J. Physiol. (1987), **392**, *pp.* 273–299
With 12 text-figures
Printed in Great Britain

SODIUM AND POTASSIUM CURRENTS INVOLVED IN ACTION POTENTIAL PROPAGATION IN NORMAL BOVINE LACTOTROPHS

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(*Received 17 June 1986*)

SUMMARY

1. The properties of whole-cell and single-channel Na^+ and K^+ currents in immunocytochemically identified bovine lactotrophs were studied using the patch-clamp technique.

2. In the whole-cell, current-clamp mode, cells had membrane potentials of $-94{\cdot}7 \pm 6{\cdot}7$ mV and input resistances of 2–17 GΩ. Current-induced action potentials were recorded with a threshold around −35 mV and amplitude of 40–65 mV. Repetitive firing was not sustained at frequencies greater than 1–2 Hz without total inactivation.

3. Under voltage clamp, action potentials were shown to be composed of an inward TTX-sensitive Na^+ current and an outward K^+ current that was abolished by internal Cs^+.

4. The isolated Na^+ current had a threshold for activation around −35 mV and rapidly inactivated to a steady state during a test voltage pulse. Inactivation was strongly voltage-dependent, with the Na^+ current being half-inactivated at −20 mV.

5. Recovery from inactivation was voltage dependent and at a holding potential of −60 mV, 50% reactivation was achieved after 420 ms. The implications of this long reactivation time on sustained action potential frequency are discussed.

6. Single Na^+ channel activity was examined with the outside-out patch configuration and yielded single-channel conductances of 22·5 pS. Reconstruction of the voltage and time dependence of single-channel currents provided an accurate picture of the whole-cell Na^+ current.

7. Whole-cell outward current carried by K^+ in the absence of Na^+ and Ca^{2+} had a large conductance, was slowly activated and demonstrated no inactivation. A second, more rapidly activating Ca^{2+}-dependent K^+ current could also be demonstrated.

8. Ensemble analysis of whole-cell K^+ currents in the absence of Ca^{2+} showed underlying single-channel amplitudes of 1·2 pA at +10 mV, with the lactotroph having about 350 active channels at this potential.

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9. Recordings of single K^+ channels also demonstrated two classes of channel: a small (50 pS) voltage-activated channel and a higher-conductance (100 pS) Ca^{2+}-sensitive channel.

10. Prolactin secretion was shown to be TTX-insensitive but sensitive to membrane potential, demonstrated as increased release following increased external K^+ but not Na^+ concentration.

11. The failure to sustain high-frequency Na^+ action potentials and the TTX-insensitivity of secretion are discussed in terms of stimulus–secretion coupling in the lactotrophs.

INTRODUCTION

Electrophysiological properties of many endocrine cell types have provided evidence to suggest that the electrical properties of their membranes play a central role in the secretory process (for reviews, see Petersen, 1980; Poisner & Trifaro, 1985). Studies using both normal pituitary cells (Taraskevich & Douglas, 1977; Ingram, Bicknell & Mason, 1986) and the GH3 tumour cell line (Kidokoro, 1975; Ozawa & Sand, 1978; Ozawa & Kimura, 1979; Sand, Haug & Gautvik, 1980; Taraskevich & Douglas, 1980; Ozawa, 1981, 1985) have demonstrated that pituitary cells are able to generate spontaneous action potentials, the frequency of which is regulated by hypophysiotrophic releasing and inhibiting factors.

More recently the patch-clamp technique (Hamill, Marty, Neher, Sakmann & Sigworth, 1981) has been applied to the study of the whole-cell and single-channel currents of the GH3 and GH4 pituitary cell lines (Hagiwara & Ohmori, 1982; Dubinsky & Oxford, 1984; Fernandez, Fox & Krasne, 1984; Matteson & Armstrong, 1984). These studies have demonstrated a number of voltage-sensitive, ion-specific currents that may underlie spontaneous electrical activity. The advantage of examining electrophysiological properties in GH3 cells is that they provide a homogeneous cell population secreting specific hormone products. However, doubts have been raised as to the degree to which these cells have retained their normal function, and variations in the electrophysiological and secretory properties of different strains have been observed (Taraskevich & Douglas, 1980; Dubinsky & Oxford, 1984).

We have, therefore, concentrated on an examination of primary pituitary cell cultures. Previously, studies of normal anterior pituitary cell types have not been possible since the gland is a mixture of at least six different hormone-containing cell types and this heterogeneity restricts the extent to which properties can be ascribed to individual cell species. For the present study, we have developed a technique for the isolation of a population of anterior pituitary cells specifically enriched for prolactin-secreting lactotrophs (Mason & Ingram, 1986) and combined this with post-recording immunocytochemistry to confirm the identity of the recorded cells.

In this paper we describe the characteristics of both whole-cell and single-channel recordings of Na^+ and K^+ currents that are responsible for the propagation of action potentials in lactotrophs and attempt to correlate this activity with the secretory process. In particular, we have sought to examine the importance of Na^+–K^+ action potentials for prolactin secretion. Some of these data have been published in a preliminary form (Cobbett, Ingram & Mason, 1986*a*, *b*).

METHODS

Cell preparation

Lactotrophs were prepared from the pars distalis of bovine pituitary obtained from a local abattoir, using a previously described technique (Mason & Ingram, 1986). Briefly, fresh pituitary glands were enzymatically dispersed, and a lactotroph-rich population was produced by separation of cells on a discontinuous Percoll density gradient. Cells from the interface of the 1·062 and 1·074 mg/ml density layers were removed, washed and plated in 35 mm plastic Petri dishes (Nunc) at a density of 250000 cells per dish. The cells were maintained in Dulbecco's modified Eagle's medium with 10% newborn calf serum (Uniscience, Cambridge), at 37 °C in a humidified atmosphere containing 5% CO_2. This cell population contains approximately 70% lactotrophs as determined by immunocytochemistry for prolactin (see below), and few fibroblastic cells. Recordings were made 2–14 days following dispersion: no difference was detected in the properties of cells in relation to time in culture, and the data are therefore treated as such.

Electrophysiological recordings

Whole-cell, outside-out and cell-attached patch recordings were obtained, using the methods described by Hamill *et al.* (1981), to record membrane potentials and voltage-activated currents. Electrodes had resistances of 2–10 MΩ for whole-cell recordings and 2–25 MΩ for patch recordings. At the time of the recording, the culture medium was removed from the Petri dish and substituted by an appropriate recording medium (see Table 1) and the dish was mounted on the stage of an inverted microscope.

Using phase-contrast optics, we selected to record from single cells that were spherical and 12–17 μm in diameter. These characteristics are highly correlated with bovine prolactin-containing cells in culture (Ingram *et al.* 1986). Formation of a satisfactory seal (cell-attached patch) was observed as a large increase in resistance as measured by the current response to a small (4 mV) applied voltage pulse. Following appropriate compensation of the fast component of the capacitative current further suction was applied to rupture the membrane patch and produce the whole-cell recording configuration, seen as a sudden increase in the capacitative current transients. The series capacitance and series conductance, were then adjusted to compensate for this additional (whole-cell) capacitative current. Lastly the series resistance was compensated: typically the series resistance was 5·1 MΩ and could be 80% compensated before oscillation of the amplifier. In these conditions the mean maximum voltage drop during maximal Na^+ current activation (mean 283 pA) was 0·4 mV. In some experiments, outside-out patches were formed after retraction of the electrode from the cell during a whole-cell recording. In other experiments, the electrode was withdrawn before achieving the whole-cell mode to produce an inside-out patch.

To measure voltage-activated currents during voltage-clamp recordings, depolarizing test pulses were applied from a holding potential (−60 to −80 mV). To measure membrane potential, the amplifier was used in the current-clamp mode after the whole-cell configuration had been achieved.

Membrane currents and potentials were recorded with a List EPC-7 amplifier and stored on magnetic video tape (Lamb, 1985) at a recording bandwidth of 16 kHz. For analysis, records were first filtered using an 8-pole Bessel or Butterworth characteristic filter (Kemo type VBF8-03, Beckenham, Kent). Signals were digitized at appropriate frequencies using a PDP 11/73 computer and a Cambridge Electronic Design 502 analogue–digital interface with direct memory access to an 80 MB Winchester hard disc (see Figure legends for details of filtering and digitizing frequencies used). Computer acquisition of data, current averaging, and subtraction of capacitative and linear leakage currents were performed using two suites of programs. The first was the ANADISK and DA23 library of programs compiled by Dr T. D. Lamb (Physiological Laboratory, University of Cambridge), which were used for acquisition of data, capacitative–leak current subtraction, and average and variance computations. Currents obtained from negative voltage pulses of 20 or 40 mV were scaled to subtract capacitative and/or linear leak currents from responses to positive voltage pulses (all records illustrated are thus subtracted unless stated). A second suite of programs produced by J. D. Dempster (Department of Physiology and Pharmacology, University of Strathclyde, Glasgow) was used to compute single- and double-exponential decays using a Levenberg–Marquadt iterative least-squares curve-fitting routine on averaged data captured at 10–25 kHz. Other curve-fitting routines for single and double Lorentzian power density spectra were run under the maximum likelihood (MLP) data-analysis package on the Agrenet VAX network.

