

# Properties of Calcium and Potassium Currents of Clonal Adrenocortical Cells

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**ABSTRACT** The ionic currents of clonal Y-1 adrenocortical cells were studied using the whole-cell variant of the patch-clamp technique. These cells had two major current components: a large outward current carried by K ions, and a small inward Ca current. The Ca current depended on the activity of two populations of Ca channels, slow (SD) and fast (FD) deactivating, that could be separated by their different closing time constants (at  $-80$  mV, SD, 3.8 ms; and FD, 0.13 ms). These two kinds of channels also differed in (a) activation threshold (SD,  $\sim -50$  mV; FD,  $\sim -20$  mV), (b) half-maximal activation (SD, between  $-15$  and  $-10$  mV; FD between  $+10$  and  $+15$  mV), and (c) inactivation time course (SD, fast; FD, slow). The total amplitude of the Ca current and the proportion of SD and FD channels varied from cell to cell. The amplitude of the K current was strongly dependent on the internal  $[Ca^{2+}]$  and was almost abolished when internal  $[Ca^{2+}]$  was  $< 0.001$   $\mu$ M. The K current appeared to be independent, or only slightly dependent, of Ca influx. With an internal  $[Ca^{2+}]$  of  $0.1$   $\mu$ M, the activation threshold was  $-20$  mV, and at  $+40$  mV the half-time of activation was 9 ms. With 73 mM external K the closing time constant at  $-70$  mV was  $\sim 3$  ms. The outward current was also modulated by internal pH and Mg. At a constant pCa, a decrease of pH reduced the current amplitude, whereas the activation kinetics were not much altered. Removal of internal Mg produced a drastic decrease in the amplitude of the Ca-activated K current. It was also found that with internal  $[Ca^{2+}]$  over  $0.1$   $\mu$ M the K current underwent a time-dependent transformation characterized by a large increase in amplitude and in activation kinetics.

## INTRODUCTION

The electrophysiological properties of adrenocortical cells are poorly studied although it has been known for over a decade that they can generate spike-like depolarizations (Matthews and Saffran, 1973; Natke and Kabela, 1979). Recently, action potentials of over 70 mV in amplitude have been recorded in clonal Y-1 murine adrenocortical cells (Tabares and López-Barneo, 1986). These action potentials are tetrodotoxin (TTX)-resistant and disappear after removal of external Ca or after introduction of Ca channel blockers. These cells have a dense and uniform distribution of Ca-activated maxi-K channels, the properties of which have been studied at the single-channel level (Tabares et al., 1985; López-Barneo et al., 1986).

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Cells dispersed from the rat adrenal cortex also exhibit Ca-dependent electrical excitability (Quinn et al., 1987) and when subjected to voltage clamp, they can generate Ca and K currents (Payet et al., 1987; Tabares et al., 1988).

Several endocrine cells generate Ca- and Na-dependent action potentials that are responsible for the Ca entry required for exocytosis (for a review see Petersen, 1980), and in some secretory cells the ionic conductances underlying membrane excitability have been studied (Fenwick et al., 1982; Hagiwara and Ohmori, 1982; Dubinsky and Oxford, 1984; Matteson and Armstrong, 1984; Cota, 1986; Rorsman and Trube, 1986; Hiriart and Matteson, 1988). Adrenocortical cells are unusual because stimulation by adrenocorticotropin (ACTH) and other secretagogues primarily increase hormone biosynthesis, and the release of newly synthesized steroids can occur by diffusion across the lipid phase of the membrane (Jaanus et al., 1970; Sibley et al., 1981). However, it is well established that external Ca is required for optimal steroid production and output in response to ACTH (Birmingham et al., 1953; Jaanus et al., 1970; Sayers et al., 1972; Fakunding et al., 1979) and that secretagogues increase Ca uptake in adrenocortical cells (Leier and Jungmann, 1973; Yanagibashi, 1979; Kojima and Ogata, 1986). In adrenal cells cytosolic Ca may be important for key enzymatic steps and cytoskeleton rearrangement involved in steroidogenesis (Hall et al., 1979; Cheitlin and Ramachandran, 1981). Thus, the characterization of the ionic currents present in these cells, which are possibly regulated by secretagogues and intracellular mediators, will surely be of importance for the understanding of the action of hormones and drugs on steroid biosynthesis and secretion.

We have studied the properties of ionic currents present in Y-1 cells. These are a transformed line of murine adrenocortical cells that secrete glucocorticoids in response to ACTH and other secretagogues, and that are broadly used in biochemical studies on the mechanisms underlying the production and release of steroid hormones (Mrotek and Hall, 1977; Clark and Shay, 1981; Hall et al., 1979; Mattson and Kowal, 1982). In this article it is shown that clonal adrenocortical cells have voltage-dependent Ca and K currents. The Ca current is mediated by two different types of Ca channels, and the K current appears to be essentially due to the activity of Ca-activated K channels. It is also shown that this K conductance is modulated by cytosolic pH and  $Mg^{2+}$ .

Preliminary accounts of some of these results have appeared elsewhere (López-Barneo and Tabares, 1987; Tabares and López-Barneo, 1988).

## METHODS

### *Cell Culture*

Y-1 cells were purchased from the American Type Cell Collection (Rockville, MD) and cultured in Ham's F-10 medium (Gibco, Grand Island, NY) supplemented with 5% fetal bovine serum (Gibco), penicillin (100 U/ml), and streptomycin (100  $\mu$ g/ml; Flow Laboratories, Inc., Rockville, MD). In some experiments 1% glutamine (Sigma Chemical Co., St. Louis, MO) was added to the culture medium. Cells were plated on slivers of glass coverslips coated with poly-L-lysine (Sigma) placed inside 35-mm plastic Petri dishes. Cells were kept in a  $CO_2$  incubator at 37°C until use (4–72 h after plating).

*Solutions*

At the beginning of each experiment a coverslip was placed in a chamber with a volume of ~0.2 ml and filled with the recording external solution. The pipette filling solution is referred to as internal solution since, as previously observed in other preparations (Fenwick et al., 1982; Matteson and Armstrong, 1984), it equilibrated rapidly with the cell cytoplasm. In the text and figure legends solutions are indicated as external//internal. The compositions of the recording solutions are shown in Table I. In some experiments TTX (1  $\mu\text{g/ml}$ ) and 1 mM  $\text{CdCl}_2$  were added to the standard external solution, or 10 mM NaCl was replaced by an equimolar concentration of tetraethylammonium (TEA) chloride. 2 mM Mg-ATP was added to the 70 Cs internal solution to retard wash-out of Ca channels (Kostyuk, 1984; Forscher and Oxford, 1985; Cota, 1986). Unless otherwise noted the pH of the external and internal solutions were adjusted to 7.2 and 7.2–7.3, respectively. In experiments designed to test the effect of internal  $\text{Ca}^{2+}$  and pH, Ca/EGTA buffers were added to the standard internal solution (Table II). The compositions of these buffers were obtained with the aid of a short computer program that calculates pCa ( $\log 1/[\text{Ca}^{2+}]$ ) and  $[\text{Mg}^{2+}]$  at a given pH, given the total concentrations of Ca ( $\text{Ca}_t$ ), EGTA ( $\text{EGTA}_t$ ), and Mg ( $\text{Mg}_t$ ). Stability constants for EGTA, EGTA/Ca and EGTA/Mg (at 20°C and 0.1 M ionic strength) were taken from Martell and

TABLE I  
*Composition of Recording Solutions\**

External	NaCl	KCl	$\text{CaCl}_2$	$\text{MgCl}_2$	$\text{BaCl}_2$	HEPES	
Standard	130	5	0–10	1	—	10	
10 Ca	140	—	10	—	—	10	
10 Ba	140	—	—	—	10	10	
73 K	62	73	10	1	—	10	
Internal	KCl	$\text{MgCl}_2$	CsCl	NMGCl	HEPES	EGTA	Mg-ATP
Standard	140	2	—	—	10	0–10	—
140 Cs	—	2	140	—	10	0–10	—
70 Cs	—	2	70	70	10	10	2

\*All values are in millimolar.

Smith (1974). Solutions were passed through a 0.2- $\mu\text{m}$  filter (Millipore, Bedford, MA) before use. Experiments were performed at 20–25°C.

