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$GABA_B$ receptor-mediated mechanisms in human intestine in vitro

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The spontaneous motility of longitudinal muscle of human jejunum was recorded and the effect of γ-aminobutyric acid-ergic (GABAergic) drugs was tested. GABA and (−)-baclofen (10^{-6}–10^{-4} M) dose dependently reduced the amplitude and frequency of the spontaneous contractions; muscimol and 3-aminopropanesulfonic acid (3×10^{-5} M) were ineffective. The effect of 3×10^{-5} M GABA was reduced by 3×10^{-3} M 5-aminovaleric acid but not by 3×10^{-5} M picrotoxin. The dose–response curve for GABA was shifted to the right by 3×10^{-3} M 3-aminopropanesulfonic acid. Tetrodotoxin 3×10^{-7} M prevented the GABAergic action, whereas various receptor antagonists tested did not affect it. GABAergic drugs did not influence the spontaneous motility of either circular or longitudinal muscles of human colon. It is suggested that $GABA_B$ receptor activation induces the inhibition of human jejunum longitudinal muscle motility by a neurogenic mechanism. The possible involvement of postganglionic cholinergic neurons is to be evaluated by other techniques.

GABA (γ-aminobutyric acid); Baclofen; Jejunum (human); Colon (human); Intestinal motility

1. Introduction

Interest in γ-aminobutyric acid (GABA) as a peripheral neurotransmitter has increased greatly in recent years. Peripheral GABA receptors have been demonstrated in a variety of organs of different species. The gut has been the most studied and the presence of GABA neurons in the myenteric plexus has been emphasised (for a review see Jessen et al., 1987). These neurons – the existence of which has been demonstrated definitively by immunohistochemical techniques (Jessen et al., 1986; Beatge and Gershon, 1986; Saito and Tanaka, 1986) – are responsible for a specific process of GABA uptake (Jessen et al., 1979, 1983; Krantis and Kerr, 1981; Krantis et al., 1986), are able to release GABA by stimulation with K^+ ions or electrical current (Taniyama et al., 1982; 1983; Jessen et al., 1983; Kerr and Krantis, 1983) and seem to possess the enzymes able to metabolize this amino acid (Jessen et al., 1979; Miki et al., 1983).

Moreover there is increasing pharmacological evidence regarding GABA receptors in this organ for: (1) the presence of two pharmacologically distinguishable GABA receptors called $GABA_A$ and $GABA_B$ receptors (Bowery et al., 1981; Kaplita et al., 1982; Giotti et al., 1983; Ong and Kerr, 1983; Krantis and Harding, 1987); (2) the modulatory role of these receptors in the release of neurotransmitters (Kaplita et al., 1982; Giotti et al., 1983; Ong and Kerr, 1983; Kleinrock and Kilbinger, 1983; Maggi et al., 1984; Manzini et al., 1985); (3) the ability of GABAergic antagonists and/or GABA receptor desensitization to affect the peristaltic reflex (Kerr and Ong, 1986; Frigo et al., 1987; Schwörer and Kilbinger, 1988).

This evidence points to the existence of a modulatory GABAergic system in the mammalian gut. However the situation differs at various levels of the gut, or in separate species. For example the guinea-pig ileum has both $GABA_A$ and $GABA_B$ receptors that modulate the release of acetylcholine (ACh) (Kaplita et al., 1982; Giotti et al., 1983; Ong and Kerr, 1983; Kleinrock and Kilbinger, 1983), whereas only $GABA_B$ receptors have been described in the guinea-pig jejunum (Ong and Kerr, 1987). In the rat small intestine, in addition to less functionally important $GABA_B$ receptors (Krantis and Harding, 1987), $GABA_A$ receptors have been found that modulate the release of a purinergic (Maggi et al., 1984; Manzini et al., 1985) or cholinergic (Krantis and Harding, 1987) neurotrans-

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mitter. At another level of the gut, i.e. in the colon, mainly $GABA_B$ receptor-mediated effects have been evidenced in the guinea-pig (Giotti et al., 1985), while only $GABA_A$ receptors have been described in the cat colon (Taniyama et al., 1987).

Nothing is known at present of the effects of GABA in the human gut and, on the basis of the above described discrepancies between species, it is impossible to speculate about what the effects could be. For this reason we decided to evaluate the effects of GABAergic drugs in segments of human jejunum and colon.

