A facilitatory effect of bicuculline on the enteric neurones in the guinea-pig isolated colon

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- 1 Changes in the efficiency of the peristaltic reflex, acetylcholine (ACh) output and motor responses to transmural and periarterial nerve stimulation produced by bicuculline and γ -aminobutyric acid (GABA) receptor desensitization were investigated in the guinea-pig isolated colon.
- 2 Bicuculline, at concentrations unable to affect spontaneous colonic motility and lacking anticholinesterase activity, produced a dose-dependent increase of both the efficiency of the peristaltic reflex and the stimulated ACh output. Such effects could not be observed in GABA-desensitized preparations.
- 3 A frequency-dependent potentiation of the cholinergic excitatory and non-adrenergic non-cholinergic (NANC) inhibitory responses to transmural stimulation was also observed in the presence of bicuculline. Conversely bicuculline exhibited an inhibitory effect on the relaxation induced by periarterial nerve stimulation.
- 4 Acute GABA-desensitization was unable to affect the contractile responses to transmural stimulation, the ACh output and the efficiency of the peristaltic reflex. On the contrary, desensitization was able to mimic the effects of bicuculline on the inhibitory responses to both transmural and periarterial nerve stimulation.
- 5 Our results are consistent with a significant role played by an intrinsic GABAergic pathway in the modulation of both cholinergic excitatory and NANC inhibitory neurones. The hypothesis is advanced that a feed-back modulation carried out through bicuculline-sensitive GABAergic synapses could operate during the propagation of peristaltic motor activity.

Introduction

Since high affinity uptake of γ -aminobutyric acid (GABA) by enteric neurones has been demonstrated by Jessen *et al.* (1979), evidence has accumulated that GABA may serve as a transmitter in the enteric nervous system. Besides being widely distributed along the intestine together with its synthesizing and metabolic enzymes (Miki *et al.*, 1983; Krantis & Harding, 1986) and strongly taken up by intrinsic neurones (Jessen *et al.*, 1983), GABA has been shown to be released during depolarization of enteric neurones (Taniyama *et al.*, 1983a). Moreover enteric neurones have been shown to be sensitive to GABA receptor stimulation (Krantis *et al.*, 1980; Giotti *et al.*, 1983; Cherubini & North, 1984). However, the involvement of the postulated GABAergic pathway in the neurogenic control of intestinal motor function is still a matter of speculation. In particular little is

known about the participation of the putative GABAergic neurones in the multineuronal pathways which subserve the intestinal intrinsic reflexes. All the hypotheses on the possible functional role of endogenous GABA are still mainly based on the analogy to the pharmacological effects of exogenous GABA (Giotti *et al.*, 1985). Little attention has been devoted to the study of the functional effects of GABA receptor blockade which has so far yielded conflicting results (Krantis *et al.*, 1980; Krantis & Kerr, 1981a; Ong & Kerr, 1983).

In the present paper the effects of the GABA receptor antagonist, bicuculline, and of the desensitization to exogenous GABA on the efficiency of the peristaltic reflex, acetylcholine output and nerve mediated responses of the longitudinal muscle have been investigated in the guinea-pig distal colon. In

addition, the ability of the motor enhancing concentrations of bicuculline to exhibit anticholinesterase activity was also tested.

Methods

Experiments were carried out in the guinea-pig isolated distal colon. The models used to measure the nerve-mediated motor activities were the peristaltic reflex and the responses of the longitudinal muscle to nerve stimulation. Moreover, the activity of cholinergic neurones was investigated by measuring the stimulated acetylcholine (ACh) output. Isolated specimens of terminal colon 5–7 cm long were taken from male guinea-pigs weighing 300–400 g, mounted in organ baths perfused with Tyrode solution and loaded with 1–1.5 g through an isotonic force transducer.

