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CALCIUM COMPONENT TO ACTION POTENTIALS IN RAT PARS INTERMEDIA CELLS

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SUMMARY

1. The ionic dependence of the action potential of rat pars intermedia cells was investigated by using intracellular recording techniques.

2. In the presence of tetrodotoxin (TTX, 5×10^{-6} M), the action potentials evoked by passing depolarizing current through the recording electrode were abolished, confirming that they are mainly dependent on Na; however, when tetraethylammonium (TEA, 10 mM) was added to the TTX-containing solution the imposed depolarizations triggered all-or-none regenerative potentials indicative of involvement of another ion.

3. These TTX-insensitive regenerative potentials persisted when the cells were perifused with Na-free solution but were severely reduced or abolished by Ca-free solution. This suggests that the ion producing these potentials is Ca.

4. These Ca action potentials were suppressed by Ni, Co and Mn in concentrations that did not suppress the 'Na spikes' recorded in the absence of TTX and TEA.

5. Sr and Ba could substitute for Ca in maintaining the action potentials recorded in the presence of TTX. These ions also prolonged the duration of these action potentials.

6. The demonstration of a Ca component to the predominantly Na-dependent action potentials of pars intermedia cells heightens the possibility that these action potentials participate in the regulation of secretion.

INTRODUCTION

Action potentials whose frequency is increased or decreased by hypophysiotropic factors that stimulate or inhibit secretion have been demonstrated in adenohypophysial cells of both the pars distalis (Taraskevich & Douglas, 1977, 1978) and the pars intermedia (Davis & Hadley, 1978; Douglas & Taraskevich, 1978; Taraskevich & Douglas, 1979) in various species. Moreover, there are indications that the frequency of action potential discharge is higher in adenohypophysial cells that secrete spontaneously at relatively high rate such as teleost prolactin cells (Taraskevich & Douglas, 1978) and rat pars intermedia cells (Douglas & Taraskevich, 1978) than in other adenohypophysial cells whose secretory rate is comparatively low such as the cells of anole pars intermedia (Taraskevich & Douglas, 1979). Such findings are consonant with the view that action potentials may be involved in stimulus-secretion

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coupling in adenohypophysial cells and that it may be by initiating, suppressing or modulating these action potentials that the brain, by way of hypophysiotropic factors, regulates secretion (Taraskevich & Douglas, 1977). In adenohypophysial cells, as in many others, calcium ions seem to act as mediators in stimulus-secretion coupling (see reviews by Douglas, 1968, 1978; Katz, 1969; Geschwind, 1971; Vale, Rivier & Brown, 1977) and action potentials in adenohypophysial cells have therefore been supposed to provide an adequate stimulus to secretion by promoting an influx of Ca ions. Such an influx of Ca can indeed be inferred from demonstrations of a Ca component to action potentials recorded from several types of adenohypophysial cells from different species (Taraskevich & Douglas, 1977, 1978, 1979; Ozawa & Sand, 1978) as well as from neoplastic cells of anterior pituitary origin (Kidokoro, 1975; Biales, Dichter & Tischler, 1977; Ozawa & Miyazaki, 1979; Taraskevich & Douglas, 1980). Our present purpose was to seek a Ca component to action potential activity in pars intermedia cells of the mammal (rat) where heretofore Na has been the only ion identified as carrying charge (Douglas & Taraskevich, 1978).

METHODS

Pars intermedia cells were isolated from male Sprague-Dawley rats (250–350 g), maintained in culture and manipulated during recordings as previously described (Douglas & Taraskevich, 1978).

Intracellular recording and current passing were done through high resistance (60–100 MΩ) glass ('Kwik-fil', single barrel, W. P. Instruments, Connecticut) micro-electrodes filled with 4 M-K acetate (buffered to pH 7·2 with acetic acid) by use of a high input impedence amplifier with a bridge circuit (M4A, W. P. Instruments, Connecticut).

The recording solution contained (mM): NaCl, 142; KCl, 5; $CaCl_2$, 10; glucose, 5·5 and HEPES (4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid) buffer, 5 (pH 7·2); with the addition of tetrodotoxin (TTX, 5×10^{-6} M); tetraethylammonium (TEA, 1×10^{-2} M) and bovine serum albumin, 1 mg/ml. except during experiments shown in Figs. 1*A-C* and 3*J-L* where both TTX and TEA were omitted. Cells were perifused with test solutions of ionic composition different from that of the recording solution by means of a gravity flow delivery pipette (Taraskevich & Douglas, 1977). In 'Na-free' solutions NaCl and HEPES were replaced with an equiosmotic amount of Tris (2-amino-2-(hydroxymethyl)-1,3-propanediol). In 'Ca-free' solutions $MgCl_2$ (10 mM) was substituted for $CaCl_2$. In solutions containing Ni, Co, or Mn, 10 mM of the Cl salt of the particular ion was added to the recording solution. All experiments were done at room temperature (20–23 C).

