

Effects of short-term treatment with CaCl₂ or EDTA on the parathyroid glands in pregnant golden hamsters, with special reference to large vacuolar bodies

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Summary. The large vacuolar bodies in the parathyroid glands of pregnant golden hamsters after administration of CaCl₂ or EDTA were investigated. In the parathyroid glands of the pregnant animals 15 min after administration of CaCl₂, the mean serum calcium concentration was significantly high when compared to that of the control animals. In the parathyroid glands of the pregnant animals 15, 30 and 60 min after administration of EDTA, the mean serum calcium concentration was significantly low when compared to that of the control animals. In the parathyroid glands of the pregnant animals 15 min after administration of CaCl₂, the percentage area occupied by large vacuolar bodies was significantly increased when compared to that of the control animals. In the parathyroid glands of the pregnant animals 15 min after administration of EDTA, the percentage area occupied by large vacuolar bodies was significantly decreased when compared to that of the control animals.

Key words: Parathyroid gland, Large vacuolar body, CaCl₂, EDTA, Golden hamster

Introduction

It is well known that the serum calcium concentration of animals is elevated after administration of calcium and lowered after administration of ethylenediaminetetraacetic acid (EDTA). Morphological studies have indicated that the secretory process in the parathyroid glands of animals was suppressed by treatment with calcium (Wild and Becker, 1980; Setoguti et al., 1981, 1985; Wild et al., 1982) and stimulated by treatment with EDTA (Tanaka et al., 1969; Isono and Shoumura, 1973; Soji et al., 1974; Isono et al., 1976; Setoguti et al., 1981).

The parathyroid gland of the golden hamster contains a small number of large vacuolar bodies, besides many secretory granules and a few large secretory granules (Emura et al., 1988, 1989, 1994a,b, 1995; Shoumura et al., 1988a-c, 1989a,b, 1991a,b, 1992). However, with respect to the large vacuolar bodies, some questions still remain to be answered. Furthermore, no report on the effects of calcium or EDTA on the parathyroid gland of the pregnant hamsters has been done.

This investigation was undertaken to study the effects of pregnancy on the large vacuolar bodies in the parathyroid glands of golden hamsters after short-term administration of calcium or EDTA.

Materials and methods

Four- to 6-month-old pregnant golden hamsters were used in this study. The age of gestation was estimated from the time of ovulation. A male golden hamster was caged with a female golden hamster between 9.00 and 17.00 h, and then the animals were separated. Pregnant golden hamsters were detected by palpation 15 days later, as parturition normally occurs between 15 and 18 days. Seventeen CaCl₂-treated and 16 EDTA-treated pregnant golden hamsters were divided into three groups, respectively. These groups were given a 4% CaCl₂ or 4% EDTA solution intraperitoneally at a dose of 150 mg/kg body weight and sacrificed at 15, 30 and 60 min after injection. The control group of 7 pregnant golden hamsters was given saline intraperitoneally. The parathyroid glands of the control, CaCl₂-treated and EDTA-treated groups were removed under sodium pentobarbital anesthesia. The glands were immersed in a mixture of 2.5% glutaraldehyde and 2% OsO₄ in Millonig's buffer at pH 7.4 for 1 h, dehydrated through increasing concentrations of acetone and embedded in Epon 812. Thin sections were cut on a Porter-Blum MT-1 ultramicrotome, stained with uranyl acetate and lead salts, and examined with a Hitachi H-700 H electron

Effects of CaCl₂ or EDTA in pregnant hamster

microscope.

In all animals from each group, 20 micrographs (final magnification x 12,000) were taken from different regions of the parathyroid glands. The areas of cytoplasm and large vacuolar bodies was estimated with the aid of an image analyser (Digigrammer Model-G, Mutoh).

The serum calcium levels of all animals were measured using a Corning calcium analyser 940.

All values are presented as means±SEM. In the serum calcium concentration and the percentage area occupied by large vacuolar bodies in each group, mean

values were compared by Anova (one-way analysis of variance) followed by Duncan's multiple-comparison test.

