J. Membrane Biol. 71, 109–118 (1983)

The Journal of
Membrane Biology

Regulation of Excitation-Secretion Coupling by Thyrotropin-Releasing Hormone (TRH): Evidence for TRH Receptor-Ion Channel Coupling in Cultured Pituitary Cells

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Summary. The electrophysiological and secretory properties of a well-studied clonal line of rat anterior pituitary cells (GH_3) have been compared with a new line of morphologically distinct cells derived from it (XG-10). The properties of the latter cells differ from the parent cells in that they do not have receptors for thyrotropin-releasing hormone and their basal rate of secretion is substantially higher (ca. three- to fivefold). While both cell types generate Ca^{++} spikes, the duration of the spike in XG-10 cells (ca. 500 msec) is about 2 orders of magnitude longer than that in GH_3 cells (5–10 msec). The current-voltage characteristics of the two cell types are markedly different; the conductance of GH_3 cells is at least 20-fold higher than XG-10 cells when cells are depolarized to more positive potentials than the threshold for Ca^{++} spikes (~ -35 mV). While treatment of GH_3 cells with the secretagogues tetraethylammonium chloride or thyrotropin-releasing hormone decreases the conductance in this voltage region to approximately the same as that for XG-10 cells, the electrophysiological and secretory properties of XG-10 cells are unaffected by treatment with either of these agents. Results of this comparative study suggest that XG-10 cells lack tetraethylammonium-sensitive K^+ channels. The parallel loss of thyrotropin-releasing hormone receptor binding activity and of a K^+ channel in XG-10 cells implies that the thyrotropin-releasing hormone receptor may be coupled with, or be an integral part of, this channel. Apparently thyrotropin-releasing hormone, like tetraethylammonium chloride, acts by inhibiting K^+ channels resulting in a prolongation of the action potential, promoting Ca^{++} influx and subsequently enhancing hormone secretion.

Key Words excitation-secretion coupling · thyrotropin-releasing hormone · pituitary cells · potassium channels · electrophysiological measurements · calcium action potentials

Introduction

The regulation of hormone secretion from pituitary cells is under the direct control of a number of effectors which act at the level of the plasma membrane [26, 27]. Among these effectors are hypothalamic polypeptide factors, such as thyrotropin-releasing hormone (TRH)[1] and luteinizing hormone-releasing hormone, which act to stimulate secretion [12, 13, 18]. Various lines of rat anterior pituitary cells [20, 30, 32], such as the GH_3 line, have been used as models to investigate the regulation of hormone release. TRH interacts with GH_3 cells in a concentration-dependent fashion via receptors [4, 15] which are localized in the plasma membrane [34]. The short term effect of TRH is to stimulate the release of both prolactin (PRL) and growth hormone (GH). Longer term effects include down regulation of TRH receptors [16, 30] and stimulation of PRL mRNA synthesis [5] by mechanisms which are not yet known. Several working hypotheses have emerged to describe some of the membrane events that control secretion [6, 35]. The rate of secretion is believed to be controlled by intracellular Ca^{++} levels. These levels are regulated by the influx of extracellular calcium which occurs during spontaneously firing Ca^{++} action potentials [8–11, 17, 31]. Secretagogues, such as TRH, enhance the rate of secretion by increasing the duration and/or frequency of the spontaneous spikes as well as by increasing the number of cells that are spontaneously generating Ca^{++} spikes [7, 21, 25, 28, 29]. A similar increase in duration was achieved by inhibition of voltage-sensitive K^+ channels by TEA [7] and 4-aminopyridine [25]. However, from the available data, the molecular mechanisms by which Ca^{++} influx is enhanced are not clear.

This paper reports a comparative study of GH_3 cells with a new, morphologically distinct cell line, XG-10, derived from the GH_3 cell line (*manuscript in preparation*). Although the electrophysiological data gathered with GH_3 cells are similar to that previously reported [17, 21, 25, 29], comparison with data from the XG-10 cells has allowed new insights into the mechanism of TRH-receptor-me-

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[1] *Abbreviations used:* TRH, thyrotropin releasing hormone; TEA, tetraethylammonium chloride; PRL, prolactin; GH, growth hormone; E_M, membrane potential; R_E, input resistance.

0022-2631/83/0071-0109 $02.00

diated release of hormones. The XG-10 cells lack TRH receptors (*unpublished observations*) and do not exhibit any of the TRH-mediated secretory responses. The Ca^{++} action potentials in XG-10 cells are markedly prolonged and are unaffected by either TRH or TEA. However, treatment of GH_3 cells with either or both TRH and TEA results in electrophysiological properties similar to those recorded from XG-10 cells. These observations suggest that the TRH receptor regulates or is part of a TEA-sensitive K^+ channel. Regulation of membrane potential by this channel modulates the activity of the voltage-sensitive Ca^{++} channels.

Materials and Methods

Cells

GH_3 cells were obtained from the American Type Culture Collection and were maintained in monolayer culture with a modification of conditions described by Tashjian et al. [32]. The cells were routinely grown in a Hepes-buffered medium described by Higuchi [14] with the addition of 15% horse serum and 2.5% fetal bovine serum. Cells for hormone secretion experiments or for electrophysiological measurements were prepared by trypsinization of the stock monolayer cultures with subsequent plating into 35 mm tissue culture dishes. The cells were grown at 37 °C in a humidified atmosphere without CO_2 and the media was replenished every 2–3 days. The cells were cultured for 3–8 days before use.

