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Effect of metoclopramide in guinea-pig ileum longitudinal muscle: evidence against dopamine-mediation

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SUMMARY The investigation examines the hypothesis that metoclopramide-induced potentiation of gastrointestinal motility is mediated through dopamine receptors. *In vitro* studies on the longitudinal muscle of the guinea-pig ileum were performed. Metoclopramide, in concentrations comparable with those seen in plasma after therapeutic doses in man, selectively potentiated the cholinergic response. Dopamine (1–100 μM) inhibited cholinergic transmission by inhibiting neuronal acetylcholine release. The inhibitory action of dopamine was antagonised by phentolamine, an α-adrenoceptor antagonist, but not by the dopamine receptor antagonists metoclopramide or pimozide. Bromocriptine inhibited cholinergic responses by a postsynaptic mechanism which was not antagonised by metoclopramide, pimozide, or phentolamine. The results are consistent with the view that metoclopramide-induced potentiation of gastrointestinal motility does not involve local dopamine receptors.

Metoclopramide (4-amino-5-chloro-2-methoxy-N-2-diethylaminoethylbenzamide) is used clinically for preventing emesis and increasing gastrointestinal motility[1]. Its most prominent effect on the gastrointestinal tract of various mammalian species is enhanced motor activity, an action generally attributed to potentiation of intrinsic cholinergic mechanisms of the gastrointestinal tract by metoclopramide. Some have suggested that it acts by promoting the release of acetylcholine from cholinergic nerve terminals,[2] while others[3 4] have proposed that metoclopramide sensitises muscarinic receptors.

Metoclopramide can block dopamine, and its antiemetic effect probably involves antagonism of dopamine centrally.[5 6] The presence of dopamine in gastrointestinal nerve[7], raises the possibility tested here that metoclopramide acts peripherally by antagonising dopamine.

Methods

Experiments were carried out on longitudinal muscle strips from guinea-pig ileum. Male guinea-pigs, 300–500g, were killed by a blow on the back of the head, bled out, and the terminal 30–40 cm ileum after discarding the last 20 cm was removed. Longitudinal muscle strips 4–5 cm long, were prepared from the terminal ileum.[8] Each strip was suspended under a load of 0·25 g in a 5 ml organ bath with built-in platinum electrodes. The bath contained Krebs-Henseleit solution: (mM) (NaCl, 113; KCl, 4·7; $CaCl_2$, 2·5; $MgSO_4$, 1·2; KH_2PO_4, 1·2; $NaHCO_3$, 25; glucose, 11·5), maintained at 37°C and bubbled with 95% O_2 and 5% CO_2. Electrical stimulation was delivered from an electronic stimulator[9], through the built-in platinum electrodes with a pulse duration of 0·2 ms, frequency 0·1 Hz and supramaximally effective voltage. Longitudinal muscle tone was monitored continuously using an isometric transducer and a pen-recorder. All experiments were performed a minimum of three times.

DRUGS

The following drugs were dissolved in distilled water: acetylcholine chloride, atropine sulphate, dopamine hydrochloride, hexamethonium bromide, histamine chloride (Sigma); metoclopramide hydrochloride (Beecham Pharmaceuticals); phentolamine mesylate (Ciba); tetrodotoxin (Sigma).

Bromocriptine methansulphonate (Sandoz) was dissolved in 30% ethyl alcohol to make 1·3 mM stock solution; further dilutions were made in Krebs solution. Pimozide (Janssen Pharmaceuticals) was dissolved in citric acid solution to make 5·5 mM stock solution and further diluted with distilled water.

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Results

EFFECT OF METOCLOPRAMIDE ON CHOLINERGIC TRANSMISSION AND DRUG-INDUCED CONTRACTIONS OF LONGITUDINAL MUSCLE

The innervated longitudinal muscle preparation responded to single-pulse electrical stimulation with a brief contraction. It also contracted after administration of histamine or acetylcholine. Electrically evoked contractions were neurogenic because they were readily prevented by tetrodotoxin 0·62 μM. Electrically evoked contractions were unaffected by hexamethonium 280 μM but antagonised by atropine 0·1 μM, indicating that they originated in postganglionic cholinergic nerves.

