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EFFECT OF PARATHYROID HORMONE ON $^{45}Ca^{2+}$ ACCUMULATION NEUROSECRETORY CELLS AND ON BLOOD VASOPRESSIN LEVELS AFTER PARATHYROIDECTOMY AND INJECTION OF PARATHYROID EXTRACT

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Despite the important role of calcium ions in secretory processes the role of the Ca-regulating hormonal system in activity of the hypothalamohypophyseal neurosecretory complex still remains largely unstudied. Changes in functional activity of the supraoptic nucleus (SON) under conditions of specific parathyroprival hypocalcemia, discovered by the writers previously [1], suggests that under these circumstances the synthesis of vasopressin (VP) and its release into the blood stream are disturbed.

The aim of this investigation was to study the parathyroid hormone-dependent accumulation of $^{45}Ca^{2+}$ in hypothalamic neurosecretory cells and to determine the blood BP level after parathyroidectomy and administration of parathyroid extract.

EXPERIMENTAL METHOD

Experiments were carried out on 55 male albino rats weighing 180-200 g, divided into four groups: 1) intact (control), 2) parathyroidectomized by electrical coagulation 5 days before the experiment, 3) animals receiving parathyroid extract intramuscularly in a dose of 0.5U/100 g body weight daily for 7 days, 4) animals receiving the same dose of parathyroid extract but in a single injection. The animals of group 4 were decapitated 30 min after injection of the hormone. The total serum calcium concentration was determined spectrophotometrically, and ionized calcium was determined on a Kone-Microlit ion-selective analyzer (Finland), inorganic phosphorus by means of the Bio-La-Test set of reagents (Czechoslovakia), and VP by radioimmunoassay using kits from "Buhlmann Laboratories" (Switzerland). To study parathyroid hormone-dependent entry of $^{45}Ca^{2+}$ into neurosecretory cells, the anterior hypothalamic region was separated, weighed,

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TABLE 1. Effect of Parathyroidectomy and Injection of Parathyroid Extract on Serum Levels of Total and Ionized Calcium, Phosphorus, and Vasopressin in Rats (M ± m)

Parameter	Control (n = 10)	Parathyroidectomy (n = 7)	Parathyroid extract daily for 7 days (n = 9)	Parathyroid extract, single injection (n = 8)
Total calcium, mM	2,07±0,03	1,68±0,01*	1,98±0,04	2,02±0,03
Ionized calcium, mM	0,77±0,01	0,38±0,01*	0,93±0,02*	0,79±0,03
Phosphorus, mM	2,03±0,4	2,37±0,02*	1,55±0,2	1,99±0,1
Vasopressin, pg/ml	5,7±0,2	16,85±1,5*	17,87±1,9*	31,1±2,1*

Legend. Here and in Table 2, asterisk indicates values for which $p < 0.001$.

and incubated in a special medium containing 10^{-10} M parathyroid substance (PTS, from "Sigma," USA), and 1 μCi $CaCl_2$ with specific activity of 12.4 TBq/mole ("Izotop," USSR). The control samples did not contain PTS. The effect was evaluated 15 sec and 30 min later, taking account of the results of previous investigations [6]. The quantity of $^{45}Ca^{2+}$ taken up was expressed in pg/mg weight of tissue. The results were subjected to statistical analysis by Student's test.

EXPERIMENTAL RESULTS

The investigations showed that in the early period after parathyroidectomy (5 days) serum levels of ionized calcium were lowered and of phosphorus raised (Table 1), and functional activity of the neurosecretory cells of SON was enhanced, the content of Gomori-positive neurosecretion (the morphological equivalence of the nonapeptide hypothalamic hormone) in the neurohypophysis was reduced from 0.91 ± 0.041 in the control to 0.825 ± 0.073 conventional unit of optical activity ($p < 0.05$). This is evidence of the intensity of secretion of these neurohormones into the general blood stream [4]. In fact, as was shown by radioimmunoassay, the blood VP level under these circumstances was raised to 16.85 ± 0.15 compared with 5.76 ± 0.2 pg/ml in the control ($p < 0.001$).

