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Modulatory activity of $GABA_B$ receptors on cholinergic tone in guinea-pig distal colon

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1 The effect of γ-aminobutyric acid (GABA) administration was studied in both *in vitro* and *in vivo* preparations of the guinea-pig distal colon.

2 In *in vitro* preparations GABA (10^{-7} – 10^{-3} M) elicited a dose-dependent relaxation; a decrease in the spontaneous contractions was sometimes observed.

3 The effect of GABA was mimicked by (−)-baclofen, which gave a dose-response curve overlapping that of GABA, while (+)-baclofen was about one hundred times less potent.

4 The relaxation responses induced by the above drugs were antagonized by 5-aminovaleric acid (5×10^{-4} M), which did not affect adenosine-induced relaxation, but they were insensitive to bicuculline (10^{-5} M) and picrotoxin (10^{-5} M). Moreover, they were prevented by tetrodotoxin (6×10^{-7} M). In hyoscine (10^{-7} M)-pretreated preparations, GABA still evoked a small relaxation response (approx. 10% of the maximum) that was bicuculline-sensitive.

5 Desensitization to GABA (10^{-5} M) was observed. A specific cross-desensitization occurred between GABA (10^{-5} M) and (−)-baclofen (10^{-5} M).

6 In *in vivo* preparations, GABA (10 μmol kg^{-1}) and (−)-baclofen (5 μmol kg^{-1}) produced a dose-related inhibition of basal tone, while (+)-baclofen (5 μmol kg^{-1}) had much less effect (about 25%). A decrease in the spontaneous contractions was sometimes observed.

7 The relaxant effect of GABA and (−)-baclofen persisted in guinea-pigs pretreated (1–2 min) with picrotoxin (1.6 μmol kg^{-1}), whereas it was significantly reduced in animals injected 1 min beforehand with 5-aminovaleric acid (0.2 mmol).

8 The maximal relaxant effect induced by GABA and (−)-baclofen did not differ from that of atropine (0.9 μmol kg^{-1}) and after atropine administration GABA had no further inhibitory effect.

9 Relaxation responses induced by GABA and (−)-baclofen still occurred after blockade of nicotinic receptors by hexamethonium (0.17 mmol kg^{-1}), which itself caused an increase in the basal tone.

10 When the tone was increased by topical application of physostigmine (40 μg), GABA and (−)-baclofen induced a greater relaxation than that obtained in basal conditions.

11 It is concluded that GABA, both *in vitro* and *in vivo* administration, inhibits cholinergic tone in guinea-pig distal colon and that this effect is mediated mainly by activation of $GABA_B$ receptors. Further experiments are required to ascertain the possible physiological role of a GABA-releasing neuronal system in the colon *in vivo*.

Introduction

In the last few years, evidence has accumulated on the physiology and pharmacology of γ-aminobutyric acid (GABA)-ergic neurones and GABA receptors in the peripheral nervous system. Most of the pharmacological papers deal with the effects of exogenously administered GABA and of other drugs showing agonistic or antagonistic properties on GABA receptors at the central level. The findings obtained with peripheral nervous tissue led to the differentiation of two subtypes of GABA receptors, a 'classical' bicuculline-sensitive GABA receptor ($GABA_A$-receptor) and a bicuculline-insensitive receptor ($GABA_B$-receptor) (Bowery & Brown, 1974; Bowery *et al.*, 1981; 1984). This distinction was later demonstrated in the

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central nervous system also, through binding studies and receptor autoradiography (Hill & Bowery, 1981; Wilkin *et al.*, 1981; Bowery *et al.*, 1984).

In the ileum, GABA, muscimol and homotaurine behave as $GABA_A$ receptor agonists, causing bicuculline-sensitive contractions, while additionally GABA (and also (−)-baclofen) causes a bicuculline-insensitive relaxation and inhibition of the twitch response. Both actions have been shown to occur through a presynaptic modulation of cholinergic neuronal function (Giotti *et al.*, 1983; Bowery *et al.*, 1984).

In the distal colon *in vitro*, exogenously-applied GABA caused a bicuculline-insensitive reduction in the amplitude and frequency of spontaneous cholinergic contractions of the circular muscle (Ong & Kerr, 1983a). In hyoscine-pretreated preparations of longitudinal muscle, Krantis *et al.* (1980) demonstrated a weak bicuculline-sensitive relaxant effect of GABA.

A physiological role of GABA in the gut has also been suggested. GABA-ergic neurones have been identified through autoradiography in the myenteric plexus of taenia coli and in cultured neurones from the myenteric plexus (Jessen *et al.*, 1979). Moreover, two different research groups have pointed out that the content of endogenous GABA is higher in the myenteric plexus than in smooth muscle (Jessen *et al.*, 1979; Miki *et al.*, 1983). Furthermore, the GABA synthesizing enzyme, L-glutamic acid decarboxylase (GAD; EC 4.1.1.15), has been found in homogenates of myenteric plexus (Jessen *et al.*, 1979) and the ability of dissected myenteric ganglia to synthesize [^{3}H]-GABA from [^{3}H]-glutamic acid has also been shown (Jessen *et al.*, 1979).

Further evidence of a physiological role of GABA in enteric neurotransmission arises from studies on evoked GABA-release. Taniyama *et al.*, (1982) found an electrically-evoked, Ca^{2+}-dependent GABA-release in cat colon preloaded with [^{3}H]-GABA. More recently Jessen *et al.* (1983) produced direct evidence for the Ca^{2+}-dependent, tetrodotoxin-sensitive, neuronal release of [^{3}H]-GABA both from cultured enteric neurones and guinea-pig taenia coli.

In this paper we present further data on the pharmacological activities of GABA agonists and antagonists in the guinea-pig distal colon *in vitro* and *in vivo*. Our results, both *in vitro* and *in vivo*, indicate a modulatory role for GABA on cholinergic neurones which is exerted mainly through $GABA_B$ receptors.

Methods

In vitro *preparations*

Male guinea-pigs (weighing 300–500 g) were killed by a blow on the head; segments of distal colon were quickly removed and placed in a modified Krebs solution of the following composition (mM): KH_2PO_4 1.3, KCl 3.4, NaCl 134.7, $CaCl_2$ 2.8, $MgSO_4$ 0.6, $NaHCO_3$ 16.3, glucose 7.7.

