ACTIVATION OF HUMAN SYNOVIAL MEMBRANE ADENYLATE CYCLASE BY THYROID STIMULATING HORMONE (TSH)

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SUMMARY

A TSH - responsive adenylate cyclase has been demonstrated in human synovium providing the first indication of a possible role for cyclic adenosine - 3',5'-monophosphate (cyclic AMP) in synovial membrane function. TSH activation of the synovial membrane preparation was completely inactivated by TSH antibodies. These findings indicate a possible mechanism for the pathogenesis of myxedema arthritis and perhaps the presence of other hormonal controls for the biosynthesis and secretion of synovial fluid.

INTRODUCTION

Myxedema is associated with rheumatic complaints and synovial effusions (1,2). Such findings raised the possibility that synovial membrane might contain receptor sites for thyroid and/or pituitary hormones. In recent years it has become clear that the mediator of hormone effects on many tissues is the adenylate cyclase-cyclic 3',5'-AMP system (3). We now report the presence of adenylate cyclase activity in normal human synovial membrane which is activated by thyroid stimulating hormone.

METHODS AND MATERIALS

Synovial membrane was obtained at surgery from patients undergoing tibial tubercle transplant for recurrent dislocating patellas. None of these patients had had a patellar dislocation for a period of six months prior to surgery, and the synovial membrane was normal by gross and light microscopic examination. A thin, mono-

layer of synovial membrane was dissected rapidly from underlying subsynovial connective tissue and homogenized at 4°C in 0.25 M sucrose, pH 7. The particulate fraction of synovial membrane recovered after centrifugation in the cold (4°C at 12,000 x G) for 15 minutes was used as the enzyme source. Adenylate cyclase activity was assayed by the method of Krishna, Weiss and Brodie (4) measuring the conversion of α-labeled ATP^{32} to cyclic 3',5'-AMP^{32}. The incubation conditions have been previously described (5). Incubations were performed at 37°C for 5 minutes. The data are expressed as picomoles of cyclic adenosine - 3',5'-monophosphate formed / mg of protein / 5 min $\pm$ a S.E. of the mean. Control incubations lacked TSH. Bovine TSH (2.21 USP units/mg) was a gift of the Endocrinology Study Section of the National Institutes of Arthritis and Metabolic Diseases. Burro anti-bovine TSH antibody was a gift from Dr. Ira Pastan, National Cancer Institute, National Institutes of Health and was prepared as previously described (6).

RESULTS AND DISCUSSION

The mean resting adenylate cyclase activity for three separate synovial membrane preparations was 430 $\pm$ 55, whereas TSH, in graded doses, produced a 100 to 150 per cent increase above control values of mean adenylate cyclase activity (Table 1). To determine the specificity of the TSH response, experiments were undertaken using burro anti-bovine TSH antibodies (Table 2). Burro serum and TSH antibody mixtures had no significant effect on resting adenylate cyclase activity (850 $\pm$ 150 and 530 $\pm$ 150, respectively). Paired incubations containing control sera or TSH antibody demonstrated that TSH antibody totally abolished adenylate cyclase activity in response to TSH stimulation (2530 $\pm$ 620 vs 310 $\pm$ 120) $p < 0.0001$.

Recent evidence has been presented which indicates the possible contamination of NIH-TSH preparations with other compounds

TABLE I

EFFECT OF GRADED DOSES OF TSH ON SYNOVIAL MEMBRANE ADENYLATE CYCLASE ACTIVITY

TSH (mU/ml)	Picomoles cyclic 3',5'-AMP accumulated/mg protein/5 min
0.0	430 ± 55
0.7	333 ± 67
3.5	633 ± 317
7.0	742 ± 125
35.0	878 ± 61
70.0	1117 ± 370
140.0	875 ± 75

TABLE 2

EFFECT OF TSH ANTIBODY ON THE TSH-MEDIATED ACTIVATION OF SYNOVIAL ADENYLATE CYCLASE

	Picomoles cyclic 3',5'-AMP accumulated/mg protein/5 min*
Control	
+ Control Serum	850 ± 150
+ TSH Ab	530 ± 150
TSH (35 mu/ml)	
+ Control Serum	2530 ± 620
+ TSH Ab	310 ± 120

* Each value represents the mean ± SE of three samples.

capable of stimulating DNA synthesis in New Zealand rabbit chondrocytes in tissue culture (7). These conclusions were based on the absence of chondrocyte DNA stimulation when purer TSH preparations

were utilized, and the authors suggested the possible occurrence of a new pituitary hormone which is a contaminant of the NIH-TSH preparation with the capacity to initiate chondrocyte proliferation. Since the pure TSH preparations were not available to us, we have been unable to test the activation of synovial membrane by these hormones. Thus, at this time, we cannot determine whether human synovial membrane adenylate cyclase is activated by a new pituitary hormone identical or similar to the one proposed by other workers for the rabbit chondrocytes (7). Nonetheless, our data clearly demonstrate the presence of adenylate cyclase activity in human synovial membrane, its activation by NIH-TSH preparation, a concentration - response curve for the NIH-TSH, and complete inhibition of TSH activation in the presence of burro antibovine TSH antibodies. The presence of a TSH - responsive adenylate cyclase in human synovial membrane suggests a role for this pituitary hormone in the production of increased synovial fluid in myxedema and may indicate a broader role for pituitary hormones in the normal control of biosynthesis and secretion of synovial fluid by synovial cells.

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