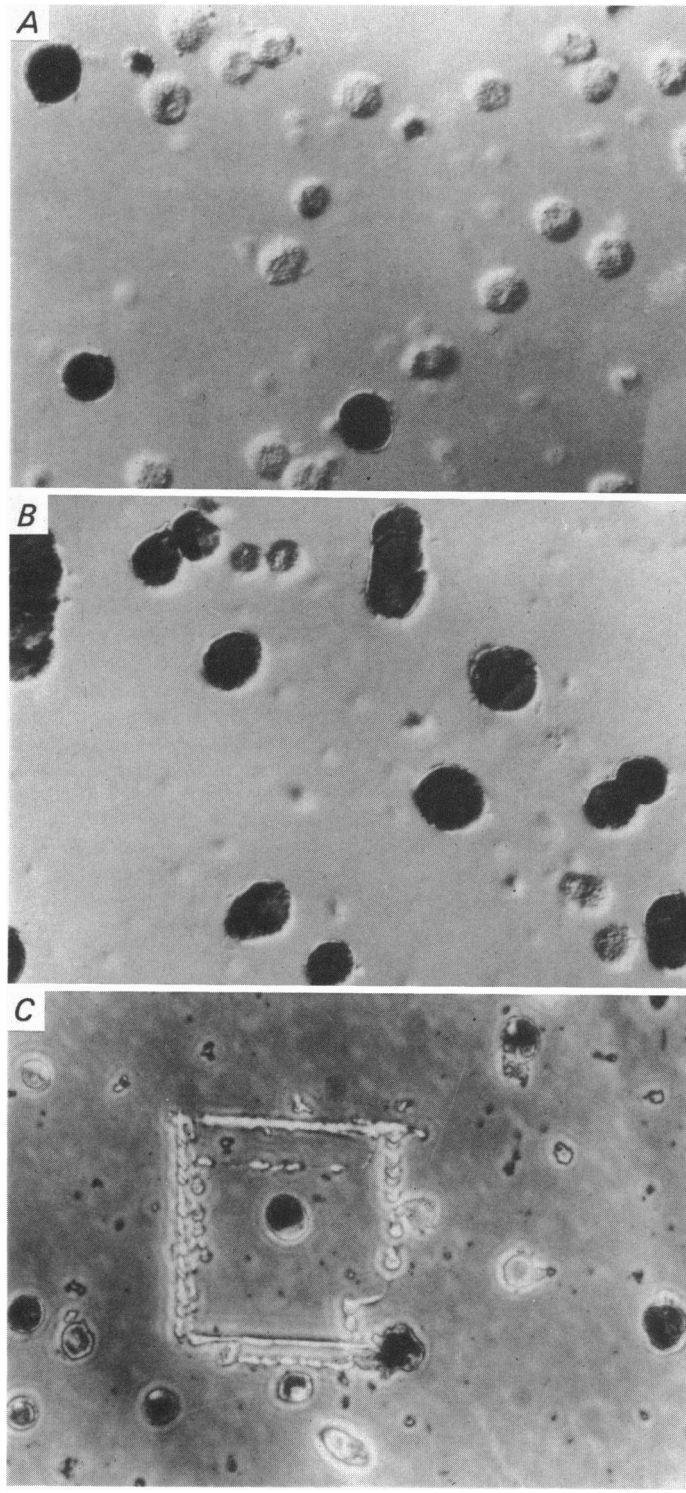

Fig. 1. For legend see opposite.

Immunocytochemistry

After a recording was completed, the Petri dish was scratched to indicate the position of the recorded cell. Cells were fixed in 4% w/v paraformaldehyde in phosphate-buffered saline (PBS, pH 7·3) for 1–2 h, and subsequently incubated in an antiserum to bovine prolactin (L 134) at a dilution of 1:20000. This antiserum shows full cross-reaction between ovine and bovine prolactin and is specific (with less than 0·3% cross-reaction with bovine growth, thyroid stimulating, luteinizing and follicle stimulating hormones). For immunocytochemical detection, a second biotinylated antibody directed against rabbit immunoglobulin was employed and was in turn detected with an avidin–biotin–horseradish peroxidase complex, both steps using reagents from Vectastain (Burlingame, CA, U.S.A.). Final visualization was with 3',3-diaminobenzidine (50 mg/100 ml PBS) and H_2O_2 (2 μl/100 ml), to give a dense brown reaction product. All immunoreactivity was abolished by pre-incubation with 10 μg prolactin/ml but not growth hormone.

Hormone secretion measurements

Techniques for measurement of prolactin secretion from monolayers of primary cell cultures were essentially as described by Ingram *et al.* (1986). Radioimmunoassay for prolactin used the same antibody as described above.

RESULTS

Identification of lactotrophs

Figure 1*A* shows an unpurified cell culture typical of those obtained by collagenase dispersion of bovine pituitary and immunocytochemically stained for prolactin. Figure 1*B* shows a culture obtained from the same dispersion following enrichment for lactotrophs using Percoll density gradients. With this technique, prolactin-positive cells constitute approximately 70% of this prolactin-rich fraction. It can also be seen that prolactin cells are significantly larger (12·2 $\pm$ 0·18 μm diameter, $n = 159$) than unstained cells (9·8 $\pm$ 0·16 μm, $n = 105$), and this difference was used in the selection procedure to increase the likelihood of recording from a lactotroph. Post-recording immunocytochemical identification with anti-prolactin antibody was routinely performed to confirm the prolactin content. A typical cell identified following whole-cell recording is shown in Fig. 1*C*.

General electrical properties

After initial seal formation, single-channel currents were observed, as well as spontaneous 'action currents' similar to those described in adrenal chromaffin cells (Fenwick, Marty & Neher, 1982). These action currents were biphasic in shape and were frequently activated by hyperpolarizing voltage pulses which would be expected to depolarize the remainder of the cell.

Fig. 1. Prolactin immunolabelling of pituitary cell cultures. *A*, unpurified fraction of pituitary cells plated prior to enrichment for prolactin cells. Fixed, immunostained and photographed at 4 days in culture. Only 30–40% of the cells are prolactin positive. *B*, cells from the density-gradient fraction enriched for prolactin used in these studies, stained immunocytochemically for prolactin. Note the large proportion of stained cells and their larger diameter compared to unstained cells. *C*, the bottom micrograph shows a cell marked following a whole-cell recording, and subsequently fixed and immunocytochemically stained for prolactin. This technique was used to stain the recorded cells in this paper, confirming their identity as lactotrophs. Magnifications: *A* and *B*, × 700; *C* × 450.

After rupture of the membrane patch, as monitored by an increase in capacitative current, lactotrophs were current and/or voltage clamped, and currents underlying action potential generation were studied in detail.

Whole-cell recordings: current clamp

Under current-clamp recording conditions with the electrode containing K^+ as the major cation (internal solution 2, external solution 1, Table 1), lactotrophs had resting potentials of $-94{\cdot}7 \pm 6{\cdot}7$ mV (mean ± S.E. of mean; $n = 38$). Spontaneous depolarizing potential fluctuations of up to 30 mV were recorded but spontaneous action potentials were absent in most cells. However, in four of thirty-eight cells

Table 1. Composition of solutions used for recording experiments

	External solutions (mM)			
Solution	NaCl	Choline	KCl	TEA
1	135	—	2	—
2	—	125	—	20
3	145	—	—	—
4	—	135	—	20
5	—	140	5	—

	Internal solutions (mM)		
	KCl	CsCl	EGTA
1	—	140	10
2	150	—	10

All external solutions additionally contained 5·6 mM-glucose, 1 mM-$MgCl_2$ and 10 mM-HEPES. Internal solutions contained only 10 mM-HEPES. All solutions were buffered to pH 7·3 with NaOH or KOH as appropriate. CsCl solution was buffered with TEA OH.

spontaneous action potentials were recorded and these appeared to be superimposed upon the depolarizing potential fluctations. In the remaining cells, one to three action potentials were evoked by injection of depolarizing current pulses (Fig. 2*Aa*). Threshold for these action potentials was between -40 and -30 mV and the first action potential peaked at a membrane potential (V_m) between 0 and $+25$ mV. This initial action potential had a duration of $11{\cdot}8 \pm 4{\cdot}5$ ms at one-third amplitude. The amplitude of successive action potentials during a steady current pulse was increasingly reduced until complete spike failure was observed. After returning the membrane to its resting potential (no applied current) for a 1–5 s period, a subsequent current pulse once again evoked action potentials. Even with a large depolarizing current injection, action potentials were not observed at frequencies in excess of about 2 Hz, before complete spike failure occurred. Action potentials were not affected by addition of EGTA (5 mM) to the bath but were completely abolished by 5–30 μM-TTX (tetrodotoxin) (data not shown).

Voltage responses to applied current were characterized by a membrane time constant of $67{\cdot}1 \pm 24{\cdot}2$ ms. The steady-state voltage response to these pulses produced a current–voltage (I–V) relation which exhibited a small outward rectification (Fig. 2*Ab*). Input resistances calculated from the response to hyperpolarizing pulses were in the range of 3–15 GΩ, approximately 10–50 times greater than obtained

during conventional intracellular recordings with high-resistance microelectrodes (Ingram *et al.* 1986).

Whole-cell recording: voltage-clamp recording of mixed currents

In the same cells from which current-clamp recordings were made, mixed inward and outward currents were recorded under voltage-clamp conditions (Fig. 2*Ab*). From a holding potential of −70 mV, voltage pulses to potentials more positive than

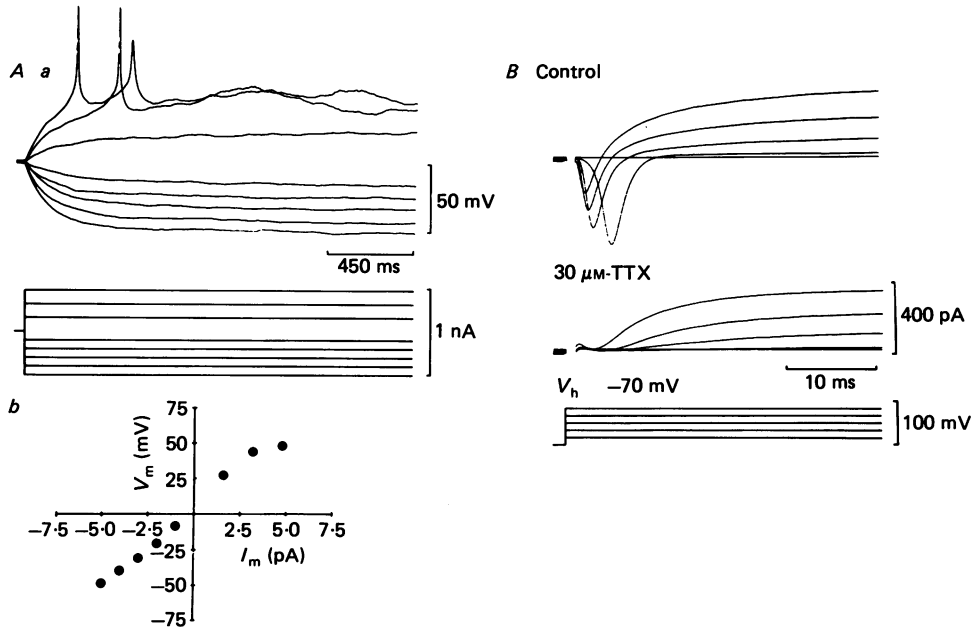

Fig. 2. Action potentials and underlying compound currents in bovine lactotrophs. *Aa*, cell recorded in current-clamp mode showing potential responses to applied depolarizing and hyperpolarizing current pulses (records digitized at 2 kHz). The initial action potential evoked by repeated current injection was of constant amplitude but subsequent spikes had a reduced amplitude. *Ab*, from the response to pulses of increasing hyperpolarizing current the input resistance of the cell was calculated to be 11·6 GΩ. *B*, upper panel shows the same cell recorded under voltage-clamp conditions. Application of voltage pulses from a holding potential of −70 mV evoked first a rapid inward current, and at more depolarized potentials a slow sustained outward current. Lower panel shows that the inward component of the mixed current (control) was blocked by TTX but the outward component was unaffected (30 μM-TTX). V_h, holding potential. (For *A* and *B*, records filtered at 3 kHz and digitized at 5 kHz.)

