

# EFFECT OF THYROCALCITONIN ON THE BLOOD CALCIUM LEVEL AND DEGREE OF ADHESION OF THE HEPATOCYTES

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The effect of hyper- and hypocalcemia on the adhesion of rat hepatocytes was studied by the fluid disintegration method. Hypercalcemia was produced by intravenous injection of calcium gluconate, hypocalcemia by injection of EDTA or thyrocalcitonin and by thyro-parathyroidectomy. Hypercalcemia increases but hypocalcemia reduces adhesion of the hepatocytes. Injection of thyrocalcitonin into thyro-parathyroidectomized animals does not aggravate the hypocalcemia produced by the operation, nor does it lower the adhesion of the hepatocytes below the level attained by thyro-parathyroidectomy.

The role of calcium ions in the mechanism of cell adhesion is generally known. After removal of calcium ions the ultrastructure of the cell contacts is altered [5, 14], the degree of adhesion is reduced [9], and the permeability of the plasma membranes is increased [12]. Considering the role of thyrocalcitonin (TCT) in the regulation of calcium metabolism, it might be supposed that this hormone could regulate the degree of adhesion between cells through a change in the calcium concentration in the fluid media of the body or by its direct action on the contact system.

The investigation described below was carried out to study this problem.

## EXPERIMENTAL METHOD

Experiments were carried out on 155 male Wistar rats. The animals were transferred to a calcium-deficient diet 5 days before the experiment began and were kept on that diet throughout the experiment. In the experiments of series I, 76 rats weighing 150–200 g were divided into two groups: hypercalcemia was induced in the rats of group 1 by intravenous injection of 10% calcium gluconate solution in a dose of 0.2 ml/100 g body weight, while in group 2 a state of hypocalcemia was produced by the intravenous injection of 1% EDTA solution in a dose of 0.1 ml/100 g body weight. The animals were killed in batches 10, 20, 90, and 180 min after injection of the preparations. The experiments of series II were carried out on 79 young rats weighing 45–55 g, for young animals are much more sensitive to TCT [3, 10]. Hypocalcemia was induced in half of them by removal of the thyroid and parathyroid glands. From the day of the operation until the experiment (6th day) these rats received thyroid extract, suspended in water, daily by mouth in a dose of 5 mg/100 g body weight [4]. All the animals of this series received a single intravenous injection of TCT in a dose of 1.25 µg/100 g body weight. The rats were killed 5, 30, and 60 min after injection of the hormone. At the time of sacrifice, blood was taken from all the rats and the calcium concentration determined in the serum from it by titration of the complex formed with 0.001 N EDTA solution using murexide as indicator. The degree of adhesion of the hepatocytes was determined by the fluid disintegration method [1]. The disintegrator chamber was loaded with eight standard pieces of liver which were washed with a mixture of 0.067 M phosphate

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TABLE 1. Dynamics of Serum Calcium Concentration and Degree of Adhesion of Hepatocytes Following Injection of Calcium Gluconate and EDTA ( $M \pm m$ )

Time after injection of compound (in min)		Calcium gluconate		EDTA	
		calcium conc. (in mg %)	index of adhesion	calcium conc. (in mg %)	index of adhesion
Control		8,8±0,26	56,0±4,7	8,8±0,26	56,0±4,7
10		12,8±0,32	19,0±1,96	7,3±0,29	96,0±6,32
	P	<0,001	<0,001	<0,001	<0,001
20		11,2±0,11	24,4±3,7	7,0±0,31	81,1±4,5
	P	<0,001	<0,001	<0,001	<0,001
45		9,8±0,57	19,5±1,97	8,3±0,24	78,7±4,6
	P	>0,05	<0,001	>0,05	<0,001
90		9,2±1,02	12,7±2,13	10,1±0,63	67,2±3,61
	P	>0,05	<0,001	>0,05	>0,05
180		9,4±0,9	30,0±1,62	9,1±0,22	60,1±6,78
	P	>0,05	<0,001	>0,05	>0,05

