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5-Hydroxytryptamine desensitization fails to modify GABA-induced inhibitory responses in the guinea-pig colon

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In segments of guinea-pig colon, treated with 1 μM hyoscine, a 5-HT desensitization procedure that decreased the potency of 5-HT 41-fold in relaxing the longitudinal musculature, failed to modify the non-adrenergic GABA-induced inhibitory responses, suggesting that the action of GABA in this preparation is not 5-HT-mediated. These results are at variance with those obtained in the guinea-pig ileum where 5-HT seems to play a role in GABA-induced cholinergic contractions.

In a previous paper we provided evidence that γ-aminobutyric acid (GABA)-induced cholinergic contractions of the guinea-pig proximal ileum are mediated by interneuronal liberation of 5-hydroxytryptamine (5-HT) (Tonini et al 1983) which represents the transmitter responsible for the final activation of cholinergic nerves. 5-HT, however, like GABA (Krantis et al 1980) may also activate the intrinsic inhibitory nerves of the intestine through a non-nicotinic mechanism (Furness & Costa 1973; Costa & Furness 1979; Gershon 1981). We have examined whether 5-HT involvement could be extented to GABA-induced non-adrenergic inhibitory responses in the guinea-pig distal colon. Since in the absence of specific antagonists of 5-HT nerve-mediated responses, desensitization to 5-HT appears to be the most specific antagonistic treatment available (Huidobro-Toro & Foree 1980; Gershon 1981), concentration-response curves for GABA before and after 5-HT desensitization were compared.

Materials and methods

Guinea-pigs of either sex (250–350 g) were stunned and bled. Segments of distal colon were removed and placed in Tyrode solution 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. Pieces of intestine 3 cm in length were mounted isotonically (load applied 1 g) in 20 ml organ baths containing Tyrode solution kept at 36 °C and gassed with 95% oxygen + 5% carbon dioxide. Mechanical activity of the longitudinal musculature was recorded after a 60 min equilibration period by using an Ugo Basile recording microdynamometer.

In pilot experiments we observed that 5-HT induced a contractile response which was followed by transient relaxation. Since the blockade of muscarinic receptors could unmask an immediate relaxation due to activation of enteric inhibitory nerves, concentration-response curves for the inhibitory effect of 5-HT (and also for GABA) were constructed in the presence of hyoscine (1 μM) by exposing the tissue to increasing concentrations of the agonist, left in contact with the tissue for 30–60 s before drug removal by washing. The interval between two subsequent exposures of the agonist was 15 min. A second concentration-response curve for both 5-HT and GABA was constructed in the presence of 5-HT desensitization leaving a 10 μM priming dose of 5-HT in contact with the tissue for 6 min (time required for the tone to return to the baseline value). After this time a second dose of 5-HT or GABA was applied without intervening washings. Since desensitization was rapidly and completely reversible by washing and could be re-established after subsequent additions of 10 μM 5-HT, a given preparation was used to assess the response to at least 3 doses for each agonist in the presence of 5-HT desensitization.

Responses to agonists were expressed as means ± s.e.m. percentages of the maximal response. Antagonists were left in contact for 10 min before agonist addition.

Drugs used were 5-hydroxytryptamine creatinine sulphate (5-HT) (Sigma), γ-aminobutyric acid (GABA) (BDH), hyoscine hydrobromide (BDH), bicuculline methyliodide (Sigma), hexamethonium iodide (Fluka), guanethidine sulphate (Ciba-Geigy), isoprenaline bitartrate (Sigma), methysergide bimaleate (Sandoz) and tetrodotoxin (TTX) (Sankyo).

Results

Addition of 5-HT (1–100 μM) to the bath caused the colon to contract rapidly, and this was usually followed by a slow developing transient relaxation. The original tone was resumed within 6 min. In a few preparations, a brief relaxation preceded the contractile response. Methysergide (20 μM) did not significantly alter 5-HT-induced responses (5 expts), while hyoscine (1 μM) completely prevented the initial contractile effect caused by 5-HT. In the presence of hyoscine 5-HT elicited mainly a relaxation, usually followed by a contraction large enough to induce recovery of the basal tone. TTX (0·3 μM) prevented both the contractions and the relaxations evoked by 5-HT in the absence and the presence of hyoscine respectively (5 expts) while hexamethonium (20 μM) was without effect on both responses (6 expts). Guanethidine (5 μM in 5 expts) was

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without effect on 5-HT evoked relaxations, indicating that these were mediated by activation of non-adrenergic inhibitory neurons.

