SHORT COMMUNICATION

CALCIUM SHIFT INTO BONE: A CALCITONIN-RESISTANT PRIMARY ACTION OF PARATHYROID HORMONE, STUDIED IN RATS

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SUMMARY

1. Parathyroid hormone (PTH) injected intravenously into rats caused a small rapid uptake of simultaneously administered radioactive calcium into the skeleton.

2. This skeletal uptake of calcium occurs in many other species, including the monkey and probably man. It appears to be an initiating event in PTH action on bone.

3. Although calcitonin prevents PTH-induced bone resorption, it did not affect this early calcium shift.

4. This evidence is discussed in connection with the possibility of combining calcitonin with some other agent for the treatment of osteoporosis.

Key words: parathyroid hormone, calcitonin, bone, calcium, osteoporosis, vitamin D.

We have reported elsewhere that parathyroid hormone (PTH) causes, in all species studied, a small transient uptake of calcium into bone, beginning within a minute of intravenous injection (Parsons & Robinson, 1971; Parsons, Neer & Potts, 1971). Like the activation of adenyl cyclase (Chase & Aurbach, 1968; Chase, Fedak & Aurbach, 1969) this appears to be a primary action of the hormone. More recent evidence (Parsons & Robinson, 1972a, b) tends to confirm that the calcium shift increases intracellular calcium concentration. The increases in intracellular calcium and cyclic AMP probably mediate the later phases of the bone response to PTH by mechanisms such as those suggested by Talmage, Cooper & Park (1970), Rasmussen (1970) and Rasmussen & Tenenhouse (1970). We now describe experiments that show that this calcium shift into bone is not antagonized by calcitonin (CT).

METHODS AND MATERIALS

Wistar rats weighing 80-100 g were starved overnight. Groups of six rats were injected intravenously with 0.3 ml of a solution containing 1 μCi of 47Ca in an iso-osmotic medium with cysteine and albumin, buffered to pH 4 (Parsons & Robinson, 1971). The hormones to be...
tested were added to the same solution, rats receiving either 60 units of parathyroid hormone or 50 m-units of calcitonin, or both. We found this dose of parathyroid hormone submaximal (Parsons & Robinson, 1971) and the dose of calcitonin supramaximal (Parsons & Reynolds, 1968) in other experiments with similar rats.

Each rat was anaesthetized and bled 10 min ± 20 s after injection. Plasma calcium concentration was measured by atomic-absorption spectrophotometry (Parsons, Dawson, Callahan & Potts, 1970) and 47Ca by scintillation spectrometry as described by Parsons & Robinson (1971).

Bovine PTH, purified by gel filtration on Sephadex, was a gift from Dr H. T. Keutmann and Dr J. T. Potts and had a specific activity of 1500 units/mg [determined by the rat assay of Munson (1961)] in terms of M.R.C. Research Standard A for this hormone. Porcine calcitonin (batch No. K423079) was a gift from Dr R. J. Schlueter (Armour Pharmaceutical Company) and had a stated specific activity of 88 M.R.C. units/mg.

RESULTS

Control rats received only 47Ca and the plasma obtained 10 min after injection contained (per ml) a mean of 2.7% of the injected dose of isotope. Fig. 1 shows the mean isotope concentrations in hormone-treated rats in proportion to this control value, which is set equal to 100%.

![Graph showing isotope concentration in control rats and rats treated with PTH, CT, PTH+CT, CT, and PTH+CT](image)

**Fig. 1.** Effect of adding parathyroid hormone, with or without calcitonin (CT), to an intravenous injection of radioactive calcium. The radioactive calcium remaining in plasma after 10 min is expressed as a proportion of that in the control group, which is assigned a value of 100%. Values are the means from groups of six rats; vertical bars show standard errors. The statistical significance of differences between the groups is reported in the text.

