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An Inactivating Inward-rectifying K Current Present in Prolactin Cells from the Pituitary of Lactating Rats

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Abstract. Primary cultures containing a high percentage of lactotrophs were obtained by dissociating the pituitary of rats following 14–18 days of lactation. Lactotrophs with a distinctive appearance were recorded after 1–35 days in vitro and identified by immunocytochemical staining for prolactin. Whole-cell voltage clamp measurements in isotonic KCl solution from a holding potential of −40 mV revealed the presence of inward-rectifying K currents with a time-dependent, Na^+-independent inactivation at potentials negative to −60 mV. The time for complete inactivation was strikingly different between lactotrophs, varying between 1 sec and more than 5 sec at −120 mV, and was not related to time in culture. The reversal potential shifted 59 mV (25°C) for a tenfold change in external K^+ concentration, demonstrating the selectivity of the channel for K^+ over Na^+. The inward-rectifying K current was blocked by 5 mM Ba^{2+} and partially blocked by 10 mM TEA. Chloramine-T (1 and 2 mM) produced a total block of the inward-rectifying K current in lactotrophs. Thyrotropin-releasing hormone (500 nM) significantly reduced the inward-rectifying K current in about half of the lactotrophs. This current is similar to the inward-rectifying K current previously characterized in clonal somatomammotrophic pituitary cells (GH_3B_6). The variability of the rate of inactivation of this current in lactotrophs and its responsiveness to TRH is discussed.

Key words: Pituitary — Lactotroph — Inward-rectifying K current — Current inactivation — Chloramine-T — Thyrotropin-releasing hormone

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Introduction

Ever since the initial discovery of ''anomalous rectification'' in K-depolarized skeletal muscle by Katz (1949), inward-rectifying K currents have been described in a wide range of different cell types including starfish eggs (Hagiwara, Miyazaki & Rosenthal, 1976) and heart muscle cells (Hall, Hutter & Noble, 1963). Molecular cloning has revealed that most inward-rectifying K channels belong to a family of structurally similar proteins (Bond et al., 1994). Distinct tissue distributions of these channel proteins as well as differences in the kinetics and the pharmacology of the currents they produce hint at a functional diversity. K inward rectifiers are thought to be important for setting and stabilizing the resting membrane potential of cells and for permitting long depolarizing responses. Additional studies of inward rectifiers in a variety of particular cell types are needed, though, to clarify the scope of possible functions.

We have previously characterized an inward-rectifying K current in GH_3B_6 clonal rat pituitary cells (Bauer, Meyerhof & Schwarz, 1990). This current is present at the resting potential of these cells (−40 to −60 mV) and starts to inactivate below this potential range, suggesting that it is a major determinant of the membrane potential (reviewed in Corrette, Bauer & Schwarz, 1995). Thyrotropin-releasing hormone (TRH) reduces the inward-rectifying current in GH_3B_6 cells, and this modulation could underlie an increase in the frequency of action potentials. GH_3B_6 cells, though, are tumor-derived and are known to be aberrant. We have therefore investigated lactotrophs in primary culture and now show that a similar but more variable inactivating inward-rectifying K current is present in these cells. In particular, the rate of the time-dependent inactivation of this current is much more variable in lactotrophs than in GH_3B_6 cells. This current is also reduced by TRH, but a

TRH effect is only present in about half of the lactotrophs.

Materials and Methods

Cell Culture

Primary cultures were prepared from the pituitary of female Lewis A rats after 14–18 days of lactation. The pituitary gland was removed following decapitation, cut into 8–12 pieces and dissociated at 37°C for 30 min. No attempt was made to separate anterior, posterior and intermediate lobes of the pituitary. The dispersion medium consisted of Hank's balanced salt solution (Sigma, Deisenhofen, Germany) containing 0.1% bovine serum albumin, 10 mM HEPES and 10 mM glucose, adjusted to pH 7.3–7.4 with NaOH. The following enzymes and inhibitor were added to this dispersion medium just prior to use: 0.5 mg/ml collagenase A (Boehringer, Mannheim, Germany), 1 μg/ml trypsin-chymotrypsin inhibitor, 10 μg/ml DNAase I, and 2 mg/ml hyaluronidase (Type 1-S). The dispersed cells were sedimented and resuspended in the dispersion medium. Small amounts of the suspension were pipetted into the center of twenty 35 mm plastic culture dishes (Nunc, Wiesbaden, Germany), which had been coated with poly-D-lysine (25 μg/ml). The dishes were placed in an incubator in a water-saturated atmosphere of 95% air and 5% CO_2 at 37°C for 1 hr, resulting in a dense layer of cells in the center of each dish. The dishes were then filled with culture medium consisting of Dulbecco's modified Eagle's medium (Gibco/Life Technologies, Eggenstein, Germany) containing 10% fetal calf serum (Biother, Kelkheim, Germany), 2 mM L-glutamine and antibiotics (20 U/ml penicillin and 0.02 mg/ml streptomycin). The medium was changed every 3–4 days.

Electrophysiological Recordings

Membrane currents were recorded in the whole-cell configuration (Hamill et al., 1981) using an EPC9 patch clamp amplifier in conjunction with stimulation and data acquisition software (PULSE, HEKA elektronik, Lambrecht, Germany). The patch electrodes (resistance: 2.5–4 MΩ) were fabricated from 1.5-mm diameter borosilicate glass capillaries (BioLogic, Science Products, Hofheim, Germany) using a L/M-3P-A puller (List Electronic, Darmstadt, Germany). Tips of the electrodes were polished by holding them near a glowing platinum wire. Recordings were made from primary culture cells between 1 and 35 days after dissociation and plating. Access resistance of the recordings was 6–10 MΩ. All potential measurements were corrected for liquid junction potentials. Data were low-pass filtered at 3 kHz and have been compensated for both fast and slow capacity transients, but current traces are shown without correction for leakage currents. All experiments were conducted at room temperature (RT).

Solutions

A high-K^+, low-Ca^{2+} extracellular recording solution designed to increase the currents through inward-rectifying K channels (Bauer et al., 1990) and to reduce Ca^{2+} influx and activation of Ca-dependent K channels (Ritchie & Lang, 1989) was used for all experiments. This external isotonic KCl solution contained (in mM): 140 KCl, 4 $MgCl_2$, 1 $CaCl_2$, 2.5 EGTA, 10 HEPES, 10 glucose and had a free Ca^{2+} concentration of 75 nM (estimated using EQCAL, Biosoft, Cambridge, UK). The pipette solution contained (in mM): 140 KCl, 2 $MgCl_2$, 1 $CaCl_2$, 2.5 EGTA, 10 HEPES and had an estimated free Ca^{2+} concentration of 66 nM, which is similar to the basal values of the intracellular Ca^{2+} concentration in lactotrophs (Reid et al., 1995). Both solutions were titrated with 10 mM KOH to pH 7.3, yielding a final K^+ concentration of 150 mM. Tetrodotoxin (500 nM) was added to all extracellular solutions to block Na^+ channels. The osmolarity of all solutions was adjusted to 315 mosM for external and 300 mosM for the internal solution by the addition of glucose.