Metoclopramide 0·3–300 μM potentiated these cholinergically mediated electrically evoked contractions. The threshold concentration for potentiating effect of metoclopramide was around 0·3 μM (Fig. 1A), and a maximum potentiation was usually seen at 30–150 μM (Fig. 1C, E). Metoclopramide increased the spontaneous activity and 30–300 μM also caused a modest increase in tone (Fig. 1C, E). Metoclopramide 0·3–300 μM potentiated electrically evoked contractions in a concentration-related fashion, but its effect on acetylcholine-evoked contractions was not uniform. Concentrations up to 30 μM tended to increase the response to acetylcholine (Fig. 1C) but higher concentrations caused depression (Fig. 1E).

Histamine-evoked contractions were not significantly altered by metoclopramide up to 30 μM (Fig. 2) but were reduced with higher concentrations.

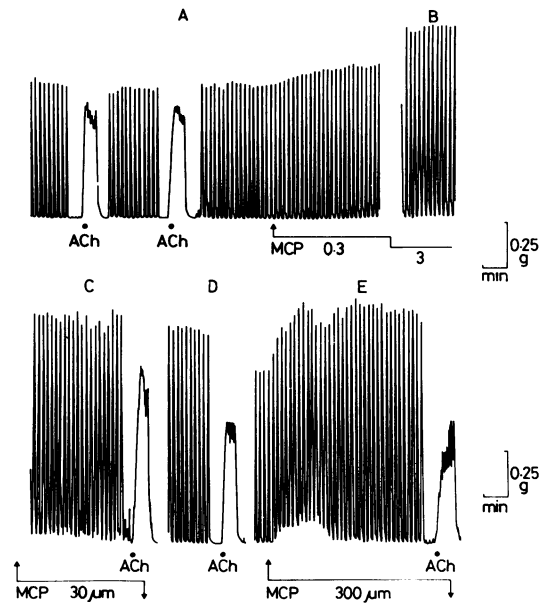

Fig. 1 *Effect of metoclopramide (MCP) 0·3–300 μM on the contractions of the longitudinal muscle preparation of the guinea-pig ileum to electrical field stimulation or acetylcholine (ACh) 0·1 μM. Note that MCP up to 30 μM potentiated electrically-evoked twitches and ACh-induced contractions almost equally.*

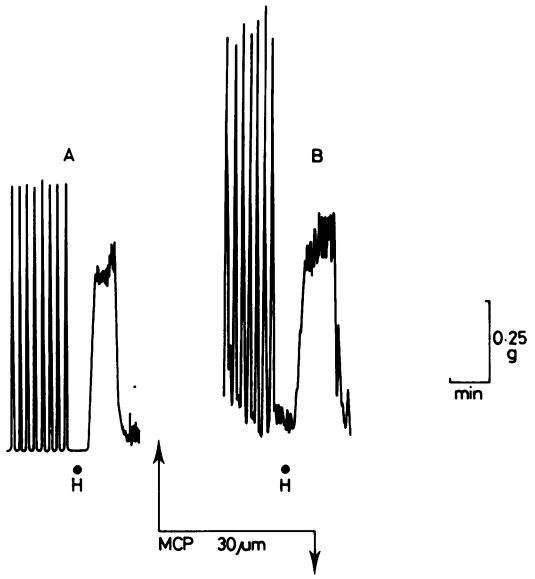

Fig. 2 *Metoclopramide (MCP) potentiated electrically-evoked cholinergic contractions and had little effect on contractions induced by histamine 0·15 μM (H). A: control responses to electrical stimulation and histamine. B: responses to electrical stimulation and histamine in the presence of MCP 30 μM.*

EFFECT OF METOCLOPRAMIDE ON NON-CHOLINERGIC TRANSMISSION

The longitudinal muscle of guinea-pig ileum receives non-cholinergic motor nerves which are not blocked by atropine.[10 11] Contractions elicited in atropinised (0·5 μM) longitude muscle preparations (trains of 10 pulses of 0·2 ms at 20 Hz once every 60 seconds) were blocked by tetrodotoxin, confirming their neurogenic nature. This non-cholinergic transmission was unaffected by metoclopramide at 0·3 μM but was depressed in a dose-related fashion at 3–300 μM (Fig. 3).

