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TSH-STIMULATED ELECTRICAL EXCITATION IN THYROID CELLS

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This report demonstrates TSH-stimulated electrical excitation in cultured porcine thyroid cells. TSH depolarizes the thyroid cell membrane potentials and causes the appearance of action potentials, which occur in a burst. The burst is preceded by depolarization and after the burst, during which usually 2 spikes are seen, a repolarization occurs. This TSH-induced electrical excitation is associated with iodide discharge.

It has been suggested that change in membrane permeability is an important initial step in stimulus-secretion coupling(1,2). Stimulus has been postulated to cause electrical excitation in gland cells(2). All nerve and muscle cells are electrically excitable, i.e., a membrane potential above a certain threshold causes action potentials. It is now apparent that some gland cells share this property with nerve and muscle cells; pancreatic islet β-cells are electrically excitable(3). The primary effect of thyrotropin(TSH) on thyroid cells most likely occurs at the level of the cell membrane. This view is supported by the finding that TSH affects the transmembrane potentials of the thyroid cells. Unfortunately the effects of TSH has not been studied hitherto during continuous monitoring of the membrane potentials with an indwelling electrode.

Thyroid cell cultures have been used for electrophysiological studies of thyroid cells(4,5). We will show that TSH depolarizes thyroid cell membrane potentials and causes the appearance of action potentials. This TSH-induced electrical excitation is associated with iodide discharge.

MATERIALS AND METHODS

Thyroid cell culture: Thyroid cells were obtained from porcine thyroid glands as described previously(6). Freshly isolated cells were suspended(3×10^6 cells /ml) in Eagle's minumum essential medium(MEM) supplemented with 10% new-born

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calf serum and antibiotics(penicillin, 200 units/ml; streptomycin, 50 μg/ml). Cells were incubated at 37°C in a 95% air: 5% CO_2 water-saturated atmosphere in the presence of 0.1 mU/ml TSH for 6 days.

Electrophysiology: The electrophysiological method used in this study was described previously(5). The culture dish was mounted on the stage of a Nikon inverted phase-contrast microscope. Identification of the cells and the microelectrode tip was performed under x400 magnification. Glass capillaries filled with 3 M KCl were used as recording electrodes, which were driven by a hydraulic system mounted on micromanipulators. Potential changes were measured by a bridge circuit. The dish was perfused continuously with Eagle's MEM warmed to 37°C. The pH of the medium was maintained with 5% CO_2 in air.

Iodide discharge: Measurements of iodide discharge was performed after loading the cells with iodide(7). Aliquots of the washed cell suspensions were incubated in the presence of 1 mM methylmercaptoimidazole(MMI) in air for 30 min in a final volume of 500 μl phosphate buffered-saline glucose(PBSG)(6) containing $Na^{127}I$(0.5 μM, final) and 0.1 μCi $Na^{125}I$. After 30 min incubation, 50 μl of TSH were added and the incubation was continued. It was stopped by adding 5 ml of PBSG rapidly, and the tubes were centifuged at 1,500xg for 3 min. The supernatants were aspirated and the cell pellets were washed twice with PBSG. The radioactivity of the washed cell pellets was measured in a well-type scintillation counter. The absolute amounts of iodide were calculated from the specific activities of the original iodide solution.

Materials and etc.: TSH was obtained from Armour Pharmaceutical Company(Phoenix, Arizona, U.S.A.); new-born calf serum and Eagle's MEM from Flow Laboratories (Irvine, Scotland, U.K.) and $Na^{125}I$ from New England Nuclear(Boston, Massachusetts, U.S.A.). All other chemicals were of the highest purity available commercially. Experiments were conducted at least 4 times. Typical data and the final concentration of TSH are shown in the text and figures.

RESULTS

TSH-stimulated electrical excitation in thyroid cells(Fig. 1) Porcine thyroid cells were cultured in the presence of 0.1 mU/ml TSH for 6 days. Alterations in membrane potentials after administration of 10 mU/ml TSH were recorded(Fig. 1). The resting membrane potentials were -70 mV; 10 mU/ml TSH depolarized the membrane potentials and caused the appearance of action potentials, which occurred in a burst. The burst was preceded by depolarization, and after the

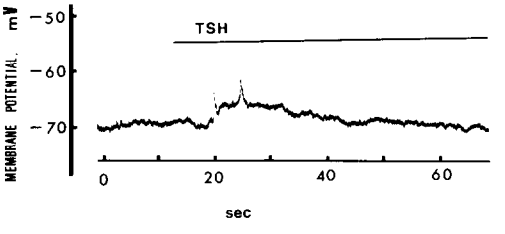

Fig. 1. TSH-stimulated electrical excitation in thyroid cells; depolarization and action potentials. Thyroid cells were cultured in the presence of 0.1 mU/ml TSH for 6 days and then 10 mU/ml TSH-induced electrical activity was studied. The start of the horizontal bar indicates the time of switching to the solution containing the 10 mU/ml TSH. A solution containing no additional TSH did not induce electrical excitation.