−35 mV produced an inward current which when maximally activated had a peak amplitude of more than 200 pA. At potentials between −35 and −20 mV, current activation and inactivation were slow. At more positive potentials, activation and inactivation of this inward current became more rapid and it was followed by a slowly developing, non-inactivating outward current (Fig. 2*B*). This latter current reached a maximum amplitude approximately 50 ms after onset of the voltage pulse and showed a near-linear relationship with applied voltage. Blockade of the action potential by TTX, noted above for current-clamped cells, was accompanied by

blockade of the inward current in voltage-clamp recordings, leaving the outward current unaffected (Fig. 2*B*).

To perform more detailed analyses of the two currents attributed to Na^+ and K^+ current flow and which underlie the action potential in lactrotrophs, currents were isolated by internal perfusion of the cell under appropriate recording conditions.

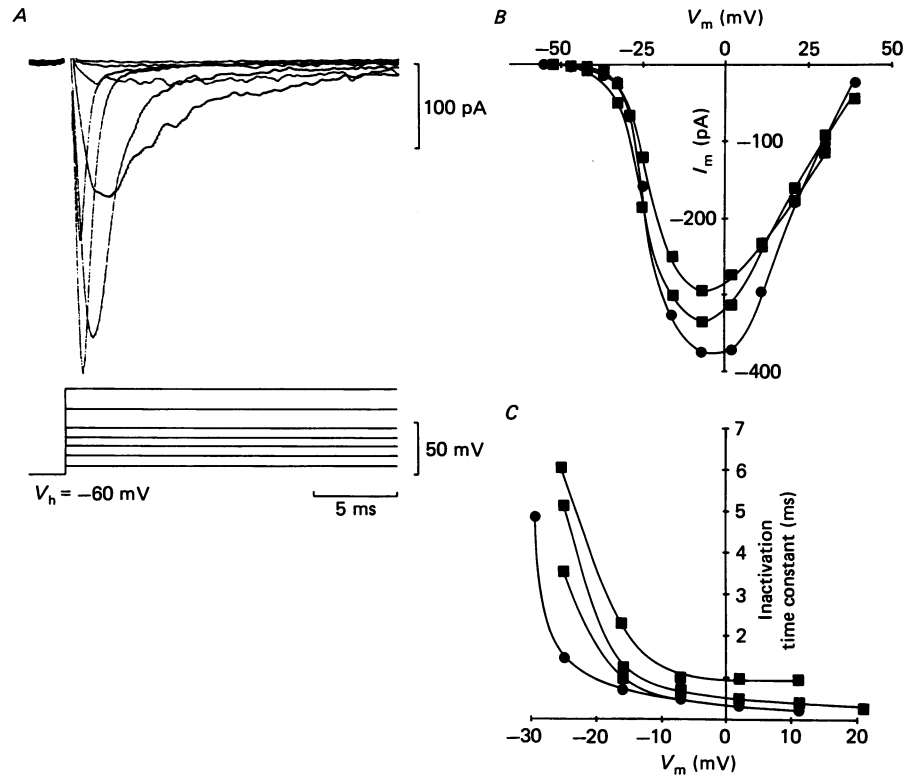

Fig. 3. Voltage dependence of I_{Na} in bovine lactotrophs. *A*, current records for test voltage pulses (average of five responses per potential) applied from a holding potential (V_h) of −60 mV. (Digitizing frequency 8 kHz, filter frequency 2 kHz.) *B*, *I–V* relationship of peak I_{Na}, for three representative cells, showing activation at potentials above −30 mV and peak activation at around +8 mV. *C*, plot showing the decline of the inactivation time constant at more positive test potentials in four representative cells. The values shown represent the time constant for the fast decay of the double-exponential fit and were obtained from non-subtracted current records. In *B* and *C* filled circles represent data plots from records shown in *A*.

Na^+ currents

To record isolated Na^+ currents, K^+ was replaced by Cs^+ as the principal intracellular cation (internal solution 1) and the extracellular medium contained high Na^+ and 20 mM-TEA (external solution 2) to prevent Cs^+ movement through K^+ channels. Under these conditions, positive test pulses (40 ms duration) applied from a holding potential of −60 mV activated an inward current with no evidence of any outward current (Fig. 3). The threshold for current activation was −40 to −30 mV and the current was maximal in the range of −10 to +10 mV (Fig. 3*A* and *B*),

declining towards zero at potentials between +40 to +60 mV. The current was rapidly activated, reaching peak amplitude within 5 ms, and then displayed a rapid inactivation to a steady-state level, usually about 5% of the peak current. These currents were completely abolished by addition of 10–30 μM-TTX, suggesting they were entirely carried through voltage-activated Na^+ channels.

The mean peak inward current was 283 ± 37 pA ($n = 8$). For an average lactotroph cell diameter of 12·2 μm, this yields a Na^+ current density of 60·6 $\mu A/cm^2$. The amplitude of inward current was dependent on external Na^+ concentration (data not shown), and when all external Na^+ was replaced by choline no inward current was observed. This confirms that Na^+ was indeed the current carrier responsible for this fast inward current.

During test pulses of increasing amplitude, the Na^+ current was observed not only to change in amplitude but also to change its activation and inactivation characteristics. The activation time (time to peak) of the Na^+ current was clearly dependent on the voltage applied and decreased at more positive potentials (see Fig. 3*A*). However, because onset of the ionic current was partially obscured by the capacitative current transient, a reliable study of the activation kinetics could not be undertaken. On the other hand, inactivation could be conveniently examined using either exponential-curve fitting of individual responses or two-pulse protocols.

Time- and voltage-dependent inactivation of Na^+ currents

Using computerized curve fitting of the current the time course of inactivation of the Na^+ current during a prolonged (40 ms) test pulse was best described by a double-exponential decay to a steady-state level. The slower component of this double exponential was small (less than 10% of the fast component) and was voltage independent. In contrast, the time constant for the rapid phase of the decay was voltage dependent and decreased sharply at potentials more positive than −30 mV (Fig. 3*C*).

The time course of inactivation was studied in more detail using a two-pulse technique in which each test pulse was preceded by a conditioning pulse of variable duration and amplitude (Fig. 4). The amplitude of the current activated during the test pulse was proportional to the fraction of I_{Na} that had not been inactivated during the conditioning pulse. In Fig. 4*C* this non-inactivated fraction of the Na^+ current is expressed as a fraction of the total current recorded in the absence of a conditioning pulse. The voltage and time dependence of inactivation were initially examined by applying conditioning pulses of increasing duration to two potentials, −17·3 and −26·1 mV (Fig. 4*A* and *B*). Inactivation was observed to increase with conditioning-pulse duration: with a conditioning-pulse potential of −26·1 mV 50% of the current was inactivated within 10 ms (Fig. 4*C*). The time course of inactivation was sigmoidal with a lag of 1–1·5 ms after onset of the test pulse before the beginning of significant inactivation, after which the non-inactivated fraction of the current declined to a steady-state level (~20%) after about 20 ms. In contrast, at −17·3 mV a conditioning pulse of 2·5 ms was sufficient to produce 50% inactivation (Fig. 4*C*). In this case, the lag between test-pulse onset and onset of inactivation was reduced to only 0·5 ms, I_{Na} was totally inactivated with conditioning pulses of 7 ms, and steady-state inactivation was more complete (5% non-activated).

The voltage dependence of this Na^+ current inactivation was demonstrated by

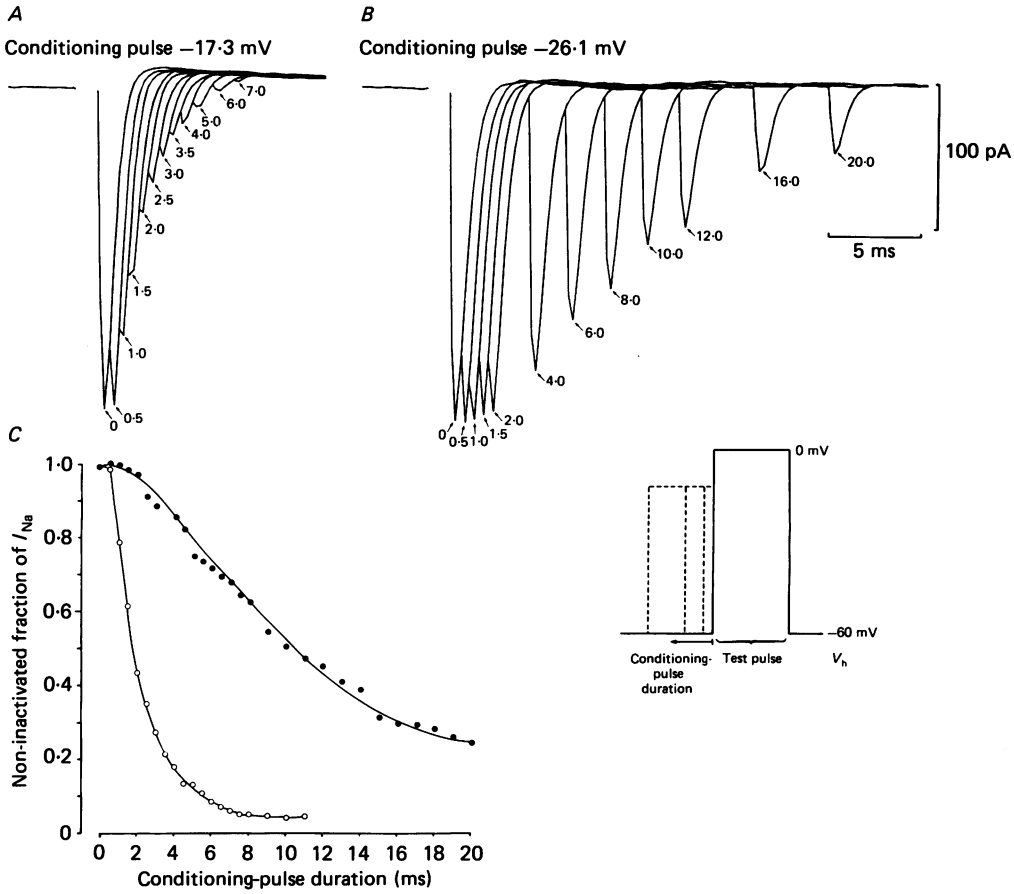

Fig. 4. Time course of inactivation of I_{Na} demonstrated with a two-pulse protocol. I_{Na} was recorded during a test pulse (to 0 mV) which was preceded by a conditioning pulse of different durations and two amplitudes (see inset): pulses applied at 0·33 Hz. *A*, currents during test pulses showing I_{Na} for different duration conditioning pulses to a potential of −17·3 mV. Each record was averaged from ten responses and was displaced relative to the conditioning-pulse duration (ms) which is given next to each peak. (Records were filtered at 2 kHz and digitized at 5 kHz.) *B*, current records obtained during test pulses for a conditioning-pulse potential to −26·1 mV. *C*, peak currents, plotted as the non-inactivated fraction of I_{Na}, against conditioning-pulse duration. It can be seen that there is a delay of 0·5–1·0 ms before any inactivation takes place, and that it has a sigmoid decay to a steady-state current. Steady-state inactivation is more complete at the more depolarized conditioning potential. Conditioning-pulse potentials: ●, −26·1 mV; ○, −17·3 mV. V_h, holding potential.

