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THE DIFFERENTIAL EFFECT OF THYROTROPIN ON THE ELECTRICAL RESPONSES OF THYROID CELLS IN MONOLAYER CULTURES OF VARYING DURATION

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Summary

The acute effects of thyrotropin on the membrane potential of thyroid cells maintained in the presence or absence of thyrotropin (0.2 U/ml) in the culture medium was determined. Monolayer cultures were prepared from porcine thyroid glands and cultured for 4—17 days after which the culture medium was exchanged for a buffered salt solution for intracellular measurements of the membrane potential. Cells were serially impaled with a microelectrode, first in the absence and then in the presence of 10 mU/ml thyrotropin. Cells cultured for 4—9 days depolarized from −29.6 ± 1.7 (mean ± S.E.) to −19.3 ± 1.3 mV within 10 min after acute addition of 10 mU/ml thyrotropin. From 11 to 17 days of culture, basal membrane potentials were lower and, in most instances, cell hyperpolarization occurred within 30 min in response to thyrotropin. There was no difference in the electrical response of cells maintained in culture with or without thyrotropin. However, cells cultured with thyrotropin formed follicle-like structures in contrast to the monolayer formation of cells cultured without thyrotropin. The changes in the basal and stimulated electrical responses occur within a time frame similar to that reported for changes in the biosynthetic capacity of thyroid cells in culture. The data further emphasize the possible regulatory role of the cell membrane in stimulus-secretion coupling in the thyroid.

Introduction

The primary effect of thyrotropin (TSH) on the thyroid cell most likely occurs at the level of the cell membrane [1—6]. This view is supported by the

Abbreviations: TSH, thyrotropin; $TPMP^+$, triphenylmethylphosphonium; Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid.

findings that TSH rapidly affects the transmembrane potential in the isolated intact thyroid [7,8] or cultured thyroid cells [5]. In fact, in the latter system, the changes in potential, as indicated by the uptake of the lipophilic cation, triphenylmethylphosphonium ($TPMP^+$), may depend on a specific interaction of TSH with a receptor on the cell surface. With vesicle preparations of thyroid cell membranes, the TSH-induced uptake of $TPMP^+$ also preceded the increase in adenylate cyclase activity [5].

Monolayer cultures of thyroid cells have been increasingly used to study the acute or chronic effects of TSH on thyroid cell morphology [10—15] and various metabolic and functional aspects [12—19]. The purpose of this study was to determine the acute effects of TSH on the membrane potential of cells cultured in the presence or absence of TSH in an effort to further clarify the role of the plasma membrane of cultured thyroid cells in the recognition of TSH as a physiological stimulant of events culminating in the release of the secretory product.

Methods

Monolayer cultures were prepared from isolated cells of porcine thyroid glands using the collagenase technique [20,21]. Collagenase (0.3%, w/v, in Ca^{2+}-Mg^{2+}-free Hank's balanced salt solution) was used to digest the tissue to obtain a suspension of isolated cells. The isolated cells were separated from undigested tissue and rinsed three times before resuspension in the culture medium consisting of M199 with Earle's salts supplemented with 20% fetal calf serum, 100 units penicillin/ml and 100 μg streptomycin/ml. Some of the cells were cultured with 0.2 U/ml TSH (Armour Pharmaceutical). The cells were cultured at 37°C in a humidified atmosphere of 95% O_2 and 5% CO_2. After three days the cells attached and spread to confluency on the bottom of the petri dish. The culture medium was changed every 3—4 days.

For short term electrical experiments after the culture period, the culture medium was replaced by a Krebs-Henseleit salt solution containing 25 mM Hepes in order to maintain a stable pH without gassing with CO_2. Glucose, 5.6 mM, was added to the medium to provide a substrate. The thyroid cells were viewed with a Reichert inverted phase contrast microscope equipped with a thermoelectric stage for maintaining the temperature of the medium at 35°C. Intracellular electrical responses of thyroid cells were measured by using glass microelectrodes filled with 3 M KCl; the tip resistance varied from 50 to 100 MΩ. The input was led into a Mentor N-950 amplifier via a Ag|AgCl wire placed in the microelectrode. A Ag|AgCl electrode immersed in the bathing solution contained in the culture dish served as the reference electrode. The amplifier possesses systems for negative capacitance neutralization and for measuring the electrode resistance. The electrical responses were monitored on a Tektronix 5031 oscilloscope and simultaneously recorded by a Honeywell 906C oscillograph. The system can be calibrated by injecting a 10 mV square wave signal from the amplifier onto the oscilloscope screen and oscillograph paper.

