CALCITONIN: ANTAGONISM AT INTESTINAL MUSCARINIC RECEPTORS

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The action of calcitonin was studied on the motility of isolated innervated segments of rabbit and guinea-pig intestines as well as longitudinal muscle with adherent myenteric plexus dissected from the guinea-pig ileum. Calcitonin (0.25 μM) antagonized contractile responses to acetylcholine and the cholinergic response to electrical field stimulation. This hormonal effect was relatively specific since it was not observed at nicotinic receptors or adrenoceptors, nor did calcitonin act as a local anaesthetic or directly on the contractile machinery of smooth muscle. Perivascular adrenergic and intrinsic non-adrenergic inhibitory responses also were unaffected by calcitonin. However, calcitonin did have antihistaminic properties directed against H_1-receptors. The concentration of calcitonin required to achieve muscarinic antagonism in our experiments is not reached at the resting level of circulating hormone.

Introduction Calcitonin lowers the calcium concentration of blood primarily by inhibition of bone resorption, but the hormone also inhibits renal tubular calcium reabsorption and intestinal calcium absorption (Haymovits & Rosen, 1972). Recent studies have indicated that calcitonin may have additional effects on gastrointestinal function besides inhibition of calcium absorption. For example, in the cat and in man, calcitonin decreases basal, gastrin, and histamine-stimulated gastric acid secretion (Hesch, Hufner, Hasenjager & Creutzfeldt, 1971; Becker, Konturek, Reeder & Thompson, 1973; Becker, Reeder, Scurry & Thompson, 1974). There is also evidence that calcitonin decreases release of gastrin (Becker *et al.*, 1973; Becker *et al.*, 1974) and inhibits exocrine secretion of pancreatic enzymes (Schmidt, Hesch, Hufner, Paschen & Creutzfeldt, 1971). Moreover, gastrointestinal symptoms have been reported by 10% to 20% of patients receiving calcitonin for treatment of Paget's disease (Haymovits, Wright, Ling, Hobitz & Tayag, 1975). These additional effects on secretion, and perhaps the symptoms associated with calcitonin therapy, could be explained if calcitonin either antagonized the release or the action of acetylcholine and histamine. In order to test the involvement of acetylcholine in the gastrointestinal actions of calcitonin, the effects of the hormone on the motility of innervated intestinal smooth muscle preparations was studied *in vitro*.

Methods Adult male Hartley guinea-pigs and New Zealand rabbits were used. Two preparations were obtained from guinea-pigs, a segment of intact ileum, and a segment of isolated longitudinal muscle with adherent myenteric plexus (Paton & Zar, 1968). Rabbit ileum was mounted to permit transmural (Paton, 1955) or perivascular nerve stimulation (Finkelman, 1930). All preparations were studied in an organ bath containing oxygenated Krebs solution at 37°C and mechanical activity was recorded isotonically. Intact ileum was stimulated transmurally and the longitudinal muscle-myenteric plexus preparation was stimulated between two stainless steel wire mesh electrodes positioned on either side of the tissue. Rectangular pulses each of less than 0.2 ms duration were used to deliver supramaximal stimuli. These pulses activate nerve but not smooth muscle.

Concentrations of drugs given in the text refer to the final concentration in the bath. The drugs used were: acetylcholine iodide, hyoscine hydrobromide, histamine diphosphate, nicotine tartrate, and (–)-noradrenaline bitartrate. Either purified human calcitonin (Haymovits & Levin, 1975) prepared by solid phase peptide synthesis, and standardized against pure synthetic calcitonin obtained from CIBA Pharmaceutical Co. (Sieber, Brugger, Kamber, Riniker & Riettel, 1968), or the CIBA product was used. Studies performed with either preparation yielded identical results. Stock solutions of calcitonin contained 5% albumin. The diluent alone had no effect on any of the preparations studied.

Results The action of calcitonin (0.25 μM) on the activity of the intact rabbit and guinea-pig intestine is shown in Figure 1. The rabbit intestine shows

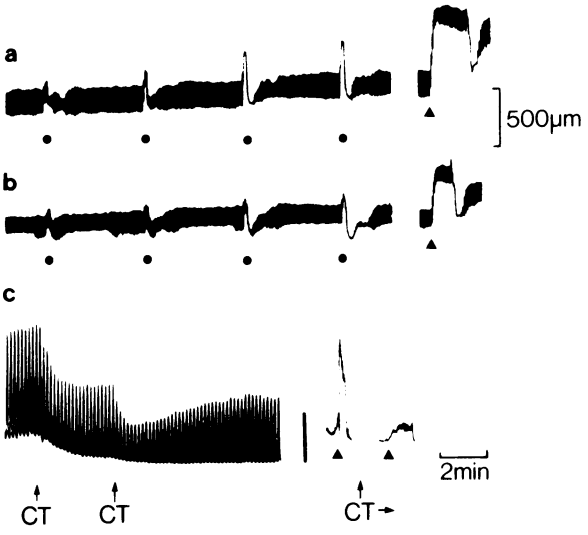

Figure 1 Effects of calcitonin on spontaneous and electrically stimulated intestinal motility *in vitro*. (a) and (b) Rabbit ileum stimulated transmurally at the dots for 10 s at successively 5, 10, 20 and 30 Hz: (a) control, (b) in presence of calcitonin (0.25 μM); (▲) acetylcholine (0.37 μM). (c) Guinea-pig ileum, each contraction is the response to a single shock. Calcitonin (CT) was added twice, first to give a concentration of 0.25 μM, then 0.5 μM. Acetylcholine (▲, 3.7 nM) was tested in the presence and absence of calcitonin (0.25 μM). Similar records were obtained from intact ileum and isolated longitudinal muscle-myenteric plexus.

