Effects of Calcitonin on Osteoclasts in vivo
An Ultrastructural and Histochemical Study*

Ulf Lucht
Department of Cell Biology, Institute of Anatomy, University of Aarhus, Aarhus, Denmark

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Summary. Osteoclasts from the tibial metaphyses of young rats treated with porcine calcitonin were studied by electron microscopy. The animals were sacrificed 1½, 4, 8 or 12 hours after injection of the hormone. In survey sections examined by light microscopy the osteoclasts appeared smaller than in control animals. At the ultrastructural level the osteoclasts showed the following alterations: 1) The typical ruffled border was absent. 2) Acid phosphatase was not present in the extracellular space between cell and bone. 3) The number of large vacuoles was decreased and there was no local accumulation of vacuoles in the cytoplasm. 4) The vacuoles did not contain bone crystals. 5) Vacuoles with cell organelles were increased in number. The majority of these vacuoles were identified as autolysosomes because they contained acid phosphatase and the enclosed cell organelles were partially digested. The above changes were present at all time intervals studied.

The findings suggest that calcitonin decreases or inhibits bone resorption by osteoclasts. A decreased function of the osteoclasts may contribute to the hypocalcemic effect of the hormone. The increased number of autolysosomes is evidence of an enhanced autophagocytosis. Possible origins of the autolysosomes in osteoclasts are discussed.

Key words: Osteoclasts — Calcitonin — Bone resorption — Acid phosphatase — Autophagocytosis — Electron microscopy.

Introduction

Calcitonin is a hypocalcemic hormone which in mammals is located in the C cells of the thyroid gland (see reviews by Rasmussen and Pechet, 1970; Copp, 1972; Anast and Conaway, 1972). Several experimental studies have shown that calcitonin decreases or inhibits bone resorption, both in vivo and in vitro (see review by Anast and Conaway, 1972). It is a possibility that the reduction in bone resorption after calcitonin may be caused by a change in the number and/or function of the osteoclasts since osteoclasts are generally believed to resorb bone (see review by Hancox, 1972). Thus, the number of these cells has been reported to be reduced after treatment with calcitonin, both in vitro (Reynolds, 1968) and in vivo (Foster et al., 1966). Evanson et al. (1967) on the contrary found an increase in the number of osteoclasts after prolonged infusion of calcitonin in intact animals, but this has been explained as the effect of a secondary hyperthyroidism (Rasmussen and Pechet, 1970). Recent electron microscope studies indicate that osteoclasts lose their ruffled border after extended treatment with calcitonin in vivo (Zichner, 1971) or after brief incubation with calcitonin in vitro (Kallio et al., 1972).

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The aim of the present study was to investigate whether calcitonin promotes any early ultrastructural changes in osteoclasts *in vivo* and if so to try to evaluate which cellular functions may be influenced by the hormone treatment. The present study represents a continuation of previous ultrastructural reports on osteoclasts (Lucht, 1971, 1972a, b, e, 1973; Lucht and Maunsbach, 1973) and these papers can be consulted for detailed descriptions of the ultrastructure of normal osteoclasts in young rats.

**Material and Methods**

1–2 week old rats from the Wistar strain were used. Five animals served as controls while 12 animals were injected intraperitoneally with porcine calcitonin (Armour Pharmaceutical Co., Eastbourne, England) in 16% gelatine. The treated animals were divided into four experimental groups each containing three animals. The rats of the first group received 10 MRC units (Medical Research Council units, for definition see Copp, 1972) and were anaesthetized and sacrificed by perfusion-fixation 1 1/2 hour after the injection. The animals of the second group received 10 MRC units and were similarly sacrificed 4 hours after the injection. The animals of the third group received 10 additional MRC units 4 hours after the initial dose of 10 MRC units and were sacrificed 8 hours after the first dose. The animals of the fourth group received 10 MRC units at 4 hours and 8 hours after the initial dose of 10 MRC units and were sacrificed 12 hours after the first dose. For the perfusion-fixation 4% glutaraldehyde in 0.1 M sodium cacodylate buffer was used. The tibial metaphyses were removed and immersion-fixed in the same glutaraldehyde solution for 2–4 hours followed by fixation in 1% osmium tetroxide for 1 hour. Before fixation in osmium tetroxide some pieces of bone were decalcified in 10% EDTA, cut on a tissue sectioner and incubated for 30 minutes in a modified Gomori medium for the demonstration of acid phosphatase, as previously described (Lucht, 1971). The bone pieces were dehydrated in increasing concentrations of alcohol and embedded in Epon 812. The thin sections were stained with lead citrate or with uranyl acetate followed by lead citrate and studied in a JEM 100B electron microscope.

A total of 100 osteoclasts from hormone treated animals were studied. The osteoclasts were equally divided between the four experimental groups and derived within each group from three animals. Further details concerning the present method for the preparation of bone tissue for electron microscopy have been given elsewhere (Lucht, 1972b).

**Observations**

Treatment with calcitonin induced several changes in the osteoclasts. In the survey sections studied by light microscopy the cells in general were smaller than controls. Also a larger number of elongated profiles were encountered in the cross sections. After 12 hours the number of osteoclasts was clearly reduced as judged by counting the cells in random sections.

At the ultrastructural level alterations occurred in the ruffled border and the cytoplasmic vacuoles as well as in the location of acid phosphatase. The changes were already present after 1 1/2 hour and were seen at all subsequent time intervals studied here.

**The Cell Membrane Adjacent to Bone.** In normal rats the majority of osteoclasts have a ruffled border consisting of numerous slender cytoplasmic projections separated by extracellular channels. This border has a close relation to bone under resorption which is characterized by 1) the absence of the electron dense border of bone and 2) the presence of isolated collagen fibers and numerous free bone crystals.