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Modification of electrical field stimulation-induced contractions in the guinea-pig ileum by metoclopramide and ICS 205–930 depends on the integrity of the mucosa

B. COSTALL, S. J. GUNNING, R. J. NAYLOR*, F. D. TATTERSALL

Postgraduate School of Studies in Pharmacology, University of Bradford, Bradford BD7 1DP, UK

Contractions induced by electrical field stimulation of guinea-pig ileum longitudinal muscle strips were enhanced by metoclopramide and ICS 205-930 at concentrations similar to those required to antagonize at 5-hydroxytryptamine 'M' receptors. The enhancement of contraction was observed in intact ileum strips but was not recorded in the longitudinal muscle myenteric plexus preparation or from the ileum with the mucosal layer removed. It is concluded that an intact mucosal layer is required for metoclopramide and ICS 205–930 to enhance electrical field stimulation-induced contractions of the guinea-pig ileum.

The actions of metoclopramide and ICS 205-930 in enhancing cholinergically mediated contractions of the intestine (see review by Kilbinger & Weihrauch 1982; Bradbury et al 1986) may reflect the drugs' ability to antagonize 5-hydroxytryptamine (5-HT) function, which may normally regulate the release of acetylcholine (Gintzler & Musacchio 1974; Gershon et al 1983; Kilbinger & Pfeuffer-Friederich 1985). The intestinal site of action of metoclopramide and ICS 205-930 as 5-HT receptor antagonists is uncertain. The presence of 5-HT-like immunoreactivity, 5-HT binding sites and the synthesis of 5-HT within the intrinsic enteric neurons, would allow an immediacy of 5-HT action and interaction with the antagonists on the cholinergic myenteric neurons (see Branchek et al 1984). However, 5-HT and 5-HT binding sites are also found in high concentration in the mucosa-submucosa (see Branchek et al 1984; Bradbury et al unpublished data) and the mucosal application of 5-HT to modify reflex activity (Bülbring & Lin 1958) may indicate an alternative or additional site of 5-HT receptor antagonism to modify intestinal contractions. In the present study we have investigated the ability of metoclopramide, ICS 205-930 and reference 5-HT antagonists to modify field stimulation contractions in three preparations of the guinea-pig ileum.

METHODS

Preparation of the ileum longitudinal muscle strips

Male Dunkin-Hartley guinea-pigs (600–800 g) were killed by cervical dislocation and the gastrointestinal tract removed. Ileum segments 2 cm long were obtained 30 cm from the pyloric sphincter and were prepared as three different types of longitudinal muscle strips. The first preparation, the intact ileum, was secured, as were the other tissues, to allow movement of the bathing fluid to the mucosal and serosal surfaces. In the second preparation, the ileum was incised in the longitudinal plane and the mucosal layer eased away. In the final preparation a glass rod (6·0 mm diameter) was inserted into the lumen of an ileum segment and the longitudinal muscle layer incised. The tissue was stroked tangentially away from the circular muscle with a wad of moistened cotton wool to remove the longitudinal muscle layer and adherent myenteric plexus, i.e. the LMMP preparation.

Preparations were mounted in 30 ml organ baths containing 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 bubbled with 5% carbon dioxide in oxygen. The strips were placed under a resting tension of 1 g and were allowed to equilibrate for 45 min before being subjected to electrical stimulation (stainless steel wire electrodes placed parallel to the longitudinal axis of the tissue and 6 mm apart) using 0·1 ms pulse width, 0·1 to 10 Hz and a voltage adjusted to give maximal contractions. 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 (0·1–10 Hz) was initially constructed in the absence of drug and then in the presence of potential interacting drug (30 min pretreatment); the second curve was related to the

* Correspondence.

first to assess the degree of change. Different tissues were used to determine the effects of various concentrations of drugs and the significance of differences between treatments was assessed by using the Mann-Whitney U-test

Atropine sulphate (Sigma), ICS 205–930 ([3α-tropanyl]-1*H*-indole-3-carboxylic acid ester) (Sandoz), methysergide hydrogen maleinate (Sandoz), metoclopramide monohydrochloride (Beecham) and tetrodotoxin were dissolved in distilled water; ketanserin (Janssen), haloperidol (Janssen) and methiothepin maleate (Glaxo) were dissolved in the minimum amount of glacial acetic acid and sulpiride (SESIF) in the minimum amount of hydrochloric acid neutralized with sodium bicarbonate.

