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THE EARLY EFFECTS OF EDTA, COLCHICINE AND AZETAZOLAMIDE ON THE NUMBER OF OSTEOCLASTS AND THE CALCIUM^{S} IN RATS

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Changes in the number of osteoclasts in rats after injection of ethylene diamine tetra-acetic acid (EDTA), azetazolamide or colchicine were studied using succinic dehydrogenase staining of osteoclasts. As early as 10 minutes after injection EDTA caused a significant increase in the number of osteoclasts. Azetazolamide and colchicine resulted in a decrease in the number of osteoclasts, apparent after 30 minutes and 60 minutes, respectively. The changes in total serum calcium after EDTA and azetazolamide administration took place at the same rate. However, azetazolamide had an effect which was the reverse of the effect of EDTA. It caused an increase in calcium^{s} in spite of a decreased number of osteoclasts. The results of this investigation confirm those of earlier studies, showing very rapid changes in the number of osteoclasts caused by substances giving rapid changes in serum calcium.

Key words: azetazolamide; colchicine; EDTA; osteoclasts

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Tatevossian in 1972, using the succinic dehydrogenase activity in osteoclasts, showed that an increase in the number of osteoclasts in the metaphyseal area of mice was already visible 1 hour after injection of parathyroid hormone (PTH) extract. This method of counting osteoclasts is, therefore, quite sensitive, although it may involve some errors. On the other hand, other authors (Raisz & Niemann 1967, Reynolds & Dingle 1970) first observed changes in the number of osteoclasts several hours after PTH administration in *in vitro* experiments. Mobilization of calcium from the calvarial bones was apparent after a few hours. Qualitative changes, such as an increase in the size of the ruffled borders or the clear zones, become visible as early as 30 minutes after PTH injection in both *in vivo* (Holtrop et al. 1979) and *in vitro* experiments (King et al. 1978). Recently, Baron & Vignery (1981) showed that PTH injected into rats increases the number of osteoclasts and the number of nuclei per osteoclast after 30 minutes and 60 minutes, respectively. Calcitonin, on the other hand, caused a decrease in the number of osteoclasts after 30 minutes and a decrease in the number of nuclei per osteoclast after 15 minutes.

Injections of ethylene diamine tetra-acetic acid (EDTA) in rats result in a decline in the total amount of ionized calcium after 10 minutes (Perris & Whitfield 1967). It is also known that colchicine has a hypocalcemic effect on rats (Heath et al. 1972). Colchicine is considered to inhibit the microtubular function of osteoclasts, which stops bone resorption, but, according to the same authors, it is also conceivable that the drug inhibits the differentiation of osteoclasts.

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The sulphonamide azetazolamide (diamox), which is a carbonic anhydrase inhibitor, causes inhibition of the PTH-induced resorption of bone (Maren et al. 1954, Minkin & Jennings 1972, Waite 1972). We have found that different types of trauma and antigenic challenge result in a rapid increase in the number of mitoses in bone marrow and thymus. At the same time, the number of osteoclasts in the rib metaphysis increases from 24 to 72 hours after the traumatic or immunological event (Johnell & Hulth 1977, Hulth & Johnell 1978), when using the method described by Pearse (1960) and Tatevossian (1973). Because of the contradictory results of previous investigations we were interested to discover, using Tatevossian's method (Tatevossian 1973), how rapidly we could disclose changes in the number of osteoclasts after administration of EDTA, colchicine or diamox, in relation to changes in the total calcium content of serum. We used a considerable number of rats in order to clarify the very early changes occurring from 10 minutes to 3 hours after the injections.

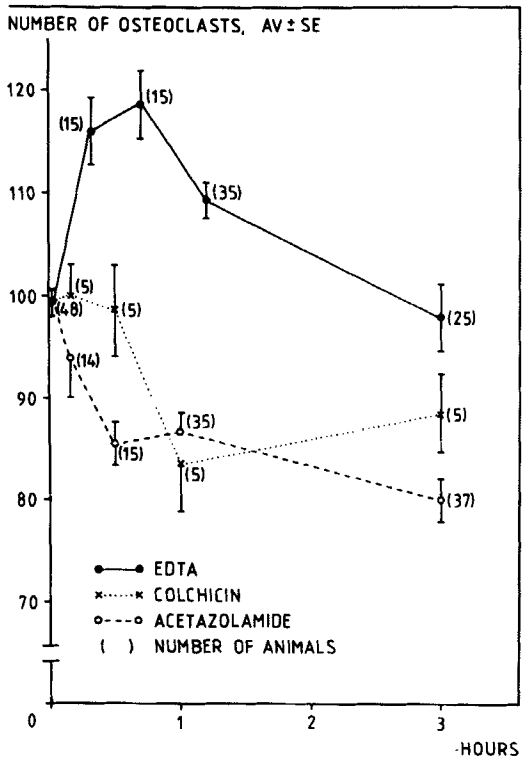

Figure 1. The change in the number of osteoclasts as a percentage of that in the control animals, 3 hours after single injections of EDTA, colchicine or azetazolamide.

MATERIAL AND METHODS

Rats weighing approximately 100 g were used. EDTA was administered in a dose of 30 mg/100 g body weight, colchicine in a dose of 0.05 mg/100 g body weight and azetazolamide (diamox) was given in a dose of 7.0 mg/100 g body weight. All drugs were injected intraperitoneally. The animals were sacrificed in groups of five to seven at different times, from 10 minutes until 3 hours after the injections. (For the calcium[s] determinations, the group of animals was observed for 24 hours after injection.) In the experiments with EDTA and diamox we used several groups of animals, each time with new controls. The total number of animals is shown in Figure 1.

