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Activation of a myenteric 5-hydroxytryptamine-like receptor by metoclopramide

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Cholinergically mediated contractions were evoked by electrical field stimulation (EFS) of guinea-pig distal ileum longitudinal muscle-myenteric plexus strips. Metoclopramide, 0·1–100 μM, dose-dependently increased the contractions, probably by increasing acetylcholine (ACh) release; contractions evoked by exogenous ACh in the presence of tetrodotoxin were not increased by metoclopramide. 5-Hydroxytryptamine (5-HT) 0·3–30 nM similarly increased the height of the EFS-evoked contractions, although the maximum increase was less than for metoclopramide; higher concentrations of 5-HT (3 and 30 μM) had no effects or caused inhibition. The compound, ICS 205-930, 0·001–1 μM, had no effect on the EFS-evoked contractions and caused inhibition at higher concentrations. Preincubation of the tissues with 5-HT, 0·3–30 nM, did not affect the increase in EFS-evoked contractions caused by metoclopramide, 1 or 100 μM, whereas 5-HT, 3 and 30 μM, prevented the response caused by metoclopramide 1 μM, but not 100 μM. ICS 205-930, 0·1 μM, had no effect on the increase in EFS-evoked contractions caused by metoclopramide 1 or 100 μM. The drug, at least in low concentrations, may therefore increase cholinergically mediated contractions of guinea-pig ileum by stimulating 5-HT-like receptors within the myenteric plexus, which differ from those antagonized by ICS 205-930.

Metoclopramide (Maxolon: Beecham Pharmaceuticals) is thought to increase gastrointestinal motility mostly by increasing acetylcholine (ACh) release from the nerves of the gut (see Sanger 1984). This action may be due to an interaction with 5-hydroxytryptamine (5-HT) 5-HT_3 receptors (Bradley et al 1986), which modulate ACh release (Bianchi et al 1970; Kilbinger et al 1982; Gunning & Naylor 1985; Sanger 1985a). However, there are major inconsistencies in the evidence presented. In particular, 5-HT tachyphylaxis prevents the increase in cholinergically mediated contractions caused by metoclopramide in guinea-pig isolated stomach (Gunning & Naylor 1985), but not in the ileum (Kilbinger et al 1982; Massingham et al 1985). In contrast, high concentrations of 5-HT (Sanger 1985a) or 5-hydroxytryptophan (Sanger & McClelland 1986) mimic the actions of metoclopramide in rat isolated forestomach.

Interpreting results obtained using 5-HT tachyphylaxis is complicated not only by the different species and regions of the gut used, but also because certain actions of 5-HT are not clearly sensitive to tachyphylaxis. For example, in guinea-pig isolated ileum, low and high concentrations of 5-HT, respectively, increase and decrease submaximal cholinergically mediated contractions; these effects are maintained during the continued presence of 5-HT (Kilbinger & Pfeuffer-Friederich 1985; Sanger 1985b). Such actions of 5-HT must therefore interfere with the actions of other substances, such as metoclopramide, which act via the same cholinergic pathways. For this reason, the interactions between metoclopramide and 5-HT have now been re-evaluated using the longitudinal muscle-myenteric plexus preparation of guinea-pig ileum. This preparation was chosen to minimize problems associated with penetration of substances to the nerves of the myenteric plexus. The effects of metoclopramide are compared with the actions of ICS 205-930, an antagonist of 5-HT_3 receptors (Richardson et al 1985).

MATERIALS AND METHODS

Male guinea-pigs (300–450 g) were used. Distal ileum was removed at least 10 cm proximal to the caecum and longitudinal muscle-myenteric plexus preparations 2–3 cm long were dissected (Ambache & Freeman 1968). The muscle-nerve strips were suspended under a 0·5 g load in 10 mL tissue baths containing Krebs solution (NaCl 121·5, $CaCl_2$ 2·5, KH_2PO_4 1·2, KCl 4·7, $MgSO_4$ 1·2, $NaHCO_3$ 25·0, dextrose 5·6 mM) bubbled with 5% CO_2 in O_2 and maintained at 37 °C. Responses were registered and magnified 6–18 times with isotonic transducers. For electrical field stimulation (EFS) of the myenteric neurons, bipolar rectangular pulses were passed between 2 platinum wire electrodes 25 mm long and 5 mm apart, suspended either side of the muscle