RESULTS

Electrical field stimulation (FS) of the three ileum preparations caused frequency-related contractions in most tissues which were maximal at 5 Hz and which could be repeated over a 3 h period. The contractions in all three preparations consisted of a rapid twitch response which was followed by an almost immediate decline, stabilizing at frequencies higher than 1·0 Hz, at approximately 50% of the original contraction height. The spectrum of contraction was similar in all three preparations and an example trace showing data obtained from the intact ileum is shown in Fig. 1. The tension changes in the three preparations were of a similar magnitude, for example at 1 Hz, 2·8 ± 0·2, 3·2 ± 0·15 and 2·3 ± 0·2 g for the intact ileum, the ileum with the mucosal layer removed and the LMMP preparation. All electrical FS-induced contractions were abolished by tetrodotoxin (10^{-7} M) and atropine (5×10^{-8} M).

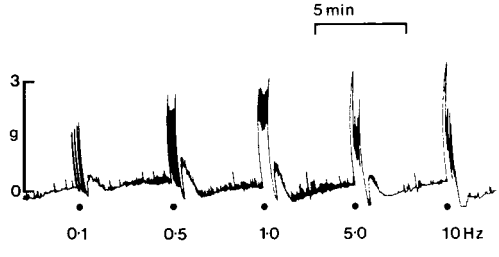

FIG. 1. Example of trace of the contractions obtained to electrical field stimulation of the 'intact' ileum longitudinal muscle.

The effect of all drug treatments was assessed in all tissues throughout the frequency range (0·1–10 Hz) and the ability of a compound to enhance or reduce FS-induced contractions was observed at each frequency of stimulation. Thus in the intact ileum preparation, metoclopramide (10^{-8}–10^{-5} M) and ICS 205–930 (10^{-9}–10^{-5} M) enhanced electrical FS-induced contractions at all frequencies, to a maximum of approximately 200–300%. Since the spectrum of action was the same at each frequency of stimulation, representative data obtained at 0·1 Hz is shown in Fig. 2. The higher concentration of ICS 205-930 (10^{-5} M) was significantly less effective than the lower concentrations to enhance the contractions ($P < 0·01$). In the ileum with the mucosal layer removed the LMMP preparations, both metoclop-

