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Effects of metoclopramide and domperidone on cholinergically mediated contractions of human isolated stomach muscle

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The experiments examine the actions of metoclopramide and domperidone on the responses evoked by electrical field stimulation or by acetylcholine, in longitudinal muscle strips obtained from human stomach. Electrical field stimulation evoked contractions which were predominantly cholinergically mediated; metoclopramide 0·28–28 μM caused a concentration-dependent increase in the height of these contractions. In the presence of atropine and barium chloride, electrical stimulation evoked relaxations of the stomach muscle, probably by stimulating non-adrenergic, non-cholinergic inhibitory nerves; metoclopramide 28 μM had no effect on these relaxations. Metoclopramide 0·003–2·8 μM had no effect on contractions evoked by exogenous acetylcholine, although higher concentrations of the drug increased the contractions. The results suggest that in human isolated stomach, low concentrations of metoclopramide may increase electrically evoked cholinergic activity by increasing the release of neuronal acetylcholine. Stimulation by metoclopramide of cholinergic activity in the gut may therefore be an important mechanism by which the drug increases gastrointestinal motility during therapy. Cholinergically mediated contractions were not increased by domperidone, and other mechanism(s) of action may therefore be important for this drug.

In guinea-pig and rat isolated stomach or small intestine low concentrations of metoclopramide increase cholinergically-mediated contractions, probably by increasing the release of neuronal acetylcholine (Anderson et al 1977; Hay 1977; Hay & Man 1979; Kilbinger et al 1982; McClelland & Sanger 1982; Costall et al 1983). It has been proposed that a similar action of metoclopramide is important in man (Harrington et al 1983), but this has not been proved. The actions of metoclopramide on cholinergically mediated contractions have therefore been investigated in human isolated stomach strips, and compared with domperidone, which is another dopamine antagonist that stimulates human gastric motility (Brogden et al 1982). Some of these results have been published as an abstract (McClelland & Sanger 1984).

Materials and methods

Specimens of human stomach were obtained at surgery for benign or malignant disease. Samples were taken at least 6 cm away from any macroscopic lesion, and were macroscopically normal. Tissue was used after storage for one or two nights at 4 °C in 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) equilibrated with 5% CO_2 in O_2. Human gastrointestinal tissues obtained and stored in this way do not lose their ability to respond to nerve stimulation, and the sensitivities to many drugs are similar to those obtained using fresh tissue (Bennett & Whitney 1966; Bucknell 1966).

The mucosal layers were removed from the muscle by dividing along the submucosal plexus. Muscle strips approximately 4 mm wide and 30 mm long were cut parallel to the longitudinal muscle fibres. Each strip was suspended under a 1 g load in 10 ml tissue baths containing Krebs solution maintained at 37 °C and bubbled with 5% CO_2 in O_2. Responses were magnified 6–18 times and registered with isotonic transducers.

For electrical field stimulation of the intrinsic nerves, muscle strips were suspended between two platinum wire electrodes 25 mm long and 5 mm apart, insulated on entry to the bathing solution. Bipolar rectangular pulses of 1 ms duration were given every 10 min for 30 s, at 5 Hz frequency and at maximum-effective voltage (80–120 V cm^{-1}). After each period of stimulation the tissue was washed by replacing the bathing solution.

In experiments with exogenous acetylcholine (ACh) or carbachol, dose-response curves were first obtained with either drug. Contact times were 30 s and the cycle time was 10 min. Doses were then chosen to give muscle contractions which were approximately 50% of maximum. For ACh and carbachol these concentrations were respectively 4–400 μM (median 12 μM) and 1·6–55 μM (median 16 μM).

Results are expressed in ranges, or medians with semi-quartile ranges in parentheses, and analysed using the Wilcoxon matched pairs test.

Drugs used: The following drugs were dissolved in 154 mM saline: acetylcholine perchlorate, atropine sulphate, carbachol (BDH), phentolamine mesylate (Ciba), propranolol hydrochloride (ICI) and tetrodotoxin (Sigma). Barium chloride ($BaCl_2$; BDH) was dissolved in distilled water. Noradrenaline bitartrate (Koch-Light) was freshly dissolved in 0·57 μM ascorbic acid solution. Metoclopramide hydrochloride (Beecham) was freshly dissolved in Krebs solution. Domperidone, 28·2 mM (synthesized in house), was freshly prepared by dissolving finely ground powder in distilled water adjusted to pH 4 with tartaric acid; subsequent dilutions were in distilled water.

Results

Specimens were obtained from the fundus, body and antrum of the stomach. There were no obvious differences in the results obtained using the tissues from the different regions, and the results are combined. The

numbers of experiments were too small to determine how disease, age or sex may affect the responses.

