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Evidence that 5-hydroxytryptamine may exert both facilitatory and inhibitory control of electrical field stimulation-evoked contractions in longitudinal muscle taken from the body of guinea-pig stomach

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Electrical field stimulation (FS) of guinea-pig stomach body longitudinal muscle strips caused frequency-related contractions mediated via cholinergic mechanisms. Metoclopramide (10^{-8}–10^{-5} M), MDL 72222 (10^{-9}–10^{-7} M) and 5-hydroxytryptophan (5-HTP) (3×10^{-7}–$2{\cdot}4 \times 10^{-4}$ M) enhanced these contractions in all tissues, whereas 5-hydroxytryptamine (5-HT) (3×10^{-8}–3×10^{-5} M) enhanced the contractions, but only in approximately 15% of the tissues tested. FS-induced contractions were also enhanced in tissues treated in-vitro with *p*-chlorophenylalanine ($2{\cdot}5 \times 10^{-7}$–$2{\cdot}5 \times 10^{-5}$ M) or monofluoromethyldopa (6×10^{-7}–10^{-4} M) or in tissues taken from animals having received *p*-chlorophenylalanine or monofluoromethyldopa. It is concluded that cholinergic-mediated contractions of stomach strips are subject to 5-HT modulation in two ways. The predominant action of endogenous 5-HT is to exert an inhibitory tone mediated via a metoclopramide and MDL 72222-sensitive 5-HT neuronal receptor. Exogenously applied 5-HT has little overt action to increase the essentially maximal inhibitory action of endogenous 5-HT, but acts on a 5-HT facilitatory receptor system to enhance contractions. Therefore, the actions of 5-HT agonists and antagonists to modify contractions in stomach strips will reflect the balance between 5-HT inhibitory and facilitatory influences, and the specificity of action of the compounds for the two 5-HT receptor systems.

There is considerable evidence to suggest that the ability of metoclopramide in enhancing motility or smooth muscle contractions in the upper gastrointestinal tract reflects an increased cholinergic activity (Hay & Man 1979; Kilbinger et al 1982; see reviews Kilbinger & Weihrauch 1982; Daniel 1982; Roberts 1982; Sanger 1984). How metoclopramide achieves this effect is uncertain, although it has been hypothesized that it may interact via 5-hydroxytryptamine (5-HT) receptors to increase the release of acetylcholine (Kilbinger et al 1982). However, metoclopramide has been reported to antagonize and also to mimic the actions of 5-HT in preparations from the gastrointestinal tract (Kilbinger et al 1982; Kilbinger & Pfeuffer-Friedrich 1982; Gunning & Naylor 1985; see review by Sanger 1984).

An additional complexity has been an inadequate knowledge of the nature of the neuronal receptors for 5-HT in the enteric nervous system (see Branchek et al 1984), combined with a lack of selective 5-HT antagonists for such receptors (see Sanger 1985a).

In the present study we have used a stomach smooth muscle preparation to clarify further the nature of the interaction of metoclopramide with 5-HT receptors in regulating neuronally mediated contraction responses in the gastrointestinal tract.

METHODS

Male Dunkin-Hartley guinea-pigs (450–550 g) were killed by cervical dislocation, the stomachs removed and one gastric body longitudinal muscle strip (20 mm long, 5 mm wide) taken from each animal and placed in tissue baths containing oxygenated (95% O_2, 5% CO_2) Krebs-Henseleit solution (NaCl 118·0, KCl 4·75, KH_2PO_4 1·2, $MgSO_4$ 1·2, $CaCl_2$ 2·5, $NaHCO_3$ 25·0, glucose 10·0 mM) at 37 °C. Tension (1 g) was applied to the tissues which were allowed to equilibrate for 45 min before electrical field stimulation using platinum wire electrodes placed parallel to the tissue and approximately 5 mm apart (supramaximal voltage, 0·1 ms pulse width). Tissues were stimulated for 30 s every 5 min. Tension changes were detected by Grass tension transducers and displayed on a Grass recorder. A frequency-response curve (0·1–10 Hz) was initially constructed

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in the absence of drug and then in the presence of the potentially interacting drug(s) (40 min pretreatments); the second curve was related to the first to assess the degree of change.

