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Evidence against an acetylcholine releasing action of cisapride in the human colon

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The effect of cisapride (R51619) on intrinsic cholinergic nerve activity of human sigmoid taenia coli muscle strips (taeniae) was assessed using radiolabelling techniques. Taeniae previously incubated with [^{3}H]-choline were exposed to cisapride (0.41 and 4.1 μM), placebo solution and electrical field stimulation (EFS; 10 Hz, 1 ms, 200 mA for 480 pulses). Cisapride did not affect unstimulated (basal) release of radioactive material nor the release evoked by EFS. It was concluded that cisapride did not demonstrate a cholinomimetic effect in human sigmoid taenia coli muscle strips.

Keywords human colon cisapride cholinergic nerves

Introduction

Cisapride (R51619) causes increased and coordinated antroduodenal motility in the dog (Schuurkes & Van Nueten, 1984). The compound does not antagonise dopamine receptors, but it may enhance the release of acetylcholine (Van Nueten *et al.*, 1983). Studies in volunteers (Lee *et al.*, 1984) and patients with idiopathic constipation indicate a motor stimulating property of cisapride in the human large intestine (Reboa *et al.*, 1984).

The enhanced release of acetylcholine brought about by cisapride from the guinea-pig ileum either results from a direct releasing effect or from increasing the excitability of intramural nerves to nerve impulses (Schuurkes, personal communication). To determine whether cisapride has such an action on the intrinsic cholinergic nerves of the human colon we have employed the radiolabelling technique used by Wikberg (1977) which shows sensitivity and reliability in the detection and measurement of acetylcholine release.

Methods

Macroscopically normal strips of human sigmoid taenia coli (taeniae) consisting of muscularis externa without attached mucosa and submucosa, were obtained from specimens of colon resected for carcinoma. Taeniae were incubated for 60 min in 2 ml of Krebs fluid containing 4 μCi ml^{-1} of [^{3}H]-choline (15 Ci mmol^{-1}, Amersham). The taeniae were then superfused, at 2.0–2.2 ml min^{-1}, with Krebs fluid containing hemicholinium (34.8 μM). Incubation and superfusion of individual taenia preparations were carried out in the same small volume bath which was fitted with vertical platinum wire electrodes (0.5 mm diameter) to allow electrical field stimulation of the tissue from a constant current stimulator. After 90 min taeniae were exposed to a low then a high dose of cisapride (0.4 and 4.1 μM). Taeniae were also challenged with electrical field stimulation (EFS 1 ms, 10 Hz, 480 pulses, submaximal current strength of 200 mA) in the presence and absence of cisapride (4.1 μM). For timing of these various challenges see Figure 1. Superfusion fluid was collected every 4 min and samples prepared for liquid scintillation counting. Efficiency of counting was determined by the automatic external standard channels ratio method.

Radioactive content of the superfusion fluid samples was expressed as disintegrations per minute per mg of tissue per sample (d min^{-1} mg^{-1} tissue).

For challenge with cisapride or placebo two sample collections were made immediately before (basal release) and four during and after the challenge (stimulated release). For EFS two

basal and three stimulated samples were collected. The evoked release of radioactive material, collected during or after challenge with EFS, cisapride or placebo, was calculated from the difference between the 'calculated' basal release and the stimulated release (i.e. stimulated release = calculated basal release + evoked release). Calculated basal release was obtained by fitting a regression line through observed basal values measured immediately before the three challenges. The sum of evoked values for a given challenge was expressed as a fraction of the radioactivity present in the tissue at the beginning of the challenge.

Statistical analysis of results was by a two-tailed unpaired *t*-test.