The proximal part of the distal colon (Elliot & Barclay-Smith, 1904) was used; this segment of colon shows a higher tone than that near the rectum and so is more suitable for studying relaxation responses. Segments were mounted in an organ bath containing Krebs solution, bubbled with a mixture of 5% CO_2 and 95% O_2 and maintained at 37°C. An isotonic lever was used to record longitudinal muscle tone and motility on a smoked drum. Preparations were allowed to equilibrate with a resting tension of 2 g for 40 min before drug administration. Preparations showing a low basal tone were discarded as not being useful for the study of relaxation responses. Agonists were administered in the organ bath in a volume which never exceeded 1% of total bath volume (12 ml) and, unless otherwise indicated, they were left in contact with the preparation for about 30 s. This 'contact time' was chosen because the effect of agonists was achieved in 15–20 s. Antagonists were added to the perfusion medium 20 min before retesting the agonists.

Since it is known that actions mediated by peripheral GABA-receptors can exhibit tachyphylaxis (desensitization) (Kaplita *et al.*, 1982; Giotti *et al.*, 1983; Ong & Kerr, 1983a), we first sought the conditions for avoiding its occurrence. With an interval of 20–30 min between two 30 s exposures GABA 3×10^{-6}M ($n=3$) or 10^{-4}M ($n=3$), responses were unchanged, indicating that desensitization had not occurred. Shorter intervals between doses were not tested. Therefore, in experiments where single doses of GABA were tested, 20 min were left to elapse between doses.

The possibility of avoiding desensitization by performing cumulative concentration-effect curves for GABA-mimetic drugs in *in vitro* preparations was also studied. The methodology of 'cumulative administra-

Table 1 Comparison of GABA-induced relaxations of guinea-pig distal colon *in vitro* obtained through either single or cumulative administration*

	Non-cumulative administration	*Cumulative administration*
GABA 3×10^{-6}M	38 ± 3.3	25 ± 6 (NS)
GABA 10^{-4}M	100	97 ± 5.3 (NS)

NS = not significant; $n = 4$.
*Time interval between doses was 20 min or 30 s for non-cumulative and cumulative administration respectively.
Figures represent the percentages (± s.e.mean) of the maximal effect elicited by GABA 10^{-4}M administered as a single dose.

Table 2 Cumulative concentration-response curves of GABA-induced relaxation in guinea-pig distal colon at different intervals between each administration

Interval between cumulatively administered doses	*GABA*						
	3×10^{-7} M	10^{-6} M	3×10^{-6} M	10^{-5} M	3×10^{-5} M	10^{-4} M	10^{-3} M
30 s	11.3 ± 2.1	25 ± 6	39 ± 4	64.5 ± 5.2	93.3 ± 3.5	95 ± 4.5	96.6 ± 2.8
60 s	9.7 ± 1.5	26.6 ± 3	38 ± 4.3	59.6 ± 3.5	88.6 ± 3.4	89 ± 5.6	93 ± 1.3
120 s	13.3 ± 1.8	29.6 ± 2	35 ± 3.3	58 ± 5.5	59.6 ± 5.4***	57.6 ± 5.6*	51.3 ± 4.9**

Effects are expressed as percentage of the maximal relaxation response elicited by a single dose of GABA (10^{-4} M) administered 20 min before (a) and after (b) the cumulative curve (difference between (a) and (b) = 5.3 ± 1.6). *P < 0.05; **P < 0.005; ***P < 0.001, compared to 30 s.

tion' seemed useful for our quantitative experiments because it was difficult to maintain a constant tone in the preparation long enough (100–120 min) to perform a concentration-response curve leaving 20 min intervals between drug administrations.

To check whether desensitization occurred during the cumulative exposures, we administered GABA 3×10^{-6} M to the preparation and, after 20 min washing, GABA 10^{-4} M; finally, after another 20 min of washing, we performed a cumulative concentration-response curve. Since the maximal effect of GABA was reached after 15–20 s, we initially chose 30 s as the interval between administrations for the cumulative curve. The concentration-response curves consisted of seven cumulative doses of GABA to produce final bath concentrations of 10^{-7} M to 10^{-3} M. Each successive higher dose was administered 30 s after the preceding one without intervening washing. Thus the curves were completed in 3 min. The effects of GABA were expressed as a percentage of the maximal effect obtained with non-cumulatively administered GABA 10^{-4} M. Statistical analyses of the results demonstrated that there was no significant difference between the relaxation obtained with GABA 3×10^{-6} M administered during the cumulative dose-effect curve and that given as a single dose (Table 1). The same experiments were performed with (−)-baclofen, and these yielded similar results. Therefore, we considered it appropriate to study the concentration-effect curves of GABAergic drugs in this preparation through cumulative curves constructed using 30 s intervals between each dose. Moreover, to obtain information on the time at which the desensitization phenomenon occurred, we decided to perform cumulative curves, increasing the time-interval between each drug administration. Cumulative curves were performed with intervals between successive doses of 30, 60 or 120 s, so the curves were completed in 3 or in 6 or in 12 min. Cumulative curves constructed with intervals of 30 and 60 s were not significantly different. On the other hand, with intervals of 120 s a significant fading of the responses to GABA occurred (Table 2).

In vivo *preparations*

Male albino guinea-pigs weighing 250–300 g were anaesthetized with subcutaneous urethane (1.5 g kg^{-1}) and the left jugular vein cannulated with a polyethylene tube for drug injection. Urethane was used as its lack of significant depressant properties on ganglionic transmission (Larrabee & Pasternack, 1952) allows neuronal input to the target organ to be maintained (Maggi *et al.*, 1983).

Through a midline incision of the abdomen, the proximal part of the distal colon was exposed and occluding silk ligatures were applied at a distance of 2 cm from each other, taking great care to avoid any lesion to the vascular and nervous supply. Through a small incision the flanged tip of a polyethylene tube (1 mm i.d., 1.5 mm o.d.) was inserted into the lumen of this pocket-like space and secured by means of a purse-string ligature. The free end of the tube was connected to a pressure transducer (Hewlett-Packard 1280 C) and the whole system filled with saline. To evaluate changes of tone and motility, intraluminal pressure and its variations were recorded on a Hewlett-Packard 7754 four-channel polygraph. The segments were inflated with a small volume (0.2–0.4 ml) of saline to obtain a pressure of 8 to 12 mmHg. After 15 min equilibration the effects of GABAmimetic drugs injected intravenously were studied. It was noted that the development of the maximum relaxant effects following intravenous (−)-baclofen was slower than that following intravenous GABA. Therefore, to obtain a quantitative evaluation, the effects of these substances on both basal and stimulated (physostigmine) tone were determined at two times after their administration, i.e. at a time (20 s) when the effect of GABA reached its maximum and at a time (3 min) when the effect of (−)-baclofen reached its maximum.