The peristaltic reflex was elicited by a radial distension of the lumen applied at the proximal end of the specimens by means of an intraluminal balloon. Longitudinal muscle movements and displacement of the balloon towards the distal end of the colon together with the surface electrical activity were recorded. Since the velocity of propulsion is dependent on the degree of distension (Frigo & Lecchini, 1970), supramaximal stimulation was employed and the maximal velocity of propulsion taken as a measure of the efficiency of the peristaltic reflex. The improvement or the inhibition of peristalsis was indicated as the percentage variation of the propulsion velocity with respect to the control value and was estimated as described by Frigo *et al.* (1984). Electrical activity was recorded from the serosal surface by means of two extracellular glass electrodes (Frigo *et al.*, 1972; Tonini *et al.*, 1974).

ACh release was measured by incubating the specimens in a 3 ml organ bath with physostigmine sulphate (1.5×10^{-5} M) during 20 min collection periods, and by applying rectangular pulses of supramaximal strength, 1 ms duration and 1 Hz frequency delivered by coaxial silver wire electrodes for the first 10 min in a way similar to that described by Frigo *et al.* (1984). The 1 Hz frequency of stimulation was chosen because of its relative selectivity in stimulating the enteric cholinergic neurones (Cowie *et al.*, 1978). In order to measure ACh release from GABA-desensitized preparations, a preincubation period of 20 min with GABA (1×10^{-4} M) was allowed before starting the collection of the ACh and GABA (1×10^{-4} M) was then present throughout the experiment. ACh was assayed on the guinea-pig isolated ileum incubated with morphine sulphate (6.6×10^{-6} M) and physostigmine sulphate (7.7×10^{-9} M) in a way similar to that described by Paton & Vizi (1969). To allow for any effects that

modifying drugs might have on the ACh assay, the concentration of such drugs in the test samples were duplicated in the standard ACh solutions during the assay. The effects of drugs on the ACh release were expressed as percentage variations in respect to the control level.

Field-stimulation was used to stimulate intrinsic nerves by delivering trains of rectangular pulses of supramaximal strength, 0.5 ms duration and frequency ranging from 0.2 to 20 Hz lasting 20 s, through coaxial silver wire electrodes. The non-adrenergic, non-cholinergic (NANC) component of the response to the intrinsic nerve stimulation was revealed by applying transmural stimulation to specimens pretreated with hyoscine sulphate (3×10^{-6} M) and guanethidine sulphate (3×10^{-6} M). The sympathetic supply to the colon was stimulated by applying trains of rectangular pulses of supramaximal strength, 0.1 ms duration and frequency ranging from 0.2 to 20 Hz lasting 20 s, by means of bipolar silver wire electrodes placed around the periarterial plexus of the inferior mesenteric artery. Electrical stimulation was applied at 10 min intervals and either excitatory or inhibitory responses of the longitudinal muscle are given as percentage of the maximal responses. The maximal response was considered the highest response independent of the frequency employed. Frequency-response relationships for both excitatory and inhibitory responses to transmural nerve stimulation were obtained over the range of 0.2–8 and 0.2–3 Hz, respectively. The range of frequencies used to construct frequency-response relationships for periarterial nerve stimulation was 0.5–10 Hz. For all kinds of stimulations, the frequency able to produce half maximum effect (F_{50}) was calculated.

The ability of bicuculline to modify the efficiency of the peristaltic reflex, the ACh output and the longitudinal muscle responses to nerve stimulation was investigated both in naïve and in GABA-desensitized preparations. The effects of bicuculline were evaluated after a preincubation period of at least 20 min for each concentration of the drug. Desensitization to exogenous GABA, unless otherwise indicated, was achieved by cumulative administrations of GABA (1×10^{-4} M) at 10 min intervals (Krantis *et al.*, 1980) and assessed by the lack of any relaxation of the longitudinal muscle in response to the drug. In separate experiments, the ability of acute GABA-desensitization to modify the efficiency of the peristaltic reflex, ACh output and the longitudinal muscle responses to nerve stimulation was investigated.