A variant clone of the GH_3 cells, designated XG-10, arose spontaneously two years ago and has been maintained under conditions identical to those used for the GH_3 cells. This clone is characterized by its distinctly elongated shape as compared to the GH_3 cells and has maintained this property through repeated cloning procedures.

Hormone Secretion Studies

PRL and GH secretion by the GH_3 and XG-10 cells was measured with a standardized protocol designed to minimize variations in secretion rates due to unequal numbers of cells in each dish and to the stimulation of release that occurs upon media replacement. Hormone released in these experiments was quantitated by RIA kits generously provided by Dr. A. Parlow, NIAMDD, National Pituitary Hormone Distribution Program, NIH. TRH receptors on these cell lines were measured with ^{3}H-TRH (New England Nuclear, 100 Ci/mmol) as previously described [36]. Protein concentrations were estimated by the method of Lowry et al. [19] after dissolution of the cells with 0.1 M NaOH. Cell volumes were measured by the method of Rottenburg [24] and were 1.6 and 1.5 µl/10^6 cells for the GH_3 and XG-10 cells, respectively. Diameters of the generally spherical GH_3 cells were 10–20 µm.

Electrophysiological Measurements

Intracellular recordings were made from GH_3 and XG-10 cells at room temperature (20–24 °C) in a basic medium composed of (mM): 140 NaCl, 1 KCl, 10 $CaCl_2$, 5 Tris-Cl, pH 7.3, with 1.0 mg/ml glucose, unless noted otherwise. In some cases, the concentration of KCl was adjusted to 5 mM with a corresponding decrease in the concentration of NaCl; Na-free solutions were made by substituting stoichiometric amounts of either choline Cl or Tris-Cl for NaCl. Test agents were added to the dishes which contained 4 ml of solution from concentrated stocks so that salt concentrations were not changed by more than 2.5%. All chemicals were obtained from commercial sources and were of the highest purity available.

After replacing the growth medium with the experimental solutions, the culture dish was mounted on the stage of an Olympus CK inverted microscope. Microelectrodes were pulled on an Industrial Science Associates puller and filled with 3 M KCl. Resistance of the electrodes ranged from 90 to 200 MΩ. A single microelectrode was used to current clamp and to monitor potential of the membrane. This was achieved with a high input resistance amplifier (10^{12} Ω) designed by Mr. E. Amatniek of Columbia University that included circuitry for injecting current and provision for balancing the voltage drop due to the microelectrode resistance. This voltage drop was nulled by an output bridge circuit before penetration and was checked after the recordings. If the resistance of the electrode changed by more than 5%, the data were discarded. The ground return from the bath was an agar gel-filled pasteur pipette containing an Ag–AgCl electrode. The pipette was filled with a heated solution of 3% agar in standard saline and subsequently cooled to 5 °C. Most electrophysiological recordings were made on a Beckman rectilinear pen recorder with a frequency response of 50 Hz. The peaks of fast action potentials therefore were attenuated. When fidelity was required, photographs were taken from a Tektronix storage oscilloscope.

Results

Comparison of Membrane Electrical Properties of GH_3 and XG-10 Cells

Membrane potential (E_M) and input resistance (R_E) were measured in cells bathed in Na^+ or Na^+-free medium (Table). For comparison of XG-10 and GH_3 cells, the recordings were made in 1 mM K^+. The low K^+ condition was chosen in order to amplify potential changes associated with an increase in K^+ permeability (e.g., undershoot of the action potential), one of the parameters of interest in this study. R_E values were considerably higher in XG-10 cells than in GH_3 cells and increased markedly in Na^+-free medium. The R_E values decreased upon increasing extracellular K^+ from 1 to 5 mM. In the presence of Na^+, about 60% of the XG-10 cells had negative E_M values (−30 to −70 mV) while the other 40% were depolarized (−10 to −15 mV). However, no depolarized cells were observed in Na^+-free medium. The depolarized cells were quiescent, but when they were repolarized to −40 mV or more negative, they could spontaneously fire action potentials or respond with spikes either to a depolarizing stimulus or on the break of a hyperpolarizing pulse.

Characteristics of Action Potentials in GH_3 and XG-10 Cells

The action potentials recorded in GH_3 cells are either spontaneous or elicited by a depolarizing

Table. Electrophysiological properties of GH_3 and XG-10 cells in various media[a]

Solution	GH_3 Cells					XG-10 Cells					
	N	E_M (mV)	R_E (MΩ)	Action potentials		N	E_M (mV)	R_E (MΩ)	Action potentials		Plateau potentials (%) (−10 to −15 mV)
				Spontaneous (%)	Nonspontaneous (%)				Spontaneous (%)	Nonspontaneous (%)	
Na^+-media, 1.0 mM K^+	22	−40.1±1.5	208±22	27	42	34	−46.8±1.5	435±38	25	64	41
Na^+-Free media, 1.0 mM K^+	15	−47.0±1.1	243±25	13	40	20	−56.5±3.2	626±131	10	60	0
Na^+-media, 5 mM K^+						13	−38.3±2.4	181±12	0	38	38
Na^+-free media, 5 mM K^+						40	−44 ±1.2	309±24	23	25	0

[a] The average of the membrane potentials ($E_M \pm$SEM) do not include cells with potentials between −10 and −15 mV (plateau potentials). Included, however, in this average are measurements which were made from spontaneously firing cells. In this case, E_M was determined in the interval between spikes when the rate of change of E_M was minimal. The average of the membrane resistance ($R_E \pm$SEM) did not include the plateau potential cells. Cells displaying action potentials fall into two categories: (a) cells firing spontaneously upon penetration and (b) cells capable of generating action potentials (nonspontaneous). The latter category includes plateau potential cells, since they are capable of generating action potentials when their membrane is hyperpolarized with a holding current.

