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$GABA_A$ and $GABA_B$ receptor-mediated effects in guinea-pig ileum

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1 The effects of γ-aminobutyric acid (GABA) and related substances were examined in guinea-pig ileum longitudinal muscle.

2 GABA at doses ranging from 10^{-7} M to 3×10^{-6} M elicited a relaxation while at higher doses (3×10^{-6} M – 10^{-4} M), as previously described, it caused a contraction followed by relaxation.

3 GABA-induced relaxation was bicuculline-insensitive, was mimicked by (−)-baclofen but not by homotaurine and muscimol. The effect of baclofen was stereospecific. GABA- and (−)-baclofen-induced relaxations were dose-dependent and their ED_{50} values were similar. A specific cross-desensitization occurred between GABA and (−)-baclofen.

4 The bicuculline-insensitive relaxation induced by GABA and (−)-baclofen was prevented by tetrodotoxin and hyoscine but not by phentolamine plus propranolol, naloxone or theophylline.

5 In preparations in which the muscle tone was raised by histamine or prostaglandin $F_{2\alpha}$, GABA and (−)-baclofen induced relaxation to the same extent as before increasing the tone. If the tone was raised by DMPP, a greater bicuculline-insensitive relaxation occurred.

6 Contraction caused by GABA was bicuculline-sensitive and was mimicked by homotaurine and muscimol. Contraction was dose-dependent and muscimol was about three times more potent than GABA or homotaurine. A specific cross-desensitization occurred between the contractile effects of GABA and those of homotaurine or muscimol.

7 Bicuculline competitively antagonized the contractile effects of GABA, homotaurine and muscimol and gave closely similar pA_2 values. The slope of the Schild plot for the above drugs was near 1, confirming the competitive nature of the antagonism.

8 The bicuculline-sensitive contraction induced by GABA, homotaurine and muscimol was abolished by tetrodotoxin and was non-competitively antagonized by hyoscine, while it was unaffected by hexamethonium, mepyramine and methysergide.

9 It is concluded that two receptors mediate the GABA effects in guinea-pig ileum: a bicuculline-sensitive $GABA_A$ receptor, which elicits contraction through an excitatory action on cholinergic post-ganglionic neurones; and a bicuculline-insensitive $GABA_B$ receptor which causes relaxation through an inhibitory presynaptic action on cholinergic post-ganglionic neurones. We confirm that GABA, homotaurine and muscimol are $GABA_A$ agonists, while GABA and (−)-baclofen are $GABA_B$ agonists.

Introduction

GABA (γ-aminobutyric acid) is a well-known inhibitory neurotransmitter in the mammalian central nervous system (Curtis & Johnston, 1974). GABA 'receptors' are also present in the peripheral nervous system but the observations existing on the actions of GABA agonists at this level are still rather scattered. Particularly significant are the studies of Bowery & Brown (1974) who observed a transient, bicuculline-sensitive GABA-induced depolarization in the rat superior cervical ganglia by means of recording with surface electrodes. This effect was shared by homotaurine (3-aminopropansulphonic acid). Bowery, Doble, Hill, Hudson, Shaw, Turnbull & Warrington (1981), studying the evoked release of [^{3}H]-noradrenaline from rat isolated atria, and the twitch responses of guinea-pig vas deferens and ileum, found a bicuculline-insensitive inhibitory action of GABA and (−)-baclofen, while homotaurine at this level was ineffective. From these studies, the presence of an atypical bicuculline-insensitive GABA-

receptor on peripheral autonomic nerve terminals has been inferred. This receptor ($GABA_B$) can be distinguished from the classical GABA receptor, which is bicuculline-sensitive ($GABA_A$). Interestingly, the atypical $GABA_B$ receptor has also been identified by the same group in the central nervous system (Hill & Bowery, 1981; Wilkin, Hudson, Hill & Bowery, 1981).

At the intestine level, GABA-induced contraction and relaxation in the guinea-pig were first described by Hobbiger (1958) and more recently by Krantis, Costa, Furness & Orbach (1980). The latter authors found that GABA-induced contraction in the ileum is bicuculline-sensitive and can be blocked by tetrodotoxin and hyoscine, thus indicating a GABA excitatory action mediated by cholinergic neurones.

Furthermore, the presence of GABA-ergic neurones in the myenteric plexus of the guinea-pig has been established by Jessen, Mirsky, Dennison & Burnstock (1979).

The studies of Kaplita, Waters & Triggle (1982), showed that of the GABA agonists, muscimol and homotaurine mimic the GABA-contracting action in guinea-pig ileum, while baclofen does not. Some of us have recently been interested in studying the binding properties of some GABA derivatives on rat brain membranes (Galli, Zilletti, Scotton, Adembri & Giotti, 1979). During these studies we thought it useful to set up a model to study peripheral GABA actions. On the basis of the above-mentioned studies we selected the guinea-pig ileum and we describe the results of experiments with this tissue.

