Aldosterone secretion, measurements of membrane potential and intracellular potassium activity in the isolated adrenal zone glomerulosa

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Cell membrane potential and intracellular potassium activity (microelectrodes filled with ion-sensitive liquid ion exchanger) were measured in the zona glomerulosa of superfused hemi-adrenals of rats kept on different diets. Simultaneously, samples of the superfusate were collected and analyzed by radioimmunoassay for aldosterone content. Cell membrane potential and intracellular potassium activity were not influenced by high sodium, low sodium or high potassium diet. However, aldosterone secretion significantly changed. These results suggest that membrane potential and intracellular potassium activity per se may not be linked to changes in aldosterone secretion.

Aldosterone secretion *Membrane potential* *Intracellular potassium activity* *Adrenal zona glomerulosa* *Changes in diet*

1. INTRODUCTION

The main components of the control system of aldosterone secretion have been well established. Alterations in aldosterone secretion in vivo and in vitro have been shown to follow a variety of stimuli such as adrenocorticotropin, sodium deprivation and potassium loading and, most important, infusion of angiotensin II [1–4]. Recently, interest has been focussed on the cellular mechanisms by which aldosterone secretion is exerted. It has been postulated that intracellular potassium and/or changes in membrane potential could be the initial common steps in the effect of all stimuli of adrenal function. The rationale for this hypothesis is: (a) the fact that in man as well as in animal studies, infusion of minute amounts of potassium resulted in aldosterone stimulation even without changing extracellular potassium concentration [5,6]; and (b) that in a number of hormonally active cell systems changes in membrane potential are involved in the process of stimulus-secretion coupling [7]. In adrenal tissues from rabbits, rats and cats no direct relation between corticosteroid production and membrane potential could be demonstrated [8–10]. On the other hand, from recordings of membrane potentials in the zona glomerulosa of cats it was suggested that membrane potential is important in regulating the stimulus-secretion coupling of aldosterone [11]. Contradictory results have also been reported when attempts have been made to relate adrenal corticosteroid activity to intracellular potassium content of the adrenal cortex. Depending on the methods used a correlation between intracellular potassium concentration and aldosterone secretion [12–14] and no such a correlation have been described [15–19]. Thus, we have measured simultaneously cell membrane potential and, for the first time, intracellular potassium activity in the adrenal zona glomerulosa of rats kept in conditions known to change

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aldosterone secretion. The results obtained show no evidence of a direct relationship between intracellular potassium activity and aldosterone secretion. The results obtained show no evidence of a direct relationship between intracellular potassium activity and aldosterone secretion.

2. MATERIALS AND METHODS

Adrenal glands were obtained from male Sprague–Dawley rats (140–160 g) kept on different diets. One half of the adrenals was used for micropuncture, the other one for measurement of aldosterone secretion. The animals were anesthetized with halothane/pentobarbital and the adrenals excised, fixed in 3% agar/Ringer and bisected with a razor blade. The hemi-adrenals were incubated in 1 ml oxygenated Ringer solution (containing in mmol/l; 123 NaCl; 4 KCl; 1 KH_2PO_4; 0.9 $MgSO_4$; 1.7 $CaCl_2$; 28 $NaHCO_3$; 5.1 glucose) at 21°C.

Microelectrodes were drawn from inner glass fibres containing borosilicate glass capillaries on a horizontal puller. When filled with 0.5 M KCl and immersed into control Ringer's solution, their resistance ranged from 30–50 $\times$ 10^6 Ω. Of the resulting pair of electrodes one was prepared for measuring the transmembranal potential difference, the other one was used for the potassium-dependent transmembranal potential difference as in [21]. In principle, the microelectrodes were silanized (2% silicone 1107, Dow Corning) and filled with a potassium liquid ion exchanger (Corning 477317). The space above the exchanger was filled with 0.5 M KCl. The potassium-sensitive microelectrodes exhibited the following properties: tip resistance of 5–30 $\times$ 10^9 Ω, slope of 57 mV per 10-fold change of potassium activity, mean selectivity coefficient for K/Na of 0.015. The intracellular potassium activity was calculated according to the equation given before [21]. Conventional and potassium-sensitive microelectrodes were advanced separately by a stepping hydraulic microdrive into the cell layer of the zona glomerulosa close to the capsule. Usually, 2–5 measurements were performed per hemi-adrenal.

During the electrophysiological experiments identical hemiadrenals were incubated in Ringer's solution and analysed for aldosterone secretion every hour. The incubation medium was collected and stored frozen for subsequent analysis. Aldosterone was extracted and assayed by a radioimmuno-assay technique [22]. 20 samples were assayed after paper chromatography of the dichloromethane extracts of incubation media. Since parallel assays without chromatography revealed negligible differences only chromatography was omitted in further assays. The antiserum for aldosterone and the procedure for its use was provided by the National Institute of Health, Bethesda. The cross-reaction of aldosterone with rat corticosterone was 0.3%.

The animals were fed on a control diet containing 0.2% Na^+ and 1% K^+. Experimental diets: high Na^+ (control diet, tap water replaced by 150 mM/l NaCl for 7 days), low Na^+ (0.03% Na^+ and 0.23% K^+ for 21 days), high K^+ (control diet, tap water replaced by 300 mM/l KCl, 15 mM/l NaCl and 250 mM/l glucose for 7 days).

Estimates of the variance are presented as standard error of the mean ($\pm$ SEM). Statistical significances are calculated with the Wilcoxon *U*-test.