The dependence of the recorded currents on external K^+ concentration was investigated by reducing the K^+ concentration in the recording solution from the normal 150 mM in the isotonic KCl solution to 15 mM by replacing 126 mM KCl with NaCl and by titration to pH 7.3 with 1 mM KOH and 9 mM NaOH. 200 μM $NiCl_2$ was added to this solution to block possible steady currents through dihydropyridine-sensitive Ca channels (Scherübl & Hescheler, 1991). This concentration of $NiCl_2$ was not found to affect the inward-rectifying current.

To examine the effect of external Na^+ on the inward-rectifying K current, the external solution was modified by replacing 100 mM KCl with either NaCl or choline chloride. These modified external solutions contained 52 mM K^+ following titration to pH 7.3 with KOH.

Ba^{2+}, chloramine-T, tetraethylammonium chloride (TEA), thyrotropin-releasing hormone (TRH) were added to the extracellular isotonic KCl solution. The osmolarity and pH of these solutions were adjusted as necessary. External solutions were changed by perfusion of the 35-mm culture dish or by superfusion of the cell with a solution from a 100 μm diameter pipette tip (Gel-loader, Eppendorf, Hamburg, Germany) placed about 500 μm from the cell. Both perfusion and superfusion of solutions were driven by a gravity-fed system controlled by an electronic valve. For the TRH experiments, TRH was pipetted directly into the bath. When not otherwise stated, all chemicals were purchased from Sigma.

Immunocytochemistry

Following a recording, the tip of the pipette was manipulated to scratch marks onto the bottom of the plastic culture dish to allow identification of the recorded cell following immunocytochemical staining for prolactin. The recording solution was replaced with 0.05 M Tris-HCl-buffered saline (TBS, pH 7.5). TBS contained (in mM): 149 NaCl, 40 Tris-HCl and 10 Tris-base and was used as the basic solution for all steps of the staining procedure. Following the brief rinse in TBS, the cells were fixed for 5 min in 10% formaldehyde. The culture dishes were then filled with TBS and held at 4°C. For the final staining, the cells were permeabilized with 0.1% Triton X-100, washed, treated with 1% hydrogen peroxide and washed, each step being performed for 5 min at RT with agitation. The cells were then incubated for 1 hr with 8% normal pig serum and overnight at 4°C with antibodies against rat prolactin (anti-rPRL-IC-5 provided by NIDDK, Baltimore, MD) used at a final dilution of 1:10,000. On the next day, the cells were returned to RT and washed 5 times for 5 min. This step was followed by a 1-hr incubation with a biotinylated secondary antibody (porcine anti-rabbit $F(ab')_2$ fragment, DAKO, Hamburg, Germany) at a dilution of 1:500, 2 washes and a 30 min-incubation in the dark with a streptavidin-biotin-horseradish peroxidase complex (DAKO). Final visualization was obtained with a 3,3′-diaminobenzidine (0.7 mg/ml) and hydrogen peroxide (2 mg/ml) solution. Controls were performed by omitting the primary antibody and by staining pituitary cells which lack prolactin (AtT-20 clonal pituitary cells).

Results

Identification of Lactotrophs

Figure 1 shows representative micrographs of cells after 14 days in a primary culture derived from the pituitary of

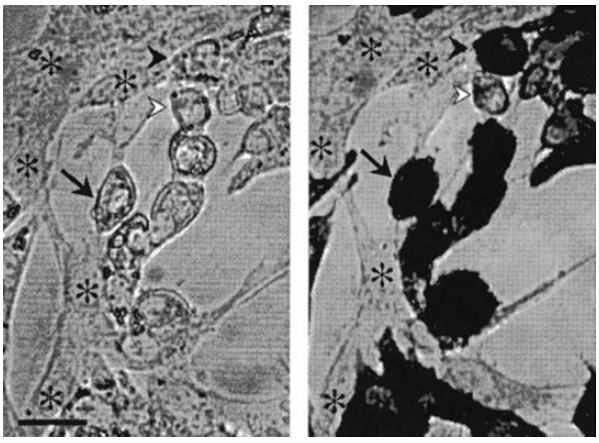

Fig. 1. Morphology and identification of lactotrophs used in this study. The two micrographs show the same field in a primary culture dish (14 days after dissociation) before (left) and after (right) immunocytochemical staining with an antibody against rat prolactin. Lactotrophs (identified by heavy staining), folliculo-stellate cells (marked by asterisks) and nonstaining pituitary cells (open arrowhead) can be seen. The morphology of the group of lactotrophs selected for experiments was well defined. Following 2–3 days in culture, these cells (*see* example marked by arrow) were flattened and elongated, had a thick dark membrane border and contained small membrane vacuoles, and were very granular. A large number of additional lactotrophs were present in these cultures but were not selected for recording (filled arrowhead), since they could not always be distinguished from nonlactotrophs (open arrowhead). Calibration bar: 20 μm.

a lactating rat. The same field of cells is shown before (Fig. 1 left) and after (right) immunohistochemical staining for prolactin, used to identify lactotrophs. With the exception of the extensive agranular folliculo-stellate cells (asterisks in Fig. 1), the large majority of granular cells in these cultures are lactotrophs, i.e., stain heavily for prolactin. After 2–3 days in culture, some of the granular lactotrophs had a distinctive outer membrane and contained small membrane vacuoles, which permitted a positive identification of these cells prior to staining. In a test, 21 cells were selected as lactotrophs based only on appearance (*see* cell marked by the arrow). In subsequent immunocytochemical staining, all 21 of these cells were positively identified as lactotrophs. Both nonstaining granular cells (open arrowhead) and other lactotrophs which did not meet all the selection criteria (filled arrowhead) were also present in these cultures. The distinct appearance of these lactotrophs was used to simplify selection of cells for electrophysiological experiments. An attempt was made to corroborate the identification of all cells as lactotrophs by staining following an experiment. This was possible for 76% of the cells whose data have been evaluated and all of these cells were found to stain heavily for prolactin. The remaining 24% of the cells were identified as lactotrophs based on appearance, but were lost either following withdrawal of the pipette or during the staining procedure.

