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EARLY EFFECTS OF PARATHORMONE AND CALCITONIN ON THE NUMBER OF OSTEOCLASTS AND ON SERUM-CALCIUM IN RATS

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Using succinic dehydrogenase staining of osteoclasts, the authors have studied the early effects on these cells of parathormone and calcitonin in rats. Thirty minutes after injection of the hormones the number of osteoclasts had increased (parathormone) or decreased (calcitonin), associated with inverse changes in total serum-calcium. The results confirm earlier studies showing the remarkably rapid changes in the number of osteoclasts after substances acting on serum calcium.

Key words: calcitonin; osteoclasts; parathyroid hormone; serum calcium

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Using the succinic dehydrogenase activity of osteoclasts according to Tatevossian (1973), we showed in an earlier paper that this method is quite sensitive enough to indicate changes in the activity of osteoclasts in ribs of rats weighing 100 g. We tested substances with known effect on S-calcium: ethylene diamine tetraacetic acid (EDTA), azetazolamide and colchicine, which act on the osteoclasts in different ways. We therefore found it worthwhile to study the hormones governing serum calcium, parathyroid hormone and calcitonin. Numerous earlier studies of intact or parathyroidectomized rats carried out to evaluate the effects of these hormones have produced differing results. Tatevossian (1973), using succinic dehydrogenase staining, found that PTE (parathyroid extract) resulted in an increase of S-calcium after 1 h and a rise in the number of osteoclasts 2 h after PTE injection in mice. Baron & Vignery (1981) found in histological studies of rat alveolar bone that PTH first increased the number of osteoclasts and then the number of nuclei per osteoclasts. Calcitonin first reduced the number of nuclei and then the number of osteoclasts. Miller (1978), using quantitative electron-microscopic methods on egg-laying Japanese quail during the period when the medullary bone was inactive, found 20 min after the administration of PTH that over 70% of the osteoclast profiles had ruffled borders. The ruffled borders very rarely occurred on the unstimulated cells.

MATERIAL AND METHODS

Rats weighing approximately 100 g were used. Parathormone was administered in a dose of 1.5 IE/g BW in 0.15 M NaCl, 1 mM HCl and 0.2 mg bovine serum albumin subcutaneously. Calcitonin was given in a dose of 0.2 MRC intraperitoneally. The animals were sacrificed in groups of ten, 10 min, 30 min, 1 h and 3 h after injection. The control groups, 10 animals each, were different for the two experiments.

Blood was collected for the analysis of S-calcium. The right fourth and fifth ribs were removed. The bone was decalcified for approximately 20 h in a 10% solution of EDTA containing 0.1 M tris buffer. The bone was then washed in cold saline solution, quickly frozen in liquid nitrogen and cut at 30 μm intervals in order to minimize the possibility of the appearance of the same osteoclast in different sections. The sections were

stained for succinic dehydrogenase by the method of Pearse (1960) with nitroblue tetrazolium salt as the H-acceptor.

The osteoclast count was carried out in the trabecular bone of the metaphysis of the rib (the entire metaphysis of the rib corresponding to one visual field) and periosteally and endosteally along a predetermined length of cortex of the metaphysis. The length was determined using a scale engraved on the eye-piece of the microscope.

RESULTS

PTH injections resulted in an increase in the number of osteoclasts, which had risen significantly 30 and 60 min after the administration (Figure 1). The S-calcium reacted in the same way but a little more slowly. It was unchanged at 10 min but had risen significantly 30 and 60 min after the administration and normalized at 3 h (Figure 1).

Calcitonin resulted in a decrease in the number of osteoclasts visible at 30 and 60 min after the administration (Figure 2). The number of osteoclasts was unchanged at 10 min after the injection and normalized at 3 h. The S-calcium reacted much more slowly than the osteoclasts. After 1 h there was a significant decrease in the calcium value, which remained low even after 3 h (Figure 2).

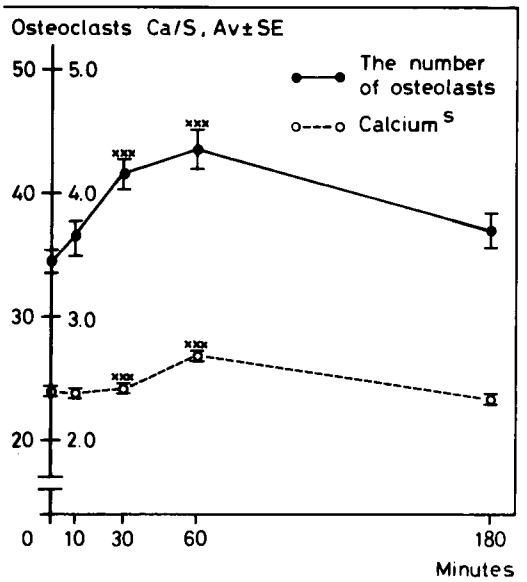

Figure 1. The effect of parathyroid hormone on the number of osteoclasts and on serum calcium from 10 to 180 min after injection.