A dose-response curve was obtained for the effects of GABA (0.1–10 μmol kg^{-1}) evaluated at 20 s, and of atropine (3–900 nmol kg^{-1}). Atropine was injected cumulatively, the next dose being administered when the effects of the preceding one had reached a steady-

state. The GABA concentration-response curve was obtained by the non-cumulative injection of increasing doses at 15 min intervals. This period of time was shown in preliminary experiments to give reproducible responses to GABA (10 μmol kg^{-1}). In another series of experiments, after a 15 min equilibration period, the tone was elevated by the topical application of physostigmine (40 μg in 0.2 ml of warm saline, i.e. 0.65×10^{-3} M) onto the outer surface of the segment of distal colon from which intraluminal pressure variations were recorded (Maggi & Meli, 1982). This produces an increase in tone and an activation of peristaltic avtivity which developed fully within 20 min of topical physostigmine administration and remained constant for at least 30 min. About 20–30% of the preparations were discarded because they failed to develop a suitable increased tone after topical physostigmine. Atropine, GABA, (−)- or (+)-baclofen were injected intravenously when the increase in tone induced by physostigmine had reached a steady level.

In other experiments, performed in non-pharmacologically-stimulated conditions, the effects of the $GABA_A$-receptor antagonist, picrotoxin, and the $GABA_B$-receptor antagonist, 5-aminovaleric acid, on GABA-induced relaxations were investigated. Either picrotoxin (1.6 μmol kg^{-1}) or 5-aminovaleric acid (0.2 mmol kg^{-1}) was injected intravenously 1–2 min before the second challenge with GABA (10 μmol kg^{-1}). The effects of these two antagonists on (−)-baclofen-induced relaxation were investigated in separate experiments from those showing control responses.

Drugs

The following drugs were used: γ-aminobutyric acid (GABA) (Sigma), bicuculline methiodide (Pierce), hyoscine hydrobromide (BDH), tetrodotoxin (Sigma), (−)- and (+)-β-(*p*-chlorophenyl)-γ-aminobutyric acid, (−)- and (+)-baclofen (kindly supplied by Dr W. Bencze, Ciba-Geigy), 1,1-dimethyl-4-phenylpiperazinium iodide (DMPP; Fluka), phentolamine hydrochloride (Ciba), propranolol hydrochloride (ICI), naloxone hydrochloride (Endo), theophylline (Carlo Erba), hexamethonium bromide (Sigma), papaverine hydrochloride (Eli Lilly), atropine sulphate (Sigma), picrotoxin (Sigma), physostigmine sulphate (Sigma).

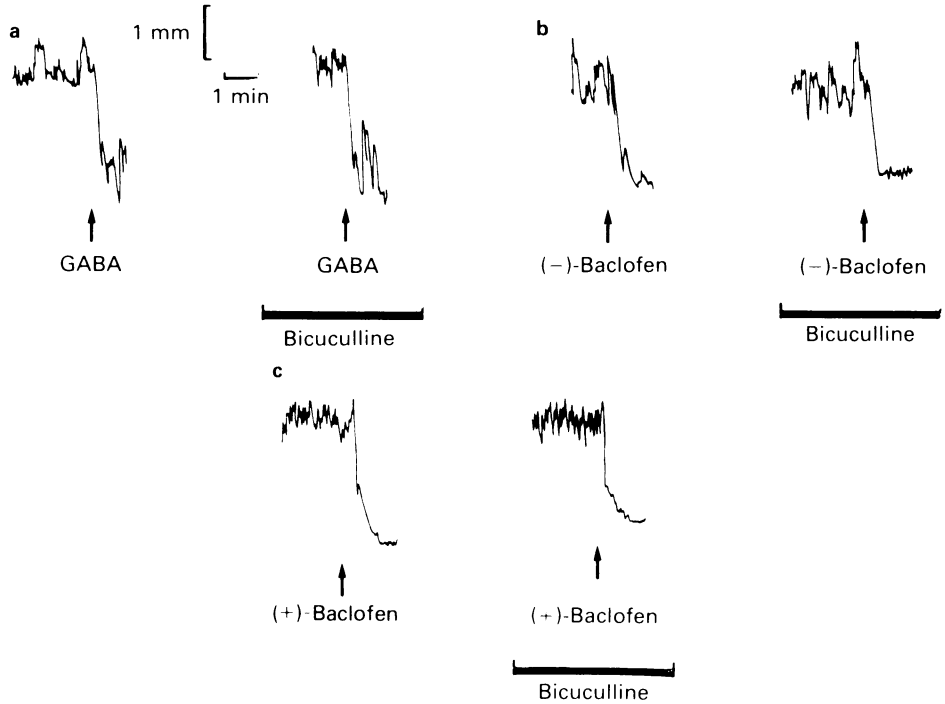

Figure 1 Effects of GABA 3×10^{-6} M (a), (−)-baclofen 3×10^{-6} M (b) and (+)-baclofen (10^{-4} M (c) on segments of guinea-pig distal colon in the absence and presence of bicuculline methiodide (10^{-5} M) added to the perfusion medium 20 min before the agonists were retested. In control experiments ($n = 3$) responses to the agonists were not significantly modified when the doses of agonists were repeated after 20 min in the absence of bicuculline.

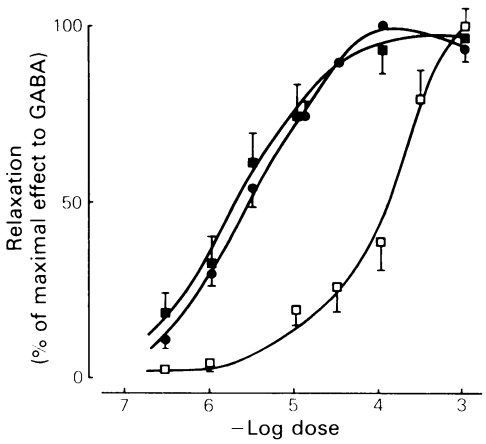

Figure 2 Log dose-response curves for relaxation responses elicited by GABA (●), (−)-baclofen (■) and (+)-baclofen (□) in distal colon *in vitro*. Each symbol is mean of at least 5 observations; vertical lines show s.e.means. Effects are presented as percentage of the maximal effect elicited by GABA.