2. Materials and methods

2.1. Methods

Longitudinal strips of human jejunum were cut from macroscopically normal specimens obtained from 11 patients (3 females and 8 males, average age 59 years) with malignant tumors (gastric or pancreatic or Vaterian cancer, gastric lymphoma). Muscle strips of human teniae coli and colonic circular muscle were cut from macroscopically normal specimens of descending colon resected for local malignancy in 4 patients (3 females and 1 male, average age 65 years).

The specimens were immediately put into Krebs solution at 4°C, where they were left for about 15 min. Muscle strips about 1 cm long and 3 mm wide were then cut and the mucosa was removed.

The strips were set up in an isolated organ bath in Krebs solution plus 10^{-7} M choline, equilibrated with 95% oxygen and 5% carbon dioxide at 37°C, and placed under a tension of 1 g. The strips were connected to MARB isometric transducers and recordings were made by a MARB polygraph. The preparations were left for about 1 h by which time spontaneous activity had usually developed.

Drugs were added in a volume not exceeding 1% of the total bath volume (4 ml). An interval of 20 min between administrations was found to avoid tachyphylaxis. Tetrodotoxin (TTX), picrotoxin, hexamethonium, phentolamine plus propranolol, naloxone, ATP, 5-aminovaleric acid (5-AVA) and 3-aminopropanesulphonic acid (3-APS) were added to the perfusion medium.

The drug effects were measured by the evaluation of a motility index, calculated as the frequency multiplied by the amplitude of the contractions.

2.2. Drugs

The sources for all drugs used in the study were the following: GABA (Sigma), (+)- and (−)-baclofen (Ciba-Geigy), 3-aminopropanesulphonic acid (Sigma),

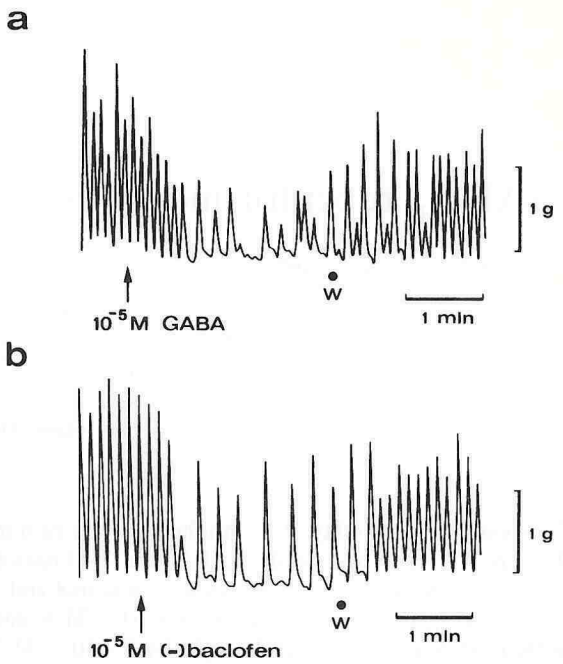

Fig. 1. Effect of $GABA_B$ receptor agonists on human jejunum in vitro. Reduction in amplitude and frequency of contractions caused by 10^{-5} M GABA (a) or 10^{-5} M (−)-baclofen (b).

muscimol (Serva) choline chloride (Carlo Erba), taurine (Sigma), β-alanine (Sigma), tetrodotoxin (Sigma), picrotoxin (Sigma), 5-aminovaleric acid (Sigma), ATP (Sigma), phentolamine (Sigma), propranolol (Sigma), naloxone (Sigma), hexamethonium bromide (Sigma), dimethylphenylpiperazinium (Sigma), glutamic acid (Sigma).

3. Results

3.1. The effect of GABA agonists on spontaneous motility of human jejunum

Human jejunum generally exhibited spontaneous motility consisting of high-amplitude contractions with a maximal frequency of 8.5–10.3 cycles/min, which lasted for several hours after the stabilization period. The administration of GABA at concentrations ranging from 10^{-6} to 10^{-4} M caused a temporary reduction in the amplitude and frequency of contractions (fig. 1a) up to a blockade of spontaneous motility at the highest doses of GABA. This effect was mimicked stereospecifically by the $GABA_B$ agonist, baclofen (fig. 1b), while the $GABA_A$ agonists, 3-APS (3×10^{-5} M) and muscimol (3×10^{-5} M), were ineffective.

