# Effect of Parathyroidectomy on Fluctuations in Calcium Transport between the Blood and Mineralized Tissues of Rats

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Parathyroidectomy increases the degree of $^{45}Ca$ fluctuations between the blood and mineralized tissues (bones and teeth) in rats, which is associated with a decrease in $^{14}C$-glycine incorporation into bone proteins. Disturbances in $Ca^{2+}$ metabolism and transport during hyperparathyroidism are partly prevented by parathyroidectomy. It is mainly related to variations in the interaction of parathyroid hormone, calcitonin, $1.25(OH)_2D_3$, and other bioactive substances, but not to initiation of mineralization with protein matrixes.

**Key Words:** *parathyroidectomy; calcium fluctuations; mineralized tissues; bones; collagen*

The most prevalent causes of parathyroid hormone deficiency accompanied by hypocalcaemia are parathyroidectomy (PTEC) for hyperparathyroidism with or without renal failure, damage to the parathyroid gland during thyroidectomy, and radical cervical surgery. In animals and humans PTEC is followed by changes in the content of C- and N-terminal peptide markers of type I collagen and $Ca^{2+}$-binding noncollagen proteins in the blood and urine [4-8,12,13].

Here we studied $Ca^{2+}$ transport between the blood and mineralized tissues. $Ca^{2+}$ fluctuations in mineralized tissues and blood were compared with protein synthesis in mineralized tissues after PTEC.

## MATERIALS AND METHODS

PTEC was performed in 20 albino rats (80-120 g) under hexenal anesthesia. Twenty control animals were sham operated (skin on the neck was cut and sutured). $^{45}CaCl_2$ or 2-$^{14}C$-glycine in 0.9% NaCl (5000 and 10,000 cpm per g body weight) was injected subcutaneously into the thigh 24 h before the end of the study. The animals were decapitated 4, 7, 11-14, and 20-21 days after PTEC. The blood was sampled before killing.

Label fluctuations between the blood and mineralized tissues were studied by the previously described method [2] with minor modifications. Plasma $Ca^{2+}$ concentration was measured by the complexonometric method using ethylenediaminetetraacetic acid and murexide. Tissue $Ca^{2+}$ concentration was estimated with $^{45}Ca$. Protein metabolism was assayed using 2-$^{14}C$-glycine. This study involved radiometers and sensors with low specific density of mica to increase the effectiveness of $^{14}C$ counting. The results were expressed in percent of label incorporation (ratio of the number of pulses per g mineralized tissue over 1 min to the number of pulses per g body weight over 1 min). Specific radioactivity (SRA) was calculated as the ratio of percentage incorporation of $Ca^{2+}$ to the number of pulses per g tissue over 1 min and plasma $Ca^{2+}$ concentration (mg/100 ml).

The predominant transport between the blood and mineralized tissue (SRA difference coefficient, $DC_{SRA}$) was calculated as follows [1]: $DC_{SRA1}=SRA_2$-

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$SRA_1$, $DC_{SRA2}=SRA_3-SRA_2$, $DC_{SRA3}=SRA_4-SRA_3$, *etc*. First we calculated $SRA_1$, $SRA_2$, $SRA_3$, *etc*.

## RESULTS

Small fluctuations in plasma $Ca^{2+}$ (from 9.5±0.2 to 10.8±0.9 mg/100 ml) were revealed in control animals on days 4-21 after administration of the isotope (Table 1). Plasma $Ca^{2+}$ fluctuations in PTEC rats significantly differed from those in control animals ($p<0.01$-$p<0.001$).

Percent of $^{45}Ca$ incorporation into bones of PTEC rats was slightly lower than in control animals in the same period of study (Fig. 1). In control animals with constantly growing incisors the percent of label incorporation was similar to that in the bones, on day 4 after PTEC it was 1.5-fold below the control, but then sharply increased and on day 21 surpassed the corresponding parameter on day 4 by more than 2 times (Fig. 1). These changes were probably associated with genetically programmed transport of $Ca^{2+}$ to constantly growing incisors and sharp decrease in $Ca^{2+}$ release from the incisors after PTEC. As distinct from the incisors, the molars are not characterized by a constant growth. On days 4-21, the percent of label incorporation into the incisors of control and PTEC rats was 3-5-fold lower than in the molars (Fig. 1).

Variations in SRA were similar to those in percent of label incorporation (Table 2), but SRA in PTEC rats was higher than in control animals.

Until the 4th day, transport of the label from the blood into bone exceeded its reverse transport. These specific features were related to the absence of radioisotope before injection. Starting from days 4-7, fluctuations in the predominant transport alternated in the opposite directions. $DC_{SRA}$ in the lower jaw of control and treated rats sharply decreased from days 0-4 to days 4-7 and 7-4. Therefore, $^{45}Ca$ was mainly transported from the bone to the blood (Fig. 1). Changes in the direction of the predominant transport were observed on days 14-21. $DC_{SRA}$ in the femurs significantly decreased from days 0-4 to days 7-14. A less significant decrease in $DC_{SRA}$ was found in the molars of control rats. $^{45}Ca$ transport from the blood to mineralized tissues of all bones and constantly growing incisors dominated on days 14-21 after PTEC. $^{45}Ca$ fluctuations probably concern surface crystals of hydroxyapatite and more soluble carbonate apatite.

