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ELECTROPHYSIOLOGY OF NEUROSECRETORY CELLS FROM THE PITUITARY INTERMEDIATE LOBE

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Summary

One of the goals in studying the electrical properties of neurosecretory cells is to relate their electrical activity to the process of secretion. A central question in these studies concerns the role of transmembrane calcium ion flux in the initiation of the secretory event.

With regard to the secretory process in pituitary cells, several research groups have addressed this question *in vitro* using mixed primary anterior pituitary cell cultures or clonal cell lines derived from pituitary tumours. Other workers, including ourselves, have used homogeneous cell cultures derived from the pituitary intermediate lobes of rats to examine the characteristics of voltage-dependent conductances, the contribution of these conductances to action potentials and their role in stimulus–secretion coupling.

Pars intermedia (PI) cells often fire spontaneous action potentials whose frequency can be modified by the injection of sustained currents through the recording electrode. In quiescent cells action potentials can also be evoked by the injection of depolarizing current stimuli. At around 20°C these action potentials have a duration of about 5 ms. Although most of the inward current during action potentials is carried by sodium ions, a calcium ion component can be demonstrated under abnormal conditions. Voltage-clamp experiments have revealed that the membrane of these cells contains high-threshold, L-type, Ca^{2+} channels and low-threshold Ca^{2+} channels.

Since hormone release from PI cells appears not to be dependent on action potential activity but does depend on external calcium ions, it is not clear what role these Ca^{2+} channels play in stimulus–secretion coupling in cells of the pituitary pars intermedia. One possibility is that the low-threshold Ca^{2+} channels are more important to the secretory process than the high-threshold channels.

Introduction

Our ideas about the process of neurotransmitter release at synapses in both the peripheral and central nervous systems are well formed and supported by a very large body of evidence. The arrival of a Na^+-dependent action potential at a presynaptic nerve terminal causes the brief opening of voltage-dependent Ca^{2+}

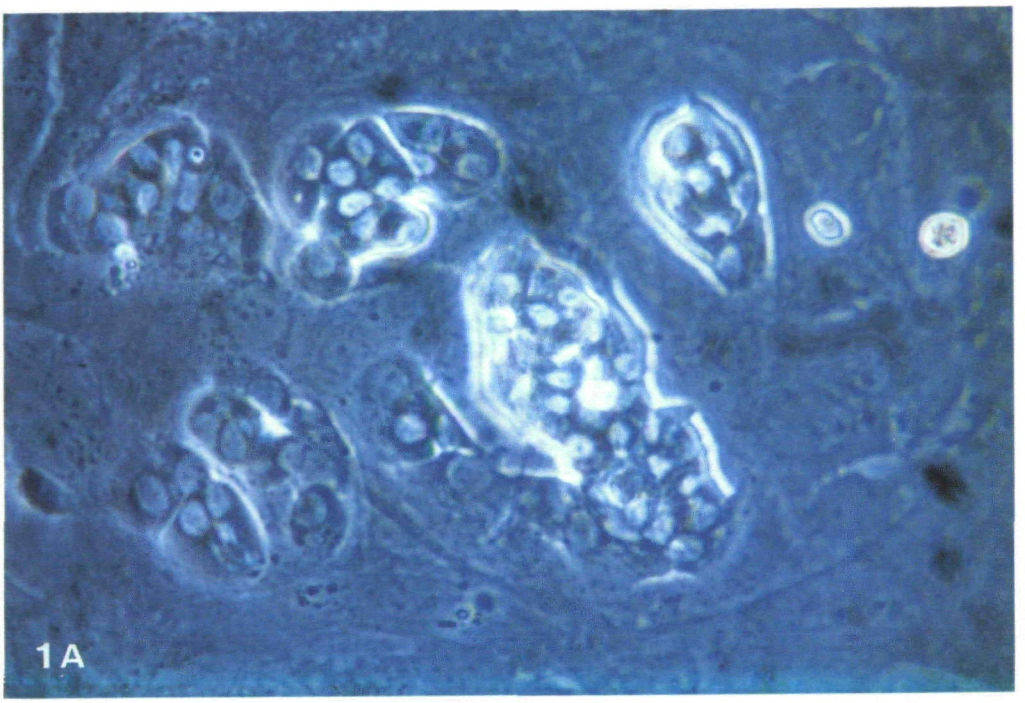
Key words: neurosecretion, pars intermedia, electrophysiology, ion channels.