Electrical field stimulation (EFS) caused contraction of muscle strips from all stomach specimens. Contractions could be prevented by tetrodotoxin 0·1 μM (n = 3) or atropine 1·4 μM (n = 6). In the presence of atropine 1·4 μM and $BaCl_2$ 0·48 mM (to raise muscle tone and facilitate detection of relaxations), EFS caused muscle relaxations which were reduced by 48–50% by tetrodotoxin 0·1 μM and prevented by tetrodotoxin 0·5 or 1 μM (n = 3 each). The relaxations evoked by EFS were not reduced by phentolamine 1·3 μM plus propranolol 0·9 μM; EFS-induced relaxations were 100–129% of those obtained before incubation of the tissue with the antagonists, whereas muscle relaxations evoked by noradrenaline 30 or 148 μM were reduced by 90–100% using phentolamine and propranolol (n = 4). In general, these experiments confirm those of others (Bennett & Stockley 1975; De Carle & Pye 1980) and suggest that muscle contractions evoked by EFS are mostly cholinergically mediated, dominating the activity of non-adrenergic, non-cholinergic inhibitory neurons which are simultaneously stimulated by EFS.

Consistent contractions were obtained in response to EFS, and then an increasing concentration of metoclopramide or domperidone was added every 10 min. Metoclopramide 0·28, 2·8 and 28 μM increased the contractions evoked by EFS, whereas lower (0·0028 and 0·028 μM) and higher (282 μM) concentrations had little or no effect (Fig. 1). In contrast, the contractions were not affected by domperidone 0·0028–28 μM and were reduced by domperidone 282 μM (Fig. 1). Usually neither drug affected resting muscle tone, but domperidone, 282 μM, increased the muscle tone in one of the 6 preparations and domperidone 28 and 282 μM reduced muscle tone in 2 preparations. In 3 of the 9 preparations metoclopramide 28 and 282 μM increased muscle tone or the amplitude of spontaneous contractions.

In the presence of atropine 1·4 μM (to block contractions to EFS) and $BaCl_2$ 0·48 μM (to raise muscle tone), the relaxations evoked by EFS were unaffected by 10 min incubation of the tissue with metoclopramide 28 μM; relaxations were 100 (96–100)% of the relaxations obtained before adding metoclopramide to the bathing solution (n = 6; $P > 0·05$).

In preparations not stimulated electrically, no atropine or $BaCl_2$ was added. Consistent submaximal contractions were obtained to ACh or carbachol, and then increasing concentrations of metoclopramide were added every 10 min. Metoclopramide 0·0028–2·8 μM had no effect on the contractions to ACh whereas metoclopramide 28 and 282 μM increased the contractions (Fig. 2). In contrast, metoclopramide 0·0028–28 μM had little or no effect on the contractions evoked by carbachol, and metoclopramide 282 μM caused a reduction (Fig. 2). In 4 other experiments, contractions to both ACh or carbachol were prevented by atropine 1·4 μM.

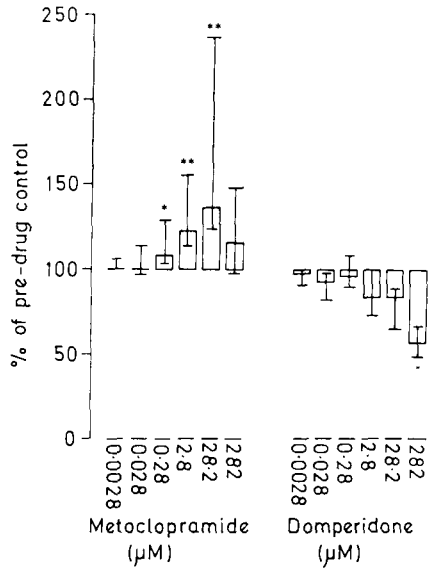

FIG. 1. Effects of metoclopramide and domperidone on the contractions evoked by electrical field stimulation in human stomach longitudinal muscle. Results are expressed as a percentage of the control contractions obtained before adding metoclopramide or domperidone to the bathing solution; the columns represent medians and the vertical bars semiquartile ranges. $*P < 0·05$, $**P < 0·01$, compared with controls; n = 9 for metoclopramide, n = 6 for domperidone. Solvents for metoclopramide or domperidone did not affect the contractions evoked by electrical stimulation.