[b] This value is determined from a sample that excludes the plateau potential cells.

current, or on the break of a hyperpolarizing current (Figs. 3, 5, 6 and 7) and are similar to those previously reported. The duration of the spike is 5–10 msec (half amplitude) and peaks approach zero or in a few cases overshoot zero by as much as 5–10 mV. In all studies the action potentials are found to persist after removal of Na^+ and they are blocked by agents that are known to abolish Ca^{++} currents. The agents tested in this study included verapamil (10 μM), nifedipine (1 μM), Mn^{++} (5 mM) and Co^{++} (2.5 mM). These data are not shown.

Some of the characteristics of action potentials generated in XG-10 cells are shown in Fig. 1. Figure 1 *A* shows recordings beginning with penetration of a cell while applying a train of hyperpolarizing pulses (lower trace). Measurements of E_M and R_E indicated that about 30 sec is required to obtain stable values. During this time, the chart speed was changed several times (indicated by dots). After termination of the hyperpolarizing pulses, the cell continued to fire spontaneously. The spontaneous action potentials of another cell, initially quiescent (E_M at −10 mV), were recorded shortly after E_M was increased to about −55 mV by applying a holding current (Fig. 1 *B*). At this holding potential the cell responded in a similar

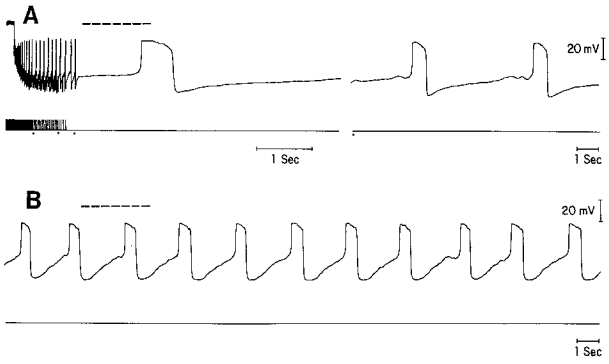

Fig. 1. Spontaneous action potentials recorded in XG-10 cells bathed in Na^+ solution with 1 mM K^+. The procedures used to penetrate cells and to monitor E_M and R_E are illustrated in *A*. Hyperpolarizing current pulses (5.0×10^{-11} amps, lower trace) are passed through the microelectrode before penetration. Upon contact of the electrode with the cell surface, small voltage steps are generated by current flow in the contact resistance (heavy baseline at beginning of trace). Penetration of the cell is caused by a gentle tap of the electrode holder. After penetration, the current pulses are maintained until R_E and E_M are stable. The cell continued to discharge spontaneously. The data were recorded at three different chart speeds (dots) during the 30-sec period required for stabilization. Afterwards the spontaneously firing action potentials were recorded at 2 different chart speeds. *B*. The E_M of the XG-10 cell was −10 mV. Spontaneous action potentials occurred upon applying a holding current to increase E_M to about −55 mV. Zero potential is indicated by dashed lines in both *A* and *B*

manner as the cell of Fig. 1*A*. Spikes occurred at 2- to 5-sec intervals, the peaks were between −10 to −15 mV, the durations were approximately 500 msec, and the undershoots were 10–20 mV in amplitude. With a different holding potential these parameters would change and with large holding currents spontaneous activity ceases. Similar results were obtained in Na^+-free media. These action potentials in XG-10 cells were blocked by the same agents at the same concentrations that are effective in GH_3 cells (*data not shown*).

Current-Voltage Characteristics of GH_3 and XG-10 Cells

In some experiments current-voltage relationships were obtained by the method employed in Fig. 2. First, the cell was brought to a predetermined potential by the application of a hyperpolarizing current (holding current). Then the current was varied in a linear manner so as to depolarize and repolarize the cell (current ramps). Figure 2*A* shows the results from an XG-10 cell in Na^+-containing medium with an E_M initially in the plateau region (−12 mV). After applying a holding current to hyperpolarize E_M to −70 mV, a current ramp was used to determine R_E, which was nearly constant from −70 to −40 mV. At −35 mV, E_M regeneratively attained the plateau potential. With further changes in current, E_M increased linearly. Upon reversal of the current ramp, a change in the slope of E_M was also observed. This type of I vs. E_M relationship was obtained in all XG-10 cells tested and is similar to that reported for GH_3 cells bathed in a Ba^{++}-containing solution [22]. In four experiments the R_E values for E_M in the −15 to 0 mV region were between 40 and 100 MΩ. R_E values for cells with E_M values more negative than −40 mV are included in the Table.