applying conditioning pulses of constant duration but variable amplitude before a test pulse to 0 mV from holding potentials of −48, −66·5 and −87·5 mV. Individual data for a holding potential of −87·5 mV are shown in Fig. 5*B*, demonstrating that at more positive potentials an increasing proportion of the current is inactivated during the conditioning pulse. Figure 5*B* shows combined data from all holding potentials tested. Inactivation was not dependent on the holding potential suggesting

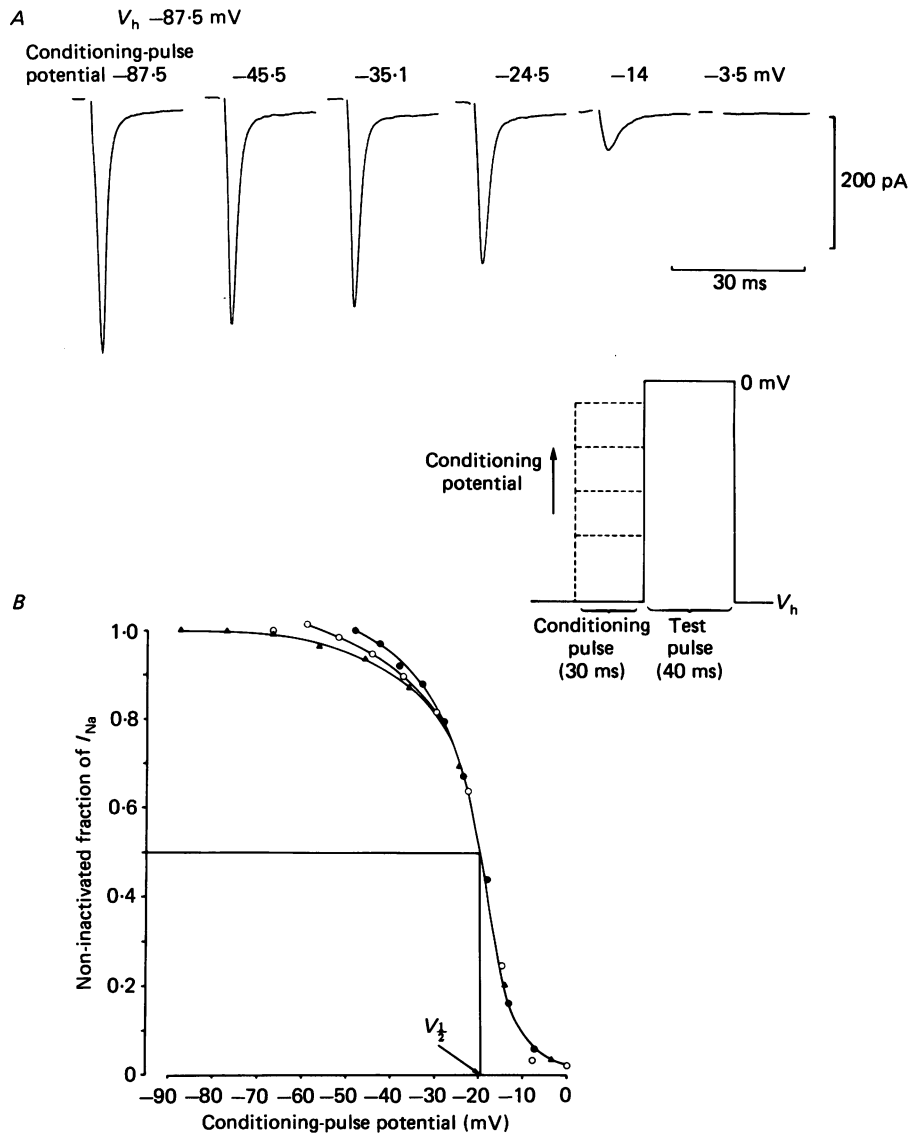

Fig. 5. Steady-state inactivation of I_{Na} was examined by measuring I_{Na} during a test pulse following a 30 ms conditioning pulse of variable amplitude (see inset). *A*, current records obtained with conditioning pulses, to the given potential, from a holding potential of −87·5 mV. Each record is the average of ten responses and currents were corrected, in this case, for capacitative and leakage currents by subtraction of the residual current after addition of 30 μM-TTX. (Digitizing frequency 5 kHz, filter frequency 2 kHz.) *B*, I_{Na} normalized to the peak current obtained for test pulses in the absence of a conditioning pulse. Control pulses (no conditioning pulse) both before and after each series of experiments were used to correct the data for small linear run-down of the current. It can be seen that the voltage dependence of steady-state inactivation is independent of the holding potential (V_h) before the conditioning pulse. The potential for half-maximal steady-state inactivation ($V_{\frac{1}{2}}$) was −19·5 mV in each case. Holding potential: ●, −48 mV; ○, −66·5 mV; ▲, −87·5 mV.

there is only a single activatable state. However, inactivation was principally determined by the conditioning-pulse amplitude: in this experiment the potential at which 50% of the current remained non-inactivated was −19·5 mV (Fig. 5*B*) and similar values were observed in other experiments. At −35 mV, the average threshold for the Na^+ action potential obtained in current-clamp experiments reported above, the majority of the current was still available for activation (~10% inactivation).

Recovery of Na^+ current from inactivation

After inactivation to the steady-state level during a test pulse, the Na^+ current could only be fully reactivated by returning to the holding potential (−60 mV). The time course of this reactivation process was studied by two different methods. The first of these employed trains of ten test pulses of constant amplitude and duration but variable frequency to examine steady-state reactivation during repetitive current activation. It was assumed that all recovery from inactivation took place during the interpulse interval (time spent at the holding potential). Figure 6*A* shows the averaged current responses to the first eight identical test pulses (to 0 mV) at three different frequencies, each averaged from five trains at each frequency. During a train of pulses, the peak current amplitude evoked by successive pulses declined to an equilibrium (Fig. 6*A* and *B*). This equilibrium value was proportional to the fraction of I_{Na} reactivated during the interpulse interval and can be seen to decrease for smaller intervals (i.e. higher frequencies). In Fig. 6*B* the decay to the equilibrium non-inactivated value is shown during trains of ten pulses at eight different frequencies. The plot of the equilibrium fraction of I_{Na} as a function of the interpulse interval shows that the current reactivated slowly, being only 50% complete after 420 ms (Fig. 6*C*). This suggests that the balance between inactivation and reactivation (at these potentials) is shifted in favour of inactivation.

It is also interesting to note that the current does not reach the equilibrium state during the second pulse, but decays only gradually over the first six pulses (Fig. 6*B*). If the relationship between inactivation and reactivation were a simple two-state (open/closed) mechanism and if most channels were activated on the first pulse then, since all pulses were identical, it would be expected that equilibrium would be reached in the second pulse. However, this slowly developing inactivation suggests the existence of two non-conducting states, with inactivation being the sum of two kinetic processes, one rapid and predominating over one pulse and one slow, becoming more apparent over several pulses.

The second method for examining reactivation employed pulses of constant frequency (1·7 Hz) but varied duration. The results of this method (Fig. 6*C*, triangles) gave a similar half-maximal recovery time (450 ms) to that of the first method. This similarity indicates that the time spent at the holding potential, not the frequency or duration of current activation *per se*, affects the time course of reactivation from equilibrium inactivation. However, in this experiment the equilibrium value was reached by the second pulse (data not shown) which suggests that the second component of inactivation was complete during the first 550 ms pulse.