When a 10 μM dose of 5-HT (both in the absence or in the presence of hyoscine) was left in the bath, a second addition of the same dose 6 min later failed to elicit any response. This antagonistic effect of 5-HT upon 5-HT responses (desensitization) was quickly reversed by washing and was specific in that the isoprenaline (1–10 μM)-induced relaxations were not modified. Fig. 1 shows the dose-response curve for 5-HT (in the presence of 1 μM hyoscine) in inhibiting the musculature

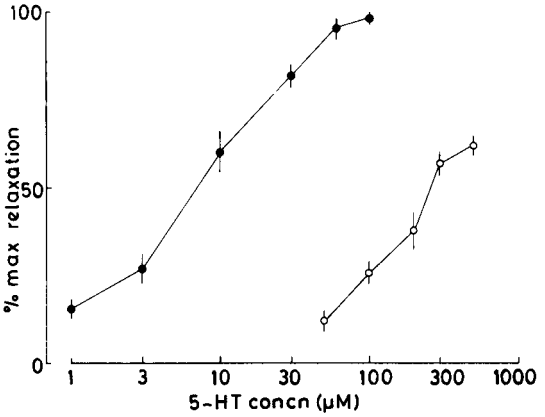

FIG. 1. Guinea-pig distal colon. Dose-response curves for 5-HT in inhibiting longitudinal muscular tone in the presence of 1 μM hyoscine. (●), Control; (○), after desensitization to a 10 μM priming dose of 5-HT. Each point represents the mean ± s.e.m. of 6 experiments.

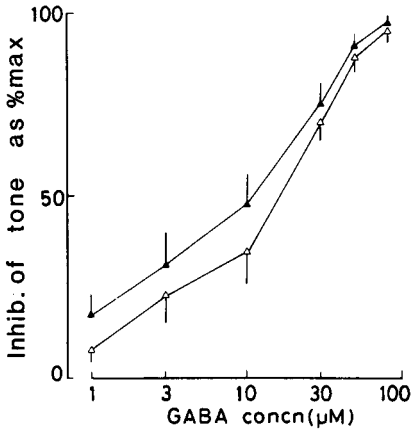

FIG. 2. Dose-response curves for GABA in inhibiting muscular tone in 1 μM hyoscine pretreated guinea-pig distal colon. (▲), Control; (△), after desensitization to a 10 μM priming dose of 5-HT. Each point represents the mean ± s.e.m. of 6 experiments.

in the absence and in the presence of 5-HT desensitization obtained by using a 10 μM priming dose of 5-HT (see methods). Dose-ratio (after/before desensitization) calculated at the ED50 level gave a value of 40·9 ± 3·0 (n = 6).

GABA (1–80 μM) invariably relaxed the preparations. These responses were unaffected by pretreatment with 1 μM hyoscine. Relaxations were rapid in onset and the peak response was achieved within 60 s. The basal tone was spontaneously resumed after 5–8 min, depending on the dose used. These inhibitory responses showed marked tachyphylaxis. If GABA was left in the bath, a second addition of the same concentration (after the tone was resumed) failed to elicit any response. GABA-induced relaxations were largely prevented by TTX (0·3 μM) in 4 expts), according to the data of Krantis et al (1980) in the same preparation, and unaffected by guanethidine (5 μM in 4 expts) and hexamethonium (20 μM in 4 expts). Bicuculline (5 μM) completely antagonized GABA (1–50 μM)-induced inhibitory responses in six preparations. The antagonism appeared to be reversible on washing and surmountable by increasing the concentration of the agonist. Fig. 2 shows that desensitization to 5-HT (produced by a 10 μM priming dose exposure) in hyoscine (1 μM)-treated preparations caused a non-significant shift to the right of the dose-response curve for GABA in inhibiting the longitudinal musculature of the colon.