The electrical responses of cells in monolayer formation were determined first in the absence and then in the presence of 10 mU/ml TSH. Only poten-

tails maintained at a stable level for at least 10—15 s were recorded. When the potential fell sharply towards baseline within a few seconds, impalement was considered unsatisfactory. Cells impaled for a period of 1 min or more did not give an average potential value different from that obtained for shorter periods. The shorter period was necessary in order to obtain a sufficient number of stable measurements to reflect the average membrane potential of the cell population.

The cells were viewed with a magnification of 160—250×, allowing impalement of the cells under direct visual control. Precaution was taken to avoid impaling any cell more than once. This was done by initially positioning the culture dish in an area in which there was a large number of confluent cells in monolayer formation. The microscope stage and the electrode were moved so that cells were impaled in a directed fashion within the area. For each cell successfully impaled the transmembrane potential was recorded. The average membrane potential was determined for each test period.

Results

In these studies cultures of thyroid cells were maintained up to 17 days in the presence or absence of 0.2 U/ml of TSH added to the culture medium. As observed by other investigators [10—15], cells cultured with TSH formed follicle-like structures (Fig. 1) in contrast to the monolayer formation of cells cultured without TSH. For determining the acute effects of TSH on the membrane potentials of both types of cultures, the cells were allowed to equilibrate for 10 min after exchanging the culture medium for the salt solution. Consequently, an average of 30 cells (range 15—60) were serially impaled during a

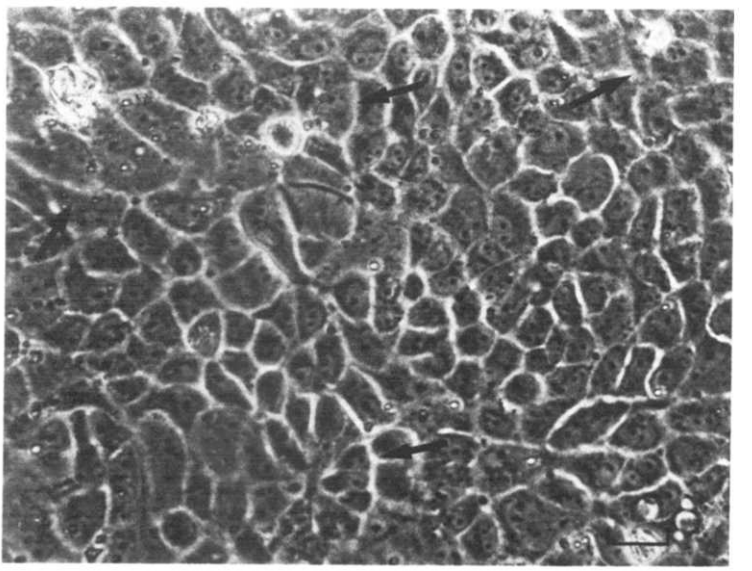

Fig. 1. Phase micrograph of a monolayer of living porcine thyroid cells in culture. The cells were cultured with 0.2 U/ml TSH for 7 days. The cells formed follicle-like structures as indicated by the arrows. The calibration scale indicates 6 μm.

TABLE I

EFFECTS OF TSH ON MEMBRANE POTENTIAL OF PORCINE THYROID CELLS IN MONOLAYER CULTURE

Thyroid cells were cultured in M199 with Earle's salts in the absence or presence (values in italics) of 0.2 U/ml TSH. After culture the medium was exchanged for Krebs-Henseleit salt solution containing Hepes buffer. Cells were impaled for 30 min in the absence and 30 min in the presence of 10 mU/ml TSH. About 30 cells (range 15—60) were impaled during a 30 min period. The values indicate the mean intracellular potential (mV) ± S.E. The columns (A—E) represent data obtained from five series of experiments, each consisting of dishes of cultured thyroid cells prepared on the same day.

Day	A		B		C		D		E	
	—TSH	+TSH	—TSH	+TSH	—TSH	+TSH	—TSH	+TSH	—TSH	+TSH
4			*36.1 ± 3.5*	*23.4 ± 1.9*					31.6 ± 1.4	17.3 ± 0.9
5										
6	*37.9 ± 3.6*	*23.9 ± 1.6*			*21.8 ± 1.3*	*16.2 ± 0.7*	28.1 ± 1.1	16.2 ± 0.8		
7					24.9 ± 1.9	16.6 ± 1.2	28.7 ± 1.5	16.0 ± 1.8		
					37.4 ± 3.8	*18.3 ± 1.2*				
8	35.0 ± 3.5	29.5 ± 3.2	24.6 ± 1.1	15.7 ± 1.2						
	38.2 ± 3.3	28.3 ± 2.2	22.6 ± 1.2	13.7 ± 0.8						
9							18.1 ± 1.6	16.1 ± 0.9		
10										
11			16.0 ± 1.0	20.8 ± 0.8						
			13.6 ± 0.8	19.2 ± 0.8						
12	25.0 ± 2.6	39.8 ± 2.9			*33.3 ± 1.8*	*25.1 ± 1.7*				
	26.2 ± 2.9	*46.8 ± 3.1*								
13							*24.2 ± 2.1*	*17.5 ± 1.8*		
							21.7 ± 1.4	23.9 ± 0.8		
14					19.5 ± 1.9	19.9 ± 0.8				
15							*18.5 ± 1.0*	*20.7 ± 1.1*		
							14.2 ± 0.7	22.6 ± 0.8		
16									*16.6 ± 1.3*	*19.2 ± 1.6*
17	23.9 ± 2.2	30.7 ± 3.3							*19.7 ± 1.1*	*30.0 ± 2.2*
									18.8 ± 0.2	30.3 ± 0.2