Percent of 2-$^{14}C$-glycine incorporation into bone proteins of treated rats decreased more significantly than in control animals (Fig. 2). Comparative study on days 4 and 20 showed that this index in control and PTEC rats decreases by 3 and 25%, respectively.

**TABLE 1.** Plasma $Ca^{2+}$ Concentration in Intact and PTEC Rats (mg/100 ml)

| Rats | Period, days | | | |
|---|---|---|---|---|
| | 4 | 7 | 14 | 21 |
| Intact | 9.8±0.5 | 10.2±0.4 | 10.8±0.6 | 10.5±0.9 |
| PTEC | 5.1±0.4 | 5.9±0.6 | 6.4±0.3 | 5.3±0.7 |

**Note.** Significant differences compared to intact rats ($p<0.001$).

**TABLE 2.** SRA of Bone/Blood and Tooth/Blood $^{45}Ca$ in the Bones and Teeth of Intact Rats and Animals Examined 4, 7, 14, and 21 Days after PTEC

| Object | | Period, days | | | |
|---|---|---|---|---|---|
| | | 4 | 7 | 14 | 21 |
| Lower jaw | intact | 309.39 | 356.86 | 267.03 | 317.24 |
| | PTEC | 511.96 | 451.0 | 415.16 | 586.23 |
| Femur | intact | 417.24 | 444.9 | 353.05 | 406.48 |
| | PTEC | 695.1 | 692.37 | 482.81 | 603.02 |
| Molars | intact | 71.94 | 81.08 | 57.96 | 54.86 |
| | PTEC | 126.27 | 114.75 | 133.13 | 145.66 |
| Incisors | intact | 356.12 | 321.86 | 309.72 | 290.57 |
| | PTEC | 412.16 | 490.17 | 570.78 | 729.43 |