Methods

Male guinea-pigs (weighing 300–500 g) were killed by a blow on the head; segments of terminal ileum were quickly removed and placed in a modified Krebs 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 and glucose 7.7. Strips of ileum lon-

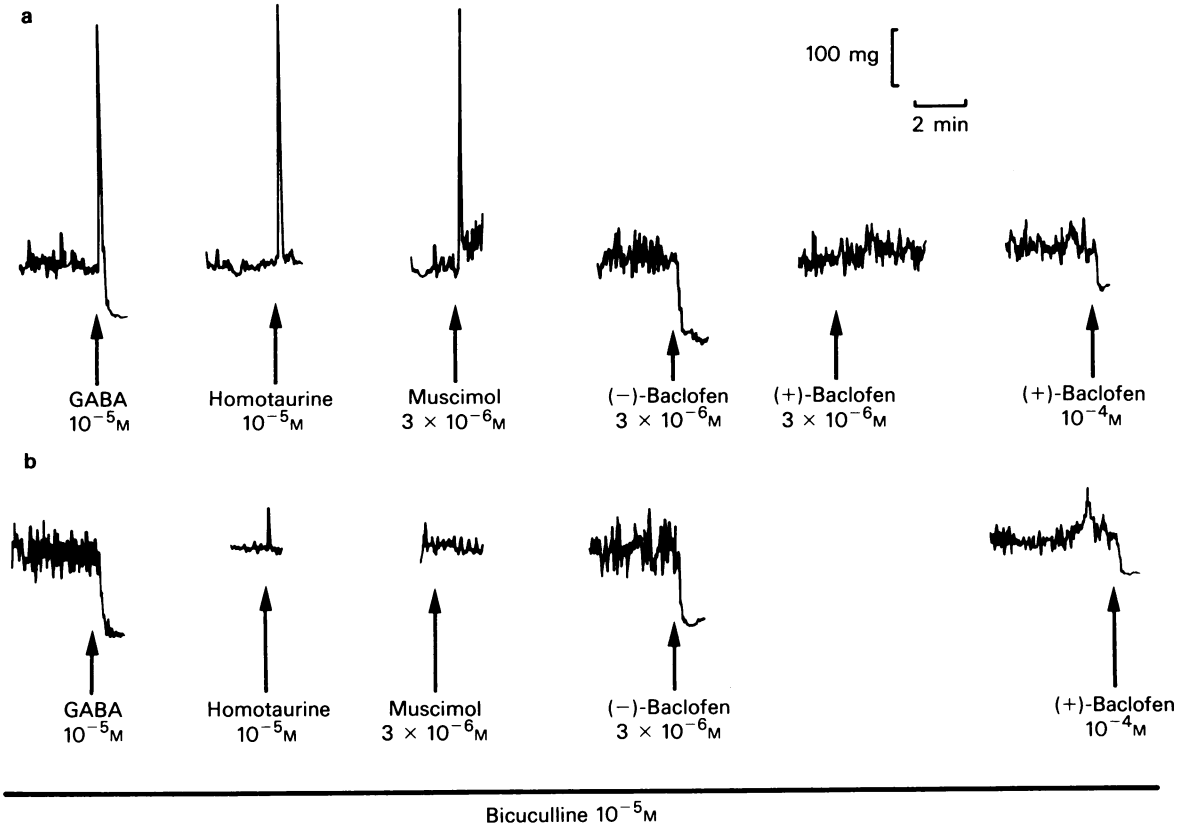

Figure 1 Effects of GABA, homotaurine, muscimol, (−)-baclofen and (+)-baclofen on strips of longitudinal muscle of terminal ileum in the absence (a) and presence (b) of bicuculline methiodide (10^{-5} M) added to the perfusion medium 60 min before the agonists were retested. In control experiments responses to the agonists were not significantly modified when the doses of agonists were repeated after 60 min in the absence of bicuculline.

gitudinal muscle were obtained following the method described by Paton & Zar (1968). Segments were mounted in an organ bath, bubbled with a mixture of 5% CO_2 and 95% O_2 and maintained at 37°C. The ileum was connected to a MARB isometric transducer under a resting tension of 0.5 g and responses were recorded on a Battaglia-Rangoni FP 2400 polygraph. Preparations were allowed to equilibrate for 60 min before drug administration. Drugs were given in a small volume which never exceeded 1% total bath volume (4 ml). An interval of 20–30 min (with intervening washings) between single submaximal doses of GABA, homotaurine, muscimol and (−)-baclofen completely avoided any desensitization ($n = 5$). Desensitization of responses occurred at less than 10-min intervals between doses for contraction and less than 3-min intervals for relaxation. Evaluation of drug antagonism was performed when possible following the pA_2 method as reported by Schild (1947) and Arunlakshana & Schild (1959). All data obtained with agonists were expressed as a percentage of the maximal effect elicited by GABA 10^{-3} M tested 20 min before and after completing the dose-response curve. Student's t test was used to compare differences in the values.

Drugs

The following were used: γ-aminobutyric acid (GABA) (Sigma), muscimol (Serva), bicuculline methiodide (Pierce), 3-amino-1-propanesulphonic acid (homotaurine) (kindly supplied by Prof G. Adembri), hyoscine hydrobromide (BDH), tetrodotoxin (Sigma), (−)- and (+)-β-(*p*-chlorophenyl)-γ-aminobutyric acid (−)- and (+)-baclofen (kindly supplied by Dr W. Bencze: CIBA-GEIGY), acetylcholine chloride (Sigma), histamine dihydrochloride (Carlo Erba), phentolamine hydrochloride (CIBA), propranolol hydrochloride (ICI), naloxone hydrochloride (Endo), theophylline (Carlo Erba), hexamethonium bromide (Sigma), noradrenaline bitartrate (Merck), morphine hydrochloride (Sigma), adenosine (Merck), prostaglandin $F_{2\alpha}$ (Sigma), 1,1-dimethyl-4-phenylpiperazinium iodide (DMPP) (Fluka), papaverine hydrochloride (Eli Lilly), mepyramine maleate (Gianni), methysergide dimaleate (Sandoz).