Results

Serum calcium level

The mean serum calcium concentrations (mg/100 ml) of the control and calcium-treated groups are shown in Table 1. In the experimental group 15 min after administration of CaCl₂, the mean serum calcium concentration was significantly high ($p<0.05$) when

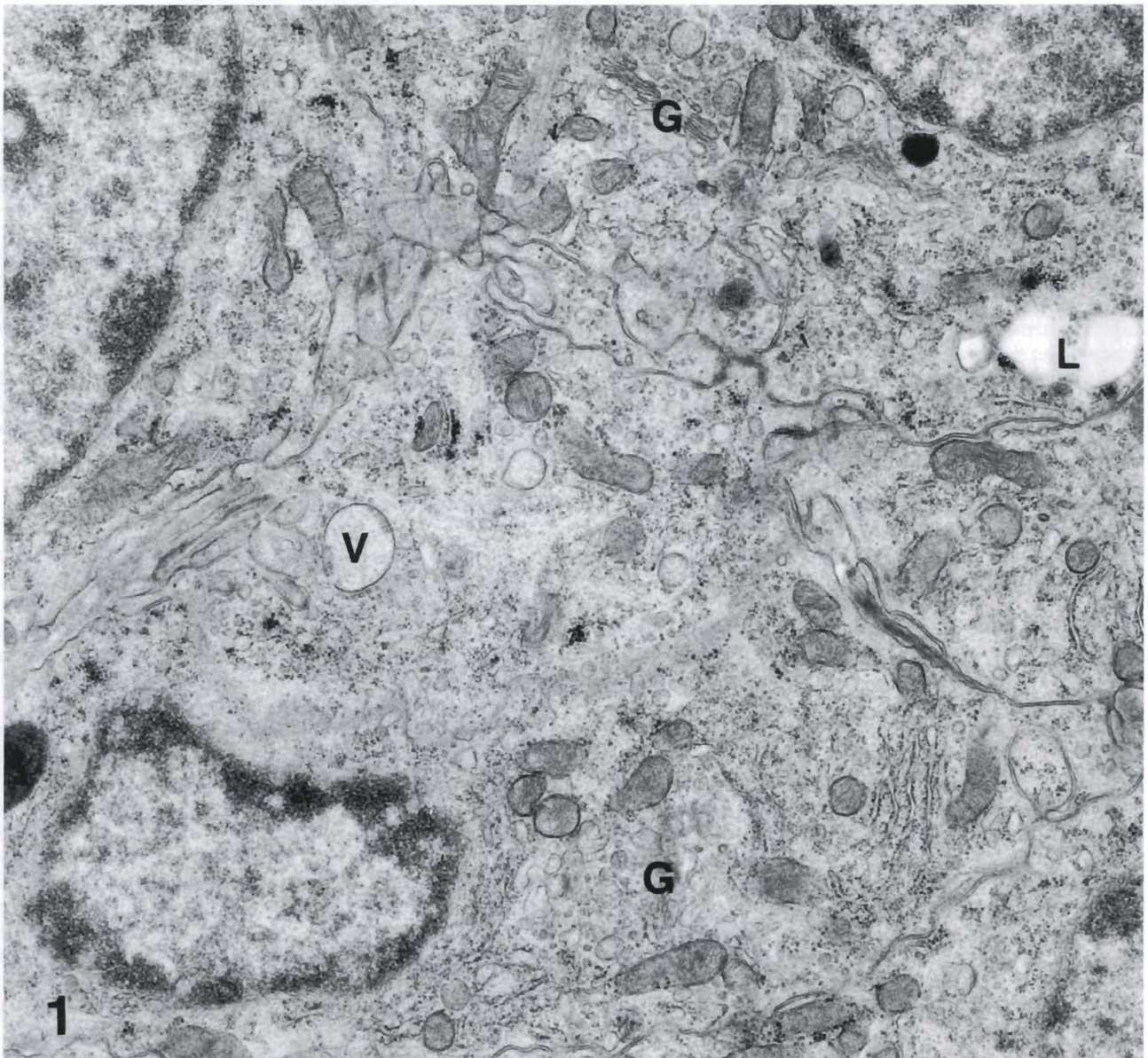


Fig. 1. Parathyroid chief cells from a control pregnant hamster. Relatively well-developed Golgi complexes (G), a large vacuolar body (V) and lipid droplets (L) are observed. x 17,000

Effects of CaCl₂ or EDTA in pregnant hamster

compared to that of the control group. In the experimental groups 15, 30 and 60 min after administration of EDTA, the mean serum calcium concentration was significantly low ($p < 0.05$) when compared to that of the control group.

