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MEASUREMENT OF INTRACELLULAR POTASSIUM IN DISPERSED ADRENAL CORTICAL CELLS

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SUMMARY

Micro-methods for the measurement of water spaces and intracellular K^+ were applied to dispersed rat adrenal cortical cells in order to follow changes in intracellular K^+ after alteration of steroidogenesis *in vitro*. Using unpurified zona glomerulosa cells, both serotonin and high [K^+] medium (8·4 mmol/l) increased corticosterone output but only the latter stimulus increased intracellular K^+. Zona glomerulosa cells purified by unit gravity sedimentation showed no changes in intracellular K^+ with serotonin or angiotensin but both stimulated corticosterone output.

Conversely, ouabain at 10^{-5} mol/l depressed intracellular K^+ of glomerulosa cells but did not alter basal or serotonin stimulated corticosterone output. A higher dose of ouabain, 5×10^{-4} mol/l, further depressed intracellular K^+ and inhibited steroidogenesis in both the glomerulosa and fasciculata cells.

Glomerulosa and fasciculata cells showed the same pattern of changes in intracellular K^+ with all of the agents tested. A variety of situations were observed where changes in intracellular K^+ of glomerulosa cells did not parallel those in steroidogenesis.

These measurements therefore do not support the hypothesis that intracellular K^+ is the sole mechanism regulating steroidogenesis in these experiments.

INTRODUCTION

Intracellular potassium has been postulated to be a final common mechanism whereby a variety of diverse agents might act to alter aldosterone production from the adrenal zona glomerulosa cell [1, 2]. However, the evidence to support this view is largely indirect [3] or based on potassium measurements of whole adrenal cortical tissue [1] where the potassium content of the fasciculata tissue would greatly predominate and the contribution of extracellular potassium was unknown. The use of dispersed cell suspensions [4, 5] offers the possibility of overcoming some of these difficulties since reproducible samples of known cell type can be prepared and the contribution of extracellular potassium accurately measured to enable valid measurements of intracellular potassium.

The present study was undertaken to investigate the changes of intracellular potassium accompanying altered steroidogenesis from isolated zone glomerulosa and fasciculata-reticularis cells during *in vitro* incubation.

EXPERIMENTAL

A detailed description of the methods will be published elsewhere [6]. Briefly, adrenal capsular cells (predominantly zona glomerulosa) or zona fasciculata-reticularis cells were prepared by collagenase dispersion of adrenal capsular strippings [4] or decapsulated rat adrenal glands [5]. In some experiments, the zona glomerulosa cells were purified by unit gravity sedimentation [7]. The cells were incubated with Krebs–Ringer Bicarbonate medium containing glucose and albumin in a total volume of 140 μl for 90 min at 37°C. Six replicate incubation mixtures were prepared for each experimental manipulation. In four of the replicates, the water space markers—tritiated water and [^{14}C]-hydroxymethylinulin—were added and incubation continued for a further 30 min to allow time for equilibration. The two other tubes were used to determine corticosterone output by radioimmunoassay [4]. Agents added to the glomerulosa incubations to alter steroidogenesis included serotonin, 1×10^{-4} mol/l, high [K^+] medium, 8·4 mmol/l, and angiotensin II, 800 μg/ml.

Ouabain was used with or without the stimulating agents at concentrations of 10^{-5} mol/l and 5×10^{-4} mol/l. With fasciculata cells ACTH, 10 mU/ml and cyclic AMP, 10 mmol/l were also used to stimulate steroidogenesis. After incubation, the suspensions containing the isotopic water space markers were aspirated into siliconized glass capillary tubes and centrifuged to separate the cells and media. The bulk of medium was aspirated from above and the cell pellet expelled and lysed in distilled water and perchloric acid. The ^{14}C and ^{3}H-radioactivity of deproteinized extracts of both the cells and media was measured by liquid scintillation counting and the extracellular water space ([^{14}C]-hydroxymethylinulin) and total water space ([^{3}H]-water) of the cell pellet calculated. After diluting the samples to a constant sodium concentration, potassium was measured in extracts of both the media and cells by flame spectrophotometry.