*Recording Techniques and Electronics*

Ionic currents were recorded from over 300 adrenocortical cells (12–16  $\mu\text{m}$  in diameter) using the whole-cell variant of the patch-clamp technique (Hamill et al., 1981). The average cell capacitance was  $17.78 \pm 7.24$  pF (mean  $\pm$  SD,  $n = 50$ ). Patch electrodes were fabricated from either soft hematocrit capillaries (Hirschmann, West Germany) or Kimax 51 borosilicate (Kimble Division, Toledo, OH) by a double pull on a puller (David Kopf Instruments, Tujunga, CA) and fire polished on a microforge. In the experimental conditions used the currents recorded with both types of electrodes were indistinguishable. Electrode resistance varied between 0.5–2 M $\Omega$ . In most experiments we used a patch-clamp amplifier built by us following the standard design (Hamill et al., 1981). In this amplifier the current-to-voltage converter is a Burr-Brown OPA 111 (Tucson, AZ). Frequency response was improved by using low-resistance electrodes, series resistance compensation, and a relatively low (100 M $\Omega$ ) feedback resistance. Because the recording of the small and fast Ca tail currents present in Y-1 cells requires better time resolution than our amplifier provides, a set of experiments was

done at the laboratory of Dr. Clay M. Armstrong (University of Pennsylvania) using a technique that enhances the speed with which a voltage step is applied to the cell membrane (for details see Armstrong and Chow, 1987; Swandulla and Armstrong, 1988). Apart from the difference in time resolution the properties of the Ca currents recorded in both laboratories were the same.

#### *Data Acquisition and Analysis*

An IBM-PC/AT computer was used for pulse generation and for acquisition, storage and analysis of the data. The pulse generator was built by us on an IBM prototype card, using an eight-bit digital-to-analog converter (DCA 0808; Analog Devices Inc., Norwood, MA) and a programmable peripheral interface (PPI 8255A-5; NEC Microcomputers, Paris, France). The

TABLE II  
*Ca/EGTA Buffers Added to the Standard Internal Solution\**

Variable pCa at constant pH				
pCa	[Ca <sub>i</sub> ]	[EGTA <sub>i</sub> ]	pH	[Ca <sup>2+</sup> ]
				<i>μM</i>
6.34	4.14	5	7.2	0.45
6.64	3.75	5	7.2	0.23
6.94	2.78	5	7.2	0.11
7.04	2.51	5	7.2	0.09
8.04	0.45	5	7.2	0.009
Variable pH at constant pCa				
pH	[Ca <sub>i</sub> ]	[EGTA <sub>i</sub> ]	pCa	
6.8	0.098	5	8	
7	0.238	5	8	
7.2	0.485	5	8	
7.6	1.669	5	8	
6.7	0.455	5	7	
7.4	3.576	5	7	
7.7	4.316	5	7	

\*All values, except the concentration of free Ca<sup>2+</sup>, are in millimolar. The concentration of free Mg<sup>2+</sup> is in all cases between 1.85–2 mM.

pulse generator triggered a 10-bit digital oscilloscope 5223; (Tektronix, Inc., Beaverton, OR) which was used to digitize the current signal. The oscilloscope was interfaced to the computer by an IEEE-488 card (National Instrument Co., Inc., Baltimore, MD). The system used for acquisition of Ca tail current was based on a LSI-11/73 computer (Digital Equipment Corp., Marlboro, MA) (Matteson and Armstrong, 1984). In all cases linear ionic and capacity currents were subtracted. Ca and K tail currents were fitted with one or the sum of two exponentials using a least-squares procedure.

## RESULTS

### *Major Current Components*

The major components of ionic current recorded in Y-1 cells are shown in Fig. 1. With high K in the pipette, depolarization to positive voltages generated in all cells a

large outward current that activated slowly and, at a membrane potential of +50 mV, reached a maximum in ~50 ms (Fig. 1 A). The outward current was mainly carried by K ions since it disappeared when all the K of the pipette solution was replaced by Cs or a mixture of Cs and *N*-methyl-D-glucamine (NMG). Furthermore, this current was greatly reduced when 10 mM TEA was added to the external solution.

With the standard external and internal solutions inward currents were observed in only a few cells and in a membrane voltage range at which K currents were not too large. On the other hand, inward currents, smaller than 200 pA, were clearly recorded under conditions that abolished the K current. Fig. 1 B shows, in a cell dialyzed with the 140 Cs solution, an inward current elicited by a step depolarization to -10 mV from a holding potential of -70 mV. The current reaches a maximum in 7-8 ms and thereafter partially inactivates. Inward tail currents are not clearly detected at the end of the 50-ms pulse due to the large fraction of the current that is already inactivated and the relatively low time resolution of the recording system (see Methods). The inward current was a result of the activity of Ca chan-

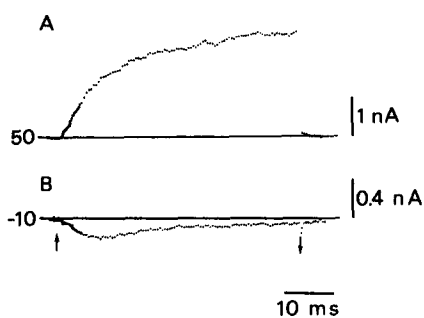


FIGURE 1. Whole-cell currents in clonal adrenocortical cells. (A) Outward current during a voltage step to +50 mV from a holding potential of -70 mV. Standard, 5 Ca//standard, 0.5 EGTA. Experiment 09MZ87C. (B) Inward current during a pulse to -10 mV from a holding potential of -70 mV. The arrows indicate the onset and the end of the voltage step. Standard, 10 Ca//140 Cs, 10 EGTA. Experiment 08JN87A.

nels since it was not affected by the addition of TTX (1  $\mu$ g/ml) to the external solution, and it disappeared in the absence of external Ca or in the presence of 1 mM external Cd. A similar current was recorded when external Ca was replaced by Ba. These results indicate the existence of Ca and K channels in Y-1 adrenocortical cells.

#### *Properties of the Ca Current*

**Current-voltage relations.** Fig. 2 A illustrates Ca currents recorded with high time resolution (see Methods) at various membrane potentials. Current generated during the pulse, which is indicated between the arrows, activates more rapidly at more depolarized levels. At the instant of repolarization to the holding potential, the driving force for Ca ions suddenly increases and inward tail currents are recorded. These tails are due to the flow of current through the Ca channels that were opened by the pulse, and their time course reflects that of the closing of the channels. After small depolarizations the decay of the tails follows a simple, slow

time course but after steps to potentials more positive than 0 mV a fast component clearly appears. Both the slow and the fast components of the tails grow larger as the pulse potential is made more positive. These findings suggest the existence in adrenocortical cells of at least two types of Ca channels with different activation thresholds and deactivation kinetics (see below).

The relation between the Ca current measured at the end of a 10-ms pulse and the step membrane potential is illustrated in Fig. 2 *B*. The line drawn through the filled symbols is the  $I/V$  curve obtained with 10 mM Ca in the external solution. In this cell, the inward current became detectable at  $-60$  mV, it reached a maximum

