contractions produced by (−)-tartaric acid in the doses used with the histamine antagonist. In the presence of the prostaglandin synthesis inhibitor indomethacin (25 μg ml^{-1}), the activity of (−)-tartaric acid was abolished, although the tissue remained sensitive to applications of PGE_2.

Collier (1974) and Bennett (1978) have reviewed the role of PGs in diarrhoea. Recent investigations on the mechanism of the laxative activity of ricinoleic acid (Beubler & Juan 1979; Capasso et al 1984) have shown that this compound stimulates PG synthesis.

In conclusion, the laxative properties of (−)-tartaric acid may also be due to its PG synthesis stimulation as well as histamine liberation.

Since (−)-tartaric acid is present in many foods and plants, it will be useful to determine its possible relation to PG synthesis by in-vivo analytical studies.

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Three different ways in which 5-hydroxytryptamine can affect cholinergic activity in guinea-pig isolated ileum

G. J. SANGER, *Beecham Pharmaceuticals Research Division, Harlow, Essex, UK*

The effects of 5-hydroxytryptamine (5-HT) have been studied on electrically-evoked contractions mediated by cholinergic nerves in guinea-pig isolated ileum. Low concentrations of 5-HT (0·0001–0·01 μg ml^{-1}) caused a sustained increase in submaximal, electrically-evoked contractions. Higher concentrations of 5-HT (0·1–10 μg ml^{-1}) initially evoked a fast, rapidly-fading contraction of the muscle. Subsequently, 5-HT 0·1–10 μg ml^{-1} caused a sustained reduction in the height of the electrically-evoked contractions. The effects of 5-HT 0·01 and 0·1 μg ml^{-1} on the electrically-evoked contractions were not blocked by methysergide 0·1 μg ml^{-1} or by hexamethonium 10 μg ml^{-1}, and may be due to changes in neuronal acetylcholine (ACh) release, since contractions evoked by exogenous ACh were unaffected by 5-HT. The results therefore imply that 5-HT can affect gut cholinergic activity in at least three different ways, two of which may modulate evoked ACh release by mechanisms which may be insensitive to tachyphylaxis.

5-Hydroxytryptamine (5-HT) can affect gastrointestinal tissues by acting either on receptors located at the muscle membrane (5-HT D receptors) or on receptors which modulate neurotransmitter release (5-HT M receptors; Gaddum & Picarelli 1957). However, different types of responses can be evoked by 5-HT acting on D or M receptors, and these could indicate the existence of subtypes of receptor. For example in human and cat intestine, 5-HT acts on D receptors to cause muscle contraction or relaxation (Burleigh 1977; Ouyang & Cohen 1982), and in myenteric neurons of guinea-pig ileum, electrophysiological studies revealed three different responses to 5-HT (see North 1982). In the present study, three ways in which 5-HT can affect cholinergic activity are decribed for guinea-pig isolated ileum.

Methods

Albino guinea-pigs of either sex, 400–500 g, were used. Segments of distal ileum 3–4 cm long, were excised at least 10 cm proximal to the caecum. Each segment was prepared for transmural electrical stimulation, with the lumen of the intestine closed to the surrounding bathing solution (Paton 1955). Segments were suspended under an initial tension of 1 g in a 10 ml tissue bath containing Krebs solution (NaCl 7·1, $CaCl_2.6H_2O$ 0·55, KH_2PO_4 0·16, KCl 0·35, $MgSO_4.7H_2O$ 0·29, $NaHCO_3$ 2·1, dextrose 1·0 g litre^{-1}) maintained at 37 °C and bubbled with 5% CO_2 in O_2. Isometric responses were measured using transducers and pen recorders.

Contractions mediated by cholinergic nerves were evoked by transmural electrical stimulation, using rectangular bipolar pulses of 0·5 ms duration and 0·1 Hz frequency. Voltage was adjusted to give contractions which were maximal (15–42 V; measured using an oscilloscope) or approximately 50% of maximum (1·7–16·0 V). A single concentration of 5-HT was added to the bath 15 min after washout and replacement of the bathing solution. The effects of 5-HT on the muscle tension and on the electrically-evoked contractions were then recorded for a further 15 min. In experiments to test the actions of drugs on the 5-HT-induced responses, tissues were incubated with the drug before adding 5-HT to the bath, and the results were compared with similar experiments in which tissues obtained from the same animal were preincubated with the drug solvent.

The effects of 5-HT on contractions evoked by exogenous acetylcholine (ACh) were studied using

cumulative dose-response curves for ACh, obtained before and then after 10 min preincubation of the tissue with a single concentration of 5-HT. The concentrations of ACh ranged from 0·1 ng ml^{-1} to 10 μg ml^{-1}, with 2 min intervals between each addition.

