The effect of calcium on potassium-induced depolarization of adrenal glomerulosa cells

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1. INTRODUCTION

Although it has long been known that a change in membrane potential is one of the primary events involved in the transfer of information from the cell surface to the cell interior in nerve cells, it is only recently that evidence has begun to accumulate showing that changes in membrane potential also occur in a variety of hormonally responsive cell types [1,2]. Of particular interest to the present investigation are studies on the effects of ACTH and cAMP upon membrane potential in cells of the adrenal cortex [2], and of K^+ and angiotensin II on cells of the zona glomerulosa [3]. In the zona fasciculata, ACTH causes a calcium-dependent depolarization [1,2] that is blocked by calcium channel blockers. In the zona glomerulosa, increases in extracellular $[K^+]$ lead to a calcium-dependent activation of aldosterone biosynthesis [4–6]. This is associated with increased calcium uptake into the cells, and is blocked by drugs which block calcium entry [5,7]. Although it is known that large increases in extracellular $[K^+]$ induce cell membrane depolarization in nerve and muscle cells, the unique feature of the adrenal glomerulosa cells is that small changes in extracellular $[K^+]$ lead to an activation of the cell. It was, therefore, of interest to measure whether such small changes in $[K^+]$ can also lead to a depolarization of the plasma membrane in this type of adrenal cells. Here, we have studied the effect that small changes in extracellular $[K^+]$ have on the distribution of the potential-sensitive lipophilic cation, tetraphenylphosphonium [8,13], both in the presence and absence of extracellular Ca^{2+}. The result obtained shows that small changes in extracellular $[K^+]$ lead to a calcium-dependent depolarization of the membrane.

2. MATERIALS AND METHODS

Adrenal cortex cells were prepared from beef adrenal glands obtained from a local abattoir and dispersed as in [5]. Briefly, the glands, freed of fat, were sliced in thin sections. The outermost portion, the capsular layer, was considered to contain mainly glomerulosa cells, while the inner portion contained the zona fasciculata and reticularis. This was confirmed by measuring the steroids secreted from these different portions of the gland. The outermost section was then finely minced, washed several times with Krebs-Ringer bicarbonate, and finally suspended in fresh Krebs Ringer that contained 2 mg collagenase/ml (Worthington Biochemical Co.), 10 μg ribonuclease/ml type II (Sigma Chemical Co.) and 4 mg BSA/ml (Sigma). The tissue was then incubated with shaking at 37°C for 30 min under 95% O_2, 5% CO_2 in a Dubnoff incubator. Cells dispersed during the 30 min incubation were separated from the tissue using nylon mesh and the remaining tissue mechanically dispersed in fresh Krebs Ringer. The dispersion

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process was stopped when a translucent filtrate was obtained after separating the remaining tissue through nylon. The filtrates from the successive dispersions were collected and the cells pelleted by centrifugation at 200 × *g* for 5 min. The soft pellet was resuspended in Krebs-Ringer bicarbonate to ~2 × 10^6 cells/ml. The changes in membrane potential were obtained measuring the uptake of tetraphenylphosphonium under control and experimental conditions.

Cells were incubated in Krebs-Ringer bicarbonate in the presence of 10 μM [^{3}H]TPP (New England Nuclear, Boston MA) 0.1 Ci/mmol and aliquots were transferred at selected times to 0.5 ml polyethylene microfuge tubes that contained 0.25 ml of a mixture of dibutylphtalate and vaseline (5:1; v/v) and immediately centrifuged in a Beckman II Microfuge for 45 s. The tip of the tube was cut above the cell pellet and placed in scintillation vials that contained 2 ml 15% SDS. The vials were shaken overnight and the radioactivity measured in a Tracor Analytic Dalton 300 liquid scintillation counter.

The membrane potential was calculated as in [9] according to the Nernst equation:

$$\Delta\Psi = 59 \log \frac{(TPP)^e \text{ cell associated}}{(TPP)^c \text{ cell associated}}$$

in which $(TPP)^e$ cell associated and $(TPP)^c$ cell associated are the amount of tetraphenylphosphonium cation accumulated in the cells in experimental and control conditions, respectively.

Aldosterone production was measured in the incubation medium using a direct radioimmunoassay as in [7].

3. RESULTS AND DISCUSSION

The plasma membrane in nearly all eukaryotic cells is depolarized when the external potassium concentration is increased. Attempts to correlate potassium-induced changes in membrane potential with the production of aldosterone by zona glomerulosa cells have been made [3]. However, the minimal [K^+] necessary to observe a change in membrane potential was 20 mM, while much smaller changes in K^+ (e.g., 6 mM) were able to induce significant increases in aldosterone production [4].

