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Electronmicroscopic studies of parathyroid storage granules in the mouse (II). — Effects of hypercalcemia

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SUMMARY : Short-term effects of CaCl_2 -treatment on parathyroid cells of the mouse, especially on their storage granules, were studied at the ultrastructural level. After an injection of 2% CaCl_2 , serum calcium levels (SCL) rapidly increased from 9.9 mg/dl (controls) to a maximum of 12.4 mg/dl at 30 min, while the mean number of type-I storage granules which have a large core (NSG-I) rapidly decreased and that of type-II storage granules which have a small core (NSG-II) increased significantly at 20 and 30 min. Vacuolar bodies containing no or small amounts of content also increased accompanying the augmented type-II storage granules. On the other hand the sum of NSG-I and NSG-II was restricted within narrow limits. Acid-phosphatase activity was occasionally found in storage granules of both types, especially in those of type-II and in vacuolar bodies. Such activities were more frequently detected in calcium-treated animals than in controls. It is concluded that type-I storage granules may be transformed into vacuolar bodies via type-II granules as a result of hydrolysis, and that these processes may be accelerated by hypercalcemia.

INTRODUCTION

In the first report we have observed that in the mouse parathyroid glands under low serum calcium concentrations small secretory granules less than 300 nm in diameter are easily discharged, but larger secretory granules are not released until the serum calcium concentration becomes lowed to a certain level, and have concluded that also in this animal species secretory granules of the parathyroid can be divided into two populations, i. e. abundant small secretory granules and a few large storage granules.

Furthermore, it was supposed that the latter may be released as an emergency supply of parathormone in the case of rapid hypocalcemia. Recently it has been reported that in the rat parathyroid the storage granules, when not

discharged, are subjected to lysosomal degradation and that this process is accelerated by hypercalcemia (SETOGUTI *et al.*, 1985). The aim of this study is to elucidate whether there are similar phenomena in the mouse parathyroid gland which has many secretory granules in contrast with the rat.

MATERIALS AND METHODS

Thirty-six healthy, adult male mice (DDK strain), weighing 40-50 g, were used for this study. They were separated into two main groups, A and B. Group A (28 mice) was used for conventional electron microscopy and subdivided into five small groups; Group A1 served as controls was injected with 0.1 ml normal saline into the peritoneal cavity and sacrificed after 10 min; groups 2-5 were given an injection

of 2% CaCl₂ in 0.1 ml saline into the peritoneal cavity and sacrificed after 10, 20, 30, and 90 min, respectively (Table 1). According to the same method as detailed in the first report, thin sections of the parathyroid chief cell were made by perfusion fixation and the serum calcium level was measured by the OCPC method.

In order to demonstrate the activity of acid-phosphatase, Group B (eight animals) was divided into two subgroups of four mice each: normal control mice and mice 20 min after an injection of 2% CaCl₂. The parathyroid glands of both subgroups were fixed by perfusion with 1/2 Karnovsky's fixative, which contained 0.1 M cacodylate buffer instead of 0.1 M phosphate buffer that was used in group A. The tissues were immediately immersed in 3 % glutaraldehyde in cacodylate buffer for one hour at 4°C and then washed in the buffer containing 7 % sucrose for one hour. These tissues were then sliced by use of a Vibratome (Oxford Laboratory) into 40 μm thick sections. Most of the sections were incubated in the modified medium of Gomori (ERICSON and TRUMP 1965) at 37°C for 40 min; the others were incubated in the above medium lacking substrate and used as controls. The incubated sections were postfixed in 1 % OsO₄ in Millonig buffer for one hour and then processed in the same way as described in the first report. All sections in groups A and B were double stained with uranyl acetate and lead citrate and examined with a JEOL-100B electron microscope.

Table 1. Experimental treatment

Group	Treatment	Number of animals	Time after CaCl ₂ injection
A-1	Solvent only	10	control
2	2% CaCl ₂ 0.1 ml	5	10 min
3	"	4	20 min
4	"	5	30 min
5	"	4	90 min
B-1	Solvent only	4	control
2	2% CaCl ₂ 0.1 ml	4	20 min

RESULTS

As described in the first report, in the mouse parathyroid chief cells numerous small secretory granules and a few large secretory granules, so-called storage granules, are present. The storage granules are membrane-bounded, round or ovoid in shape, ranging 300-600 nm in diameter. On the basis of width of the halo, they could be classified into two types: type-I granules had a core which was greater than two thirds of their diameter; type-II granules contained a core smaller than two thirds of their diameter. In the present work, these two types of storage granules were counted in cell sections containing a nucleus. Granules lacking a content or having only small amounts of content or membranous fragments, were described as "vacuolar bodies" and excluded from type-II granules.