Gastric body longitudinal muscle strips were also obtained from guinea-pigs pretreated for 5 h with monofluoromethyldopa (10 or 40 mg kg^{-1} i.p.) or *p*-chlorophenylalanine (40 mg kg^{-1} i.p.).

Levels of 5-HT in treated and non-treated stomach strips were determined using HPLC with electrochemical detection.

Metoclopramide monohydrochloride (Beecham), MDL 72222 (1αH, 3α, 5αH-tropan-3-yl-3,5-dichlorobenzoate) (Merrell), acetylcholine chloride (Sigma), atropine sulphate (Sigma), tetrodotoxin (Sigma), monofluoromethyldopa (Merrell), *p*-chlorphenylalanine (Sigma), 5-hydroxytryptophan (5-HTP) (Sigma), 5-hydroxytryptamine bimaleate (5-HT) (Koch-Light) were disolved in distilled water.

The significance of differences between treatments were assessed using the Mann Whitney U-test.

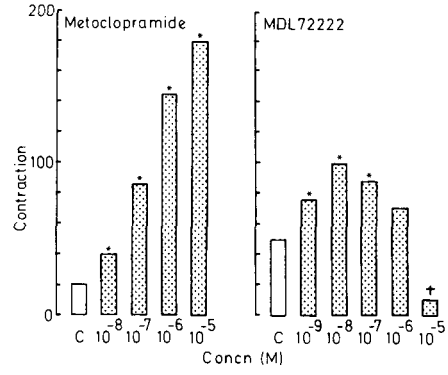

FIG. 1. Modification by metoclopramide and MDL 72222 of FS (1·0 Hz)-induced contractions of longitudinal smooth muscle taken from the body of the guinea-pig stomach. The control (C) contractions are indicated in the open columns, the effect of the drug treatments (molar concentrations indicated) in the stippled columns. All values are expressed as a percentage of the control contractions occurring at 10 Hz which are designated as 100%. n = 6, s.e.m.s on original data <11%. Significant enhancement of the contractions is indicated as $^{*}P < 0{\cdot}05$–$0{\cdot}001$, significant reduction as $\dagger P < 0{\cdot}001$ (Mann-Whitney U-test).

RESULTS

Electrical field stimulation (FS) (0·25–10 Hz) of the stomach strips caused frequency-related contractions that were repeatable at least six times over a 3 h period and which could be reversed to relaxations by atropine (5×10^{-8} M) (see also Costall et al 1984). Tetrodotoxin (10^{-7} M) was shown to abolish the contractions and relaxations (see also Costall et al 1984).

Metoclopramide (10^{-8}–10^{-5} M) and the neuronal 5-HT receptor antagonist MDL 72222 (10^{-9} to 10^{-8} M) caused concentration-related enhancements of the FS-induced contractions throughout the frequency range, without a significant effect on resting tension. However, increasing concentrations of MDL 72222 (10^{-7} and 10^{-6} M) were less effective and at 10^{-5} M the FS-induced contractions were abolished. Representative data obtained at 1 Hz are shown in Fig. 1. Notwithstanding the failure of MDL 72222 (10^{-7} to 10^{-5} M) in further enhancing FS-induced contractions, only the highest concentration of MDL 72222 (10^{-5} M) was able to reduce the concentration-related contractions of smooth muscle caused by exogenously applied acetylcholine (5×10^{-8} to 2×10^{-7} M) (results not shown, n = 5, $P < 0{\cdot}01$).