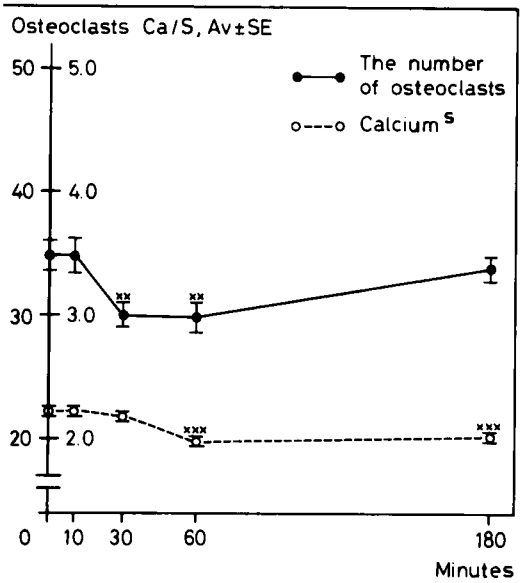

Figure 2. The effect of calcitonin on the number of osteoclasts and on serum calcium from 10 to 180 min after injection.

DISCUSSION

The number of osteoclasts in rat ribs responds very rapidly to different drugs with known effects on the S-calcium balance. In an earlier paper we have shown that EDTA results in an increase in the number of osteoclasts and in the calcium level of serum already 10 min after the administration. Colchicine and azetazolamide, on the other hand, cause a decrease in 30 and 10 min, respectively (Hedlund et al. 1982). The method used is therefore quite sensitive and it was thus a matter of course to study the effects of the hormones which regulate the calcium balance using the same method. The changes which appeared after administration of parathormone and calcitonin were significant at 30 min. The S-calcium had risen concurrently with the increase in osteoclasts after parathormone, while the effect of calcitonin

on S-calcium was delayed, first appearing 60 min after administration. The difference in the effect of hormones and toxic substances is obvious but not surprising.

The rapid effect of parathormone and calcitonin is in accordance with a recent study by Baron & Vignery (1981) which showed that the number of osteoclasts after administration of parathormone and calcitonin had increased and decreased, respectively, at exactly the same time as we found, namely after 30 min. The serum calcium (total) in their study, however, did not increase until 2 h after the dose of PTH and had already decreased 15 min after calcitonin injection. An electron-microscopic investigation by Miller (1978) on avian medullary bone showed that ruffled borders on the osteoclasts had formed 20 min after PTH injection. Tatevossian (1973) was the first to use succinic dehydrogenase staining to demonstrate osteoclasts. He found the first significant rise in the number of osteoclasts 2 h after injection of parathyroid extract in 4-week-old mice, while the peak of the S-calcium was 1 h after injection.

The differences between our investigation and the above-mentioned studies are not easy to explain. Tatevossian (1973) used mice as experimental animals and used extract of parathyroid gland, whereas we used parathyroid hormone. Baron & Vignery (1981), whose dosage we used in our investigation, gave 1 mg calcium as calcium lactate before PTH. The common finding in the above-mentioned studies was that the hormones which regulate the calcium balance act very rapidly on the effector cells, the osteoclasts. This finding is very interesting, since it contradicts the belief that bone tissue is an inert tissue. The bone surfaces must react immediately to a changed level of PTH or calcitonin in the blood, attracting and repulsing, respectively, monocytes which circulate in the blood. It is also interesting to note the study of Mundy et al. (1978) which showed that bone treated with PTH is chemotactic for monocytes. After calcitonin the number of nuclei in the osteoclasts rapidly diminishes, as Baron & Vignery (1981) have shown. The authors believe that this could be due to fission of osteoclasts into indistinguishable mononucleated cells.

Our finding, in comparison with that of Baron & Vignery (1981), suggests that the succinic dehydrogenase staining shows real changes in the number of osteoclasts and not only variations in the enzyme activity of the existing cells. The enzyme succinic dehydrogenase is active in the resorption of bone, and therefore the method used reflects the very resorptive effect of the osteoclasts in response to changes in the hormones acting on them.

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