Results

(1) *Effect of GABA, (−)-baclofen and (+)-baclofen in isolated distal colon; its antagonism with bicuculline, picrotoxin and 5-aminovaleric acid*

In this preparation GABA elicited a rapid relaxation response (Figure 1a). A decrease in amplitude of spontaneous contractions was sometimes observed. The same effect was shown with (−)-baclofen (Figure 1b) and at higher doses with (+)-baclofen (Figure 1c). The maximal relaxation response induced by the above substances was about 75% of the relaxation induced by papaverine (2.5×10^{-5} M). Log-dose-response curves for relaxation responses induced by GABA, (−)-baclofen and (+)-baclofen were parallel and reached the same maximum (Figure 2). ED_{50} values for GABA and (−)-baclofen were very similar ($2.7 \pm 0.8 \times 10^{-6}$ M for GABA and $2.7\,0.9 \times 10^{-6}$M for (−)-baclofen) whereas (+)-baclofen was about 100 times less potent (ED_{50} $1.26 \pm 0.8 \times 10^{-4}$M). Relaxation responses induced by the above drugs (3×10^{-6} M) were unaffected by the GABA$_A$ antagonists bicuculline (10^{-5} M and 10^{-4}M; Figure 1b) and picrotoxin (10^{-5} M) but were antagonized by the putative GABA$_B$ antagonist 5-aminovaleric acid (5×10^{-4} M; Figure 3). The latter did not affect the relaxation response to adenosine 10^{-5} M (Figure 3).

(2) *Desensitization of the relaxant effects of GABA and (−)-baclofen in isolated distal colon*

When GABA (10^{-5} M) was left in the organ bath, there was a slow recovery of tone that was completed in about 10 min. If, at this time, the preparation was challenged again with GABA (10^{-5} M) no effect was observed (Figure 4a). The same results were obtained with (−)-baclofen (10^{-5} M; Figure 4b). Moreover a cross-desensitization between GABA (10^{-5} M) and (−)-baclofen (10^{-5} M) was demonstrated, as shown in Figure 4b, but the preparation still showed a relaxation response to adenosine (10^{-5} M) (not shown).

(3) *Further pharmacological analysis of the relaxation responses induced by GABA and (−)-baclofen in isolated distal colon*

The effects of GABA and (−)-baclofen were tested in

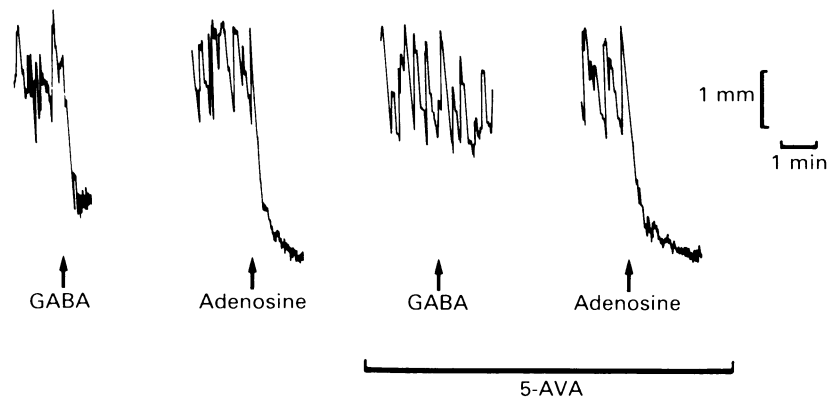

Figure 3 Effects of GABA (3×10^{-6} M) and adenosine (5×10^{-5} M) on guinea-pig isolated distal colon preparation in the absence and presence of 5-aminovaleric acid (5-AVA; 5×10^{-4} M) added to the perfusion medium 20 min before the agonists were retested. In control experiments ($n = 5$) responses to the agonists were not significantly modified when the doses of agonists were repeated after 20 min in the absence of 5-aminovaleric acid (5-AVA). The readings have been aligned so that the relative tone in the preparation at each time is correctly represented.

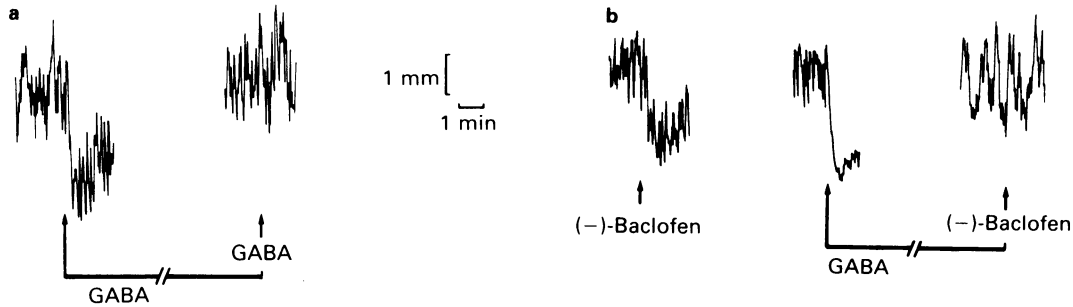

Figure 4 Desensitization of the distal colon *in vitro* to GABAmimetic drugs. (a) Desensitization of GABA effect. GABA (10^{-5} M) was left in the organ bath 10 min (horizontal line with arrow); then the preparation was challenged again with GABA (10^{-5} M). (b) Cross-desensitization between GABA and (−)-baclofen (10^{-5} M). The relaxant effect of (−)-baclofen (10^{-5} M) was determined before and 10 min after administration of GABA (10^{-5} M). GABA was administered 20 min after the first challenge with (−)-baclofen.

the presence of tetrodotoxin. Tetrodotoxin (6×10^{-7} M) greatly lowered the tone of the distal colon and almost abolished its spontaneous motility. Under these conditions there was no further relaxant effect of GABA (10^{-4} M) and (−)-baclofen (10^{-4} M), but papaverine (2.5×10^{-5} M) still showed some effect.

Relaxation responses induced by GABA (3×10^{-6} M) and (−)-baclofen (3×10^{-6} M) were not modified by phentolamine (3×10^{-6} M; $n = 4$) plus propranolol (3×10^{-6} M; n = 4), naloxone (10^{-5} M; $n = 4$), theophylline (5×10^{-5} M; $n = 4$) or hexamethonium (3×10^{-4} M; $n = 4$). When hyoscine (10^{-7} M) was added to the organ bath, there was a clear