Inclusion of the L-aromatic amino acid decarboxylase inhibitor monofluoromethyldopa (Fozard et al 1980) (6×10^{-7} to 10^{-4} M) and the tryptophan hydroxylase inhibitor *p*-chlorophenylalanine (Koe & Weissman 1968) ($2{\cdot}5 \times 10^{-7}$ to $2{\cdot}5 \times 10^{-5}$ M) in the bathing medium also caused concentration-related enhancements of the FS-induced contractions over the frequency range (Fig. 2). Stomach strips taken from guinea-pigs treated with monofluoromethyldopa 10 and 40 mg kg^{-1} i.p., 5 h pretreatment (which significantly reduced 5-HT levels by 42 and 54%, respectively, in comparison with control levels in longitudinal muscle of 516 ± 78 pg mg^{-1} wet wt; n = 7–10; $P < 0{\cdot}05$–$0{\cdot}001$, Student's *t*-test), responded to FS with frequency-related contractions that were enhanced by 25–57% compared with responses obtained in non-treated tissues. Representative results from the use of 40 mg kg^{-1} i.p. monofluoromethyldopa and a stimulation frequency of 1 Hz are shown in Fig. 3; this treatment is also shown to exaggerate the ability of metoclopramide (10^{-7}–10^{-5} M) to enhance FS-induced contractions. (Tissues taken from guinea-pigs receiving a 5 h pretreatment with *p*-chlorophenylalanine, 40 mg kg^{-1} i.p., also showed (a) significant reductions in 5-HT levels (ca 40%), (b) enhanced contractions to FS (ca 20%) and (c) an exaggeration (between 20 and 70%) of the action of metoclopramide to increase FS-induced contractions).

5-HT (3×10^{-8}–3×10^{-5} M) generally failed to modify FS-induced contractions, although in approximately 10–15% of tissues examined enhancements of FS-induced contractions were recorded throughout the frequency range (3×10^{-9} to 3×10^{-7} M

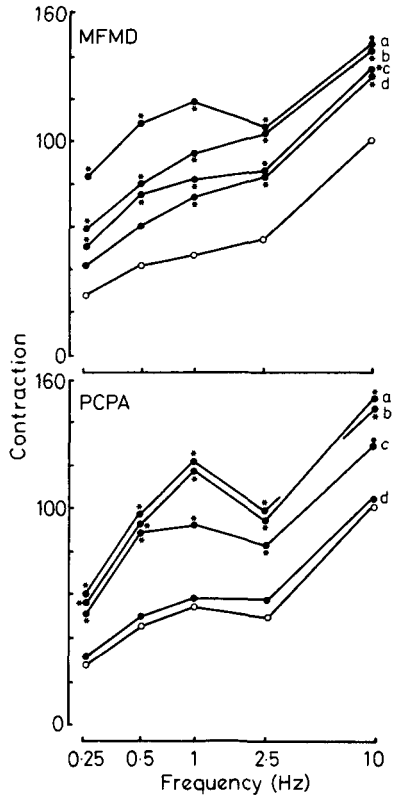

FIG. 2. Enhancement by monofluoromethyldopa (MFMD) and *p*-chlorophenylalanine (PCPA) of FS (0·25–10 Hz)-induced contractions of longitudinal smooth muscle taken from the body of the guinea-pig stomach. Control responses (○), the effect of the drug treatments (●,) (for MFMD, a = 10^{-4}; b = $2{\cdot}4 \times 10^{-5}$; c = 6×10^{-6}; d = 6×10^{-7} M and for PCPA, a = $1{\cdot}3 \times 10^{-5}$; b = $2{\cdot}5 \times 10^{-5}$; c = $2{\cdot}5 \times 10^{-6}$; d = $2{\cdot}5 \times 10^{-7}$ M). All values are expressed as a percentage of the control contractions occurring at 10 Hz which were designated as 100%. n = 6, s.e.m.s on original data <13%. Significant enhancement of the contractions is indicated as $^*P < 0{\cdot}05$–$< 0{\cdot}001$ (Mann-Whitney U-test).

