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5-Hydroxytryptamine receptor antagonism by metoclopramide and ICS 205-930 in the guinea-pig leads to enhancement of contractions of stomach muscle strips induced by electrical field stimulation and facilitation of gastric emptying in-vivo

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Contractions induced by electrical field stimulation of isolated circular muscle strips, taken from the guinea-pig stomach, were enhanced by metoclopramide, ICS 205-930 and MDL 72222 at concentrations similar to those shown to antagonize at neuronal 5-hydroxytryptamine receptor sites in a variety of preparations. Metoclopramide, MDL 72222 and ICS 205-930 also facilitated gastric emptying in-vivo. The abilities of metoclopramide, MDL 72222 and ICS 205-930 to enhance stomach muscle contraction processes and to facilitate gastric emptying may be the consequence of 5-hydroxytryptamine receptor antagonism.

The action of certain substituted benzamide drugs in facilitating gastric emptying may reflect their influence both on central sites and peripherally to enhance stomach muscle contraction, the effect involving an enhancement of cholinergic activity (Costall et al 1983, 1985). Although substituted benzamides are often potent dopamine receptor antagonists, there is no evidence to link this property with a potential to facilitate gastric emptying and to enhance stomach muscle contractions (McClelland & Sanger 1983; Costall et al 1984). Therefore, other neurotransmitter mechanisms by which the substituted benzamides may act to enhance cholinergic activity have been sought.

Some authors have suggested that the substituted benzamides may act via 5-hydroxytryptamine (5-HT) receptors to enhance contractions in the gastrointestinal system (Bianchi et al 1970; Kilbinger & Weihrauch 1982; Roberts 1982), and it has recently been shown that 5-HT can antagonize the ability of metoclopramide to enhance contractions in guinea-pig stomach strips induced by field stimulation (Gunning & Naylor 1985). However, the nature of the interaction of metoclopramide with the 5-HT receptor(s) and the type(s) of 5-HT receptor(s) involved is not clear (see review by Sanger 1984). In the present study we have used compounds established as 5-HT 'M' receptor antagonists (according to the classification of Gaddum & Picarelli 1957) and 5-HT_1 and 5-HT_2 receptor antagonists (characterized in radioligand binding and other assays; see reviews by Peroutka 1984; Leysen et al 1984). The aim has been to determine whether 5-HT receptor blockade can enhance contraction responses of the stomach to field stimulation and facilitate gastric emptying and, if so, to indicate the 5-HT receptor type(s) involved.

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Methods

Male, Dunkin-Hartley guinea-pigs (450–550g) were used. For in-vitro experiments they were killed by cervical dislocation, the stomachs removed and a strip of gastric body circular muscle (20 mm long, 5 mm wide) dissected from each. Strips were placed in 30 ml 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). 1g 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 long axis of the tissue and approximately 5 mm apart (supramaximal voltage, 0·1 ms pulse width). Tissues were stimulated for 30 s every 5 min and then washed. Tension changes were detected by Grass tension transducers and displayed on a Grass recorder. A frequency-response curve (0·25–10 Hz) was initially constructed in the absence of drug and then in the presence of the potential interacting drug (40 min pretreatment); the second curve was related to the first to assess the degree of change. The significance of differences between treatments was assessed by using the Mann-Whitney U-test.

In the in-vivo studies measurement of gastric emptying was achieved by X-ray location (50 kV, 40 mA, 0·5–0·8 s) using Kodak plates (NS-2T, 13 × 8 cm) of polystyrene-coated barium sulphate spheroids (approximately 30, 1 mm in diameter) (see Cox & Ennis 1980) which were swallowed by the guinea-pigs when placed at the back of the mouth in 0·2 ml of 1% carboxymethylcellulose with 0·05 ml glycerol to initiate prompt and voluntary swallowing. The passage of the spheroids was followed for 2 h. During this period, animals were placed in their normal housing cages, 4 guinea-pigs per cage with free access to food and water, and were only removed 5 min before X-ray (at 60 min intervals) when they were placed in individual Perspex holding cages that held the animal comfortably in a stable position. Spheroids located above thoracic and lumbar vertebrae T12 and L1, in an area usually shown in darker image on the X-ray plates, were taken as being within the stomach. The significance of differences between treatments were assessed using the Mann Whitney U-test.

