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Secoverine hydrochloride is a muscarinic antagonist in human isolated gastrointestinal muscle and myometrium

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Secoverine is a new antimuscarinic agent thought to be selective for smooth muscle. We have found it to be a potent anticholinergic antagonist of acetylcholine in strips of human gastrointestinal and uterine muscle. The pA_2 values in gut longitudinal and circular muscle and myometrium were 9·1, 9·3 and 8·6 respectively.

Secoverine hydrochloride potently blocks muscarinic receptors in various animal intestinal muscles, but seems less active at many other sites, including salivary glands, the eye, and urinary bladder (Zwagemakers & Claassen 1980a,b). This supports the suggestion that there may be different subclasses of muscarinic receptor in different tissues (Barlow et al 1976, 1980; Marshall & Ojewole 1979; Hammer et al 1980; Li & Mitchelson 1980).

The ability of secoverine to antagonize certain smooth muscle responses to acetylcholine is potentially of clinical importance. We now report the first studies of secoverine hydrochlorine on human isolated gastrointestinal muscle and myometrium, and compare the results with the previously published animal work (Zwagemakers & Claassen 1980a,b).

MATERIALS AND METHODS

Macroscopically normal specimens of human stomach, jejunum, terminal ileum, colon or myometrium were obtained at operation for benign or malignant disease. These were used immediately or stored overnight at 4 °C in Krebs solution equilibrated with 5% CO_2 in O_2. In other experiments (Bucknell & Whitney 1964; Bennett & Whitney 1966a) overnight storage did not affect the responses to acetylcholine or various other drugs. Strips of gastrointestinal muscle 4–5 mm wide and 3–4 cm long, were cut parallel to the longitudinal or circular muscle fibres (the taenia was used as the colonic longitudinal muscle) after cutting away the mucosa and submucosa. Strips of myometrium about 4 × 4 × 50 mm were cut approximately parallel to the longitudinal uterine axis.

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Each strip was suspended under a load of 1 g in bathing solution at 37°C bubbled with 5% CO_2 in O_2. For gastrointestinal muscle, the bathing fluid was usually Krebs solution (NaCl 7·1; $CaCl_2$ $6H_2O$ 0·55; KH_2PO_4 0·16; KCl 0·35; $MgSO_4$ $7H_2O$ 0·29; $NaHCO_3$ 2·1; dextrose 1·0 g litre^{-1}), but for myometrium (and sometimes gut muscle for comparison with the uterus) a low-calcium Krebs solution ($CaCl_2$ $6H_2O$ 0·02–0·11 g litre^{-1}) was used to inhibit spontaneous contractions (Sanger & Bennett 1979).

In each experiment, rough dose-response curves to acetylcholine (ACh) and KCl were obtained. Consistent, submaximal contractions to ACh (0·05–5 and 0·05–3 μg ml^{-1} for gut longitudinal and circular muscle respectively, and 0·1–10 μg ml^{-1} for myometrium) were then compared with approximately equal, consistent submaximal contractions to KCl (1–10, 2–15 and 1–7 mg ml^{-1} respectively). In some preparations (usually myometrium) consistent responses could not be obtained and the experiment was abandoned. After addition of secoverine hydrochloride to the bathing solution, consistent responses to the same concentration of excitatory agonists were re-obtained, replacing the secoverine after each washout. Subsequently, higher concentrations of ACh were used to match the original contractions. The degree of ACh inhibition by secoverine was then assessed by determining the increase in the dose of agonist needed to restore the contraction height (dose-ratio). Contact times for ACh or KCl were 30 s, cycle times were usually 10 min, and only one concentration of secoverine was tested on each preparation. Isotonic responses (magnified 8–18 times) were measured using transducers and pen recorders.

Results are expressed as medians with semiquartile ranges in parentheses, or ranges where specified, and analysed statistically using the Wilcoxon

matched pairs test or the Mann-Whitney U-test. pA_2 values were determined by fitting a regression line to an Arunlakshana & Schild (1959) plot.

Drugs used were: acetylcholine perchlorate, potassium chloride, 1-cyclohexyl-4 [ethyl (*p*-methoxy-α-methylphenetyl) amino]-1-butanone hydrochloride (secoverine hydrochloride), and atropine sulphate.

RESULTS

Acetylcholine contracted strips from all the specimens studied. KCl contracted strips of myometrium, stomach or jejunum, but the colon sometimes responded with an initial relaxation followed by contraction on washout. This relaxation occurred in taenia from 4 of 14 specimens and in 2 of 6 specimens of colonic circular muscle; it seemed unrelated to disease, sex or age. Concentrations of KCl which caused an initial relaxation were usually 2–5 mg ml^{-1}, with higher concentrations causing less relaxation; KCl was rarely tested below 2 mg ml^{-1}.