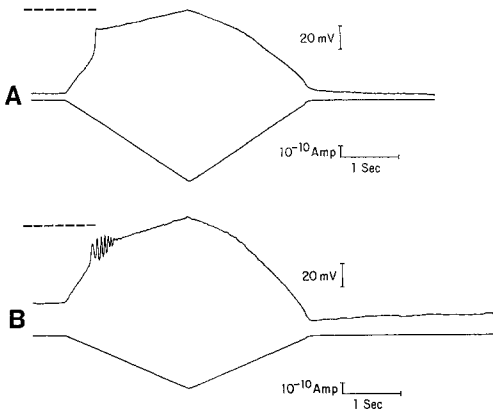

Fig. 2. Current-voltage characteristic of XG-10 cells bathed in Na^+ (*A*) and Na^+-free (*B*) solutions. The use of current ramps (lower trace) to obtain R_E values over various membrane potential regions is described in the text. In *A*, the membrane resistances are essentially constant below and above the regenerative step from −40 to −15 mV. For this XG-10 cell in Na^+ solution, E_M was at the plateau potential (−12 mV). A holding current increased E_M to −70 mV before application of the current ramp. In *B*, the response is shown for an XG-10 cell in Na^+-free solution. The E_M of this cell was −50 mV before a holding current increased it to −65 mV. When E_M passes from the high to the low resistance region during application of the depolarizing current ramp, low frequency and small amplitude oscillations are observed. Upon cessation of the current ramp, a long lasting undershoot (18 mV) occurred. The dashed lines indicate zero membrane potential

The results from a similar experiment with an XG-10 cell in Na^+-free solution are shown in Fig. 2*B*. In this cell, the initial E_M was −50 mV and a holding current was applied to increase E_M to −65 mV. Upon depolarization, a transition accompanied by slow oscillations occurred between −35 to −10 mV and E_M changed linearly between −10 and +5 mV. The R_E values before the transition were larger than those recorded in Na^+-containing media. R_E values for cells with low E_M (−15 to 0 mV) ranged from 50 to 100 MΩ (7 experiments); the R_E values for E_M values between −70 and −40 mV are included in the Table.

Current-voltage relationships in GH_3 cells obtained by current ramps (Fig. 3) differ markedly from those recorded in XG-10 cells (Fig. 2). The recordings from a GH_3 cell in Na^+-containing medium upon application of four current ramps of different rates are shown in Fig. 3*A–D*. With a holding current applied to bring the cell to −60 mV (E_M initially at −42 mV), a linear change in E_M occurs with a small depolarizing current which does not reduce E_M to spike threshold (Fig. 3*A*). With a slowly rising depolarizing current approaching threshold, small action potentials (miniatures) followed by all-or-none action potentials with large undershoots are elicited (Fig. 3*B*). These miniature action potentials may also occur during the falling phase of the ramp current (Fig. 3*C* and *D*). Miniatures are not observed when the membrane is rapidly depolarized to spike threshold by pulses rather than by slow current ramps (Fig. 3*E*). Due to the train of spikes it is difficult to obtain R_E values from the E_M vs. I data of Fig. 3*B–D*. However, if a steeper ramp current is applied to GH_3 cells, the action potentials rapidly inactivate and R_E measurements can be obtained for E_M values near threshold (*see* Fig. 5).

Comparison of the Secretory Properties of XG-10 and GH_3 Cells

To correlate the electrophysiological response of these cells to their secretory properties, the release

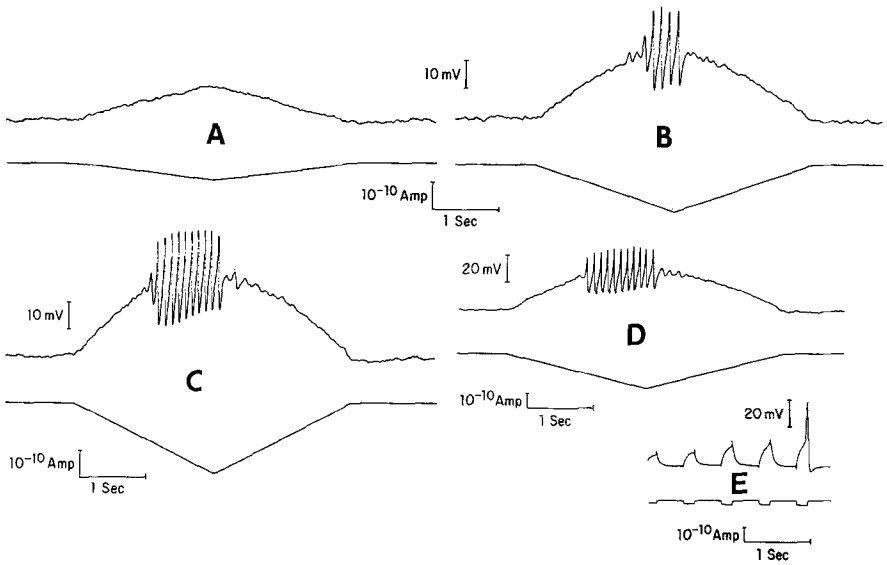

Fig. 3. E_M responses to depolarizing current ramps in GH_3 cells bathed in Na^+ solution. E_M depolarizes and repolarizes linearly when current ramps of small slopes are applied to GH_3 cells (*A*). When spike threshold is approached by applying a steeper ramp (*B*), "miniature" spikes precede a train of all-or-none spikes. With still steeper ramps (*C*), "miniature" spikes are also elicited and the firing frequency of all-or-none action potentials increases. The E_M response to a ramp identical in slope to that in *C* is shown in *D*, but both E_M and current are recorded at half gain. Initially, E_M was -42 mV but was increased to -60 mV by a holding current. In *E* another GH_3 cell with an initial E_M of -42 mV was subjected to depolarizing steps with current pulses (ca. 150 msec). No holding current was applied. A spike was elicited with threshold at about -35 mV. "Miniature" spikes and oscillations are not observed. The applied currents are shown in the lower traces of each recording