Results

Actions of GABA, homotaurine, muscimol and baclofen on guinea-pig ileum and antagonism with bicuculline

At doses ranging from 3×10^{-6} M to 10^{-4} M, GABA elicited in this preparation a contraction followed by a relaxation (Hobbiger, 1958; Krantis *et al.*, 1980) (Figure 1a), while at lower doses (from 10^{-7} M to 3×10^{-6} M) we observed only a relaxation. GABA-induced relaxation was not maintained, and the preparation recovered its basal tone in about 4 min. The GABA agonists muscimol and homotaurine elicited a similar contraction (Figure 1a) but they elicited only a slight relaxation in about 10% of the preparations tested. Homotaurine- and muscimol-induced relaxations were never seen at doses that did not elicit contraction. The putative $GABA_B$ agonist, (−)-baclofen elicited only relaxation, while (+)-baclofen was effective only at higher doses (Figure 1a). GABA-induced relaxation usually began at about 10 times lower doses than those eliciting contraction and

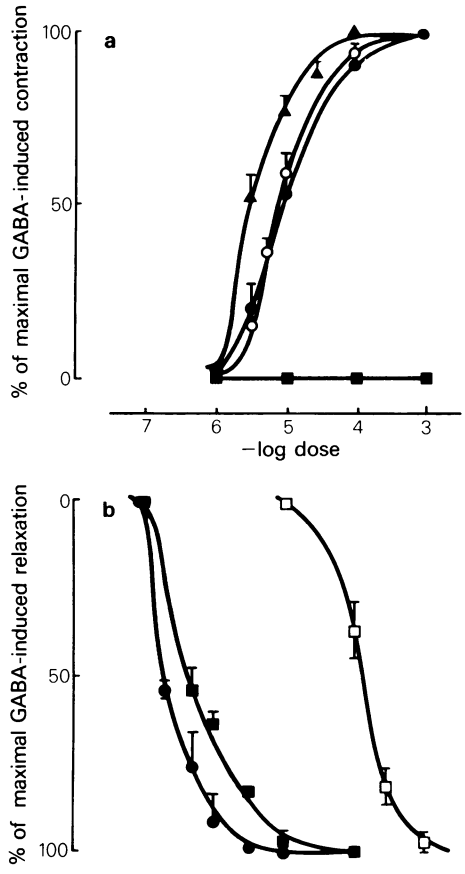

Figure 2 Log dose-response curves for contractions (a) induced by GABA (●), homotaurine (○) and muscimol (▲) and for relaxations (b) elicited by GABA (●), (−)-baclofen (■) and (+)-baclofen (□). Each symbol is mean of at least 5 observations; vertical lines show s.e.mean. Effects are presented as percentage of the maximal effect elicited by GABA. The maximal relaxant effect represented in absolute values is 14% of the maximal contractile effect.

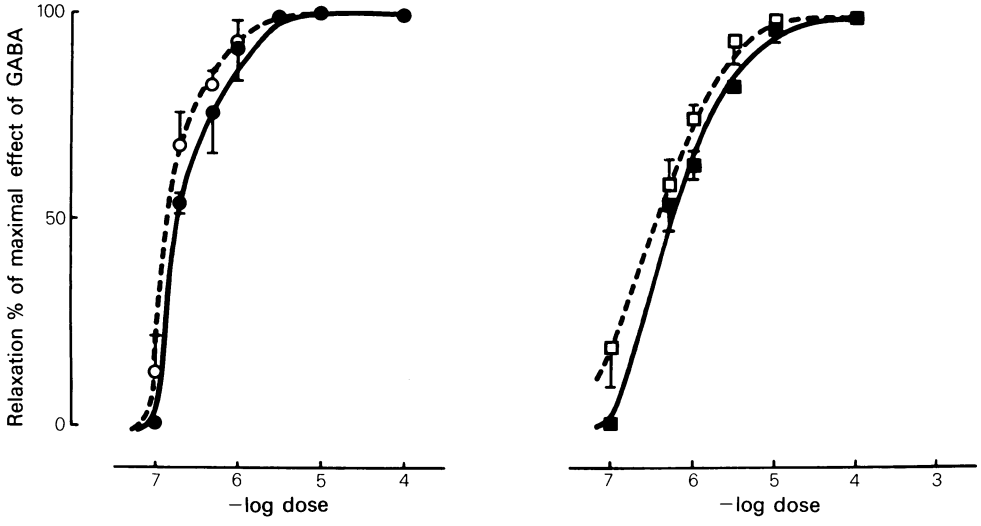

Figure 3 Log dose-response curves for relaxations induced in strips of ileum longitudinal muscle by GABA (●) and (−)-baclofen (■); 60 min after perfusing with bicuculline (10^{-5} M) dose-response curves were again obtained with GABA (○) and (−)-baclofen (□). Each symbol is mean of at least 5 observations; vertical lines show s.e.mean. In control experiments responses to the agonists were not significantly modified when the doses of agonists were repeated after 60 min in the absence of bicuculline.

reached its maximum at 3×10^{-6} M (Figure 2a and b). Log dose-response curves for relaxation elicited by GABA (ED_{50} $1.7 \pm 0.7 \times 10^{-7}$ M; $n = 6$), (−)-baclofen (ED_{50} $2.8 \pm 0.5 \times 10^{-7}$ M; $n = 6$), and (+)-baclofen (ED_{50} $1.2 \pm 0.9 \times 10^{-4}$ M; $n = 5$) were parallel and reached the same maximum (Figure 2b). GABA (ED_{50} $1.22 \pm 0.4 \times 10^{-5}$ M; $n = 21$), homotaurine (ED_{50} $9.5 \pm 1.5 \times 10^{-6}$ M; $n = 16$) and muscimol (ED_{50} $3.7 \pm 0.8 \times 10^{-6}$ M; $n = 19$) elicited a dose-dependent contraction. Log dose-response curves were parallel and reached the same maximum (Figure 2a). Muscimol was about 3 times more potent than GABA and homotaurine.

