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5-Hydroxytryptamine can antagonize the ability of metoclopramide and SCH 23390 to enhance electrical field stimulation-evoked contractions of guinea-pig isolated stomach muscle

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Electrical field stimulation of guinea-pig stomach strips caused frequency related contractions that were enhanced by metoclopramide and SCH 23390 (10^{-7}–10^{-5} M) and antagonized by atropine (5×10^{-8} M. 5-HT (3×10^{-8}–3×10^{-5} M) antagonized the ability of metoclopramide and SCH 23390 to enhance the contractions. It is concluded that metoclopramide and SCH 23390 can enhance electrical field stimulation-induced contractions in the stomach strips by a 5-HT receptor blockade which can facilitate cholinergic mediated contractions.

The cerebral dopamine antagonists metoclopramide and SCH 23390 have been shown to facilitate electrical field stimulation (FS)-induced contractions of isolated smooth muscle preparations taken from the gastrointestinal system (Harrington et al 1983; Costall et al 1984a, b). However, the relevance of the dopamine receptor antagonist actions to the ability to facilitate electrically evoked muscle contractions has been increasingly questioned, since this property is not shared by other potent dopamine antagonists (Costall et al 1984a, b).

A number of authors have forwarded the hypothesis that metoclopramide and other substituted benzamides may interact via 5-hydroxytryptamine (5-HT) receptors to enhance contractions of the gastrointestinal musculature (Bianchi et al 1970; Kilbinger & Weihrauch 1982; Roberts 1982). In the present experiments we investigate the possibility that 5-HT receptor mechanisms may be involved with the ability of metoclopramide and SCH 23390 to enhance electrical field stimulation-induced contractions in longitudinal muscle strips obtained from the body of the guinea-pig stomach.

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. 1 g tension was applied to the tissues which were allowed to equilibrate for 45 min before electrical 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 in the absence of drug and then in the presence of the potential interacting drug(s) (40 min pretreatments); the second curve was related to the first to assess the degree of change. (±)-Propranolol hydrochloride (ICI), atropine sulphate (Sigma), prazosin hydrochloride (Pfizer), methysergide hydrogen maleinate (Sandoz), metoclopramide monohydrochloride (Beechams) and tetrodotoxin (Sigma) were dissolved in distilled water. Haloperidol (Janssen) and SCH 23390 ((5)-(±)-8-chloro-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1H-3-benzazepin-7 ol hemimaleate; Schering) were dissolved in 0·1% lactic acid and 0·1% acetic acid respectively (each neutralized with sodium bicarbonate).

The significance of differences between treatments was assessed using two-way ANOVA followed by Dunnett's test.

Results

Electrical field stimulation (FS) of the stomach strips caused frequency-related contractions that were repeatable at least 6 times over a 3 h period, and which could be reversed to relaxations by atropine (5×10^{-8} M) (Costall et al 1984b). Tetrodotoxin (10^{-7} M) was shown to abolish the contractions and relaxations (Costall et al 1984b). Metoclopramide and SCH 23390 (10^{-7}–10^{-5} M) caused concentration-related enhancements of the contractions over the entire frequency range without a significant effect on the resting tension (Fig. 1). Prazosin, propranolol, methysergide or haloperidol (5×10^{-7} M) failed to modify FS-induced contractions or the enhancement of FS-induced contractions caused by metoclopramide and SCH 23390 ($n = 5$, $P > 0.05$). 5-HT (3×10^{-8}–3×10^{-5} M) also failed to significantly modify FS-induced contractions in most tissues, although approximately 10% of tissues examined showed an enhancement of FS-induced contractions of 25–50% when using 5-HT at 3×10^{-8}–3×10^{-7} M; this effect of 5-HT was not concentration-related and data from such tissues were not included. 5-HT (3×10^{-8}–10^{-5} M) also failed to alter the resting tension in all tissues. However, the enhanced contractions caused by metoclopramide and SCH 23390 were antagonized in a

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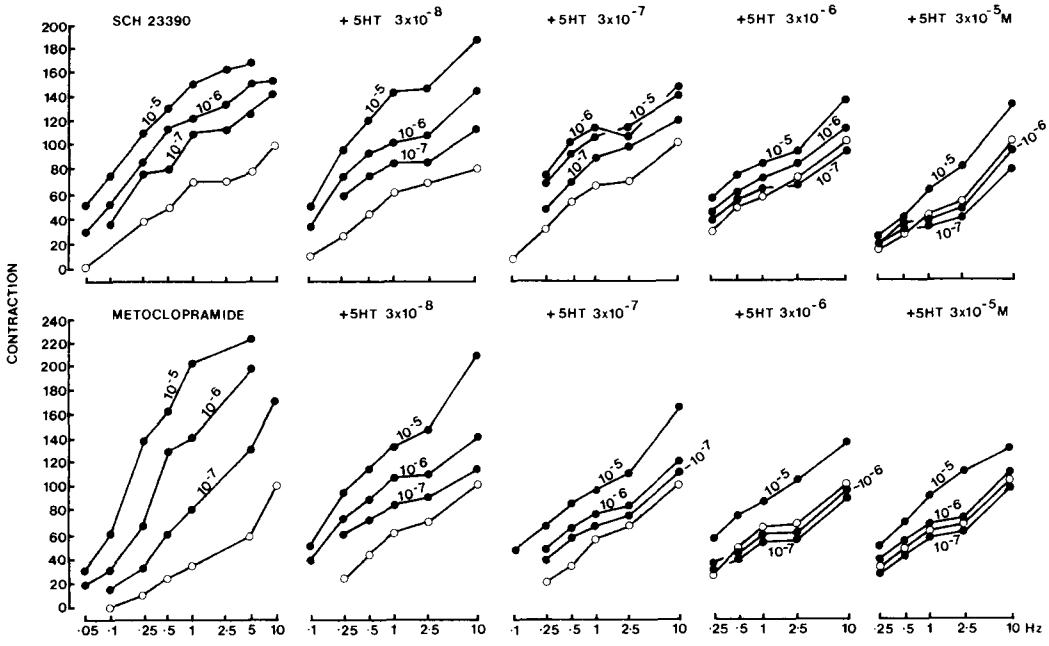