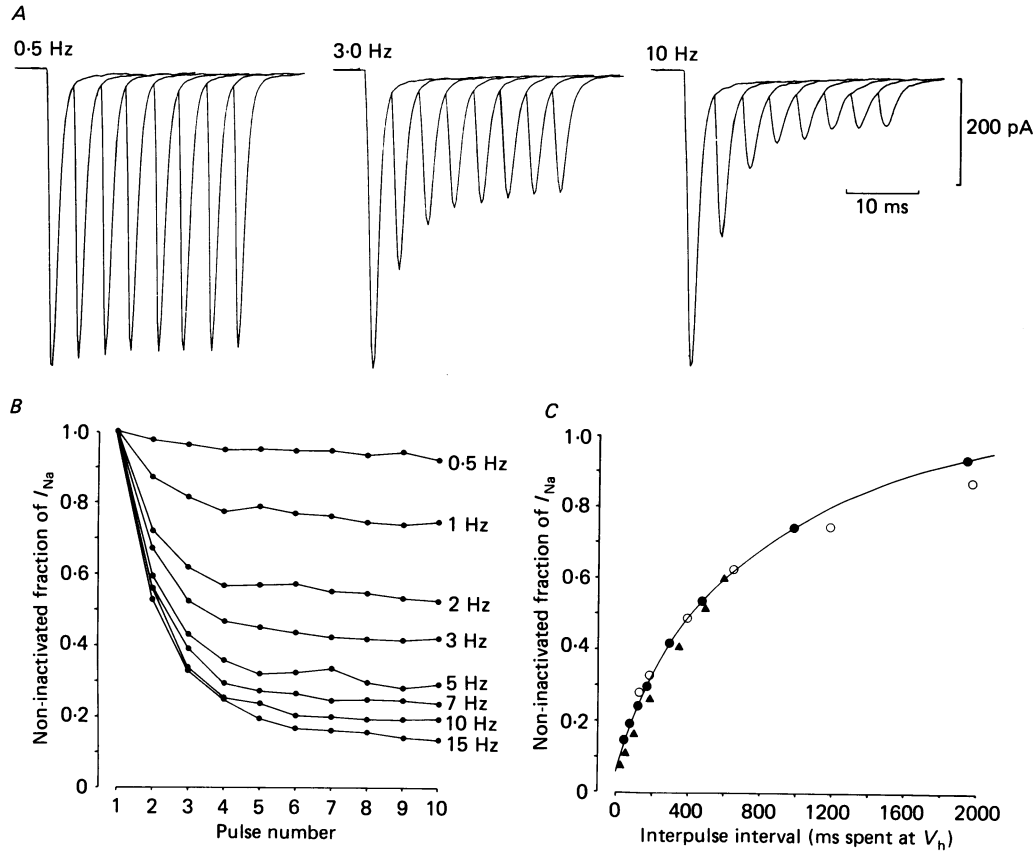

Fig. 6. Time course of reactivation of I_{Na} in bovine lactotrophs. *A*, sequential current responses to repeated test pulses (15 ms to 0 mV) at three different frequencies. Each record is averaged from five trains at that frequency and is displaced for display purposes. Each train of pulses was separated by a period of 30 s to allow for total recovery to occur. (All currents are corrected for leakage and capacitative currents by subtraction of the residual current present after adding 30 μM-TTX.) *B*, peak currents during successive pulses plotted as the fraction of the response obtained during the first pulse for eight different frequencies. *C*, non-inactivated fraction of I_{Na} plotted as a function of the interpulse interval. Symbols represent the data from *A* and *B* (closed circles), a second cell analysed in the same way (open circles) and a third cell analysed by the method described in the text (triangles). The curve is fitted to the data from parts *A* and *B* and shows that 50% reactivation has occurred for an interpulse interval of approximately 420 ms. Holding potential (V_h) = −60·0 mV; test potential = 3·7 mV; test-pulse duration = 20 ms.

Single-Na^+-channel currents

To observe single-Na^+- channel currents underlying the whole-cell current, we used outside-out patches.

This approach revealed single Na^+ channels which appeared rapidly after the onset of a voltage pulse (Fig. 7*A–D*), and varied in size dependent on test-pulse potential. Single-Na^+-channel inward currents of −1·8 to −0·8 pA were observed during po-

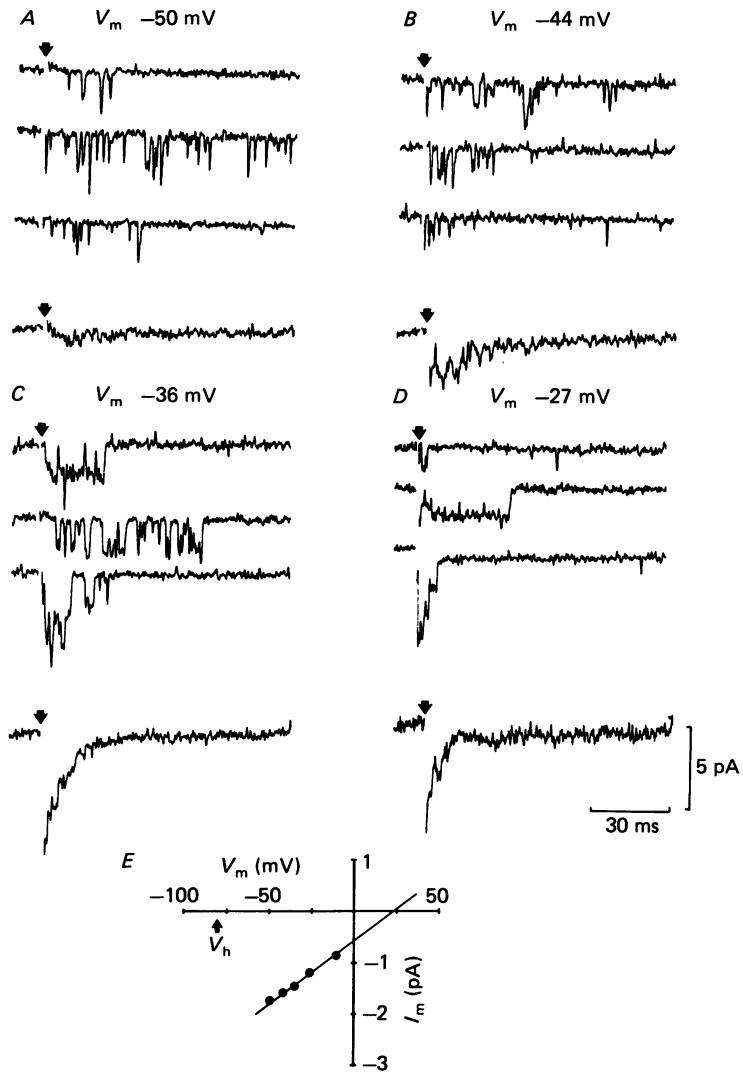

Fig. 7. Single-Na^+-channel currents recorded in an outside-out membrane patch. Each group of three records shows currents recorded at test potentials: *A*, −50; *B*, −44; *C*, −36; and *D*, −27 mV (holding potential (V_h) of −80 mV). The arrow shows the onset of the test pulse. Beneath each group is the average current record obtained from fifty individual records, and gives an indication of the similarity of the time and voltage dependency of single-channel and whole-cell current (see Fig. 3*A*). (Digitizing frequency 8 kHz, filter frequency 4 kHz.) *E*, the *I–V* relation derived from this single-channel data, indicated a slope conductance of 23 pS and a reversal potential near to +25 mV.

tential changes from a holding potential of −80 mV to test potentials of −50 to −10 mV. The reversal potential, calculated from the *I–V* relation (Fig. 7*E*), was close to +25 mV and near to the predicted Na^+ equilibrium potential for these recording conditions. The variation of single-channel current amplitude was nearly linear with voltage over this range and yielded a slope conductance of 22·5±2·1 pS

($n = 3$). Averaging of more than fifty responses for each test potential yielded current profiles similar to those for macroscopic currents recorded in the whole-cell mode (see average trace below each set of individual records, Fig. 7*A–D*), with respect to the voltage and time dependence of activation and inactivation.

K^+ currents

The inward current described above is responsible for the depolarizing phase of the action potentials. Repolarization will be partly the result of inactivation of these currents and partly due to voltage activation of outward currents. These currents were examined after replacing Na^+ with choline (external solution 3) in the extracellular solution and using K^+ as the major intracellular cation (internal solution 2).

In the absence of external Ca^{2+} test pulses to a potential more positive than −30 mV from a holding potential of −60 or −70 mV activated an outward current which had a near-linear relationship with applied potential (Fig. 8*A* and *D*). The threshold for the isolated K^+ current was similar to that for the outward component of the mixed current (see Fig. 2*B*). The K^+ current was slowly activated (reaching a maximum level 50 ms after onset); however, inactivation was not observed even when pulses of longer than 2 s were applied. Close to the threshold potential, activation of outward current has a slow and sigmoidal shape (Fig. 8*A*), while at more positive potentials, activation became more rapid. It was unaffected by omitting EGTA from the pipette solution or addition of Ca^{2+} channel blockers (including 5 mM-Mn^{2+} or 5 mM-Cd^{2+}) to the external solution (data not shown). This Ca^{2+}-independent current has been termed $I_{K(V)}$.

Addition of Ca^{2+} to the external solution at concentrations greater than 5 mM led to the appearance of an initial transient current superimposed on $I_{K(V)}$ (Fig. 8*B*). This current was blocked by 5 mM-Mn^{2+} or Cd^{2+} (Fig. 8*C*). The amplitude of this Ca^{2+}-sensitive current, termed $I_{K(Ca)}$, varied between cells but was always detected by a marked increase in the initial rate of activation of outward currents.

Figure 8*D* shows the *I–V* relation for the outward current detected at the end of each 400 ms test pulse in these records, a point at which the contribution of $I_{K(Ca)}$ might be expected to be small. The effect of increasing concentration of divalent cations (including Ca^{2+}, Mn^{2+} and Cd^{2+}) to the external solution was to cause a positive potential shift in the activation characteristics of the late $I_{K(V)}$ (Fig. 8*D*). This shift ranged from 12 mV for 5 mM to 34 mV for 15 mM total divalent cation concentration.

The voltage-activated current ($I_{K(V)}$) detected in the absence of extracellular Ca^{2+} (Fig. 9*A*) was almost totally blocked by the addition of the K^+ channel blocker TEA (15 mM) to the external solution (Fig. 9*B*). This block was complete over the potential range of −30 to 0 mV which would be expected to be activated during the action potential. Subsequent addition of Ca^{2+} in excess of 5 mM activated a rapid outward current (Fig. 9*C*) which had a temporal nature similar to $I_{K(Ca)}$. This implies that $I_{K(Ca)}$ is partially TEA insensitive.

No outward current was observed when K^+ was replaced by Cs^+ as the principal intracellular cation. This effect was apparent within 15–30 s following establishment of the whole-cell recording mode and suggests that exchange of the pipette contents with the cytoplasm was rapid. However, outward current was efficiently carried by

Rb^+, although we have not compared the selectivity of the outward-current channels for K^+ *vs.* Rb^+.