Results

The effect of cisapride (0.4 and 4.1 μM) on release of tritiated [^{3}H] material has been compared with placebo. Additionally, release of [^{3}H] material by EFS has been determined in the presence and absence of cisapride (4.1 μM, Figure 1). Cisapride (0.4 and 4.1 μM) did not significantly alter [^{3}H] release when compared to placebo ($P > 0.7$, $P > 0.5$ respectively, Table 1). The increased release of [^{3}H] material produced by EFS was not significantly changed by cisapride (4.1 μM, $P > 0.6$, Table 1). At the start of the first challenge the absolute radioactivity in the muscle strips was 17,728 ± 1,381 d min^{-1} mg^{-1} tissue (equivalent to 0.074 ng choline mg^{-1} tissue) for tissues challenged with placebo and EFS ($n = 6$). For tissues challenged with cisapride (0.41 and 4.1 μM) the absolute radioactivity in the muscle strips was 19,227 ± 1368 d min^{-1} mg^{-1} tissue (equivalent to 0.081 ng choline mg^{-1} tissue). Cisapride (0.4 and 4.1 μM) had no action on the tone or spontaneous rhythmic activity of the muscle strips.

Table 1 Effect of various challenges on release of tritiated [^{3}H] material from human taeniae muscle strips. EFS – electrical field stimulation at 10 Hz, 1 ms, 200 mA for 480 pulses. Each cisapride dose required its own placebo to allow for the different concentration of vehicle constituents. Release is expressed as a percent of radioactivity remaining in the tissue at the beginning of the challenge and is given as mean ± s.e. mean. Negative values for [^{3}H] release represent a decrease compared to the calculated basal value.

Challenge	*[^{3}H] release as % radioactivity remaining*	n
Cisapride (0.41 μM)	0.02 ± 0.08	9
Placebo	−0.03 ± 0.19	6
Cisapride (4.1 μM)	−0.01 ± 0.03	9
Placebo	0.03 ± 0.07	6
EFS	0.52 ± 0.07	6
EFS plus cisapride (4.1 μM)	0.60 ± 0.12	9

Discussion

The observations that cisapride stimulates human colonic motility in constipated patients is not unequivocal as the observed change although marked did not reach the accepted level of significance :$P = 0.081$; Reboa *et al.*, 1984). However the compound did enhance colonic transit in volunteers (Lee *et al.*, 1984).

To confirm or disprove a local cholinomimetic effect of cisapride in the human colon *in vitro* techniques have been employed. Conventional mechanical recordings of muscle strip motility and responses to electrical field stimulation are hampered by the unpredictable and changing spontaneous motility of the taeniae muscle strips (Bucknell & Whitney, 1966) and a complex response to electrical field stimulation (Stockley & Bennett, 1974). We therefore decided to use release of acetylcholine as an indicator of cholinergic nerve activity. The technique was devised by Wikberg (1977) who showed that the use of radioactive tracers could effectively substitute for bioassay in the accurate quantitative detection of acetylcholine release. Szerb (1976) and Wikberg (1977) have shown that the evoked release of tritiated [^{3}H] material by electrical field stimulation (after previous incubation of the tissue with [^{3}H]-choline) gives an accurate estimate of [^{3}H]-acetylcholine release. Use of submaximal current strength (Cowie *et al.*, 1978) and low frequencies of stimulation (Vizi *et al.*, 1984) have been found necessary to demonstrate a neuromodulatory role of opioid receptor stimulation. A similar approach was therefore adopted in the present study.

Electrical stimulation of cholinergic nerves in taeniae muscle strips, previously incubated with [^{3}H]-choline, resulted in a marked release of tritiated material. This release was unaffected by cisapride as too was the basal release from the tissue. The doses of drug chosen were optimum for producing an enhanced release of acetylcholine in guinea-pig ileum (Schuurkes, personal communication). That chemical, as well as electrical, stimulation is capable of releasing acetylcholine from intrinsic nerves was shown by the action of DMPP in increasing release of tritiated material from human colonic muscle strips