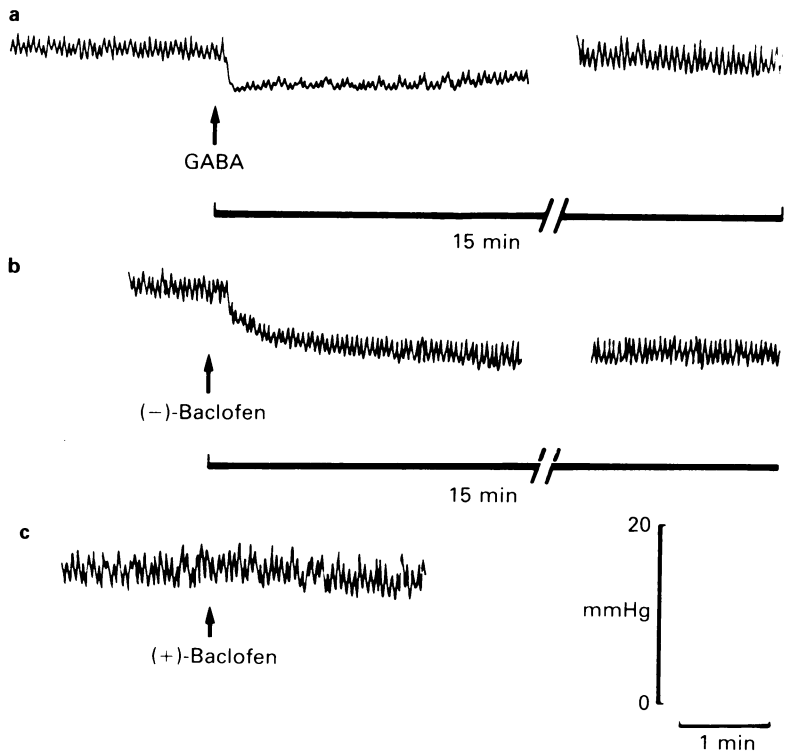

Figure 5 Effects of GABA, (−)-baclofen and (+)-baclofen administered intravenously on the resting tone of guinea-pig distal colon *in vivo*. (a) Effect of GABA 10 μmol kg^{-1}. (b) Effect of (−)-baclofen 5 μmol kg^{-1}. (c) Effect of (+)-baclofen 5 μmol kg^{-1}. Tracings (a), (b) and (c) were recorded in three different animals.

Table 3 Inhibitory effects of GABA and (−)-baclofen in guinea-pig distal colon *in vivo* measured 20 s and 3 min after intravenous administration

	GABA (10 μmol kg^{-1}) (n = 15)	*(−)-Baclofen* 5 μmol kg^{-1}) (n = 6)
20 s after i.v. drug administration	33.4 ± 3.5	14.5 ± 4.0
3 min after i.v. drug administration	15.0 ± 2.2	37.2 ± 4.1

The results show the % inhibition of resting tone (mean ± s.e.mean). The absolute values of resting tone were 9.3 ± 1.4 mmHg and 10.5 ± 1.6 mmHg in experiments with GABA and (−)-baclofen respectively.

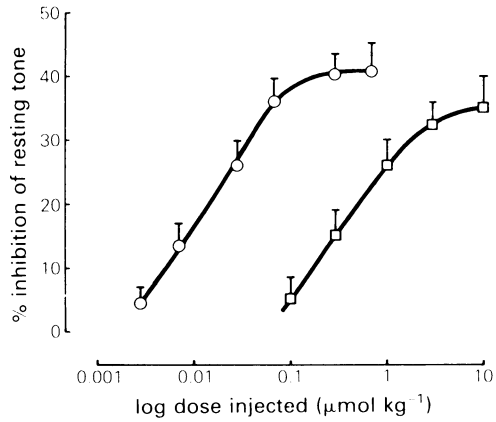

Figure 6 Log dose-response curves for inhibition of resting tone of guinea-pig distal colon *in vivo* elicited by intravenously administered atropine sulphate (○) and GABA (□). Each point is the mean of at least 8 observations. Vertical lines show s.e.means. Effects are represented as % inhibition of resting tone obtained by an imposed intraluminal pressure.

loss of tone and spontaneous motility in the preparation. Hyoscine was left in the medium 20 min before retesting GABA (10^{-4} M). Under these conditions, as previously described by Krantis *et al.* (1980), GABA still evoked a small relaxation response (about 10% of that found in the absence of hyoscine). We also found this relaxation response to be sensitive to bicuculline (10^{-5} M).

(4) *Effect of GABA, (−)-baclofen, (+)-baclofen and atropine on resting tone of guinea-pig distal colon* in vivo

The value of the resting tone of the preparations under study was 10.7 ± 0.8 mmHg (mean ± s.e.mean; n = 27). Most preparations exhibited a regular, low amplitude (1–4 mmHg) spontaneous activity with a frequency of 10–20 contractions min^{-1}. GABA (10 μmol kg^{-1}) produced a relaxant effect that was rapid in onset (time to peak, 20 s); (−)-baclofen (5 μmol kg^{-1}) also induced rapid relaxation followed by further slow decay of tone reaching its maximum within 3–4 min of its administration (Figure 5; Table 3). The relaxation response induced by GABA tended to last only a few minutes and a complete recovery of tone occurred within 10–15 min after GABA administration (Figure 5a). On the other hand, (−)-baclofen induced relaxations lasted throughout the period of observation (10–15 min) (Figure 5b). At the same dose level (+)-baclofen was barely effective (n = 4) (Figure 5c).

A transient depression of amplitude of spontaneous contractions could be observed in some GABA- or (−)-baclofen-treated preparations. Atropine (900 nmol kg^{-1})-induced relaxations were rapid in onset and lasted for several minutes. A complete suppression of spontaneous activity could usually be observed.

Both atropine (3–900 nmol kg^{-1}) and GABA (0.1–10 μmol kg^{-1}) inhibited the resting tone of guinea-pig distal colon in a dose-dependent manner (Figure 6).

Table 4 Ineffectiveness of picrotoxin on the inhibitory effect of GABA in guinea-pig distal colon *in vivo*

	GABA (10 μmol kg^{-1})	*Picrotoxin + GABA* (1.6 μmol kg^{-1}) (10 μmol kg^{-1})
20 s after i.v. drug administration	31.0 ± 3.7	35.3 ± 5.5 (NS)
3 min after i.v. drug administration	12.0 ± 3.1	19.6 ± 5.3 (NS)

Effects of GABA were measured 20 s and 3 min after i.v. administration. Picrotoxin was administered 1–2 min before GABA challenge.
The results show the % inhibition of resting tone (mean ± s.e.mean n = 5). The absolute values of resting tone were 10.0 ± 1.6 mmHg and 10.2 ± 1.1 mmHg in experiments with GABA and GABA plus picrotoxin, respectively.