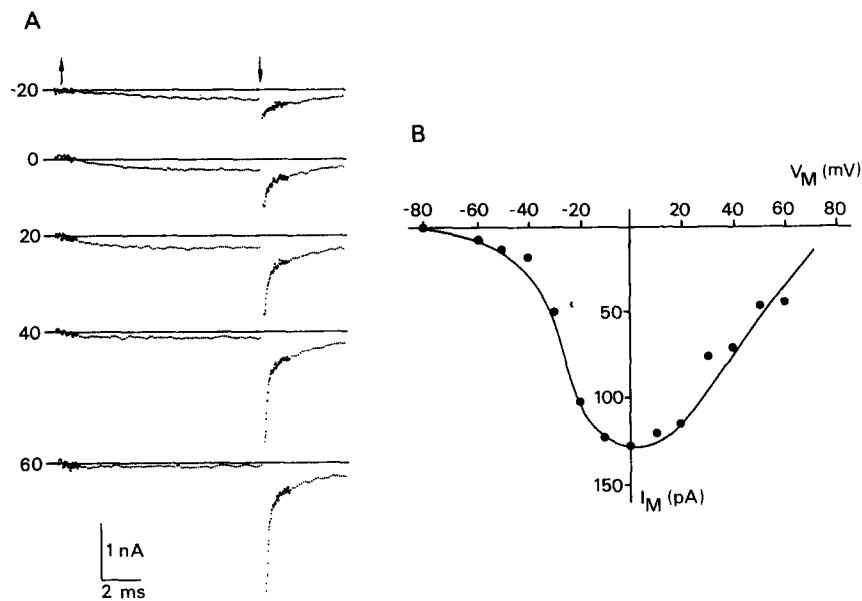


FIGURE 2. (A) Ca currents recorded with high time resolution by depolarizations to the membrane potentials given in millivolts to the left of each trace. The onset and the end of the voltage steps (10-ms duration) are indicated by the arrows. Upon repolarization tail currents with a biphasic decay are observed. (B) Current-voltage relation of inward current. Current amplitude (ordinate) was measured 10 ms after the beginning of voltage steps of variable amplitude (abscissa). The continuous line was fitted by eye. Holding potential  $-80$  mV. 10 Ca//70 Cs. Experiments 0C1587N and 0C2487N.

between  $-10$  and  $+20$  mV, and with larger depolarizations the amplitude of the current decreased as the driving force for Ca ions became progressively smaller.

*Identification of two Ca channel types.* As shown before, at least two components of Ca current could be clearly distinguished in the tail currents generated after depolarizing voltage steps. The isolation of the two components was done following the method shown in Fig. 3 (see also Matteson and Armstrong, 1986). A illustrates a Ca tail current recorded on return to a holding potential of  $-80$  mV after a depolarization to  $+40$  mV lasting 10 ms. After the initial jump in the current there is a biphasic decay. An exponential with a time constant of 3.78 ms was fitted to the slow

component and extrapolated back to the instant of the repolarization (Fig. 3 A). The difference between the tail current and the exponential yielded a separate fast component that was fitted by another exponential with a time constant of 0.13 ms (Fig. 3 B). Thus, the slow and the fast components are indications of the existence in adrenocortical cells of two different Ca channel types that differ in their activation threshold and closing kinetics. Similar results have been found in a number of endocrine cells and the two Ca channels classified as fast (FD) and slow (SD) deactivating channels (cf. Matteson and Armstrong, 1986). FD channels also have a higher threshold and inactivate more slowly than SD channels. In Y-1 cells the closing time constants measured at  $-80$  mV after 10-ms steps to  $+40$  mV were  $0.13 \pm 0.031$  ms for FD channels (mean  $\pm$  SD,  $n = 6$ ), and between 3 and 4 ms for SD channels.

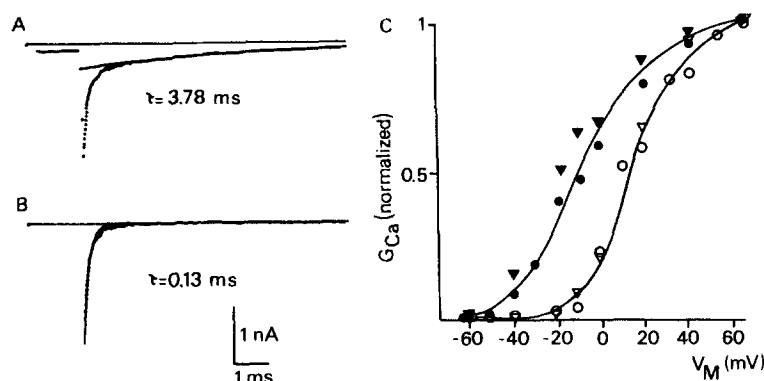


FIGURE 3. Identification of two types of Ca channels. (A) Tail current recorded at the end of a voltage step to  $+40$  mV. The repolarization potential was  $-80$  mV. The tail had a biphasic decay with a slow and a fast component. The slow component, due to the activity of SD channels, was fitted by an exponential with a time constant of 3.78 ms extrapolated back to the instant of repolarization. (B) Subtraction of the slow exponential from the current yielded an isolated fast component which was due to the activity of FD channels. The fast component was fitted by another exponential with a time constant of 0.13 ms. In A and B the baseline was fitted to the current level before the pulse step. (C) Conductance-voltage relations of SD (filled symbols) and FD (open symbols) channels. The amplitude of the slow and fast exponentials normalized to their respective maximal values are plotted as a function of the step potential. Measurements are from two cells, each one is represented by a different symbol. The lines were fitted by eye. 10 Ca//70 Cs. Experiments 0C1587N and 0C2487N.

Fig. 3 C shows the conductance-voltage relations for SD (filled symbols) and FD (open symbols) channels. Normalized SD and FD conductances, which are proportional to the amplitude of the slow and fast tail components, respectively, are plotted as a function of the step membrane potential. The two curves illustrate some of the differential properties of SD and FD channels suggested in the previous section. The activation threshold is lower for SD ( $\sim -50$  mV) than for FD ( $\sim -20$  mV) channels and half-maximal activation is between  $-15$  and  $-10$  mV for SD channels, and between  $+10$  and  $+15$  mV for FD channels.

*Conductance changes during a maintained depolarization.* SD and FD channels also differ in their behavior during a maintained depolarization. This is illustrated in

Fig. 4, *A–D* by Ca tail currents recorded upon repolarization to the holding potential of  $-80$  mV after voltage steps to  $+40$  mV. The duration of each pulse, in milliseconds, is indicated next to each trace. The slow component of the tail current is almost absent after a pulse of short duration (*a*), it appears when the pulse lasts 7 ms (*b*), and is abolished after a long pulse (*c*). After this sequence of variable pulse duration, a large slow component is recorded again when the voltage step is made shorter (*d*). These results suggest that SD and FD channels have different activation and inactivation time courses. In Fig. 4 *E*, normalized conductance is plotted as a

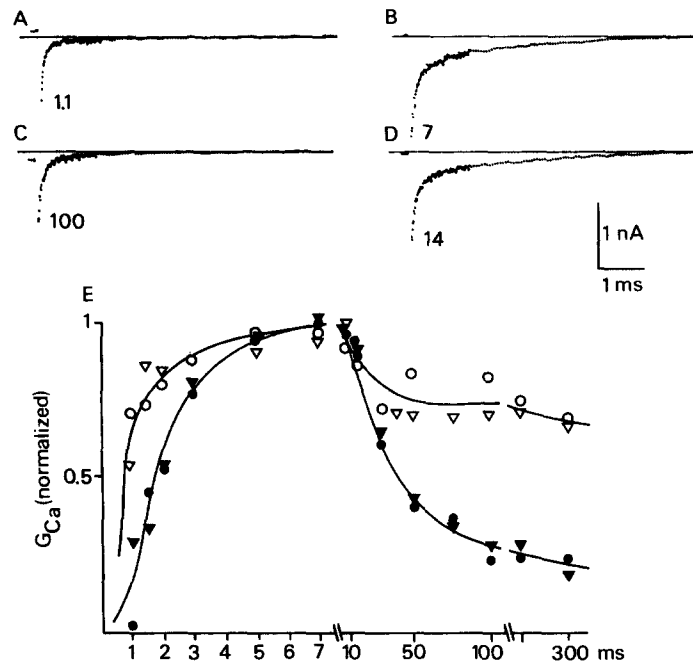


FIGURE 4. Differences in the activation and inactivation time course of SD and FD Ca channels. (*A–D*) Tail currents recorded after depolarizations to  $+40$  mV lasting (*A*) 1.1, (*B*) 7, (*C*) 100, and (*D*) 14 ms. The repolarization membrane potential was  $-80$  mV. (*E*). Time course of SD (filled symbols) and FD (open symbols) channel conductance during maintained depolarizations. Normalized amplitudes of slow and fast exponentials (ordinate) fitted to tails recorded after voltage pulses to  $+40$  mV are plotted as a function of the pulse duration (abscissa). The lines drawn through the data points were fitted by eye. Measurements are from two experimental protocols applied to the same cell. 10 Ca//70 Cs. Experiment 0C2487N.

function of pulse duration. While at this membrane voltage ( $+40$  mV) half of the maximal conductance of FD channels (open symbols) was reached in  $\sim 0.8$  ms, the same parameter for SD channels (filled symbols) was near 1.6 ms. However, SD channels tend to inactivate more rapidly than FD channels. After a 300-ms depolarization only 30% of FD conductance was inactivated whereas SD conductance progressively decreased after depolarizations lasting more than 10 ms, and at the end of a 300-ms pulse fell to 20% of the peak value.