Relaxations induced by GABA, (−)-baclofen or (+)-baclofen were insensitive to bicuculline 10^{-5} M, while contractions induced by GABA, homotaurine or muscimol were antagonized by bicuculline (Figure 1b). In the presence of bicuculline 10^{-5} M, log-dose-response curves for GABA- and (−)-baclofen-induced relaxation were not modified (Figure 3). By

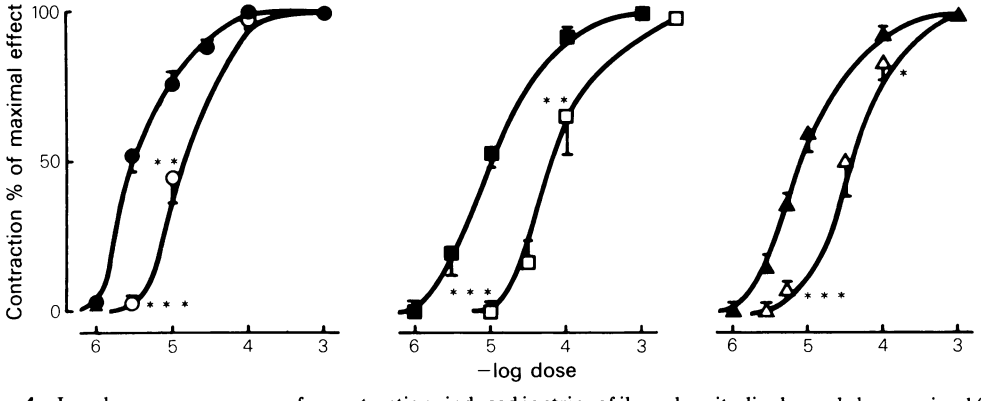

Figure 4 Log dose-response curves for contractions induced in strips of ileum longitudinal muscle by muscimol (●), GABA (■) and homotaurine (▲); 60 min after perfusion with bicuculline (10^{-5} M), dose-response curves were again obtained with muscimol (○), GABA (□) and homotaurine (△). Each symbol is mean of at least 4 observations; vertical lines show s.e. mean. Effects are presented as a percentage of the maximal effect elicited by GABA. Responses obtained in the presence and absence of the antagonist were compared statistically: $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.

contrast, bicuculline 10^{-5} M competitively antagonized the contractile effect of GABA, muscimol and homotaurine (Figure 4), while it did not antagonize the effect of ACh 10^{-8} M and histamine 10^{-7} M. At concentrations ranging from 10^{-6} M to 3×10^{-5} M, bicuculline gave closely similar pA_2 values, with GABA (5.91 ± 0.33; $n = 12$), homotaurine (5.91 ± 0.04; $n = 11$) and muscimol (5.74 ± 0.2; $n = 12$) as stimulant drugs. The slope of the Schild plot was 1 for GABA, 0.96 for homotaurine and 0.98 for muscimol, thus confirming a competitive antagonism.

Further pharmacological analysis of bicuculline-insensitive relaxation evoked by GABA-agonists

Bicuculline-insensitive relaxations induced by GABA and (−)-baclofen were abolished by tetrodotoxin (3×10^{-7} M; $n = 4$) and by hyoscine (2×10^{-7} M; $n = 4$) but were not modified by phentolamine (3×10^{-6} M) plus propranolol (3×10^{-6} M) ($n = 5$), naloxone (10^{-5} M; $n = 5$) or theophylline (5×10^{-5} M; $n = 4$). These drugs antagonized the effects of noradrenaline (10^{-6} M), morphine (10^{-7} M) and adenosine (10^{-6} M) respectively. The relaxations were not modified by hexamethonium (3×10^{-4} M; $n = 3$) (Table 1).

Cross-desensitization experiments on the relaxant action of GABA and (−)-baclofen

When the preparation was maintained for 5 min in the presence of GABA 10^{-6} M, responses to GABA (10^{-6} M; $n = 4$) and (−)-baclofen (10^{-6} M; $n = 4$) were abolished, while adenosine still induced a relaxation (Figure 5). This suggested that a specific cross-desensitization had occurred between GABA and (−)-baclofen.

Action of GABA and (−)-baclofen in pharmacologically-evoked hypertone

In a set of experiments the relaxant effects of GABA and (−)-baclofen were studied in the basal state and after having increased tone with drugs (Figure 6). Relaxant drugs were administered after having allowed the tone to stabilize at a higher level (usually after 2–4 min). Following the administration of histamine (10^{-7} M; $n = 4$) and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$, 10^{-5} M; $n = 4$), GABA and (−)-baclofen induced a relaxation similar in size to that obtained before the increase in tone. This relaxant effect was abolished by hyoscine (2×10^{-7} M; $n = 3$) both in the basal state and after histamine or $PGF_{2\alpha}$ presumably because of the reduction in cholinergic tone. When the tone was raised by the ganglionic stimulant drug DMPP (3×10^{-6} M; $n = 6$), GABA and (−)-baclofen in-

Table 1 Effect of pharmacological antagonists on the relaxation induced by GABA agonists

	Tetrodotoxin (3×10^{-7} M)	*Hyoscine* (2×10^{-7} M)	*Hexamethonium* (3×10^{-4} M)	*Phentolamine* (3×10^{-6} M) + *Propranolol* (3×10^{-6} M)	*Theophylline* (5×10^{-5} M)	*Naloxone* (10^{-5} M)	*Bicuculline* (10^{-5} M)
GABA 10^{-6} M	B (4)	B (5)	NB (3)	NB (5)	NB (4)	NB (3)	NB (5)
(−)-Baclofen 10^{-6} M	B (4)	B (5)	NB (3)	NB (5)	NB (4)	NB (3)	NB (5)
Noradrenaline 10^{-6} M	NT	NT	NT	B (5)	NT	NT	NB (5)
Morphine 10^{-7} M	NT	NT	NT	NT	NT	B (3)	NT
Adenosine 10^{-6} M	NT	B (3)	NT	NT	B (4)	NT	NB (5)
Papaverine 2.5×10^{-5} M	NB (4)	NB (5)	NT	NT	NT	NT	NB (3)

B = blocked; NB = not blocked; NT = Not tested.
In parentheses: number of observations.