channels in the surface membrane of the terminal and the resulting influx of calcium ions is the necessary and sufficient condition for initiating the process whereby vesicles containing the chemical transmitter substance discharge their contents into the synaptic cleft (Katz, 1969; Kandel, 1985). Although this scheme, which links a depolarizing event to the activation of Ca^{2+} channels, applies to synapses and perhaps to some secretory cell types which generate action potentials, such as chromaffin cells (Douglas, 1968), it is far from clear whether it applies to the secretory cells of the pituitary gland. Results obtained in some pituitary cell types (e.g. ovine gonadotrophs, Mason & Waring, 1985) suggest that, although hormone release stimulated by releasing factors depends on external Ca^{2+}, Na^{+}-dependent action potentials might not play an essential role in this process.

One difficulty in investigating stimulus–secretion coupling in pituitary cells is that the gland itself contains several different secretory cell types that secrete different peptide hormones in response to a variety of stimuli, generally unique to each cell type. If one wants information about the cellular mechanisms underlying the secretory process in one class of pituitary cell it is necessary to develop a preparation which contains just one cell type or in which particular cell types can be identified so that the appropriate stimuli can be applied to the cell(s) under investigation and, for release studies, so that an assay can be made for the appropriate hormone. The application of techniques such as immunohistochemistry and the reverse haemolytic plaque assay (see other chapters in this volume), density centrifugation (Cobbett, Ingram & Mason, 1987) and fluorescence-activated cell sorting (Chen, St John & Barker, 1987) to the identification of cell types is now making it possible for us to gain insight into the stimulus–secretion coupling process in particular anterior pituitary cell types. Furthermore, the revolution in the electrophysiological investigation of small cells *in vitro* afforded by the introduction of whole-cell and patch-clamp recording techniques (Hamill *et al.* 1981) allows us to gain detailed information about the electrical characteristics of individual secretory cells that might be relevant to stimulus–secretion coupling.

An alternative approach to obtaining a homogeneous population of a pituitary cell type is to use a clonal cell line (such as GH_3 cells, Tashjian, Bancroft & Levine, 1970) with concomitant problems in relating the results obtained to any non-tumour cell type of the pituitary. One further tactic that has attracted a small number of groups is to use an anatomically identifiable region of the pituitary gland which contains a homogeneous population of hormone-secreting cells. One such region is the pars intermedia (PI) or intermediate lobe of the rat pituitary. It is readily dissected free of the anterior and posterior lobes and yields a population of cells known as melanotrophs whose secretory products include α-melanocyte-stimulating hormone (α-MSH), corticotropin-like intermediate lobe polypeptide (CLIP), and a variety of other products of the precursor molecule pro-opiomelanocortin (Eberle, 1981).