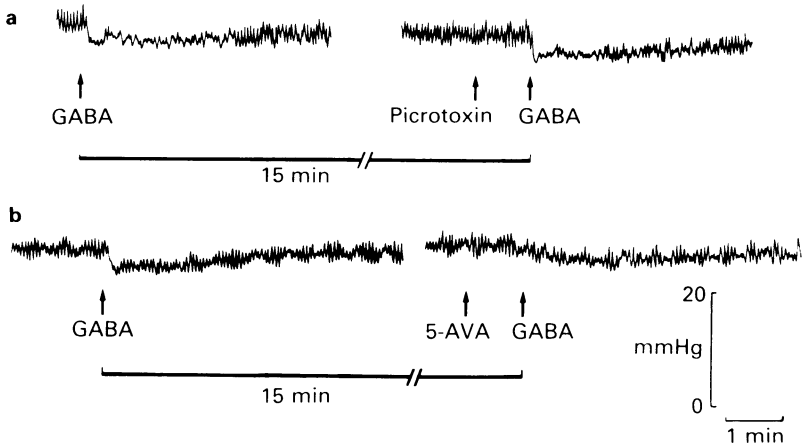

Figure 7 Effect of GABA antagonists on GABA-induced relaxation of basal tone in guinea-pig distal colon *in vivo*. The effect of GABA 10 μmol kg^{-1} was recorded in the absence and presence of (a) picrotoxin 1.6 μmol kg^{-1} or (b) 5 aminovaleric acid (5-AVA) 0.2 mmol kg^{-1} (horizontal lines). Fifteen minutes were allowed to elapse between the first and second administration of GABA. Tracings were recorded from two different animals.

After the administration of atropine (900 nmol kg^{-1}) GABA (10 μmol kg^{-1}; $n = 5$) had no further inhibitory effect.

(5) *Effect of picrotoxin and 5-aminovaleric acid on GABA- and (−)-baclofen-induced relaxations in guinea-pig distal colon* in vivo

Relaxation responses produced by a maximal dose of GABA (10 μmol kg^{-1}, i.v.) subsided within 10 min of GABA administration. A second challenge 15 min after GABA administration resulted in a reproducible response ($n = 5$). Picrotoxin (1.6 μmol kg^{-1}) intravenously administered 1–2 min before the second GABA challenge did not modify the responses to GABA (Figure 7; Table 4).

5-Aminovaleric acid (0.2 mmol kg^{-1}) intravenously administered 1 min before the second GABA challenge significantly antagonized the inhibitory effects of GABA (Figure 7; Table 5). Pretreatment with 5-aminovaleric acid (0.2 mmol kg^{-1}), 1 min before, also significantly reduced the inhibition of basal tone induced by (−)-baclofen (5 μmol kg^{-1}) (Table 6). Picrotoxin pretreatment did not modify (−)-baclofen inhibition of basal tone (Table 6).

(6) *Effect of GABA, (−)-baclofen and atropine in hexamethonium-treated preparations*

Intravenously administered hexamethonium (0.17 mmol kg^{-1}) produced a sudden but moderate (2–6 mmHg) increase in tone in guinea-pig distal colon which was maximal within 1 min of its administration. In most preparations the tone stabilized at a slightly higher level than that seen before hexamethonium administration, while in a few preparations tone recovered 1–2 min after hexamethonium. To check

Table 5 Antagonism by 5-aminovaleric acid (5-AVA) of the inhibitory effect of GABA in guinea-pig distal colon *in vivo*

	GABA (10 μmol kg^{-1})	*5-AVA + GABA* (0.2 mmol kg^{-1}) (10 μmol kg^{-1})
20 s after i.v. drug administration	32.0 ± 4.3	11.0 ± 3.7
3 min after i.v. drug administration	24.0 ± 5.7	8.5 ± 2.2

Effects of GABA were measured 20 s and 3 min after i.v. administration; 5-AVA was administered 1 min before GABA challenge.
The results show the % inhibition of resting tone (mean ± s.e.mean, $n = 6$)
The absolute values of resting tone were 8.5 ± 1.5 mmHg and 8.3 ± 1.4 mmHg in experiments with GABA and GABA plus 5-AVA, respectively.

Table 6 Influence of picrotoxin and 5-aminovaleric acid (5-AVA) on the inhibitory effect of (−)-baclofen in guinea-pig distal colon *in vivo*

	(−)-Baclofen (5 μmol kg^{-1}) (*n* = 6)	*Picrotoxin* (1.6 μmol kg^{-1}) + *(−)-baclofen* (5 μmol kg^{-1}) (*n* = 4)	*5-AVA* (0.2 mmol kg^{-1}) + *(−)-baclofen* (5 μmol kg^{-1}) (*n* = 5)
20 s after i.v. drug administration	14.5 ± 4	21.0 ± 5.2	6.0 ± 3.2
3 min after i.v. drug administration	37.2 ± 4.1	34.0 ± 3.8	7.0 ± 4.4

Three different sets of experiments were carried out. Effects of (−)-baclofen were measured 20 s and 3 min after i.v. administration; picrotoxin was administered 1–2 min and 5-AVA 1 min before (−)-baclofen injection.
The results show the % inhibition of resting tone (mean ± s.e.mean). The absolute values of resting tone were 10.5 ± 1.6 mmHg, 11.6 ± 1.9 mmHg and 11.8 ± 1.8 mmHg in experiments with (−)-baclofen, (−)-baclofen plus picrotoxin and (−)-baclofen plus 5-AVA, respectively.

the degree of nicotinic receptor blockade under these conditions, we investigated the effects of DMPP (0.3 μmol kg^{-1} i.v.; *n* = 7) before and at various times after hexamethonium. Under control conditions intravenous DMPP produced relaxations (4.1 ± 0.8 mmHg) of guinea-pig distal colon. The effects of DMPP were maximal within 1 min and thereafter tone recovered. DMPP-induced relaxation responses were almost completely abolished in the first 5 min after hexamethonium administration, while after 15 min the relaxant effect of DMPP had only slightly recovered (20%). In hexamethonium-treated preparations both GABA (10 μmol kg^{-1} i.v.;) and (−)-baclofen (5 μmol kg^{-1} i.v.) produced relaxation responses which were maximal within 20–30 s (Figure 8); interestingly, no further relaxation was caused by (−)-baclofen. The effects of GABA tended to subside and, at 3–4 min, mean % inhibition was less than at 20–30 s. The effects of (−)-baclofen were more persistent (Table 7). Intravenously administered atropine (300–900 nmol kg^{-1}) produced a long-lasting inhibition of the tone of guinea-pig distal colon in hexamethonium-pretreated animals.