Discussion

When tested in non-stimulated guinea-pig intestinal preparations GABA may exert different effects depending on the intestinal regions considered. A predominant excitatory effect (through activation of cholinergic nerves) is usually observed in the small intestine, while an inhibitory response (due to stimulation of intrinsic inhibitory nerves) is the common motor pattern in colonic preparations (Krantis et al 1980; present results). GABA induced effects are independent of nicotinic transmission, and until recently it was not clear if GABA could stimulate intrinsic neurons directly (through activation of $GABA_A$ bicuculline sensitive receptors) or indirectly by releasing a transmitter acting through a non-nicotinic mechanism (Krantis & Kerr 1981).

We have recently observed, by using a methodology similar to that employed in the present study, that the action of GABA on the guinea-pig ileum involves interneuronal release of 5-HT, that in turn determines cholinergic activation (Tonini et al 1983); The present results, however, show that 5-HT, which through a non-nicotinic mechanism may also stimulate intrinsic inhibitory nerves, is not implicated in the GABA-mediated inhibitory response of the guinea-pig distal colon. In fact, in the colon, a 5-HT desensitization procedure which caused a 41 fold shift to the right of 5-HT dose-response curve failed to cause any significant variation on GABA dose-response curve. Taken

altogether these data indicate a regional difference on GABA action and that bicuculline sensitive receptors ($GABA_A$ receptors) are present at ganglionic level probably both in motor- and inter-neurons.

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Monoamine oxidase inhibition by the tremorogenic drug—LON 954

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N-Carbamoyl-2-(2,6-dichlorophenyl)acetamidine HCl (LON 954), a tremorogenic drug, inhibited MAO activity in various tissue preparations in a reversible, competitive manner showing some degree of selectivity towards type-B MAO.

The tremorogenic drug LON 954 (*N*-carbamoyl-2-(2,6-dichlorophenyl) acetamidine HCl) (Coward et al 1977) potentiates the effects of some biogenic monoamines, e.g. tyramine, in in-vitro pharmacological studies. This prompted us to investigate whether it has any monoamine oxidase (MAO) inhibitory activity.

Methods

Mitochondrial preparations from various tissues were used as the enzyme source. Rat brain mitochondrial preparations, with either MAO type selectively inhibited, were obtained as described by Mitra & Guha (1980). Rat liver mitochondrial preparations with one MAO type selectively inhibited in-vitro, were prepared according to Tipton et al (1982) with slight modification. Rat liver mitochondrial suspensions were preincubated with either 0·3 μM clorgyline (37 °C for 60 min) or 0·3 μM selegiline (deprenyl) (37 °C for 90 min) to effect complete titration MAO-A or -B respectively. Then after centrifugation at 20000*g* and resuspension the mitochondrial pellet now showed exclusive activity of one MAO type, tyramine deamination was assayed by estimating aldehyde production as described by Mitra & Guha (1978). In some preliminary studies MAO activity was also assayed by manometric measurement of oxygen uptake (Creasy 1956) or spectrophotometric measurement of benzaldehyde production (Turski et al 1973).

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Results and discussion

LON 954 inhibited MAO activity in different tissue preparations with tyramine as substrate (Table 1). The degree of inhibition was observed to vary from preparation to preparation which may have some relationship with the type of MAO involved in the reaction. With preparations showing exclusively or predominantly MAO-A activity, a moderate degree of inhibition was observed. With the type-B rich preparations the inhibition was more marked. That this observed inhibition is enzyme inhibition and not an artifact of the assay procedure was confirmed by manometric measurement of oxygen uptake and spectrophotometric measurement of benzaldehyde production, respectively.

The possibility of some discrimination between the two MAO types by LON 954 (Table 1), was explored

Table 1. In-vitro inhibition by LON 954 of MAO activity in different tissue preparations. Crude mitochondrial preparations of each tissue was used as enzyme source. There was a 10 min preincubation of the tissue preparation with 10^{-3} M (final concentration) LON 954 in an otherwise complete reaction mixture before addition of substrate (0·01 M tyramine). For each tissue preparation, enzyme concentration and incubation period were chosen within the linear range.

Tissue preparation	MAO	Per cent inhibition of MAO
Rat liver	MAO A and B	70
Brain tissue from rats pretreated with clorgyline	B	94
Brain tissue from rats pretreated with pargyline	A	45
Rabbit liver	B predominant	99
Guinea-pig brain	B predominant	82
Guinea-pig kidney	A predominant	57