The potential anticholinesterase activity of bicuculline was assayed on *Electrophorus electricus* purified acetylcholinesterase (AChE) and on horse serum butyrylcholinesterase (BuChE). The Michaelis-Menten constants for substrate transformation (K_m), the concentrations responsible for 50% cholinesterase

inhibition (IC_{50}), the equilibrium dissociation constants for drug-enzyme combination (K_i), or that for the interaction with enzyme-substrate complex K_i were calculated as previously described by Galli *et al.* (1984). The time-dependency of the drug-enzyme interaction and the reversibility of the drug's anticholinesterase activity were also evaluated. IC_{50} values were calculated from semilogarithmic plots of drug concentration against percentage inhibition of the enzyme activity. The dissociation constants were calculated from double reciprocal plots of rate against substrate concentration according to Dixon & Webb (1979).

The Tyrode solution was of the following composition (mM): NaCl 136.9, KCl 2.7, $CaCl_2$ 1.8, $MgCl_2$ 1.04, $NaHCO_3$ 11.9, NaH_2PO_4 0.4 and glucose 5.5.

The drugs used were: acetylcholine chloride (ACh, Sigma Chemical); acetylcholinesterase from *Electrophorus electricus* (AChE, Boehringer); acetylthiocholine iodide (ATCh, Boehringer); hyoscine hydrochloride (Sigma Chemical); bicuculline methiodide (Sigma Chemical); butyrylcholinesterase from horse serum (BuChE, Sigma Chemical); butyrylthiocholine iodide (BuTCh, Boehringer); 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB, Boehringer); γ -aminobutyric acid (GABA, Sigma Chemical); guanethidine sulphate (Ciba); morphine sulphate (Carlo Erba); physostigmine sulphate (BDH); tetrodotoxin (TTX, Sankyo).

Linearization of the frequency or concentration-response relationships was obtained by log transformation and the least squares method in order to calculate the frequencies and the concentrations able to produce half maximum effect. Statistical sig-

nificance of differences between groups was analysed by applying Student's *t* test for unpaired data.

Results

Effect of desensitization to exogenous GABA

The efficiency of the peristaltic reflex in 9 out of 12 preparations was not significantly modified by desensitization achieved by cumulative administration of GABA (1×10^{-4} M). In fact the mean velocity of propulsion ($\pm$ s.e.mean) was similar before (2.18 ± 0.21 mm s $^{-1}$) and after GABA-desensitization (2.34 ± 0.19 mm s $^{-1}$). In the remaining three preparations after addition of GABA the peristaltic reflex could no longer be elicited. Either the frequency and amplitude of spikes and spontaneous contractions or the resting tone of longitudinal muscle were similar before and after GABA desensitization.

Similarly, desensitization to exogenous GABA did not affect the release of ACh. The mean ACh output ($\pm$ s.e.mean, $n = 8$) before and after GABA desensitization was 56.50 ± 12.11 ng g $^{-1}$ min $^{-1}$ and 54.67 ± 10.40 ng g $^{-1}$ min $^{-1}$, respectively.

The effect of GABA desensitization was tested on the hyoscine- and TTX-sensitive excitatory responses of longitudinal muscle to transmural stimulation and on the TTX-sensitive inhibitory responses to transmural or periaxillary nerve stimulation. The ability of GABA-desensitization to modify the nerve-mediated responses varied according to the kind of stimulus applied. The frequency-response relationships before and after GABA-desensitization are shown in Figure 1. The excitatory responses to transmural nerve

Figure 1 Effect of desensitization to exogenous GABA on the guinea-pig colon longitudinal muscle responses to nerve stimulation. Log frequency-response relationships for excitatory (a) and NANC inhibitory (b) responses to transmural stimulation and for inhibitory responses to periaxillary nerve stimulation (c) before (●) and after GABA-desensitization (○). Frequency of stimulation is plotted against percentage of the maximal response. Each point represents the mean of 5 experiments. Vertical bars indicate s.e.mean.

stimulation were not modified by acute GABA desensitization at all the frequencies tested, the F_{50} values with 95% confidence limits before and after GABA desensitization being 1.68 (0.94–2.98) and 1.87 (1.12–3.10) Hz, respectively. The effect of desensitization on the NANC inhibitory responses was frequency-dependent, inhibitory responses being significantly increased only at the lower frequencies (0.2–0.5 Hz). The F_{50} values with 95% confidence limits before and after GABA desensitization were 0.41 (0.27–0.62) and 0.31 (0.17–0.59) Hz, respectively. The inhibitory responses to periarterial nerve stimulation were reduced by GABA desensitization, the F_{50} values with 95% confidence limits before and after GABA desensitization being 1.71 (1.40–2.07) and 3.13 (2.07–4.32) Hz, respectively.

