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Effect of secoverine and atropine on intestinal secretion and motor activity in the rat small intestine in-vivo

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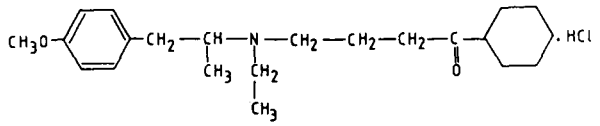
The actions of secoverine and atropine on bethanechol-induced intestinal secretion, hypermotility and transintestinal potential difference were investigated in the rat jejunum in-vivo. Both secoverine (10^{-7} mol kg^{-1}) and atropine ($1{\cdot}2 \times 10^{-9}$ mol kg^{-1}) inhibited motility at doses that did not affect secretion or transintestinal potential difference. However, secoverine was a less potent antagonist of all the bethanechol-induced changes than atropine. Increases in transintestinal potential difference were more closely related to production of fluid secretion than to increases in motility.

Secoverine, 1-cyclohexyl-4-C [ethyl (*p*-methoxy-α-methylphenylethyl) amino]-butazone hydrochloride (I), is thought to be a selective muscarinic antagonist



I. Secoverine hydrochloride.

which inhibits cholinergically-induced gastrointestinal motility (Sanger & Bennett 1981), at doses which have no effect on salivary and gastric secretions (Zwagemakers & Claassen 1980). This suggests that the drug may be useful clinically in treating conditions of intestinal hypermotility such as diverticular disease or colonic spasm, without causing dryness of the mouth or the passage of hard dry stools. However, since these studies were carried out in different experiments, often in different animals, using different routes of administration, it is difficult to be sure whether they represent a selectivity of action. We have investigated the action of secoverine on the increases in motility and secretion induced in the rat small intestine by a submaximal dose of bethanechol (10^{-4} mol kg^{-1}) and compared it with that of the anticholinergic agent, atropine. Bethanechol was chosen because, unlike acetylcholine, it acts purely on muscarinic cholinoreceptors, and has been shown to induce secretion (Tidball 1961; Hubel 1977) and increase muscular contraction (Weinbeck 1972; Snape 1981) in the small intestine.

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MATERIALS AND METHODS

Animals

Male Wistar albino rats 230–260 g, obtained from the Sheffield Field Laboratories were used and allowed free access to food (diet 86, Oxoid, London) and water. They were anaesthetized with intraperitoneal (i.p.) sodium pentobarbitone (Sagatal, May and Baker, Dagenham, U.K.), 60 mg kg^{-1}. At the end of the experiment the rats were killed.

Chemicals

Secoverine was generously supplied by Duphar BV, Weesp, The Netherlands. Bethanechol and atropine sulphate were obtained from Sigma Chemicals, Poole, Dorset, UK, and [^{14}C]PEG from the Radiochemical Centre, Amersham.

Experimental Designs

The effects of secoverine and atropine on bethanechol-induced fluid transport, hypermotility and changes in transintestinal potential difference (PD) were assessed in-vivo using loops of rat small intestine. Fluid transport and motility measurements were obtained in a separate series of experiments, though PD was measured in both.

In preliminary experiments (10^{-4} mol kg^{-1}) bethanechol and either secoverine or atropine (10^{-9} to 10^{-3} mol kg^{-1}) were administered together i.p. immediately before the test period, as shown in Design 1 (Fig. 1). However, secoverine was ineffec-

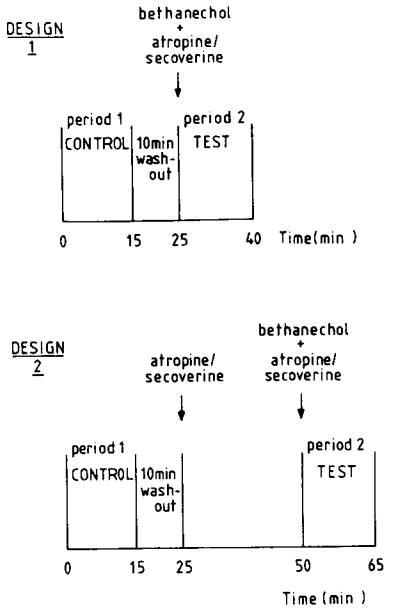

FIG. 1. Experimental designs (see text for details).