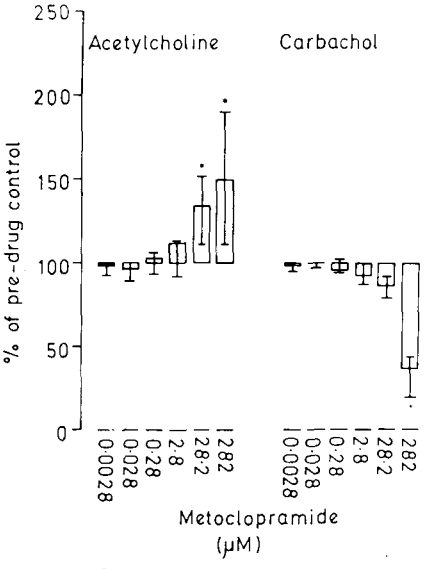

FIG. 2. Effects of metoclopramide on submaximal contractions to ACh or carbachol in human stomach longitudinal muscle. Results are expressed as a percentage of the control contractions obtained before adding metoclopramide to the bathing solution; the columns represent medians and the vertical bars semiquartile ranges. $*P < 0·05$, compared with controls; n = 8 for ACh, n = 6 for carbachol.

Discussion

High concentrations of metoclopramide occasionally stimulated the resting tone in muscle strips cut from human stomach or the lower oesophageal sphincter (Burleigh 1979). However it seems unlikely that this inconsistent response to abnormal concentrations of metoclopramide is relevant to the therapeutic mechanism of action. In contrast, low concentrations of metoclopramide (0·28–28 μM) consistently increased the contractions evoked by electrical field stimulation. Since metoclopramide did not reduce non-adrenergic, non-cholinergic inhibitory nerve activity, metoclopramide may augment the EFS-induced contractions of human isolated stomach by increasing the cholinergic activity.

The question of whether metoclopramide stimulates cholinergic activity by acting prejunctionally (to increase neuronal ACh release) or postjunctionally (to increase the response to ACh) was examined by testing the effects of metoclopramide on contractions evoked by exogenous ACh. Whereas EFS-induced contractions were increased by metoclopramide 0·28–28 μM, only high concentrations of metoclopramide (28 and 282 μM) increased the contractions to ACh. Similar results were obtained by Eisner (1968), using ACh and strips of human isolated stomach (the muscle layer was not defined). However, in the present experiments, metoclopramide did not increase the contractions evoked by carbachol. Since carbachol is resistant to hydrolysis by acetylcholinesterase, these results may be explained if high concentrations of metoclopramide increase ACh-induced contractions by inhibiting the breakdown of ACh by acetylcholinesterase. Such an action of metoclopramide is supported by experiments with extracts of acetylcholinesterase (Huizing & Beckett 1980), but other possibilities cannot be excluded, such as a difference in the receptors activated by ACh or carbachol.

The results therefore suggest that low concentrations of metoclopramide (0·28–2·8 μM) may increase cholinergic activity in human isolated stomach mostly by increasing neuronal ACh release. Higher concentrations of metoclopramide (28–282 μM) may increase cholinergic activity by an additional mechanism involving inhibition of acetylcholinesterase. However, these high concentrations are unlikely to be of therapeutic importance, since they are greater than those found in human blood plasma after a normal therapeutic dose (Bateman 1982), or even after injection of the high doses of metoclopramide required to treat cisplatin-evoked emesis (Taylor & Bateman 1983). In addition, side-effects of metoclopramide which may be due to inhibition of acetylcholinesterase are not detected in man (Harrington et al 1983).

The conclusions drawn from the experiments with human isolated stomach are therefore consistent with similar experiments using guinea-pig and rat isolated stomach or small intestine (see Introduction for references). The same low concentrations of metoclopramide (0·28–28 μM) have also been shown to increase the cholinergically-mediated contractions which follow electrical stimulation of human isolated lower oesophageal sphincter (Marshall et al 1982). Increased cholinergic activity, due mostly to increased release of ACh, may therefore make a major contribution to the way in which metoclopramide stimulates gastrointestinal motility in man.

It is not clear how metoclopramide may increase neuronal ACh release in the gut. In guinea-pig isolated ileum or stomach, the action of metoclopramide seems unrelated to its ability to antagonize responses mediated by dopamine (Spedding 1981; Kilbinger et al 1982; Zar et al 1982; Costall et al 1983), but could be related to an effect of metoclopramide on receptors for 5-hydroxytryptamine responsible for modulating ACh release (Kilbinger et al 1982).

In contrast to metoclopramide, domperidone did not increase cholinergically mediated contractions in either human or rat (McClelland & Sanger 1982) isolated stomach muscle. Similarly, domperidone does not affect other nerve-mediated responses evoked by electrical stimulation in human isolated lower oesophageal spincter (Marshall et al 1982) or colonic circular muscle (Rennie et al 1980). In those patients for whom domperidone is found to be an effective stimulant of gastric motility, other mechanisms of action therefore appear to be important, such as antagonism of α_1-adrenoceptors or dopamine (Brogden et al 1982).

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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.