Inward-rectifying K Currents in Lactotrophs

Whole-cell voltage-clamp measurements in isotonic KCl solution revealed the presence of hyperpolarization-activated currents in identified lactotrophs. Examples of the currents elicited by the standard pulse protocol (Fig. 2*A*) from a holding potential of −40 mV are shown in Fig. 2*Ba–Bf* for six lactotrophs. At the holding potential of −40 mV part of the inward-rectifying K current is already activated. Hyperpolarizing potential steps induced an instantaneous K current followed by a potential-dependent current activation and inactivation. The time course of the activation of these inward currents is similar from lactotroph to lactotroph. As seen in the examples of Fig. 2, maximum inward current occurred about 20 msec after the onset of the hyperpolarizing pulse at −120 mV, ranging from 10 to 50 msec. The latency of activation increases with weaker hyperpolarization. Inactivation occurred at potentials equal or negative to −60 mV. The rate of this inactivation was strikingly different between lactotrophs. For most cells, the inactivation was not complete at the end of the 1.5-sec test pulse even at −120 mV (Fig. 2*Ba–Be*) and was observed to continue longer than 5 sec in particular experiments (*see* −120 mV pulse in Fig. 8*Ca*). In other cells, essentially complete inactivation had occurred within 1 sec at −120 mV (Fig. 2*Bf*). This variability in rate of inactivation from lactotroph to lactotroph was not the simple result of some gradual change related to time in culture, since it was present following both short (Fig. 2*Ba,b,c*) and long (Fig. 2*Bd,e,f*) times in culture. Current-voltage relationships for the six cells in Fig. 2*B* are shown in Fig. 2*Ca–f*. Peak and late currents (measured at the end of the test pulse when steady state had not been reached in most cases) are shown. The voltage-dependence of the activation and inactivation of this inward-rectifying K current was similar for all lactotrophs. Taking into account the membrane capacities of these cells (14–24 pF), the peak current densities (at −120 mV) varied between 86 and 125 $pApF^{-1}$. Due to differences in the rate of inactivation, the amplitude of the late current was variable, but the voltage range over which inactivation developed (−60 to −120 mV) was similar for all lactotrophs.

A small, fast activating and inactivating current component appeared in many lactotrophs upon repolarization at the end of strong hyperpolarizations (Fig. 2*Ba–Be*). This current was also observed in GH_3B_6 cells (Bauer et al., 1990), and is assumed to be the inactivating K current described by Oxford and Wagoner (1989).

Dependence on the Concentration of External K^+

The ion selectivity of the hyperpolarization-activated current was examined in a series of experiments in which

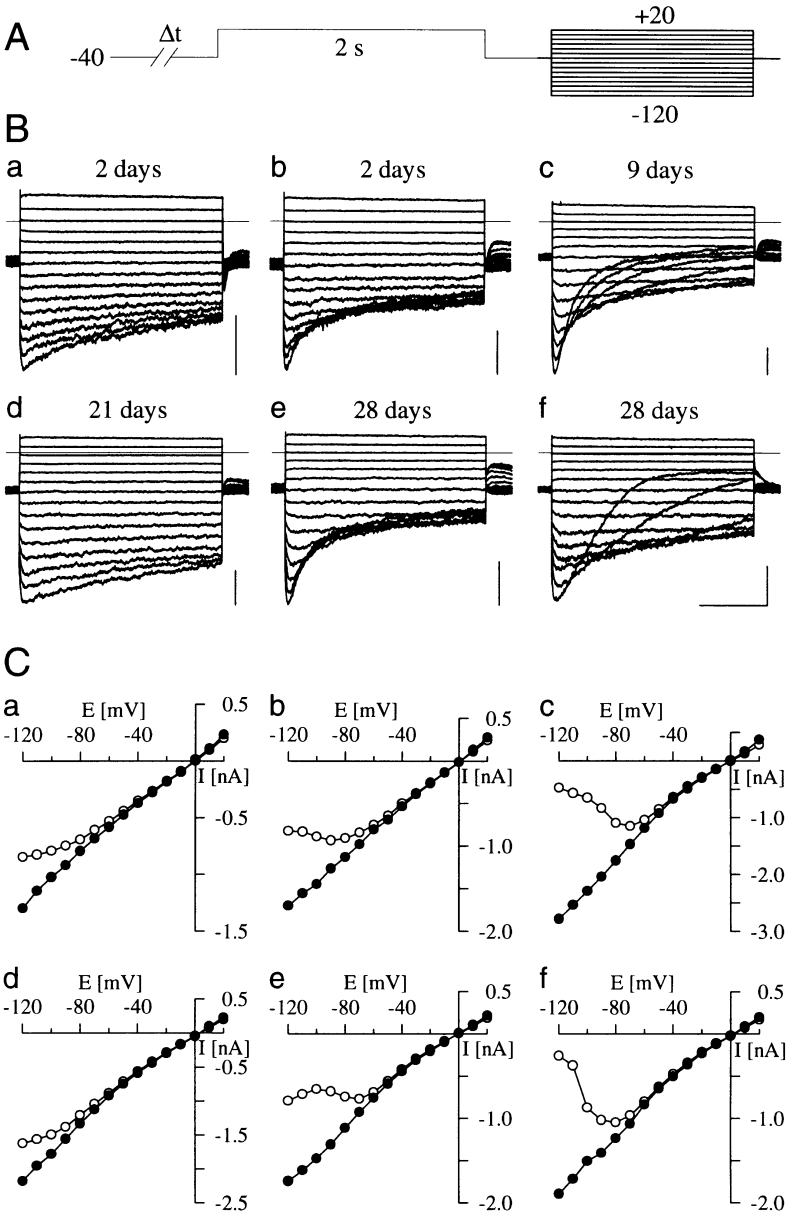

Fig. 2. Variability of inward-rectifying K currents in lactotrophs. (*A*) Standard pulse protocol used to measure the inward-rectifying K currents. Voltage-clamp measurements were made in an external isotonic KCl solution from a holding potential of –40 mV with 1.5-sec test pulses to potentials between +20 and –120 mV in –10 mV steps (thick lines). Three conditioning pulse segments (thin line) preceded these test pulses: a 0.8-sec segment at –40 mV which was increased exponentially in duration at subsequent voltage steps ($\Delta t = 1.23^{step-2}$ sec, range: 0 to 12 sec), a 2-sec depolarization to +20 mV and a brief 0.5-sec return to –40 mV. These pulse segments were designed to insure that recovery from inactivation would occur following larger hyperpolarizations, while holding the total time for a series of test pulses to a minimum. (*Ba–Bf*) Membrane currents of six different lactotrophs elicited by the pulse protocol shown in (*A*). Thin lines indicate zero current levels. Vertical bars denote 0.5 nA, horizontal bar in *Bf* denotes 500 msec and is valid for *Ba–Bf*. (*Ca–Cf*) Current-voltage relationships for examples shown in *Ba–Bf*, respectively. Peak currents (filled circles) and late currents (open circles) at the end of the 1.5-sec test pulses are shown as a function of test pulse potential. Although most lactotrophs have inward-rectifying currents with an intermediate rate of inactivation (examples in *b, e*), lactotrophs with slowly (*a, d*) or rapidly (*c, f*) inactivating currents were also measured. This variability in the rate of inactivation is observed in lactotrophs either after short (*a, b, c*) or long (*d, e, f*) periods in culture.