tive under these conditions and in subsequent experiments secoverine (10^{-8} to 10^{-4} mol kg^{-1}) or atropine ($1{\cdot}2 \times 10^{-10}$ to $1{\cdot}2 \times 10^{-5}$ mol kg^{-1}) were administered i.p. immediately after the 15 min control period, and again 25 min later together with bethanechol. This was followed by the test period (Design 2, Fig. 1).

In a further series of experiments, secoverine (10^{-9} to 10^{-5} mol kg^{-1}) or atropine ($1{\cdot}2 \times 10^{-14}$ to $1{\cdot}2 \times 10^{-9}$ mol kg^{-1}) were administered intravenously (i.v.) with bethanechol (5×10^{-6} mol kg^{-1}) immediately before the test period, similar to Design 1.

At least 5 rats were treated with each dose of antagonist for all experiments.

Measurement of fluid transport and transintestinal potential difference in-vivo

Measurements of fluid transport in the rat jejunum in-vitro were determined using the cannulated loop preparation, described by Hardcastle et al (1981), with polyethylene glycol as an inert fluid marker.

Fluid transport was measured over 15 min incubation periods and during each period the transintestinal potential difference (PD) was measured by two salt bridge electrodes (1M KCl in 3% agar), one in contact with the luminal fluid and the other in contact via a wick with the peritoneal cavity fluid. The electrodes were connected via calomel half cells to a high impedance electrometer (Vibron, model 33B-2), its output being displayed on a chart recorder (model 700, Telsec Instruments Ltd., Oxford, UK). Readings were taken every minute during each 15 min incubation period. The contractile activity of the intestinal segment and the degree of lacrimation or salivation of the animal were also noted throughout the experiment.

Immediately after each experiment, the intestinal segment was removed from the animal, dried and weighed. The fluid movements were then expressed as ml g^{-1} dry wt 15 min^{-1}.

Measurements of intestinal motility

Intestinal motility was recorded in a separate series of experiments on cannulated jejunal loops, arranged to allow unobstructed passage of fluid. Pre-warmed saline was perfused through the test segment at a rate of 0·15 ml min^{-1}.

As contraction of intestinal muscle leads to an increased resistance to flow, a greater head of pressure is required to maintain the original flow, so the pressure in the proximal cannula was used to give an index of the contractile activity of the intestinal segment. Intraluminal pressure was recorded by a pressure transducer (Elcomatic EM750, Ormed Engineering Ltd, Welwyn, U.K.) situated in the infusion line. The output was amplified and recorded on a chart recorder. Transintestinal PD was recorded during the same experiments using the method described above.

Changes in PD and motor activity were analysed by planimetry. The results were then expressed as a percentage of the average response induced by bethanechol alone, and given as the mean ± s.e.m. with the number of experiments (n) in brackets. Significance of the results was assessed by using Student's paired or unpaired *t*-test, on the original data.

RESULTS

Under control conditions, the intestinal segment secreted a small volume of fluid (0·12 ± 0·05 ml g^{-1} dry wt 15 min^{-1}, n = 9), and generated a PD of 3.2 ± 0·5 mV, the serosal side of the tissue being positive with respect to the mucosal side. Preliminary experiments showed that there was no significant difference ($P>0{\cdot}1$) in the volume of fluid transported and the PD generated during three consecutive control periods.