Secoverine hydrochloride was tested on strips of gastrointestinal longitudinal and circular muscle (21 and 14 strips respectively) and on 19 strips of myometrium. The strips were taken from 3 specimens of stomach, 1 jejunum, 13 transverse or sigmoid colons and 16 uteri. Secoverine 0·01–1 μg ml^{-1} in the bathing fluid did not usually affect the muscle tone or spontaneous activity, although both were reduced in one specimen of sigmoid colon (resected for diverticular disease) and the spontaneous activity was reduced in the longitudinal muscle from jejunum (1 and 0·1 μg ml^{-1} secoverine respectively).

Secoverine 0·01–1 μg ml^{-1} greatly reduced contractions to ACh in both gastrointestinal muscle and myometrium, without significantly affecting the contractions or initial relaxations to KCl (Fig. 1).

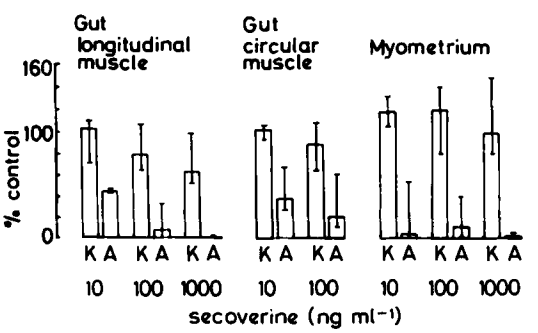

FIG. 1. Secoverine inhibited contractions of human gastrointestinal muscle or myometrium to acetylcholine (A) with relatively little effect on contractions to KCl (K).

Maximum antagonism of contractions to ACh were registered 10–70 min after the first addition of secoverine, with higher concentrations usually acting more quickly. In further experiments on taenia coli (2 specimens) and colonic circular muscle (1 specimen), contractions to ACh or KCl were unaltered by secoverine hydrochloride in the low concentration of 1 ng ml^{-1} or were slightly bigger.

The concentration of ACh required to restore the response after the addition of secoverine 0·01 μg ml^{-1} was increased by approximately 4 times in both longitudinal and circular gastrointestinal muscle, and by about twice in myometrium (Fig. 2). Increasing the concentration of secoverine dose-dependently increased the dose of ACh required to restore the response (dose-ratio) in each of the three tissues. For the gastrointestinal tissues, the dose-ratios for ACh in the stomach were usually higher than those obtained with the colon (Fig. 2). Although this could indicate regional differences in the potency of secoverine, the small numbers of stomach studied make a firm conclusion inappropriate, and the pA_2 values were calculated from the combined results of all gastrointestinal tissues. These gave regression line slopes which were 0·91, 0·77 and 0·93 for gut longitudinal, gut circular and uterine muscle respectively. The pA_2 values for these tissues were respectively 9·1, 9·3 and 8·6 (Fig. 2). These values should be considered with some caution. If regional differences of potency occur with secoverine, this would introduce error into our calculations. In addition, the slope of the regression line for gut circular muscle might indicate non-competitive antagonism in this tissue. However, the range of secoverine concentrations and the number of circular muscle preparations studied were less than for gut longitudinal muscle or myometrium.

In four similar experiments with gastrointestinal longitudinal muscle (3 taenia coli, 1 terminal ileum) using a low-Ca^{2+} Krebs solution (0·04 g $litre^{-1}$) similar to that used in the experiments with the myometrium, secoverine 0·1 μg ml^{-1} inhibited contractions to 0·1–0·5 μg ml^{-1} ACh with a dose-ratio of 27 (range 9 to 62), not significantly different from that obtained in normal Krebs solution ($P = 0·91$). Contractions to KCl were more difficult to obtain in the low-Ca^{2+} Krebs solution, and maximum responses were less than those obtained with ACh. Initial relaxation of taenia coli in response to KCl was not seen in the few preparations studied, but the low-Ca^{2+} Krebs solution reduced the muscle tone and made relaxations difficult to detect.