Ultrastructure of the parathyroid gland

The chief cells were polygonal or oval in shape (Fig. 1). The intercellular spaces were generally narrow, and occasional enlargements contained floccular or finely particulate material. The cytoplasm was scattered

diffusely with free ribosomes and randomly with abundant mitochondria (Fig. 1). Cisternae of the granular endoplasmic reticulum were sometimes arranged in parallel arrays, randomly distributed in the cytoplasm (Fig. 1). Most Golgi complexes were relatively well developed and contained some prosecretory granules (Fig. 1). Secretory granules, large vacuolar bodies, lipid droplets and lysosomes were sometimes observed in the cytoplasm (Fig. 1)

Percentage area occupied by large vacuolar bodies in the parathyroid gland

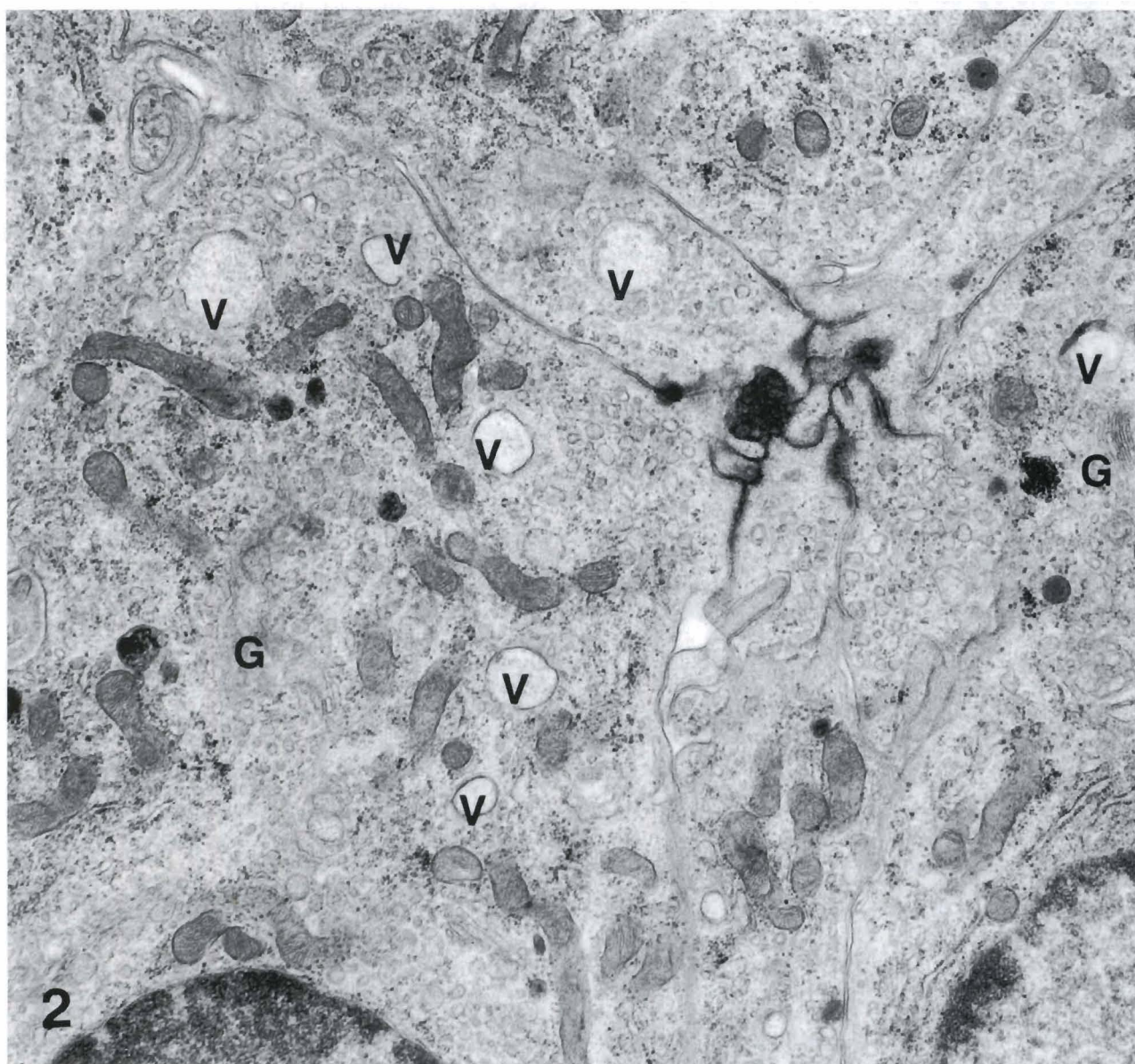


Fig. 2. Parathyroid chief cells from an experimental hamster 15 min after calcium administration. Many large vacuolar bodies (V) are observed, and one of them is seen near the Golgi areas (G). x 17,000

Effects of CaCl₂ or EDTA in pregnant hamster

Table 1. Percentage area occupied by the large vacuolar bodies (VB) and serum calcium level (mg/100ml).