*Variability of Ca currents.* Indications of the existence of SD and FD channels were observed in all Y-1 cells subjected to voltage clamp, but the relative contribution of each channel type to the total current varied from cell to cell. Clonal adrenocortical cells also showed a large variability in the size of the total Ca current. This might be related to some unknown physiological phenomena and could be an aspect of interest for future experimental work.

The results so far presented demonstrate that Y-1 adrenocortical cells have voltage-dependent Ca conductances. There appear to be two types of Ca channels (FD and SD channels) that differ in their activation thresholds and kinetic properties.

#### *Properties of the K Current*

It was shown in Fig. 1 that clonal adrenocortical cells can generate a K current of several nanoamperes of amplitude. During the initial experiments designed to study the properties of this current we observed dramatic changes in its amplitude and kinetic with time unless internal  $[Ca^{2+}]$  was maintained at  $0.1 \mu M$  or lower. Thus, the study of the properties of this current was performed in conditions that yielded stable and reproducible recordings. The time-dependent changes in the K current that occurred when internal  $[Ca^{2+}]$  was higher are presented in a separate section at the end.

*Current-voltage relations.* Fig. 5 illustrates K currents recorded in a cell dialyzed with a standard solution where free  $Ca^{2+}$  was  $0.1 \mu M$ . The traces shown in *a* were obtained within the first 2 min after the beginning of the whole-cell recording by depolarizing steps to the indicated membrane potentials. In this experiment a relatively large inward Ca current could be observed at membrane voltages below 0 mV. Larger depolarizations elicited slow outward K currents with a typical sigmoid activation time course at all membrane voltages. Part *b* of the figure shows another family of traces recorded at the same membrane potentials but obtained 20 min after breaking into the same cell. In both sets of recordings the K currents are almost identical but the inward current is absent in *b* due to wash-out of Ca channels. Plot *c* illustrates the current-voltage relation for the recordings shown in *a* (dots) and *b* (squares). The lines represent the  $I/V$  curves of the K current in the presence (continuous line) and in the absence (discontinuous line) of functional Ca channels.

Although the outward current of Y-1 cells was strongly dependent on internal  $[Ca^{2+}]$  (see below), we never observed clear N-shaped  $I/V$  relations that resembled those of Ca-activated K currents described in other preparations (Meech and Standen, 1975; Bolsover, 1981; Dubinsky and Oxford, 1984; Thomas, 1984; Marty and Neher, 1985). In several experiments small "humps" in the current-voltage relation similar to the one shown in the example of Fig. 5 *C* (continuous line) were observed. This, however, could be a result of the large scattering in the time course of current records obtained with large depolarizations and therefore may have no functional significance. In many other cells that were dialyzed with solutions in which pCa was between 6 and 8 and in the presence of 5–10 mM external  $Ca^{2+}$ , no appreciable inflexions were seen.

*Time course of activation.* The time course of the outward K current is illustrated in Fig. 6. Trace *a* is a recording generated by a voltage step to +50 mV

showing the slow activation time course of the current that reaches the maximum by the end of the 50-ms pulse. The voltage dependence of activation kinetics is summarized in *d* where, with measurements done in three different cells, the time to reach half-activation ( $t_{1/2}$ ) is plotted vs. membrane potential. Traces *b* and *c* are recordings obtained with longer pulses and illustrate the time course of the K current during a maintained depolarization. In this experiment there was only a slight decay of the current during the long depolarization but in other cells a fall of 10–20% of the peak current was observed at the end of 500-ms pulses. Pulses of 1 s or longer

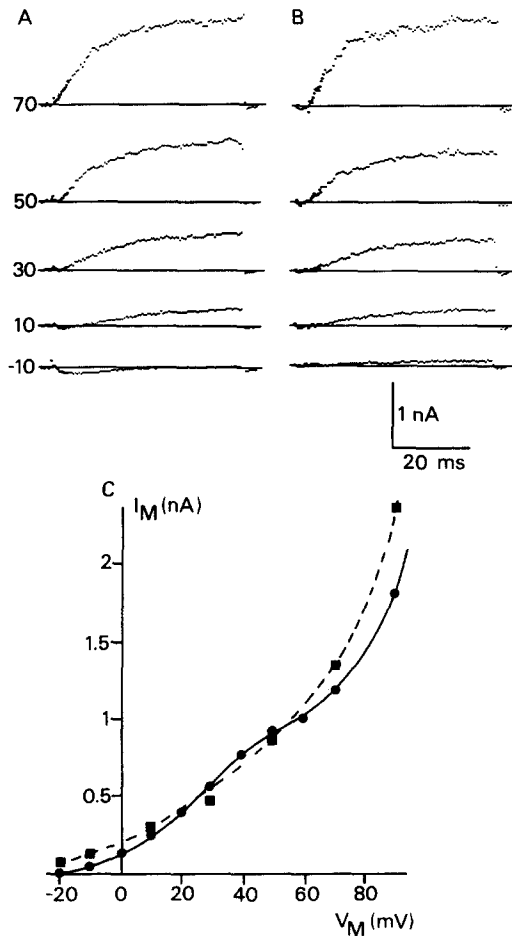


FIGURE 5. Current-voltage relation of K current. (A and B) Outward currents recorded in the same cell (A) 2 and (B) 20 min after going into the whole-cell recording mode. The membrane potential of the voltage step is given, in millivolts, by the number drawn near to each trace. The holding potential was  $-70$  mV. In C, current amplitude, measured at 50 ms from the onset of the pulses, is plotted as a function of the step membrane potential. Each symbol belongs to  $I/V$  curves taken around 2 (dots) and 20 (squares) min from the beginning of the experiment. The lines were drawn by eye. Standard, 5 Ca//standard,  $0.1 \mu\text{M}$  Ca. Experiment 03JN87A.

caused in all cells a substantial inactivation of the outward current, however, this phenomenon was not studied in detail.

**Closing kinetics.** The closing kinetics of the K channels were studied by recording tail currents in cells bathed with a high K solution. Fig. 7 A shows a set of K currents activated by depolarizing steps to various voltages in the presence of high external K. The pulse current was similar to the ones recorded with the standard external solution, but at the instant of the repolarization the driving force for K ions

suddenly changed in direction and large inward tail currents were generated. The decay of the tails reflects the time course of K channel closing. In Fig. 7 B, tail currents were recorded after a step to +30 mV lasting 50 ms. Membrane potential after the step is indicated in the figure. Tails were inward at potentials more negative than  $E_K$  and outward at more positive potentials. The amplitude of the tails is represented in *c* as a function of membrane potential. The curve is the instantaneous  $I/V$  relation for the K channels, which has a constant slope between -60 and -10 mV but decreases with more positive potentials. The intercept with the abscissa gives a value for  $E_K$  in this experiment (-14 mV) that was only 2 mV away from the value predicted by the Nernst equation (-16 mV). In these experiments a stable  $E_K$  value was attained within the first 30 s after breaking into the cells, which indicates that, as reported before (Fenwick et al., 1982; Matteson and Armstrong, 1984), the pipette filling solution rapidly equilibrated with the cytoplasm. At all membrane

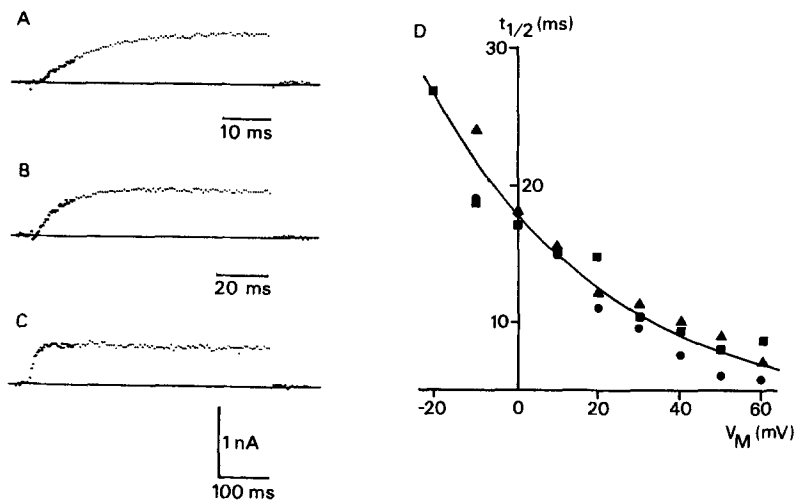


FIGURE 6. Activation time course of the K current. (A-C) Outward currents recorded in a cell by pulses to +50 mV of 50, 100, and 500 ms. The holding potential was -70 mV. (D) Time to reach half-activation ( $t_{1/2}$ , ordinate) as a function of step membrane potential. Measurements are from three cells each one represented by a different symbol. The line was drawn by eye. Standard, 5 Ca//standard, 0.09  $\mu$ M Ca. Experiments 25MY87A and 25MY87B.

potentials tail currents were well fitted by a single exponential and an example is shown by the trace at -60 mV in Fig. 7 B. The closing time constant was voltage-dependent, and was faster as the repolarizing membrane potential became more negative (Fig. 7 D).