3. RESULTS AND DISCUSSION

Intracellular potassium modulates a wide spectrum of cellular activities. New information on the importance of intracellular potassium has been obtained by intracellular recordings with microelectrodes filled with ion-sensitive liquid-resins [23]. With this method the biological relevant intracellular activity of ions can be measured. It has been demonstrated that activity measurements of intracellular potassium disagree with measurements based on chemical or electron microprobe approaches [23].

The membrane potential from cells of the zona glomerulosa (fig.1) was -60 mV and was constant over the experimental period of 3 h indicating a stable biological preparation. Similar data have been reported for newborn rats, rabbits and kittens [8–10], adult kittens [11], rats [24] and normal human adrenocortical tissue [25]. The mean intracellular potassium activity of 34 cells of the zona glomerulosa was 63.1 $\pm$ 8.2 mmol/l during the first hour of incubation. Again, intracellular potassium activity did not change significantly during the experimental period. Assuming an ac-

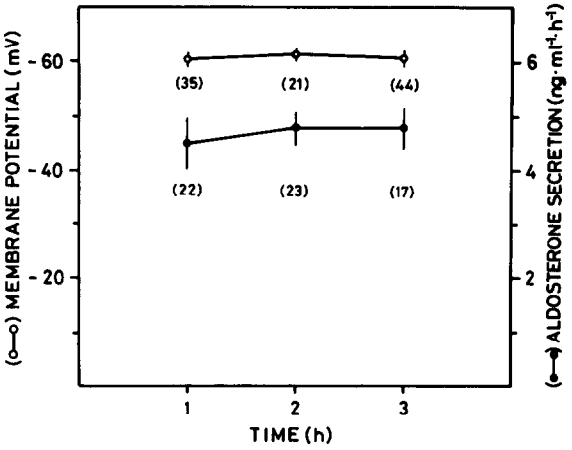

Fig.1. Time-course of intracellular membrane potential of zona glomerulosa cells and aldosterone secretion. Number of experiments is given in parenthesis. Mean values ± SEM.

tivity coefficient of 0.76, an intracellular activity of 63 mmol/l corresponds to a cellular concentration of 87 mmol/l, a value approximately a quarter to a third less than reported values based on chemical determinations [12–20]. However, the activity measurements reported here are within the range of activity measurements in epithelial tissues [23]. Fig.1 also summarizes the control data on aldosterone output. Aldosterone output per hemiadrenal was with 4.5 to 4.7 $ng \cdot ml^{-1} \cdot h^{-1}$ essentially unchanged during the 3-h control period.

Previous studies in man and in experimental animals have convincingly demonstrated that alterations in dietary sodium and potassium intake can influence aldosterone secretion [1–6]. Thus, this well established model has been used to correlate aldosterone production and the electrophysiological parameters. Since the membrane potential depends largely upon the extracellular potassium concentration [8–10] and changes in membrane potential may play a role in the stimulus-secretion coupling of aldosterone [11], the potassium concentration of the extracellular bathing medium for the isolated adrenals was kept constant under all experimental conditions. As expected, intracellular membrane potential (mean values during a 2-h observation period) of zona glomerulosa cells in adrenal taken from control animals did not differ from data obtained in high sodium, low sodium or high potassium animals

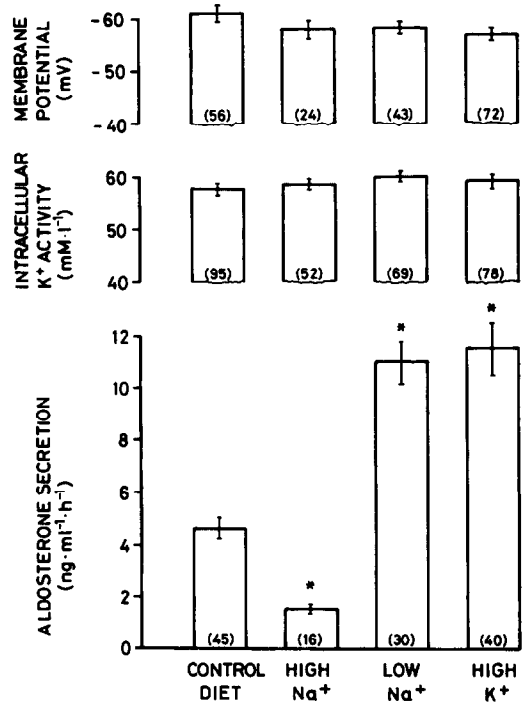

Fig.2. Effect of dietary changes on membrane potential, intracellular potassium activity and aldosterone secretion. * $P < 0.001$.

(fig.2). On the other hand, aldosterone secretion decreased ($P < 0.001$) in high sodium and increased ($P < 0.001$) in low sodium or high potassium animals. These results suggest that changes in membrane potential per se may not be linked to changes in corticosteroid secretion [8–11,25]. However, most important is the observation that intracellular potassium activity of zona glomerulosa cells was unchanged despite marked differences in aldosterone production. Since the method of determination of intracellular potassium activity is very sensitive [23] it is unlikely that changes in intracellular potassium of >5 mmol would not have been detected. Contradictory results have been reported on the possible correlation between intracellular potassium and corticosteroid secretion. All methods used so far to investigate this problem (flame photometry [12–14,20]; atomic absorption spectrophotometry [15,16,18]; electron microprobe X-ray microanalysis [17,19]) determined the potassium content or concentration in the total adrenal cortex or zona glomerulosa cells. From all these measurements

the potassium activity, which is the biological relevant intracellular parameter of potassium, could not be calculated. Thus, from our results it can be concluded that the known dietary stimuli to aldosterone secretion do not act by significantly changing intracellular potassium activity of zona glomerulosa cells. More complex mechanisms must be considered, including intracellular calcium as an important mediator in the steroidogenesis of the zona glomerulosa cells [26–28].

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