Effect of the drugs given intraperitoneally

Effect of bethanechol on fluid transport and transintestinal PD

Bethanechol (10^{-4} mol kg^{-1}) caused a significant increase in fluid secretion of 0·42 ± 0·01 ml g^{-1} dry wt 15 min^{-1} ($P<0·01$), increased the PD to 8.6 ± 1·0 mV ($P<0·01$), and induced lacrimation, salivation and defaecation. PD remained above control levels throughout the 15 min test. At 5 × 10^{-5} mol kg^{-1} bethanechol did not produce a sustained elevation in PD while 1·5 × 10^{-4} mol kg^{-1} resulted in a greater response in fluid secretion and PD but caused significant mortality.

Effect of secoverine or atropine on bethanechol-induced secretion and PD

Secoverine up to 10^{-3} mol kg^{-1} i.p. failed to inhibit bethanechol-induced fluid secretion when given together with the agonist while atropine (1.2 × 10^{-6} to 1.2 × 10^{-5}) significantly inhibited the response (Fig. 2).

When antagonists were administered 25 min before bethanechol (Design 2), the doses of secoverine required to significantly inhibit fluid secretion (10^{-5} and 10^{-4} mol kg^{-1}, $P<0·025$ and 0·005 respectively) were higher than those of atropine (1·2 × 10^{-6} and 1·2 × 10^{-5} mol kg^{-1}, $P<0·001$). Very low doses of atropine (1.2 × 10^{-8} and 1·2 × 10^{-7} mol kg^{-1}) actually enhanced the bethanechol-induced fluid secretion ($P<0·01$). Salivary secretion was inhibited by both secoverine and atropine at the same doses required to inhibit bethanechol-induced intestinal secretion.

Similarly, a higher dose of secoverine (10^{-4} mol kg^{-1}) was required to significantly inhibit bethanechol-induced hyperpolarization ($P<0·001$) compared with atropine (1·2 × 10^{-6} and 1·2 × 10^{-5} mol kg^{-1}, $P<0·001$) (Fig. 3).

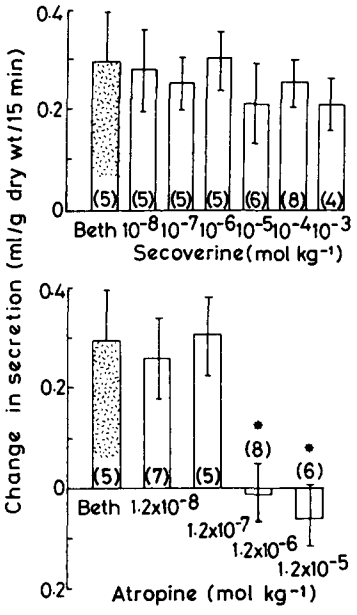

FIG. 2. The effect of secoverine (10^{-8} to 10^{-3} mol kg^{-1}) and atropine (1·2 × 10^{-8} to 1·2 × 10^{-5} mol kg^{-1}) on the changes in intestinal fluid secretion induced by bethanechol (10^{-4} mol kg^{-1} i.p.) [Beth and stippled bars] when the two drugs were given simultaneously via the i.p. route. Numbers within the histograms refer to the number of experiments. * = statistically significant ($P<0·05$).

Effect of secoverine or atropine on bethanechol-induced jejunal motility

Bethanechol (10^{-4} mol kg^{-1}) caused increases in intestinal motility and transintestinal PD, which were maintained throughout the 15 min test period (Figs 4, 5). Secoverine (given 25 min before bethanechol) significantly inhibited bethanechol-induced hypermotility at lower doses (10^{-7} mol kg^{-1}) ($P<0·005$) than those that inhibited PD and secretion and caused complete inhibition at 10^{-4} mol kg^{-1} ($P<0·001$) (Figs 3, 4) whereas atropine inhibited bethanechol-induced hypermotility at 1·2 × 10^{-9} mol kg^{-1} to 1·2 × 10^{-5} mol kg^{-1} ($P<0·001$) (Figs 3, 4), again much lower than the doses required to inhibit PD and secretion.

Effect of drugs given intravenously

Preliminary experiments showed that i.v. administration of bethanechol (5 × 10^{-6} mol kg^{-1}) induced increases in fluid secretion and PD, which were of similar magnitude to those induced by 10^{-4} mol kg^{-1} bethanechol i.p.