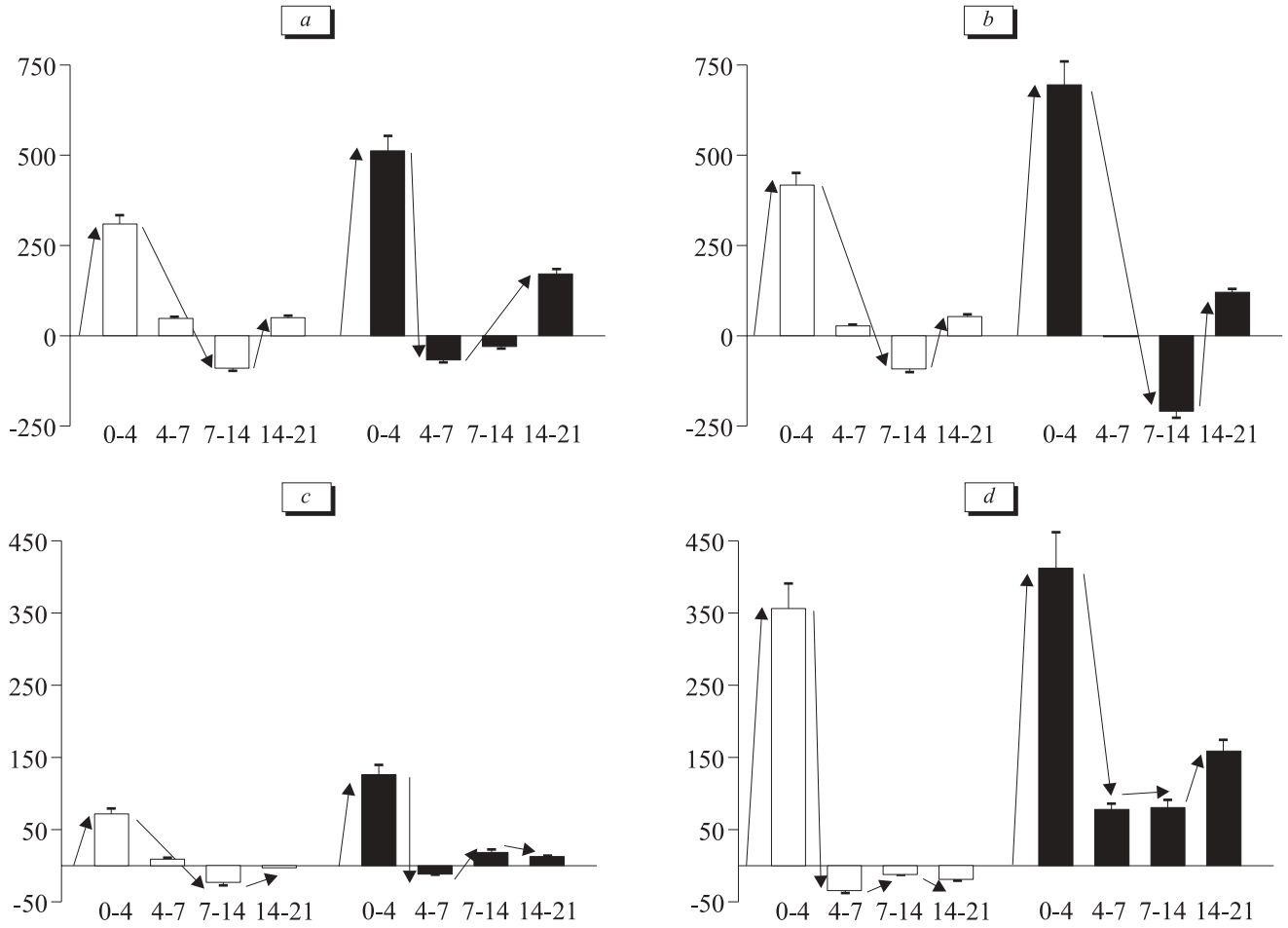


**Fig. 1.** $DC_{SRA}$ in intact (light bars) and PTEC rats (dark bars): lower jaw (*a*), femur (*b*), molars (*c*), and incisors (*d*).

These data show that PTEC increases the reduced percent of $^{45}Ca$ incorporation into mineralized tissues, but decreases the percent of 2-$^{14}$C-glycine incorporation into bone protein during hyperparathyroidism. Type I collagen constitutes 95% of bone protein. The amount of glycine in type I collagen accounts for 30% of amino acids [9]. PTEC decreases the hypocalcaemic effect of osteoclastogenesis inhibitor osteoprogerin [11,14] belonging to the family of tumor necrosis factor-α (TNF-α) receptors. Published data show that interleukin-6 (IL-6) concentration increases in the plasma, but decreases in biopsy specimens from the bones [5]. The concentrations of IL-1β, TNF-α, transforming growth factor-β, and basic fibroblast growth factor-β in biopsy specimens from the bones increases by 3-5 times during hyperparathyroidism [11]. However, the concentrations of hepatocyte growth factor and insulin-like growth factor-1 remain high.

Total and tartrate-resistant activity of bone alkaline phosphatase decreases in the plasma [4,5]. The contents of hydroxyproline (collagen marker) and osteocalcin decreases in the plasma and urine [4]. The amount of type I collagen N-telopeptides decreases, while the content of procollagen C-propeptides increases in the plasma and urine [5,6-8,13].

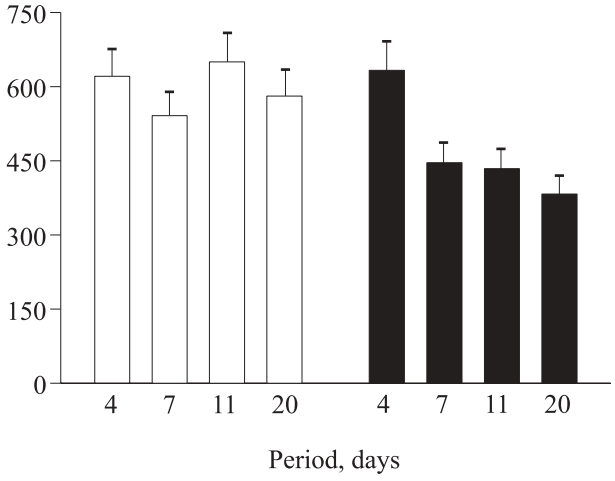


**Fig. 2.** Percent of 2-$^{14}$C-glycine incorporation into the femur of intact arts (light bars) and animals examined 4, 7, 11, and 20 days after PTEC (dark bars).

The polypeptide chain of bone Gla protein contains 49 amino acid residues, including 3 residues of γ-carboxyglutamic acid that bind $Ca^{2+}$. It is unlikely that the 3-fold decrease in plasma Gla protein concentration after PTEC [12] contributes to the increase in $^{45}Ca$ incorporation into mineralized tissues (similarly to the decrease in bone collagen synthesis after PTEC). It should be emphasized that anionic groups of glutamate and aspartate, as well as protonated groups of lysine and arginine in collagen, may bind $^{45}Ca$ after phosphorylation.

Our results indicate that a change in $Ca^{2+}$ metabolism and transport during hyperparathyroidism is partly prevented by PTEC. It is mainly related to variations in the interaction of 3 $Ca^{2+}$-regulating hormones (parathyroid hormone, calcitonin, and $1.25(OH)_2D_3$) and other biologically active substances, but not to the initiation of mineralization with protein matrixes.

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