RESULTS

The action potential evoked in rat pars intermedia cells is abolished by TTX (1 to 5×10^{-6} M) and there remains little, if any, indication of a residual regenerative component in the membrane response to imposed depolarizations (Fig. 1*A*, *B*). From this we have concluded that these action potentials result primarily from an increase in membrane permeability to Na (Douglas & Taraskevich, 1978). However, we find that a regenerative potential becomes apparent when TEA is added to the TTX-containing solution to reduce potential-dependent K conductance. This regenerative potential varied in size from cell to cell: sometimes it appeared as a small graded response (e.g. Fig. 1*C*) whereas in other instances it was a large all-or-none action potential (e.g. Fig. 1 *D–F*). These two responses do not seem to indicate the presence of two cell types with different electrical characteristics, rather, it seems

that the small responses result from cell damage. Thus, the two responses were sometimes obtained from the same cell. Furthermore, when this occurred the small graded responses were recorded either immediately after impalement, when membrane resistance and potential tended to be low, or toward the end of a prolonged impalement when these membrane parameters had declined from higher values. Moreover, it was often possible to convert a graded response recorded in a cell with a low resting

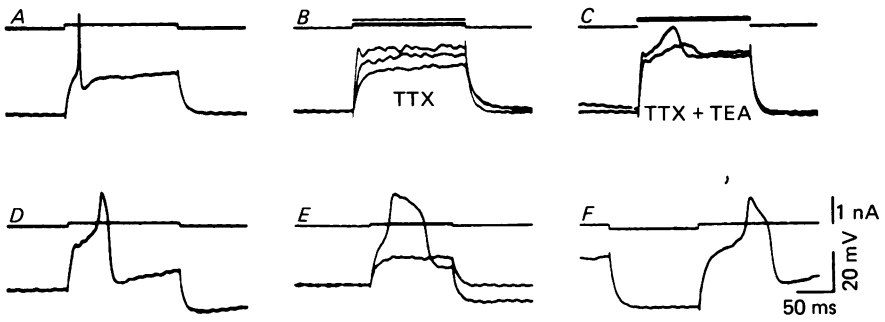

Fig. 1. Intracellular records of evoked potentials in pars intermedia cells demonstrating a TTX-resistant regenerative component. *A–C*, responses to imposed depolarizations. *A*, control action potential in absence of TTX and TEA. *B*, abolition of the action potential upon perifusing the cell with TTX (5×10^{-6} M). *C*, appearance of regenerative component upon perifusing the cell with TEA (10 mM) in addition to TTX. Membrane potential in each case held at -60 mV by imposing a sustained hyperpolarizing current except during the depolarizing pulses. *D–F*, regenerative responses from three different cells after adding both TTX (5×10^{-6}M) and TEA (10 mM) to the bathing medium. *D* and *E*, all-or-none responses to depolarizing current pulses. *F*, all-or-none response produced by terminating a hyperpolarizing current pulse in a cell with a relatively low resting potential. Top trace in each pair of records in this and other Figures indicates current passed during pulse and zero level of membrane potential unless otherwise stated.

potential (less than -40 mV) to an all-or-none potential by imposing hyperpolarizing current to maintain the membrane potential at about -60 mV (a value that may be close to the true resting potential, see Douglas & Taraskevich, 1978) before eliciting the response. The all-or-none action potentials had a threshold near a membrane potential of -20mV, a peak amplitude which overshot the zero level potential, and a duration (at half amplitude) which ranged from 15 to 180 ms in different cells.

