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Evidence for a direct anti-inflammatory action of calcitonin: inhibition of histamine-induced mouse pinnal oedema by porcine calcitonin

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Porcine calcitonin (PCT) caused a potent inhibition of histamine-induced vascular leakage in the mouse pinna. These effects were observed at doses of PCT lower than those required to affect plasma calcium concentrations. In contrast PCT did not inhibit responses of the mouse pinna to 5-hydroxytryptamine despite observed falls in plasma calcium concentrations. It would appear that PCT inhibits histamine-induced oedema in the mouse by a direct action which may partially or fully explain the anti-inflammatory action of PCT, observed by other workers, in some acute inflammatory conditions.

Calcitonin has been shown to have anti-inflammatory actions in man (Velo et al 1976) and acute and chronic animal experiments (Riesterer & Jaques 1969; Bobalik et al 1974; Abdullahi et al 1975, 1977). Most workers have suggested that its actions may be related to its ability to alter plasma calcium concentrations. Riesterer & Jaques (1969), however, have proposed that calcitonin may have direct anti-inflammatory actions on dextran-induced rat paw oedema in addition to any indirect action via plasma calcium concentrations.

We have investigated the action of porcine calcitonin (PCT) on histamine-and 5-hydroxytryptamine (5-HT)- induced oedema in the pinna of the mouse. In this preparation acute changes in vascular permeability are measured by the extravascular leakage of the dye pontamine sky blue, which is strongly bound to plasma proteins.

MATERIALS AND METHODS

Procedure

Mouse pinnal oedema was developed according to a modification of the method of Church & Miller (1975). Albino mice, CFLP or CPLA strains, of either sex, which had been allowed free access to tap water and 'modified rat-mouse breeding diet cube' (Heygate and Sons Ltd, Northampton, U.K.) were injected intravenously, via the tail vein, with 0·2 ml of a 10 mg ml^{-1} solution of pontamine sky blue and immediately afterwards their pinnae were pierced through a drop of either vehicle or agonist solutions, with a 21 gauge hypodermic needle. In each mouse, one ear was used as a control and the contralateral ear as a test, using either histamine or 5-HT as the agonist. Porcine calcitonin was administered immediately before the dye via the same tail vein as the dye. Mepyramine and methysergide were administered intraperitoneally 30 min before the dye.

Blood samples were taken by cardiac puncture 30 min after administration of the dye and then the animals were killed by cervical dislocation. All operational procedures were performed under light ether anaesthesia.

The blood samples were placed in heparinized tubes and cooled to 4°C. The plasma was separated within 6 h of collection, and stored at −20°C.

Determination of plasma calcium concentrations

Plasma calcium concentrations were determined by atomic absorption.

Analysis of results

The pinnae were removed and the surface areas of the blue spots produced by dye leakage were measured. The areas of blueing were estimated by circumscription on to cellulose acetate sheets, and then the areas were enlarged using an overhead projector. Responses were calculated as the response in the control subtracted from the response in the test ear. For each ear three cellulose acetate sheets were prepared, and each value is the mean of these estimations.

Statistically significant differences were calculated according to the U test of Mann-Whitney (Campbell 1967).

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Materials

Drugs used were: porcine calcitonin batch XM 0503 (Armour), supplied in the lyophilized form in vials containing 160 Medical Research Council Units (MRC U): histamine dihydrochloride (Sigma): 5-hydroxytryptamine creatinine sulphate complex (Sigma): mepyramine maleate (May and Baker): methysergide hydrogenmaleinate (Sandoz): pontamine sky blue 6BX (R. A. Lamb). All drugs were dissolved in 154 mM sodium chloride solution. Bovine serum albumin (Sigma) at a concentration of 1 mg ml^{-1} was added to the PCT solution to prevent binding of the hormone to glass. Between doses, PCT was stored at 0–5°C and warmed to room temperature immediately before injection. Long term storage of the hormone was in the frozen solution at −20°C.

RESULTS

Inflammatory actions of histamine and 5-HT

Both histamine and 5-HT produced concentration-related increases in the leakage of dye into the extravascular space of the mouse pinna. 5-HT (0·01–1 mM) was up to 100 times more potent than histamine (0·1–100 mM), threshold responses to histamine and 5-HT occurred at concentrations of 20 and 0·2 mM respectively. The sensitivity of the pinna to histamine varied over the period of investigation and consequently each series of experiments included a control group receiving histamine. Injection of the vehicle used for calcitonin alone did not affect the response to histamine or 5-HT. There was no statistically significant difference in the response to histamine or 5-HT between sexes.