a tenfold difference in the external K^+ concentration was produced by exchanging most of the K^+ in the isotonic KCl solution with Na^+. Changing the K^+ concentration from 15 to 150 mM decreases outward currents and increases both the peak and late inward currents (Fig. 3*A*). The reversal potential, though, shifts by about only 50 mV (Fig. 3*B*). Taken into account a liquid junction potential of 3.7 mV using the 15 mM K^+ solution (calculated from the generalized Henderson equation, Barry & Lynch, 1991), the resulting difference in reversal potentials was 54 mV instead of the 59 mV (25°C) predicted by the Nernst equation for a K^+-selective current. More accurate measurements of this shift in the reversal potential were made by activating the inward-rectifying K current with a 5-msec potential step to –120 mV and then applying a rapid 50-msec ramp voltage spanning from –120 to +20 mV (Fig. 4). This more complicated ramp procedure was necessary to minimize the effects of inactivation on these measurements. With 15 mM K^+, the mean reversal potential was –59.0 ± 0.8 mV (mean ± SEM, $n = 7$). In isotonic KCl (150 mM K^+), a mean reversal potential of –0.1 ± 0.3 mV ($n = 8$) was measured. The difference in the mean reversal potentials (–58.9 mV) is almost exactly the –59 mV change in E_K predicted by the Nernst equation, and demonstrates the high selectivity of the recorded current for K^+ over Na^+.

Lack of Effect of External Na^+

To examine possible effects of Na^+ on the inward-rectifying K current, 100 mM K^+ in the isotonic KCl

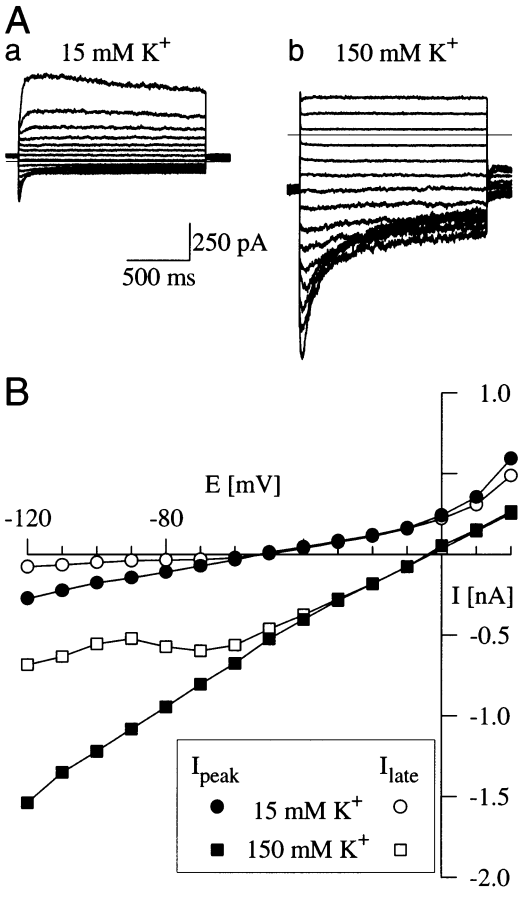

Fig. 3. Influence of external K^+ concentration on the inward-rectifying current. Families of currents elicited by the standard pulse protocol (*see* Fig. 2*A*) before and after a tenfold increase in the external K^+ concentration from 15 mM (*Aa*) to 150 mM (*Ab*). (*B*) Current-voltage relationships for the peak and late currents shown in *Aa* and *Ab*. As seen in this example, a large increase in the amplitude of inward currents and a shift in the voltage dependence of the currents occur when the external K^+ concentration is increased from 15 mM (reversal potential: –54 mV; not corrected for a liquid junction potential of 3.7 mV) to 150 mM (reversal potential: –4 mV).

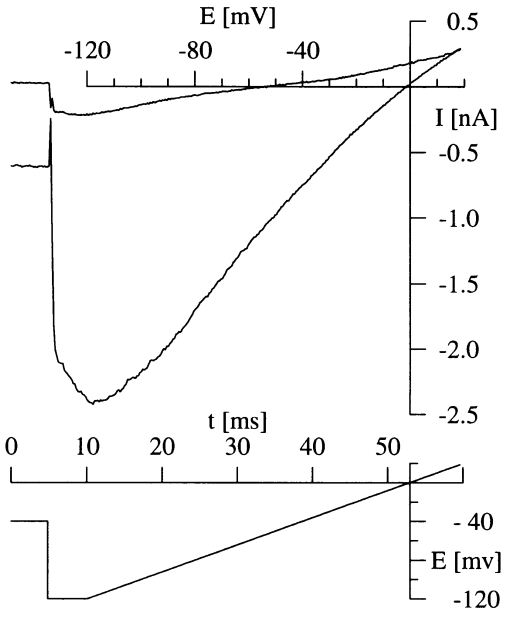

Fig. 4. A ramp pulse (*see* protocol at bottom) was used to directly measure the reversal potentials in an external K^+ concentration of either 15 mM (upper trace) or 150 mM (lower trace). To minimize the effects of inactivation of the inward-rectifying K current, this current was activated using a standard prepulse followed by a brief 5-msec hyperpolarization from –40 to –120 mV before making measurements with a short 50-msec 140-mV ramp. The reversal potentials of these currents in 15 mM K^+ and in 150 mM (iso) K^+ were –55 and –1 mV, respectively, without correction for the liquid junction potential of 3.7 mV in 15 mM K^+.

solution was replaced with either choline chloride or NaCl. As seen in Fig. 5 *Aa*, an inward-rectifying K current similar to that in isotonic KCl could still be measured in the 52-mM K^+ solution containing 100 mM choline and 0 mM Na^+. Exchange of this solution with one containing no choline and 100 mM Na^+ (Fig. 5*Ab*) produced little change in the currents recorded. At more negative potentials (–100 to –120 mV), a linear component became apparent with Na^+ instead of choline in the external solution. More importantly, though, the voltage-dependent inactivation of the inward-rectifying K current starting at about –70 mV (Fig. 5*B*) was not influenced by external Na^+ (n = 8).