period of 30 min before, and 30 min after addition of 10 mU/ml TSH. Table I provides data on the acute effects of TSH on the membrane potentials of five batches of cultures prepared at different times. Fig. 2 shows the frequency distribution of the values of membrane potentials recorded from cells before and after the acute addition of TSH. Cells cultured for 4—9 days exhibited an average basal membrane potential of —29.6 ± 1.7 mV (mean ± S.E.); addition of TSH resulted in cell depolarization to a value of —19.3 ± 1.3 mV ($p <$ 0.001). Mean values between cultures incubated with and without TSH during 4—9 days of culture were not significantly different (Table I). After 11—17 days of tissue culture, basal membrane potentials were significantly lower ($P < 0.001$) than those cultured from 4 to 9 days. Contrary to what was observed in younger cultures, the acute addition of TSH resulted, in most instances, in cell hyperpolarization (Table I, Figs. 2 and 3). For all preparations the basal membrane potential was —20.8 ± 0.8 mV and the potential recorded from cells in the presence of TSH was —26.2 ± 2.0 mV ($P < 0.001$). Upon inspection of Table I (columns C and D) it is apparent that cells that were cultured with TSH from the beginning had a tendency to exhibit higher basal potentials and depolarized after the acute addition of TSH. However, these cultures finally behaved electrically as cultures maintained without TSH. In fact, a comparison of the values of the membrane potentials recorded from 11 to 17-day cultures maintained without TSH to those maintained with TSH show no statistical difference. Fig. 3 shows the rapidity of the electrical

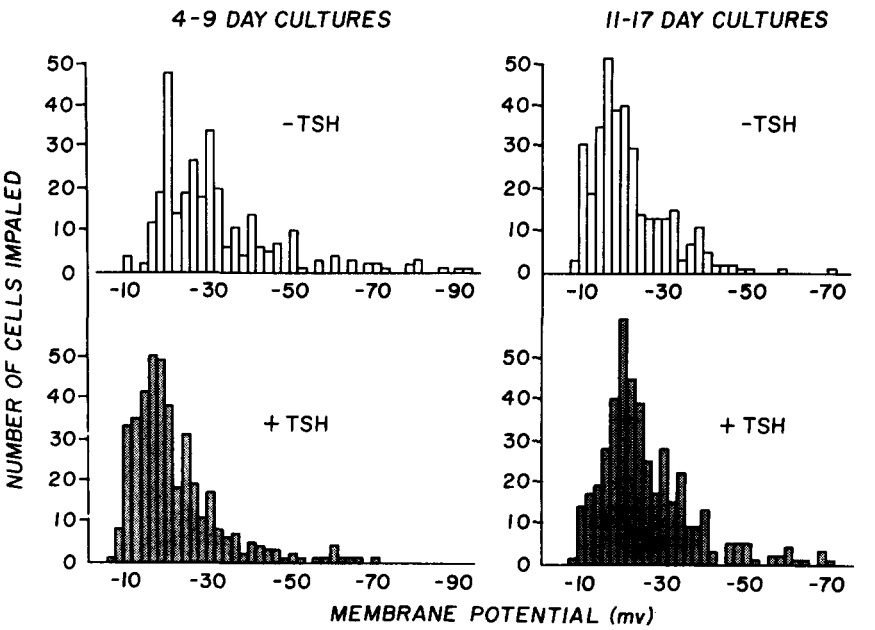

Fig. 2. Frequency distribution of membrane potentials recorded from porcine thyroid cells in monolayer culture. The cells were cultured in (A) for 4—9 days and (B) for 11—17 days with or without 0.2 U/ml TSH added to the culture medium. After the culture period the culture medium was withdrawn and replaced by Krebs-Henseleit salt solution containing Hepes buffer and 5.6 mM glucose. Cells were serially impaled for 30 min in the absence and 30 min in the presence of 10 mU/ml TSH. See Table I for results of individual experiments.