Atropine sulphate (Sigma), ICS 205–930 ([3α-tropanyl]-1*H*-indole-3-carboxylic acid ester) (Sandoz), mesulergine hydrochloride (Sandoz), methysergide hydrogenmaleinate (Sandoz), MDL 72222 (1α*H*, 3α, 5α*H*-tropan-3-yl-3,5-dichlorobenzoate methyl sulphate hemihydrate) (Merrell), metoclopramide monohydrochloride (Beecham), and tetrodotoxin (Sigma) were dissolved in distilled water. Ketanserin (Janssen), methiothepin maleate (Glaxo) and spiperone (Janssen) were dissolved in the minimum amount of glacial acetic acid and each neutralized with sodium bicarbonate. Doses administered peripherally are expressed as the base.

Results

Electrical field stimulation (FS) of circular muscle strips taken from the body of the guinea-pig stomach caused frequency-related contractions (0·25–10 Hz) that were repeatable at least 6 times over a 4 h period and which were reversed to relaxation by atropine (5×10^{-8} M). Tetrodotoxin (10^{-7} M) abolished the contractions and relaxations (see also Costall et al 1984).

Metoclopramide (10^{-7}–10^{-5} M) and ICS 205–930 (10^{-10}–10^{-8} M) caused concentration-related enhancements of the FS-induced contractions over the entire frequency range. The maximum enhancement of FS-induced contractions by metoclopramide and ICS 205–930 was 200% of control values. A higher concentration of ICS 205–930 (10^{-7} M) less effectively enhanced contractions (Fig. 1). MDL 72222 (10^{-9}–10^{-8} M) also caused a concentration-related and significant increase in FS-induced contractions, but at the lower frequencies (0·25–2·5 Hz). Also, the enhanced contraction responses were more modest than when using metoclopramide or ICS 205–930 and did not exceed the control contraction reponses recorded at 10 Hz. Further, the use of higher concentrations of MDL 72222 (10^{-7}–10^{-5} M) was associated with a concentration-related reduction in the drug's ability to facilitate FS-induced contractions and of the contractions themselves (Fig. 1). Methysergide, methiothepin, spiperone, mesulergine and ketanserin (10^{-8}–10^{-6} M) failed to enhance or reduce FS-induced contractions (n = 6–10, $P > 0.05$). EC50 values for metoclopramide, MDL 72222 and ICS 205–930 enhancement of FS-induced contractions at 1 Hz are given in Table 1.

Metoclopramide (1–10 mg kg^{-1} i.p.) and ICS 205–930 (0·01–1·0 mg kg^{-1} i.p.) caused dose-related increases in gastric emptying in the guinea-pig (Fig. 2). MDL 72222 at 0·01 mg kg^{-1} i.p. also enhanced gastric emptying but a lower dose (0·001 mg kg^{-1} i.p.) and higher doses (0·1 and 1·0 mg kg^{-1} i.p.) were ineffective. The comparative potencies of the three compounds in facilitating gastric emptying are shown in Table 1.

Discussion

ICS 205-930 is a potent and highly selective antagonist at 5-HT receptors located on peripheral nerves (Donatsch et al 1984: Buchheit et al 1985) and MDL 72222 has a similar though less potent action (Fozard 1984a). The main finding of the present study is that ICS 205–930 potently enhanced field stimulation (FS)-induced contractions of circular muscle strips obtained from the body of the guinea-pig stomach while MDL 72222 was less potent. Since their relative potencies in enhancing FS-induced contractions are comparable with their abilities to antagonize the actions of 5-HT at 'neuronal' receptors (i.e. 5-HT 'M' receptors) in other systems, for example on the vagus nerve, rabbit heart and the Von Bezold-Jarisch reflex (see Table 1), the most reasonable conclusion is that ICS 205–930 and MDL 72222 are acting as antagonists at neuronal 5-HT receptors in the guinea-pig stomach and thereby facilitating FS-induced contractions. In addition, the potency of metoclopramide in enhancing FS-induced contractions is comparable to its affinity for neuronal 5-HT receptors in other systems (see also Fozard et al 1977; Fozard & Host 1982), indicating that metoclopramide may also act as a 'neuronal' 5-HT receptor antagonist in the stomach thereby facilitating FS-induced contractions. Therefore, the ability of 5-HT to antagonize the actions of metoclopramide on the stomach strip (Gunning & Naylor 1985) may reflect a direct interaction at the 5-HT receptor.