Drugs used were: acetylcholine perchlorate, atropine sulphate, 5-hydroxytryptamine creatinine sulphate, nicotine hydrogen tartrate (BDH), methysergide bimaleate, hexamethonium bromide (Sandoz) and tetrodotoxin in citrate buffer (Sigma). All were dissolved in 0·96% saline and were added to the Krebs solution bathing the serosal surface of the ileum. Concentrations refer to the salt listed above. Results are given as medians with semiquartile ranges, and analysed using the Mann-Whitney U-test and the Wilcoxon matched pairs test.

Results

Maximal, electrically-evoked contractions of guinea-pig ileum could be prevented by tetrodotoxin 0·2 or atropine 1 μg ml^{-1}. Hexamethonium 10 μg ml^{-1}, a concentration which reduced by 34–72% (median 52%, n = 5) the submaximal contractions evoked by nicotine 0·1 μg ml^{-1}, had no consistent effect on the maximum contractions to electrical stimulation (n = 4) but reduced the submaximal contractions by 20 (10–33)% ($P = 0·05$; n = 6).

During maximal and submaximal electrical stimulation, 5-HT 0·1–10 μg ml^{-1} initially evoked a fast, rapidly fading contraction; 5-HT 0·01 μg ml^{-1} also evoked a contraction in some, but not all tissues (Figs 1, 2). However in the present experiments, these and lower concentrations of 5-HT subsequently affected the contractions caused by electrical stimulation (Figs 1, 2).

5-HT 0·0001–0·01 μg ml^{-1} increased the contractions evoked by submaximal electrical stimulation, whereas 5-HT 0·1–10 μg ml^{-1} reduced the contractions (Fig. 1 for examples). These effects of 5-HT usually became consistent within 3–10 min after their addition to the bath, regardless of the concentration (there were four exceptions which took longer to become consistent, and each was of a different concentration). Thereafter, the new heights of contractions were maintained for the remainder of the 15 min recording time. The results are shown in Fig. 2. Methysergide 0·1 μg ml^{-1} pre-incubated with the tissue for at least 120 min, did not affect the increase or decrease in the heights of the electrically-evoked contractions caused, respectively by 5-HT 0·01 and 0·1 μg ml^{-1} ($P > 0·7$; n = 6 each). Similarly, 30 min pre-incubation of the tissue with hexamethonium 10 μg ml^{-1} did not affect the responses to 5-HT 0·01 or 0·1 μg ml^{-1} ($P > 0·9$, n = 6 each).

Maximum contractions evoked by electrical stimulation were not affected by 5-HT 0·0001–0·1 μg ml^{-1}. However, 5-HT 1 or 10 μg ml^{-1} reduced the contractions (Fig. 2), and this response was usually consistent within 2–10 min after adding 5-HT to the bath (there was one exception which was longer).

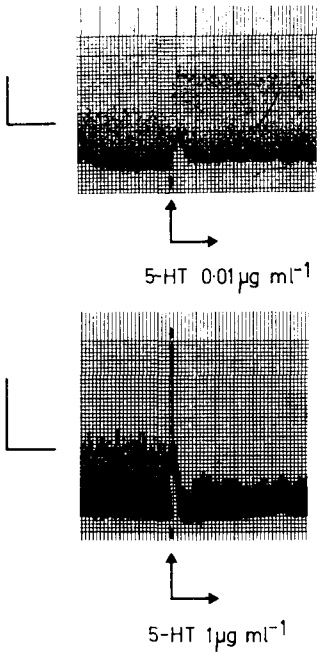

FIG. 1. Records showing the effects of 5-HT on muscle tone and on submaximal electrically-evoked, contractions mediated by cholinergic nerves in guinea-pig isolated ileum. Electrical stimulation was with bipolar, rectangular pulses of 0·5 ms duration, 0·1 Hz frequency and with voltages which gave contractions which were approximately 50% of maximum. The presence of 5-HT in the bathing solution is shown by the horizontal line below each trace. For each trace, the calibration is for 1 g tension (vertical calibration) and for 5 min (horizontal).

5-HT, 0·0001, 0·001, 0·01, 0·1, 1 or 10 μg ml^{-1} had no consistent effects on the contractions evoked by each concentration of exogenous ACh, and the EC50 values for ACh were not changed by any concentration of 5-HT ($P > 0·5$; n = 6 each).