strip. EFS was given as 0·5 ms pulses at 0·1 Hz frequency and at a voltage which evoked maximum muscle contractions (25–40 V). Consistent contractions with EFS were achieved 10 min after washout and replacement of the bathing solution and then concentration-response curves for metoclopramide or ICS 205-930 were constructed by adding cumulative concentrations to the bathing solution at 5 min intervals. In experiments to test the effects of 5-HT on the ability of metoclopramide to increase EFS-evoked contractions, tissues were preincubated for 10 min with a single concentration of 5-HT, and metoclopramide, 0·1 and 100 μM, was then cumulatively added at 5 min intervals. The effects of 5-HT were statistically compared with experiments in which metoclopramide was tested in the presence of saline. Similarly, to test the effects of a single concentration of ICS 205-930 on the increase in EFS-evoked contractions caused by metoclopramide, tissues were preincubated with ICS 205-930 or its solvent for 30 min before metoclopramide was added to the bath.

To study the effects of drugs on contractions evoked by exogenous acetylcholine (ACh), concentrations of ACh (0·04–0·4 μM) were chosen to evoke contractions which were approximately equal in height to those caused by EFS; the contractions were 39 ± 3% of maximum contractions evoked by higher concentrations of ACh (n = 12). Contractions were evoked every 10 min using 30 s contact times, during which the contractions reached their maximum and the tissues began to relax. After obtaining consistent contractions, increasing concentrations of metoclopramide or 5-HT were added to the bathing solution after each dose of ACh. In similar experiments, metoclopramide and 5-HT were tested against ACh during the presence of tetrodotoxin 0·1 μM; tissues were preincubated with tetrodotoxin for 30 min before adding metoclopramide or 5-HT.

Results are expressed as means ± standard errors of the mean and were analysed statistically using the Student's *t*-test.

Drugs used: The following were dissolved in 0·54 mM saline: acetylcholine perchlorate, atropine sulphate (BDH), hexamethonium bromide (May and Baker), 5-hydroxytryptamine creatinine sulphate (protected from light; Sigma), metoclopramide hydrochloride (Beecham) and tetrodotoxin in citrate buffer (Sigma). ICS 205-930 20 mM ((3α-tropanyl)-1*H*-indole-3-carboxylic acid ester, synthesized in house) was dissolved in distilled water adjusted to pH4 with tartaric acid, and further dilutions were in distilled water.

RESULTS

Contractions evoked by EFS were prevented by atropine 1·4 μM (n = 6) or by tetrodotoxin 0·1 μM (n = 4). Hexamethonium 28 μM reduced the contractions by 10 ± 4% ($P < 0·05$, n = 6). EFS-evoked contractions may therefore be predominantly due to activation of post-ganglionic cholinergic neurons.

Metoclopramide, 0·1–100 μM, dose-dependently increased the height of the EFS-evoked contractions, whereas a higher concentration (1 mM) reduced the contractions (Fig. 1). The effects of each concentration of metoclopramide reached their maximum during the 5 min dosing intervals and were sustained thereafter. Contractions evoked by exogenous ACh were also increased with metoclopramide 1–100 μM, in the absence, but not in the presence of tetrodotoxin 0·1 μM (Table 1). 5-HT, 0·3–30 nM, caused a small, sustained increase in the height of the EFS-