The occurrence of such action potentials in pars intermedia cells exposed to TTX (as well as TEA) did not appear to be accounted for by Na influx through TTX-resistant Na channels of the sort occasionally encountered in some other cells (Ritchie & Rogart, 1977) for we found that the potential was little, if at all, affected when the cells were perifused with a Na-free solution (Fig. 2*A–C*). On the other hand, the potentials were severely depressed or abolished when the cells were perifused with a Ca-free solution (Fig. 2*D–F*) suggesting that they are Ca-dependent action potentials reflecting inward flux of Ca ions through voltage-dependent Ca channels. This led us to test the effects of Ni, Co and Mn which are known to block such Ca channels and thereby suppress Ca-dependent action potentials in various other cells and to do so at concentrations that have little effect on Na-dependent action

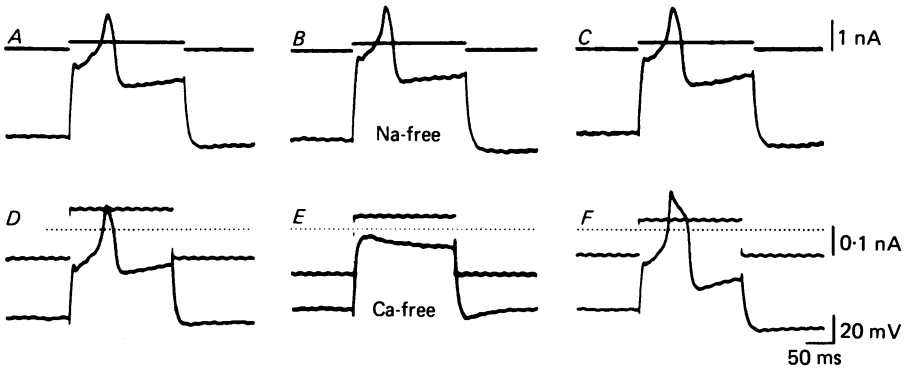

Fig. 2. Regenerative potential evoked in the presence of TTX and TEA requires Ca, not Na. *A–C*, responses before (*A*), during (*B*), and after (*C*) perifusion with Na-free solution. Membrane potential held at −60 mV by hyperpolarizing current between depolarizing pulses. *D–F*, records from a second cell before (*D*), during (*E*) and after (*F*) exposure to Ca-free solution. In *D–F* the dotted line indicates the zero level of membrane potential and the reference level for current passed. The gain of the current trace has been increased thus revealing the amount of hyperpolarizing current used to maintain the potential at −60 mV as well as the amplitude of the depolarizing pulses.

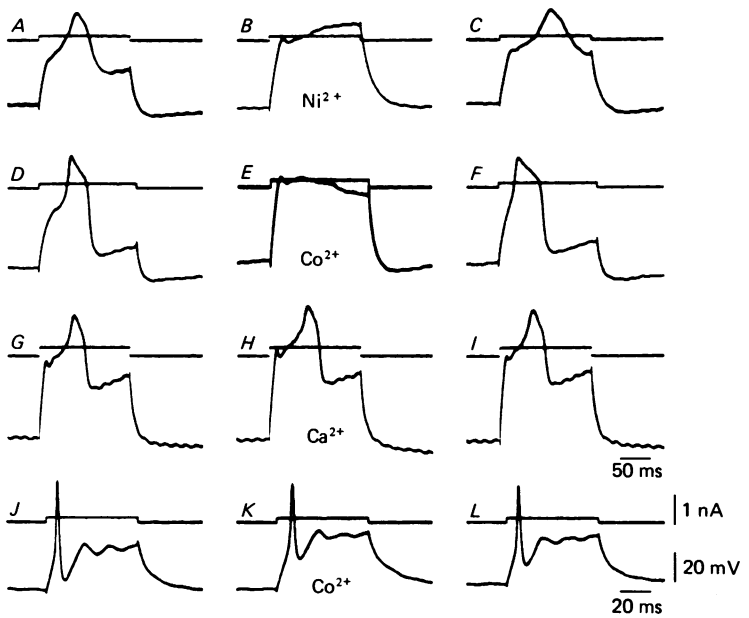

Fig. 3. Effect of divalent ions on the regenerative potentials. *A–C*, inhibitory effects of Ni. Responses recorded before (*A*), during (*B*) and after (*C*) perifusion with recording solution to which Ni (10 mM) was added. *D–F*, corresponding effect of Co (10 mM) demonstrated similarly in another cell. *G–I*, comparable experiment on a third cell demonstrating that Ca (10 mM) similarly administered has no inhibitory effect. *J–L*, potentials ('Na spikes') recorded from a fourth cell in the absence of TTX and TEA showing that Co (10 mM) does not suppress these 'Na spikes.' Membrane potential of cells shown in *A–I* held at −60 mV throughout. Voltage and current calibrations apply to all records. Time calibration in *I* applies to records *A–I*. Time calibration in *L* applies to records *J–L*.