TABLE 2. Dynamics of Serum Calcium Concentration and Degree of Adhesion of Hepatocytes After Injection of TCT ( $M \pm m$ )

Time after injection of compound (in min)		Injection of TCT into intact animals		Injection of TCT into thyro-parathyroidectomized animals	
		calc. conc. (in mg %)	index of adhesion	calc. conc. (in mg %)	index of adhesion
Control		9,0±0,47	13,0±0,77	9,0±0,16	12,1±0,31
Thyro-parathyroid-ectomy		—	—	7,4±0,5	23,1±2,0
	P	—	—	<0,05	<0,001
5		8,7±0,24	15,0±5,1	7,3±0,8	20,8±6,6
	P	>0,05	>0,05	>0,05	>0,05
30		7,4±0,22	17,0±1,73	7,7±0,2	18,0±0,46
	P	<0,05	<0,05	>0,05	>0,05
60		7,25±0,31	20,4±1,0	7,6±1,7	20,0±2,2
	P	<0,01	<0,01	>0,05	>0,05

buffer, pH 7.4, and 5% sucrose solution (1:1). The degree of adhesion of the hepatocytes was judged from the index of cell adhesion: the number of cells detached in a known time interval from a measured area of surface of the tissue when rinsed with a jet of isotonic fluid under constant hydrodynamic conditions [2]. The number of detached cells is inversely proportional to the degree of adhesion of the cells [7].

## EXPERIMENTAL RESULTS

The results of the experiments of series I are given in Table 1. Injection of calcium gluconate led to a substantial increase in the blood calcium level during the first 20 min. By the 45th minute the calcium level was back to normal. Hypercalcemia was accompanied by a sharp increase in adhesion of the hepatocytes. High values of adhesion continued to be obtained for a long time even after restoration of the normal blood calcium level.

For the first 20 min, EDTA significantly lowered the blood calcium level, which returned to normal by the 45th minute of the experiment. Hypocalcemia was accompanied by a decrease in adhesion of the hepatocytes which lasted for some time after restoration of the normal blood calcium level. These results thus indicate a direct relationship between the degree of adhesion and the blood calcium level.

Injection of TCT (Table 2) into the intact animals lowered the blood calcium level from the 30th to the 60th minute. During this period a marked decrease in the adhesion of the hepatocytes was found. Removal of the thyroid and parathyroid glands led to stable hypocalcemia and, at the same time, reduced the degree of adhesion of the hepatocytes. The attempt to produce a further decrease in the blood calcium level by injecting TCT into the thyro-parathyroidectomized rats was unsuccessful. This time the TCT likewise did not change the adhesion of the cells when reduced as a result of removal of parathormone and TCT from the circulation.

Even the temporary hypercalcemia evoked by injection of calcium gluconate was thus accompanied by an increase in cell adhesion. Regardless of its genesis (injection of EDTA, TCT, or thyro-parathy-

roidectomy), hypocalcemia was accompanied by a decrease in adhesion of the hepatocytes. In both hyper- and hypocalcemia the change in the adhesiveness of the cells persisted for some considerable time after restoration of the normal blood calcium level.

The problem of the site of action of TCT requires special examination. Some workers consider that the target for TCT is the bone tissue cells [8, 13]. Meanwhile, the possibility of its direct action on the cell surface of other somatic cells cannot be ruled out. Although injection of TCT into thyro-parathyroidectomized rats had no effect on hepatocyte adhesion, there are nevertheless grounds for considering that TCT cannot act directly on plasma membranes. Injection of TCT did not aggravate the hypocalcemia induced by thyro-parathyroidectomy, confirming data in the literature [11]. It may be that under these conditions the injected TCT is more intensively bound with the tissue proteins and serum proteins, forming inactive complexes which liberate the active hormone only much later than after 60 min (the longest period of these observations) [6].

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