Effect of bicuculline in naïve and in GABA-desensitized preparations

Peristaltic reflex The mean velocity of propulsion ($\pm$ s.e.mean, $n = 8$) in naïve and in GABA-desensitized preparations was $2.03 \pm 0.19 \text{ mm s}^{-1}$ and $2.14 \pm 0.23 \text{ mm s}^{-1}$, respectively. Bicuculline at concentrations of $2 \times 10^{-6} \text{ M}$, $5 \times 10^{-6} \text{ M}$ and $1 \times 10^{-5} \text{ M}$ was able to increase significantly the velocity of propulsion in naïve but not in GABA-desensitized preparations. The effect of bicuculline was concentration-dependent, the log concentration-response relationship being shown in Figure 2. The maximum average percentage increase ($\pm$ s.e.mean, $n = 6$) of the velocity of propulsion was 37.14 ± 6.5 and was observed at the concentration of $1 \times 10^{-5} \text{ M}$. The concentration with 95% confidence limits producing half maximum effect in increasing the velocity of propulsion was $2.21 (1.12\text{--}4.38) \times 10^{-6} \text{ M}$. At the concentrations able to increase the efficiency of the peristaltic reflex, bicuculline did not affect either the frequency and amplitude of spikes and spontaneous contractions or the resting tone of longitudinal muscle (Figure 3). Similar concentrations were ineffective also in GABA-desensitized preparations. Bicuculline $1 \times 10^{-4} \text{ M}$ was able to prevent the onset of the peristaltic reflex both in naïve and in GABA-desensitized preparations.

Acetylcholine output The mean ACh output ($\pm$ s.e.mean, $n = 8$) in naïve and in GABA-desensitized organs was 49.03 ± 7.20 and $53.33 \pm 10.18 \text{ ng g}^{-1} \text{ min}^{-1}$, respectively. Bicuculline at concentrations of $5 \times 10^{-6} \text{ M}$ and $1 \times 10^{-5} \text{ M}$ was able to increase significantly the ACh output in naïve but not in GABA-desensitized organs. The effect of bicuculline was concentration-dependent, the log concentration-response relationship being shown in Figure 2. The maximum average percentage increase ($\pm$ s.e.mean, $n = 6$) of the ACh output was 19.71 ± 2.65 and was

observed at a concentration of $1 \times 10^{-5} \text{ M}$.

Nerve mediated excitation and inhibition of longitudinal muscle The effect of bicuculline on the frequency-

Figure 2 Effect of bicuculline on the efficiency of the peristaltic reflex and on the acetylcholine output in naïve (●—●) and in GABA-desensitized preparations (●---●) of guinea-pig colon. Log concentrations are plotted against percentage variation of velocity of propulsion (a) and of stimulation-induced acetylcholine (ACh) output (b). Unless otherwise indicated, each point represents the mean of 6 experiments. Vertical bars show s.e.mean. The asterisks indicate significant difference from the control value: (* $P < 0.05$; ** $P < 0.01$).

Figure 3 Effect of bicuculline on the distension-induced peristaltic reflex in the guinea-pig isolated colon. In each panel, from top to bottom: electrical activity recorded from surface electrodes placed on the oral (upper tracing) and the aboral (lower tracing) side in respect to the site of stimulation; longitudinal movements; oral-aboral displacement of the bolus. The mark (●) indicates distension of the intraluminal balloon. In (b) the reflex was elicited after 20 min incubation with bicuculline 1×10^{-5} M. Note the higher velocity of the propulsion as deduced from the steeper slope of the lower tracing in the presence of bicuculline.