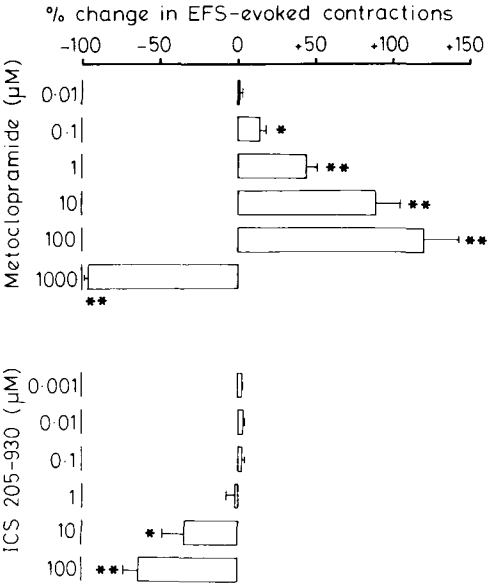

FIG. 1. Effects of metoclopramide and ICS 205-930 on EFS-evoked contractions of longitudinal muscle-myenteric plexus preparations from guinea-pig ileum. Electrical stimulation was at 0·1 Hz, 0·5 ms pulse duration and at maximum effective voltage. Cumulative concentrations of metoclopramide, ICS 205-930 or their respective solvents were added to the bath every 5 min, beginning 10 min after washout and replacement of the bathing solution. The results were calculated as a % increase or decrease in the heights of the EFS-evoked contractions obtained prior to addition of drug. The columns represent mean values, the bars standard errors of the mean. The respective solvents for metoclopramide and ICS 205-930 had no effects on the EFS-evoked contractions ($P > 0·05$ for each dose, n = 6 each) and compared with the solvent, $^{*}P < 0·05$, $^{**}P < 0·001$; n = 6 each.

Table 1. Effects of metoclopramide and 5-HT on ACh-evoked contractions of guinea-pig ileum, in the absence and presence of tetrodotoxin 0·1 μM. The contractions evoked by ACh were submaximal and approximately equal in height to the maximum contractions evoked by EFS at 0·1 Hz. Contractions were obtained every 10 min using 30 s contact times and increasing concentrations of metoclopramide or 5-HT were added after each challenge with ACh. Results are expressed as the % increase or decrease in the height of the contractions obtained before addition of metoclopramide or 5-HT.

Drug	μM	Control	+Tetrodotoxin 0·1 μM
Metoclopramide	0·01	+4 ± 8	−5 ± 6
	0·1	+5 ± 3	+10 ± 9
	1·0	+37 ± 9*	+3 ± 5
	10·0	+56 ± 15*	+10 ± 8
	100·0	+58 ± 23*	+7 ± 4
	1000·0	−93 ± 2*	−68 ± 10*
5-HT	0·0003	0 ± 6	+6 ± 3
	0·003	+4 ± 8	+9 ± 5
	0·03	+20 ± 7*	+3 ± 7
	0·3	+11 ± 7	+6 ± 8
	3·0	−10 ± 6	−18 ± 8
	30·0	−18 ± 6*	−21 ± 8*

* $P < 0·05$, n = 6 each.

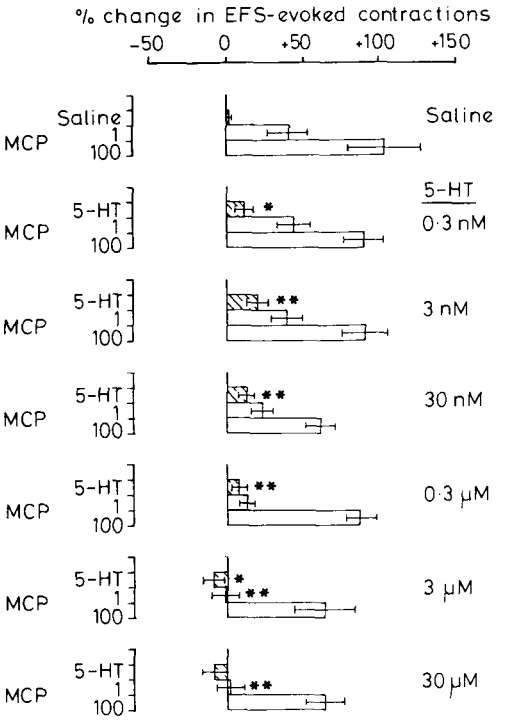

FIG. 2. The effects of 5-HT on the increase in EFS-evoked contractions caused by metoclopramide in longitudinal muscle-myenteric plexus preparations from guinea-pig ileum. Electrical stimulation was as described for Fig. 1. Saline or 5-HT 0·3 nM–30 μM were added to the bath 10 min after washout of the bathing solution and 10 min before metoclopramide (MCP), 1 and 100 μM, which were added at 5 min intervals. In each experiment, only saline or a single concentration of 5-HT was tested. The results were calculated as a % increase or decrease in the heights of the EFS-evoked contractions obtained before addition of saline or 5-HT. The columns represent mean values, the bars standard errors of the mean. The effects of 5-HT and of metoclopramide in the presence of 5-HT were compared with the experiments in which metoclopramide was tested in the presence of saline, *$0·1 > P > 0·05$, **$P < 0·05$; n = 10 for saline controls, n = 6 for each 5-HT.

