. Thus, although the adjacent sphincter zone possesses opposite cholinergic and adrenergic contractile properties (Pelckmans et al., 1990), a number of reports characterize the terminal part of the ileum as a region with diminished inhibitory transmission and powerful contractile activity which is ensured by mainly cholinergic neurotransmission (Nowak and Harrington, 1985; Nowak and Harrington, 1987; Atanassova et al., 1990). Thus we suggest that the increased potency of GABA to facilitate or inhibit cholinergic neurotransmission in the terminal part of the ileum or the distal to proximal decreasing pattern of GABA action along the ileum is an essential part of the neurogenic control of motility, which ensures propulsion in the intestine. We, however, failed to define the target neurones which mediate the biphasic or monophasic $GABA_A$-induced changes. The present results suggest that neither opioids nor noradrenaline are candidates as the final neurotransmitter in all ileal regions. Our recent data (Pencheva, 1997) for the terminal part of the ileum have shown nitric oxide and to a smaller extent ATP to be possible candidates. Evidence for the dual excitatory and inhibitory effect of nitric oxide on peristalsis (Holzer et al., 1997) and on acetylcholine release (Hebeiss and Kilbinger, 1996) in the intestine, and for colocalization of $GABA_A$ receptors and nitric oxide synthase (Krantis et al., 1995) encourage further investigation on the role of nitric oxide in the diversity of GABA action along the cat ileum.

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