Peripherally administered ICS 205–930 strongly enhanced gastric emptying, the drug being approximately 10–50 times more potent than metoclopramide. The enhanced gastric emptying may involve action of the drug on the stomach tissue to facilitate cholinergic-mediated contraction processes directly, an action on the central nervous system, or a combination of such actions (Costall et al 1985). In contrast, MDL 72222 failed to facilitate gastric emptying markedly. This failure may be explained by the in-vitro experiments in which MDL 72222 was only modestly effective in enhancing FS-induced contractions and this action

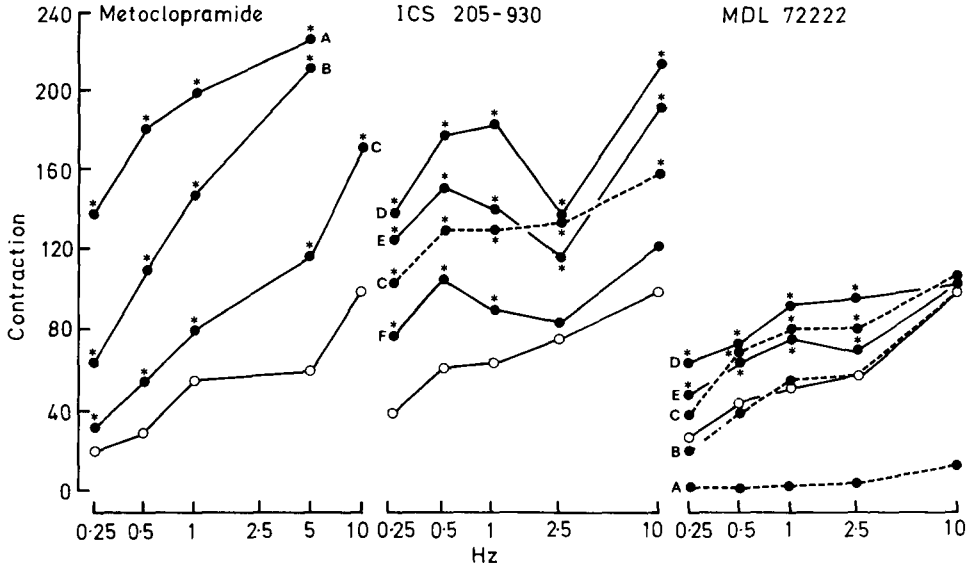

FIG. 1. Modification by metoclopramide, ICS 205–930 and MDL 72222 of contractions induced by electrical field stimulation (0·25–10 Hz) of circular muscle taken from the body of the guinea-pig stomach. (○——○) Control values; (●——●) dose-related enhancements of contractions by drug treatments; (●- - - -●) enhancements reduced as concentrations are increased. All values are expressed as a % of the control contractions occurring at 10 Hz which were designated as 100%. n = 6, s.e.m.s on original data <12%. Enhancement of the contractions significant to $*P < 0{\cdot}05$–$P < 0{\cdot}001$ as analysed by the Mann Whitney U-test. Concentrations (M): A 10^{-5}, B 10^{-6}, C 10^{-7}, D 10^{-8}, E 10^{-9}, F 10^{-10}.

Table 1. A comparison of the potencies of metoclopramide, MDL 72222 and ICS 205–930 in enhancing FS-induced contraction responses of guinea-pig stomach strips (circular muscle, gastric body), in facilitating gastric emptying, and in inhibiting the actions of 5-HT on neuronally located 5-HT receptors.