potentials (Hagiwara, 1975). Each of these divalent ions suppressed the action potentials recorded from pars intermedia cells in the presence of TTX and TEA. This is illustrated for Ni and Co in Fig. 3 (*A–C* and *D–F* respectively): the effect of Mn was similar. This inhibitory effect seemed to result from the specific blocking

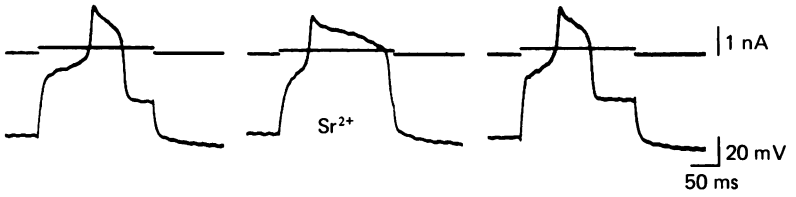

Fig. 4. Regenerative potentials elicited in the presence of TTX and TEA showing the effects of replacing Ca with Sr. Sr sustains and prolongs the regenerative response. Records before (left) during (centre) and following (right) perifusion of cell with Sr-containing solution (10 mM-Sr replacing 10 mM-Ca). Cell held at −60 mV by hyperpolarizing current.

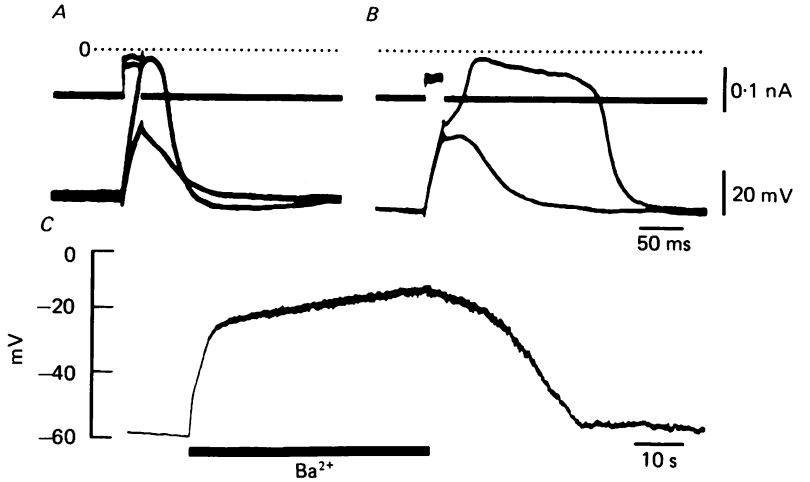

Fig. 5. Effects of replacing Ca with Ba on the regenerative potential and the resting potential. *A* and *B*, Ba sustains and prolongs the regenerative potential recorded in presence of TTX and TEA. Records before (*A*) and during (*B*) perifusion of cell with Ba-containing solution (10 mM-Ba replacing 10 mM-Ca). In both *A* and *B* two successive traces are superimposed. The first is the response to a subthreshold pulse of current. The second is the regenerative potential elicited by a suprathreshold pulse (the current pulses used here are much briefer than in the previous records). Cell held at −70 mV by hyperpolarizing current. *C*, Ba-induced depolarization in a second cell. Cell perifused with Ba containing solution (10 mM-Ba replacing 10 mM-Ca; TTX but not TEA present) for time indicated by bar beneath record.

action of Ni, Co and Mn on Ca channels rather than from any unspecific 'membrane stabilizing' action of divalent cations (Hagiwara, 1973; Reuter, 1973). In the first place, no such suppression of this response was observed when the divalent ion concentration was correspondingly increased with Ca (Figs. 3*G–I*). Secondly, at the concentrations used neither Ni nor Co nor Mn reduced the 'Na-spike' recorded from these pars intermedia cells in the absence of TTX and TEA (Figs. 3*J–L*).

In most cells that produce Ca-dependent action potentials Sr and Ba can substitute for Ca in maintaining the action potential (Fatt & Ginsborg, 1958; Hagiwara, 1973). The action potential recorded from pars intermedia cells in the presence of TTX and TEA not only persisted when Ca was replaced with either Sr (Fig. 4) or Ba (Fig. 5*A*, *B*) but also increased in duration. The different durations of the action potentials seen with these different divalent cations may reflect the different effects of these ions on K conductance. All three (Ca, Sr and Ba) are capable, in varying degrees, of activating or blocking different K currents in various cell membranes (see for example Gorman & Hermann, 1979). A prolongation of the action potential by Sr and Ba in the presence of TEA has also been observed in crustacean muscle fibres (Fatt & Ginsborg, 1958). In addition, Ba (Fig. 5*C*), and to a lesser extent Sr, tended to depolarize the pars intermedia cells whether or not TTX, TEA or Na were present. In most cells the depolarization was 'K-like' (e.g. Fig. 5*C*) whereas in some others exposure to Ba produced repetitive action potential activity. The actions of Ba and Sr on the pars intermedia cell membrane are evidently complex, but the apparent ability of these ions to substitute for Ca in maintaining the action potential is consistent with their known ability to act as charge carriers through Ca channels (Reuter, 1973; Hagiwara, 1975).