Ensemble analysis of whole-cell K^+ currents

To examine the single-channel events underlying K^+ currents in lactotrophs, we applied the technique of ensemble analysis to isolated K^+ currents in the absence of Ca^{2+}. Averaging was achieved using at least fifty current responses to test pulses to

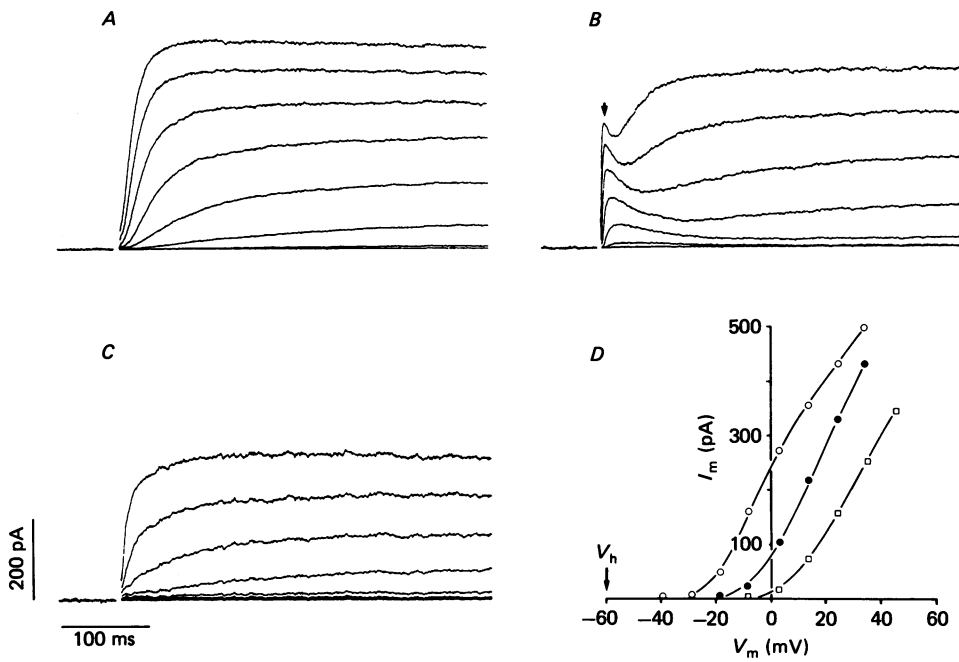

Fig. 8. Voltage-dependent and Ca^{2+}-dependent whole-cell K^+ currents in a lactotroph. *A*, when the intracellular solution contained K^+ test pulses from a holding potential of −60 mV evoked a slowly activating outward K^+ current which did not inactivate during long voltage pulses. Test potentials were −30, −19, −8, 3, 14, 25 and 36 mV. *B*, after addition of 10 mM-Ca^{2+} to the external solution, a multicomponent outward current was obtained with an initial rapidly activating and inactivating current (arrowed). *C*, this rapid current was abolished by the presence of 5 mM-Cd^{2+} in the external solution. (All records are averages of five responses. Digitizing frequency was 5 kHz and filter frequency was 2 kHz.) *D*, *I–V* relationships showing a positive shift in the voltage activation characteristics for the current at the end of records in *A* (open circles), *B* (closed circles) and *C* (open squares). V_h, holding potential.

0, +20 and +40 mV from a holding potential of −70 mV. For each test potential, the mean current (I_m) and variance of the ensemble current (σ^2) were calculated and plotted to obtain an estimate of the underlying single-channel current (intercept with the ordinate). Figure 10*A* shows such analysis for a test potential of 0 mV and Fig. 10*B* shows similar data for a test potential of +40 mV. Linear-regression analysis of the lower plots of σ^2/I_m *vs.* I_m implied a single-channel current (i) of about 0·38 pA at 0 mV and of 1·52 pA at +40 mV. Taken together with values at +20 mV for this cell (data not shown, i = 1·04 pA), a slope conductance of about 25 pS was derived

for the underlying K^+ currents. From the slope of the line of the same data, a total of about 350 open channels was derived at 0 mV.

Single-K^+-channel currents

In order to compare these statistical estimates obtained from analysis of whole-cell currents with the unitary current amplitude, single-channel K^+ currents were recorded in the outside-out or cell-attached patch configuration. Outside-out patches

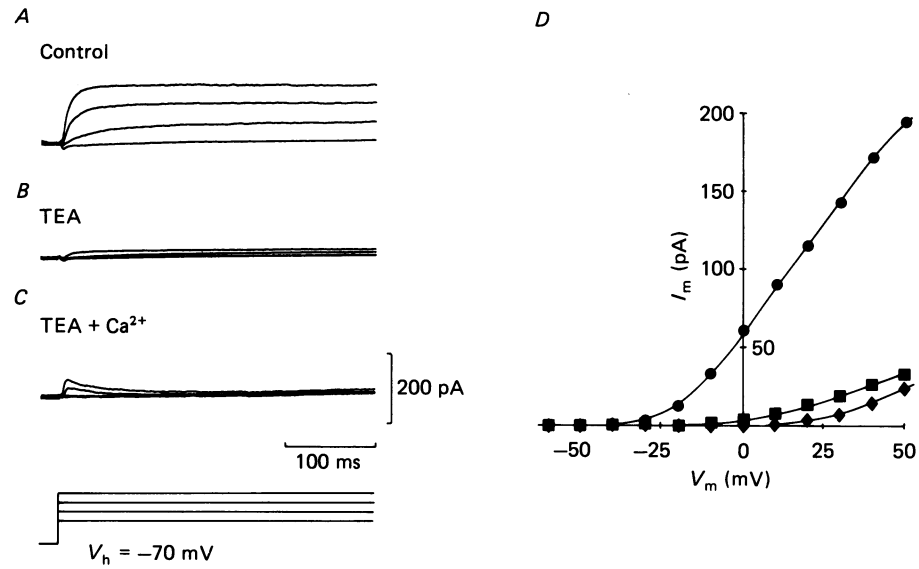

Fig. 9. TEA sensitivity of voltage-activated K^+ currents. *A*, control records show typical large outward currents in response to test pulses from a holding potential of −70 mV. *B*, addition of 15 mM-TEA to the bath suppressed the peak outward current by about 85% for this cell. *C*, addition of 5 mM-Ca^{2+} in the presence of TEA revealed a rapidly activating Ca^{2+}-sensitive component of K^+ current which was inactivated with time to a steady-state level similar to that seen in the absence of Ca^{2+} and presence of TEA. (Records were digitized at 4 kHz, filtered at 2 kHz.) *D*, *I–V* relationship at the end of the 400 ms pulse under conditions in *A* (circles), *B* (squares) and *C* (diamonds). V_h, holding potential.

were obtained from cells after demonstrating the presence of the whole-cell ionic current. We observed at least two distinct channel types, distinguished on the basis of single-channel conductance and Ca^{2+} sensitivity.

The first K^+ channel recorded in outside-out patches had a small conductance, usually less than 50 pS, and was not Ca^{2+} sensitive. This small-conductance channel was commonly observed in outside-out patches where the internal Ca^{2+} was buffered to a low value (less than 10^{-8} M) and as with the whole-cell recordings, single-K^+-channel currents had a threshold of −30 mV and channel inactivation did not occur during pulses of more than 1 s. Examination of single-channel current amplitude examined at various membrane potentials yielded a slope conductance of 42 pS.

The second K^+ current had a conductance in excess of 100 pS and was Ca^{2+} sensitive. Figure 11 shows this larger-conductance K^+ channel recorded in a cell-

attached patch following establishment of a seal. Its slope conductance was calculated to be 110 pS. In other experiments not reported here, this channel has been shown to be sensitive to Ca^{2+} on the internal membrane face (W. T. Mason & P. Cobbett, unpublished data), and probably represents the commonly found Ca^{2+}-activated channel permeable to K^+.

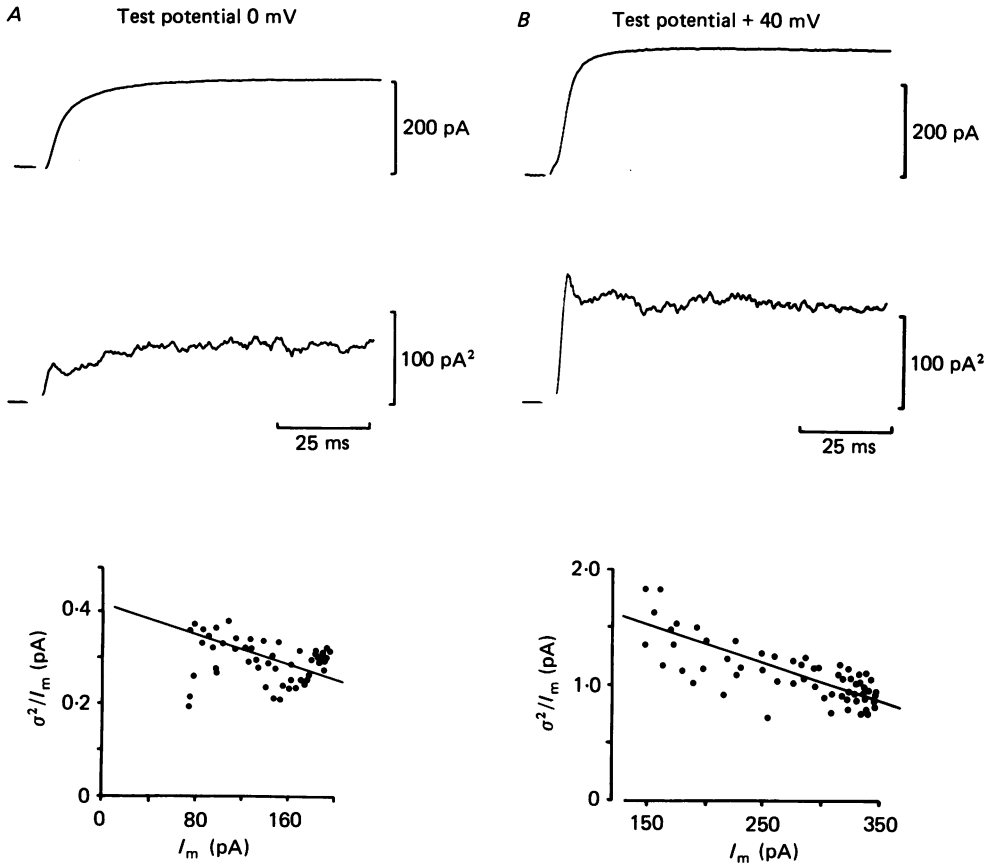

Fig. 10. Ensemble analysis of voltage-activated K^+ currents recorded in the absence of Ca^{2+}. Data collected for test pulses to −10 mV (*A*) and +30 mV (*B*). The top record of *A* and *B* shows current and the bottom record shows the computed variance of the current for fifty test pulses. The relationship between current and the ratio of variance to current is plotted for each potential and indicates a single-channel current of 0·38 pA at −10 mV and 1·52 pA at +30 mV. Regression lines fitted to the data indicated a slope consistent with the number of open channels being 265 at −10 mV and 330 at +30 mV. Respective correlation coefficients were $r = 0{\cdot}92$ and $r = 0{\cdot}87$. (Digitized at 4 kHz, filtered at 2 kHz.)

Hormone secretion

To examine the possible role of voltage-activated Na^+ and K^+ currents in regulation of prolactin release from lactotrophs, secretion from lactotroph-rich cultures was measured. Experiments were performed using a modified recording medium which contained 5 mM-Ca^{2+} and 125 mM-Na^+.