response relationships of longitudinal muscle to transmural and periarterial nerve stimulation both in naïve and in GABA-desensitized preparations is shown in Figures 4 and 5. The ability of bicuculline to shift frequency-response relationships was tested at the concentration able to produce the maximum increase in both the propulsion velocity and the ACh output (1×10^{-5} M). Such a concentration was devoid of any effect on the longitudinal muscle resting tone. A potentiating effect of bicuculline 1×10^{-5} M on the excitatory responses of the longitudinal muscle to transmural stimulation was observed at the lower frequencies (0.2–2 Hz). Frequency-response relationship curve was shifted to the left, the F_{50} values with 95% confidence limits being 1.95 (1.17–3.24) Hz before and 0.84 (0.47–1.50) Hz after bicuculline administration. Bicuculline was able to increase significantly the NANC inhibitory responses at the lower frequencies (0.2–1.5 Hz), the F_{50} values with 95% confidence limits before and after bicuculline administration being 1.40 (0.49–3.94) and 0.29 (0.09–0.95) Hz, respectively. The inhibitory effect of periarterial nerve stimulation was reduced by bicuculline. The frequency-response relationship was shifted to the right, the F_{50} values with 95% confidence limits in the presence and in the absence of bicuculline being 1.04 (0.42–2.54) and 3.24 (2.50–4.21) Hz, respectively. No effect of bicuculline on either transmural or periarterial nerve stimulation could be observed in GABA-desensitized preparations.

Effect of bicuculline on cholinesterase activity

Bicuculline inhibited both electric eel AChE and horse serum BuChE in a mixed, competitive and non-competitive, manner (Figure 6). The slope inhibition constants, K_i , and the intercept inhibition constants, $K_{i'}$, are given in Table 1. The competitive nature of the inhibition, as can be deduced by comparing K_i and $K_{i'}$ values, was slightly higher for the bicuculline effect on AChE than on BuChE. Bicuculline was significantly more effective in inhibiting AChE than BuChE, the mean IC_{50} values ($\pm$ s.e.mean, $n = 4$) being $130 \pm 18 \mu\text{M}$ and $200 \pm 21 \mu\text{M}$, respectively. In the same conditions, physostigmine exhibited IC_{50} values as low as 0.02 and $0.06 \mu\text{M}$ on eel AChE and horse serum BuChE, respectively, proving to be about 3000–9500 times more potent than bicuculline. In the experiments carried out to evaluate the time-inhibition relationship, the mean AChE inhibition ($\pm$ s.e.mean, $n = 4$) produced by 0.1 mM bicuculline ($42 \pm 2\%$) was not modified by increasing the preincubation period from 0 to 30 min. In the experiments carried out to evaluate the reversibility of the bicuculline-induced AChE inhibition, rapid dilution of bicuculline concentration from 0.3 mM to $3 \mu\text{M}$ was able to decrease the mean AChE inhibition ($\pm$ s.e.mean, $n = 4$) from 69 ± 2 to $3 \pm 0.4\%$. No further enzyme recovery was observed by allowing up to 30 min time periods to elapse before performing the assay.

Figure 4 Effect of bicuculline on the longitudinal muscle responses to nerve stimulation in naïve and in GABA-desensitized preparations of guinea-pig isolated colon. The lines indicate log frequency-response relationships in naïve (a) and in GABA-desensitized preparations (b) for the excitatory (E) and NANC inhibitory (NANC) responses to transmural stimulation and for the inhibitory responses to periarterial nerve stimulation (P) before (●) and after (○) adding bicuculline 1×10^{-5} M. Frequency of stimulation is plotted against percentage of the maximal response. Each point represents the mean of 5 experiments. Vertical bars indicate s.e.mean.

Discussion

Measurement of the functional effects of GABA receptor blockade by competitive antagonists is considered to be a reliable pharmacological tool to assess

the role of GABAergic neurotransmission in the CNS (Enna, 1983). Bicuculline, although not completely devoid of non-specific effects, is still commonly employed as the reference drug for competitive antagonism on GABA receptors (Curtis *et al.*, 1971;

Figure 5 Effect of bicuculline on the guinea-pig colon longitudinal muscle responses to nerve stimulation. In the upper tracing (E), trains of pulses (supramaximal strength, 0.5 ms duration) at increasing frequencies delivered through coaxial electrodes were applied for 20 s periods. In the middle tracing (NANC), transmural stimulation was applied after pretreatment with hyoscine hydrochloride (3×10^{-6} M) and guanethidine sulphate (3×10^{-6} M). In the lower tracing (P), trains of pulses (supramaximal strength, 0.1 ms duration) delivered through bipolar electrodes around the periarterial plexus were applied. Between (a) and (b) bicuculline 1×10^{-5} M was added to the bath. Note that both excitatory and inhibitory responses to transmural stimulation were increased while responses to periarterial nerve stimulation were decreased in the presence of bicuculline.