Secoverine (10^{-5} mol kg^{-1}) inhibited bethanechol-induced hyperpolarization ($P<0·001$) while much smaller doses were able to inhibit bethanechol-induced hypermotility (10^{-7} mol kg^{-1}) ($P<0·01$) (Fig. 6). Similar results were obtained with atropine, but at much lower concentrations than secoverine (Fig. 6). Bethanechol-induced secretion and hyperpolarization were inhibited by 1·2 × 10^{-11} mol kg^{-1} atropine ($P<0·05$) and above whereas hypermotility was inhibited by doses of 1·2 × 10^{-13} mol kg^{-1} ($P<0·01$).

DISCUSSION

Our results showed that the muscarinic antagonist secoverine was able to inhibit bethanechol-induced

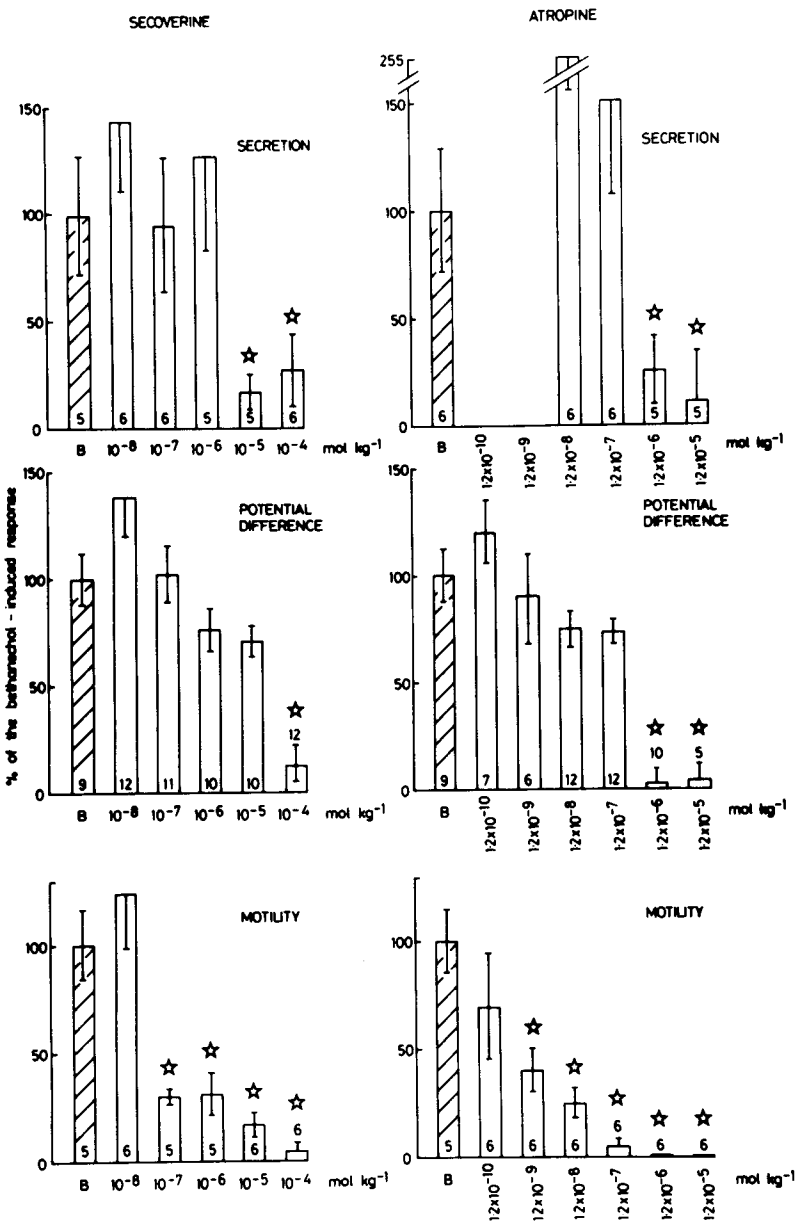

FIG. 3. The effect of secoverine (10^{-8} to 10^{-4} mol kg^{-1}) and atropine ($1{\cdot}2 \times 10^{-10}$ to $1{\cdot}2 \times 10^{-5}$ mol kg^{-1}) on the changes in intestinal fluid secretion, transintestinal potential difference (PD) and intestinal motility, induced by bethanechol (10^{-4} mol kg^{-1}) [Beth and hatched bars]. Numbers within the histograms refer to the number of experiments. * = statistically significant ($P<0{\cdot}05$).