FIG. 1. Antagonism by 5-HT molar concentrations indicated) of the enhancement of electrical field stimulation (0·1–10 Hz)-induced contractions of longitudinal smooth muscle, taken from the body of guinea-pig stomach, by SCH 23390 and metoclopramide (●——●, molar concentrations indicated), ○——○ control values. 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 <14%. Reductions in the responsiveness to each concentration of SCH 23390 and metoclopramide with increasing concentrations of 5-HT were significant to $P < 0{\cdot}05$–$P < 0{\cdot}001$ as analysed by two-way ANOVA.

concentration-dependent manner by 5-HT (3×10^{-8}–3×10^{-5} M) (Fig. 1). 5-HT (3×10^{-8} M) caused modest reductions (approximately 10–30%) in FS-induced contractions caused by SCH 23390 although these generally did not achieve significance whilst 5-HT (3×10^{-8} M), throughout the frequency range, reduced the enhanced contractions caused by metoclopramide (10^{-6} and 10^{-5} M). The higher concentrations of 5-HT (3×10^{-7}, 3×10^{-6} and 3×10^{-5} M) caused increasing reduction of the metoclopramide and SCH 23390-induced enhancement of the contractions, the higher concentrations of 5-HT abolishing the effects of metoclopramide (10^{-7}–10^{-6} M) and SCH 23390 (10^{-7}–10^{-6} M) (Fig. 1). The ability of 5-HT (3×10^{-6} M) to antagonize the metoclopramide response was not affected by the inclusion of haloperidol, prazosin or propranolol (5×10^{-7} M) in the bathing medium (n = 5, $P > 0{\cdot}05$).

Discussion

Electrical FS of longitudinal muscle obtained from the body of the guinea-pig stomach causes contractions mediated via cholinergic systems; metoclopramide and SCH 23390 can enhance the cholinergic mediated response (Costall et al 1984a, b). The precise mechanism by which these potent neuroleptic drugs can achieve this effect has remained uncertain, with a division of opinion as to the importance of a dopamine or 5-HT receptor antagonist action to facilitate the cholinergic function (Van Nueten 1980; Kilbinger & Weihrauch 1982; Roberts 1982; Harrington et al 1983; Costall et al 1984a, b). The present study provides experimental data to support previous hypotheses advocating an involvement of 5-HT.

The critical finding is that 5-HT was found to antagonize the actions of metoclopramide and SCH 23390 in a concentration-related manner, and at higher concentrations to abolish the ability of the two compounds to enhance FS-induced contractions. That the actions of 5-HT were mediated at 5-HT receptors is inferred by the failure of the α- and β-adrenoceptor antagonists prazosin and propranolol and the dopamine antagonist haloperidol to modify the inhibitory effects of 5-HT.

The studies provoke two questions. Firstly, does the 5-HT interaction with metoclopramide and SCH 23390 reflect a direct 5-HT receptor antagonism by metoclopramide and SCH 23390? Evidence to support a 5-HT receptor antagonist action has been shown in other systems: (a) metoclopramide has antagonist action on 5-HT receptors located on the cardiac sympathetic nerves (Fozard et al 1977) and receptors mediating the Bezold-Jarisch reflex (Fozard & Host 1982), (b) SCH 23390 can antagonize the contractile effects of 5-HT in the rat tail artery preparation (Langer

1982) and in binding studies has been shown to possess high affinity (albeit less than for the D_1-receptor) for the 5-HT receptor (Hyttel 1983) and (c) most relevant of all, Sanger (1984) has recently reported that tachyphylaxis of the 5-HT receptors in the rat stomach enhanced FS-induced contractions and precluded a further enhancement by metoclopramide. However, we do not suggest that metoclopramide and SCH 23390 may necessarily interact with the same type of 5-HT receptors as identified in these studies.

Secondly, if it is accepted that 5-HT may normally exert an inhibitory influence on acetylcholine release from the nerves of the stomach musculature (see also Bülbring & Gershon 1967), it may be questioned why acute treatment with 5-HT itself failed to modify FS-induced contractions. We suggest that endogenous 5-HT may normally exert a maximal effective inhibitory response to preclude a further enhancement of the inhibitory response by exogenous 5-HT. That both metoclopramide and SCH 23390 can induce contractions at low rates of FS where contractions are not usually observed would support a tonic inhibitory role for 5-HT.

In summary, the evidence of the present study suggests that metoclopramide and SCH 23390 may enhance electrically evoked smooth muscle contractions in the guinea-pig stomach by a 5-HT receptor blockade which can facilitate cholinergic-mediated contraction processes. The precise location of the receptor, presumably presynaptic, and the classification of the 5-HT receptor type is not known. In any event, metoclopramide and SCH 23390 may prove useful tools in the identification and delineation of the 5-HT receptor types in the gastrointestinal system.

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