In addition to containing a homogeneous population of hormone-secreting cells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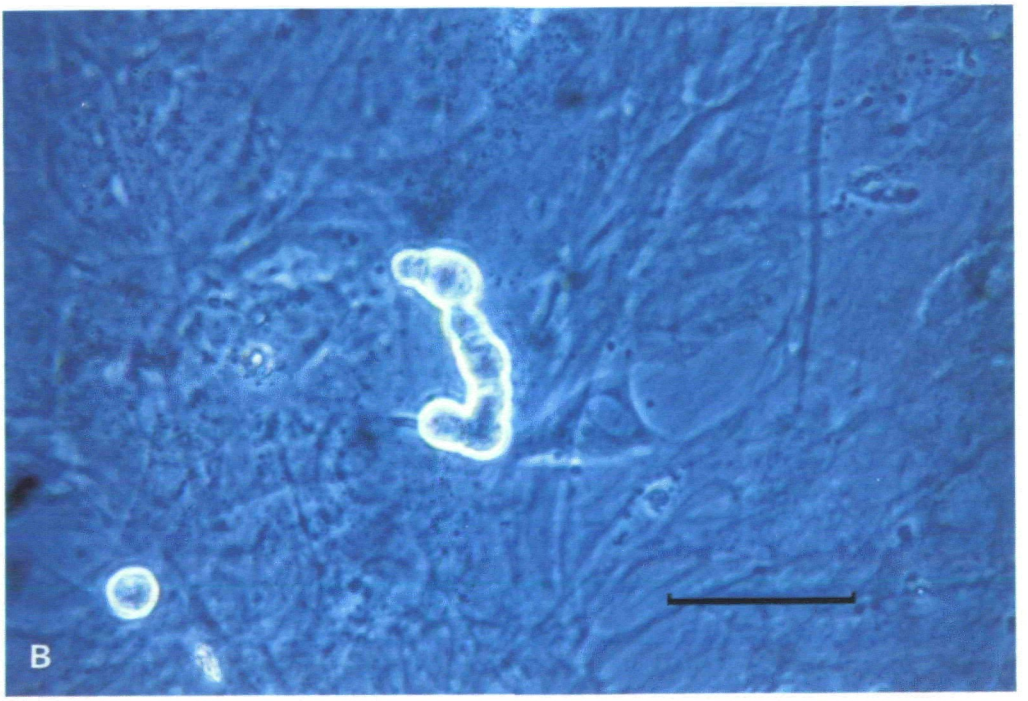
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this region of the pituitary gland is unique in that the secreting cells are innervated. Histochemical studies have shown that the terminals of this innervation contain a wide variety of neurotransmitters and neuropeptides and from this finding one might infer that the cells of the pars intermedia are subjected to a far more complex control regime than anterior pituitary cells. However, since we know relatively little about the functions of the pars intermedia and its hormones, it is dangerous to draw any such conclusions. Nevertheless, because this region of the pituitary can be isolated and studied *in vitro*, either intact or as dispersed cells, it is an excellent source for an identified pituitary cell type which has the bonus of possessing a wide range of neurotransmitter receptors and their signal transduction mechanisms.

Following the lead of Douglas, Taraskevich, their colleagues and a small number of other groups, we have studied the electrophysiological properties of single cells from the rat pituitary pars intermedia which were maintained in dispersed cell culture (for methods see Tomiko, Taraskevich & Douglas, 1984; Cota, 1986; Kehl, Hughes & McBurney, 1987). This article is a brief overview of the current state of knowledge about the electrophysiological properties of pars intermedia cells and is a commentary on present understanding of stimulus–secretion coupling in these cells.

Pars intermedia cells in cell culture

The process of dispersing the intermediate lobe produces small 'rafts' of cells and isolated cells (Fig. 1). The isolated cells are about 15 μm in diameter and very suitable for use with whole-cell recording techniques (Cota, 1986; Kehl *et al.* 1987). The advantages of using isolated cells in cell culture as opposed to acutely isolated glands for both electrophysiological studies and hormone release experiments are the good visibility of the cells for electrophysiology and the fact that in investigations of electrically stimulated and/or releasing-factor (transmitter)-induced cellular events one can be confident the action of these stimuli is only on the secretory cells and not on any aspect of their innervation (see for example Tomiko, Taraskevich & Douglas, 1984; Taraskevich, Tomiko & Douglas, 1986). The major disadvantage of this *in vitro* preparation is the loss of innervation, which may lead to the expression of abnormal cellular properties (Cota & Armstrong, 1987).

Fig. 1. Light micrographs taken under phase-contrast optics of cells derived from the pituitary intermediate lobe of adult rats and maintained in monolayer culture. Lobes were broken up, as described in Kehl, Hughes & McBurney (1987), into clumps of cells and single cells then plated onto a confluent layer of rat cerebral cortical astrocytes (phase-dark background cells). Some of the cells flattened on the background layer losing their phase-bright appearance (A) whereas other cells, both isolated and in 'rafts', maintained an approximately spherical shape (B). The cells we have used for most experiments were isolated, phase-bright cells like the one in the lower left corner of B. Scale bar, 50 μm.