Actions of PCT

PCT (4 MRC mU kg^{-1} –120 MRC U kg^{-1}) administered intravenously immediately before the dye gave a dose-related inhibition of the response to histamine; a 50% inhibition of the response occurred at 15 MRC mU kg^{-1} (Fig. 1).

PCT (1 MRC mU kg^{-1}–40 MRC U kg^{-1}) administered intravenously immediately before the dye did not affect the response of the mouse pinna to 5-HT (Fig. 2).

PCT lowered plasma calcium concentrations in both series of experiments. Threshold responses to PCT on plasma calcium concentrations occurred at doses of 1 MRC U kg^{-1} (Figs 1 and 2).

Actions of mepyramine and methysergide

Mepyramine (0·025–10 μmol kg^{-1}) gave a dose-related inhibition of the response to histamine.

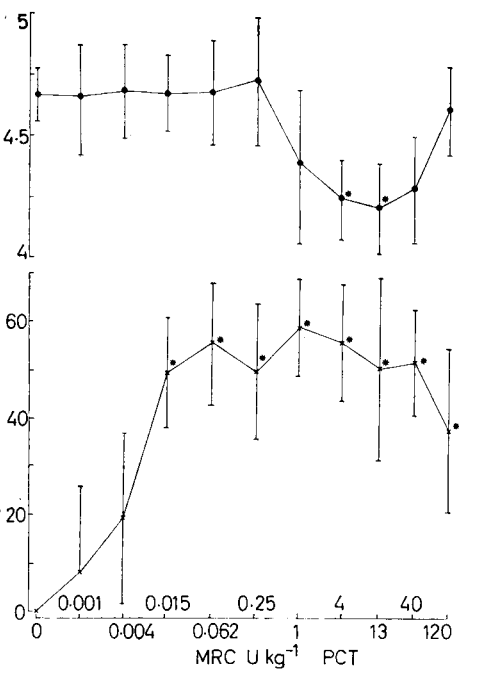

FIG. 1. The effect of PCT on (upper curve) plasma calcium concentrations (ordinate: m equiv $litre^{-1}$) (n = 4–10) and (lower curve) % inhibition (ordinate) of histamine-induced mouse pinnal oedema (n = 8–10). Values are expressed as means and standard errors of means. Statistically significant differences from control values are shown at the $P < 0{\cdot}05$ level (*).

Doses of 0·025, 0·5 and 10 μmol kg^{-1} mepyramine gave 36 ± 17, 78 ± 9 and 89 ± 5% inhibitions of the response to histamine respectively (means ± s.e.m., n = 8–10).

Methysergide (0·03–3 μmol kg^{-1}) gave a dose-related inhibition of the response to 5-HT. Doses of 0·03, 0·3 and 3 μmol kg^{-1} methysergide gave 50 ± 12, 88 ± 6 and 93 ± 2% inhibitions of the response to 5-HT respectively (means ± s.e.m., n = 8–10).

DISCUSSION

The increase in vascular permeability caused by histamine in the mouse pinna was antagonized by PCT, but changes in vascular permeability caused by 5-HT were not affected by PCT. It is probable that this effect of PCT on histamine-induced oedema is not secondary to the changes in the concentration of plasma calcium, as the development of oedema was antagonized by doses of PCT which did not affect plasma calcium concentrations. Furthermore the oedema induced by 5-HT was not antagonized by