The total K^+ content of the cell pellet so obtained was corrected for the contribution of K^+ from

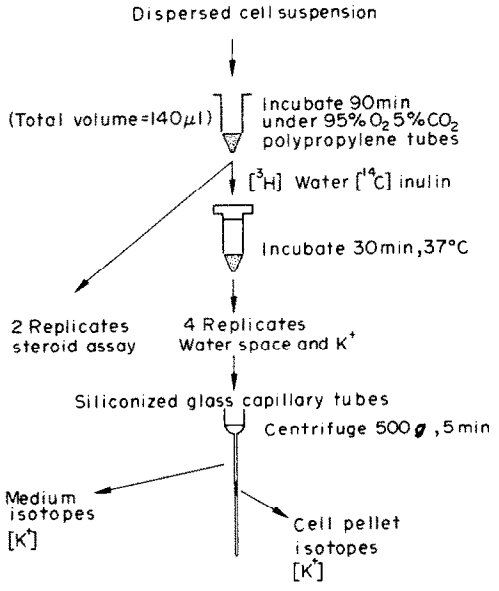

Fig. 1. Flow chart of the methods used to measure water spaces, intracellular K^+ and corticosterone output of dispersed adrenal cortical cells.

trapped medium in order to calculate intracellular potassium content. Figure 1 is a flow chart which summarizes the methods used.

RESULTS

A detailed report of experiments undertaken in order to establish the validity of the methods and of results with adrenal cortical cells will be published elsewhere [6, 8]. Table 1 summarizes experiments performed in an attempt to validate the methods.

1. *Effects of serotonin and high* $[K^+]$ *medium*

(*a*) *Unpurified zona glomerulosa cells.* As shown in Table 2, both serotonin and $[K^+]$ 8·4 mmol/l medium significantly increased corticosterone output. However, intracellular K^+ did not alter with serotonin but was elevated after high $[K^+]$ medium.

Since it might be argued that the presence of contaminating fasciculata cells (up to 5%) might obscure significant changes in the glomerulosa population, these experiments were repeated using zona glomerulosa cells purified by unit gravity sedimentation which are virtually free from fasciculata cells [7].

(*b*) *Purified glomerulosa cells.* An essentially similar pattern was observed using purified cells (Table 3). Again both stimuli increased corticosterone output but intracellular K^+ was unaltered by serotonin and was elevated after high $[K^+]$ medium.

2. *Effects of angiotensin II on purified zona glomerulosa cells*

Purified cells were necessary to investigate the action of angiotensin II since this agent may stimulate steroidogenesis in both the fasciculata and glomerulosa. As shown in Table 4, both angiotensin II and high $[K^+]$ medium increased corticosterone output but angiotensin II, unlike high $[K^+]$ medium, had no consistent effect on intracellular K^+.

Table 1. Validation of methods

A. *Water space measurements*
1. Correlation of ECW measured with [^{14}C]-Hydroxymethyl inulin and total Na content.
2. Correlation of ICW and number cells.
3. Value of ICW for human erythrocytes: 70% of total cell volume.

B. *Potassium measurements*
1. Correlation of $[K^+]$ measurements in cell extracts by flame emission spectrophotometry on two independent instruments.
2. Correlation of $[K^+]$ measurements in cell extracts by flame emission and atomic absorption spectrophotometry.
3. Recovery of K^+ added to cell extracts (initial mean content 10·4 nmol).
   (a) 1·5 nmol K^+: 84 ± 22% (S.E.M.), $n = 9$
   (b) 10 nmol K^+: 100·8 ± 2·2%, $n = 7$

C. *Both methods*
1. Value of $[K^+]$ for human erythrocytes: 140·8 ± 4 mmol/l
2. Correlation of intracellular K^+ of glomerulosa and number of cells.

Table 2. Effects of serotonin and high $[K^+]$ medium on unpurified zona glomerulosa cells

	Control $[K^+]$ 3·6 mmol/l	Serotonin, 10^{-4} mol/l $[K^+]$ 3·6 mmol/l	$[K^+]$ 8·4 mmol/l
Intracellular K^+, nmol/adrenal equivalent	20·0 ± 0·9 $n = 11$	19·8 ± 1·0 $n = 8$	23·5 ± 0·9 $n = 8$
P-value for comparison with control		$P > 0·6$	$P < 0·01$
Corticosterone output ng/rat equivalent hr	17·4 ± 1·5 $n = 10$	27·9 ± 2·4 $n = 8$	35·1 ± 3·0 $n = 7$
P value for comparison with control		$P < 0·001$	$P < 0·001$

Values are mean ± S.E.M.
Paired students *t*-test.