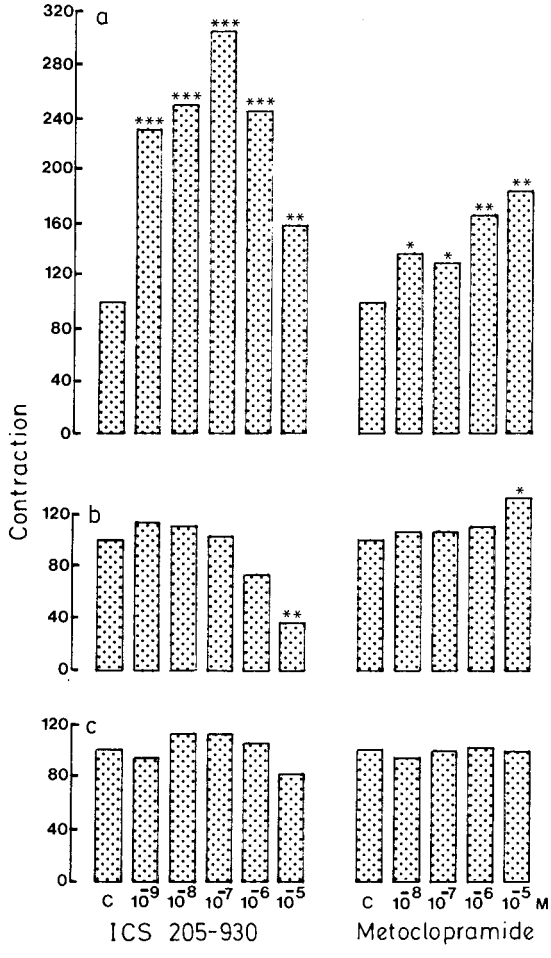

FIG. 2. Modification by metoclopramide and ICS 205-930 (molar concentrations indicated) of the contractions of the (a) intact ileum, (b) longitudinal muscle myenteric plexus (LMMP) and (c) mucosal stripped ileum preparations to electrical FS (0·1 Hz). Control responses (C) were designated 100% and other values related to these. n = 6; s.e.m.s on original data <11%. Enhancement or reduction of the contractions significant to $^{*}P < 0·05$, $^{**}P < 0·01$, $^{***}P < 0·001$ (Mann-Whitney U-test).

ramide (10^{-8}–10^{-5} M) and ICS 205-930 (10^{-9}–10^{-5} M) failed to enhance electrical FS-induced contractions (0·1–10 Hz), indeed ICS 205-930 (10^{-7}–10^{-5} M) reduced the contractions to approximately 30% (Fig. 2).

Methiothepin, methysergide and ketanserin (10^{-8}–10^{-5} M) failed to enhance electrical FS-induced contractions (0·1–10 Hz) consistently in any of the three tissue preparations. The highest concentration of methiothepin (10^{-5} M) reduced the contractions in all three preparations and ketanserin (10^{-5} M) also reduced the contraction in the LMMP preparation, representative data again obtained at 0·1 Hz being shown on Fig. 3. Sulpiride and haloperidol (10^{-7}–10^{-5} M) failed to enhance the electrical FS-induced contraction responses in the intact ileum preparation.

DISCUSSION

The study has shown that metoclopramide, a 5-HT and dopamine receptor antagonist (see Costall et al 1984; Fozard 1984), and ICS 205-930, a highly selective 5-HT 'M' receptor antagonist (Richardson et al 1985) can enhance cholinergically mediated contractions in the intact guinea-pig ileum preparation. A comparable action has been shown using guinea-pig stomach strips (Buchheit et al 1985), and metoclopramide and ICS 205-930 are effective at the same concentrations in both tissues where their potencies correlate with their abilities to antagonize at the 5-HT receptors located on the peripheral nerves (see Buchheit et al 1985). Therefore, it is concluded that in the stomach and intestine metoclopramide and ICS 205-930 enhance electrical FS-induced contractions by antagonism at the 5-HT 'M' receptor. It is unlikely that a dopamine receptor antagonism contributed to the effect of metoclopramide since sulpiride and haloperidol were ineffective. The selectivity of action of metoclopramide and ICS 205-930 for the 5-HT 'M' receptor in the ileum is emphasized by the ineffectiveness of ketanserin, methiothepin and methysergide in enhancing electrical FS-induced contractions.

5-HT has been shown to reduce electrically evoked contractions of the guinea-pig ileum (Gintzler & Musacchio 1974; Kilbinger & Pfeuffer-

