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Selectivity of bethanechol on muscarinic receptors

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Bethanechol is a cholinergic agonist with little if any nicotine actions (Goodman & Gilman, 1975; Martindale, 1977). Evidence for this selective action is based on the absence of any pressor activity of bethanechol in the atropinized cat (Simonart & Simonart, 1935) or dog (Farber, 1936). Although bethanechol is used to demonstrate the effects of muscarinic stimulation in the gastrointestinal tract its selectivity of action at this site has not been established. A suitable gastrointestinal preparation for demonstrating selective stimulation of muscarinic receptors is the isolated human colon. Circular and longitudinal muscle layers of the colon contain both muscarinic and nicotinic receptors, the former mediating contraction the latter relaxation (Fishlock & Parks, 1963; Bucknell & Whitney, 1964).

Circular muscle strips about 20 mm long and 2 mm wide were taken from specimens of colon removed at operation. Muscle strips prepared only from healthy tissue consisted of the full thickness of bowel wall with the mucosa removed. The strips were suspended in Krebs bicarbonate solution at 37° aerated with a mixture of 5% CO_2 in oxygen. Movements of the muscle were recorded by a Devices or Rikadenki recorder using an isotonic transducer (load 0·5–1 g). The Krebs bicarbonate solution contained (mM) Na 140, K 5·9, Ca 2·5, Mg 1·2, Cl 122, HCO_3 25, HPO_4 1·2, SO_4 1·2, dextrose 11·5. Circular colonic muscle strips usually lost tone before completion of the experiment, thus making it difficult to obtain relaxation to nicotine receptor stimulation. This was avoided by incubating muscle strips with barium chloride ($BaCl_2$) which maintained the tone of the strips at a higher level and made it possible for nicotine receptor stimulation to relax the muscle strip. One experiment was completed without addition of $BaCl_2$ (Fig. 1) and the results were similar to those experiments using $BaCl_2$.

Acetylcholine (0·2–7·1 × 10^{-6} M) and bethanechol (0·6–40 × 10^{-6} M) contracted circular colonic muscle strips, this effect was blocked by hyoscine (4·6 × 10^{-6} M). Increasing the concentration of acetylcholine (0·9–4·4 × 10^{-4} M) in the presence of hyoscine (4·6 × 10^{-6} M) caused a biphasic response (relaxation followed by contraction); the relaxation was blocked by hexamethonium (35·6 × 10^{-6} M, Fig. 1). In contrast when the concentration of bethanechol was increased in the

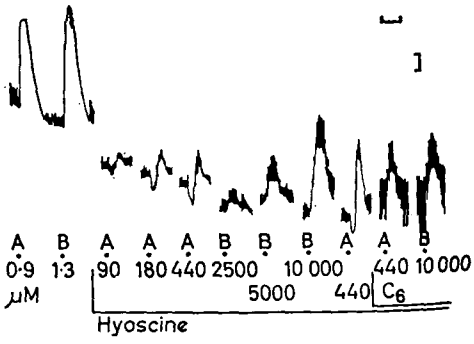

FIG. 1. Human circular sigmoid colon: showing responses to acetylcholine (A) and bethanechol (B) before and after hyoscine (4·6 × 10^{-6} M). Hexamethonium (C_6, 35·6 × 10^{-6} M) was then tested against bethanechol and the modified response to acetylcholine. A 2 min contact time was used for the agonists. Horizontal scale: 5 min; vertical scale: 1 cm.

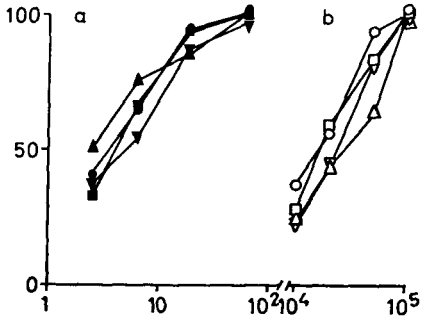

FIG. 2. The effect of hyoscine ($4{\cdot}6 \times 10^{-6}$ M) on bethanechol log dose-response curves of human circular colonic muscle. The results from four muscle strips are shown. a: represents responses before, b: responses after, the addition of hyoscine. Ordinate: Response (% of maximum). Abscissa: Concn (μM).

presence of hyoscine ($4{\cdot}6 \times 10^{-6}$ M), muscle strips gave only contractions (Fig. 2) which were unaffected by hexamethonium ($35{\cdot}6 \times 10^{-6}$ M). Preliminary experiments with longitudinal (taenia coli) muscle strips have given similar results.

When muscarinic receptors were blocked by hyoscine, acetylcholine relaxed colonic muscle strips by stimulation of nicotinic receptors. However, bethanechol did not appear to stimulate nicotinic receptors as when its concentration was increased in the presence of hyoscine, muscle strips only gave contractions. These results confirm earlier work, using the blood pressure of atropinized cats and dogs, that bethanechol selectively stimulates muscarinic receptors.

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