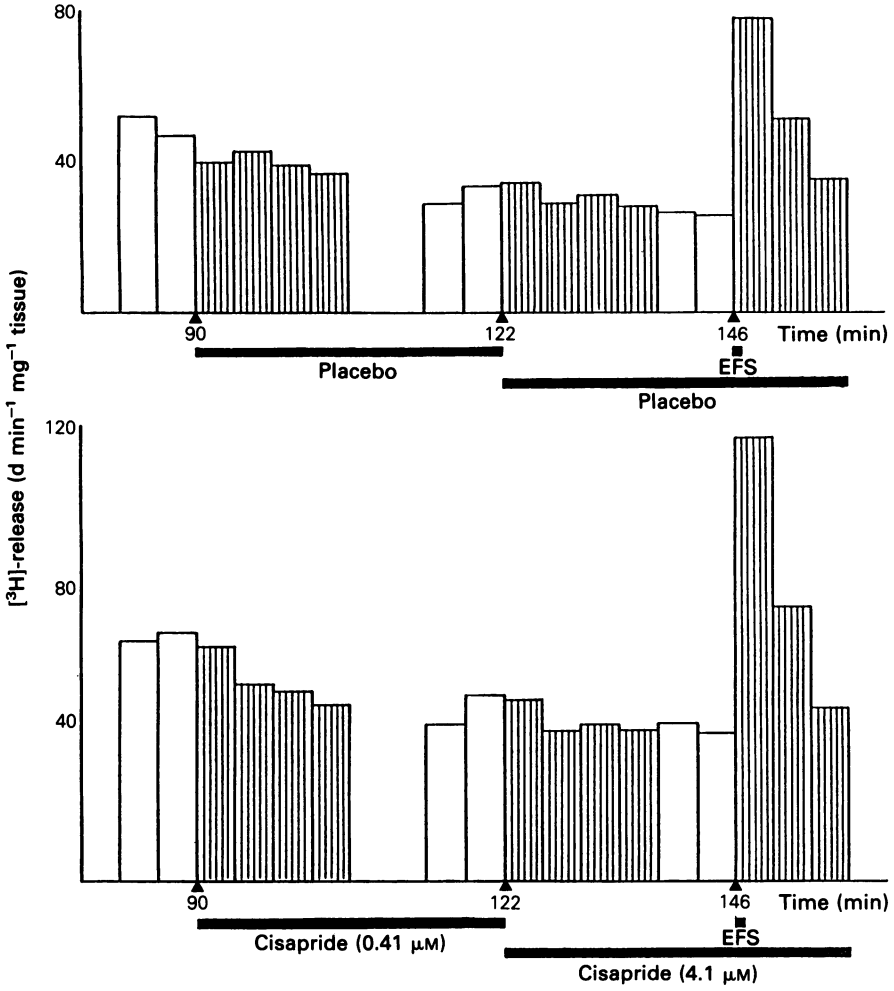

Figure 1 Typical records of changes in release of tritiated material with placebo or cisapride (0.41 and 4.1 μM) and the effect of cisapride (4.1 μM) on electrical field stimulation (EFS, 10 Hz, 1 ms, 480 pulses at 200 mA). □ Collections to estimate basal release; ▥ Collections to estimate stimulated release, i.e. basal plus evoked release.
Vertical axis: $[^3H]$ release measured as d min^{-1} mg^{-1} tissue/sample.
Horizontal axis: time after incubation with $[^3H]$-choline stopped.

previously incubated in $[^3H]$-choline (Burleigh & Trout, 1983).

In conclusion *in vitro* radiolabelling experiments using human sigmoid taenia coli muscle have failed to indicate an enhanced release of acetylcholine by cisapride. Reboa *et al.* (1984) showed cisapride stimulated colonic motor activity in constipated, but not normal individuals. If this motor effect can be shown to be significant and reproducible it is possible that constipation affects the responsiveness of cholinergic nerves to cisapride. Evidence for constipation affecting cholinergic mechanisms in the human gut has been described by Paskins *et al.* (1982). In contrast to the findings of Reboa *et al.* (1984), Lee *et al.* (1984) did observe a stimulatory effect of cisapride in non-constipated volunteers. Enhancement of acetylcholine release, which has been shown in the guinea-pig ileum, may not explain the action of cisapride on the human colon *in vivo*.

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