Using the distribution of the lipophilic cation

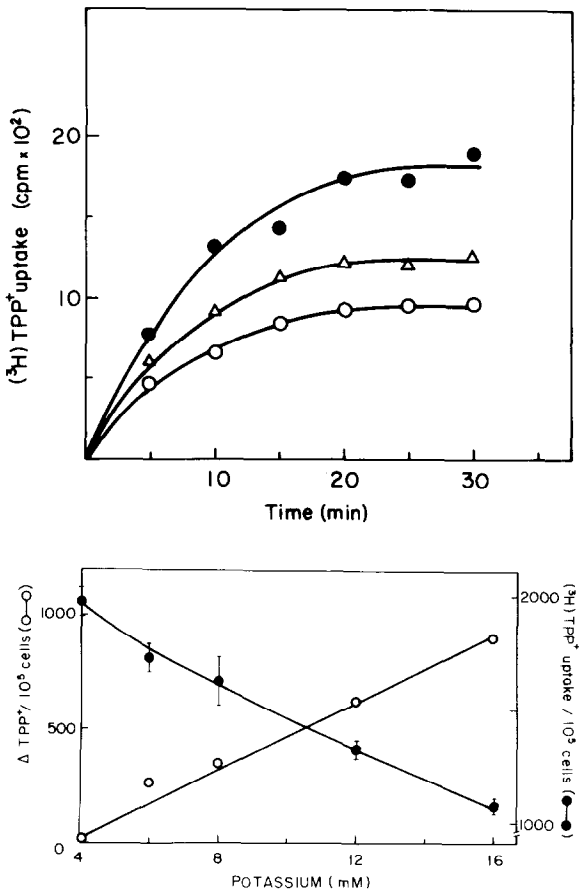

Fig. 1. (a) Time-course of TPP^+ accumulation by glomerulosa cells at different [K^+]. Experimental conditions were as described in section 2: control (4 mM K^+); 8 mM K^+ (△); 12 mM K^+ (○). Each point is the mean of quadruplicate samples from one experiment. (b) Effects of extracellular [K^+] on TPP^+ accumulation by glomerulosa cells. Cells were incubated with 10 μM [^{3}H]TPP in the presence of 4–16 mM K^+ for 30 min. The data are presented as the [^{3}H]TPP uptake ± SE (●) and as the difference between [^{3}H]TPP^+ uptake in the control and the experimental conditions (○).

tetraphenylphosphonium (TPP) across the cell membrane for estimating membrane potentials, it is now possible to measure accurately membrane potential changes during cell activation. Fig. 1a shows the time course of the cellular accumulation of [^{3}H]TPP at 4.8 and 12 mM potassium in the incubation medium. It can be seen that the rate of [^{3}H]TPP uptake clearly depends on [K^+]. It is highest at 4 mM K^+ and decreases, as expected,

with increasing external K^+. The maximal accumulation of [^{3}H]TPP at any given [K^+] was reached at 20 min incubation. Fig. 1b depicts [^{3}H]TPP uptake at 30 min when 4, 6, 8, 12 and 16 mM potassium were present in the incubation medium. The data are replotted in the same figure as the difference between the uptake of [^{3}H]TPP at 4 mM potassium and the uptake when 6, 8, 12 and 16 mM potassium were present. This magnitude of change in membrane potential is seen in other cell systems only when the change in external potassium exceeds 50 mM [3,8,11].

Once an equilibrium value for [^{3}H]TPP uptake is reached at 4 mM K^+, addition of extra K^+, sufficient to raise [K^+] to 12 mM, leads to a prompt fall in membrane potential (fig. 2).

Because the steroidogenic response in these cells is highly dependent upon external calcium, we studied the role of calcium in the depolarizing effect of potassium. Table 1 and fig. 3 depict the depolarizing effect of 12 mM K^+ at different [Ca^{2+}]. It is important to note that at a Ca^{2+}/EGTA ratio

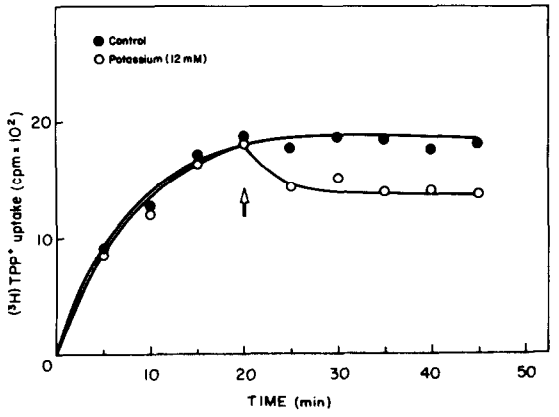

Fig. 2. Change in TPP^+ accumulation by glomerulosa cells induced by 12 mM K^+ added at equilibrium. Each point represents the mean of quadruplicate samples from one experiment.