EFFECT OF DOPAMINE ON CHOLINERGIC TRANSMISSION

Dopamine, 1–100 μM caused an inhibition (Table) of the electrically evoked cholinergic twitch (maximum % inhibition 46·1 ± 7·8 SEM). The threshold con-

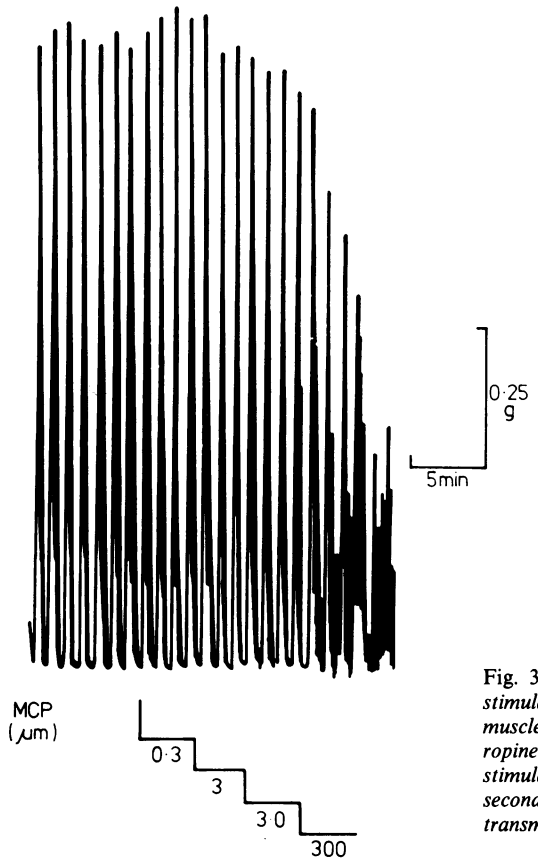

Table *Inhibitory effect of dopamine on response to cholinergic nerve stimulation in longitudinal muscle preparation of guinea-pig ileum (10 experiments)*

Dopamine concentration (μM)	*% inhibition of electrically evoked twitch (mean ± SEM)*
1	2·7±0·7
10	18·5±3·8
50	46·1±7·8
100	42·9±6·9

centration of dopamine for inducing inhibition was around 1 μM and maximum inhibition was usually achieved by 50 μM. The presynaptic nature of dopamine-induced inhibition of the electrically evoked twitch was indicated by the finding that acetylcholine-evoked contraction remained unaffected by dopamine (Fig. 4).

Neither metoclopramide 3–300 μM nor another dopamine-blocker, pimozide, 1 μM antagonised the dopamine inhibition of electrically evoked twitches.

Inhibitory α-adrenoceptors are located on the cholinergic nerve supply to the longitudinal muscle of the guinea-pig ileum.[12] The possibility that do-

Fig. 3 *Effect of metoclopramide (MCP) on responses to stimulation of non-cholinergic motor nerves in a longitudinal muscle preparation of the guinea-pig ileum treated with atropine 0·5 μM. Contractions were evoked by electrical field stimulation (trains of 10 pulses at 20 Hz once every 60 seconds). Note the lack of potentiation of non-cholinergic transmission by MCP.*