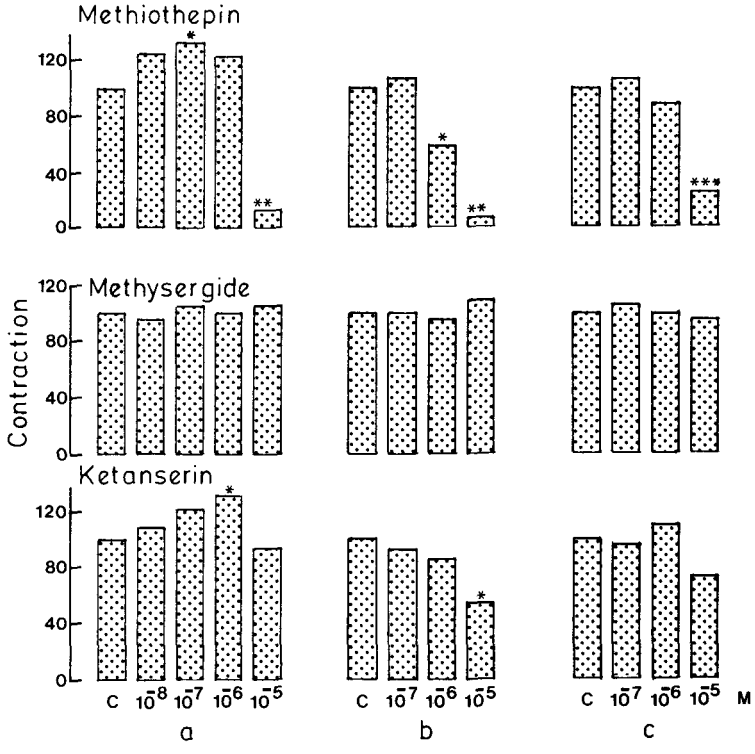

FIG. 3. Modification by methiothepin, methysergide and ketanserin (molar concentrations indicated) of the contractions of (a) intact ileum, (b) longitudinal muscle myenteric plexus (LMMP) and (c) mucosal stripped ileum preparations to electrical FS (0·1 Hz). Control responses (C) were designated 100% and other values related to these. n=6; s.e.m.s on original data < 9%. Reduction or enhancement of the contractions significant to $^{*}P < 0{\cdot}01$, $^{**}P < 0{\cdot}001$ (Mann-Whitney U-test).

Friederich 1982; Sanger 1985) which may reflect changes in the release of acetylcholine from postganglionic neurons. An antagonism of this action by metoclopramide and ICS 205-930 would explain their ability to enhance electrical FS-induced contractions. However, the site of interaction between 5-HT and the antagonistic drugs remains uncertain, since 5-HT may have a neurotransmitter/neuromodulatory role within the myenteric plexus or mucosa-submucosa or submucosal plexus (see introduction). Therefore it was an important finding of the present study that in ileum preparations with the mucosal layer removed, metoclopramide and ICS 205-930 failed to enhance electrical FS-induced contractions. This indicates that metoclopramide and ICS 205-930 require the influence of a neurotransmitter or endogenous factor from the mucosa-submucosa to enhance electrical FS-induced contractions. There may be an analogy to the findings in vascular muscle that endothelial derived factors may modify muscle tone (see review by Furchgott 1984). However, within the ileum, and assuming that the absence of 5-HT is a critical determinant to annul the effects of metoclopramide and ICS 205-930, the most immediate site of action for 5-HT release from within the mucosa would be the neurons in the submucosal plexus, which have been shown to be depolarized by 5-HT (Hirst & Silinsky 1975). An action of the 5-HT antagonists to prevent this effect would modify the neuronal activity of the intrinsic afferent neurons which may subsequently influence the enteric neurons in the myenteric plexus to modify the contraction response.

However, it is possible that 5-HT released within the mucosa-submucosa may diffuse to influence neurons within the myenteric plexus directly. Certainly, 5-HT has been shown in electrophysiological studies to modify the activity of such neurons, although in different ways (see North 1982). It may be relevant to such observations that Sanger (1985) has shown that 5-HT can enhance or reduce FS-induced contractions in the intact ileum preparation. In these experiments it is interesting that the intact ileum was prepared with the lumen closed, to reduce the access of 5-HT to the mucosal surface. Sanger (1985) emphasized that different responses to 5-HT might be obtained dependent on access to the mucosal or serosal surface.

The present studies clearly indicate that an elucidation of the mechanism of action of 5-HT antagonists on the ileum must consider not merely the stimulation parameters employed (Sanger 1985; Bradbury et al 1986) but the nature of the preparation used. A mucosal factor, possibly 5-HT, is essential for the actions of metoclopramide and ICS 205-930 to enhance electrical FS-induced contractions. The importance of the nature of the preparation was also emphasized by the results obtained using the higher concentrations of ICS 205-930 which, although less effective than lower concentrations in enhancing electrical FS-induced contractions in the intact ileum, actually reduced the contractions in the LMMP preparation. The significance of these observations remains to be determined.

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