jejunal motor activity at doses that were 100 times lower than those required to inhibit secretion and hyperpolarization in the same tissue. Thus, these results were compatible with those of Zwagemakers & Claassen (1980, 1981), who showed that secoverine inhibited ileal motility at doses which did not appear to affect gastric or salivary secretion. However, this property of selectivity is not unique to secoverine but is shared by atropine at much lower doses.

On the basis of their studies, Zwagemakers and his colleagues (1980, 1981) suggested that the differ-

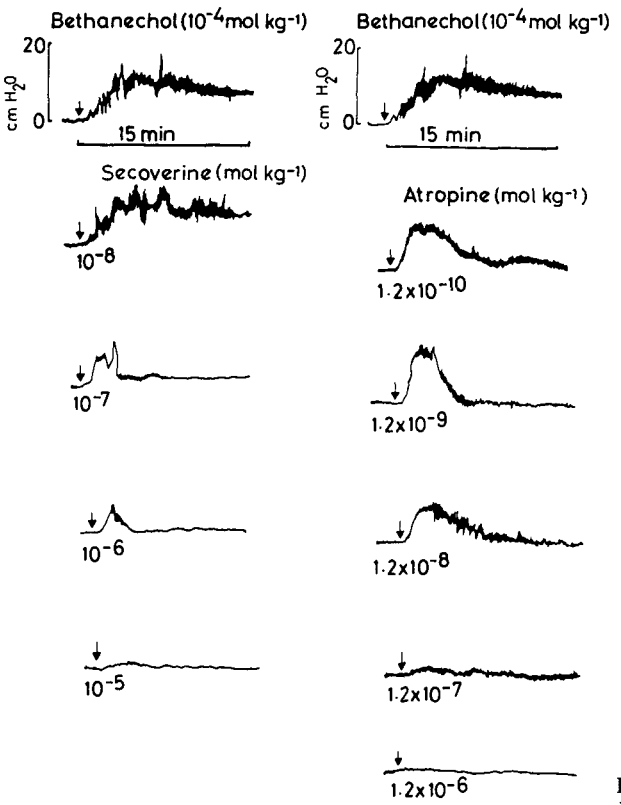

FIG. 4. The typical inhibitory effects of secoverine (10^{-8} to 10^{-5} mol kg^{-1}) and atropine ($1{\cdot}2 \times 10^{-10}$ to $1{\cdot}2 \times 10^{-6}$ mol kg^{-1}) on increases in small intestinal motility induced by bethanechol (10^{-4} mol kg^{-1}).