Other amino acids related to GABA, such as glutamic acid (10^{-4} M) (n = 4), taurine (10^{-4} M) (n = 6) and β-alanine (10^{-4} M) (n = 4), were also ineffective. The inhibitory effects of GABA and (−)-baclofen were

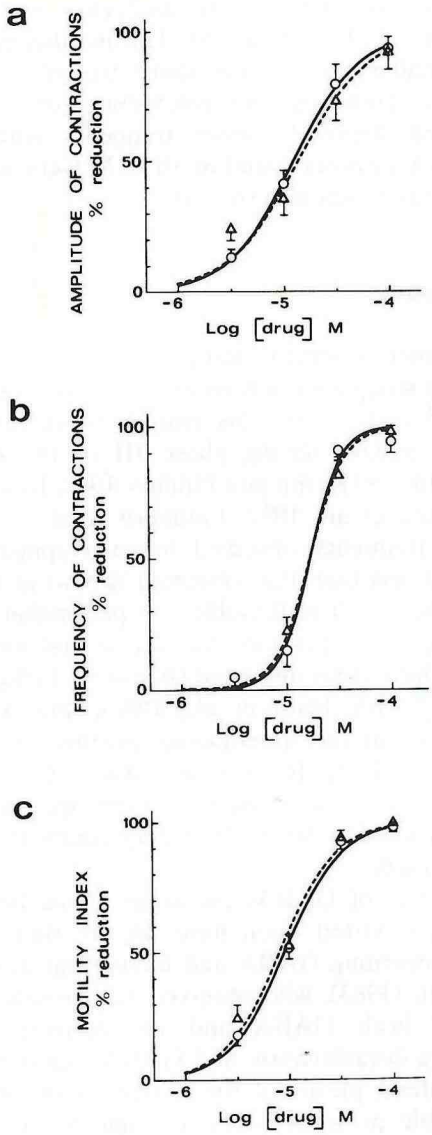

Fig. 2. Dose dependence of the inhibitory effects of GABA (○) and (−)baclofen (△) on spontaneous motility in human jejunum. Dose-related curves respectively for amplitude (a) and frequency (b) of contraction and for motility index (c).

dose-dependent (fig. 2a and b). Evaluation of the motility index showed that the dose–response curves of the two drugs were almost superimposable (fig. 2c), the ED_{50} being 9×10^{-6} M for GABA and 8×10^{-6} M for (−)-baclofen.

The jejunum strips sometimes showed no or only sporadic spontaneous contractions. In these cases dimethylphenylpiperazinium (DMPP) (10^{-6}–10^{-5} M) caused high-amplitude and regular contractions lasting for a few minutes (fig. 3a). GABA (10^{-4} M) interrupted the series of contractions, or, when administered before the DMPP, prevented their development (n = 6) (fig. 3b).

Previous administration of GABA prevented subsequent responses to the same drug, indicating that desensitization occurred; also cross-desensitization was observed between GABA (3×10^{-5} M) and (−)-baclofen (3×10^{-5} M).

3.2. Effect of GABA antagonists on GABA inhibition of spontaneous motility

Different putative $GABA_A$ and $GABA_B$ antagonists were used in an attempt to prevent the GABA inhibitory effect. Picrotoxin (3×10^{-5} M) was ineffective (n = 4), while the weak $GABA_B$ antagonist, 5-AVA, at its effective dose (3×10^{-3} M), partially antagonized the GABA (3×10^{-5} M) effect on both frequency and amplitude of contractions and therefore on the motility index (n = 6) (fig. 4). Instead 3-APS, at the effective dose of 3×10^{-3} M, was able to inhibit the GABA effect on all the parameters, shifting the dose–response curve for GABA to the right (fig. 5), which showed its ED_{50} on the motility index at 7.9×10^{-5} M.

3.3. Effects of various antagonists on GABA inhibition of spontaneous motility

In the presence of 3×10^{-7} M TTX in the perfusion medium, spontaneous motility was only temporarily reduced and it recovered after an about 15-min perfusion, indicating the development of non-neural contractions. GABA was found to be ineffective under these conditions (n = 4).