Parathyroid hormone caused a decrease to 88%, a fall which was highly significant ($P<0.001$). Calcitonin caused no decrease and the combination of calcitonin and parathyroid hormone caused a fall ($P<0.001$) similar to that due to PTH alone. Even when calcitonin was given (also intravenously) 5 min before the injection of isotope, it neither affected the 10 min concentration of radioactive calcium ($P>0.05$), nor prevented the response to parathyroid hormone ($P<0.001$).
Action of parathyroid hormone on bone

With respect to total plasma calcium concentration, the mean fall caused by PTH in this experiment amounted to 2.5%, close to that reported elsewhere (Parsons & Robinson, 1971) though not statistically significant with these relatively small groups. However, calcitonin did cause a decrease (6% at 10 min) which was just significant.

DISCUSSION

It is established that calcitonin lowers the calcium concentration in plasma principally by inhibiting bone resorption (Milhaud, Perault & Moukhtar, 1965; Johnston & Deiss, 1966; Hirsch, 1967; Robinson, Martin, Matthews & MacIntyre, 1967; O'Riordan & Aurbach, 1968). One of the actions of parathyroid hormone is to accelerate bone resorption, and CT and PTH thus act antagonistically on the skeleton (Raisz & Niemann, 1967; Hirsch & Munson, 1969).

Existing evidence on the biochemical mechanism of PTH–CT antagonism is of a preliminary and negative kind. Aurbach (1972) reported that calcitonin does not prevent the activation of adenylyl cyclase observed when PTH is added to isolated bone cell membranes. Indeed it causes a further small activation. The present experiments show that calcitonin does not inhibit the small flow of calcium into the skeleton which begins within a minute of PTH injection. A third possibility, that the two hormones interact at the PTH receptor, was discounted by Hirsch & Munson (1969), because in some circumstances calcitonin lowers the plasma calcium in parathyroidectomized animals. In any case, the criteria that would suggest competition at a single receptor site (Schild, 1947) are lacking, since a parallel shift of the PTH log dose–response line is seen only at low doses of calcitonin (Copp, Rafferty, Robinson & Parsons, 1971) whereas higher doses abolish the hypercalcaemia (Hirsch, 1967; Copp et al., 1971).

Thus calcitonin appears not to antagonize any of the three actions of PTH which can confidently be associated with bone cell membranes. It does, however, prevent calcium mobilization and the increase in number and activity of osteoclasts (Gaillard, 1967; Matrajt, Bordier, Tun-Chot, Hioco, Foster & Doyle, 1967), which are among the later consequences of exposing bone to PTH. PTH–CT antagonism may therefore be tentatively located within bone-resorbing cells, osteocytes (Belanger, 1965) as well as osteoclasts (Barnicot, 1948; Gaillard, 1955), or at the bone crystal surface.

Perhaps the simplest hypothesis to account for these facts is that of Rasmussen & Tenenhouse (1970), who proposed that calcitonin increases calcium transport out of bone cells, thus opposing the PTH-induced increase in intracellular calcium. Our evidence does not conflict with this possibility, because the additional radioactive calcium admitted to the skeleton by PTH probably mixes with a large exchangeable calcium pool. The estimated PTH-induced calcium shift was 1.2 μmol (50 μg) (Parsons & Robinson, 1971) whereas approx. 100 μmol of bone calcium is exchangeable within 10 min in such rats. Pumping of calcium out of cells under the influence of calcitonin could therefore occur without diminishing the alteration in distribution of radioactive calcium which is the clearest sign of the PTH-induced calcium shift (Parsons & Robinson, 1972a).

Not all the changes that develop in bone exposed to PTH are blocked by calcitonin. Further studies of this dissociation may advance our knowledge of the biochemical mechanisms involved. For example the release of lysosomal enzymes is unaffected (Reynolds, Dingle, Gudmundsson & MacIntyre, 1968) and so is the liberation of hydroxyproline from collagen.
newly formed by foetal bone in tissue culture (Raisz, Brand, Au & Niemann, 1968; Heersche, 1969). (The diminution in urinary excretion of hydroxyproline caused by calcitonin in vivo may be secondary to inhibition of calcium removal from more fully mineralized bone.) The resistance to calcitonin of these constituents of the PTH response could readily be explained on the Rasmussen–Tenenhousen (1970) hypothesis if they were an independent consequence of the adenyl cyclase activation and were not mediated by an increase in intracellular calcium concentration. Such a possibility requires testing in a bone tissue-culture system.