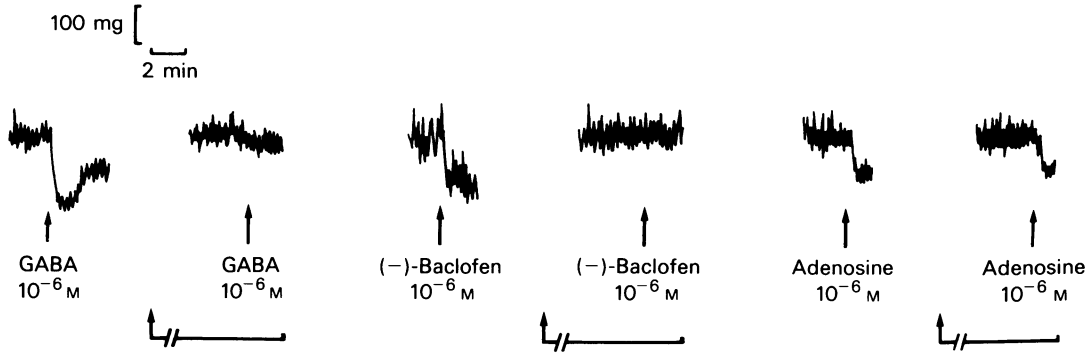

Figure 5 Cross-desensitization between GABA and (−)-baclofen but not adenosine-mediated relaxation of the ileum. The relaxant effects of GABA 10^{-6} M, (−)-baclofen 10^{-6} M and adenosine 10^{-6} M were determined before and 5 min after administration of GABA 10^{-6} M (horizontal lines with arrows). Twenty minutes were allowed to elapse between doses of drugs administered alone or in presence of GABA. The readings have been aligned so that the relative tone in the preparation at each time is correctly represented.

duced greater relaxations, that were also insensitive to bicuculline 3×10^{-5} M ($n = 4$).

In high potassium medium (40 mM), GABA and (−)-baclofen had no effect on the tonic component of the KCl-induced contraction ($n = 4$), while papaverine (2.5×10^{-5} M), as expected, relaxed the preparation.

Desensitization of bicuculline-sensitive contraction induced by GABA, homotaurine and muscimol

Five minutes after the administration of a submaximal dose of GABA (10^{-4} M), the preparation showed desensitization to GABA (10^{-4} M), homotaurine (10^{-4} M) and muscimol (10^{-4} M), while responses to histamine (10^{-7} M) and acetylcholine (10^{-7} M) remained unchanged (Figure 7).

Effect of other pharmacological antagonists on contraction induced by GABA agonists

GABA-, homotaurine- and muscimol-induced contractions were abolished by tetrodotoxin (3×10^{-7} M; $n = 3$) and were non-competitively antagonized by hyoscine (2×10^{-7} M) (Figure 8). Contractions were not antagonized by hexamethonium (3×10^{-4} M;

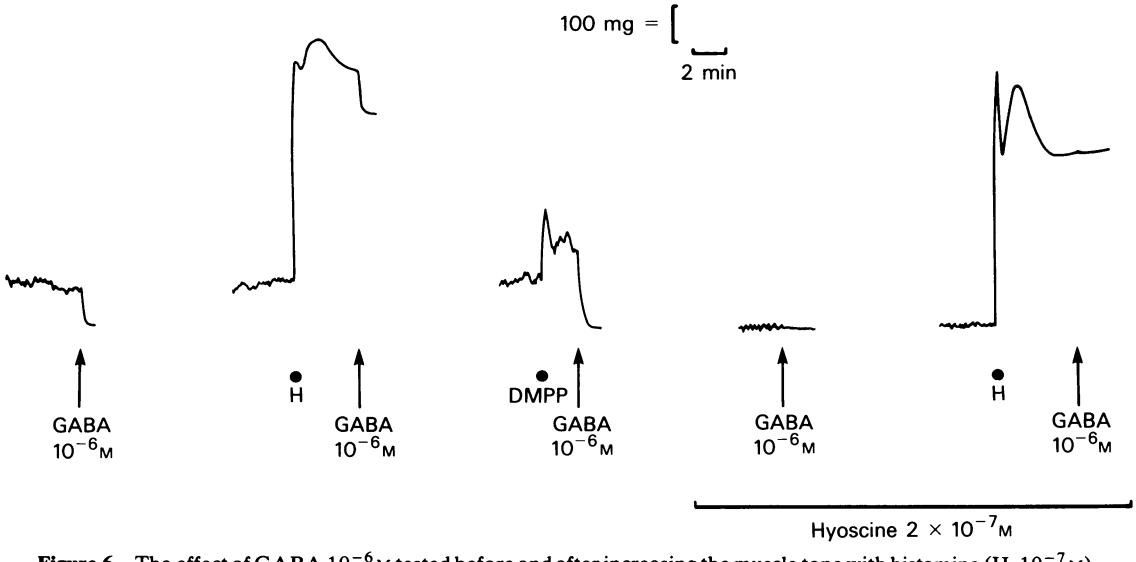

Figure 6 The effect of GABA 10^{-6} M tested before and after increasing the muscle tone with histamine (H, 10^{-7} M) and DMPP (3×10^{-6} M). After perfusing with hyoscine (2×10^{-7} M) for 20 min, GABA 10^{-6} M was retested and its effect compared with that obtained by raising the tone with histamine (10^{-7} M). The readings have been aligned so that the relative tone in the preparation at each time is correctly represented.