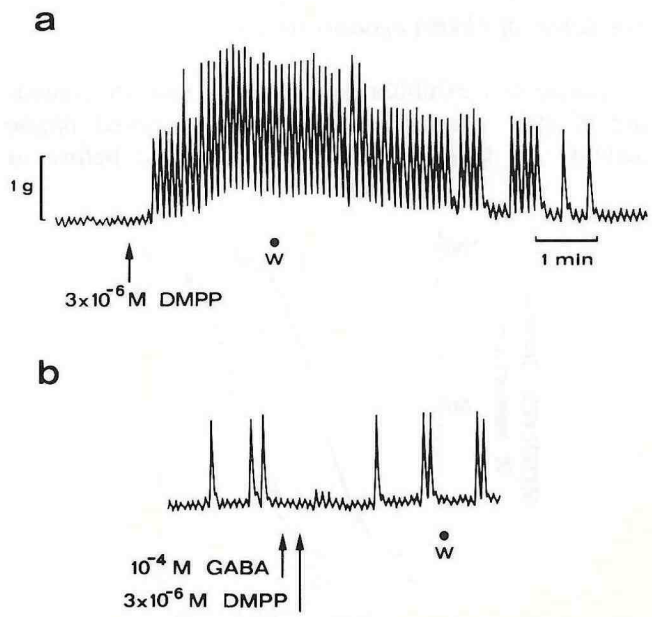

Fig. 3. Development of contractions evoked by 3×10^{-6} M DMPP (a) in a specimen of human jejunum with very low spontaneous motility and inhibitory effect of 10^{-4} M GABA on the same preparation (b). At arrow, injection of drug; w: washing.

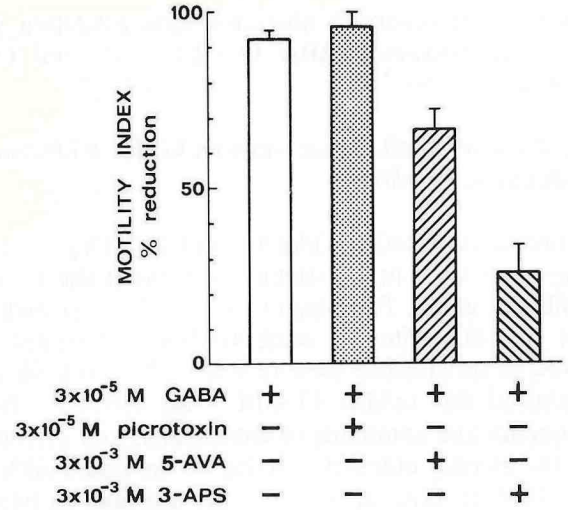

Fig. 4. Antagonism of 3×10^{-3} M 5-AVA and 3×10^{-3} M 3-APS against inhibitory effect of 3×10^{-5} M GABA on spontaneous motility expressed as motility index in human jejunum and ineffectiveness of 3×10^{-5} M picrotoxin.

Among the other receptor antagonists tested only hyoscine was effective, strongly reducing spontaneous motility (n = 4). In this case any possible prevention of the GABA effect was difficult to evaluate. On the contrary, hexamethonium (3×10^{-5} M) (n = 5), phentolamine (10^{-5} M) plus propranolol (10^{-5} M) (n = 5) and naloxone (10^{-5} M) (n = 4) affected neither spontaneous motility nor the GABA effect. Desensitization to ATP by perfusion of this drug (10^{-4} M) did not affect the inhibition of DMPP-induced motility by GABA (n = 4).

3.4. Effect of GABA agonists on human colon motility

Teniae coli exhibited spontaneous motility consisting of slow contractions, with superimposed phasic activity. All GABA agonists administered before or during the contraction were ineffective at the doses tested (10^{-6}–10^{-4} M, n = 5). The motility pattern of colon circular muscle was more irregular with low amplitude/high frequency contractions interrupted by some high amplitude/lower frequency contractions; the GABA agonists tested at 10^{-4} M were ineffective in this preparation also (n = 4).

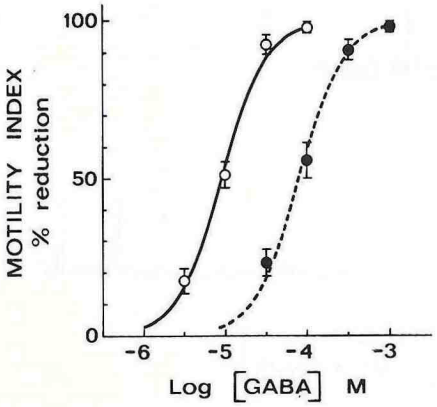

Fig. 5. The dose–response curve for the inhibitory effect of GABA on human jejunum was shifted to the right in the presence of 3×10^{-3} M 3-APS.