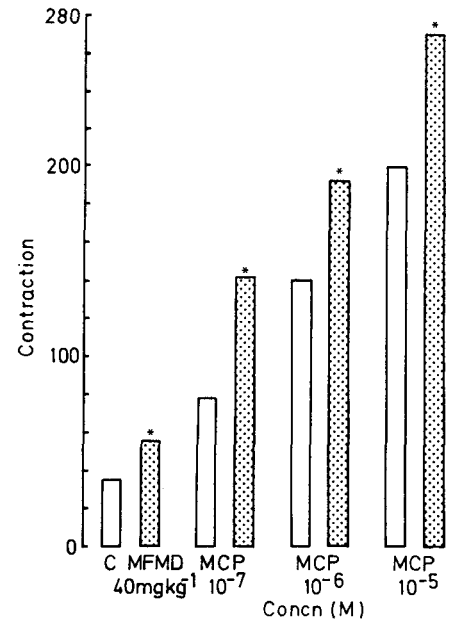

FIG. 3. Modification by monofluoromethyldopa (MFMD) treatment of FS (1·0 Hz)-induced contractions and the enhancement of contractions caused by metoclopramide (MCP, molar concentrations indicated) of longitudinal smooth muscle taken from the body of the guinea-pig stomach. Responses were obtained from tissues taken from non-treated animals (C, open columns) or animals that had received a 5 h pretreatment of MFMD, 40 mg kg^{-1} i.p. (stippled column). All values are expressed as a percentage of the control contractions occurring at 10 Hz which were designated as 100%. n = 6, s.e.m.s on original data <13%. A significant enhancement of the contractions by metoclopramide in MFMD treated tissues compared to non-treated tissues is indicated as $^*P < 0{\cdot}05$–$P < 0{\cdot}001$ (Mann-Whitney U-test).

5-HT) (representative data obtained at 1·0 Hz are in Fig. 4). At the concentrations used, 5-HT failed to alter the resting basal tension or the spontaneous activity of any tissue tested. 5-HTP (3×10^{-7} to $2{\cdot}3 \times 10^{-4}$ M) also enhanced FS-induced contractions in a concentration-related manner across the entire frequency range, without causing change in resting tension or spontaneous activity. This action was demonstrated in all tissues (Fig. 4). (The ability of 5-HTP, 3×10^{-5} M, to enhance FS-induced contractions was abolished by a 30 min pretreatment with monofluoromethyldopa, $2{\cdot}5 \times 10^{-6}$ M, which alone caused a modest increase in FS-induced contractions, results not shown).

DISCUSSION

The ability of tetrodotoxin and atropine to inhibit FS-induced contractions in the stomach body longitudinal muscle indicates mediation of the contractions via the neuronal release of acetylcholine. The results of the present study suggest that 5-HT may act through an inhibitory and facilitatory influence to modulate the contraction response.

Evidence to support a functional inhibitory role is twofold. Firstly, metoclopramide and MDL 72222 are known antagonists at 5-HT receptors located on the 'c' fibres in the rabbit vagus nerve, the terminal sympathetic neurons innervating the cat heart and on afferent nerves mediating the Bezold-Jarisch reflex (Fozard & Mobarok Ali 1978; Fozard & Host 1982; Fozard 1984b; Donatsch et al 1984) and facilitated FS-induced contractions in the stomach strips. Also, the 5-HT antagonist ICS