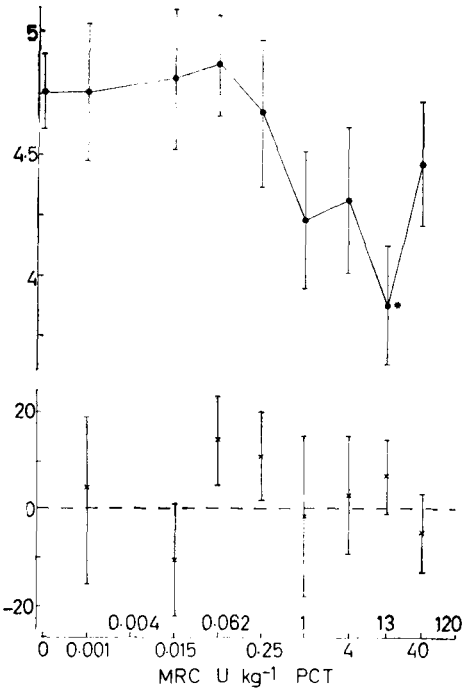

FIG. 2. The effect of PCT on (upper curve) plasma calcium concentrations (ordinate: m equiv litre^{-1}) (n = 4–10) and (lower curve) % inhibition (ordinate) of 5-HT-induced mouse pinnal oedema (n = 8–10). Values are expressed as means and standard errors of means. Statistically significant differences from control values are shown at the $P < 0.05$ level (*).

doses of PCT which produced marked falls in plasma calcium concentrations.

Several possibilities exist to explain this selective inhibition of histamine-induced oedema by PCT. These possibilities include actions on cyclic 3, 5—adenosine monophosphate (cAMP), histamine receptors and cellular calcium concentrations.

Calcitonin has been shown to increase cAMP concentrations in kidney and bone (Murad et al 1970) and to alter cAMP concentrations in other systems (Whitfield et al 1970; Minkin et al 1977; Heersche et al 1978). Increased cAMP concentrations have been demonstrated to inhibit vascular leakage (Ichikawa et al 1972). However, drugs that are known to alter cAMP concentrations do not differentially affect histamine- and 5-HT-induced changes in vascular permeability (Green 1974).

Dreyfus et al (1976) have claimed an antihistaminic action of human calcitonin on guinea-pig ileum, through an action on H_1-receptors, but did not present any evidence to substantiate their claim.

Calcitonin has an action that markedly alters cellular calcium concentrations (Borle 1975). Studies on endothelial cells have suggested that these cells may be able to contract in a similar manner to smooth muscle cells (Majno et al 1967; Constantinides & Robinson 1969). Decreasing extracellular calcium concentrations differentially affect the responses of guinea-pig colon (Botting & Turner 1966) and guinea-pig ileum (Johnson 1963) to histamine and 5-HT, but in this species the response to 5-HT is more sensitive to changes in calcium.

Although exogenous histamine has only a low potency as an inflammatory agent in rodents (Northover 1963; Maling et al 1974), it is probable that oedema induced by dextran is mediated principally by histamine, since much more histamine than 5-HT is released by drugs that induce mast cell degranulation (Maling et al 1974). This theory has been supported by the work of Nishida et al (1978) who have correlated histamine release and foot oedema development in the rat after dextran administration. Thus, the effects of calcitonin on dextran-induced foot oedema may be due to its effects on the histamine component of dextran oedema. This action would be in addition to any direct or indirect (Grosman & Diamant 1974) effect that calcitonin might have on mast cell function.

Abdullahi et al (1975) have suggested that calcitonin is inactive against the initial phase of carrageenan foot oedema, which some workers have proposed may be due to histamine and 5-HT release (Bhalla & Tangri 1970; DiRosa et al 1971). However there is considerable evidence that suggests little involvement for histamine or 5-HT in the early phase of carrageenan oedema (Doherty & Robinson 1975; Vinegar et al 1976). Consequently calcitonin might be expected to be inactive against the initial phase of this condition.

The minimal doses of PCT required to inhibit histamine-induced changes in vascular permeability were similar to the doses of the indigenous hormone that would be required to produce physiological plasma concentrations of the hormone. In the rat, physiological concentrations of the hormone have been measured at 0·03 MRC mU ml^{-1} (Gray et al 1974). Considerable variation exists in the relative potencies of the various native hormones that have been isolated (Copp 1970; Gray et al 1974), and consequently no conclusion can be made regarding the possibility that the concentrations of PCT achieved in this study would have the same activity as similar concentrations of mouse calcitonin.

In conclusion, we have shown a potent inhibitory

action of calcitonin on histamine-induced vascular leakage, which would appear to be independent of the action of calcitonin on plasma calcium concentrations. This effect of calcitonin may fully or partially account for the inhibitory action of calcitonin on acute inflammatory conditions.

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