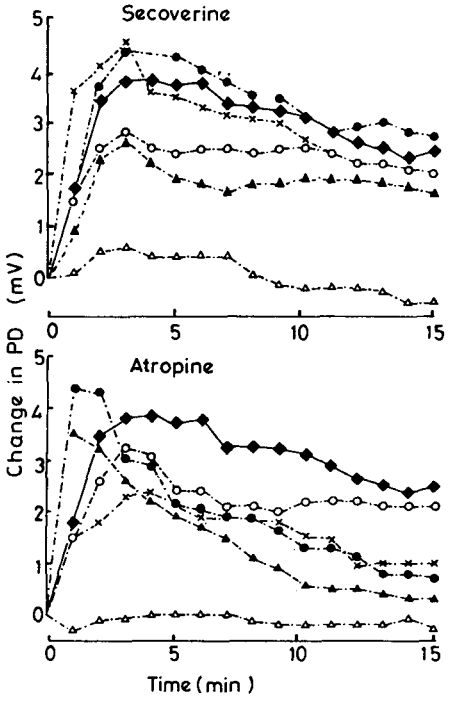

FIG. 5. The effect of secoverine (△ 10^{-4}, ▲ 10^{-5}, ○ 10^{-6} × 10^{-7}, ● 10^{-8} mol kg^{-1}) and atropine (△ $1{\cdot}2 \times 10^{-6}$, × $1{\cdot}2 \times 10^{-7}$, ○ $1{\cdot}2 \times 10^{-8}$, ▲ $1{\cdot}2 \times 10^{-9}$, ● $1{\cdot}2 \times 10^{-10}$ mol kg) on the Pd profiles induced by bethanechol 10^{-4} mol kg^{-1} ◆. Each profile represents the mean from up to twelve identical experiments. 10^{-4} mol kg^{-1} bethanechol.

ences between the effects of secoverine on different tissues may be explained by the existence of different types of muscarinic receptors. This concept was supported by the discovery of compounds with a lower affinity for muscarinic receptors in the guinea-pig atrium compared with the ileum (Barlow et al 1976, 1980) and the observation that another muscarinic antagonist, pirenzepine, inhibited cholinergically-induced gastric secretion at doses which did not antagonize salivation or gastric motility (Hammer et al 1980). However, the fact that atropine, which has not previously been regarded as a selective antagonist, exhibits a similar selectivity suggests that the differences in potency could depend on differential access to receptors in the intestinal muscle and epithelium. In addition, secoverine possesses direct spasmolytic activity, unrelated to the blockade of muscarinic receptors (Zwagemakers & Claassen 1980), and it is possible that these may have contributed to the relative potency of action of the drug on intestinal smooth muscle.

It is of interest that secretory and electrical effects of bethanechol were inhibited by the same doses of atropine while the dose of secoverine required to significantly inhibit bethanechol-induced fluid secretion was one order of magnitude lower than that required to inhibit hyperpolarization. This suggests that the changes in transintestinal PD are more closely related to epithelial events than to intestinal contractions.

The observation that secoverine is a less potent muscarinic antagonist than atropine in the rat jejunum, is similar to previous studies (Zwagemakers & Claassen 1980), which showed that secoverine has about 0·6 the potency of atropine in inhibiting carbachol-induced contractions of rat or guinea-pig ileum, and 0·01 the potency of atropine in inhibiting cholinergically-induced salivary and gastric secretion in mice and rats respectively.

Low doses of atropine ($1{\cdot}2 \times 10^{-8}$ and $1{\cdot}2 \times 10^{-7}$ mol kg^{-1}) actually enhanced the bethanechol-induced fluid secretion. Similar results were found