Table 3. Effect of serotonin and high $[K^+]$ medium on glomerulosa cells purified by unit gravity sedimentation

	Control $[K^+]$ 3·6 mmol/l	Serotonin 1×10^{-4} mol/l	$[K^+]$ 8·4 mmol/l
Intracellular K^+ (nmol)			
Experiment 1	10·7 ± 0·7	11·2 ± 0·5	13·5 ± 1·0
Experiment 2	13·0 ± 1·0	11·7 ± 0·4	15·0 ± 1·1
Corticosterone output (ng/rat equiv./hr)			
Experiment 1	10·9 ± 0·3	27·3 ± 0·2	35·5 ± 0·7
Experiment 2	8·6 ± 0·5	18·0 ± 0·1	25·9 ± 0·9

Means ± S.E.M. for measurements from single experiments.
Experiment 1 used $1{\cdot}36 \times 10^5$ cells/incubation and experiment 2 used $1{\cdot}12 \times 10^5$ cells.

Table 4. Effect of angiotensin II on purified zona glomerulosa cells

	Control $[K^+]$ 3·6 mmol/l	Angiotensin II 800 μg/ml	$[K^+]$ 8·4 mmol/l
Intracellular K^+ (nmol)	14·0 ± 0·8	14·9 ± 1·2	17·2 ± 0·8
P value for comparison with control		$P > 0{\cdot}6$	$P < 0{\cdot}005$
Corticosterone output (ng/hr)	9·5 ± 1·3	13·3 ± 0·6	17·1 ± 0·6
P value for comparison with control		$P < 0{\cdot}05$	$P < 0{\cdot}05$

Values are means ± S.E.M. for four experiments.
P-values obtained by analysis of variance.
Mean member of cells/incubation is $1{\cdot}25 \times 10^5$.

3. *Ouabain dose–response curves*

The effect of a range of concentrations of ouabain on corticosterone output and intracellular K^+ using unpurified zona glomerulosa cells is shown in Fig. 2. Low concentrations of ouabain caused a small increase in corticosterone output and this effect was reversed at higher doses. At an intermediate concentration of 10^{-5} mol/l ouabain, no net effect on steroidogenesis was observed. Intracellular K^+ showed a progressive fall with increasing concentration of ouabain. These dose response curves reveal at least two regions of dissociation between changes in intracellular K^+ and corticosterone output. Two doses of ouabain were selected for further investigation: 10^{-5} mol/l which appeared to depress intracellular K^+ with no net effect on corticosterone output; and a 50 times higher concentration, 5×10^{-4} mol/l, which depressed both parameters.

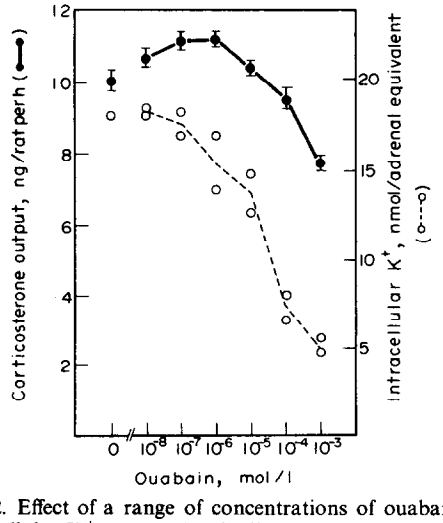

Fig. 2. Effect of a range of concentrations of ouabain on intracellular K^+ content (○, duplicates) and corticosterone output (●, mean ± S.E.M.) of purified zona glomerulosa cells.

4. *Effect of ouabain at* 10^{-5} *mol/l on unpurified glomerulosa cells*

As shown in Table 5, this dose of ouabain significantly depressed intracellular K^+ of cells incubated in $[K^+]$ 3·6 mmol/l medium with or without serotonin. However, despite these falls in intracellular K^+, basal ($[K^+]$ 3·6 mmol/l) and serotonin stimulated corticosterone outputs were not significantly altered. This result confirms the dissociation of changes in intracellular K^+ and steroid output shown in Fig. 2 and extends it to the case of serotonin stimulated cells. In the presence of $[K^+]$ 8·4 mmol/l, ouabain at the low dose did not change intracellular K^+ or corticosterone output which suggests a direct antagonism of high $[K^+]$ on the effect of ouabain.

