thiosalicylic acid[16] and monothioacetylacetone[17] showed M-S stretching vibration at 392—278 cm^{-1}. In the case of the metal chelates of 1-SH-phz, the bands at 360—312 cm^{-1} and 270—235 cm^{-1} are distinctly understood to be metal-sensitive, and the former may be considered to be associated with the metal-sulfur bond, because these bands can not be observed in the 1-SK-phz spectrum. Both the frequencies at 360—312 cm^{-1} and 273—235 cm^{-1} are in the order Zn>Ni>Co>Pb. It may be suggested that the Zn-chelate has covalent character between zinc and the sulfur atom, because the frequency of the Zn-chelate is the highest.

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UDC 615.272.2.015.42 : 615.076.9

Effect of Thyrocalcitonin on Calcium Efflux in Liver Slices of Rats

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(Received February 25, 1975)

The effect of thyrocalcitonin (TCT) on the calcium content of liver slices has been studied. At 30 min incubation period, TCT significantly ($p<0.01$) increased the accumulation of calcium in the concentration range from 0.1 to 8.0 mU/ml with half-maximal concentration occurring at about 0.1 mU/ml. During the 60 min incubation period, the effect of TCT on the liver calcium accumulation was additively enhanced. On the other hand, the radiocalcium ($^{45}CaCl_2$) efflux in the absence of the hormone increased linearly during the incubation period. The radiocalcium efflux in the presence of the hormone increased later with time than that in the absence of the hormone, and the inhibition of efflux was 25% of the control ($p<0.01$). These data suggest that TCT increased calcium accumulation in the liver cells by inhibiting the efflux of calcium.

It is known that thyrocalcitonin (TCT) increases calcium concentration in kidney cells. A number of studies on kidney cells[2,3] and on HeLa cells[4,5] have demonstrated that calcium entry is a passive phenomenon, while its extrusion is a metabolically dependent transport process. Accumulation of calcium in cultured kidney cells is enhanced by a decrease of calcium efflux with TCT,[3] and parathyroid hormone increases the calcium accumulation by an increase of calcium influx.[2] More recently we found that TCT markedly increased hepatic calcium concentration *in vivo*, and suggested that the action of TCT on liver calcium is not dependent on cyclic adenosine monophosphate (AMP).[6] The present study was therefore undertaken to investigate the effect of TCT on calcium transport in liver slices of rats, especially to examine whether TCT would inhibit the active extrusion of calcium from liver cells. The present results show that TCT increases the calcium concentration of the liver cells possibly by decreasing calcium efflux.

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Materials and Methods

Male Wistar-strain rats, each weighing approximately 120 g, were utilized in these experiments. The animals were kept at room temperature (25±1°) and fed commercial lab chow and tap water *ad libitum*. The animals were sacrificed by decapitation and the liver was perfused with cold 0.25 M sucrose solution to remove the blood.

Liver slices were prepared with a razor blade by a modification of Deutsch's method,[7] and each slice (about 70 mg wet weight per flask) was preincubated separately in 2 ml of Krebs-Ringer bicarbonate buffer (pH 7.4) containing 10 mM glucose, 2.7 mM Ca with 95% O_2–5% CO_2 as the gas phase. After preincubation at 37° for 15 min, the slice was transferred to fresh buffer (described above) with or without thyrocalcitonin (TCT) and incubated for an additional 30 or 60 min. TCT (lyophilized porcine TCT, 68 MRC U/mg protein, Armour Pharmaceutical Company, Kankakee, I11.) was dissolved in demineralized water, and was prepared fresh for each experiment. After incubation, the slice was weighed and the calcium content was determined by atomic absorption spectrophotometry after chloric-acid digestion of the tissue.[8,9]

In the experiments in which the efflux of Ca was measured, the liver slice was preincubated at 37° for 30 min in the Krebs-Ringer bicarbonate buffer (pH 7.4) in the presence of 1.25 μCi $^{45}CaCl_2$/ml of medium. Immediately after preincubation, the radioactive medium was decanted and the tissue slice which incorporated ^{45}Ca was washed and transfered to the nonradioactive Krebs-Ringer bicarbonate buffer with or without TCT (8.0 mU/ml). At 30 or 60 min of incubation, the medium in the flask was decanted and the slice was washed. The radioactivity of the medium and the slice was measured separately by a scintillation spectrometer.[10] The radioactivity of medium plus slice was taken as the total radioactivity (C_0) which was present in the slice at 0 time of incubation. The slices were labeled to the range from 9528 to 14900 cpm/70 mg tissue. The radiocalcium efflux was calculated by an equation of $Ct/C_0 \times 100$ (%) where Ct is the radioactivity which was released from the slice into the medium during the incubation period (Δt).