Blood was collected for the analysis of calcium[s], albumin and hematocrit. The right fourth and fifth ribs were removed. The bone was decalcified for approximately 20 hours in a 10 percent solution of EDTA, containing 0.1 M tris buffer. The bone was then washed in cold saline solution, quickly frozen in liquid nitrogen and then cut in a cryostat into five to six, 10 μ thick, sections. These sections were then cut at 30 μ 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 by a rule engraved on the eye-piece of the microscope.

Because the number of osteoclasts in the control animals varied between the different sets of experiments, the changes observed were designated as percentages, with normal values as 100 percent. This made it possible to combine the results of all the separate experiments, which were performed in exactly the same way.

The serum albumin was determined by the bromcresole green method. Serum calcium was also analyzed by flame photometry, and the calcium values were corrected for the albumin level. The hematocrit value was also measured. These laboratory analyses were performed only in a couple of the experimental groups.

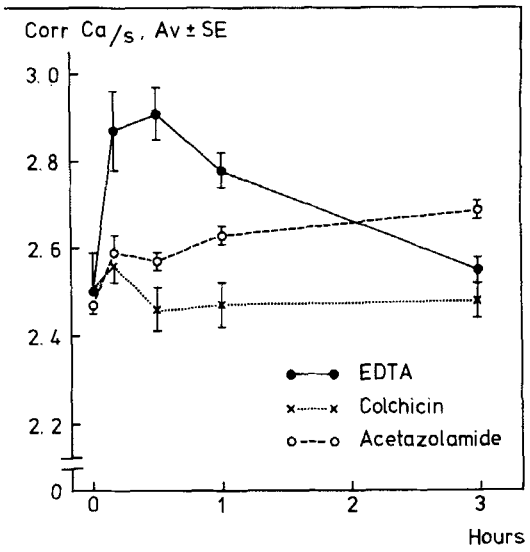

Figure 2. The change in serum calcium (corrected for albumin) during a period of 3 hours following single doses of EDTA, colchicine or azetazolamide. Each point is the average serum calcium value of at least five animals.

RESULTS

EDTA injections resulted in a rapid increase in the number of osteoclasts. Already at 10 and 30 minutes after administration, the number had risen significantly, but it returned to normal at 3 hours (Figure 1). The calcium^s also reacted rapidly. At 10 minutes calcium^s had risen from 2.52 to 2.86 and, at 30 minutes the value was even higher. The peak was reached very rapidly. By 1 hour after injection, the calcium^s value had begun to drop again (Figure 2).

Diamox caused a rapid and significant decrease in the number of osteoclasts counted after 30 minutes, reaching its minimum value at 3 hours after injection (Figure 1). Calcium^s showed an unexpected but slight increase, visible during the first hour (Figure 2). There was also a decrease in pH beginning 1 hour after the injection.

Colchicine caused a slight but significant decrease in the number of osteoclasts, appearing during the first 3 hours, and first visible 1 hour after injection (Figure 1). The corrected calcium^s rightly followed the changes of the osteoclasts, with a fall from the first to the third hour (Figure 2).

DISCUSSION

The present investigation shows that, with the aid of the counting procedure of osteoclasts stained with succinic dehydrogenase, the number of osteoclasts can be observed to change very rapidly when substances known to result in rapid changes of calcium^s are administered. This confirms Tatevossian's early results (Tatevossian 1973) as well as the results of Baron & Vignery (1981). There was a significant increase in the number of osteoclasts as early as 10 minutes after the administration of EDTA. A significant decrease in the number of osteoclasts occurred 30 minutes after diamox injection. The effect of colchicine was – probably because of the relatively low dosage – unimpressive but significant, occurring as a slight decrease after 1 hour.

The effect of injection of a chelating agent such as EDTA on calcium^s is slightly confusing. One would expect a rapid drop in calcium, as found by Perris & Whitfield (1967). On the other hand, Peacock & Care (1976) studied the problem by infusing EDTA into pigs. They found an immediate drop in ionized calcium and a rise in total plasma calcium. We did not investigate ionized calcium, but found the same rapid increase as these authors in total calcium. Thus the bone must be able to maintain the serum calcium level by an immediate release of minerals in response to decreased ionized calcium. Baron & Vignery (1981), also using total calcium determinations, found that the increase in calcium after PTH was first apparent after 2 hours.

In spite of the large decrease in the number of osteoclasts after injections of diamox there was a slight increase in calcium^s. It is known that diamox inhibits parathyroid function (Waite 1972), which explains the rapid decrease in the number of osteoclasts. Other investigators giving prolonged azetazolamide medication to intact rats (Maren et al. 1954) have not shown any changes in calcium^s. Colchicine caused a decrease in calcium^s following a decrease in the number of osteoclasts.

The crucial point of our investigation is whether the increase or decrease in the osteoclasts counted is linked to a real change in the number of osteoclasts. Our results can also be

explained as a change in the activity of the enzyme succinic dehydrogenase, which is the prerequisite for the counting procedure. This would mean that, in a period of 10 minutes, an osteoclast is capable of consistently changing the content of this enzyme. An increase in the enzyme content could possibly make the existing number of osteoclasts in the metaphyseal area easier to count. Taking into account the investigation of Baron & Vignery (1981), it is likely that changes observed in the number of osteoclasts are real, depending on a rapid formation and fission of the osteoclasts as a response to substances causing rapid changes in the serum calcium. The results of *in vitro* investigations on calvaria are in no way comparable. Only with *in vitro* experiments is there unlimited access to osteoclast progenitor cells.

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