After daily injections of parathyroid extract for 7 days the serum ionized calcium level rose whereas the phosphorus level fell (Table 1). The blood VP concentration rose to 17.87 ± 1.9 pg/ml. It is interesting to note that a single injection of parathyroid extract into intact rats led to a significant increase in the VP concentration (31.1 ± 0.7 pg/ml).

In connection with the key role of calcium ions in neurosecretory processes, in order to shed light on the mechanism of elevation of the blood VP level in response to injection of parathyroid extract, the action of PTS on $^{45}Ca^{2+}$ accumulation by cells of the hypothalamus was studied. The results are given in Table 2, and show that PTS potentiates this process. The greatest changes were observed when samples were incubated with PTS for 15 sec, the increase being by 291%. Incubating the samples with the hormone for 30 min also was accompanied by an increase in the Ca-accumulating capacity of the hypothalamic cell populations (by 240%).

Analysis of the results suggests that the increase in Ca^{2+}-accumulating capacity of the hypothalamic region in medium containing physiological concentrations of parathyroid hormone is mainly the result of activation of voltage-gated Ca-channels, as was confirmed by the similar results obtained on nerve cell membranes [3].

Since VP is synthesized mainly in neurons of SON, an increase in the level of functional activity of SON in the early period after parathyroidectomy [1] may indicate possible stimulation of synthesis and secretion of the neurohormone. A decrease in the content of neurosecretion in the posterior lobe of the pituitary indicates potentiation of synthesis, and also of the excretion of neurohormones from the pituitary gland. This conclusion is confirmed by elevation of the peripheral blood level of VP.

Thus both in the early period after parathyroidectomy and after administration of parathyroid extract, the blood VP level rises. These observations, so apparently contradictory at first glance, are based, in our view, on the unidirectional kinetics of the processes enabling increased synthesis and secretion of the hormone. This may be associated, in the first place, with stimulation of transmembrane transport and increased accumulation of Ca^{2+}, for its intracellular level determines the ultimate response of the secretory cells [8]. The results of the present investigation, and also those obtained in the writers' laboratory during the study of the Ca-accumulating capacity of the mitochondria of nerve cells in hypoparathyroidism [5] are evidence that calcium accumulation in the cell takes place in both cases, although by different mechanisms. The increase in the Ca^{2+} concentration in the mitochondria in hypoparathyroidism may be the result of redistribution of the ion under conditions of developing hypocalcemia as was shown in experiments in which a fall in the extracellular

TABLE 2. Effect of Parathyroid Hormone on $^{45}Ca^{2+}$ Concentration (in pg/mg weight of tissue) in Rat Hypothalamus (M ± m, n = 5)

Incubation time	Control	PTS (10^{-10} M)
15 sec	218,0±60,0	636,19±99,59, $p<0,001$
30 min	237,36±96,14	569,66±68,46, $p<0,001$

calcium concentration leads to an increase in its intracellular concentration [9]. This could arise as the result either of activation of processes responsible for the entry of Ca^{2+} into the mitochondria, or inhibition of its outflow. Parathyroid hormone, however, increases the intracellular calcium concentration, evidently through its action on the receptor opening the Ca-channel, which is followed by transmission of a signal to phospholipase C and to the adenylate- and guanylate-cyclase systems [3]. Considering that PTH can pass through the blood-brain barrier [7], the sharp increase in the blood VP level in response to a single injection of parathyroid hormone, without any change in the blood calcium concentration, may be evidence of the direct modulating effect of parathyroid extract on the membrane mechanisms of neurosecretory cell function.

The physiological importance of a significant increase in the blood VP concentration may lie in the need to maintain activity of the hypothalamo-hypophyseal system in order to stabilize disturbed relations between the Ca-regulating and other hormones, for VP is known to have a direct influence on secretion of ACTH and glucocorticoids [2], which have the ability to increase the resistance of the body to unfavorable factors.

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