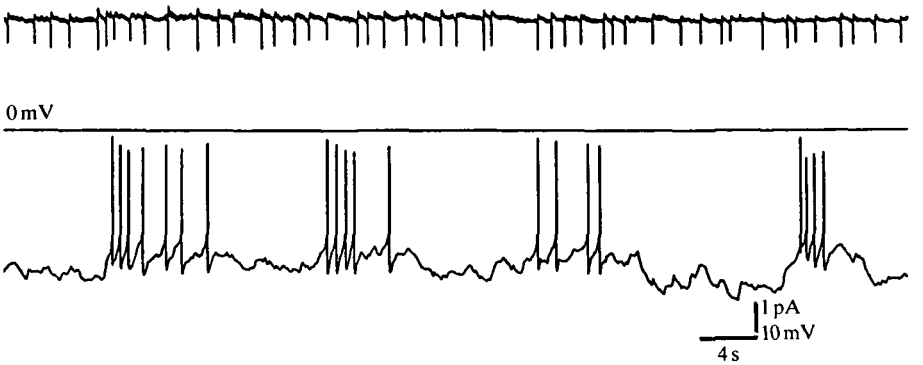

Fig. 2. Spontaneous activity recorded from a PI cell in the cell-attached (upper trace) and whole-cell (lower trace) configurations. Prior to applying a pulse of suction to obtain a whole-cell recording, the patch current showed biphasic waveforms reflecting spontaneous firing in the cell. Subsequently, in the whole-cell configuration, the resting potential fluctuated between −50 and −35 mV, the latter potential being the threshold for the activation of action potentials which overshot the 0 mV level by 30–35 mV. The peak amplitude of the action potentials shown here was attenuated by the low-frequency response of the pen recorder. The basis for the shift from a tonic (cell-attached record) to a phasic pattern (whole-cell record) of firing is unknown, but perhaps is the result of a change in the intracellular environment of the cell. The solid horizontal line in the lower trace record denotes the 0 mV level.

'Resting' properties of cultured pars intermedia cells

Early experiments, using both intracellular and extracellular recording techniques, demonstrated that isolated pars intermedia cells possess spontaneous electrical activity (Douglas & Taraskevich, 1978). This activity consists of subthreshold fluctuations in the cell's resting membrane potential and action potentials (Fig. 2). Because the input resistance of the cells is so high (around 4 GΩ, S. J. Kehl & R. N. McBurney, unpublished observations) it is possible that the voltage fluctuations arise as a result of the opening and closing of single ion channels in an asynchronous manner. If this is so both the cause of this channel activity and the nature of the ion channels involved remain unresolved.

Action potentials in pars intermedia cells

The characteristics of action potentials in these cells are relatively straightforward. They are brief depolarizing events which can overshoot 0 mV by 30–35 mV and are followed by a marked after-hyperpolarization (Fig. 3). At room temperature they have a half-width of about 5 ms and under these conditions a monotonic repolarization phase, unlike some rat sensory neurones which reveal the calcium component of their action potential as a hump in the repolarization phase (Dichter & Fischbach, 1977). In some cells which show spontaneous voltage fluctuations but no action potential activity, the injection of sustained depolarizing current can