Experimental animals

The ultrastructure of parathyroid gland of the normal mouse was described in the previous report. Comparing with control animals, in the experimental animals at 10 min after an injection of CaCl₂, the plasma membranes were usually less interdigitated and pursued a relatively straight course; the intercellular spaces were more narrow. The most prominent changes at this period were a decrease in number of type-I granules and an increase in type-II granules. At 20 min after the injection, most parenchymal cells exhibited rather angular outlines due to the remarkable decrease in the tortuosity of their plasma membranes; the adjacent two plasma membranes were closely apposed and ran straightly. Free ribosomes seemed to be less densely distributed. Type-I granules were rarely detected, while type-II granules and vacuolar bodies increased in number. At 30 min after the injection, most parenchymal cells are characterized by remarkably narrow intercellular spaces and angular contours. Type-I granules were scarce but type-II granules and multivesicular or vacuolar bodies were easily detected. In the Golgi area, prosecretory granules were not so many as in controls. Cisternae of the granular endoplasmic reticulum (RER) were shortened and dispersed more randomly; their large parallel arrangement appeared less frequently.

Free ribosomes seemed to be slightly decreased in number (Fig 1).

In the present study, the mean number of type- I granules and type- II granules per cell section (NSG- I and NSG- II respectively) in each animal group was compared with the serum calcium level (SCL) of the corresponding group (Table 2, Fig 2). After the injection, SCL promptly increased and reached the maximum 12.4 mg/dl, by 30 min, while NSG- I rapidly decreased, and NSG- II increased significantly at 20 and 30 min. Therefore, as shown in Fig 2 the curve of NSG- I crossed that of NSG- II between 10 and 20 min after the injection; the ratio of NSG- I to NSG- II, which were more than two in controls, was also reversed between 10 and 20 min after the injection; the SCL in the cross point was about 12 mg/dl.

Acid phosphatase

Acid phosphatase reaction was carried out in both the control mice and the mice at 20 min after an injection of CaCl_2 . In the parenchymal cells of both groups, almost all cisternae or saccules of the Golgi lamellae show a positive reaction (Fig 3). In the controls, storage granules of both types usually showed no reaction product, but sometimes they did. Especially, type- II granules exhibited reaction products more frequently and strongly than type- I granules

(Fig 3, inset). In contrast, in the calcium-injected mice reactive storage granules could be more easily found than in the control mice. Sometimes vacuolar bodies indicated reaction products. In addition, lysosomal dense bodies and some lipofuscin pigment granules also displayed reaction products.

DISCUSSION

Many works have been done on the fine structural response of the chief cells of the parathyroid gland to a long-term administration of calcium salts (ROTH and RAISZ 1964, 1966; NAKAGAMI 1967; ROTH *et al.* 1968; ALTENAHN 1970; CAPEN 1971; OLDFHAM *et al.* 1971; STOECKEL and PORTE 1973; ROTH and CAPEN 1974; NUNEZ *et al.* 1974; BOQUIST and FAHRÆUS 1975; CHERTOW *et al.* 1975). The present studies, however, were concerned with the short-term effects (0-90 min) of hypercalcemia on the parenchymal cells of the mouse parathyroid gland.

In the cases of a long-term administration of calcium chloride solution, it has been described that most of the chief cells show a slight decrease in cell volume. But such a finding can not be detected in the present studies. On the other hand, there were similar responses in ultrastructure such as decrease in secretory granules, low electron density of the cytoplasmic

Table 2. Mean number of storage granules per cell section and serum calcium level

Group	Mean number of storage granules			Ratio of type- I to type- II	Serum calcium level (mg/dl)
	Type I	Type II	Sum		
cont.	0.95 ± 0.37	0.43 ± 0.11	1.38 ± 0.48	2.21	9.9 ± 0.3
10 min	0.65 ± 0.16	0.55 ± 0.18	1.20 ± 0.34	1.18	11.8 ± 1.3
20 min	0.50 ± 0.12	0.75 ± 0.11 *	1.25 ± 0.23	0.67	12.2 ± 1.2
30 min	0.49 ± 0.13	0.75 ± 0.11 *	1.27 ± 0.24	0.65	12.4 ± 0.8
90 min	0.46 ± 0.17	0.52 ± 0.16	0.98 ± 0.33	0.88	10.9 ± 1.4