Effects of Ba^{2+} and TEA

An example of the block produced by the addition of Ba^{2+} to the external solution is shown in Fig. 6. 0.5 mM Ba^{2+} blocked about 15–20% of the inactivating current component measured at –120 mV (Fig. 6*Ab* and 6*B*) and shifted the voltage-dependent inactivation to less negative potentials (n = 5, Fig. 6*B*). 5 mM Ba^{2+} was required to produce a substantial block of the inactivating component (n = 5, Fig. 6*Ac* and Fig. 6*B*). Parallel experiments in GH_3B_6 cells (n = 3, *data not shown*) indicated a similar Ba^{2+} sensitivity of the inward-rectifying K current in these cells.

As seen in the current-voltage relationship shown in Fig. 6*B*, peak currents are much more weakly blocked by Ba^{2+} than the late currents, as expected for an open channel blocker. It is interesting to note that in this lactotroph, complete inactivation to a steady state current at –120 mV appears to have occurred during the 1.5-sec pulse, since Ba^{2+} produced no further decrease in this current. The Ba^{2+}-sensitive current is shown in Fig. 6*Ca*. Its current-voltage relationship (Fig. 6*b*) clearly demonstrates the inward rectification.

In other experiments, 100-msec hyperpolarizing pulses to –100 mV (to avoid substantial inactivation) were repeated every 10 sec to monitor the inward-rectifying K current in lactotrophs while changing between isotonic KCl and KCl containing either 5 or 0.5 mM Ba^{2+} (*data not shown*). A similar block by Ba^{2+} was

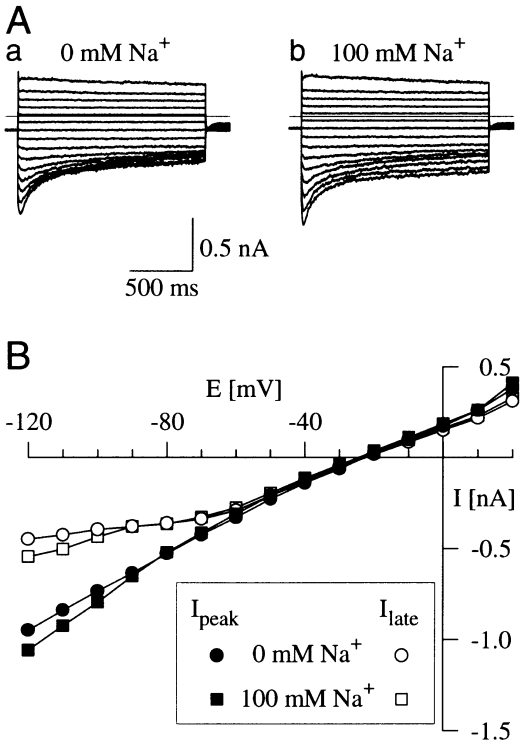

Fig. 5. The presence of 100 mM Na^+ in a high-K^+ (52 mM) extracellular solution is without effect on the inactivation of the inward-rectifying current in lactotrophs. Families of currents measured with 100 mM choline chloride (0 mM Na^+) in the external solution (*Aa*) and following substitution with 100 mM NaCl (*Ab*). (*B*) Current-voltage relationships for peak and late currents shown in *a* and *b*. External Na^+ induces a slight shift in the inward-rectifying current at very negative potentials (−100 to −120 mV), but is without effect on the inactivation.

obtained and could be completely reversed at both concentrations.

TEA (10 mM) could only partially inhibit the inward-rectifying K current in lactotrophs, producing a 35–40% reduction of the inactivating current component at −120 mV (n = 7). A typical experiment with TEA is shown in Fig. 7. Both peak and late currents were similar affected by both 5 and 10 mM TEA (Fig. 7*B*). The lactotroph in this experiment also had an inward current component which developed slowly during the test pulses at very negative potentials (−100 to −120 mV) and this unknown component appears to be insensitive to TEA.

A partial block by TEA was also reported by Barros et al. (1992) in GH_3 cells. Recent experiments in GH_3B_6 cells (n = 3) confirm this result (*data not shown*). We could not reproduce our previous results of a complete block of the inward-rectifying K current in GH_3B_6 cells (Bauer et al., 1990).

Effects of Chloramine-T

Chloramine-T has been shown to inhibit the inactivation of the inward-rectifying K current in guinea pig heart