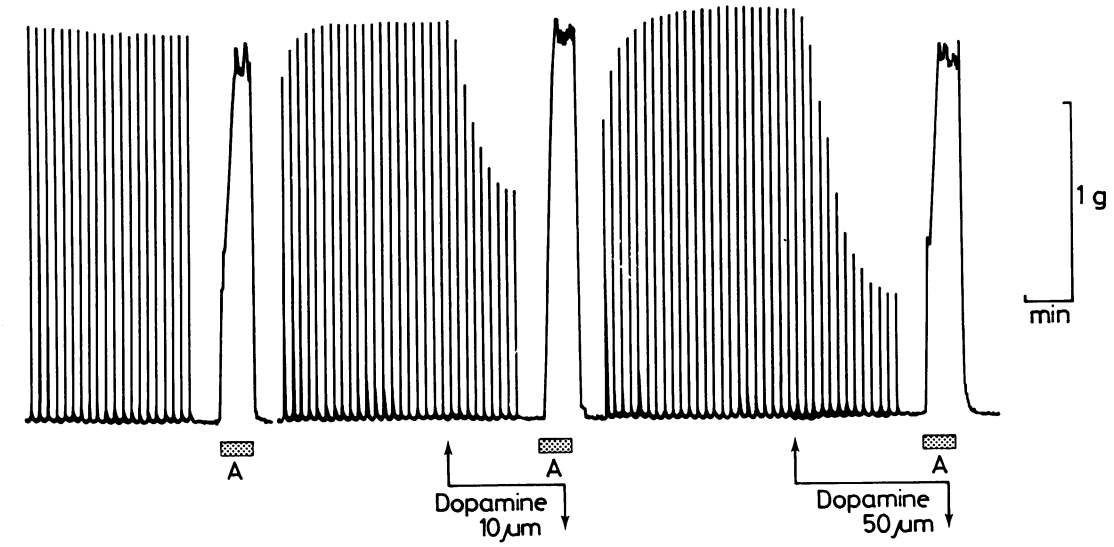

Fig. 4 *Dopamine inhibited the contractions of the longitudinal muscle preparation evoked by cholinergic nerve stimulation but not by acetylcholine 0·1 μM (A).*

pamine inhibits cholinergic transmission by activating these receptors was tested using phentolamine to block the α-adrenoceptors. Phentolamine 5 μM fully antagonised dopamine but did not prevent the augmentation of electrically evoked twitches by metoclopramide. These results are summarised in Fig. 5.

Bromocriptine 0·15–15 μM reduced the electrically evoked cholinergic contractions in parallel with a similar reduction of acetylcholine-evoked contractions, suggesting the postsynaptic site of its action (Fig. 6). The inhibition by bromocriptine was not antagonised by the dopamine receptor antagonists metoclopramide and pimozide, nor, unlike dopamine-induced inhibition, by the α-adrenoceptor antagonist phentolamine (Fig. 7).

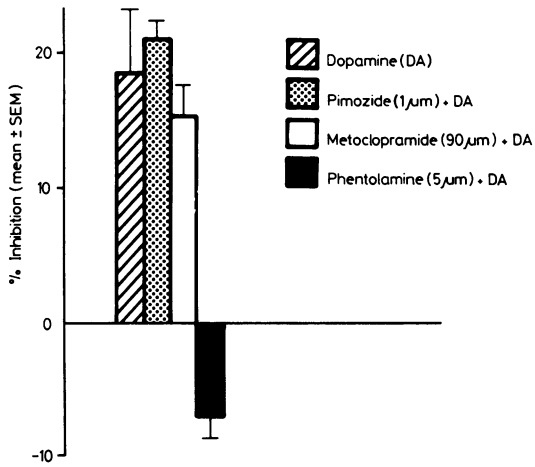

Fig. 5 *The inhibitory effect of dopamine (DA) 10 μM on the electrically-evoked contraction was antagonised by phentolamine but not by metoclopramide or by pimozide. Columns: mean results. Vertical lines: SEM (n=8–10).*

Discussion

Previous studies by other workers in this field have not used the isolated longitudinal muscle preparation of the guinea-pig ileum. As this preparation is thin and allows the drug-receptor interaction to take place rapidly, the *in vitro* drug response may mimic that which occurs when the drug is carried in the blood stream. The removal of the ileal circular muscle, while unphysiological, enables an accurate assessment of the response of the longitudinal muscle without interference from the circular layer. Metoclopramide produced enhancement of spontaneous activity, augmentation of electrically evoked cholinergic contraction, and increased tone of the longitudinal muscle. These potentiating actions were selective for cholinergic response, as they were lost