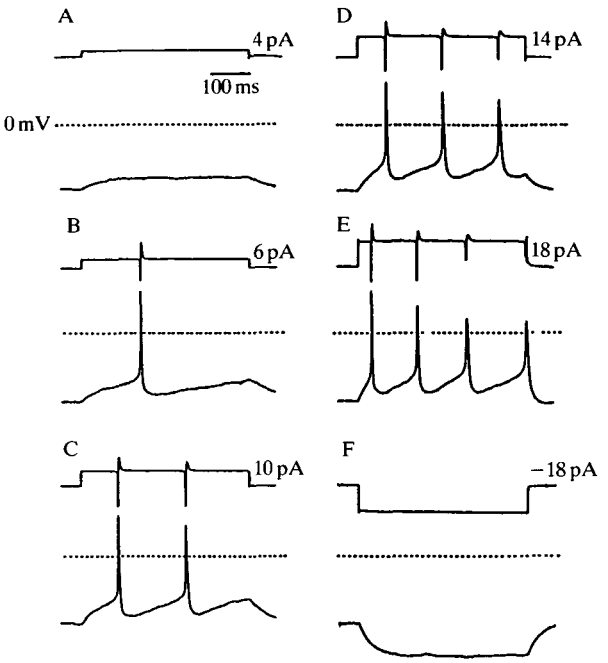

Fig. 3. Injection of depolarizing (A–E) and hyperpolarizing currents (F) into a quiescent PI cell. Each panel shows a current injection trace above a membrane voltage trace. The dashed line represents 0 mV for the membrane voltage trace. The resting potential for this cell was −60 mV. The amount of injected current is shown at the end of each current trace. The biphasic deflections on the current traces are due to inadequate control of the membrane current during action potentials. Note that the frequency of action potentials is related to the magnitude of the current injection. Note also the marked accommodation of the action potentials in C, D and particularly in E.

generate action potentials whose average frequency depends on the magnitude of the injected current (Fig. 3).

Relationship between action potential activity and secretion

When the intermediate lobe is isolated from its innervation the average frequency of action potentials in the secretory cells rises and hormone output increases. From this observation it has seemed reasonable to propose that action potentials are the important event in the secretory process and that the regulation of hormone output from the gland *in vivo* is by the modulation of a tonic inhibition mediated by the dopamine- or γ-aminobutyric acid (GABA)-containing fibres of its innervation. If this proposal were true one might expect that the basal secretion from isolated glands or cells would be suppressed in the presence of tetrodotoxin (TTX). Unfortunately TTX does not suppress basal secretion from either isolated

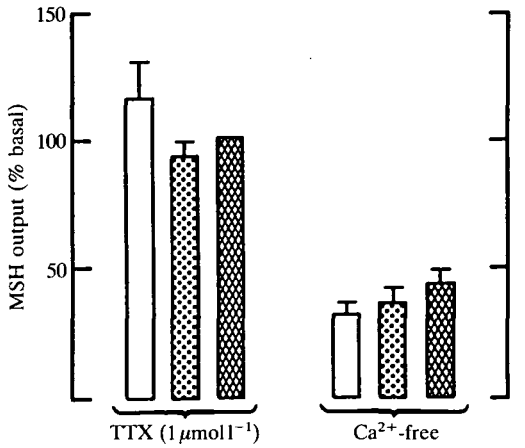

Fig. 4. Basal melanocyte-stimulating hormone (MSH) output from isolated melanotrophs or neurointermediate lobes is not inhibited by tetrodotoxin (TTX) but is inhibited by omission of Ca^{2+}. Average responses from preparations of dispersed rat pars intermedia cells ($N = 4$; empty columns), mouse pars intermedia cells ($N = 3$; stippled columns) or mouse neurointermediate lobes ($N = 2$; hatched columns); error bars denotes S.E.M. or the range. The values plotted are the outputs of MSH during the last 5 min of a 10–20 min exposure to TTX or Ca^{2+}-free solutions. These values are expressed as a percentage of the basal secretion during the 5 min that preceded the introduction of TTX or the omission of Ca^{2+}. The responses illustrated in Ca^{2+}-free solution were obtained subsequently from the same preparations on which TTX had been tested. Reproduced from Tomiko, Taraskevich & Douglas (1984) with permission.

cells or isolated neurointermediate lobes (Fig. 4). However, hormone release is reduced in Ca^{2+}-free bathing solutions (Fig. 4).

This pair of observations was the first sign that the release process in pars intermedia cells is not completely straightforward. One interpretation is that the action potentials are not associated with any membrane conductance change in response to Ca^{2+} and that the calcium entry which affords the release process a dependence on extracellular [Ca^{2+}] is through Ca^{2+} channels which are not primarily associated with action potentials. Although this idea is attractive and has a parallel in ovine gonadotrophs (Mason & Waring, 1985), there is no direct evidence to support this for rat pars intermedia cells. Furthermore, the actions of neurotransmitters such as dopamine, GABA, serotonin (5-HT) and noradrenaline on hormone output are associated with changes in the spontaneous action potential activity of pars intermedia cells (Douglas & Taraskevich, 1982; Taraskevich & Douglas, 1982, 1984; Tomiko, Taraskevich & Douglas, 1983) and it is possible to demonstrate the existence of voltage-dependent Ca^{2+} channels in these cells which might contribute a component to normal action potentials (Douglas & Taraskevich, 1980; Cota, 1986; Hughes, Kehl & McBurney, 1987; Kehl, 1987).