Atropine sulphate 0·018 μg ml^{-1} (equimolar to

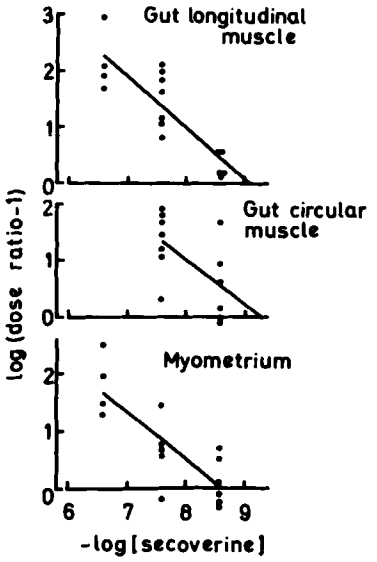

FIG. 2. The pA_2 values for secoverine against acetylcholine in gut longitudinal muscle, gut circular muscle and myometrium, as judged from the intercept of the regression line with the horizontal axis, were 9·1, 9·3 and 8·6 respectively. The slopes of the regression lines were 0·91, 0·77 and 0·93 respectively.

0·02 μg ml^{-1} secoverine hydrochloride) also antagonized contractions to ACh in longitudinal muscle strips cut from the same gastrointestinal specimens used with secoverine 0·01 μg ml^{-1}. Atropine reduced the contractions to ACh and KCl by respectively 79(69 to 93)% and 8(−7 to 9)%; n = 6 for each, the dose-ratio for ACh being 33(range: 5 to 57) n = 5. The effect of atropine 0·018 μg ml^{-1} was approximately 8 times greater than that for secoverine 0·01 μg ml^{-1} ($P < 0·004$). On a molar basis secoverine was therefore about 0·25 as potent as atropine, assuming that secoverine competitively antagonized contractions to ACh (Zwagemakers & Claassen 1980).

DISCUSSION

ACh contracts human isolated gastrointestinal muscle or myometrium by acting on muscarinic receptors. The response is increased by cholinesterase inhibitors, reduced by hyoscine or atropine, and usually unaffected by cholinergic nerve blockade (Graham 1949; Bucknell & Whitney 1964; Bennett 1965; Bennett & Whitney 1966a; Nakanishi & Wood 1971). This last point makes it unlikely that secoverine blocks ACh at its receptors in ganglia.

The actions of KCl on human colon have been reported only briefly by Stockley & Bennett (1974) who found contraction of taenia coli with KCl 2 mg ml^{-1}. The contraction is presumably due to depolarization of the cell membrane, as shown in animal tissues (Holman 1958; Marshall 1962). Our experiments with secoverine or atropine suggest that KCl does not activate excitatory cholinergic mechanisms, but the reason for the initial relaxation to KCl in some specimens of colon is not known. In other smooth muscles, KCl-induced relaxation may be due to stimulation of inhibitory nerves or to direct hyperpolarization of the cell membrane (see Ishii & Shimo 1980). The predominantly inhibitory intrinsic nerves of human colon (see Bennett & Whitney 1966b) may suggest at least a partial autonomic nerve involvement in relaxations to KCl in our experiments. The difficulty in obtaining contractions of gastrointestinal muscle to KCl using low-Ca^{2+} Krebs solution suggests that KCl-induced contractions are more dependent on extracellular calcium than are those to ACh. Such a difference has previously been reported for several other smooth muscle tissues (see Triggle 1971).

Antagonism by secoverine hydrochloride of contractions to ACh in human gastrointestinal strips was similar in the longitudinal and circular muscle. Secoverine also antagonized ACh-induced contraction of human myometrium with a potency approximately similar to that in the gastrointestinal experiments. The comparison seems valid since drug contact-time and recording conditions were the same for all experiments (see Butt 1972), and the low-Ca^{2+} Krebs solution used for the myometrium did not alter the potency of antagonism by secoverine in gastrointestinal longitudinal muscle. Similarly, the effects of hyoscine on guinea-pig ileum were not altered by varying the calcium concentration (Paton & Rothschild 1965). Muscarinic receptors in the human isolated gut muscle and in the myometrium may therefore be similar, although there might be differences that secoverine or our experimental techniques cannot show.

For the gastrointestinal longitudinal muscle, secoverine had about a quarter the potency of atropine, whereas in several animal gastrointestinal tissues secoverine was approximately 0·6 times as active as atropine (Zwagemakers & Claassen 1980a). This moderate variation may reflect species differences, but our comparison of potencies was less precise than that of Zwagemakers & Claassen (1980a). Our results on the gut accord with in vivo data. In patients with diverticular disease secoverine 0·5 mg kg^{-1} i.v. reduced or abolished neostigmine-stimulated sigmoid colonic motility (Stoddard et al 1980).

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