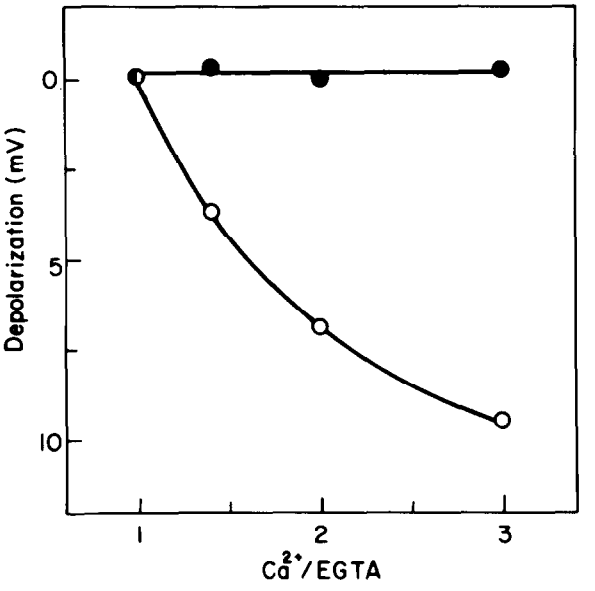

Fig. 3. Effects of extracellular Ca^{2+} on membrane potential changes of glomerulosa cells. The change in membrane potential was calculated at 4 mM (●) and 12 mM K^+ (○) using a ratio of Ca^{2+}/EGTA = 1 as control, as in section 2.

Table 1

Influence of calcium on tetraphenylphosphonium distribution and aldosterone production at different potassium concentrations

Ca^{2+}/EGTA	[^{3}H]TPP uptake (cpm . 10^5 $cell^{-1}$. 30 min^{-1})		Aldosterone (ng . 10^6 $cells^{-1}$. 2 h^{-1})	
	4 mEq . K^{-1} . L^{-1}	12 mEq . K^{-1} . L^{-1}	4 mEq . K^{-1} . L^{-1}	12 mEq . K^{-1} . L^{-1}
1	1695 ± 62	1304 ± 37	1.58 ± 0.25	1.61 ± 0.09
1.4	1731 ± 57	1135 ± 46[a]	2.13 ± 0.22	3.84 ± 0.16
2	1699 ± 43	1005 ± 109[b]	2.66 ± 0.26	4.14 ± 0.29
3	1735 ± 30	925 ± 108[b]	2.69 ± 0.16	3.15 ± 0.37

[a] $P < 0.025$; [b] $P < 0.01$

[EGTA] was maintained constant while the [Ca^{2+}] varied from 1.25–3.75 mM

of 1, there is a significant depolarization caused by 12 mM potassium, but at this [Ca^{2+}], the aldosterone production rate does not increase (table 1). No calcium effect on [^{3}H]TPP uptake was seen when the cells were incubated at 4 mM K^+, but a significant calcium effect was found at 12 mM potassium. The key point of these observations is the fact that at a [Ca^{2+}] of (Ca^{2+}/EGTA) = 1, an increase from 4–12 mM K^+ leads to a depolarization of the membrane without an increase in aldosterone production. At higher [Ca^{2+}], a greater membrane depolarization occurs as a function of [K^+].

When fasciculata cells are stimulated by ACTH the membrane potential decreases in a dose-dependent manner [2]. Because steroid production in this cell type is not activated by changes in external potassium, we have examined the effect of the same range of [K^+] on membrane potentials of both fasciculata and glomerulosa cells. Fig. 4 shows the effect of 4, 6, 8, 12 and 16 mM potassium on membrane potential in both cell types. It was found that, while an increased [K^+] had marked effect in glomerulosa cells, the effect on fasciculata cells was only very slight.

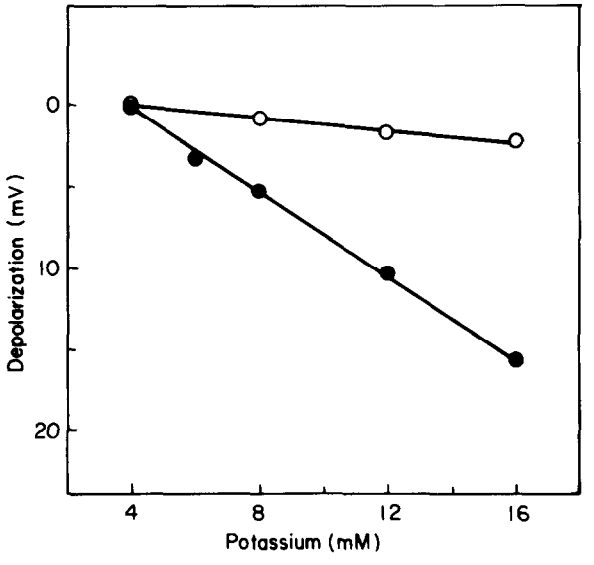

Fig. 4. Effect of extracellular K^+ on membrane potential changes of bovine glomerulosa (●) and fasciculata (○) cells. The data represent the mean of two experiments performed in different days.

The above evidence clearly shows that small changes in [K^+] produce a decrease in membrane potential of zona glomerulosa cells, and that such a decrease is calcium-dependent.

The data raise the interesting possibility that an increase in extracellular K^+ activates a specific Ca^{2+} channel in the plasma membrane of these cells, and that resulting influx of Ca^{2+} is responsible for an additional inward calcium current.

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