*Activation of K currents by internal  $Ca^{2+}$ .* It has been shown that the K current of Y-1 cells is independent of, or only slightly dependent, on external  $[Ca^{2+}]$  or  $Ca^{2+}$  influx. In this section it is shown that this current is, however, strongly affected by changes of internal  $[Ca^{2+}]$ . Fig. 8, A-D are current traces elicited by 50-ms depolarizing steps to +50 mV in four cells of equivalent size. All recordings were obtained within the first 60 s after breaking into the cells. The free Ca concentrations of the

internal solution were (in micromolar): (A) 5–10, (B) 0.45, (C) 0.09, and (D) < 0.001. The four current traces had a rather similar time course but the amplitude was drastically reduced as  $[Ca^{2+}]$  decreased. With very low internal  $Ca^{2+}$  (D) the current was almost suppressed. The current-voltage relations for the K currents at the four different internal  $[Ca^{2+}]$  indicated above are plotted in *e*. The  $I/V$  curves obtained in the presence of high (0.45 and 5–10  $\mu M$ ) internal  $[Ca^{2+}]$  had small inflexions but a clear N-shape was not observed despite the fact that the external

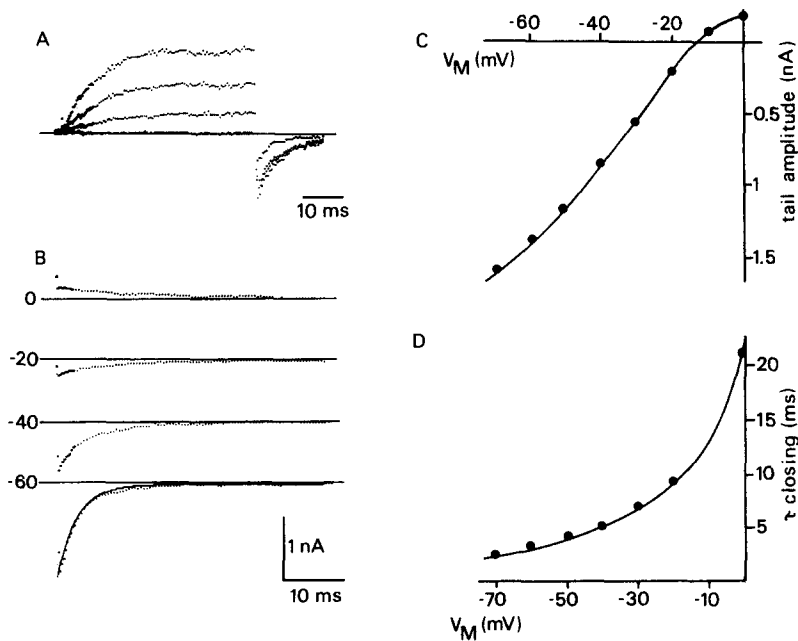


FIGURE 7. Deactivation time course of K channels. (A) Superimposed current traces recorded in a high K external solution by pulses to  $-10$ ,  $+10$ ,  $+30$ , and  $+50$  mV from a holding potential of  $-70$  mV. (B) K tail currents recorded after 50-ms pulses to  $+30$  mV from a holding potential of  $-70$  mV. In each trace the repolarization membrane potential is indicated in millivolts. In the trace at  $-60$  mV there appears superimposed an exponential with a time constant of 3.3 ms fitted to the current recording. (C) Tail amplitude as a function of the repolarization membrane potential. The point where the eye-fitted line crosses the voltages axis indicates the equilibrium potential. (D) Deactivation time constant (ordinate) as a function of the repolarization membrane potential (abscissa). 73 K//standard, 0.5 EGTA. Experiments 21MY87B and 22MY87A.

solution contained, in all cases, between 5 and 10 mM  $Ca^{2+}$ . The differences in current amplitudes were larger at more depolarized membrane potentials, which is perhaps explained by the enhancement of the Ca sensitivity of K channels with depolarization (Barrett et al., 1982; Moczydlowski and Latorre, 1983; Thomas, 1984). At extremely low  $[Ca^{2+}]$  the voltage-dependence was almost lost. Table III is a summary of the experiments in which the effect of internal  $[Ca^{2+}]$  was tested. In the Table the current is given in pA/pF to minimize variations in current amplitude due

to differences in cell size. These results suggest that the outward K current of Y-1 cells is predominantly due to the activity of Ca-activated K channels.

*Modulation of the K current by internal pH.* Cytosolic pH has a marked effect on different types of ionic conductances (for a review see Moody, 1984), and there is a report indicating that acidification of the cytoplasmic membrane surface inhibits Ca-activated K channels in pancreatic B cells (Cook et al., 1984). In several experiments we tested the effect of internal pH on the K current of Y-1 cells. At a fixed internal  $[Ca^{2+}]$  the amplitude of the current was reduced as internal pH decreased. Current-voltage relations observed at two different internal pH values are shown in Fig. 9 with internal pCa of 8 (A) and 7 (B), and Table IV summarizes the effect of internal

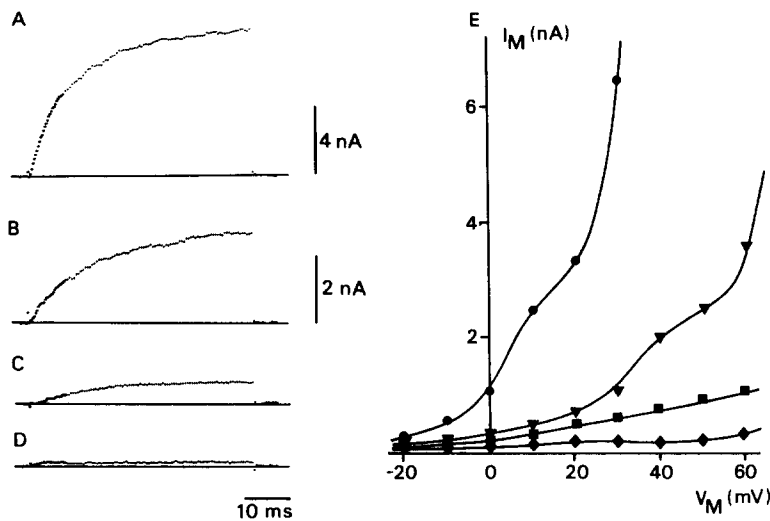


FIGURE 8. K currents at various internal  $Ca^{2+}$  concentrations. (A-D) Currents recorded by 50-ms pulses to +50 mV in four cells of similar size delivered within 60 s from the onset of whole-cell recording. The holding potential was -70 mV. The internal  $[Ca^{2+}]$ s in each cell were: (A) 5-10, (B) 0.45, (C) 0.09, and (D) <0.001  $\mu M$ . In A, no Ca was added to the solution and a contaminating free Ca concentrations of 5-10  $\mu M$  was assumed (cf. Meech and Standen, 1975). In D, the solution contained 10 mM EGTA and no Ca was added. (E) Current-voltage curves obtained by measuring current amplitude at 50 ms from the beginning of pulses of variable amplitude. Each symbol indicates a different  $[Ca^{2+}]$ : dots, 5-10  $\mu M$ ; triangles, 0.45  $\mu M$ ; squares, 0.09  $\mu M$ ; and diamonds, <0.001  $\mu M$ . The lines are drawn following the data points. Standard 5-10 Ca//standard, variable pCa. Experiments 29EN87C,D and 25MY87A,D.