evoked contractions, but did not prevent a further increase in the contraction heights caused by metoclopramide, 1 or 100 μM (Fig. 2). Higher concentrations of 5-HT (3 and 30 μM) had no effect or reduced the EFS-evoked contractions and prevented the response to metoclopramide 1 μM, but not to 100 μM (Fig. 2). In the presence of tetrodotoxin 0·1 μM, 5-HT did not increase contractions evoked by exogenous ACh (Table 1).

ICS 205-930, 1 nM–1 μM, did not affect EFS-evoked contractions; higher concentrations (10 and 100 μM) reduced the contractions (Fig. 1). ICS 205-930 0·1 μM did not affect the increase in EFS-evoked contractions caused by metoclopramide, 1 or 100 μM (n = 6, $P > 0·5$ for each concentration of metoclopramide).

DISCUSSION

Metoclopramide, 0·1–100 μM, clearly and dose-dependently increased the cholinergically mediated contractions evoked by EFS in guinea-pig ileum longitudinal muscle-myenteric plexus preparations. Its effects were sustained and therefore did not demonstrate auto-desensitization. In similar preparations of guinea-pig distal and mid ileum, others have previously reported stimulation by metoclopramide of cholinergically mediated contractions (Kilbinger et al 1982; Zar et al 1982; Massingham et al 1985), contrasting with the failure of Gunning et al (1986a) to detect excitatory actions of metoclopramide in a longitudinal muscle-myenteric plexus preparation of proximal ileum.

In the presence of tetrodotoxin, metoclopramide did not increase muscle contractions evoked by exogenous ACh. It may therefore increase cholinergic activity in guinea-pig isolated ileum, by increasing the release of ACh from neurons within the ileum (Kilbinger et al 1982). This conclusion has also been reached for several other isolated tissues from different species or regions of the gut (see Sanger 1984).

Low concentrations of 5-HT increased EFS-evoked contractions, and this was sustained during the continued presence of 5-HT. However, the maximum increase caused by 5-HT was less than that caused by metoclopramide; higher concentrations of 5-HT had no effects or reduced the contractions. The sustained increase or decrease in EFS-evoked contractions caused by 5-HT suggests an absence of auto-desensitization for both actions of 5-HT and confirms earlier observations (Kilbinger & Pfeuffer-Friederich 1985; Sanger 1985b). In the presence of tetrodotoxin, 5-HT did not increase ACh-evoked contractions. The increase in EFS-evoked contractions caused by low concentrations of 5-HT may therefore be due to an increase in ACh release from the cholinergic nerves of the gut, as previously reported using whole segments of guinea-pig distal ileum (Sanger 1985b).