DISCUSSION

Previous experiments on the pars intermedia cells of the rat revealed that the action potentials are due mainly to an increase in membrane permeability to Na in that they were suppressed in Na-free solution or by the addition of TTX (Douglas & Taraskevich, 1978). The present experiments, however, demonstrate that in the presence of TTX a regenerative potential can be elicited provided TEA is present. Presumably TEA facilitates demonstration of this regenerative potential by its familiar action of blocking countervailing K current. The ionic event underlying the regenerative potential appears to be inward Ca current for the responses were lost when Ca was omitted or when Ni, Co or Mn were added to block voltage-dependent Ca channels (Reuter, 1973; Hagiwara, 1975). The further demonstration that Ba and Sr could substitute for Ca in sustaining the response, considered along with the blocking effects of Ni, Co and Mn, indicates that these Ca channels have properties similar to those most commonly encountered in various other cells (Reuter, 1973; Hagiwara, 1975; but see, for example, Anderson, 1979; Hagiwara, 1979). Our previous inability to observe a regenerative Ca-dependent response in pars intermedia cells exposed to TEA in Na-free conditions (Douglas & Taraskevich, 1978) is probably attributable to the prolonged period of Na deprivation in these earlier experiments. Katz & Miledi (1969) have observed that prolonged Na deprivation is inimical to the demonstration of a Ca component in the presynaptic terminal of the squid giant synapse. Moreover, in the present experiments we could elicit a regenerative Ca component from TEA-treated pars intermedia cells in the absence of Na when the period of Na deprivation was short (Fig. 2*A–C*).

The occurrence of a Ca component in the predominately Na-dependent action potential in pars intermedia cells has an obvious bearing on the possibility that action potentials participate in stimulus-secretion coupling in these cells inasmuch as the Ca

component reflects an influx of Ca ions into the cell that could initiate secretion. Ca is known to provide a stimulus to secretion in many cell types (Douglas, 1968; 1978; Katz, 1969; Rubin, 1974) including various adenohypophysial cells (Bicknell & Schofield, 1976; Vale *et al.* 1977), and secretion from pars intermedia cells requires Ca (Hopkins, 1970; Bower & Hadley, 1972). It will be recalled that action potentials occur spontaneously in these spontaneously secreting pars intermedia cells (see Introduction), and, moreover, that dopamine, the physiological inhibitor of secretion, slows or arrests this discharge (Davis & Hadley, 1978; Douglas & Taraskevich, 1978).

The view that Ca entry during the action potentials is important for secretion is not inconsistent with the apparent smallness of the Ca component to the action potential. In nerve terminals where Ca entry during the action potential is recognized as the stimulus for secretion (Katz, 1969; Llinás & Heuser, 1977) the contribution of inward Ca current to the shape of the action potential is negligible in comparison with that of Na and K currents (Katz & Miledi, 1969). Likewise in adrenal chromaffin cells, where again Ca influx is the critical event in stimulus-secretion coupling (Douglas, 1975), the apparent contribution of inward Ca current to the action potential is also small (Brandt, Hagiwara, Kidokoro & Miyazaki, 1976).

The apparent contribution of Na and Ca to the action potential of adenohypophysial cells evidently varies with species and region of the gland. A much more obvious Ca component than that here described has been observed in action potentials of lizard pars intermedia cells (Taraskevich & Douglas, 1979) and rat pars distalis cells (Taraskevich & Douglas, 1977; Ozawa & Sand, 1978) as well as those of neoplastic cells derived from rat pars distalis (Kidokoro, 1975; Biales *et al.* 1977; Ozawa & Miyazaki, 1979; Taraskevich & Douglas, 1980). In fish pars distalis cells (prolactin cells), on the other hand, Na dominates (Taraskevich & Douglas, 1978) as it does in the rat pars intermedia cells. These variations aside, the significant fact remains that a Ca component is present in each instance. This encourages the view that action potentials in adenohypophysial cells participate in secretory control.

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