pH on K current amplitude. The major effect of lowering pH was a reduction of the current amplitude that was more pronounced as membrane depolarization was larger. Thus, reduction of pH had an effect very similar to an increase in internal pCa. On the other hand, activation kinetics of K currents were not much altered by modifications in internal  $[H^+]$ . Protons are known to block K channels in a voltage-dependent manner (cf. Moody, 1984) and this could explain our results, however, a more subtle mechanism is probably involved because the changes in current ampli-

TABLE III  
Dependence of  $I_K$  Amplitude on Internal  $Ca^{2+}$

pCa	[ $Ca^{2+}$ ] $\mu M$	$I_K$ $pA/pF^*$
5-5.31	5-10 <sup>2</sup>	400 ± 140 (4)
6.34	0.45	340 ± 200 (4)
6.64	0.23	205 ± 70 (3)
7.04	0.09	50 ± 10 (9)
8.05	0.009	35 ± 10 (5)

\*Values are mean ± SD and the number of cells are in parentheses.  $I_K$  was measured at the end of 50-ms pulses to +50 mV delivered 30-60 s after breaking into the cells. The pH of all solutions was 7.2 and free Mg ranged between 1.85 and 2 mM.

<sup>†</sup>In the absence of Ca/EGTA, a contaminating [ $Ca^{2+}$ ] of ~5-10  $\mu M$  was assumed (Frankenhaeuser, 1957; Meech and Standen, 1975).

tude induced by altering internal pH were also dependent on internal [ $Ca^{2+}$ ]. An equivalent decrease of pH caused a relatively larger reduction of the current amplitude with a pCa of 7 (Fig. 9 B) than with a pCa of 8 (Fig. 9 A). These results could be explained if internal protons compete for the site normally occupied by  $Ca^{2+}$ , and thus at low pH fewer channels open at a given membrane voltage.

*Effect of internal  $Mg^{2+}$ .* It is known that some Ca-binding proteins can also bind  $Mg^{2+}$ , and that in some cases binding of  $Mg^{2+}$  modifies the affinity of the protein for  $Ca^{2+}$  (Fuchs, 1971; Cox et al., 1977; Leavis and Gergely, 1984). In a broad sense the Ca-activated K channels are an example of Ca-binding protein, and thus their

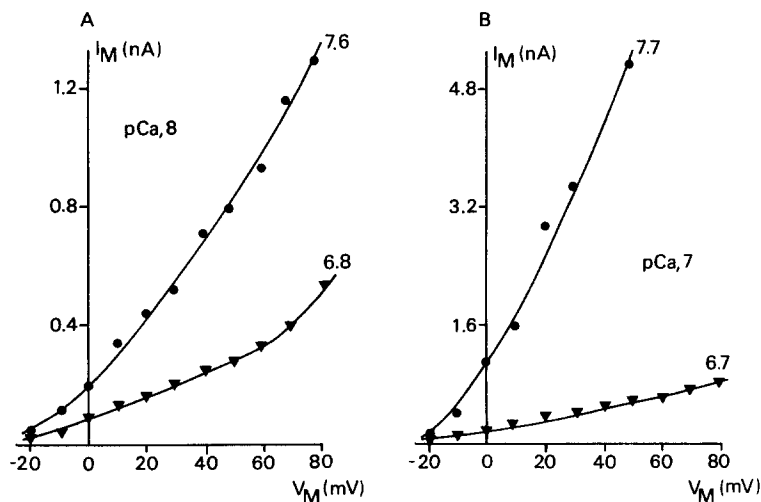


FIGURE 9. Effect of internal pH on the amplitude of K currents. (A) Current amplitude (ordinate) measured 50 ms after the onset of pulses of variable amplitude (abscissa) in two cells. Internal pH and pCa are indicated in the figure. Experiments 16JL87D and 17JL87B. (B) Similar experimental protocol as in A but with different pH and pCa values. Note the different current scales in A and B. Experiment 02JN87B,C. In A and B the lines follow approximately the data points. Standard, 5 Ca//standard, variable pCa and pH.

TABLE IV  
Dependence of  $I_K$  Amplitude on Internal pH

pH	pCa	$I_K$
		<i>pA/pF*</i>
6.8	8	21.8 ± 4 (2)
7.0	8	19.5 ± 2 (2)
7.2	8	30.0 ± 1 (3)
7.6	8	42.3 ± 1.6 (3)
6.7	7	46.0 ± 16 (2)
7.4	7	64.0 ± 7 (3)
7.7	7	209 ± 45 (3)

\*Values are mean ± SD and the number of cells are in parentheses.  $I_K$  was measured at the end of 50-ms pulses to +50 mV delivered 30–60 s after breaking into the cells. In all solutions free Mg was between 1.85 and 2 mM.

activation by internal  $Ca^{2+}$  could be influenced by internal  $Mg^{2+}$  ( $Mg_i$ ). We tested this hypothesis by measuring the K current amplitude using internal solutions with and without Mg. In all these experiments Ca/EGTA buffers were not added and contaminating internal  $[Ca^{2+}]$  was 5–10  $\mu$ M.

Fig. 10, *A* and *B* illustrates the effect of  $Mg_i$  on K currents recorded in two different cells by voltage steps to +10, +30, and +50 mV from a holding potential of -70 mV. The concentration of  $Mg_i$  is given next to each set of traces. In 0 mM  $Mg_i$  (*B*), the amplitude of the K currents were much smaller than in the control situation (2 mM  $Mg_i$ , *A*). This effect occurred at all membrane potentials as illustrated by the *I-V* curves of Fig. 10 *C*. The average peak K current amplitude elicited

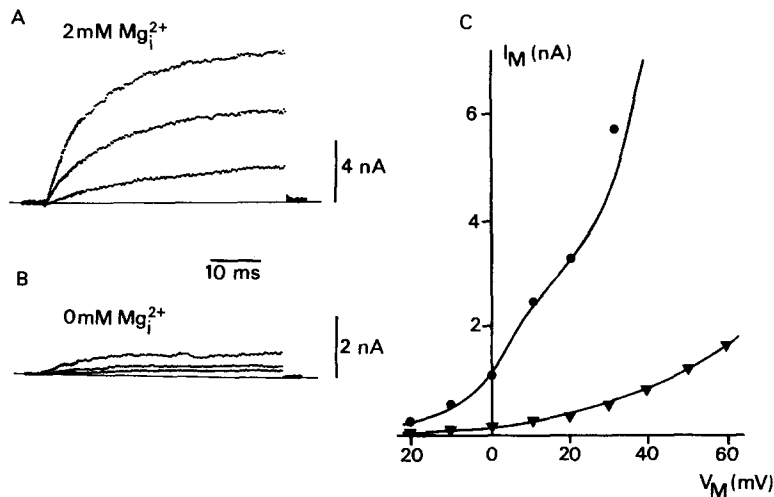


FIGURE 10. Effect of removal of internal Mg on Ca-activated K currents. (*A* and *B*) K currents recorded by depolarizations to +10, +30, and +50 mV from a holding potential of -70 mV with (*A*) 2 and (*B*) 0 mM Mg in the internal solution. The current-voltage relation is shown in *C* (dots, 2 mM internal Mg; triangles, 0 mM internal Mg). Standard 5 Ca//standard, 0 Ca, 0 EGTA. Experiments 29EN87C and 23AB87A.

by a depolarization to +30 mV was  $4.12 \pm 2$  nA (mean  $\pm$  SD,  $n = 5$ ) with 2 mM  $Mg_i$ , whereas in the absence of  $Mg_i$  this value was  $0.5 \pm 0.2$  nA ( $n = 4$ ). These results indicate that a decrease of internal  $Mg^{2+}$ , from 2 to 0 mM, causes a 10-fold reduction of the Ca-activated K current.