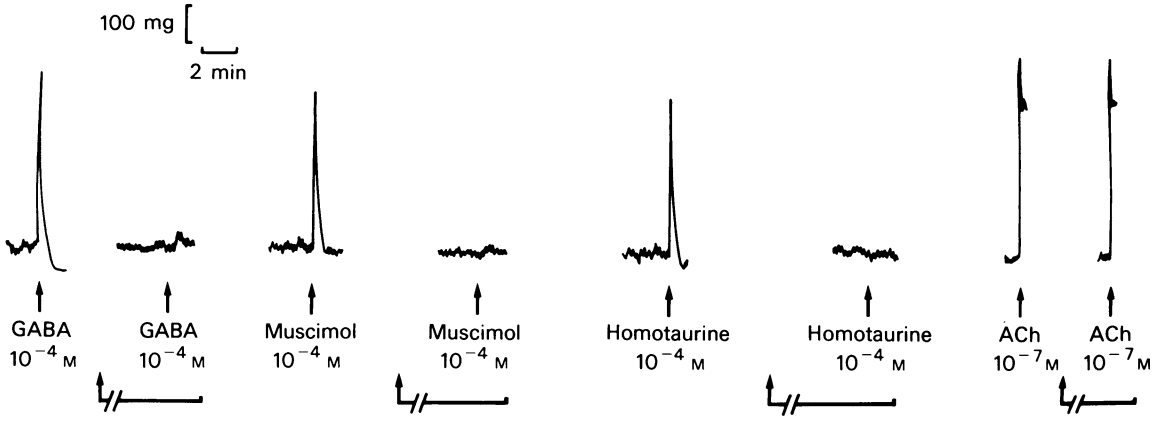

Figure 7 Cross-desensitization between GABA (10^{-4} M) and muscimol (10^{-4} M) and homotaurine (10^{-4} M) but not acetylcholine (ACh 10^{-7} M) on their contractile action in the ileum preparation. Each compound was tested before and 5 min after administration of GABA (10^{-4} M, horizontal lines with arrows). Twenty minutes were allowed to elapse between doses of drugs administered alone or in the presence of GABA. The readings have been aligned so that the relative tone in the preparation at each time is correctly represented.

$n = 3$), mepyramine (5×10^{-8} M; $n = 4$) and methysergide (5×10^{-8} M; $n = 4$) which antagonized the effects of DMPP (3×10^{-6} M), histamine (10^{-7} M) and 5-HT (10^{-7} M) respectively (Table 2).

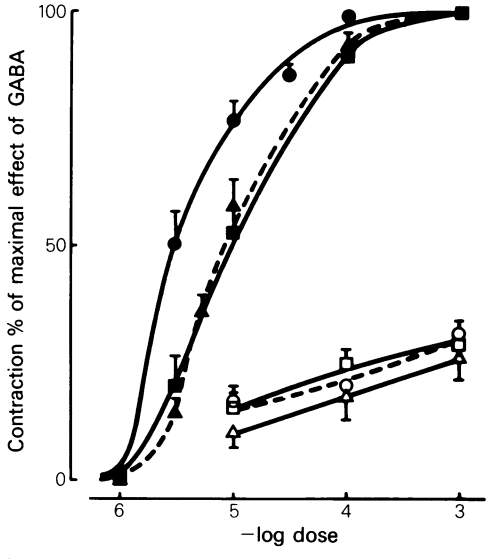

Figure 8 Log dose-response curves for contraction of strips of longitudinal muscle of ileum by GABA (■), homotaurine (▲) and muscimol (●). Sixty minutes after perfusing with hyoscine (10^{-7} M) dose-response curves were again obtained with GABA (□), homotaurine (△) and muscimol (○). Each symbol is mean of at least 5 observations; vertical lines show s.e.mean. Responses (ordinate scale) are presented as a percentage of the maximal effect elicited by GABA.

Discussion

A dual action of GABA (contraction and relaxation) in the guinea-pig intestine has previously been described (Hobbiger, 1958; Krantis *et al.*, 1980; Kaplita *et al.*, 1982).

Our data on contraction in ileum longitudinal muscle confirm results recently obtained by Krantis *et al.* (1980) and Kaplita *et al.* (1982). GABA-elicited contraction appears to be mediated by the classical $GABA_A$ receptor, since it is bicuculline-sensitive; it is also evoked by the $GABA_A$ agonists homotaurine and muscimol, and a specific cross-desensitization occurs for the contractile effects of those drugs. Log dose-response curves for GABA, muscimol and homotaurine are parallel and reach the same maximum. The pA_2 values of bicuculline with GABA, homotaurine and muscimol are similar to or match those reported by Krantis & Kerr (1981) for GABA in the same preparation. All this evidence points to a common bicuculline-sensitive receptor site. We also confirm the neurogenic cholinergic post-ganglionic nature of the GABA-induced contraction, in that it can be blocked by tetrodotoxin and hyoscine (Krantis *et al.*, 1980) but not by hexamethonium.