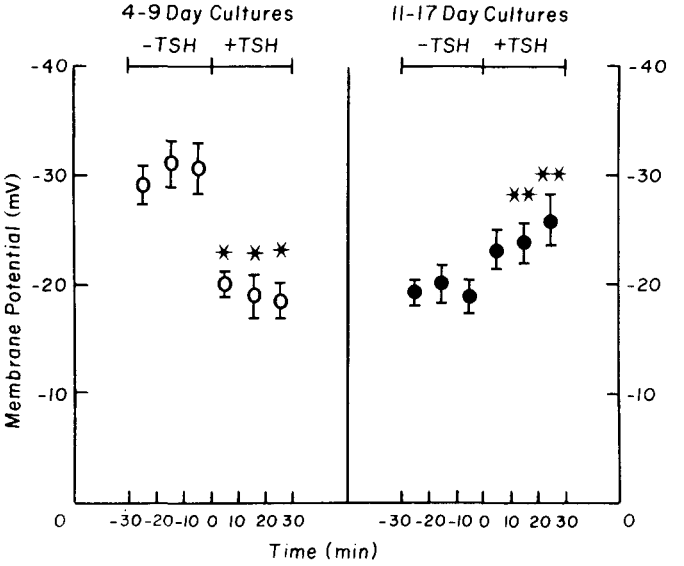

Fig. 3. Time course of changes in the average membrane potential of cultured porcine thyroid cells during sequential incubation in basal medium and medium containing 10 mU/ml TSH. Each point represents the mean ± S.E.; * $P < 0.001$; ** $P < 0.05$, compared to value obtained from −10 to 0 min.

response of the thyroid cells to the addition of TSH. For 4—9-day cultures depolarization occurred within 10 min and the level of the average potential was maintained for 30 min. However the cells of the 11—17-day cultures exhibited a more sluggish response to TSH. The cells hyperpolarized over a period of at least 20 min after which the maximum potential was achieved.

Discussion

This is the first time to our knowledge that membrane potentials have been recorded in thyroid cell cultures. Values for basal membrane potentials in young cultures (4—9 days old), are in agreement with those obtained in different preparations by other investigators [8,9]. The depolarization after addition of 10 mU/ml TSH is also in agreement with the observations of other workers. However, it is of interest that aging of thyroid cells in monolayer cultures is accompanied by a decrease of basal membrane potentials, and contrary to what is observed in young cultures, hyperpolarization occurred after addition of TSH. Hyperpolarization of thyroid cells was recorded by Batt and McKenzie [22] in the perfused mouse thyroid gland after addition of small amounts of TSH (less than 3 mU/ml), while depolarization was found with doses larger than 10 mU/ml. The significance of this remains unknown. Konno and McKenzie [9] showed that infusion of 10 mU/ml TSH resulted in depolarization concomitantly with release of trichloroacetic acid-soluble ^{125}I. Rapoport [21] consistently obtained maximal stimulation of cyclic AMP in response to 10 mU/ml TSH in cultures of thyroid cells. Based on all of these observations, we selected 10 mU/ml TSH for determining the acute effect of TSH on cultured thyroid cells.

It should be noted that thyroid cell cultures retain many in vivo organ

specific characteristics for as long as 7 days and that addition of TSH at the start of culture consistently enhances ^{131}I incorporation into iodoamino acids from the third to seventh day of culture [16]. Radioactive T_4 is recovered in free form from the incubation medium and its amount increases with time in the TSH-treated cultures. TSH also maintains the ability of the cells to synthesize thyroglobulin for at least 8 days of culture [17—19]. On the second week of culture, cells can incorporate ^{131}I into iodinated proteins, but iodotyrosines are not formed [17,18]. Therefore, the changes with aging in basal membrane potentials and in the type of electrical responses to TSH might correlate with the above biochemical changes.

It is important to make a distinction between these studies in which the acute cellular response to TSH was made and the other studies cited above [16—19] in which cells maintained with or without TSH in culture were examined in terms of their biochemical functions without acute exposure to TSH. It would be interesting to determine the ability of cells cultured without TSH to respond to TSH in terms of biochemical and other functional parameters. Recent studies demonstrated that cells maintained in culture without TSH exhibit morphological changes within 15 min in response to an acute challenge with TSH [21]. These changes included the formation of surface microvilli and cytoplasmic projections resulting from both cell retraction and active cytoplasmic extension. Although there is a correspondence in the rapidity with which the cell membrane can respond morphologically and electrically to an acute application of TSH, this may be merely coincidental without any functional relation. The data reported here further emphasize the possible regulatory role of the cell membrane in stimulus-secretion coupling in the thyroid. Hopefully, further studies utilizing the microelectrode to measure the electrical properties of the thyroid cell, as the hormonal state of the gland is varied, will reveal to what extent changes in ionic gradients and membrane permeability control or trigger other processes involved in hormonal synthesis and release.

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