(7) *Effect of GABA, (−)-baclofen, (+)-baclofen and atropine on the physostigmine-induced increased tone of guinea-pig distal colon* in vivo

Topical application of physostigmine (40 μg in 0.2 ml of saline administered onto the outer surface of colon) produced a long-lasting contraction of guinea-pig distal colon with a superimposed peristaltic activity of

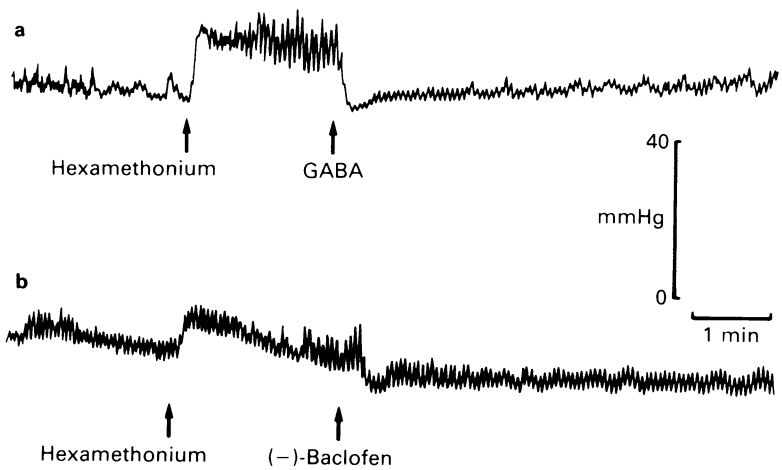

Figure 8 Effect of GABA and (−)-baclofen on distal colon in hexamethonium-treated guinea-pigs. Hexamethonium (0.17 mmol kg^{-1}) was injected intravenously and after the tone had reached a steady-state, GABA 10 μmol kg^{-1} (a) or (−)-baclofen 5 μmol kg^{-1} (b) was administered intravenously.

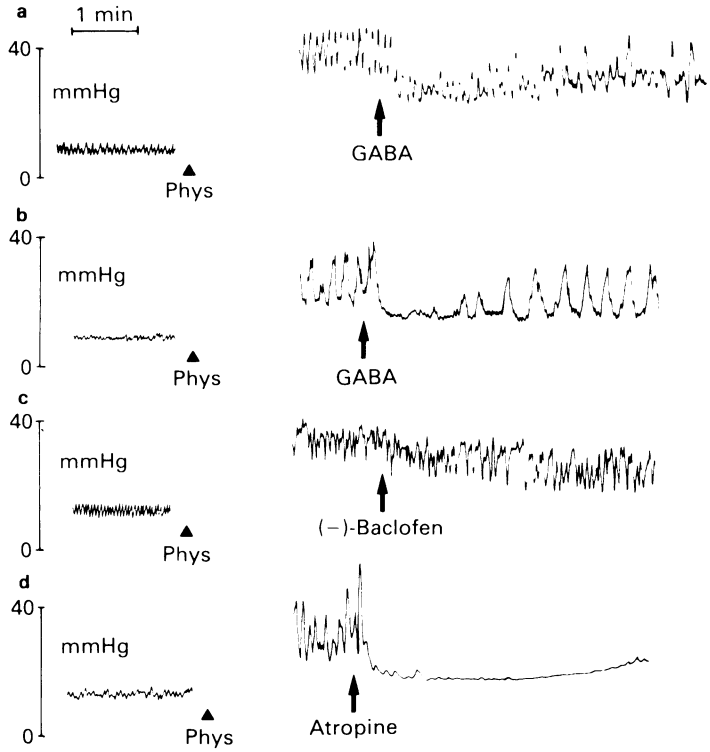

Figure 9 Effect of intravenous GABA 3 μmol kg^{-1} (a and b), (−)-baclofen 1.5 μmol kg^{-1} (c) and atropine 900 nmol kg^{-1} (d) on the increase in tone induced by physostigmine (Phys) in the guinea-pig distal colon *in vivo*. Physostigmine (40 μg in 0.2 ml of warm saline) was applied topically to the outer surface of the segment of distal colon. Tracings were recorded from four different animals before and after topical application of physostigmine.

a higher amplitude than that recorded in basal conditions. Tone increased from 10.7 ± 0.8 to 20.4 ± 1.2 mm Hg ($P < 0.001$; $n = 30$). Intravenous administration of either GABA (3–10 μmol kg^{-1}) or (−)-baclofen (1.5–5 μmol kg^{-1}) inhibited the physos-

Table 7 Effects of GABA and (−)-baclofen on the distal colon in hexamethonium-treated guinea-pigs

	GABA (10 μmol kg^{-1}) ($n = 10$)	(−)-*Baclofen* (5 μmol kg^{-1}) ($n = 12$)
20 s after i.v. drug administration	48.0 ± 4.5	27.0 ± 2.4
3 min after i.v. drug administration	19.7 ± 1.5	8.1 ± 2.1

Effects of drugs were measured 20 s and 3 min after i.v. injection and are expressed as % of the increased tone measured in the presence of hexamethonium 0.17 mmol kg^{-1}. Hexamethonium caused an increase of tone from 10.2 ± 1.0 mmHg to 11.7 ± 0.5 mmHg in experiments with GABA and from 8.7 ± 0.9 mmHg to 10.4 ± 0.5 mmHg with (−)-baclofen.

Table 8 Effects of GABA and (−)-baclofen on the increase in tone induced by physostigmine in guinea-pig distal colon *in vivo*

	GABA (10 μmol kg^{-1}) ($n = 10$)	(−)-Baclofen (5 μmol kg^{-1}) ($n = 7$)
20 s after i.v. drug administration	31.2 ± 5.1	16.4 ± 4.4
3 min after i.v. drug administration	20.0 ± 6.2	37.6 ± 3.9

Topically applied physostigmine (40 μg in 0.2 ml) caused an increase in tone from 7.0 ± 1.6 mmHg to 18.0 ± 1.8 mmHg in experiments with GABA and from 11.0 ± 1.3 mm Hg to 28.2 ± 2.1 mmHg in experiments with (−)-baclofen. After this increase in the tone had reached a steady-state, GABA and (−)-baclofen were administered, their inhibitory effects were measured 20 s and 3 min after administrations and are expressed as percentage inhibition of tone in the presence of physostigmine.

tigmine-induced increase in tone of guinea-pig distal colon (Figure 9). At the same doses, (+)-baclofen inhibition did not exceed 10% ($n = 4$).

The time-courses of the relaxant effects of GABA and (−)-baclofen on physostigmine-activated preparations were similar to those described under basal conditions, i.e. GABA-induced relaxation responses were rapid in onset and preparations tended to return to pre-drug tone values, whereas the effects of (−)-baclofen were slower in onset and persistent in duration (Table 8). In 8 out of the 10 preparations, the effects GABA on physostigmine-activated peristaltic activity were confined to a reduction in amplitude and a slowing-down of frequency while in 2 preparations suppression of phasic contractions was observed.