GABA-induced relaxation can be distinguished from GABA-evoked contraction by its bicuculline-insensitivity. GABA-induced relaxation can be further distinguished from GABA-induced contraction on the basis of the different range of doses at which they each occur. In fact, as shown in the dose-effect curves, relaxation has a lower threshold and reaches its maximum at doses of GABA which cause only slight contraction. Bicuculline-insensitive relaxation is also produced by (−)-baclofen, a

Table 2 Effect of pharmacological antagonists on GABA agonist-induced contraction in ileum

	Tetrodotoxin (3×10^{-7} M)	*Hyoscine* (2×10^{-7} M)	*Hexamethonium* (3×10^{-4} M)	*Mepyramine* (5×10^{-8} M)	*Methysergide* (5×10^{-8} M)	*Bicuculline* (10^{-5} M)
GABA 10^{-5} M	B (3)	B (7)	NB (3)	NB (4)	NB (4)	B (9)
Homotaurine 10^{-5} M	B (3)	B (6)	NB (3)	NB (4)	NB (4)	B (7)
Muscimol 3×10^{-6} M	B (3)	B (6)	NB (3)	NB (4)	NB (4)	B (7)
Acetylcholine 10^{-7} M	NT	B (7)	NT	NT	NT	NB (4)
5-HT 10^{-7} M	NT	NT	NT	NT	B (3)	NT
Histamine 10^{-7} M	NB (3)	NT	NT	B (4)	NT	NB (4)
DMPP 3×10^{-6} M	NT	NT	B (4)	NT	NT	NT

B = blocked; NB = not blocked; NT = not tested.
In parentheses: number of observations.

$GABA_B$ agonist, which never causes contraction. The action of baclofen is stereospecific since (+)-baclofen is much less potent than (−)-baclofen. Log dose-response curves of (−)-baclofen and GABA for relaxation are parallel and reach the same maximum. Furthermore, since a specific cross tachyphylaxis occurs between GABA and (−)-baclofen, we infer that in the ileum, relaxation is mediated by a $GABA_B$ receptor. Muscimol and homotaurine only rarely produce a slight relaxation, confirming that they might be regarded predominantly as $GABA_A$ agonists.

Relaxation in the ileum appears to involve a neural inhibitory presynaptic mechanism. In fact, relaxation is abolished by tetrodotoxin and is dependent on the basal neural tone of the ileum which is cholinergic in nature (Johnson, 1962). If cholinergic tone is lowered by hyoscine relaxation disappears, while if it is heightened by DMPP, a greater bicuculline-insensitive relaxation occurs. By contrast, if myogenic tone is raised by histamine or by $PGF_{2\alpha}$, GABA-induced relaxation remains unaffected. In a high K medium, when both neurones and muscle cells are depolarized, no GABA action is demonstrated. These results match those obtained by Hobbiger (1958), who demonstrated an antinicotinic effect of GABA, and by Bowery *et al.* (1981), who found a bicuculline-insensitive inhibition of twitch response in field-stimulated preparations, which they interpreted as a presynaptic action of GABA on peripheral autonomic nerve terminals.

Because of the dual effect of GABA, an interference between contraction and relaxation could take place. However relaxation is apparently not influence by contraction, since when contractions are antagonized by bicuculline (10^{-5} M), the log dose-response curve for GABA-induced relaxation remains unchanged. Hence, at concentrations up to 3×10^{-6} M, GABA-induced relaxation is maximal and can be assumed to be constant. This fact makes possible comparison of the pA_2 of bicuculline for contraction evoked by GABA analogues, even if GABA induces relaxation and the other drugs do not. On the other hand, the lack of a specific $GABA_B$ antagonist makes it difficult to evaluate the influence of relaxation on contraction. In preliminary experiments (unpublished observations) we tried to perform such an evaluation by comparing contractile responses to GABA (3×10^{-6} M, 10^{-5} M, 10^{-4} M) before and after perfusion of the preparation with (−)-baclofen (10^{-5} M). Under these conditions, $GABA_B$ receptors are desensitized. Contractile responses to GABA were only weakly increased in the presence of (−)-baclofen, apparently in the range of experimental variability. Moreover the slope of the log dose-response curve for GABA contraction was not significantly different from those of the more