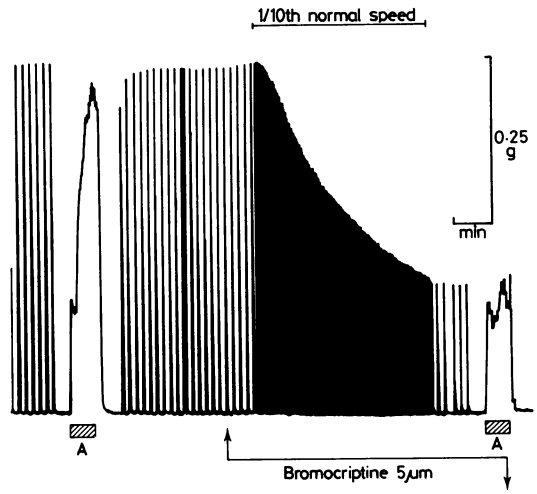

Fig. 6 *Effect of bromocriptine on electrically-evoked and 0·05 μM acetylcholine-induced (A) contractions of the longitudinal muscle of ileum. Note that the inhibitory effect of bromocriptine developed slowly and affected almost equally the electrically-evoked and acetylcholine-induced contractions.*

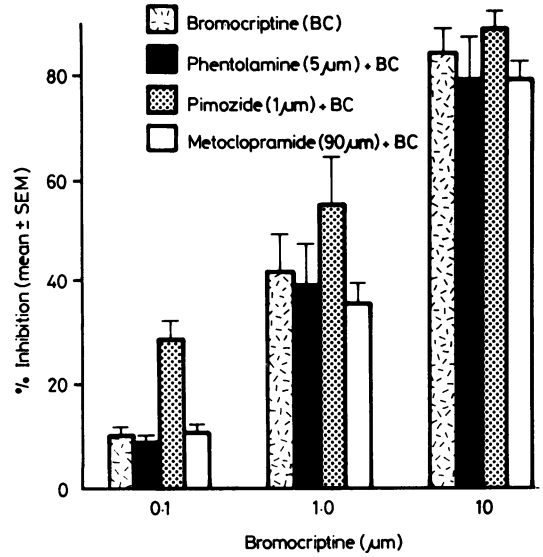

Fig. 7 *Phentolamine, pimozide, or metoclopramide given 30 minutes previously did not antagonise the inhibitory action of bromocriptine 0·1, 1·0, or 10 μM on electrically evoked cholinergic twitches. Each column represents mean percentage inhibition of four experiments. Vertical lines: SEM.*

after treatment with the muscarinic blocking agent atropine, and did not occur with contractions to histamine or stimulation of non-cholinergic nerves. Indeed, metoclopramide > 3 μM depressed the response to non-cholinergic nerve stimulation. Thus

the innervated longitudinal muscle preparation of the guinea-pig ileum displays the basic characteristics of metoclopramide action seen elsewhere on the gastrointestinal tract.[2-4 13]

Metoclopramide is a dopamine receptor antagonist[14 15] and its anti-emetic action is believed to be mediated by central dopamine receptor mechanisms.[5 6] However, although dopamine is present in gastrointestinal tissues,[16-18] it does not appear that metoclopramide stimulates gastrointestinal motility by antagonising gut dopamine receptors. The presynaptic effect of dopamine, shown by inhibition of electrically evoked cholinergic twitch but not of acetylcholine-induced contraction, was antagonised by the α-adrenoceptor blocker phentolamine, whereas metoclopramide had little effect. Presynaptic inhibitory α-adrenoceptors are known to be present on the cholinergic nerve terminals in this tissue[12] and dopamine stimulates α-adrenoceptors.[19-21] The results with pimozide and bromocriptine further confirm that, in guinea-pig ileum, dopamine acts on α-adrenoceptors rather than on dopamine receptors, suggesting a lack of dopamine receptors in this tissue. Pimozide, a potent dopamine-receptor antagonist, did not counteract the inhibitory effect of dopamine on cholinergic transmission. Bromocriptine, a dopamine-receptor agonist, inhibited both the response to cholinergic nerve stimulation and to acetylcholine at a post-synaptic site not affected either by the α-adrenoceptor blocker phentolamine or by the dopamine-receptor blockers pimozide and metoclopramide. Thus, in this experimental model, metoclopramide, in concentrations within the range seen in plasma after therapeutic doses in man[22], does not seem to act by blocking dopamine receptors but selectively facilitates cholinergic mechanisms by an action which still remains to be elucidated.

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