Discussion

It has long been known that in guinea-pig isolated ileum, 5-HT can evoke a fast, rapidly fading contraction. The contraction is sensitive to tachyphylaxis and is due mostly to stimulation of cholinergic activity (via activation of 5-HT M receptors) and partly due to activation of 5-HT D receptors (see Costa & Furness 1979 for details and references). In addition, previous authors have described the ability of 5-HT to reduce electrically-evoked contractions of guinea-pig ileum (Gintzler & Musacchio 1974; Kilbinger & Pfeuffer-Friederich 1982), but none have quantified both an excitatory and inhibitory action of 5-HT.

In the present experiments, 5-HT has been shown to increase or reduce electrically-evoked cholinergic activity in guinea-pig isolated ileum. These actions of 5-HT became consistent within 10 min and were maintained during the presence of 5-HT. Neither methysergide (a

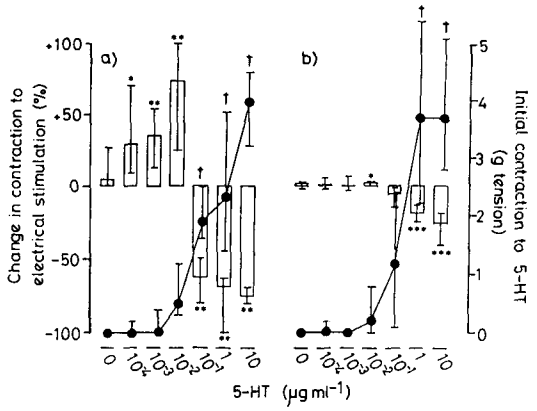

FIG. 2. Effects of 5-HT on muscle tone and on electrically-evoked, contractions mediated by cholinergic nerves in guinea-pig isolated ileum. The electrically-evoked contractions were (a) approximately 50% of maximum, or (b) maximum. 5-HT-induced changes in the heights of the electrically-evoked contractions usually became consistent within 10 min and were sustained thereafter. The mean contraction height obtained during the 13–15 min after the addition of 5-HT was therefore calculated as a percentage of the mean contraction height during the 2 min period immediately before addition of 5-HT (shown by the left-hand vertical axis); columns represent medians and the bars are semiquartile ranges. The results are analysed by comparing with the control experiments in which saline was added instead of 5-HT; $*P < 0{\cdot}1$, $**P < 0{\cdot}05$, $***P < 0{\cdot}01$. n = 6 each. The initial contractions evoked by 5-HT are expressed as median increases in g tension (●—●), with semiquartile ranges (shown by the right-hand vertical axis): † indicates that a contraction was obtained in all 6 experiments.

5-HT D-receptor antagonist; see Costa & Furness 1979) nor hexamethonium affected the increase or decrease in the height of the electrically-evoked contractions caused respectively by 5-HT 0·01 and 0·1 μg ml^{-1}. The actions of at least these concentrations of 5-HT may therefore involve changes in post-ganglionic cholinergic activity. In addition, 10 min pre-incubation of similar preparations of ileum with the same concentrations of 5-HT had no effects on the contractions evoked by exogenous ACh. The effects of 5-HT on the electrically-evoked contractions may therefore reflect changes in the release of ACh from post-ganglionic neurons. Bülbring & Crema (1958) found that 5-HT increased contractions to exogenous ACh, but in these experiments it is not clear if the lumen of the intestine was open or closed to the surrounding bathing solution. This could be an important difference between the two experiments, because 5-HT administered to the serosal or mucosal surface of guinea-pig ileum can reach different sites of action (Bülbring & Lin 1958).

The present experiments are preliminary and do not demonstrate the existence of 5-HT receptor subtypes; further studies are required for receptor characterization, in which antagonists of the tachyphylaxis-sensitive response to 5-HT (Fozard et al 1979) may prove useful However, the experiments do demonstrate that 5-HT can affect cholinergic activity of guinea-pig isolated ileum in at least three different ways. Relatively low concentrations of 5-HT (0·0001–0·01 μg ml^{-1}) cause a sustained increase in submaximal cholinergic-mediated contractions, probably by facilitating ACh release from post-ganglionic cholinergic neurons. Similar actions of 5-HT have also been described for guinea-pig isolated stomach (Yamaguchi 1972) and oesophagus (Kamikawa & Shimo 1982). 5-HT 0·01–10 μg ml^{-1} evoke cholinergic-mediated contractions which are known to be tachyphylaxis-sensitive, and 5-HT 0·1–10 μg ml^{-1} may cause a sustained reduction of contractions mediated by cholinergic nerves.

There is only a partial correlation of the present experiments and the depolarization and hyperpolarization responses to 5-HT observed by intracellular recording from myenteric neurons of guinea-pig ileum (Johnson et al 1980); hyperpolarization evoked by 5-HT was not sensitive to tachyphylaxis, but 5-HT-induced depolarizations were all subject to tachyphylaxis.

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