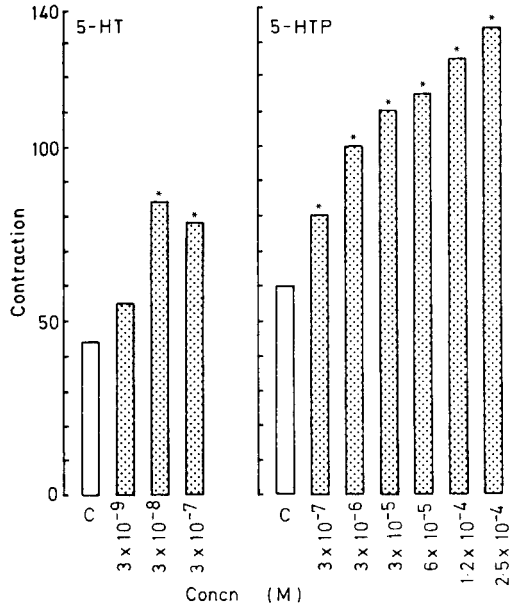

FIG. 4. Enhancement by 5-HT and 5-HTP of electrical FS (1·0 Hz)-induced contractions of longitudinal smooth muscle taken from the body of the guinea-pig stomach. Control responses (C) are shown in the open columns, the effect of the drug treatments (molar concentrations indicated) in the stippled columns. All values are expressed as a percentage of the control contractions occurring at 10 Hz which were designated 100%. The responses obtained to 5-HT were recorded in 10–15% of tissues (the remainder failed to respond), the responses obtained to 5-HTP were obtained in 100% of tissues. n = 6, s.e.m.s on original data <12%. A significant enhancement of the contractions is indicated as $^{*}P < 0{\cdot}05$–$< 0{\cdot}001$ (Mann-Whitney U-test).

205–930 [(3α-tropanyl)-1*H*-indole-3-carboxylic acid ester], having a highly selective and potent antagonist action at peripheral neuronal 5-HT receptors (Donatsch et al 1984), has been shown to facilitate potently FS-induced contractions of guinea-pig stomach strips (Buchheit et al 1985a). The relative potency of metoclopramide, MDL 72222 and ICS 205–930 in facilitating FS-induced contractions in the stomach strips is similar to their abilities in antagonizing at the 'neuronal' 5-HT receptors in the above preparations. Therefore, it is concluded that metoclopramide, MDL 72222 and ICS 205–930 act in the stomach as 5-HT receptor antagonists to enhance FS-induced contractions.

Secondly, the ability of these 5-HT antagonists to facilitate FS-induced contractions clearly implies that 5-HT must normally act to inhibit cholinergic-mediated contractions in guinea-pig stomach strips. However, 5-HT alone failed to reduce FS-induced contractions. This was an unexpected finding, particularly when 5-HT has been shown to antagonize the ability of metoclopramide in enhancing FS-induced contractions (Gunning & Naylor 1985). It was hypothesized that when 5-HT was administered alone, its failure to reduce FS-induced contractions was due to a maximally effective inhibitory endogenous 5-HT tone. Data from the present study would support this hypothesis in showing that an inhibition of 5-HT synthesis in the stomach tissues, effected by treatment in-vitro or in-vivo with the L-aromatic amino acid decarboxylase inhibitor monofluoromethyldopa (Fozard et al 1980) or the tryptophan hydroxylase inhibitor *p*-chlorophenylalanine (Koe & Weissman 1968), can enhance FS-induced contractions and the response to metoclopramide.

The major evidence in support of a facilitatory role for 5-HT in modulating FS-induced contractions is that 5-HT was able to enhance contractions in a small proportion of tissues (see also Yamaguchi 1972) and that 5-HTP, whose effects were antagonized by monofluoromethyldopa, was able to enhance contractions in all tissues. The failure of 5-HT or 5-HTP to reduce FS-induced contractions again indicates that the 5-HT inhibitory mechanism is functioning at a maximal rate. It may also be relevant that 5-HTP facilitates gastric emptying in-vivo (McLelland, personal communication, Gunning unpublished data).

MDL 72222 was shown to be less effective than metoclopramide in enhancing FS-induced contractions and as the concentration of MDL 72222 was increased, so its ability to enhance FS-induced contractions was reduced. Such effects (apart from those shown to occur at the highest concentration of MDL 72222) could be dissociated from an anticholinergic action since they occurred at concentrations 100 times less than those antagonizing acetylcholine-induced contractions. Although speculative, it is possible that the falling ability of MDL 72222 to facilitate FS-induced contractions reflects an increasing antagonism of the 5-HT facilitatory mechanism.