4. Discussion

Spontaneous jejunal motility 'in vitro' is characterized by sharp contractions at a maximal frequency of 9.5 ± 0.15 cycles/min; this pattern is similar to that observed in vivo during phase III of the migrating motor complex (Kerlin and Phillips, 1982; Kellow et al., 1986; Sarna et al., 1989; Lindberg et al., 1990). The maximal frequency observed in vitro appears to be somewhat less than that observed in vivo at the same level of the gut. It is plausible that pacemaker cells are present in our specimens, the nature and localization of which have been discussed (Faussone-Pellegrini and Cortesini, 1983; Hara et al., 1986), and which are responsible for the spontaneous motility, even in the presence of TTX. In vivo the same cells cannot be responsible for the motility in their specific region, since excitation at higher frequency comes from orally situated tracts.

The effect of GABAergic drugs in the human gut has been reported upon here. In the past the only report concerning GABA and human gut was that by Miki et al. (1983), who observed the presence of low levels of both GABA and metabolizing enzymes (glutamate decarboxylase and GABA transaminase) in the myenteric plexus of the human colon, indicating the possible presence of GABAergic neurons in this region. In preparations of jejunal longitudinally oriented muscle GABA and (−)-baclofen inhibited both the amplitude and frequency of spontaneous and DMPP-induced contractions, finally reaching a temporary break at higher concentrations (3×10^{-5}–10^{-4} M). On the other hand, $GABA_A$ agonists were ineffective. This inhibitory effect was dose-dependent and specific, since it was not induced by other GABA-related amino acids.

The suggested involvement of $GABA_B$ receptors was further proved by cross-desensitization between GABA and (−)-baclofen, and by the effectiveness of the $GABA_B$ antagonists. On the contrary the $GABA_A$ antagonist tested, picrotoxin, was found ineffective. This effect of GABA, analogous to what was observed in other species (Ong and Kerr, 1987), appears to be exerted at the neuronal level and is TTX-sensitive.

The possible involvement of some other neurotransmitting systems, such as adrenergic and enkephalinergic, in the GABAergic effect was ruled out since corre-

sponding antagonists were found not to affect the GABA action. Hexamethonium was also ineffective, ruling out the involvement of cholinergic preganglionic terminals in GABAergic mechanisms. Hyoscine could not be tested since it was strongly effective to reduce spontaneous motility. We could thus not demonstrate that the mechanism of the GABA effect was an inhibitory action on cholinergic myenteric neurons.

Nevertheless, this hypothesis (which has been demonstrated to apply in the guinea-pig ileum by Kleinrok and Kilbinger, 1983) is suggested by the apparent prevalence of the excitatory cholinergic nervous system in human small intestinal longitudinal muscle (Whitney, 1965; Bennett and Stockley, 1975) and by the effectiveness of GABA to inhibit or prevent DMPP-induced contractions, due to excitation of cholinergic postganglionic neurons which are hyoscine-sensitive (Whitney, 1965). A possible action of GABA on other neurotransmitting systems such as the purinergic, vasointestinal peptide (VIP)ergic or substance P (SP)ergic appears to be unlikely in our opinion, since desensitization to ATP did not affect the GABA effect on DMPP-induced motility. VIP up to 10^{-7} M appeared not to influence either spontaneous or DMPP-induced motility of this preparation (data not shown) and perfusion with 10^{-5} M capsaicin or 2.5 U/ml α-chymotrypsin did not prevent the GABA effect (data not shown). However the hypothesis of a GABA action mediated by cholinergic neurons remains to be evaluated by other techniques.

In conclusion, we have now observed an inhibitory response to GABA, mediated by type B receptors, on the spontaneous motility of the human jejunum longitudinal muscle. No $GABA_A$-mediated effect was evidenced. With respect to other mammalian species there is an analogy with the guinea-pig, in the jejunum of which only $GABA_B$-mediated relaxation has been described (Ong and Kerr, 1987). On the contrary, $GABA_A$ receptors are present in the same region of the rat and are responsible for an inhibitory effect on motility due to stimulation of a NANC inhibitory system (Krantis and Harding, 1987).

However the analogy with the guinea-pig appears to apply only to the jejunal tract, since in human ileum longitudinal muscle we observed a $GABA_B$ inhibitory effect similar to that evidenced in the jejunal preparation (data not shown), whereas in the guinea-pig a $GABA_A$ excitatory response appears prevalent at this level (Ong and Kerr, 1987). Moreover, unlike what is described for other animals, GABA agonists appear to be inefficient in the human colon. However further studies are necessary to exclude any $GABA_A$ action in the human small intestine as well as any drug effect in the colon that might be demonstrated under other experimental conditions.

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