* p > 0.01

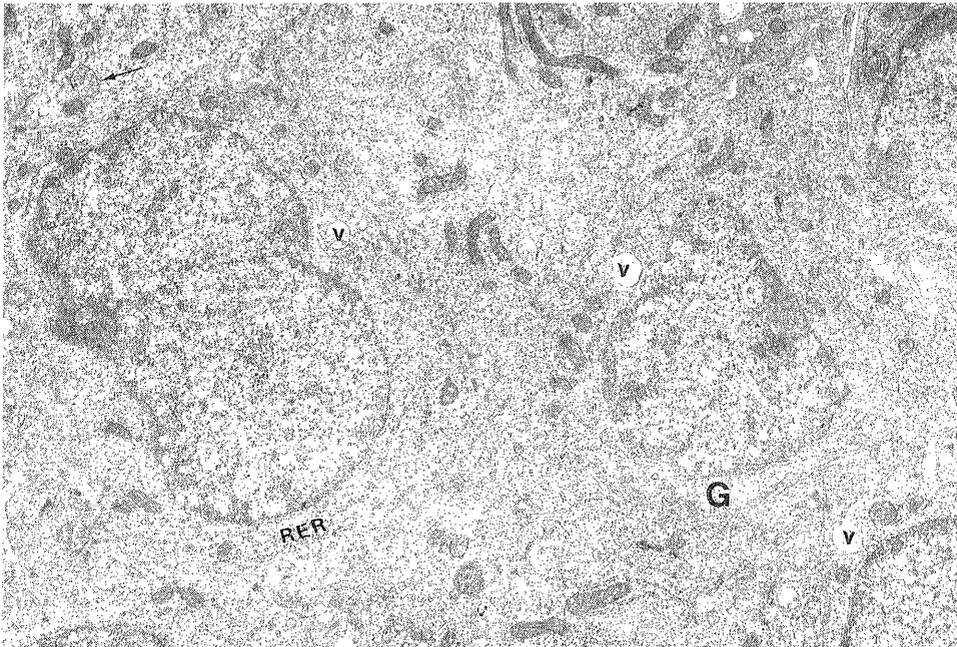


Fig 1 Parathyroid chief cells from 30 min after CaCl_2 -injection. Note remarkably narrow intercellular spaces and angular outlines. Increased vacuolar (V) or multivesicular bodies (arrow) can be detected. Cisternae of RER were shortened and dispersed more randomly. Free ribosomes seemed to be slightly decreased in number. G golgi apparatus, RER granular endoplasmic reticulum. X 7,900

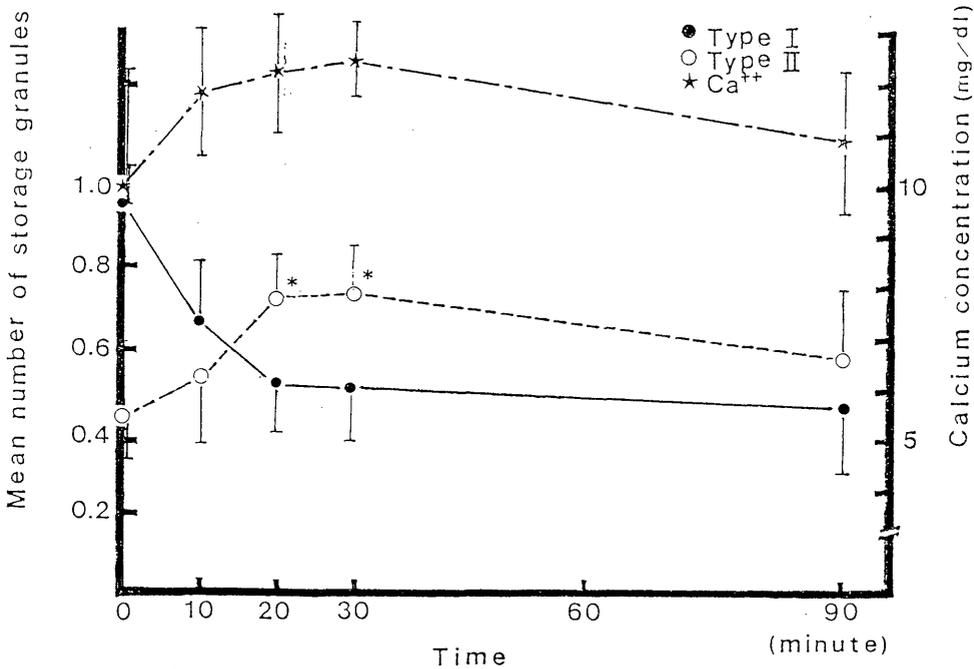


Fig 2 Effect of injection of 2% CaCl_2 on the number of storage granules per cell section and the concentration of serum Ca^{++} (mg/dl). * $p > 0.01$