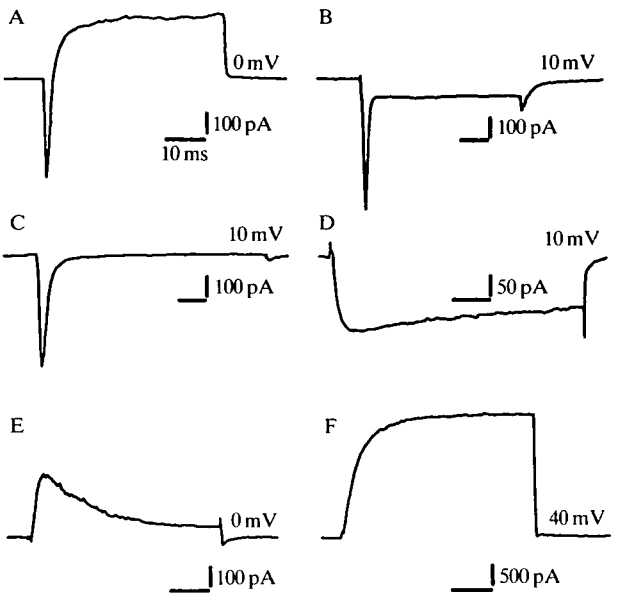

Fig. 5. Voltage-activated ionic currents in rat PI cells obtained with the whole-cell recording technique. All recordings were obtained by stepping the membrane voltage from −70 mV to the potential indicated at the end of each current trace then stepping back to −70 mV. Without going into too much detail, the following solution modifications were made to achieve the recordings shown. (A) 'Normal' membrane current response to a voltage step. No membrane ion channels intentionally blocked. (B) Inward current isolated by substituting Cs^+ for K^+ in the recording electrode solution. (C) Na^+ current revealed by the addition of 300 μmol l^{-1} Cd^{2+} to the external solution while blocking outward currents with internal Cs^+ as in B. (D) Ca^{2+} current revealed by the addition of 2 μmol l^{-1} tetrodotoxin (TTX) to the external solution while blocking outward currents as in B. (E) Fast transient K^+ current revealed by blocking Na^+ and Ca^{2+} currents with TTX and Cd^{2+}, respectively, and additionally blocking the delayed-rectifier K^+ current (see F) with 20 mmol l^{-1} tetraethylammonium in the external solution (S. J. Kehl, unpublished observation). The internal solution contained K^+ as the predominant cation. (F) The delayed-rectifier K^+ current revealed by blocking both Na^+ and Ca^{2+} currents by the methods indicated above. This cell did not display a fast transient K^+ current.

The voltage-dependent ion channels in rat pars intermedia cells

Fig. 5 illustrates the variety of membrane ionic currents that we have been able to record from isolated cells under various experimental conditions. This summary figure shows that conventional Na^+ channels and delayed-rectifier K^+ channels are present (Fig. 5C,F, respectively) and that inward Ca^{2+} currents can be elicited by a depolarizing voltage step (Fig. 5D). Fig. 5 illustrates the presence of a fast transient K^+ conductance mechanism in some PI cells (S. J. Kehl & R. N. McBurney, unpublished observations). Our findings largely confirm and extend the work of Taleb, Trouslard, Demeneix & Feltz (1986) on porcine PI cells and the