	ANIMAL, No.	VB	SERUM CALCIUM LEVEL
<i>Control group</i>	7	0.27±0.02	10.93±0.14
<i>CaCl₂</i>			
15-min-treated group	7	0.48±0.05 ^a	14.75±0.68 ^a
30-min-treated group	5	0.35±0.10	12.41±0.54 ^b
60-min-treated group	5	0.27±0.01 ^b	11.38±0.19 ^b
<i>EDTA</i>			
15-min-treated group	6	0.16±0.02 ^a	8.89±0.42 ^a
30-min-treated group	5	0.22±0.02	7.44±0.39 ^{a,b}
60-min-treated group	5	0.23±0.02	7.45±0.26 ^{a,b}

Percentages area is presented as percentage of cytoplasmic area. Values are means±SEM. ^a: p<0.05 versus control group; ^b: p<0.05 versus 15-min-treated group (Anova and Duncan's multiple-comparison test).

The results obtained from the control and experimental groups are shown in Table 1. In the parathyroid gland of the experimental group 15 min after administration of CaCl₂, the percentage area occupied by large vacuolar bodies was significantly increased (p<0.05) when compared to that of the control group and the experimental group 60 min after administration. In the chief cells of the experimental group 15 min after administration, many large vacuolar bodies were observed, and some of them were seen near the Golgi area (Fig. 2). There was no significant difference between the control and experimental groups with regard to large vacuolar bodies 30 min after administration.

In the experimental group 15 min after administration of EDTA, the percentage area occupied by large vacuolar bodies was significantly decreased (p<0.05) when compared to that of the control group. There were no significant differences between the control and experimental groups 30 min or 60 min after administration with regard to large vacuolar bodies.

Discussion

It is well known that the serum calcium level of animals is elevated after administration of calcium. It has been reported that secretion of parathyroid hormone in the chief cells is suppressed earlier than synthesis of parathyroid hormone in response to acute hypercalcemia (Oldham et al., 1971; Roth and Capen, 1974). In the present study, in the experimental group 15 min after administration of CaCl₂, the serum calcium level was significantly increased when compared to that of the control group.

In the present study, in the parathyroid glands of the experimental group 15 min after administration of CaCl₂ the percentage area occupied by large vacuolar bodies showed a significant increase when compared to that of the control group and the experimental group 60 min after administration, and in the glands of the experimental group 15 min after administration of EDTA

the percentage area occupied by large vacuolar bodies showed a significant decrease when compared to that of the control group. These findings are fairly consistent with our observations, which have reported the effects of different ages on large vacuolar bodies in the parathyroid glands of golden hamsters after short-term treatment with calcium (Emura et al., 1992). Furthermore, the percentage area occupied by large vacuolar bodies is increased in response to hypercalcemia induced by PGE₂ (Emura et al., 1994b). We think that large vacuolar bodies in the parathyroid glands of the golden hamsters are increased by acute hypercalcemia, and decreased by acute hypocalcemia. It is thought that the percentage area occupied by large vacuolar bodies shows the condition of functional activity of the parathyroid gland. In addition, the present study demonstrated that some of the large vacuolar bodies were observed near the Golgi areas. Recently, we investigated the effects of CaCl₂ treatment on large vacuolar bodies in the parathyroid glands of infant golden hamsters (Emura et al., 1994a). The results suggested that the Golgi vacuoles may change into some of the large vacuolar bodies (Emura et al., 1994a).

Protein A-gold particles are concentrated over the secretory granule but are absent over the large vacuolar bodies (Shoumura et al., 1988c). Accordingly, the secretory granules are thought to include the parathyroid hormone, but not in the large vacuolar bodies.

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Effects of CaCl₂ or EDTA in pregnant hamster

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