*Changes in the properties of the K current during intracellular dialysis.* K currents of dialyzed Y-1 cells underwent striking time-dependent changes in their amplitude and kinetic properties. This phenomenon, illustrated in Fig. 11, was observed in all cells when internal  $[Ca^{2+}]$  was higher than 0.1  $\mu$ M. In *a* there is a set of currents

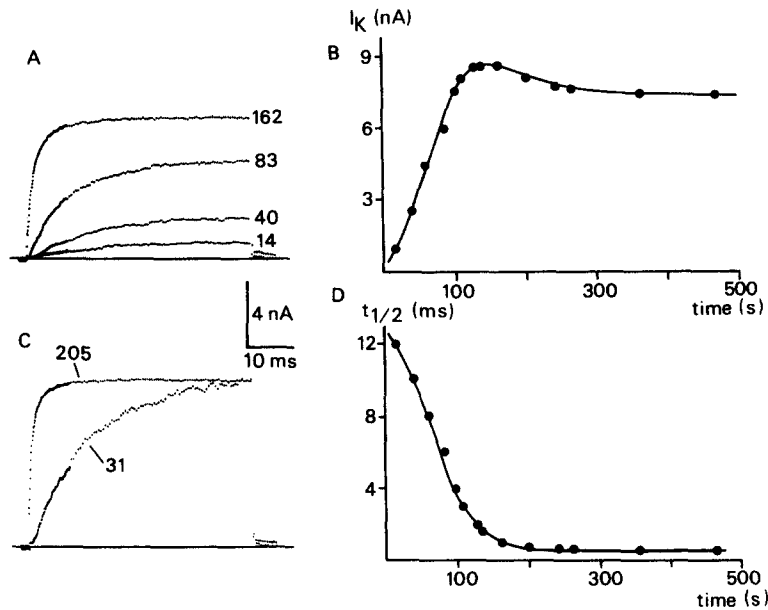


FIGURE 11. Time-dependent transformation of the outward current. (A) Currents recorded by a pulse to +30 mV delivered to a cell at various times after breaking into the cell. The time is given in seconds next to each trace. (B) Time-dependent changes in current amplitude measured 50 ms after the beginning of pulses to +30 mV. (C) Current traces recorded by a pulse to +50 mV at 31 and 205 s from the onset of whole-cell recording mode. The trace at 31 s was scaled by a factor of 1.95. (D) Time-dependent changes in the activation time course (time to reach half-activation,  $t_{1/2}$ ) of K currents recorded by depolarizations to +30 mV. Holding potential  $-70$  mV. Standard, 5 Ca/standard, 5–10  $\mu$ M Ca. Experiment 23MZ87A.

activated by pulses to +30 mV applied to a cell at various times, indicated in seconds by the number near each trace, after going into the whole-cell mode. In this experiment there was no Ca/EGTA added to the pipette filling solution and contaminating free  $Ca^{2+}$  was  $\sim 5$ –10  $\mu$ M (Frankenhaeuser, 1957; Meech and Standen, 1975). In *b*, current amplitude measured at the end of each pulse is plotted as a function of time. Both figures show a time-dependent increase in current amplitude. The current reached a more or less stable amplitude 150–200 s after the beginning of the intracellular dialysis. The recordings of Fig. 11 A, obtained without



linear subtraction, also show that the resting holding current was unaltered during the experiment. Besides the increase in amplitude, the current acquired a qualitatively different time course that was characterized by a marked acceleration of the activation kinetics. This is illustrated in *c* by two current recordings scaled to the same amplitude that were obtained by pulses to +50 mV delivered 31 and 205 s after the beginning of the experiment. Half-activation at 31 s was reached in ~10 ms, a value comparable to the one measured at the same voltage with low internal  $[Ca^{2+}]$  (see Fig. 6), whereas at 205 s the current reached half the maximal value in less than 1 ms. The change in activation time course as a function of time in this experiment is shown in plot *d*. The data shown in Fig. 11 must be taken as useful only for illustrative purposes since the time course of the transformation of the current varied from cell to cell. In many experiments the current grew progressively for several minutes and before reaching a steady value the cells were destroyed.

The origin of this phenomenon is for the moment uncertain. It is unrelated to external Ca or Ca influx since it appeared in the absence of external Ca or in cells bathed with solutions containing 1 mM Cd. However, it seems to be somehow related to internal  $[Ca^{2+}]$  because currents were stable when pCa was higher than 7 and the changes of the K current developed more slowly as internal  $[Ca^{2+}]$  was lower. Thus, the transformation of the  $K^+$  current is probably a reflection of the equilibration of cytosolic  $Ca^{2+}$  with  $Ca^{2+}$  in the pipette solution after the replenishment of intracellular  $Ca^{2+}$  stores and the depletion of cytoplasmic ATP. In previous work using whole-cell recording it has been reported a time-dependent negative shift of 15–25 mV for the kinetic parameters of Na (Marty and Neher, 1983; Fernandez et al., 1984), Ca (Cota, 1986), and K (Cahalan et al., 1985) channels. In the K current of Y-1 cells the changes expressed in terms of shifts were of 60–80 mV or larger.

These results indicate that intracellular dialysis with solutions containing high  $Ca^{2+}$  have profound effects on the K channels of Y-1 cells. In perfused cells time-dependent changes of Ca currents, which are believed to be partially due to Ca-stimulated enzymatic processes, are prevented by exogenous nucleotides (Fedulova et al., 1985; Forscher and Oxford, 1985; Byerly and Yazajian, 1986; Cota, 1986). In some Y-1 cells the addition of 2 mM Mg-ATP to the pipette filling solution appeared to retard the time-dependent modifications in the K current. However, this observation must be taken as preliminary since the effects of internal ATP were not studied in detail.

#### DISCUSSION

Much of the previous evidence concerning the existence of ionic channels in adrenocortical cells has come from intracellular recording studies. In this report we show that clonal adrenocortical cells, which can generate large action potentials (Tabares and López-Barneo, 1986), have voltage-dependent Ca and K channels. Indications of the activity of Na channels were never observed.

#### *Ca Channels*

Ca currents of Y-1 cells depend on the activity of two main populations of Ca channels that are clearly distinguished by their different voltage range of activation and

closing kinetics. SD channels have a lower threshold and inactivate faster than FD channels. The kinetic properties of these channels seem to be similar to those of the endocrine cells so far studied: GH<sub>3</sub> (Matteson and Armstrong, 1986), rat hypophyseal pars intermedia (Cota, 1986), and pancreatic beta (Hiriart and Matteson, 1988) cells. In preliminary reports, two types of Ca channels have also been described in freshly dispersed bovine (Cohen et al., 1987) and rat (Tabares et al., 1988) adrenal glomerulosa cells. Indications for the existence of two populations of Ca channels have been found in a number of preparations other than endocrine cells, including neurons (Llinas and Yarom, 1981; Carbone and Lux, 1984; Fedulova et al., 1985; Yoshii et al., 1985; Alvarez de Toledo and López-Barneo, 1988), egg cells (Fox and Krasne, 1984; Hagiwara et al., 1975), and muscle cells (Bean, 1985; Cota and Stefani, 1986). SD and FD channels of adrenocortical cells seem to be respectively similar to the T and L Ca channel types observed in dorsal root ganglion cells (Nowycky et al., 1985). These last authors have also reported the existence of a third Ca channel type (N channel), however, a current component that could represent the activity of N channels was not detected in our preparation.

Y-1 cells have a large resting potential and upon depolarization they generate large Ca-dependent action potentials repetitively (Tabares and López-Barneo, 1986). This electrical behavior is probably a reflection of the activity of the two Ca channel types described in this report. Each Ca channel type may have a different physiological role. It has been suggested that SD channels facilitate spike initiation and participate in the pacemaker mechanism that make possible the spontaneous firing of action potentials, whereas FD channels may be more specifically involved in spike generation and serve to inject Ca into the cytoplasm (Matteson and Armstrong, 1986).