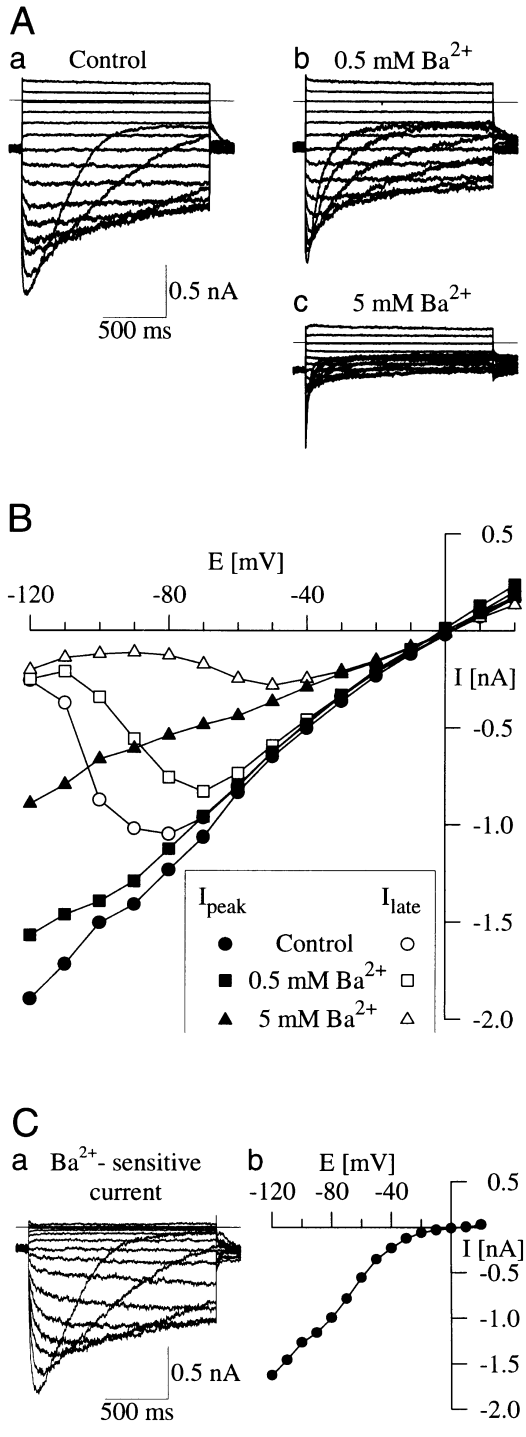

Fig. 6. Block of the inward-rectifying K current by Ba^{2+}. (*A*) Families of currents in isotonic KCl containing 0 mM Ba^{2+} (*Aa*, control), 0.5 mM Ba^{2+} (*Ab*) and 5 mM Ba^{2+} (*Ac*). (*B*) Peak and late currents of the traces in *A* as functions of test pulse potential. (*C*) Subtraction of the current traces shown in Ac from those of Aa yielded the Ba^{2+}-sensitive current (*Ca*). The current-voltage relationship (*Cb*) was obtained by plotting the peak amplitudes of the currents recorded with potential steps from −40 mV to potentials between −80 and −120 mV. For potential steps to potentials between +20 and −70 mV, current amplitudes at the end of the pulses were evaluated.

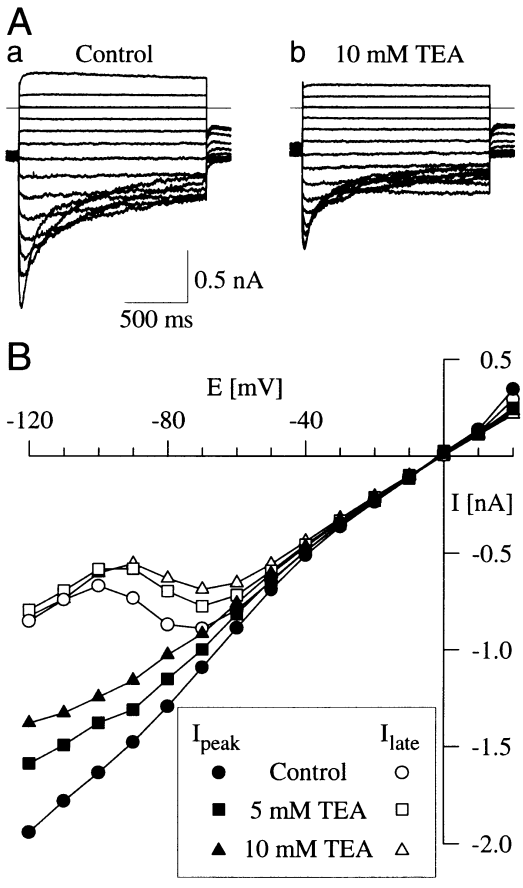

Fig. 7. Sensitivity of the inward-rectifying K current to TEA. (*A*) Current traces in normal isotonic KCl solution (*Aa*) compared with those in the presence of 10 mM TEA (*Ab*). (*B*) Peak and late currents as functions of pulse potential. Presence of either 5 or 10 mM TEA in the external solution produces only a partial block of the inward-rectifying K currents.

(Koumi, Sato & Hayakawa, 1994), and therefore its effects on the inward-rectifying K current in lactotrophs were tested. Given the strong irreversible effects produced by 2 mM chloramine-T reported in this study (Koumi et al., 1994), chloramine-T (1 or 2 mM) was only added to the recording solution for a 2-min application followed by a wash. The results of an experiment with chloramine-T are shown in Fig. 8. Chloramine-T (1 mM) increased the amplitude of depolarization-activated K currents and produced a very strong block of the inward-rectifying K currents (Fig. 8*A*,*B*). During the development of this block, no slowing in the inactivation rate was observed. Peak currents are strongly blocked, but this is not immediately apparent since activation of the inward-rectifying K current has been replaced by fast deactivating tail currents which follow increased holding currents after more negative test pulses (pair of asterisks in Fig. 8*Ab*). To better demonstrate the differing effects of chloramine-T, a series of test pulses without prepulses

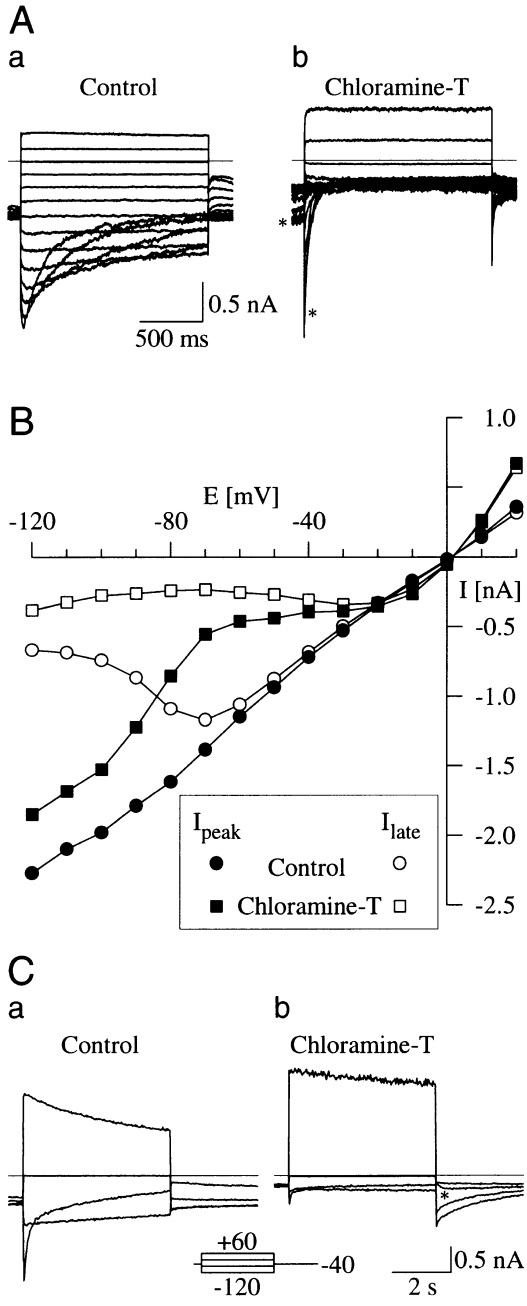

Fig. 8. Effects of chloramine-T on both outward and inward currents in isotonic KCl solution. (*A*) Currents measured before (*Aa*) and after (*Ab*) a 2-min application of 1 mM chloramine-T. A pair of asterisks indicate increased holding currents and the fast deactivating tail currents which follow them at more negative test pulses. (*B*) Current-voltage relationships for peak and late currents of the traces shown in *A*. (*C*) Four current traces elicited in the absence of the normal prepulse by two 5-sec depolarizing pulses to +60 and 0 mV and two 5-sec hyperpolarizing pulses to −60 and −120 mV (holding potential −40 mV) separated by a 15-sec pulse interval are shown for the same cell before (*Ca*) and after (*Cb*) a 2-min application of 1 mM chloramine-T. Chloramine-T blocks the inward-rectifying K current and produces a strong reduction of the inactivation of outward currents resulting in tail currents upon repolarization. An asterisk indicates activation of a current following repolarization after the hyperpolarization to −120 mV.