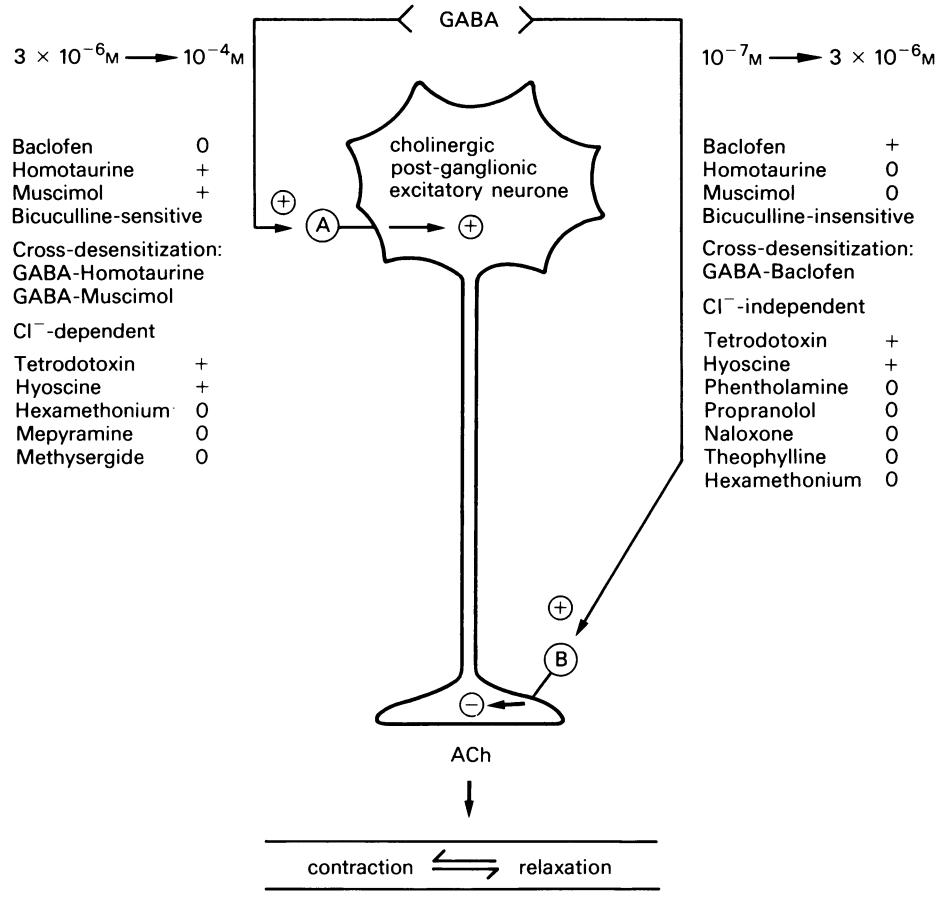

Figure 9 Diagram interpreting the dual actions of GABA as a consequence of the presence of two GABA receptors on an intramural cholinergic neurone and the effects of various drugs. Localization of receptors on the cholinergic neurone is purely arbitrary.

specific $GABA_A$ agonists studied. This indicates that an eventual interference of GABA relaxation with contraction would not be strong enough to modify the slope of the GABA contraction curve. Comparison of ED_{50} values for contraction should thus be possible.

In conclusion, the action of GABA in the intestine appears to be exerted chiefly through a modulatory action on cholinergic post-ganglionic neurones. A possible model of this modulatory effect is presented in Figure 9. GABA excites cholinergic neurones through a bicuculline-sensitive receptor ($GABA_A$), that may act by increasing the membrane permeability to chloride (Krantis & Kerr, 1981). The other site of GABA action is the bicuculline-insensitive receptor ($GABA_B$), located on cholinergic nerve-terminals. The effect mediated by this receptor is inhibitory and is chloride-independent (Bowery *et al.*, 1981). At the moment, however, it cannot be established whether the action of GABA at this level is exerted directly on the cholinergic neurone or through an eventual interneurone. Moreover, the clear parallels between the pharmacological features of GABA receptors at the central and peripheral levels suggest that peripheral preparations could provide a model for studying substances presumed to possess a GABA-ergic action.

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References

ARUNLAKSHANA, O. & SCHILD, H.O. (1959). Some quantitative uses of drug antagonists. *Br. J. Pharmac. Chemother.*, **14**, 48–57.

BOWERY, N.G. & BROWN, D.A. (1974). Depolarizing action of γ-aminobutyric acid and related compounds on rat superior cervical ganglia in vitro. *Br. J. Pharmac.*, **50**, 205–218.

BOWERY, N.G., DOBLE, A., HILL, D.R., HUDSON, A.L., SHAW, J.S., TURNBULL, M.G. & WARRINGTON, R. (1981). Bicuculline-insensitive GABA receptors on peripheral autonomic nerve terminals. *Eur. J. Pharmac.*, **71**, 53–70.

CURTIS, D.R. & JOHNSTON, G.A.R. (1974). Amino acid transmitters in the mammalian central nervous system. *Ergebn. Physiol.*, **69**, 97–188.

GALLI, A., ZILLETTI, L., SCOTTON, M., ADEMBRI, G. & GIOTTI, A. (1979). Inhibition of Na^+-independent [^{3}H]-GABA binding to synaptic membrane of rat brain by β-substituted GABA derivatives. *J. Neurochem.*, **32**, 1123–1125.

HILL, D.R. & BOWERY, N.G. (1981). [^{3}H]-baclofen and [^{3}H]-GABA bind to bicuculline-insensitive $GABA_B$ sites in rat brain. *Nature*, **290**, 149–152.

HOBBIGER, F. (1958). Effects of γ-aminobutyric acid on the isolated mammalian ileum. *J. Physiol.*, **142**, 147–164.

JESSEN, R.K., MIRSKY, R., DENNISON, M.E. & BURNSTOCK, G. (1979). GABA may be a neurotransmitter in the vertebrate peripheral nervous system. *Nature*, **281**, 71–74.

JOHNSON, E.S. (1962). A note on the relation between the resting release of acetylcholine and increase in tone of the isolated guinea-pig ileum. *J. Pharm. Pharmac.*, **15**, 69–72.

KAPLITA, P.V., WATERS, D.H. & TRIGGLE, D.J. (1982). γ-Aminobutyric acid action in guinea-pig ileal myenteric plexus. *Eur. J. Pharmac.*, **79**, 43–51.

KRANTIS, A., COSTA, M., FURNESS, J.B. & ORBACH, J. (1980). γ-Aminobutyric acid stimulates intrinsic inhibitory and excitatory nerves in the guinea-pig intestine. *Eur. J. Pharmac.*, **67**, 461–468.

KRANTIS, A. & KERR, D.I. (1981). GABA-induced excitatory responses in the guinea-pig small intestine are antagonized by bicuculline, picrotoxinin and chloride ion blockers. *Naunyn-Schmiedebergs Arch. Pharmac.*, **317**, 257–261.

PATON, D.M. & ZAR, M.A. (1968). The origin of acetylcholine released from guinea-pig intestine and longitudinal muscle strips. *J. Physiol.*, **194**, 13–33.

SCHILD, H.O. (1947). pA, a new scale for the measurement of drug antagonism. *Br. J. Pharmac. Chemother.*, **2**, 189–206.

WILKIN, G.P., HUDSON, A.L., HILL, D.R. & BOWERY, N.G. (1981). Autoradiographic localization of $GABA_B$ receptors in rat cerebellum. *Nature*, **294**, 584–587.

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