High concentrations of 5-HT did not prevent the increase in EFS-evoked contractions caused by a high concentration of metoclopramide, supporting the results of Kilbinger et al (1982) and of Massingham et al (1985), who used a similarly high concentration of metoclopramide. However, the same concentrations of 5-HT prevented the excitatory actions of a lower concentration of metoclopramide. Since the actions of both metoclopramide and 5-HT on EFS-evoked contractions are themselves not clearly tachyphylaxis-sensitive, 5-HT may therefore prevent the actions of low concentrations of metoclopramide not by tachyphylaxis, but by directly or indirectly affecting the same receptor. Gunning & Naylor (1985) suggested that metoclopramide increased cholinergically mediated contractions of guinea-pig isolated stomach by blocking an inhibitory action of endogenous 5-HT (see also Gunning et al 1986b), but for the following reasons, the mechanisms by which metoclopramide increases cholinergically mediated contractions in guinea-pig ileum are unlikely to involve antagonism of 5-HT. Firstly, the actions of any endogenous 5-HT in guinea-pig ileum may be excitatory since low concentrations of 5-HT (0·3–30 nM) increase cholinergically mediated contractions. These low concentrations of 5-HT should not be expected to cause tachyphylaxis of an inhibitory action of endogenous 5-HT for the reason already described, and because high concentrations of substances are normally required for tachyphylaxis. Secondly, and in contrast with results obtained using guinea-pig isolated stomach (Gunning & Naylor 1985) or proximal ileum (Costall et al 1986), the selective 5-HT_3 receptor antagonist ICS 205-930 did not increase EFS-evoked contractions and therefore did not in any way mimic the excitatory actions of metoclopramide or 5-HT. Furthermore, ICS 205-930 0·1 μM did not prevent the ability of low or high concentrations of metoclopramide to increase cholinergically mediated contractions. The failure to detect an increase in EFS-evoked contractions with ICS 205-930 suggests that this compound will not stimulate guinea-pig small intestinal motility by an action on enteric neurons. In conscious dogs, ICS 205-930 did not affect small intestinal myoelectric activity (Davidson & Pilot 1986). Further support for the suggestion that 5-HT_3 receptor antagonists do not necessarily stimulate enteric nerve activity is given by the lack of correlation between the ability of various compounds to stimulate gut motility (metoclopramide, BRL 20627 ((2α,6β-9aα)-(±)-4-amino-5-chloro-2-methoxy-*N*-(octahydro-6-methyl-2H-quinolizin-2-yl)benzamide hydrochloride), cisapride, BRL 24924 ([(±)-(endo)]-4-amino-5-chloro-2-methoxy-*N*-(1-azabicyclo-[3.3.1]-non-4-yl)benzamide hydrochloride)) and their potency as 5-HT_3 receptor antagonists (Dunbar et al 1986).

Finally, the increase in cholinergically mediated contractions caused by metoclopramide may also be unrelated to antagonism of the inhibitory effects of high concentrations of 5-HT. This action of 5-HT is not antagonized by metoclopramide or by MDL 72222 (1αH,3α,5αH-tropan-3-yl-3,5-dichlorobenzoate methane sulphonate), a selective 5-HT_3 receptor antagonist (Kilbinger & Pfeuffer-Friederich 1985). Instead, those authors suggested that high concentrations of 5-HT may cause inhibition by activating 5-HT_1-like receptors in the ileum. The existence of 5-HT_1-like receptors in guinea-pig ileum has since been further substantiated using 5-HT_{1A} receptor agonists and antagonists (Fozard & Kilbinger 1985; Hagenbach et al 1986).

In conclusion, it is, therefore, suggested that low concentrations of metoclopramide increased cholinergically mediated contractions in guinea-pig isolated ileum, not by antagonizing an inhibitory action of 5-HT (acting on 5-HT_3 or on 5-HT_1 receptors), but by directly or indirectly stimulating an as yet uncharacterized 5-HT-like receptor within the myenteric plexus, which in turn modulates ACh release from cholinergic nerves. A similar conclusion was reached following similar experiments with the gastric prokinetic compound BRL 24924 (Sanger 1987). These actions of BRL 24924 or of low concentrations of metoclopramide were not sensitive to antagonism by ICS 205-930 but may be mimicked by low concentrations of 5-HT. In the present experiments, it is important to note that the increase

in EFS-evoked contractions caused by 5-HT was similar in magnitude only to the increase in contractions caused by low concentrations of metoclopramide (which may be blocked by high concentrations of 5-HT), and was less than the increase caused by higher concentrations of metoclopramide (which may be unaffected by 5-HT). Metoclopramide could therefore increase EFS-evoked contractions by at least two different mechanisms, only one of which involves activation of enteric 5-HT-like receptors. In contrast, BRL 24924 increased EFS-evoked contractions of guinea-pig isolated ileum by no more than the increase caused by 5-HT (Sanger 1987). However, compared with metoclopramide, BRL 24924 may be a more potent and effective stimulant of gastric motility and emptying (Cooper et al 1986). Activation of enteric 5-HT-like receptors by low concentrations of 5-HT, low concentrations of metoclopramide or by BRL 24924 may therefore be an important mechanism by which gastric motility can be increased in-vivo.

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