was used (Fig. 8*C*). Two 5-sec depolarizing pulses to +60 and 0 mV were followed by two hyperpolarizing pulses to −60 and −120 mV. Depolarization at +60 mV elicited large, slowly inactivating outward current (Fig. 8*Ca*). The inactivation of this current was reduced by chloramine-T, resulting in large tails following repolarization from +60 and 0 mV (Fig. 8*Cb*). The control hyperpolarizing pulses to −60 and −120 mV elicited the inward-rectifying K^+ current (Fig. 8*Ca*). In this example, the initially rapid inactivation at −120 mV was still not complete at the end of the 5-sec pulse. Following application of 1 mM chloramine-T, the inward-rectifying K current is blocked (Fig. 8*Cb*). In the case of repolarization from −120 mV, the activation of a current component is apparent and marked with an asterisk (Fig. 8*Cb*). This current adds to the holding current and produces the tail currents upon subsequent hyperpolarizing pulses, as seen in Fig. 8*Ab*. It should be noted that the kinetics of these tails are different from those related to the depolarization-activated outward currents. The major effects of chloramine-T, therefore, are removal of the inactivation of depolarization-activated currents and a block of the inward-rectifying K current ($n = 7$).

TRH Effects on the Inward-Rectifying K Current

In another set of experiments, a possible modulation of the inward-rectifying K current by TRH was investigated. Cell-to-cell variability was observed in the effects of TRH (500 nM) on the inward-rectifying current of lactotrophs ($n = 13$). 100 msec hyperpolarizing pulses from −40 to −100 mV were used in these experiments to avoid inactivation of the inward current and to permit measurements every 10 sec (Fig. 9). In about half of the lactotrophs measured (6 of 13), TRH produced a substantial reduction of the inward-rectifying K current. An example of this effect of TRH is shown in Fig. 9*A*. A few seconds after application of TRH, a slight increase in the inward current occurred (Fig. 9*Ab*) relative to the controls. This increase was accompanied by an increase in the holding current and, in GH_3B_6 cells, has been assumed to result from the activation of the voltage-independent small Ca^{2+}-activated K^+ channel (SK channel, Ritchie & Lang, 1989) during the transient release of Ca^{2+} from the endoplasmic reticulum which accompanies phase 1 of the cellular TRH response (Bauer et al., 1990). Following this transient increase, a 60% reduction of the inward current occurred over about 2 min (Fig. 9*Ab*). The holding current was also reduced by TRH (Fig. 9*Aa*, top traces), but the small transient inward current activated by repolarization to −40 mV (*see above*) remained unchanged. In the remaining lactotrophs, TRH either was without effect ($n = 6$) or, in one case, the effect could not be distinguished from the run-down of the inward-rectifying K current which often occurs during whole-cell measurements. Figure 9*B* shows an example of a lactotroph in which application of 500 nM TRH was without effect on the inward-rectifying K current. No differences between cells responding or not responding to TRH were observed concerning their morphology or the inactivation of the inward-rectifying K current.

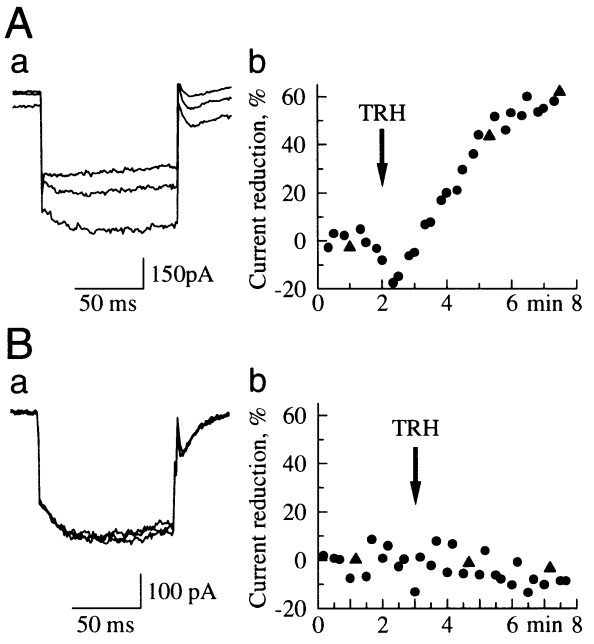

Fig. 9. The effect of TRH on the inward-rectifying K current in two different lactotrophs (*A, B*). (*Aa, Ba*) Current traces in response to 100-msec hyperpolarizing test pulses from −40 to −100 mV before and after application of 500 nM TRH. (*Ab, Bb*) Time course of the TRH responses expressed as the percentage reduction in the inactivating current estimated from long hyperpolarizing pulses. The data points corresponding to the sample traces shown in *a* are marked by triangles. (*A*) TRH produces a 60% reduction in the inward-rectifying K current in this lactotroph. (*B*) In another lactotroph, no effect of TRH on the inward-rectifying K current was observed.

Discussion

We have shown that an inward-rectifying K current is present in lactotrophs of lactating rats. This current is similar to the inward-rectifying K current previously characterized in clonal somatomammotrophic pituitary cells (GH_3B_6, Bauer et al., 1990; GH_3, Barros et al., 1992), although these cells exhibit a rapid, less variable inactivation of the current. Inactivating voltage-dependent inward-rectifying K currents have also been observed in pituitary-derived mouse AtT-20 clonal corticotrophs (Dousmanis & Pennefather, 1992) and in sheep somatotrophs (Chen et al., 1994).

The currents described here for lactotrophs share a number of characteristics with inward-rectifying K cur-

rents in cells from other tissues, especially those in porcine cerebral capillary endothelial cells (Hoyer et al., 1991). In these cells, the inward-rectifying K current is also relatively insensitive to Ba^{2+} and TEA, shows a rapid Na^+ independent inactivation over the same range of voltage and a great variability in inactivation kinetics. Analogous to the TRH-induced G protein-mediated reduction of the inward-rectifying K current in lactotrophs and GH_3 cells (Barros et al., 1992, 1994; Bauer et al., 1990, 1994), angiotensin II and arginine-vasopressin can reduce this current in part of the endothelial cells (Hoyer et al., 1991). Although endothelial cells and lactotrophs perform very different functions, both cell types have low resting membrane potentials (Hoyer et al., 1991; Lingle et al., 1986) and presumably make use of hormone-induced reduction of K conductance. Both the absence of more negative resting potentials and the transient effects of these hormones appear to be functionally correlated with the inactivating inward-rectifying K current shared by these cell types.