Results and Discussion

The effect of TCT on the calcium content of the liver slice is shown in Fig. 1. After 15 min preincubation, the liver calcium showed a mean values of 155.2±9.7 μg/g slices (obtained from the data of five experiments). By the incubation for an additional 30 min, the liver calcium increased in the absence of hormone, and was much the same that of the 60 min incubation period. At this stage calcium accumulation seemed readily to be demonstrated by addition of TCT. At 30 min incubation period, the hormone significantly ($p<0.01$) increased the accumulation of increased the accumulation of calcium through the concentration range from 0.1 to 8.0 mU/ml with half-maximal effective concentration occurring at about 0.1 mU/ml. During the 60 min incubation period, the effect of TCT on the liver calcium accumulation was additively enhanced. Thus, it was demonstrated that the amount of calcium retained by the liver slices in the presence of TCT depended on the concentration of the hormone and the length of incubation period.

To examine whether TCT inhibits the active efflux of calcium, liver slice was labeled with ^{45}Ca by the preincubation of 30 min, and the rate of release of the radioactive calcium from the slices into media was measured in the presence and absence of the hormone. The effect of TCT (8.0 mU/ml) on the radioactive calcium efflux is shown in Fig. 2. In the absence of hormone, the calcium efflux increased linearly during the incubation period, suggesting that the efflux could be caused greater than the influx in this experimental system. In the presence of TCT, the calcium efflux increased to a smaller extent than in the absence of TCT. The amplitude of the decrease of calcium efflux was about 25% of the calcium efflux in the control. The difference of the calcium efflux between the control and TCT treated slices was found statistically significant ($p<0.01$) by a paired analysis as to each of 30 and 60 min of incubation.

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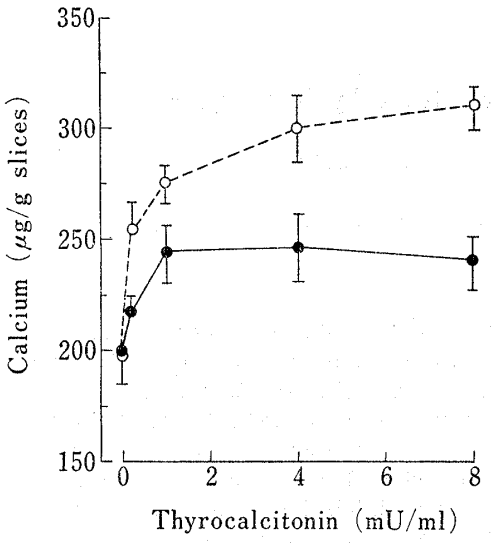

Fig. 1. Effect of Thyrocalcitonin on Calcium Content in Liver Slices from a Normal Rat

Liver slices were prepared as described in the text and incubate for 30 or 60 min in the absence or presence of thyrocalcitonin. Each point represents the mean of values of 5 or 6 flasks of liver slices. The vertical lines give the ± S.E. of means.
incubation time; ●—●, 30 min; ○---○, 60 min

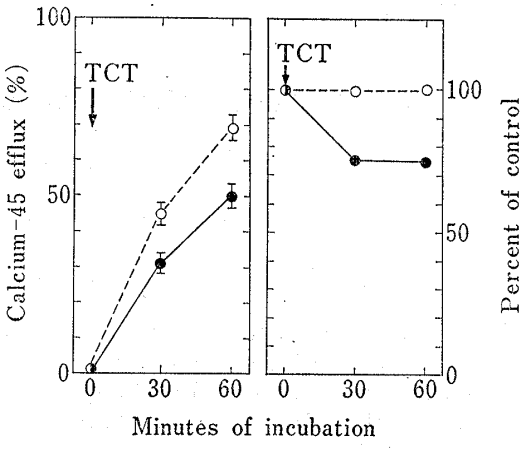

Fig. 2. Effect of Thyrocalcitonin on the Radiocalcium Efflux in Liver Slices from a Normal Rat

Liver slices were incubated with or without thyrocalcitonin (8.0 mU/ml of medium). Left: The ordinate represents the amount of released ^{45}Ca in the term of percentages of the total radioactivity which was initially present in the slices. Right: The decrease of radiocalcium efflux by thyrocalcitonin treatment is expressed as % of control at the respective time points. Each point represents the mean of values of 6 flasks of liver slices. The vertical lines give the ± S.E. of means.
●—●, thyrocalcitonin, ○---○, control

It was reported that TCT did not affect the hepatic cyclic AMP concentration,[11] and that the increased accumulation of calcium in the liver cells by TCT administration was not dependent upon cyclic AMP.[6] The inhibitory effect of TCT on the calcium efflux was exhibited in the liver cells *in vitro* by the present study in accordance with what have been shown in the experiments on kidney[3] and bone cells,[12] suggesting that liver is one of the target organs of the hormone.

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