Figure 12 shows the combined data from two separate cell preparations and demonstrates that TTX (6 μM) had no significant effect ($P < 0{\cdot}05$; Student's t test) either on basal prolactin secretion or on secretion stimulated by thyrotropin-releasing hormone (TRH), a releasing factor known to increase the frequency of action potentials (Taraskevich & Douglas, 1977; Ingram *et al.* 1986). In other experiments,

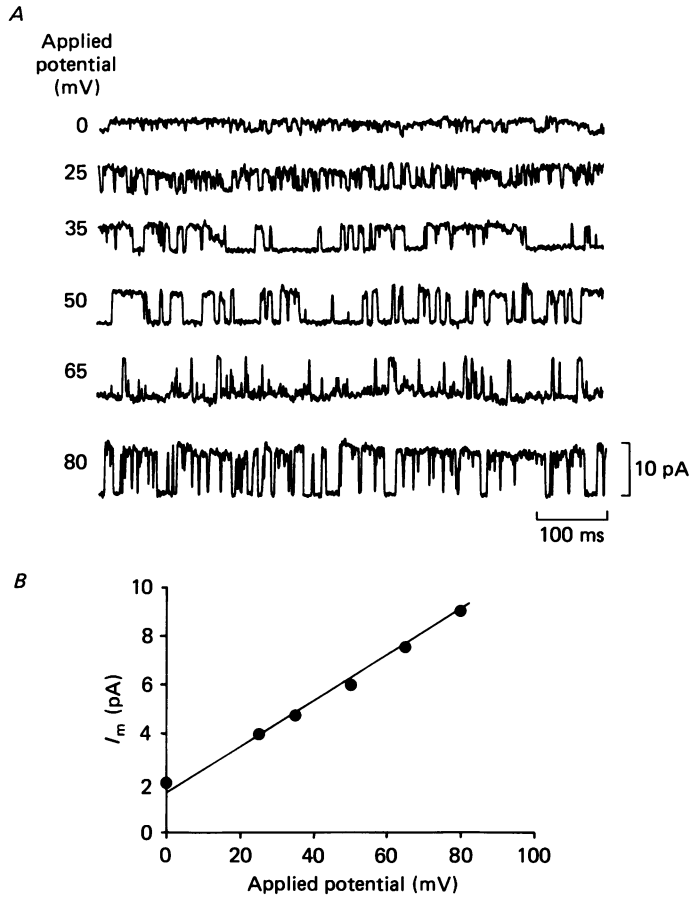

Fig. 11. *A*, single-channel K^+ currents recorded from a cell-attached membrane patch (internal solution 1, Table 1) at various applied potentials as shown for each record. *B*, the *I–V* relationship gives a slope conductance of 105 pS and a conductance of 1·65 pA at the resting potential (zero applied potential).

30 μM-TTX was also without effect on prolactin secretion. In a different group of experiments, the effect of TEA was tested on basal and TRH-induced secretion. Although TEA had no detectable effect on basal prolactin secretion, TEA did significantly ($P < 0{\cdot}01$) increase TRH-stimulated secretion by about 20% compared to TRH alone. In a final group of experiments, the effects of increased K^+ and Na^+ concentrations were tested. An increment of 60 mM-KCl, adequate to strongly depolarize the cells, evoked a highly significant increase in prolactin secretion, whereas a similar increase in NaCl was without significant effect ($P < 0{\cdot}05$).

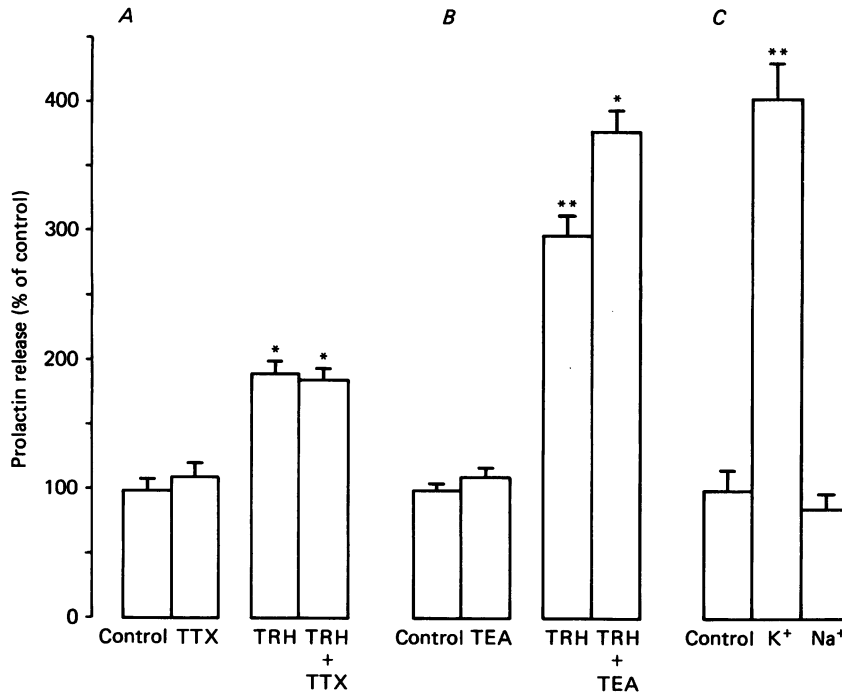

Fig. 12. Prolactin secretion from lactotroph-enriched pituitary cell cultures. *A*, the inclusion of 6 μM-TTX in the medium had no significant effect on either the basal or TRH (1 μM)-stimulated release of prolactin. (Both TRH- and TRH+TTX-stimulated prolactin secretion were significantly different from control at the 0·01 level.) *B*, TEA (20 mM) had no significant effect on basal secretion, but significantly (* $P < 0{\cdot}05$) increased TRH (1 μM)-stimulated prolactin secretion. TRH-stimulated prolactin secretion in this group was significantly greater than control at the 0·001 level (**). *C*, elevation of K^+, but not Na^+, by 60 mM to make the medium hypertonic significantly (** $P < 0{\cdot}001$) increased basal prolactin secretion. This was presumably due to the depolarizing effect of K^+ on the lactotroph. Experiments in *A* are the mean ± S.E. of mean of eight tests (eight different cell preparations) with each point in quadruplicate, whereas experiments in *B* and *C* are similar results but for four different preparations, also in quadruplicate.

DISCUSSION

Most current knowledge about the ionic mechanisms involved in control of hormone secretion from anterior pituitary cells has derived from a number of studies on clonal pituitary tumour cell lines, particularly the GH3 and GH4 subclones which secrete growth hormone and prolactin (Hagiwara & Ohmori, 1982; Dubinsky & Oxford, 1984; Fernandez *et al.* 1984; Matteson & Armstrong, 1984) which in several respects may not reflect the physiology of the normal cell.

In order to achieve an understanding of normal pituitary cell physiology we have studied immunocytochemically identified bovine lactotrophs using the whole-cell recording technique. This has made possible the first detailed study of Na^+ and K^+ channels in normal pituitary cells using voltage-clamp techniques. The major ques-

tions addressed in this paper are what the characteristics of voltage-activated Na^+ and K^+ channels in normal lactotrophs are, how these might contribute to action potentials, and how these action potentials might play a role in the secretory process.

Action potentials and Na^+ currents in bovine lactotrophs

From current-clamp experiments it has been demonstrated that bovine lactotrophs generate Na^+-dependent, Ca^{2+}-independent action potentials similar to those found in GH3 (Ozawa, Miyazaki & Sand, 1979) and pituitary pars intermedia cells (Douglas & Taraskevich, 1978, 1980). When spontaneous, these action potentials were triggered by depolarizing potential fluctuations. Similar fluctations were observed during recordings of bovine pituitary cells using conventional intracellular recording techniques (Ingram *et al.* 1986). However, in this case they were > 5 mV in amplitude. This difference results in part from the much higher seal between membrane and electrode with the whole-cell technique than the conventional technique. The high resting potentials recorded in the present study may also reflect quality of the seal and the high input resistance of these cells. However, the measured resting potential (mean $-94{\cdot}7$ mV) was in most cases lower than the K^+ equilibrium potential ($-108{\cdot}7$ mV at 18 °C). That the resting potentials in some cases were in excess of the K^+ equilibrium potential must be due to a contribution to the resting potential by non-permeable anions. In our laboratory these high values have not been recorded from cultured neurones and other cultured pituitary cells with similarly high input resistances (S. Rawlings & W. T. Mason, unpublished observations) under the same ionic conditions, thus eliminating the possibility of instrumentation error. The characteristics of the action potentials are similar to those in other secretory cell types (Fenwick *et al.* 1982), yet differ from those in neurones in having a relatively high threshold for activation and a long reactivation time. The latter is seen as failure to sustain repetitive firing of action potentials.

From voltage-clamp recordings, peak inward Na^+ current density ($60{\cdot}6$ $\mu A/cm^2$), calculated on the basis of a spherical cell with negligible infolding, was also considerably smaller than that measured in axons and in cultured rat muscle cells (1 mA/cm^2; Sigworth & Neher, 1980) but larger than in a neuroblastoma × glioma hybrid cell line ($8{\cdot}5$ $\mu A/cm^2$; Bodewei, Hering, Schubert & Wollenberger, 1985). However, current density at peak magnitude was similar to that of GH3 cells (200 $\mu A/cm^2$) and other secretory cells (Fenwick *et al.* 1982; Marty & Neher, 1983), a feature which may underlie a difference in the functional importance of the Na^+ current between neuronal and endocrine cells. The inward current density derived from these studies suggests that lactotrophs have three to five Na^+ channels per 10 μm^2 of surface area.

Although current density may vary, the properties of single lactotroph Na^+ channels are in many respects similar to those recorded in a variety of other tissues. For instance, the conductance (15–20 pS) of single-channel currents recorded in outside-out patches is similar to that recorded by other groups from cardiac cells (Cachelin, de Peyer, Kokubun & Reuter, 1983), skeletal muscle cells (Sigworth & Neher, 1980) and bovine chromaffin cells (Fenwick *et al.* 1982).