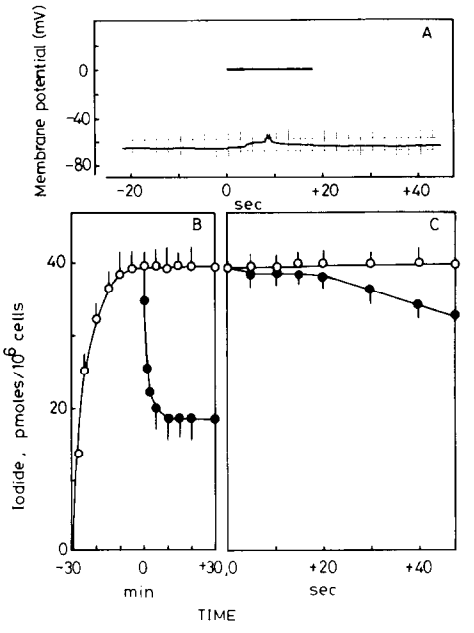

Fig. 2. TSH-induced depolarization and action potentials(A) and iodide discharge(B,C). Thyroid cells were cultured in the presence of 0.1 mU/ml TSH for 6 days and then TSH-induced electrical activity was studied(A). The start of the horizontal bar indicates the time of switching to the solution containing 10 mU/ml TSH and the end of the horizontal bar indicates the time of switching to the solution containing no additional TSH. Other thyroid cells, cultured in the presence of 0.1 mU/ml TSH for 6 days, were washed and then incubated with 0.5 μM $Na^{127}I$ and 0.1 μCi $Na^{125}I$ in the presence of 1 mM MMI(B, C); the cells took up iodide(○). After 30 min incubation with NaI, 10 mU/ml TSH(●) or buffer(○) was added(0 time) and the incubation was continued further for the indicated periods. Each point is the mean±SE of triplicate determinations.

burst, during which usually 2 action potentials were seen, repolarization occurred. The TSH-induced depolarization and action potentials were transient: continuous exposure to TSH did not cause them to reoccur.

TSH-stimulated iodide discharge(Fig. 2) Thyroid cells were cultured in the presence of 0.1 mU/ml TSH for 6 days and then incubated with iodide solution [0.5 μM $Na^{127}I$ and 0.1 μCi $Na^{125}I$(final concentrations)] in the presence of 1 mM MMI. The cells took up iodide(Fig. 2B); the uptake was maximal at 30 min, when 10 mU/ml TSH was added(0 time in Fig. 2); this caused discharge of accumulated iodide, which was observed usually within 10-20 sec(Fig. 2C) and the maximum iodide discharge was observed after about 10 min(Fig. 2B). In this experiment TSH-induced action potentials were observed 8 sec after TSH stimulation(Fig. 2A).

DISCUSSION

This is the first report to demonstrate TSH-stimulated electrical excitation in the thyroid. TSH depolarizes the thyroid cell membrane and causes the appearance of action potentials. This TSH-induced electrical excitation is associated with iodide discharge.

Electrophysiological responses to TSH have been investigated using microelectrodes(8,9,10,11,12). However, the results were controversial and depolarization(8,9,10) and hyperpolarization(11,12) after TSH stimulation were both reported. All of these earlier studies employed multiple impalement techniques allowing only statistical comparison between potential recordings obtained from cells under control and test conditions to be made. Such studies are susceptible to technical difficulties and artifacts. Continuous transmembrane recordings with an indwelling electrode enable the time course and characteristics of any electrophysiological changes resulting from TSH stimulation to be established. In view of the conflicting reports concerning the nature of the electrical response of thyroid cells to TSH stimulation, we re-investigated the effects of TSH on electrical potentials using continuous recordings with an indwelling electrode. The present study reveals that TSH depolarizes the thyroid cell membrane potentials and causes the appearance of action potentials, which resemble those of excitable cells and tend to occur in a burst. These electrical activities are similar to those observed under the influence of insulin secretagogues in β-cells of the Langerhans islets of the pancreas(3).

The observation that TSH initiates action potentials in thyroid cells immediately poses an important question, namely, what is their role? TSH causes the discharge of iodide accumulated in the cells. The discharge is obseved immediately after the action potentials, indicating that the discharge is closely related to TSH-induced action potentials, although the mechanism involved is unknown.

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