This set of experiments also confirms the earlier finding in an independent set of experiments (Table 2) that serotonin does not increase intracellular K^+.

5. *Effect of ouabain at* 5×10^{-4} *mol/l on unpurified glomerulosa cells*

This high dose of the glycoside produced profound falls in intracellular K^+ whether used alone, with serotonin or with high $[K^+]$ medium (Table 6). There was a fall in corticosterone output produced by ouabain both in the basal state ($[K^+]$ 3·6) or after stimulation with K^+ or serotonin. In cells incubated with high $[K^+]$ medium and ouabain intracellular K^+ was still profoundly depressed although corticosterone output had returned to control values. The data for incubations without ouabain in Table 6 were presented separately in Table 2 for clarity.

Table 5. Effects of ouabain 10^{-5} mol/l on unpurified zona glomerulosa cells

	Control $[K^+]$ 3·6 mmol/l	$[K^+]$ 3·6 + ouabain 10^{-5} mol/l	Serotonin 10^{-4} mol/l	Serotonin 10^{-4} mol/l + ouabain 10^{-5}mol/l	$[K^+]$ 8·4 mmol/l	$[K^+]$ 8·4 mmol/l + ouabain 10^{-5} mol/l
Intracellular K^+ (nmol/adrenal equivalent)	15·6 ± 0·7	13·2 ± 0·9	16·5 ± 0·8	13·8 ± 0·7	18·8 ± 0·6	20·5 ± 1·5
P-value for comparison with control			$P > 0·3$		$P < 0·001$	
P-value for effect of ouabain		$P < 0·02$		$P < 0·005$		$P > 0·1$
Corticosterone output (ng/rat equiv./hr)	15·4 ± 1·6	14·6 ± 3·3	24·1 ± 2·9	20·6 ± 2·4	28·5 ± 3·8	31·3 ± 6·0
P-value for comparison with control			$P < 0·02$		$P < 0·01$	
P-value for effect of ouabain		$P > 0·4$		$P > 0·1$		$P > 0·5$

Values are means ± S.E.M. of five experiments.

It is important to note that the high dose of ouabain also inhibited steroidogenesis in the fasciculata (Fig. 3) indicating that the effects of ouabain at this concentration are not specific for glomerulosa.

Intracellular water space was not significantly affected by any of the previously described manipulations indicating that the changes in intracellular K^+ content which have been presented probably reflect changes in intracellular K^+ concentration rather than swelling or shrinkage of the cells.

6. *Effects of serotonin,* $[K^+]$ 8·4 *mmol/l,* ACTH *and cyclic* AMP *on fasciculata-reticularis cells*

Serotonin and high $[K^+]$ medium had no effect on corticosterone output of fasciculata cells (Fig. 3) as is well known. However, their effects on intracellular K^+ were similar to those found using glomerulosa cells: serotonin had no consistent effect but in high $[K^+]$ medium intracellular K^+ was elevated by approximately 12% above the controls. ACTH and cyclic AMP had no effect on intracellular K^+. Corticosterone output with ACTH and cyclic AMP was measured under two sets of incubation conditions: in micro-incubations identical to those used for the measurement of intracellular K^+, the maximal steroid output was less than usually observed [5]. For this reason paired incubations were also performed in a total volume of 2·0 ml where the expected increase of corticosterone output of approximately 100-fold was observed.

7. *Effects of ouabain* 5×10^{-4} *mol/l on fasciculata-reticularis cells*

Ouabain at this high concentration depressed intracellular K^+ to approximately 50% of the control values whether used alone or with ACTH or cyclic