Drug	Guinea-pig stomach EC50 mol litre^{-1} [a]	Gastric emptying ED50 mg kg^{-1} i.p. [b]	Rabbit vagus pA_2 [c]	Rabbit heart pA_2 [d]	Von Bezold Jarisch reflex K_i μg kg^{-1} i.v. [e]
Metoclopramide	7·4	7·0	7·3 ± 0·2	7·1 ± 0	183 ± 15
MDL 72222	9·6	*	7·9 ± 0·2	8·9 ± 0·1	39 ± 7 [f]
ICS 205–930	10·4	0·019	10·2 ± 0·3	10·6 ± 0·1	0·37 ± 0·03

[a] log concentrations causing a 50% increase in the FS-induced contraction response at 1 Hz; [b] dose causing a 50% increase in gastric emptying at 1 h; pA_2 values to antagonize the actions of 5-HT on [c] "c" fibres [d] sympathetic and [e] afferent nerves. Data presented from the rabbit vagus nerve, rabbit heart and Von Bezold Jarisch Reflex were obtained from Donatsch et al (1984) and from Fozard & Host (1982) (f). * Could not be determined, see text.

declined as the concentration of drug increased until finally a reduction in FS-induced contractions was observed. ICS 205–930 also became less effective at the highest dose in facilitating contraction responses. It is possible that at the higher doses, MDL 72222 and, to a lesser extent, ICS 205–930, administered in-vivo may have an additional antagonistic effect on another 5-HT receptor type which may normally mediate in stomach tissues, and perhaps in the brain, a facilitatory influence on muscle contractions and gastric emptying (Bradbury et al 1985).

Our studies indicate that metoclopramide, MDL 72222 and ICS 205–930 may act as 5-HT 'M' receptor antagonists in facilitating gastric emptying in-vivo and/or enhancing FS-induced contractions in circular muscle strips taken from the body of the guinea-pig stomach. Their specificity of action is shown by the failure of the 5-HT-D, 5-HT_1 or 5-HT_2 receptor antagonists (methysergide, methiothepin, ketanserin, spiperone, mesulergine) to enhance gastric emptying or the FS-induced contractions. It remains to be established whether the 5-HT 'M' receptor is located on the cholinergic nerve to directly inhibit the release of acetylcholine, or on another neuron to excite the release of an inhibitory neurotransmitter which then reduces the output of acetylcholine. Also, it is emphasized that the present data were obtained from a stomach preparation and studies are in progress to determine whether

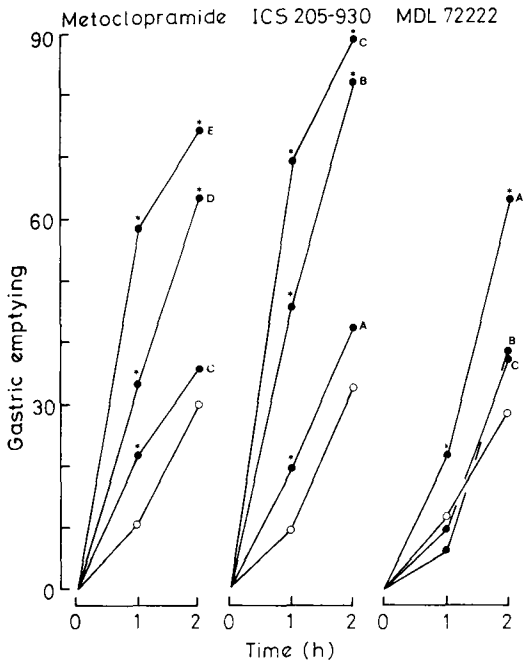

FIG. 2. Modification by metoclopramide, ICS 205–930 and MDL 72222 of gastric emptying in the guinea-pig. Gastric emptying is expressed as the % of spheroids leaving the stomach over a 2 h period. (○——○) control values, (●——●) enhancement of gastric emptying by drug treatments, significant to $^*P < 0{\cdot}005$–$P < 0{\cdot}001$ as analysed by the Mann Whitney U-test. n = 5–6, s.e.m.s. on original data <13%. Dose (mg kg^{-1}): A 0·01, B 0·1, C 1·0, D 5·0, E 10·0.

the nature of the 5-HT involvement is tissue dependent since Fozard (1984a) has reported that MDL 72222 does not increase FS-induced contractions in the guinea-pig ileum.

The role of 5-HT in peripheral and central systems is being intensively investigated and receptor classification aided by the rapid development of selective 5-HT agonists and antagonists (see reviews by Feniuk 1984; Fozard 1984b; Humphrey 1984; Peroutka 1984). The 'M' receptor is almost certainly capable of subdivision. Our studies indicate an important role for 5-HT in the control of stomach motility and the use of the above antagonists may allow the nature of the 5-HT receptor(s) to be categorized according to the unified system currently being developed.

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