of PRL under various conditions was measured by radioimmunoassay. Basal rates of secretion of PRL in XG-10 cells are generally 3–5 times higher than in GH_3 cells when normalized to protein concentrations. The effects of TEA (25 mM), TRH (100 nM) and elevated K^+ (60 mM) on the basal secretion rates for both GH_3 and XG-10 cells were determined (Fig. 4). In both cell types, elevated K^+ levels markedly stimulated PRL release. However, while TRH and TEA are both effective as stimulatory agents in GH_3 cells, neither affected XG-10 cells. Similar responses are seen when GH release is monitored (*data not shown*). The observation that TRH is without effect on the XG-10 cells is consistent with the lack of TRH receptors in these cells (*manuscript in preparation*).

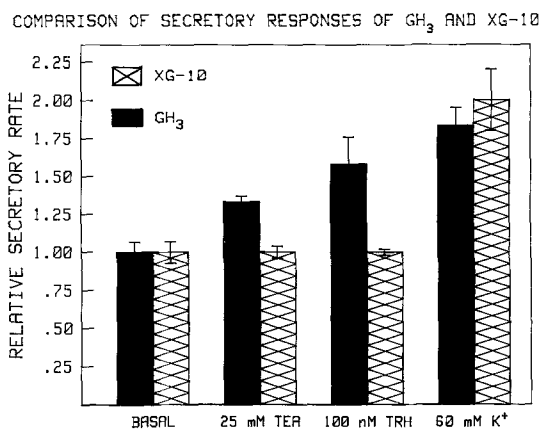

Fig. 4. Relative rates of PRL secretion from both GH_3 and XG-10 cells were measured from cells grown in 35 mm tissue culture dishes. After rinsing, 1.0 ml complete medium was added to each dish. After 1 hr, the medium was carefully aspirated and frozen. New medium containing the test compound was then added to each dish. After an additional hour, the solution was removed and stored frozen until assay for hormone levels by radioimmunoassay (RIA). The ratio of the hormone released in the second hour period to that released in the first period is the relative secretory rate for each dish. This protocol for hormone release was chosen to minimize variations between dishes due to unequal numbers of cells. Each bar represents the average (±SEM) ratio of triplicate dishes of cells. The filled bars are for the GH_3 cells while the cross-hatched bars are for XG-10 cells. Basal release rates for the GH_3 and XG-10 cells were 440 and 1606 ng/mg/hr, respectively

Effects of TRH and TEA on Membrane Resistance

The effects of TEA and TRH on the current-voltage relationships of GH_3 cells (Fig. 5) were determined by the current ramp procedure described above. Recordings were obtained from cells bathed in Na^+-containing media. In one GH_3 cell, before TEA treatment (inset *A–I*), application of a current ramp to reduce E_M to about -35 mV elicited a train of spikes followed by small oscillations. With further depolarization, the membrane exhibited a

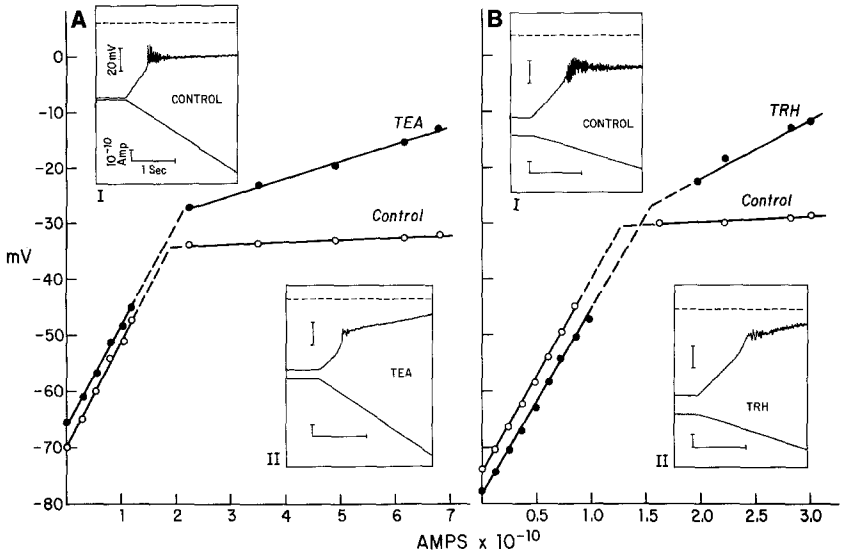

Fig. 5. Effects of TEA and TRH on I vs. E_M relationship of GH_3 cells. Trains of action potentials with decreasing amplitudes and high firing frequencies (insets *A–I* and *B–I*) are elicited in GH_3 cells subjected to more rapid rates of depolarization than those shown in Fig. 3. The spike amplitudes are attenuated due to the limited frequency response of the pen recorder. Coincident with the train of spikes is a large decrease in R_E. Both TEA (inset *A–II*) and TRH (inset *B-II*) abolish the train of spikes, and a regenerative step in E_M is observed followed by a less dramatic reduction in R_E than that which occurred in the controls. Dashed lines included in the insets are at 0 mV. The plots *A* and *B* were constructed from the I vs. E_M data of the insets by selecting I and corresponding E_M values at convenient intervals. Calibration is the same for all insets. Further description of these responses is found in the text. The lower traces in the insets are recordings of the current ramps