Table 6. Effects of ouabain 5×10^{-4} mol/l on unpurified zona glomerulosa cells

	Control, $[K^+]$ 3·6 mmol/l	$[K^+]$ 3·6 + ouabain 5×10^{-4}mol/l	Serotonin 10^{-4} mol/l	Serotonin 10^{-4} mol/l + ouabain 5×10^{-4} mol/l	$[K^+]$ 8·4 mmol/l	$[K^+]$ 8·4 + ouabain 5×10^{-4} mol/l
Intracellular K^+ (nmol/adrenal equivalent)	20·0 ± 0·9 $n = 11$	6·1 ± 0·5 $n = 10$	19·8 ± 1·0 $n = 8$	5·3 ± 1·1 $n = 3$	23·5 ± 0·9 $n = 8$	8·9 ± 0·8 $n = 4$
P value for comparison with control			$P > 0·6$		$P < 0·001$	
P value for effect of ouabain		$P < 0·001$		$P < 0·01$		$P < 0·005$
Corticosterone output (ng/rat equiv./hr)	17·4 ± 1·5 $n = 10$	12·8 ± 1·3 $n = 10$	27·9 ± 2·4 $n = 8$	8·2 ± 1·2 $n = 3$	35·1 ± 3·0 $n = 7$	18·3 ± 4·5 $n = 4$
P value for comparison with control			$P < 0·001$		$P < 0·001$	
P value for effect of ouabain		$P < 0·005$		$P < 0·05$		$P < 0·001$

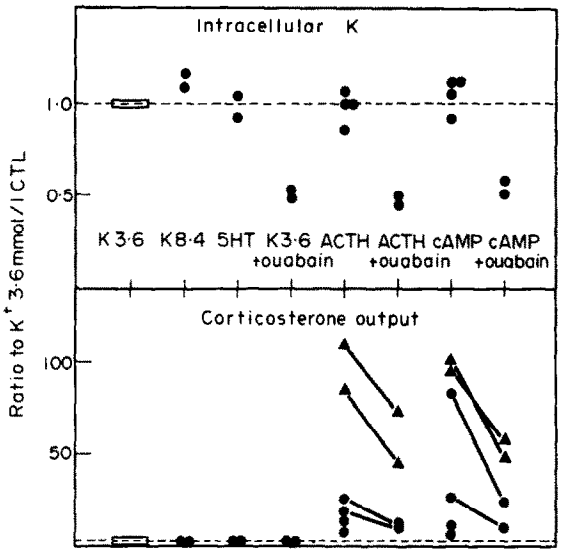

Fig. 3. Intracellular K^+ content and corticosterone output from dispersed zona fasciculata cells. Both results are expressed as a ratio of the values obtained in paired control incubations in [K^+] 3·6 mmol/l medium (open rectangles, horizontal broken lines). Stimuli used were: none (K 3·6); [K^+] 8·4 mmol/l (K 8·4); serotonin, 10^{-4} mol/l (5HT); ACTH 10 mU/ml (ACTH); cyclic AMP, 10 mmol/l (cAMP) with or without ouabain, 5×10^{-4} mol/l. Closed circles represent values obtained from incubations in total volume of 0·14 ml, triangles represent corticosterone outputs from incubations of same number of cells in total volume of 2·0 ml.

AMP (Fig. 3). Stimulated corticosterone output was depressed by ouabain at this concentration under both sets of incubation conditions (Fig. 3).

8. Effects of ouabain 10^{-5} mol/l on fasciculata-reticularis cells

The lower dose of ouabain did not affect basal or stimulated corticosterone output from fasciculata (Table 7) as was the case for glomerulosa (Table 5). This concentration of the glycoside did, however, depress intracellular K^+ of fasciculata cells.

DISCUSSION

These results show a variety of situations where changes of intracellular K^+ of glomerulosa cells do not parallel those of corticosterone output. Thus, serotonin did not alter intracellular K^+ in two sets of experiments although it was effective in stimulating corticosterone output. Similarly, angiotensin II did not consistently alter intracellular K^+ under conditions where it stimulated steroid output of glomerulosa cells. The experiments with angiotensin II may need to be interpreted with caution because the dose necessary to produce maximal steroid output was considerably higher than that reported by other groups using similar *in vitro* systems [9] and the maximum steroid output was slightly less than that produced by high [K^+] in this series of experiments.

High [K^+] medium was the only condition which increased intracellular K^+ in the glomerulosa cells in parallel with increased steroidogenesis. However, a similar effect was also observed with fasciculata cells whose steroid output is not responsive to external [K^+] [5], (Fig. 3).

When intracellular K^+ of glomerulosa cells was lowered by the use of ouabain at 10^{-5} mol/l, neither basal or serotonin-stimulated corticosterone output was altered. A higher concentration of ouabain, 5×10^{-4} mol/l, did impair steroidogenesis from the glomerulosa but this effect also occurred with fasciculata cells which casts some doubt on the specificity of the action of ouabain at this concentration and could represent a toxic effect of the drug. These experiments did not reveal any difference in the sensitivity of glomerulosa and fasciculata cells to the action of ouabain, in contrast to other workers using intact tissues [3, 10, 11]. This difference may be partly explained by differences of diffusion of the active drug to the fasciculata when using intact tissues.