The concept of two 5-HT receptor mechanisms modulating smooth muscle contraction processes in the gastrointestinal tract has been forwarded by other workers. Thus, Yamaguchi (1972) identified two types of 5-HT receptor on circular/longitudinal muscle obtained from the guinea-pig stomach. The first was located on the smooth muscle cells where it influenced resting tissue tension (i.e. the classical 5-HT–'D' receptor of Gaddum & Picarelli 1957) whilst stimulation of a second neuronal 5-HT receptor led to facilitation of electrical stimulation-induced contractions, and was antago-

nized by morphine (i.e. the 'M'-receptor of Gaddum & Picarelli 1957). In the present studies, 5-HT failed to modify basal tissue tone, but action on the second receptor type may be analogous to the facilitatory 5-HT mechanism identified in the present study. However, Yamaguchi (1972) failed to gain evidence for an inhibitory 5-HT mechanism.

Kilbinger & Pfeuffer-Friedrich (1985) have also suggested the existence of two 5-HT receptors in the guinea-pig myenteric plexus to facilitate or inhibit the release of [^{3}H]acetylcholine. However, metoclopramide actually failed to antagonize the inhibitory actions of 5-HT and, instead, antagonized the facilitatory effects. These actions on [^{3}H]acetylcholine release processes in the ileum are opposite to those that could be predicted on the basis of present studies using stomach strips: metoclopramide would be expected to inhibit the inhibitory actions of 5-HT thereby facilitating smooth muscle contraction responses. Furthermore, Kilbinger & Pfeuffer-Friedrich (1984) found that methysergide and methiothepin antagonized the inhibitory effects of 5-HT on [^{3}H]acetylcholine release in the ileum preparation whilst both antagonists failed to modify FS-induced contractions in stomach muscle (Buchheit et al 1985a).

It is interesting that Sanger (1985b) has recently found that low and high concentrations of 5-HT can respectively increase and decrease cholinergic-mediated contractions in the guinea-pig ileum, with an additional direct contractile effect on the smooth muscle cells. The 5-HT receptor types remain to be established. Buchheit et al (1985b) have also indicated that 5-HT may mediate its contractile effects in the ileum via three independent receptors. Therefore the 5-HT receptor types identified in the ileum are not the same as those identified in the present work which mediate different effects or have different locations. This is emphasized by the data from metoclopramide, MDL 72222 and ICS 205–930 which have a much lower affinity for the subclass of 5-HT receptors in ileum than for those in the stomach, or on 'c' fibres in the vagus nerve or on sympathetic nerve terminals in the rabbit heart (Donatsch et al 1984; Buchheit et al 1985b).

It is apparent that the present attempt to clarify the nature of the metoclopramide interaction with 5-HT receptors in the gastrointestinal tract by the use of stomach preparations may be compromised by the different 5-HT receptor types which may exist within different areas of the gastrointestinal system. Nevertheless, the concept that two 5-HT receptors in the stomach may be balanced to exert inhibitory and facilitatory influences on cholinergic function may provide a new perspective for the actions of metoclopramide to apparently 'mimic' the action of 5-HT in other systems where an antagonism of an inhibitory function would reveal a facilitatory influence. This would provide an alternative explanation to hypotheses requiring that metoclopramide may have partial agonist action on 5-HT receptors (see Kilbinger & Weihrauch 1982, for full discussion) but is concordant with the previous findings that metoclopramide is a 5-HT receptor antagonist (Fozard & Mobarok Ali 1978; Donatsch et al 1984). Definitive evidence for the presence of two 5-HT receptor types in the stomach to modulate cholinergic-induced contractions will require the use of selective antagonists at the 'facilitatory' receptor and the use of selective agonists at the 'facilitatory' and 'inhibitory' 5-HT receptors. The rapid development of 5-HT receptor agonists and antagonists by many groups (see reviews by Feniuk 1984; Fozard 1984a; Peroutka 1984; Leysen et al 1984; Humphrey 1984; and also Donatsch et al 1984) should allow the characterization of the 5-HT receptors in the gastrointestinal tract in accordance with the 5-HT receptor classification currently being developed.

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