low resistance (5 MΩ). After TEA treatment, (25 mM), the resistance markedly increased in the voltage region above threshold (30 MΩ) and the spikes and oscillations were essentially abolished (insert *A–II*). Comparable data were obtained in another GH_3 cell after treatment with 100 nM TRH (inset *B–II*). The data from these cells are plotted in Fig. 5. The tests were repeated on several GH_3 cells. The membrane resistance in the voltage region above threshold (1 to 6 MΩ) increased in three cells with TEA treatment (60 to 80 MΩ), as well as in five cells after TRH treatment (50 to 130 MΩ). Treatment of GH_3 cells with either agent produced a current-voltage relationship that is very similar to that obtained from current ramp studies on XG-10 cells (Fig. 2). The membrane resistance of XG-10 cells was not affected by TRH or TEA.

Effects of TRH and TEA on Action Potentials

TRH and TEA both cause a similar modification of action potentials in GH_3 cells. Figure 6*A* and *B* display action potentials of a GH_3 cell upon termination of hyperpolarizing current pulses. Addition of 100 nM TRH to the medium resulted in both brief and long duration spontaneous spikes. The hyperpolarizing current pulses were maintained in this experiment but their frequency was decreased (Fig. 6*C*). In this Figure only the long duration action potentials have undershoots (3 to 4-sec duration). Sometimes, a hyperpolarizing pulse (arrow) will be followed by another brief spike instead of a long duration spike. It should be noted that when a hyperpolarizing pulse was applied during the plateau of a long duration spike, the action potential was terminated prematurely with a corresponding diminution of the duration of the undershoot. Similar brief and long duration action potentials were obtained after treating GH_3 cells with 25 mM TEA for 15 min (Fig. 6*D*). While the brief spikes do not have undershoots, the undershoot of the long duration spike is about 13 mV. In marked contrast, neither TRH nor TEA had any effect whatsoever on the shape or duration of the action potential in XG-10 cells. The brief spikes were never observed in these cells.

The persistence of large and long lasting undershoots associated with long duration action potentials in GH_3 cells treated with TEA or TRH is further illustrated in Fig. 7. Figure 7*A* is an oscilloscope recording of spontaneously firing spikes in a GH_3 cell bathed in a Na^+-containing medium. The frequency is about 3 Hz and the amplitude of the spikes is approximately 40 mV. These spontaneously firing spikes are similar in all respects to those previously described [29]. In Fig. 7*B*, one spontaneously firing long duration spike is shown

from another GH_3 cell which had been treated with both TEA (25 mM) and TRH (1 μM) for about 10 min. While under this condition only long duration spikes were observed, an initial brief duration step is seen preceeding the peak of the action potential. To be noted is the large undershoot (20–30 mV) that followed a long lasting plateau (ca. 600 msec).

Discussion

This comparative study has demonstrated that GH_3 and XG-10 rat anterior pituitary cells have markedly different electrophysiological properties. The experiments reported suggest that XG-10 cells lack fast responding voltage-regulated K^+ channels. This single modification can account for the long duration Ca^{++} action potentials, the increased secretory activity, and the lack of sensitivity to TEA found in XG-10 cells. Furthermore, loss of both TRH binding and biological response to TRH in XG-10 cells, coupled with electrophysiological evidence that TRH inhibits TEA-sensitive K^+ channels in GH_3 cells, provides compelling evidence that the TRH receptor regulates and, perhaps, is part of a voltage-regulated K^+ channel.

Membrane Potential (E_M) and Resistance (R_E)

The data of the Table show that the membrane potentials and the input resistances of the XG-10 cells are dependent upon Na^+ and K^+ ions. Both GH_3 and XG-10 cells hyperpolarize and R_E increases upon removing Na^+ from the medium.

In Na^+-containing and Na^+-free solutions, increasing external K^+ causes membrane depolarization, but in neither solution did the membrane behave as an ideal K^+-electrode. In three experiments on XG-10 cells in which the K^+ concentration was increased from 5 to 100 mM (not included in the Table), E_M decreased by only 25 to 30 mV. These results, approximately half the theoretical value for a K^+-selective membrane, are similar to those reported by Taraskevich and Douglas [29].

Action Potentials

In GH_3 cells brief duration (5–10 msec) action potentials persist in Na^+-free media, Ca^{++} current blockers abolish them [17, 29] and agents known to block outward K^+ currents, TEA [7, 29] and 4-aminopyridine [25], prolong them.

The action potentials in XG-10 cells also persist in the absence of Na^+ and are likewise abolished by Ca^{++} blockers but they are unaffected by TEA. The action potentials are of long duration (ca.