Perez *et al.*, 1985). Indeed, the facilitatory effects of bicuculline on neuronal firing or neurotransmitter release has been interpreted as evidence of the involvement of GABA as a transmitter in the tonic modulation of CNS activity (Willette *et al.*, 1984). The effect of bicuculline on peripheral neurotransmission in the autonomic nervous system is less well-known although morphological and functional evidence for a GABA involvement in the neuronal modulation at autonomic synapses is accumulating (Bowery *et al.*, 1984). In particular it has been suggested that GABA plays a role as a neurotransmitter at the autonomic synapses of the gastrointestinal tract (Jessen *et al.*, 1983). However, the direct involvement of GABA in the integrated neuronal activity subserving the reflex

motor responses has so far been poorly substantiated by experimental evidence.

The transient effects of exogenously applied GABA, due to the rapidly induced desensitization, does not help much in interpreting the possible role of the endogenously released GABA in the onset and propagation of peristaltic motor activity. Indeed the peristaltic reflex develops at a fairly slow rate through a highly coordinated activation of both excitatory and inhibitory pathways (Costa & Furness, 1982). Therefore, it is uncertain to what extent the GABA-induced changes in propulsive activity ought to be ascribed to the agonist effect itself or to the consequence of GABA receptor-desensitization. Moreover, it is very doubtful that high concentrations

Figure 6 Anticholinesterase activity of bicuculline evaluated by the inhibition of electric eel AChE and horse serum BuChE. Double reciprocal plots of the substrate (ATCh or BuTCh) concentration ($1/S$) against the rate of transformation ($1/V$) as derived from the thiocholine related changes in the absorbance during a 30 s period. (a) The lines indicate double reciprocal plots for control (●) and in the presence of 25 μM (Δ), 50 μM (□) and 100 μM (○) bicuculline. (b) The lines indicate double reciprocal plots for control (●) and in the presence of 37.5 μM (Δ), 75 μM (□) and 150 μM (○) bicuculline. The mean K_m values ($\pm$ s.e.mean) were 90 ± 17 μM for eel AChE and 240 ± 32 μM for horse serum BuChE. Each point represents the mean of 4 experiments performed in duplicate.

of exogenous GABA applied to the whole preparation could simulate the hypothetical effect of a localized activation of the postulated GABAergic pathway during the propagation of peristaltic activity.

In our experiments bicuculline seems to be able to affect the responses to the activation of the different neuronal pathways involved in the control of colonic

Table 1 Anticholinesterase activity of bicuculline: K_i and K_f values for the inhibition of electric eel acetylcholinesterase (AChE) and horse serum butyrylcholinesterase (BuChE)

Inhibition constants	AChE (μM)	BuChE (μM)
K_i	52 ± 7.1	140 ± 30
K_f	186 ± 27	238 ± 37

K_i and K_f values ($\pm$ s.e.mean) were calculated from the double reciprocal plots shown in Figure 6 by replotting the slopes and the intercepts on $1/V$ axis against the different bicuculline concentrations (Dixon & Webb, 1979): the intercepts on the x axis gave K_i and K_f , respectively. Assays were performed in duplicate. Each value represents the mean of 4 assays.

motility. In preparations desensitized by cumulative administration of exogenous GABA, bicuculline failed to produce the effects observed in naïve preparations. Although the consequence of the combined effect of bicuculline and acute desensitization on the responsiveness to endogenously released GABA cannot be predicted on the grounds of the present knowledge of GABA receptor mechanisms (Enna, 1983), the failure of bicuculline to act in GABA-unresponsive preparations is highly suggestive for the effect of bicuculline being entirely dependent on the availability of functional GABA receptors.

