EFFECT OF THYROCALCITONIN ON STATE OF THE BONDS BETWEEN LIVER CELLS

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The action of thyrocalcitonin (TCT) on changes in intercellular bonds in vitro in a calcium-free medium and in a solution containing calcium ions was investigated. The results showed that TCT can affect processes determining the level of adhesion between the liver cells. Under the influence of TCT the dissociating action of a calcium-free solution on hepatocytes was weakened. Meanwhile the effect of calcium on the tissue, which is to strengthen intercellular bonds, was reduced by TCT. Spectrophotometry and pH measurement demonstrated the formation of complexes of TCT with calcium in solution, and this evidently explains the weakening of the calcium effect by TCT.

Since the discovery of thyrocalcitonin (TCT) information on its biological properties and mode of action has been accumulated [1, 3, 8]. However, too little study has been given to the effect of TCT on metabolism in nonosseous tissues.

The state of the intercellular bonds in the tissues is a parameter which can be used to characterize calcium metabolism. According to the literature, Ca++ participates in intercellular bonding by stabilizing the intercellular "cement" and changing the charge on the surface membranes of the cell [5, 7, 9].

In the investigation described below the strength of the bonds between hepatocytes was used in order to estimate the action of TCT on soft tissues. The effect of TCT was studied on the process of dissociation of cells taking place during incubation of the liver in vitro under various conditions.

EXPERIMENTAL METHOD

The method of fluid disintegration suggested by Arkhipenko and Chuich [2, 6], by which the strength of the intercellular bonds in the tissues can be estimated, was used in this investigation.

Changes in the state of the intercellular bonds in these experiments were produced by incubating the liver in ordinary physiological saline and also in the same solution containing calcium ions. Two concentrations of Ca++ were used: one corresponded to hypercalcemia in the intact organism (20 mg%, normal blood calcium 10 mg%), while the second (50 mg%) was considerably higher than the allowable physiological level. TCT was added to the incubation medium in a dose of 1.5 MRC unit/ml solution. A lyophilized preparation of TCT, prepared in the Department of Technology of Endocrine Preparations, All-Union Antibiotics Research Institute, with an activity of 300 MRC units/mg [4], was used in the investigation.

The experiments were carried out on the liver of 37 healthy male Wistar rats weighing 120-150 g. In the tests of group 1, the liver was removed from a rat immediately after sacrifice and placed in a Petri dish with 0.9% sodium chloride, while in group 2 the liver was incubated in a solution of 0.9% sodium

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chloride containing Ca++ in a concentration of 20 mg%, in group 3 the Ca^{++} concentration in the physiological saline was 50 mg%. In the experimental series the liver was placed in the above-mentioned solutions together with TCT. The number of tests in the groups varied from nine to 16.

To secure contact between the hormone and Ca^{++} over a wide area of liver tissue, during the first 15 min of incubation the solutions were injected slowly (60 ml in 15 min) into the vascular system of the isolated liver through the gaping vessels by means of a syringe. After this had been done the tissue was kept in the incubation medium for a further 15 min (the experiments were carried out at room temperature). Six pieces of liver, weighing 250 mg altogether, were placed in the chamber of a disintegrator and the reservoir was filled with 7 ml 0.9% sodium chloride solution irrespective of the character of the experiment. After 10, 20, and 30 strokes of the plunger (periods I, II, and III of the investigation respectively) the number of separated cells was counted in a Goryaev's chamber.

EXPERIMENTAL RESULTS

Considerable accumulation of hepatocytes in the solution (up to 695 ±46 cells in the Goryaev's chamber) was observed by fluid disintegration of the tissue after incubation of the liver in calcium-free solution. The dynamics of the increase in number of cells in this case was characterized by a decline in the absolute increase in the number in the course of disintegration. If the number of cells in the solution after the first period of disintegration was taken as 100%, the increase in the number of cells at the second stage was 58.2% and at the third stage 32.7% of the initial number. The decrease observed in the rate of increase in the number of hepatocytes can be explained by assuming that some cells which were in the solution at the beginning of disintegration had been destroyed as the result of repeated exposure to the jet of liquid, so that the ratio between the number of newly separated cells and the number of cells destroyed was shifted in favor of the latter [6].

Addition of TCT to the calcium-free medium led to changes in the character of the increase in number of cells in the reservoir of the disintegrator. At the first stage the difference between the number of detached cells in the test with and without hormone was very small (310 ±25 and 364 ±47 cells respectively; $P > 0.05$). However, the subsequent increase in the number of cells was significantly less in the group with added TCT. For instance, the number of cells at the second stage of the investigation in this group was increased by 106 ±25 compared with 212 ±31 in the control, of 34.2 and 58.2% of the initial number of cells in the experiment and control respectively ($P < 0.001$). Comparison of the results obtained at the third period of the investigation is particularly interesting. By contrast with the control group, in which the increase in the number of cells continued to decline, reaching 32.7% of the initial level at the end of disintegration, in the experimental group this index was actually a little higher (40.0%). This last observation suggests that under the influence of TCT the resistance of the surface membranes of the cells to mechanical action is increased, and as a result of this, fewer of the cells in the solution were broken up than in the experiments with the calcium-free solution and without TCT, and for this reason the increase in the number of cells remained constant after the last two stages of disintegration.

These experiments thus show that TCT prevents to a certain degree the dissociating action of the calcium-free solution on intercellular bonds, and perhaps increases the mechanical strength of the surface membranes of the hepatocytes.

In the modern view weakening of intercellular bonds in a calcium-free medium takes place as a result of the break-down of protein-calcium complexes, which play the role of a tissue cement [5, 10]. TCT evidently inhibits the process of destruction of the intercellular material and thus prevents separation of the cells which would otherwise occur under the influence of the calcium-free solution.

A study of the action of TCT when present in the solution along with Ca^{++} showed that it is directed toward weakening the effect of Ca^{++} on the tissue. The addition of Ca^{++} alone to the incubation medium sharply reduced the number of cells entering the reservoir of the disintegrator through the action of the jet of fluid (33 ±5, 86 ±9, and 173 ±25 cells with a Ca^{++} concentration of 20 mg%, and 32 ±5, 77 ±8, and 116 ± 17 cells with a Ca^{++} concentration of 50 mg%), whereas if TCT was present these figures were much higher (111 ±20, 206 ±22, and 337 ±37 cells and 35 ±6, 113 ±24, and 159 ±25 cells respectively with 20 and 50 mg% Ca^{++}). The action of the hormone was more clearly exhibited in a medium with Ca^{++} in a concentration of 20 mg%, corresponding to a state of hypercalcemia ($P < 0.01$ at the first and third and $P < 0.001$ at the second stage of the investigation). With an increase in the Ca^{++} concentration to 50 mg%, the action of TCT was weakened (the difference between the control and the experimental groups was not significant at any stage of the investigation).

Weakening of the action of Ca^{++} on the intercellular bonds in the presence of TCT was possibly due to partial binding of the ionized Ca^{++} by the hormone. Spectrophotometric tests and measurements of pH showed that when Ca^{++} and TCT reacted in solution, there was a decrease in the optical density and an increase in the pH of the TCT solution. This may serve to confirm the hypothesis put forward above. The results suggest that the formation of TCT-Ca complexes interferes with the action of Ca^{++} on the state of the intercellular bonds in the liver tissue.

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