In general, the similarities between glomerulosa and fasciculata cells in their changes in intracellular K^+ with all of the agents tested is remarkable. This similarity of the two cell types would be surprising

Table 7. Effects of ouabain (10^{-5} mol/l) on fasciculata-reticularis cells

	Control [K^+] 3·6 mmol/l	[K^+] 3·6 + ouabain 10^{-5} mol/l	ACTH 10 mU/ml	ACTH 10 mU/ml + ouabain 10^{-5} mol/l	ACTH 10 mU/ml + ouabain 5×10^{-4} mol/l
Experiment 1					
Corticosterone output (ng/rat equiv./hr)	6·6	7·8	988 (×150)	1187 (×180)	243 (×37)
Experiment 2					
Corticosterone output (ng/rat equiv./hr)	3·4	3·7	487 (×143)	494 (×145)	239 (×70)
Intracellular K^+ (nmol/adrenal equivalent)	31·7 ± 0·4	26·9 ± 0·1	31·6 ± 0·6	27·8 ± 0·5	13·2 ± 0·6

Mean ± S.E.M. of four determinations.
Values in brackets are ratios of corticosterone outputs stimulated/control.

Table 8. Mean values of intracellular K^+ concentrations in terms of intracellular water

Human erythrocytes
140·8 ± 4·0 mmol/l, $n = 10$
Fasciculata cells
125·9 ± 12·6 mmol/l, $n = 4$
Unpurified zona glomerulosa (3–5% Fasciculata)
103·4 ± 6·0 mmol/l, $n = 11$
Purified zona glomerulosa
Purified zona glomerulosa
Experiment 1: 143 ± 21 mmol/l, $n = 4$
Experiment 2: 77 ± 5 mmol/l, $n = 4$
Experiment 3: 68 ± 6 mmol/l, $n = 4$

Values are mean ± S.E.M.

if, as has been suggested [3], intracellular K^+ has a special role in the glomerulosa cell only.

Intracellular K^+ results have been expressed as contents for a given number of cells where the term "adrenal equivalent" has been used as a convenient working unit to represent the yield of cells from one adrenal (approximately $1·7 \times 10^5$ for glomerulosa and $1·1 \times 10^5$ for fasciculata). Intracellular K^+ may also be expressed in terms of the intracellular water space although this was not done for individual experiments because the variability of the intracellular water measurements was too great to make meaningful comparisons when using cell volumes of less than 0·5 μl. However, the values of intracellular K^+ concentration obtained from pooled data from several experiments (Table 8) may allow useful comparisons. The value of intracellular K^+ concentration obtained for purified zona glomerulosa cells in two experiments seems low in comparison with other published data for mammalian cells[12]. This does not appear to be due to loss of K^+ from the cells during the fractionation procedure because, although the unfractionated cells have a higher mean intracellular K^+ concentration, this preparation is contaminated by up to 5% fasciculata cells which because of their greater relative volume may represent up to 40% of the total cell volume; calculations to correct for the fasciculata contamination give a value for glomerulosa cells in the unpurified preparation which very close to that found for the purified glomerulosa cells. In an indirect attempt to test for loss of K^+ during fractionation, fasciculata cells were purified by unit gravity sedimentation. It was found (8) that intracellular K^+ concentration of these cells was not altered by the procedure which may suggest that the same is true for the glomerulosa. Whatever the explanation for the observed value of intracellular K^+ concentration of the glomerulosa cells, it does not appear to detract from the validity of the observed changes in intracellular K^+ contents between different experimental conditions.

The observed dissociations of changes in intracellular K^+ from those in steroidogenesis of glomerulosa cells is not compatible with the hypothesis [2] that intracellular K^+ is the major factor regulating steroid production in these experiments.

This does not mean that intracellular K^+ has no role in the control of aldosterone production but merely that it cannot be the sole of major factor. It seems more likely that control of steroidogenesis in the glomerulosa is exerted at several different levels, perhaps involving different mechanisms [13]. Investigation of multiple factors (e.g. cyclic AMP, Ca^{2+}, intracellular K^+) may be needed to elucidate this control system rather than consideration of one mechanism alone.

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