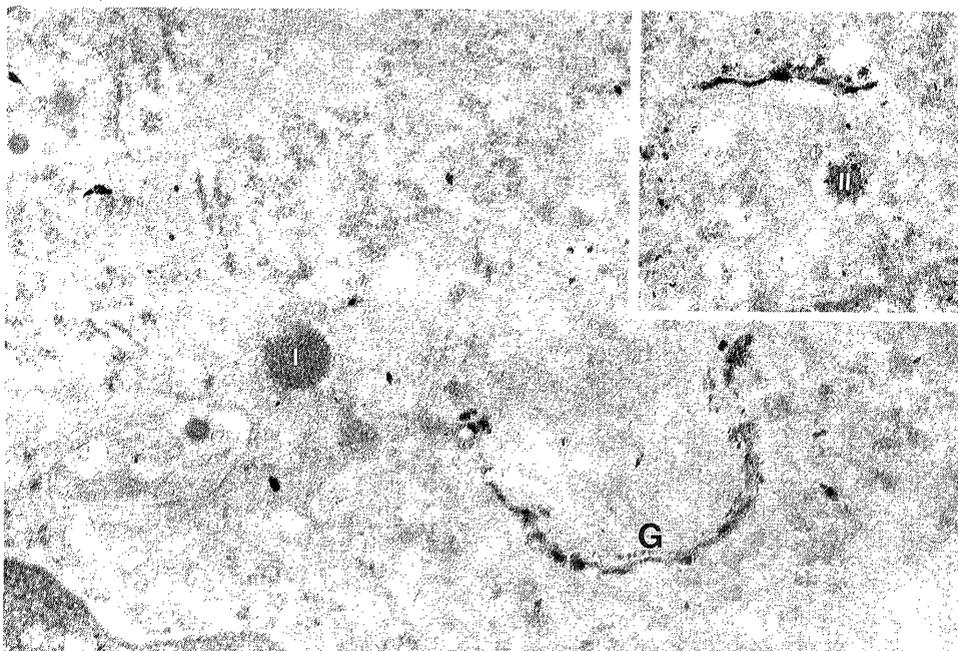


Fig 3 Acid-phosphatase activity in the parathyroid chief cells of a control mouse and a mouse 20 min after CaCl_2 injection (inset). Reactive Golgi cisternae (G) and a non-reactive, type-I storage granule (I). Inset shows a reactive type-II storage granule (II) as well as Golgi cisternae (G). X 28,000

matrix and more angular cell contour. These responses are known to be suggestive of suppressed secretory activity (NAKAGAMI 1967). But, we can stress that in the short-term treatment these may be more temporarily and physiologically as compared with the long-term administration of calcium salts, in which normal activity of chief cells may be injured.

In the present work each CaCl_2 -injection caused a decrease in NSG-I and an increase in NSG-II. In addition, between 10 and 20 min after the injection, both curves of NSG-I and NSG-II crossed each other (Fig 2) and the ratio of NSG-I to NSG-II became reversed. In combination of the increased type II granules, vacuolar bodies were also augmented in number (data not shown). Since the sum of NSG-I and NSG-II is restricted within narrow limits between 0.98 and 1.38 at each time after the injection, it can be suggested that type-I granules may be transformed into type-II granules and finally changed into vacuolar bodies.

In the present study, storage granules usually showed no reaction products of acid phos-

phatase, but some of them, in particular type-II granules, showed a positive reaction (Fig 3). Such reactive storage granules were more easily detected in the calcium-treated group than in the controls. In addition, occasional vacuolar bodies indicated reaction products. On the basis of these results, it can be suggested that the transformation of type-I granules to vacuolar bodies via type-II granules may be caused by hydrolytic process, and that this process may occur under normal calcium concentrations of serum but may be accelerated by hypercalcemia. Our results also coincided with the findings obtained in the rat parathyroid gland (SETOGUTI *et al.* 1985).

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