Pharmacology

The inward-rectifying K channel in lactotrophs exhibits a high selectivity for K^+ over Na^+ as observed for the inward rectifier in marine eggs (Hagiwara et al., 1976), but the channel in lactotrophs requires a tenfold higher concentration of Ba^{2+} (5 mM) for a current block compared to that in marine eggs (Hagiwara et al., 1978). This reduced sensitivity to Ba^{2+} has been observed for inward rectifier channels in other cells such as neurons, but in these cells it is associated with substantial permeation by Na^+ (Lynch & Barry, 1991). The inward-rectifying K current in lactotrophs was only partially blocked by high concentrations of external TEA (10 mM). Ten mM TEA also only partially blocked the inward-rectifying current in GH_3B_6 cells, as has been previously reported for GH_3 cells (Barros et al., 1992). In somatotrophs, though, a complete block of the inward rectifier current by 2 mM TEA (Chen et al., 1994) has been observed. The inward-rectifying K channels in most other cell types studied are characterized by a sensitivity to blockade by external TEA, but TEA-insensitive inward-rectifying K channels have also been reported for a number of different types of cells, including, for example, starfish eggs (Hagiwara et al., 1976), other secretory cells (Ishikawa & Cook, 1993) and glial cells (Le Dain et al., 1994). These differences make clear that the pharmacology of inward-rectifying K channels belies any simple classification scheme and needs to be carefully documented for each new channel type.

Intrinsic Inactivation

The amplitude of the inward-rectifying K current induced during a hyperpolarization to potentials equal to or more negative than –60 mV increased and subsequently decreased. This transient change in the current amplitude is described in this paper as current activation and inactivation. The rate of inactivation is extremely variable from lactotroph to lactotroph, whereas the voltage dependence of activation and inactivation remained reasonably constant from cell to cell. This inactivation is little influenced by both external Na^+ and K^+, since drastic changes in their concentrations (K^+: 15 to 150 mM; Na^+: 0 to 100 mM) were without effect, suggesting that rapid depletion of K^+ in extracellular spaces and/or voltage-dependent block by external Na^+ are not involved in the mechanism of inactivation. Voltage-dependent block by external Na^+ is known to cause the inactivation of some inward rectifier channels (Biermans, Vereecke & Carmeliet, 1987), but other inward rectifier channels inactivate in the absence of Na^+ (Harvey & Ten Eick, 1988).

The mechanism underlying inactivation and the variability in the inactivation rate of the inward-rectifying K current in lactotrophs is not known. If K channel inactivation is the result of an occlusion of the channel pore due to N-type inactivation (ball-and-chain model; Kukuljan, Labarca & Latorre, 1995), then changes in the rate of inactivation of macroscopic K current could result from the presence of different subunits forming either heteromultimeric channels or different populations of homomultimers, as suggested by the study of cloned inward rectifiers (Bond et al., 1994). The differing subunits which vary the inactivation rate could equally well be β-subunits that add a ball-and-chain component to noninactivating channel subunits (Kukuljan et al., 1995). The degree of phosphorylation of the inward rectifier channels could also influence inactivation kinetics and produce a variable rate of inactivation from cell to cell. It is known, for example, that the TRH-induced shift in the voltage dependence of inactivation of the inward-rectifier in GH_3 cells is the result of a phosphorylation/dephosphorylation step (Barros et al., 1992).

Another possibility is that the ionic channels underlying the inward-rectifying K current are activated but blocked at more positive membrane potentials. Upon hyperpolarization, relief of this block would cause an increase in the current amplitude. A subsequent deactivation of these channels would then induce a current decrease as has been suggested for a human inward rectifier (HERG; Trudeau et al., 1995).

The oxidant chloramine-T is suggested to interact with the ball-and-chain structure which results in an inhibition of channel inactivation, including the inward-rectifying K channels in guinea-pig ventricular myocytes (Koumi et al., 1994). As expected from previous experiments (Rouzaire-Dubois & Dubois, 1990), chloramine-T slowed the inactivation of outward-rectifying K currents in lactotrophs, but produced a total block of the

inactivating inward-rectifying K current. A block by chloramine-T of a transient K current without effect on the inactivation rate has also been observed in rat suprachiasmatic nucleus neurons (Huang & Yau, 1994).

A voltage-dependent inward-rectifying K channel has recently been cloned from GH_3B_6 cells (Falk et al., 1995), but the currents resulting from expression of this cloned inward rectifier channel lack intrinsic inactivation. Therefore, the molecular identity of the inward-rectifying K current in pituitary cells is uncertain.

TRH-Responsiveness

As shown here, TRH reduced the inward-rectifying K current in about half of the lactotrophs. In intact cells, closing of these K channels could result in a depolarization and/or increase in action potential frequency and be functionally significant for Ca-dependent secretion, as previously discussed for GH_3B_6 cells (Bauer et al., 1990) and for GH_3 cells (Barros et al., 1992). Studies on the effects of TRH on Ca^{2+} signaling carried out on individual lactotrophs in primary culture from our laboratory (Reid et al., 1995) showed that the majority of these lactotrophs responded to TRH with either a 1st- and/or 2nd-phase increase in cytosolic free Ca^{2+}. The 1st phase was independent of extracellular Ca^{2+}, whereas the 2nd phase which was observed in about ⅔ of the cells depended on Ca^{2+} influx into the cell which might involve the TRH effect on the inward-rectifying K current.

The lack of an effect of TRH on the inward-rectifying K current in part of the lactotrophs could be explained by a lack of some necessary element of the signal cascade underlying this response and be associated with a particular physiological state of these lactotrophs or with a particular subtype of lactotroph. A dichotomy in the responsiveness to TRH has previously been described by Lledo et al. (1991) for two groups of lactotrophs from lactating rats, obtained from light and heavy fractions of a continuous gradient of bovine serum albumin. These two subpopulations of lactotrophs could correlate with the groups found in the present study.

Culture methods, though, likely influence the functional state of individual cells and change the subpopulations present in a culture. Given the many paracrine interactions possible in the pituitary (Corrette et al., 1995), lactotrophs cultured together with a restricted group of cells of similar gradient density could be very different from those in a mixed culture obtained from the entire pituitary. Clearly, much more complete characterization of individual lactotrophs will be necessary before we can understand the functional significance of the heterogeneity in lactotrophs.

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