GABA-unrelated effects of bicuculline, such as the weak and rapidly reversible inhibition of cholinesterases, as assessed *in vitro* on purified cholinesterases, were found to develop at concentrations at least 10 fold higher than those required to improve the efficiency of peristaltic reflex. This is likely to be true also in the intestinal neuromuscular preparations, whose acetyl- and butyryl-cholinesterases have been observed to behave as typical cholinesterases (Ambache *et al.*, 1971). Moreover, bicuculline has been proved to be devoid of any potentiating effect on the ileum longitudinal muscle responses to exogenous ACh (Krantis & Kerr, 1981b). Furthermore, the time courses of bicuculline-induced acetylcholinesterase inhibition as well as of enzyme activity recovery are very different from those observed for physostigmine (Wilson *et al.*, 1960; Main & Hastings, 1966).

Bicuculline exhibited opposite effects on the peristaltic reflex depending on the concentration employed. However, only the propulsion-enhancing effect appeared to be GABA-dependent, being present in naïve but not in GABA-desensitized preparations. Bicuculline was highly effective in improving the efficiency of peristalsis, being able to produce up to 40% increase of the maximal velocity of propulsion. No propulsion improvement or biphasic effect of bicuculline was described by Krantis *et al.* (1980) and

Krantis & Kerr (1981a) in the guinea-pig isolated colon. However, the range of concentrations employed and the experimental model used to elicit and to measure colonic motility were different from those used in our experiments.

An increase in the efficiency of peristaltic reflex as measured by the propulsion velocity, could result from a facilitation of either ascending excitation or descending inhibition or both, or from an improvement in their distal propagation. On the other hand, both excitatory and inhibitory responses involve cholinergic synapses (Crema *et al.*, 1970; North & Tokimasa, 1982). Bicuculline was able to increase both the hyoscine-sensitive contractile responses of longitudinal muscle to transmural stimulation and ACh output. The effect of bicuculline seemed to be frequency-dependent. Indeed an inverse relationship could be observed between the potentiation of contractile responses by bicuculline and the frequency of stimulation, the maximum effect of transmural stimulation being not significantly modified. A facilitatory effect of bicuculline on the cholinergic neurotransmission has been described both in the CNS (Bianchi *et al.*, 1982) and in the peripheral autonomic nervous system (Kusunoki *et al.*, 1984). This has been taken as evidence for a GABAergic modulation of the cholinergic neurotransmission. Such a conclusion is apparently contradicted by the observation made in the small intestine that GABA receptor activation by exogenous GABA is able to increase ACh output (Kleinrock & Kilbinger, 1983; Taniyama *et al.*, 1983b). However, it has also been shown that GABA_A receptor activation is able to produce both excitatory and inhibitory effects on cholinergic neurotransmission both in the central and in the peripheral nervous system depending on the degree of the stimulation-induced neuronal activity (Bianchi *et al.*, 1982; Maggi *et al.*, 1985).

In our experiments, bicuculline has been observed to increase the NANC inhibitory responses to transmural stimulation. This at least in the colon contrasts with the postulated facilitatory role of GABA on non-adrenergic inhibitory neurones (Krantis *et al.*, 1980; Maggi *et al.*, 1984). Potentiation by bicuculline of NANC inhibitory responses to transmural stimulation was inversely related to the frequency of stimulation and could no longer be observed above 2 Hz. It is noteworthy that at the dose able to produce the maximum improvement of peristaltic reflex, bicuculline seemed to potentiate the responses to the excitation of NANC inhibitory neurones to a degree comparable to, or even higher than, that observed for the cholinergic excitatory responses. This could be relevant in interpreting the effect of bicuculline on peristalsis.