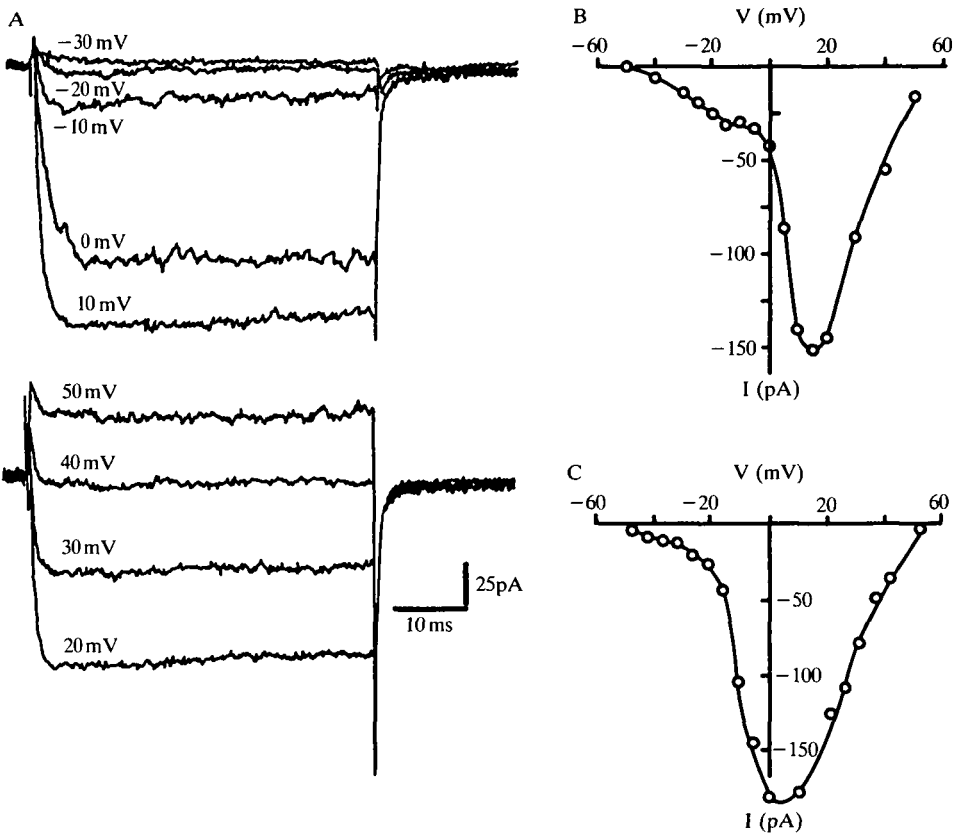

Fig. 6. The properties of Ca^{2+} currents in rat PI cells. (A) Ca^{2+} currents recorded by stepping the membrane potential from a holding potential of −70 mV to various membrane potentials indicated with the current traces. Leakage currents have not been subtracted from these records. (B) I/V relationship for Ca^{2+} currents measured at the end of 25 ms voltage pulses in a rat PI cell (from Cota, 1986). Note the characteristic shape of the I/V curve which is very similar to that shown by Carbone & Lux (1984) and indicates the contributions of two separate Ca^{2+} channel types to the recorded currents. Reproduced from Cota (1986) with permission. (C) I/V relationship for peak Ca^{2+} currents we have recorded from rat PI cells. The maximum current level of the I/V plot is similar to that shown in A but a clear separation of the two components which have been proposed to contribute to the currents is not seen.

work of Cota (1986) on rat PI cells. Clearly voltage-dependent Ca^{2+} channels are present and could conceivably contribute to action potentials and stimulus–secretion coupling in these cells.

Properties of the voltage-activated Ca^{2+} current

Cota (1986) has suggested that the voltage-activated Ca^{2+} current in pars

intermedia cells is generated by the activation of two separate ion-channel components defined in terms of their thresholds for activation and the speeds at which they deactivate after the voltage pulse has returned to the holding potential. These two components have been named the low-threshold, slowly deactivating (SD) channel and the high-threshold, fast-deactivating (FD) channel (Fig. 6B). When the properties of these Ca^{2+} channels are compared with those of Ca^{2+} channels described in vertebrate sensory neurones (Carbone & Lux, 1984) it is clear that, in terms of activation thresholds, the two classes of Ca^{2+} channel are similar in the two different cell types. However, there may be some differences in the inactivation properties of the channels between the two preparations. More experimental work will certainly be needed before proper comparisons can be made amongst different cell types on this feature of the voltage-dependent Ca^{2+} channels.