A biochemical understanding of PTH–calcitonin interaction is of more than academic interest. For example, preliminary trials of long-term administration of calcitonin for the treatment of osteoporosis have been disappointing. Although Baud, de Siebenthal, Langer, Tupling & Mach (1970) claimed a maintained improvement in histological indices of bone mineralization, this has not been confirmed by other authors. There has been general agreement that calcitonin decreases bone resorption in this condition, but bone formation is apparently also decreased. When initial improvement in calcium balance was seen, it disappeared after a few weeks (Hioco, Bordier, Miravet, Denys & Tun-Chot, 1970; Bijvoet, Van der Sluys Veer, Wildiers & Smeenk, 1970; Brown, Thin, Malone, Roscoe & Strong, 1970).

These findings suggest a trial in osteoporosis of calcitonin in combination with another agent capable of promoting bone formation. Although fully rational choice of such an agent is not possible in the present state of our knowledge, parathyroid hormone and vitamin D have both been reported to increase mineralization of the skeleton.

Parathyroid hormone is commonly thought of as an agent promoting bone breakdown. However, in rats chronic injection of low doses of parathyroid extract increases bone density, apparently by stimulating bone formation (Kalu, Pennock, Doyle & Foster, 1970). In fact, the overall physiological effect of the hormone appears to be to increase the plasma calcium concentration by a variety of means (Parsons & Potts, 1972). It promotes calcium absorption from the intestine (MacIntyre & Robinson, 1969; Wills, Wortsman, Pak & Bartter, 1970), an action unaffected by calcitonin (Robinson, Matthews & MacIntyre, 1968). Parathyroid hormone also promotes calcium retention by the kidney (Eisenberg, 1968). As already described, the action of promoting bone breakdown is inhibited by calcitonin, and a combination of PTH and calcitonin administered chronically might be expected to produce a positive calcium balance. Crude parathyroid extract has been found to lose its effect when administered chronically (Melick, Gill, Berson, Yalow, Bartter, Potts & Aurbach, 1967), but there is no evidence that this is true of the pure hormone and the discovery that synthetic fragments of the PTH molecule are biologically active (Potts, Tregear, Keutmann, Niall, Sauer, Deftsos, Dawson, Hogan & Aurbach, 1971) should soon make a trial practicable.

Excessive doses of vitamin D also have a well-established destructive action on bone (Carlsson & Lindquist, 1955). This too is inhibited by calcitonin (Mittelman, Wallach, Chausmer & Bellavia, 1968) and the vitamin has a general anabolic effect on bone at normal dietary concentrations. There is still doubt whether this is a direct or indirect action (Harris, Hoffenberg & Black, 1965). Vitamin D promotes absorption of calcium from the intestine (Wasserman & Taylor, 1969). No direct effect on renal handling of calcium or phosphate can be regarded as established, but urinary calcium loss increases in vitamin D-induced hypercalcaemia (Walser, 1969).

There seems little contraindication to a trial of calcitonin with either of these agents. Both PTH and vitamin D have been shown to promote intestinal absorption of phosphate (Borle,
Keutmann & Neuman, 1963; Harrison & Harrison, 1961) so that oral phosphate supplements should easily counterbalance the phosphaturic action of calcitonin, which is additive to that of PTH (Milhaud & Moukhtar, 1966). Chronic dosage with either PTH or vitamin D can cause ectopic deposition of calcium, but there is some evidence that this effect is opposed by calcitonin (Gabbiani, Tuchweber, Côté, Pahk & Selye, 1968) and in any case it has only been observed after grossly excessive doses. Some information could be obtained by administering calcitonin chronically in combination with either PTH or vitamin D to an animal such as the dog, whose bone metabolism resembles that of man more closely than the rat. However, only clinical trial can determine the possible benefit in human osteoporosis.

REFERENCES


Action of parathyroid hormone on bone