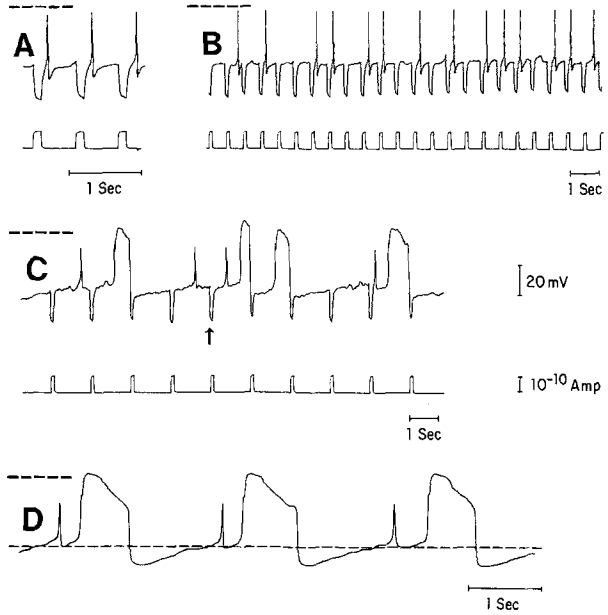

Fig. 6. Effects of TEA and TRH on action potentials of GH_3 cells. Records *A* and *B* (controls) and *C* (after exposure of cell to 100 nM TRH for approximately 10 min) are from one experiment and *D* is from another experiment in which a GH_3 cell was treated with 25 mM TEA (15 min). The heavy dashed lines indicate 0 mV. The lower traces in each part record the current

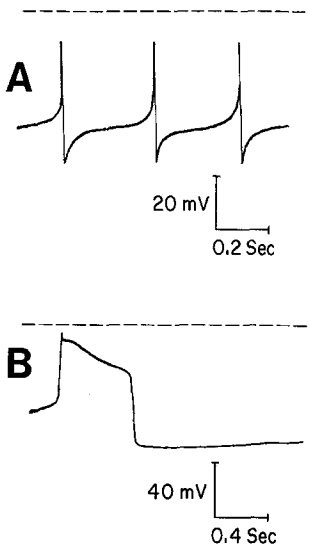

Fig. 7. Characteristics of spontaneously firing action potentials in GH_3 cells and their modification by K^+-channel blockers. Record *A* is a photograph from an oscilloscope tracing of spontaneously firing action potentials in a cell bathed in Na^+ solution (1 mM K^+). The durations of the action potentials and undershoots are brief compared to those of XG-10 cells (*see* Fig. 1). Photograph *B* is a spontaneous action potential from another cell treated with both TEA (25 mM) and TRH (1 μM) after 10 min. Note the notch at the peak of the spike, the long duration of the spike, and the large amplitude and long duration of the undershoot. Dashed lines are at 0 mV in both records

500 msec) and the late outward current that gives rise to the falling phase and undershoot of the spike is most likely transported by K^+, but probably through a K^+ channel distinct from the one that is blocked by TEA. TEA-insensitive K^+ channels have been described in other secretory cells and these channels are believed to be controlled by intracellular levels of Ca^{++} [3].

Since TEA-treated GH_3 cells have long duration spikes with large undershoots similar to those of XG-10 cells, we conclude that GH_3 cells also have a late Ca^{++}-dependent K^+ channel. Furthermore, the existence of only long duration action potentials in XG-10 cells indicates that the membranes of these cells are devoid of the early responding K^+ channels.

About 40% of the XG-10 cells bathed in Na^+-containing media (either 5 or 1 mM K^+) have low membrane potentials (about -10 mV). These low, stable potentials are not due to injury, since the membrane develops high resistance and the cells are capable of generating spikes when hyperpolarized with holding currents (Fig. 2*A*). Furthermore, these low membrane potentials (referred to as plateau potentials) have values similar to those observed in the plateau region of the long duration action potentials. Several factors may account for the high percentage of cells with plateau potentials. XG-10 cells do not have the early K^+ channels that, when activated, would repolarize E_M as in GH_3 cells (Fig. 6). In addition, since a Na^+ conductance has been demonstrated (Table), a Na^+ gradient must contribute to the depolarized state. Another factor that may be involved is a Na^+/Ca^{++} exchange mechanism (*manuscript in preparation*). With such a mechanism, intracellular Ca^{++} will be lowered in Na^+-containing media such that the Ca^{++} driving force will be increased and the Ca^{++}-regulated K^+ conductance will be decreased. Several preliminary results support this speculation. Injection of EGTA caused a depolarization of the membrane with an increase in its resistance. In addition, in Na^+-free media, small amplitude spikes are observed upon application of current ramps to XG-10 cells (Fig. 2*B*) and the undershoots are increased and prolonged under this condition (*data not shown*).

Spontaneous Activity

27% of GH_3 cells bathed in Na^+-containing media spontaneously fire action potentials while in Na^+-free media the number of active cells decreases to 13% (Table). This difference is probably due to the more positive E_M values in Na^+-containing solutions, since cells capable of generating action potentials fire repetitively when depolarized with prolonged pulses (*data not shown*) [29].

The spontaneously firing action potentials of GH_3 cells are characterized by a rapid regenerative Ca^{++} activation followed by an early K^+ current through channels opened and maintained so by the depolarization. The subsequent undershoot decays as the K^+ conductance decreases and E_M returns to the Ca^{++} threshold, whereupon the cycle is repeated (Fig. 7*A*).

In XG-10 cells, the percentage of spontaneously firing cells is also increased in Na^+-containing solutions (10 to 25%). In these cells, the long duration action potentials are terminated by a late K^+ current which is probably regulated by intracellular Ca^{++}. The subsequent large and long lasting undershoot decays (Fig. 1) as intracellular Ca^{++} is reduced. The Ca^{++}-activated K^+ conductance is thereby decreased, and E_M returns to the resting level.