Contrary to what has been observed with direct stimulation of intramural enteric neurones,

bicuculline was shown to reduce the inhibitory effect of periarterial nerve stimulation. Such an effect appeared to be frequency-independent. We cannot exclude the possibility that interference with adrenergic transmission, as suggested by Bartolini *et al.* (1985) for the vas deferens, could be responsible for the inhibitory effect of bicuculline. However the ability of both bicuculline and acute GABA-desensitization to reduce the responses to periarterial nerve stimulation together with the disappearance of effects of bicuculline in GABA-desensitized preparations does not support a bicuculline site of action other than at GABA receptors. Moreover, in the guinea-pig colon, bicuculline seems to be unable to antagonize the relaxation induced by exogenously applied noradrenaline (unpublished observation from our laboratory). It is worth noting that bicuculline-sensitive facilitatory effects of GABA on noradrenergic neurotransmission in the CNS have been observed by Bowery *et al.* (1980) and Suzdak & Gianutsos (1985).

Acute GABA-desensitization was not able to mimic the effects of bicuculline for all the kinds of responses that were examined. Comparable effects of GABA-desensitization and bicuculline could be observed for the NANC inhibitory responses to transmural stimulation and for the responses to periarterial nerve stimulation. In contrast, the contractile responses to transmural stimulation, ACh output and the efficiency of the peristaltic reflex were unaffected by acute GABA-desensitization. This could put some restrictions to the use of the combined effect of bicuculline and GABA-desensitization in order to interpret the hypothetical role of the endogenously released GABA. Discrepancies in the manner by which bicuculline and GABA-desensitization affect the responses to the stimulation of enteric neurones could rest on the different receptor specificity and on the different mechanism of receptor blockade of the two procedures. Desensitization is far less specific than bicuculline in distinguishing between GABA receptor subpopulations and the resultant effect is likely to be dependent on the existence at the intestinal level of both bicuculline-sensitive and bicuculline-insensitive GABA receptors (Giotti *et al.*, 1983; Ong & Kerr, 1983; Maggi *et al.*, 1985). The relative importance of the GABA receptor subtypes activated during nerve stimulation could differ depending on both the kind of stimulus applied and the kind of neurones involved. Indeed, activation of different GABA receptor subpopulations has been shown to produce opposite effects on cholinergic neurotransmission in the enteric nervous system (Giotti *et al.*, 1983; 1985; Kleinrock & Kilbinger, 1983).

In conclusion, if the effects of bicuculline are assumed to be entirely dependent on a selective blockade of GABA receptors, the following hypotheses could be advanced: (1) GABA receptors do not

seem to be involved to any significant extent in the local neuronal control of tone and spontaneous motility. The postulated intrinsic GABAergic pathways are unlikely to be tonically active, at least as can be inferred by the lack of any significant effect of bicuculline and GABA-desensitization either on resting tone or spontaneous electrical and mechanical activity.

(2) A bicuculline-sensitive modulation of both excitatory cholinergic and NANC inhibitory neurones through GABA receptors seems to operate during electrical activation of intrinsic enteric neurones. This is highly suggestive for a simultaneous activation of a subpopulation of enteric neurones using GABA as a transmitter to modulate the activity of both excitatory and inhibitory motor neurones. The effect of bicuculline on frequency-response relationships seems to indicate that the efficiency of GABAergic modulation is maximal at low levels of neuronal excitation.

(3) The viability of the postulated GABAergic pathways seems not to be a prerequisite for the triggering and development of peristaltic activity. Indeed, a peristaltic reflex can be elicited even after GABA receptor blockade or GABA receptor desensitization,

so that GABAergic synapses seem not to be directly involved in the activation of motor neurones responsible for the reflex responses. However, an intrinsic neural mechanism able to modulate the speed of propagation and to regulate the efficiency of propulsive activity through bicuculline-sensitive GABA receptors seems to be activated during the reflex excitation of peristalsis.

(4) In the regulation of integrated intestinal motor activity, it could be suggested that a GABAergic mechanism may be involved in the feed-back modulation of the reflex motor responses responsible for the propulsion of the intraluminal content. Beside the other tonically active modulatory systems responsible for the long-lasting control of motor neurone excitability (i.e. adrenergic modulation) a phasic feed-back modulation carried out through a GABAergic pathway could operate during activation of the peristaltic reflex. The enhancing effect exhibited by bicuculline on both excitatory and inhibitory responses to enteric neurone stimulation and its suppressive effect on the adrenergically-mediated inhibition are all compatible with such a hypothetical role.

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