#### *K Channels*

Voltage-dependent K currents recorded in Y-1 cells were mainly due to the activity of Ca-activated K channels since their amplitude was strongly dependent on internal [Ca<sup>2+</sup>] and almost disappeared when internal pCa was 9 or higher. Thus, Y-1 differ from other endocrine cells in which large K currents, apart from the Ca-activated current, can be recorded (Dubinsky and Oxford, 1984; Rorsman and Trube, 1986; López-Barneo et al., 1987). The K conductance of Y-1 cells seems to be very selective for K ions since the equilibrium potential measured with high external K was almost identical to the value predicted by the Nernst equation. The high selectivity of this conductance has been previously observed by single-channel recording in Y-1 cells (López-Barneo et al., 1986) and analyzed in detail in other preparations.

The slow activation time course of the outward K current is similar to Ca-activated K currents recorded in a number of cells, including *Helix* neurons (Meech and Standen, 1975; Lux and Hofmeier, 1982), *Tritonia* (Thompson, 1977), photoreceptors (Bolsover, 1981), neuroblastoma (Moolenaar and Spector, 1978), clonal pituitary (Dubinsky and Oxford, 1984), and chromaffin cells (Marty and Neher, 1985). However, in most preparations the *I/V* curve of Ca-dependent K currents has an N-shape, which is supposedly due to the local rise in cytosolic Ca<sup>2+</sup> that follows membrane depolarization (cf. Meech and Standen, 1975; Marty and Neher, 1985); this feature was much less pronounced, or absent, in our experiments. This suggests

that either Ca influx is small in Y-1 cells or that Ca equilibrates rapidly near the membrane. A similar current-voltage relation can be seen in Ca-activated K currents of Leydig cells (Kawa, 1987).

Closing kinetics of Ca-activated K currents have not been previously studied. We show here that channel closing is voltage-dependent, having a closing time constant of  $\sim 3$  ms at  $-70$  mV. For illustrative purposes this value can be compared with the 2.6 ms (at  $8^\circ\text{C}$  and without high external K) measured in K channels of the squid axon at the same membrane potential (Matteson and Swenson, 1986).

*Modulation of K Channel Activity by Internal  $\text{Ca}^{2+}$ , pH, and  $\text{Mg}^{2+}$*

Ca-activated K currents of Y-1 cells were modulated by internal pH ( $\text{pH}_i$ ) and Mg. In addition, with internal  $[\text{Ca}^{2+}]$  higher than  $0.1 \mu\text{M}$  the current underwent a marked time-dependent transformation characterized by a progressive increase in amplitude and an acceleration of activation kinetics.

The effects of  $\text{pH}_i$  on several K channels have been reviewed (Moody, 1984) and little is known about the effect of pH on Ca-dependent K channels. Meech (1979) reported that injection of HCl into *Helix* neurons decreased the amplitude of Ca-dependent currents, and more recently it has been shown that in pancreatic B cells lowering  $\text{pH}_i$  decreases the open probability of Ca-activated K channels without affecting the single-channel conductance (Cook et al., 1984). Although a complete explanation of the effects of  $\text{pH}_i$  requires further research, our results can be explained if internal protons compete with Ca for the same binding site and prevent the activation of the channels.  $\text{Ca}^{2+}$  and  $\text{H}^+$  are known to compete in their binding to membranes (Carvalho, et al., 1963) and cytosolic molecules (Fabiato and Fabiato, 1978).

A decrease of internal  $\text{Mg}^{2+}$ , in the range of millimolars, greatly reduces the amplitude of the Ca-activated K current of Y-1 cells. This phenomenon may have physiological interest since it is known that cytosolic  $[\text{Mg}^{2+}]$  can change under variable circumstances (Alvarez-Leefmans et al., 1986). A similar effect of Mg has been recently described on single maxi-K channels from mammalian skeletal muscle. Internal  $\text{Mg}^{2+}$  seems to have an allosteric effect increasing the affinity of the channel for  $\text{Ca}^{2+}$ . It has been suggested that elevated Mg makes accessible to  $\text{Ca}^{2+}$  additional binding sites involved in the activation process (Golowash et al., 1986). Other divalent cations that bind to Ca-binding proteins also have a similar qualitative effect on this channel (Oberhauser et al., 1988).

The dramatic time-dependent changes observed in K conductance when the internal solution had a  $[\text{Ca}^{2+}] > 0.1 \mu\text{M}$  is a phenomenon not observed previously in cells perfused with solutions containing similar Ca concentrations (Kostyuk and Krishtal, 1977; Rorsman and Trube, 1986; López-Barneo et al., 1987). In cells subjected to whole-cell patch-clamp there is a 15–25 mV negative shift of voltage-dependent parameters of Na (Marty and Neher, 1983; Fernández et al., 1984) Ca (Cota, 1986), and K (Cahalan et al., 1985) channels. Expressed in term of “shifts” the changes observed in our experiments (60–70 mV) were too large and therefore it is probable that a more specific process is involved. The time-dependent changes in the K current were also observed in the absence of internal  $\text{Mg}^{2+}$ . The transformation of the  $\text{K}^+$  current after the initiation of internal dialysis may reflect the time

required for cytosolic  $\text{Ca}^{2+}$  to equilibrate with  $\text{Ca}^{2+}$  in the pipette solution, a process that probably depends on the intracellular Ca sequestering capacity and the availability of ATP. Exogenous cyclic nucleotides seem to prevent the shift of Ca channels (Forscher and Oxford, 1985; Cota, 1986) and 2 mM ATP added to the internal solution appears to retard the transformation of the K current in Y-1 cells. However, the nature of this phenomenon and the mechanisms involved are for the moment unknown.

Thus, internal  $\text{Ca}^{2+}$ ,  $\text{H}^+$  and  $\text{Mg}^{2+}$  modulate the activity of K channels in clonal adrenocortical cells. This may be of functional relevance because it is known that the intracellular homeostasis of these ions are closely related (see for example Alvarez-Leefmans et al., 1981; Grinstein and Cohen, 1987) and their effects on K channels occur at concentration ranges around the physiological values.

*Possible Significance of Membrane Ionic Channels in the Physiology of Adrenocortical Cells*

Steroid-secreting cells differ from other endocrine cells in that they appear to have no significant amount of stored hormone and in that stimulation by secretagogues primarily increases biosynthesis, the product of which rapidly diffuses across the membrane (Jaanus et al., 1970; Sibley et al., 1981). In adrenocortical cells external Ca is required for steroid production and release, and secretagogues increase Ca uptake (Jaanus et al., 1970; Sayers et al., 1972; Fakunding et al., 1979; Yanagibashi, 1979; Kojima and Ogata, 1986). Furthermore, ACTH seems to cause a transient depolarization of fasciculata cells (Lymangrover et al., 1982) and Ca-channel agonists increase steroid secretion (Hausdorff et al., 1986). A rise of cytosolic  $\text{Ca}^{2+}$  appears to trigger key enzymatic processes that are essential for steroidogenesis. Among these processes are the activation of a Ca-calmodulin system that hydrolyzes cholesterol esters (Koletsy et al., 1983; Wilson et al., 1984; Sekimoto et al., 1984), and the reorganization of microfilaments and microtubules required for cholesterol mobilization and transport to mitochondria (Hall et al., 1979; Clark and Shay, 1981; Cheitlin and Ramachandran, 1981).

The properties of the ionic channels described here appear compatible with a significant contribution to the regulation of steroidogenesis and, perhaps, other cellular functions. Ca entry in adrenocortical cells can be mediated by SD and FD Ca channels and, therefore, regulated by membrane voltage. Secretagogues could modulate SD Ca channels and thus generate the voltage change required to open FD channels, which are well suited for fast injection of Ca into the cytoplasm. Another site of regulation could be the Ca-activated K conductance, which is the largest conductance present in these cells and which, by influencing membrane potential, determines Ca channel activity. Although this K conductance has a relatively high activation threshold ( $-10$  to  $-15$  mV) it probably participates in spike repolarization since it is known that in clonal adrenocortical cells action potentials of over 70–80 mV amplitude are usually recorded (Tabares and López-Barneo, 1986). The K conductance of Y-1 cells, modulated by  $\text{Ca}^{2+}$ ,  $\text{H}^+$ ,  $\text{Mg}^{2+}$ , and perhaps other intracellular mediators, also serves as a link between the cell metabolism and membrane potential and thus may impose a fine control of membrane ionic permeability. This is probably of importance to determine the Ca channel activity appropriate for the

different physiological states of the cells. These hypotheses may serve as guide for future experimental work.

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