Inactivation of Na^+ currents

Inactivation of I_{Na} was rapid and followed a sigmoidal time course with a brief lag before the onset of inactivation. A similar time course has been reported for inactivation of I_{Na} in squid axons (Bezanilla & Armstrong, 1977) and is probably a characteristic function of the mean open-time distribution of the Na^+ channel. The results obtained using both two-pulse protocol (Fig. 4) and single-channel data (Fig. 7) show that this open time is reduced at more positive potentials. Inactivation time constants were reduced at potentials positive to −20 mV and a similar voltage dependence has been reported for inactivation of I_{Na} in GH3 cells (Fernandez *et al.* 1984), except that in the latter the relationship is displaced approximately 10 mV more negative compared to the present relationship.

The steady-state inactivation characteristics of I_{Na} have indicated that at −38 mV, the average threshold for action potential generation, approximately 90 % of the total current is available to contribute to the action potential. This result is in marked constrast to GH3 cells in which only 20% of total I_{Na} is available at this potential (Dubinsky & Oxford, 1984; Matteson & Armstrong, 1984). It is interesting to note the difference between GH3 cells and normal lactotrophs with respect to the negative potential shift of the inactivation time constant and half-inactivation potential of the Na^+ current in GH3 cells. We suggest this is a physiological difference and not the result of a difference of ionic conditions or technique since the I–V relationships for I_{Na} in normal lactotrophs and GH3 cells are very similar.

When a holding potential of −87 mV was used, only 3% of the total current was inactivated by a conditioning pulse to −60 mV, indicating that highly negative potentials do not activate a greater number of Na^+ channels than the holding potential of −60 mV used for most of these studies. That the inactivation of the Na^+ current was independent of the holding potential (providing this was more negative than the threshold for current activation) suggests there is only a single activatable state. On the other hand, reactivation experiments suggest the existence of more than one non-conducting state. Reactivation is conventionally studied using paired pulses rather than pulse trains. We used pulse trains precisely because we were interested in steady-state reactivation. It is this exponential decay of I_{Na} during a train that indicates more than one non-conducting state. Thus, equilibrium reactivation was only achieved after the fourth, fifth or sixth pulse in a train even though the pulses and interpulse intervals were indentical.

The data obtained from the time course of reactivation of I_{Na} show that at a membrane potential of −60 mV approximately 420 ms is required for 50% recovery of I_{Na}. This is considerably longer than the 30 ms required for recovery of I_{Na} in molluscan neurones (Adams, Smith & Thompson, 1980) or 2 ms required at a holding potential of −70 mV in squid axons (Bezanilla & Armstrong, 1977). This long reactivation of I_{Na} is perhaps then the underlying mechanism responsible for the rapid inactivation of the action potential in current-clamped cells. Experiments on GH3 cells have shown that reactivation is more rapid, being 50% complete after 53 ms at −70 mV, and 5 ms at −110 mV (Matteson & Armstrong, 1984). However, the relevance of these values for GH3 cells is uncertain since studies with conventional intracellular recording techniques have shown the membrane potential of

these cells to be about −45 mV (Kidokoro, 1975; Ozawa & Miyazaki, 1979; Sand *et al.* 1980; Taraskevich & Douglas, 1980) a potential at which the time constant for reactivation should be considerably greater.

Outward currents

These data have provided evidence for significant outward currents in bovine lactotrophs carried by K^+. No outward current was observed when Cs^+ was used as the intracellular cation, suggesting that Cs^+ cannot pass through K^+ channels as has been suggested for rat lactotrophs (DeRiemer & Sakmann, 1986). The components of outward current appear to involve two different channel types differentiated on the basis of pharmacological and ionic sensitivities in whole-cell recordings and conductance in single-channel recordings.

The major current, $I_{K(V)}$, was voltage activated in the absence of Ca^{2+}, had a slow rise time and showed no inactivation during prolonged depolarization. However, in the presence of extracellular Ca^{2+} a transient current ($I_{K(Ca)}$) that increased the rate of activation of outward current could be recorded. In molluscan neurones $I_{K(Ca)}$ appears to be the major outward current (Thompson, 1977; Aldrich, Getting & Thompson, 1979) but differs from that in lactotrophs in being slowly activated and more sustained during prolonged depolarization. The short duration of $I_{K(Ca)}$ in the present whole-cell recordings could represent the inactivation characteristics of the channel carrying this current. However, cell-attached recordings of Ca^{2+}-sensitive K^+ channel currents showed that inactivation of these channels did not occur, and therefore we conclude that the short duration of $I_{K(Ca)}$ in whole-cell recordings was in fact a consequence of rapid buffering of intracellular Ca^{2+} by EGTA. $I_{K(Ca)}$ would also appear to be at least partially resistant to TEA since it was recorded after $I_{K(V)}$ was blocked by TEA. The blocking action that TEA had on $I_{K(V)}$ is consistent with its reported effects in neurones (Thompson, 1977). This effect is probably responsible for the increased duration of spontaneous action potentials in these cells seen after TEA application (C. D. Ingram & W. T. Mason, unpublished observations). The voltage-activated K^+ current, $I_{K(V)}$, would be the principal current producing repolarization in the current-clamp recordings presented here since Ca^{2+} was absent externally and buffered internally. The relatively slow activation of $I_{K(V)}$ is probably responsible for the long duration of the action potentials (11·8 ms) recorded in these conditions. However, the more rapidly activating Ca^{2+}-dependent outward current, $I_{K(Ca)}$, may also play an important role in controlling membrane repolarization under physiological conditions.

Both sustained and transient voltage-activated K^+ currents have been detected in GH3 cells, but in this case it is the slowly activating, sustained current that is sensitive to extracellular Co^{2+} and Cd^{2+} and may represent $I_{K(Ca)}$ (Dubinsky & Oxford, 1984). In contrast, the voltage-activated K^+ current ($I_{K(V)}$), insensitive to these Ca^{2+} channel blockers in GH3 cells, showed an inactivation during prolonged depolarization similar to the present $I_{K(Ca)}$. In the GH3 cells no appreciable TEA-sensitive K^+ current has been detected (Hagiwara & Ohmori, 1982). Interestingly we have failed to record a current resembling I_A (sensitive to 4-aminopyridine) in activation and inactivation characteristics which is present in neurones (e.g. Thompson, 1977). That this current may be present in some anterior pituitary cells

has been suggested since 4-aminopyridine affects spike duration in cultured rat pituitary cells (Sand *et al.* 1980). However, in the latter study the effect of 4-aminopyridine was suggested to be on a slowly activating rather than a rapidly activating current (Sand *et al.* 1980). The lack of the I_A current was probably not the result of channel inactivation at the routinely used holding potentials in the present study, since it was also absent when the holding potential was increased to -100 mV at which it would be expected to be fully activatable.

When divalent cations were added to the external solution during recordings of $I_{K(V)}$, a positive shift in the activation potential was observed. This is not due to a counteracting inward current since it was caused both by Ca^{2+} and Ca^{2+} channel blockers. Nor was it likely to be due to a change in the junction potential of the system since addition of 5–25 mM divalent cations will only affect the junction potential by 2–3 mV. However, a similar positive shift in the $I-V$ relation of I_{Ca} has been reported to occur with increasing concentrations of divalent cations in GH3 cells (Hagiwara & Ohmori, 1982) and chromaffin cells (Fenwick *et al.* 1982), and has been attributed to a screening effect on negative surface charges (Frankenhaeuser & Hodgkin, 1957). Such changes in outer surface potential would be expected to affect the voltage activation characteristics of all membrane currents and, in this respect, recent studies on the I_{Na} in these lactotrophs have suggested that divalent cations have an apparent blocking effect on whole-cell I_{Na} due to a similar positive shift in voltage activation (P. Cobbett & W. T. Mason, unpublished data). These shifts present a major technical limitation when analysis of currents in the presence and absence of divalent cations is required but, more importantly, they may partly explain the complete blocking effect of Mn^{2+} on current-evoked action potentials reported both for these (Ingram *et al.* 1986) and other pituitary cell types.

Are action potentials involved in prolactin secretion?

Given the characteristics of Na^+ currents and action potentials reported here and the effects of agents which are known to block these ionic currents, it is possible to speculate about the role of action potentials in prolactin secretion.

Failure of these cells to sustain repetitive firing of Na^+ action potentials and the long reactivation time of I_{Na} suggest that action potential frequency may not be directedly related to prolactin secretion. Furthermore, prolactin secretion was unaffected by TTX, Na^+ entry being inessential for the hormone release process. These results are in good agreement with the previous demonstration that basal prolactin secretion from rat pituitary cultures is unaffected by TTX (Thorner, Hackett, Murad & MacLeod, 1980), and that basal and TRH-induced prolactin secretion from bovine pituitary cells is independent of extracellular Na^+ (Saith, Bicknell & Schofield, 1984). Prolactin secretion is also unaffected by the alkaloid veratridine (Schofield, Bicknell & Saith, 1982) which is known to shift the voltage activation characteristics of Na^+ channels to more negative potentials and decrease Na^+ channel inactivation (Catterall, 1980).

Studies on $^{45}Ca^{2+}$ uptake into GH4C1 cells have shown Ca^{2+} entry to be voltage dependent but unaffected by TTX or $[Na^+]_o$ (Tan & Tashjian, 1984), and biochemical methods of detecting membrane potential of GH3 cells show that prolactin secretion may occur by two different pathways (Gerschengorn, 1980). First, it may be voltage

activated since elevated K^+ or added TEA depolarizes the cell and produces increased release of prolactin. Second, however, TRH could also cause prolactin secretion but not measurably affect membrane potential. This finding has been confirmed directly by our own studies with conventional intracellular recording techniques where it was found that the TRH caused less than a 5 mV depolarization (Ingram *et al.* 1986).

Finally, recent studies on the two clonal pituitary cell lines, GH3 and GH4, have demonstrated that although both are capable of prolactin secretion, the GH3 line exhibits large Na^+ currents while most GH4 cells are incapable of generating voltage-activated Na^+ currents (Dubinsky & Oxford, 1984). These results taken as a whole suggest that voltage-activated events during Na^+ action potentials may not be important in prolactin secretion as previously supposed, and that some alternative mechanism must be ultimately more important for secretion. The role and significance of Na^+ currents in the normal pituitary cell remains to be defined: a minimal involvement in secretion is indicated, but other potentially important roles may exist, such as in intercellular communication in the intact pituitary.

C.D.I. gratefully acknowledges the support of the Meat and Livestock Commission. P.C. is a Beit Memorial Fellow. The technical assistance of Miss Anna Tibbs was greatly appreciated in this work. Finally, much of the data analysis presented here would not have been possible without use of computer programs written by Dr Trevor Lamb, Physiological Laboratory, University of Cambridge, and by Mr John Dempster, Department of Pharmacology, Strathclyde University, Glasgow.

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