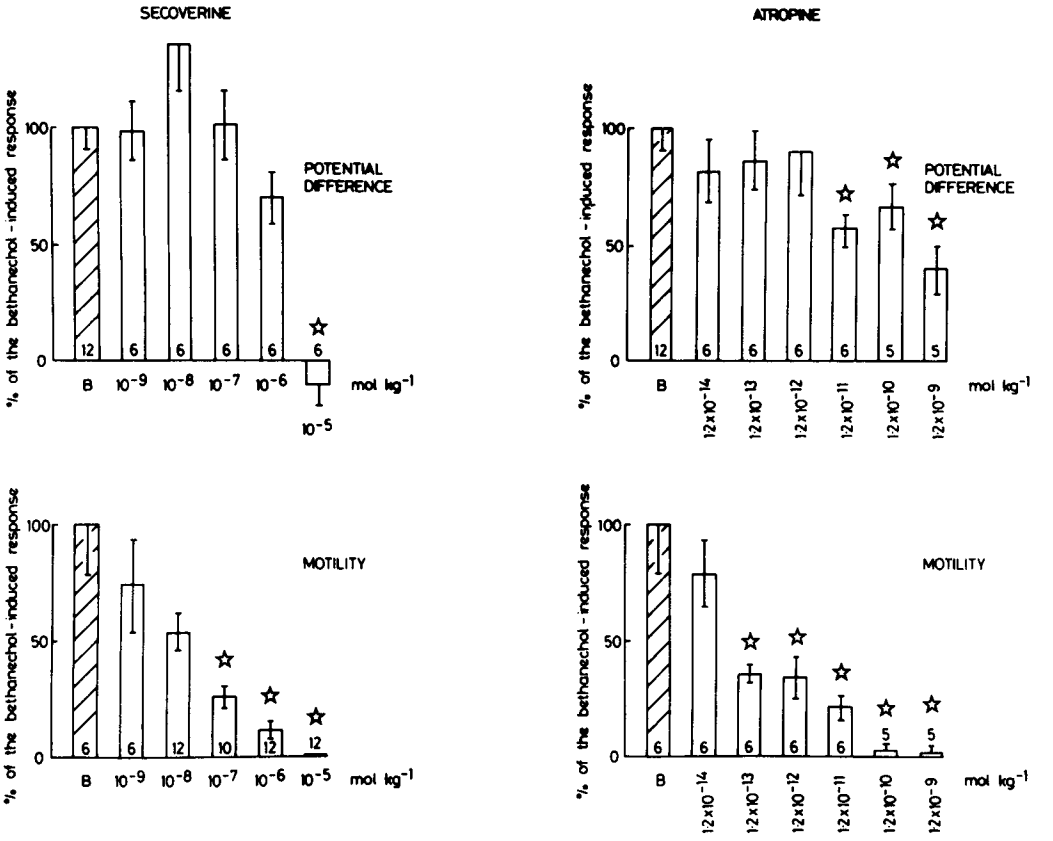

FIG. 6. The effect of secoverine (10^{-9} to 10^{-5} mol kg^{-1}) and atropine ($1{\cdot}2 \times 10^{-14}$ to $1{\cdot}2 \times 10^{-9}$ mol kg^{-1}) on the increases in intestinal motility and transintestinal PD, induced by bethanechol (5×10^{-6} mol kg^{-1}) *($P<0{\cdot}05$).

by Ashford et al (1962) who showed the low dose atropine exhibited cholinergic activity by potentiating the effect of acetylcholine. Particularly interesting are the potent inhibitory effects on intestinal motility of i.v. administration of low doses of atropine ($1{\cdot}2 \times 10^{-13}$ mol kg^{-1}) when receptor binding studies have shown that the affinity of atropine for the receptor is around 10^{-9}M. As receptor binding studies were carried out on in-vitro tissue preparations which possess no blood supply, we envisage that in the in-vivo preparation the intact blood supply provides a means of transporting atropine more efficiently to the receptor surface, so lower doses of atropine are required to produce an inhibitory effect. To our knowledge, an apparent selectivity of action has not been previously reported for atropine, probably because the drug is normally administered in doses above $1{\cdot}2 \times 10^{-11}$ mol kg^{-1}, which inhibit both motility and secretion.

Unlike atropine, secoverine only inhibited bethanechol-induced changes in motility and secretion via the i.p. route when given 25 min before the agonist, although both secoverine and atropine inhibited the effects of bethanechol on the gut when they were given at the same time as bethanechol via the i.v. route, suggesting that either secoverine is poorly absorbed from the peritoneal cavity or it is almost completely metabolized during first pass through the liver. The observation that administration of large doses of secoverine to swamp the degrading enzymes and administration of SKF 525A, a blocker of the hepatic cytochrome oxidase system (unpublished data) both failed to influence the lack of effect of i.p. secoverine on simultaneously administered bethanechol suggests that hepatic metabolism cannot be implicated. Thus, the most likely explanation would seem to be slow absorption of the drug from the peritoneal cavity.

Acknowledgements

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