The current–voltage curves (Fig. 6C) which we have derived from measuring peak calcium currents during voltage-clamp steps (Fig. 6A) do not show such a clear separation of low-threshold and high-threshold conductances. Perhaps our methodology has prevented us from clearly identifying this component. Nevertheless, our results confirm the observations of a sizeable voltage-dependent Ca^{2+} conductance in PI cell membranes. Both investigations show that this conductance begins to activate around −50 mV and is maximally activated around 0 mV.

One feature of the Ca^{2+} conductance change that is worth noting is the relatively slow activation kinetics following a step change in membrane voltage (Fig. 6). This slow activation means that during a brief (5–10 ms) action potential not all the available Ca^{2+} channels will be activated. In fact, depending on the precise relationship between the kinetics of the various ionic conductances that contribute to the action potential, the fraction of the total number of Ca^{2+} channels that is activated might be very small, especially in the case of the high-threshold channels. Nevertheless, the inward Ca^{2+} current resulting from the activation of a very small fraction of available Ca^{2+} channels might raise the cytosolic concentration of free calcium ions to levels sufficient to trigger hormone release directly or to evoke additional Ca^{2+} release from an intracellular store.

The exact relationship between action potentials, the activation of Ca^{2+} conductances and the intracellular concentration of calcium ions ($[Ca^{2+}]_i$) will only be determined by the direct measurement of $[Ca^{2+}]_i$ during action potentials in PI cells in a manner similar to that recently reported for GH_3 cells (Schlegel *et al.* 1987).

Role of Ca^{2+} channel types in the secretory process

It seems unlikely that high-threshold Ca^{2+} channels would make any contribution to the secretory process if action potentials were eliminated. Once the Na^+ channels have been blocked by TTX it is extremely difficult to evoke a Ca^{2+} spike by the artificial injection of depolarizing current under normal conditions (Douglas & Tarakevich, 1980). However, it is possible that some of the low-

threshold Ca^{2+} channels are continuously opening and closing at the 'resting' potential in PI cells. Such activity might be, in part, responsible for the spontaneous voltage fluctuations mentioned earlier (see Fig. 2).

The inward Ca^{2+} currents underlying the fluctuating membrane voltage might maintain $[Ca^{2+}]_i$ at levels sufficient to cause hormone secretion. Any transient increases in $[Ca^{2+}]_i$ associated with action potentials might, in overall terms, be relatively unimportant to secretion from PI cells. The scenario mentioned above would explain the lack of TTX-sensitivity of the release process and its dependence on extracellular calcium ions. However, since the preceding sentences in this section contain one 'possible' and three 'mights' it is clear that much experimental work will be necessary to put this suggestion on a firmer foundation.

If the low-threshold Ca^{2+} channels are more involved in the secretory process than the high-threshold Ca^{2+} channels then it is not clear why the stimulant dihydropyridine BAY K 8644 can markedly potentiate hormone secretion from PI cells in a TTX-insensitive manner (Taraskevich & Douglas, 1986). Since the dihydropyridines are relatively specific for the L-type, or high-threshold, Ca^{2+} channel in neurones (Nowycky, Fox & Tsien, 1985) one can only conclude that the Ca^{2+} channels of PI cells are not identical in their pharmacological properties to those previously studied in neurones.

The possible greater importance of low-threshold Ca^{2+} channels to the secretory process than high-threshold channels has implications for the design of experiments to investigate the modulation of secretion from PI cells. If it is necessary to use K^+ depolarizations to evoke additional secretion from cells, the K^+ concentrations used should be chosen in the light of the activation curves for the various Ca^{2+} channel types.

Conclusions

Electrophysiological studies are revealing the spectrum of voltage-dependent ion channels that are present on the surface membranes of secretory cells of the pituitary intermediate lobe. The role of these ion channels in stimulus–secretion coupling in PI cells is not clear. Future experiments, where electrical events, $[Ca^{2+}]_i$ and secretion are measured from single cells (see, for example, Penner & Neher, 1988), will undoubtedly provide much of the information needed to resolve some of the paradoxes which abound in the literature. Whether the final mechanism of stimulus–secretion coupling in PI cells is unique or can be generalized to other pituitary cell types remains to be determined.

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