Effects of TEA and TRH

The parameters affected by TEA and TRH in GH_3 cells were the I vs. E_M relationship, action potential characteristics, and rate of hormone secretion. Neither TEA nor TRH had an effect on these parameters in XG-10 cells. The essential findings are that TRH mimics TEA, a known blocker of voltage-dependent K^+ channels, and that GH_3 cells treated with either secretagogue have membrane characteristics similar to XG-10 cells.

The prominent, and similar, effect of TEA and TRH on the I vs. E_M relationship is to suppress the high conductance in GH_3 cells made more positive than -35 mV (Fig. 5) by current injection. The similarity between GH_3 cells treated with either TEA or TRH and XG-10 cells is apparent when the records of the insets of Fig. 5 are compared with Fig. 2. In view of these data and the extensive literature on TEA, it is clear that both TEA and TRH block an early voltage-dependent K^+ conductance. Consistent with this conclusion is that these agents prolong Ca^{++} spikes (50- to 100-fold) in GH_3 cells (Fig. 6) [25].

When TEA is applied intracellularly in squid [1] and frog [2] axons, the K^+ channels are blocked only after they open and a brief outward K^+ current has occurred. On the basis of these findings the pattern of brief and long duration spikes in GH_3 cells can be explained. In Fig. 6*D*, following a long duration spike and a large afterpotential, E_M returns to the Ca^{++} threshold and a brief Ca^{++} spike is initiated. The early transient

outward K^+ current terminates the spike but, because of the subsequent blockage by TEA, the falling phase is slower than for untreated GH_3 cells (Fig. 6*A*) and there is no undershoot. In the absence of an undershoot, E_M remains close to threshold and immediately another pre-spike potential develops, followed by a long duration action potential. Since recovery from TEA blockage is also voltage- and time-dependent [1, 2], some K^+ channels remain blocked. The opening of unaffected K^+ channels and their blockage by TEA produces a notch on the rising phase of the long duration spike. The resulting spike is terminated by the activation of the Ca^{++}-dependent K^+ channels (late K^+ current). During the subsequent large and long-lasting undershoot the blocking effects of TEA are totally reversed and the cycle begins again with another brief spike.

The brief followed by long duration spikes were also observed in GH_3 cells exposed to TRH (Fig. 6), extending the parallelism between the effects of TEA and TRH. While these effects can be explained on the basis of the well-described actions of TEA on axons [1, 2], the mechanisms that account for the voltage- and time-dependent blocking of K^+ channels may be different. Specifically, TRH is active, presumably, by binding to receptors on the membrane surface while the voltage/time dependence of TEA arises due to an interaction between TEA and the K^+ channel on the inner membrane surface [2, 23]. In these experiments, TEA was applied to the cells in the bathing solution; however, since some agents are rapidly accumulated into cells by fluid endocytosis [33] or passive diffusion, sufficient quantities of TEA may be available intracellularly to block the K^+ channels from within the cell. The data of Fig. 6*C* suggest that the recovery from channel blockage by TRH may also be voltage- and time-dependent as previously described for reversal of TEA blocking in axons [1]. When a hyperpolarizing pulse was applied after a brief spike (arrow, Fig. 6C) the subsequent spike was also brief. Since hyperpolarization has been shown to accelerate recovery from TEA blockage, a similar effect of hyperpolarization on TRH blockage in GH_3 cells could also explain the observed modification in the sequence of brief followed by long duration spikes.

In summary, XG-10 cells, like GH_3 cells, generate Ca^{++} spikes and spontaneously firing action potentials which provide an influx of Ca^{++} for excitation-secretion coupling. While the frequency of spikes is lower (ca. fivefold) in XG-10 cells than in GH_3 cells (Figs. 1 and 7), the duration of Ca^{++} spikes in XG-10 cells is much greater (1 to 2 orders) and hence the basal rate of secretion is higher. When GH_3 cells are treated with TRH or with agents that block early K^+ currents, such as TEA and 4-aminopyridine, the duration of spikes and I vs. E_M characteristics become similar to those of XG-10 cells. Thus, it is probable that TRH stimulates secretion in GH_3 cells by blocking these early K^+ channels. It is also possible that the TRH blocking efficiency is voltage- and time-dependent in an analogous manner, although probably by a different mechanism than that described for TEA blockage of K^+ channels. It is, therefore, concluded that XG-10 cells do not possess the K^+ channels through which the early outward K^+ current is transported. Furthermore, the apparent loss of both TRH receptors and of TEA-sensitive K^+ channels from the membranes of XG-10 cells suggests that these two entities in GH_3 cells may be functionally or structurally coupled.

Drs. Katz and Reuben were supported in part by grants from NIH (NS 11766), the Muscular Dystrophy Association, The Merck Foundation, and the Ipco Corp. Part of this work was done at the Marine Biological Laboratory, Woods Hole, Massachusetts, while Dr. Reuben was Director of the Grass Fellowship Program and Dr. Katz was a Lecturer.

We thank Drs. A. Mauro and G. Suarez-Kurtz for their help in the initial phases of this work, L. Costello, C. Telakowski-Hopkins and K. King for maintaining the cells and for technical assistance, and J. Kiliyanski for typing this manuscript. We are indebted to Drs. H. Grundfest, A. Karlin and R. Cox for reading drafts of the manuscript, for their suggestions, and their helpful discussion of the data.

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Received 12 April 1982; revised 28 June 1982