Intravenous atropine (300–900 nmol kg^{-1}) also inhibited the physostigmine-induced increase in tone. The effect of atropine was rapid in onset and persistent in duration and was characterized by an almost complete suppression of peristaltic activity.

Discussion

The actions of GABA on longitudinal muscle of *in vitro* preparations of distal colon appear to be mainly due to an activation of $GABA_B$ receptors; GABA-induced relaxation responses were bicuculline- and picrotoxin-insensitive. This effect of GABA is shared by the $GABA_B$-selective agonist baclofen, whose action is stereospecific, (+)-baclofen being much less potent than (−)-baclofen. The $GABA_B$ antagonist 5-aminovaleric acid antagonized the effects of GABA and (−)-baclofen. Moreover, a cross-desensitization between GABA and (−)-baclofen was demonstrated. The hypothesis that this GABA-inhibitory action is mainly due to an activation of $GABA_B$ receptors is further supported by the finding that the dose-response curves for GABA and (−)-baclofen are parallel, and reach the same maximum. These findings are essentially in agreement with the observations of Ong & Kerr (1983a), who demonstrated $GABA_B$-mediated inhibition of spontaneous contractions of the circular muscle of guinea-pig distal colon.

$GABA_B$-induced relaxation appears to be exerted, as in the guinea-pig ileum, through a neuronal inhibitory action on cholinergic neurones. In fact, the relaxation response to GABA disappears when neuronal tone is abolished by tetrodotoxin and is virtually non-existent when cholinergic tone is lowered by hyoscine. A similar interpretation was applied to colonic circular muscle by Ong & Kerr (1983a).

We also observed the small bicuculline-sensitive relaxation caused by GABA in hyoscine-pretreated preparations of distal colon as has previously been described by Krantis *et al.* (1980). However, this GABA-induced relaxation response appears to be only a small part (10%) of the total relaxant effect of GABA in hyoscine-free preparations, thus making the contribution of $GABA_A$ receptors to GABA-induced relaxation negligible.

It is also noteworthy that in our *in vivo* experiments, GABA possesses an inhibitory action, i.e. relaxation and reduction of spontaneous motility. Since peripherally-administered GABA does not cross the blood-brain barrier, at least at the doses used in these experiments (Roberts & Kuriyama, 1968; Kuriyama & Sze, 1971), the inhibition of colonic motility observed after its intravenous administration is likely to be due to a peripheral site of action.

Since GABA acts at various levels in the periphery, including ganglia (De Groat, 1970; Bowery & Brown, 1974), it is difficult to attribute the relaxant effect of GABA merely to a direct action on distal colon. However, the inhibitory action of GABA on ganglia previously described by De Groat (1970) and Bowery & Brown (1974), was bicuculline- and picrotoxin-sensitive, thus suggesting an involvement of $GABA_A$ receptors, whereas the relaxant effect on distal colon (this paper) appears to be mediated through $GABA_B$-receptors, since it is picrotoxin-insensitive and 5-amino-valeric acid-sensitive. Therefore, a ganglionic site of action of GABA is unlikely.

When the nicotinic ganglionic synapses were blocked by hexamethonium, inducing an increase in the tone of preparations, GABA, like (−)-baclofen, still induced a relaxation response. However, it is interesting to note that in this situation the time-course of the effect of (−)-baclofen was different from that observed in basal conditions. In fact, the relaxation response to (−)-baclofen achieved a maximum within 20–30 s; in basal conditions a slower, delayed relaxation was observed reaching its maximum after about 3 min. It is possible to speculate that hexamethonium treatment cut off the preganglionic inhibitory action of (−)-baclofen, which would be responsible for the delayed component of the relaxation response.

The possibility that the inhibitory effects of GABA and (−)-baclofen on distal colon *in vivo* could be exerted through the known cardiovascular actions of GABA (Elliott & Hobbiger, 1959) should also be considered. A decrease in blood pressure and pulse rate were observed by us in guinea-pigs after i.v. injection of GABA 10 μmol kg^{-1} (systolic BP from 72 ± 8 to 49 ± 6 mmHg; diastolic BP from 48 ± 6 to 27 ± 4 mmHg; pulse rate from 292 ± 18 to 262 ± 20 beats min^{-1}; $n = 6$), as well as a decrease in blood pressure after (−)-baclofen 5 μmol kg^{-1} (systolic BP from 75 ± 8 to 50 ± 6 mmHg; diastolic BP from 48 ± 4 to 32 ± 3 mmHg; pulse rate from 264 ± 14 to 269 ± 10 beats min^{-1}; $n = 6$). However, a comparable hypotension and decrease in pulse rate was found by us (systolic BP from 84 ± 8 to 46 ± 5 mmHg; diastolic BP from 43 ± 6 to

22 ± 4 mmHg; pulse rate from 291 ± 21 to 267 ± 24 beats min^{-1}; $n = 5$) after i.v. hexamethonium administration (0.17 mmol kg^{-1}); but unlike with GABAmimetic drugs, there was an increase in the tone of distal colon (see Results). Furthermore, the hypotension accompanied by a transient tachycardia induced by intravenously injected diazoxide 6.5 μmol kg^{-1} (systolic BP from 74 ± 9 to 60 ± 6 mmHg; diastolic BP from 52 ± 9 to 37 ± 6 mmHg; pulse rate from 259 ± 15 to 275 ± 19 beats min^{-1}) did not in any way affect the tone of the distal colon (unpublished results).

These considerations, together with the strict similarity existing between our *in vitro* and *in vivo* findings on the involvement of $GABA_B$ receptors, support the hypothesis that the effect of GABA is due to a direct action on the distal colon. This hypothesis is further strengthened by the finding that the GABA effect *in vivo*, similar to that *in vitro*, could be related to an inhibitory action on cholinergic tone. In fact, the relaxant effects of atropine and GABA were non-cumulative and the effect of GABA was more evident when cholinergic tone was increased by local application of physostigmine.

In conclusion, our *in vitro* and *in vivo* data point to a GABA receptor mediated, prevalently $GABA_B$, modulation of cholinergic tone in guinea-pig distal colon.

However, we have not investigated whether other mechanisms are also involved in the effect of GABA at this level. For example, 5-hydroxytryptamine has been implicated in the action of GABA in the ileum (Tonini *et al.*, 1983; Ong & Kerr, 1983b). Jessen *et al.* (1983) recently demonstrated in the myenteric plexus of distal colon that GAD activity and neurones specifically taking up and releasing GABA are present, and that GABA presumably exerts its functional role as a neurotransmitter. In agreement with the above-mentioned data our observations suggest, for the first time *in vivo*, a role for GABA in modulating tone and motility.

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