pendular spontaneous activity and responds to trains of transmural electrical stimuli with a biphasic response consisting of an initial contraction and, particularly at higher frequencies of stimulation, with an after-relaxation (Figure 1a). The spontaneous activity is known to be myogenic (Gershon, 1967). Both phases of the response to transmural stimulation are known to be neurogenic (Paton, 1955; Gershon, 1967). The excitatory component of the response is mediated by cholinergic neurones and the relaxant response is mediated by non-adrenergic intrinsic inhibitory neurones (Gershon, 1967; Paton & Zar, 1968; Burnstock, 1972). The guinea-pig intestine shows no spontaneous activity but responds with a contraction to single shocks delivered transmurally (Figure 1c). Calcitonin does not inhibit the myogenic pendular activity of the rabbit intestine although, like hyoscine, it decreases slightly the amplitude of the pendular movements. On the other hand, calcitonin antagonizes the contractile phase of the response to transmural stimulation while, particularly at higher frequencies of stimulation, augmenting the relaxant component (compare Figure 1a and b). The contractions of the guinea-pig ileum to transmural shocks are also inhibited by calcitonin (Figure 1c). Similarly, in both preparations the contractions of the tissue in response to the addition of acetylcholine (3.66 nM to 3.66 μM) are antagonized by calcitonin (Figure 1, right). The lowest concentration at which an effect of calcitonin could be detected consistently was 10 nM.

In order to determine whether the action of calcitonin on intestinal motility is limited to antagonism of the muscarinic action of acetylcholine, the effects of calcitonin on relaxant responses of the rabbit ileum to stimulation of adrenergic and non-adrenergic inhibitory nerves as well as addition of noradrenaline and nicotine were also studied. These experiments were all carried out in the presence of hyoscine (0.23 μM). The rabbit intestine was used because its high tone and spontaneous activity permit relaxant responses to be seen. Calcitonin (as much as 2.5 μM) has no effect on the relaxation of this preparation to noradrenaline (0.31 μM) or nicotine (20 μM). The relaxant response to nicotine is mediated by non-adrenergic intrinsic inhibitory neurones (Gershon, 1967; Burnstock, 1972). This suggests that calcitonin does not directly relax the smooth muscle, and does not act as an antagonist at adrenoceptors or nicotinic receptors. This suggestion is confirmed by the further observation that calcitonin also fails to affect responses to stimulation of the perivascular adrenergic nerves or transmural stimulation of the intrinsic non-adrenergic inhibitory nerves. These experiments also rule out the possibility that calcitonin could be acting as a local anaesthetic.

KCl (5.4 mM and 54 mM) was added to depolarize smooth muscle directly and induce myogenic contractions. Hyoscine (0.23 μM) was present in order to eliminate any possibility of a cholinergic component to the contraction. The amplitude of the response to either concentration of KCl (1.1 ± 0.2 mm; 7.7 ± 0.5 mm; mean ± s.e.) is not significantly affected by calcitonin (2.5 μM) (1.0 ± 0.1 mm, 8.4 ± 0.4 mm). Therefore, calcitonin does not appear to influence the contractile properties of smooth muscle directly.

The contractile response of the guinea-pig ileum to histamine is mediated by H_1-receptors on smooth muscle and does not have an indirect cholinergic component (Gershon, 1967; Burnstock, 1972). The concentration-effect curve to histamine (6.5 nM to 33 μM) was shifted to the right by calcitonin (2.5 μM). This suggests that calcitonin also has antihistaminic properties directed against the H_1-receptors of intestinal smooth muscle.

Discussion The results obtained in these experiments indicate that calcitonin has a weak anticholinergic effect. This antagonism, exerted at the muscarinic receptors for acetylcholine is adequate to account for almost all of the observed effects of

calcitonin on isolated innervated smooth muscle preparations. Thus, cholinergic excitatory responses to transmural electrical stimulation are diminished in amplitude and simultaneous non-cholinergic inhibitory responses are unmasked by calcitonin and appear relatively augmented. This antimuscarinic effect is relatively specific. Calcitonin has no direct action on smooth muscle contraction, and is not an antagonist at nicotinic receptors for acetylcholine or at adrenoceptors. Moreover, the hormone does not act as a local anaesthetic nor does it affect adrenergic or non-adrenergic inhibitory neurotransmission. However, calcitonin is not entirely specific in its action. The hormone also has antihistaminic properties. The possibility that calcitonin might decrease release of acetylcholine was not studied because it was not necessary to postulate this additional action to explain the effects of the hormone.

It seems reasonable to assume that the atropine-like antimuscarinic effect of calcitonin may account for the actions of the hormone on glandular secretion which were listed previously. All of these are under at least partial cholinergic control (Grossman, 1970; Hirschowitz, 1975). However, it is not likely that calcitonin exerts these effects at the concentrations at which it normally circulates in blood (about 50 pM to 250 pM: Deftos, Goodman, Engelman & Potts, 1971). Gastrointestinal actions might accompany administration of calcitonin in pharmacological doses for therapeutic purposes or its autonomous hypersecretion by medullary carcinoma of the thyroid (Gautvik & Tashjian, 1974).

Presented in part at the annual meeting of the Endocrinology Society, Chicago, Ill., U.S.A., June 20–22, 1973. Supported by NIH grants No. NS07436, No. AM12957, No. AM11031 and No. GM00895.

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(Received January 19, 1976.)