Role of adrenal renin-angiotensin system in the control of aldosterone secretion in sodium-restricted rats

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1Section of Anatomy, Department of Human Anatomy and Physiology, University of Padua, I-35121 Padua, Italy; and 2Department of Histology and Embryology, School of Medicine, PL-60781 Poznan, Poland

Mazzocchi, Giuseppina, Ludwig K. Malendowicz, Anna Markowska, Giovanna Albertin, and Gastone G. Nussdorfer. Role of adrenal renin-angiotensin system in the control of aldosterone secretion in sodium-restricted rats. Am J Physiol Endocrinol Metab 278: E1027–E1030, 2000.—This study examined the effect of the pharmacological manipulation of adrenal renin-angiotensin system (RAS) on aldosterone secretion from in situ perfused adrenals of rats kept on a normal diet and sodium restricted for 14 days. Neither the angiotensin-converting enzyme inhibitor captopril nor the nonselective angiotensin II receptor antagonist saralasin affected basal aldosterone output in normally fed rats. In contrast, they concentration dependently decreased aldosterone secretion in sodium-restricted animals, with maximal effective concentration ranging from 10–7 to 10–6 M. Captopril (10–6 M), saralasin (10–6 M), and losartan (10–7 M) counteracted aldosterone response to 10 mM K+ in sodium-restricted rats but not in normally fed animals. Collectively, these findings provide evidence that adrenal RAS plays a role in the regulation of aldosterone secretion, but only under conditions of prolonged stimulation of zona glomerulosa probably leading to overexpression of adrenal RAS.

MATERIALS AND METHODS

Reagents. The nonselective angiotensin II (ANG II) antagonist [Sar1,Val5,Ala8]-ANG II (saralasin) was obtained from Peninsula Laboratories (St. Helens, UK), the angiotensin-converting enzyme (ACE) inhibitor captopril (Capoten) was obtained from Squibb (Milan, Italy), and the AT1 receptor antagonist losartan (DuP753) was obtained from Merck Sharp & Dohme (Rome, Italy). Human serum albumin (HSA) was from Sigma Chemical (St. Louis, MO), and Medium 199 was from DIFCO (Detroit, MI). The sodium-deprived diet (0.01 meq Na+ kg–1) was purchased from Dr. Piccioni Laboratory (Milan, Italy), and RIA kits for aldosterone and corticosterone were from IRE Sorin (Vercelli, Italy) and Eurogenetix (Milan, Italy), respectively.

Animal treatment. Adult male Wistar rats (260 ± 30 g body wt) were purchased from Charles River (Como, Italy). A group of rats was sodium restricted (sodium-deprived diet and demineralized water as drinking fluid) for 14 days, and another group was maintained on a standard diet and tap water.

In situ adrenal perfusion. Sodium-deprived and normally fed rats were anesthetized with pentobarbital sodium, and the left adrenal gland was perfused in situ, as previously detailed (11). Perfusion medium was introduced via a cannula inserted in the celiac artery into an isolated segment of aorta from which the adrenal arteries arise. After flowing through the adrenal gland, medium was collected by a cannula inserted in the renal vein. Perfusion medium (tissue culture Medium 199, modified by dilution with KCl-free Krebs-Ringer bicarbonate to give a final K+ concentration of 3.9 mM and containing 0.2% glucose and 5 mg/ml HSA) was gassed with 95% air-5% CO2, maintained at 37°C, and delivered by peristaltic pump at a constant rate of 2 ml/10 min for 90 min. Perfusion pressure was monitored by a pressure transducer inserted in the arterial cannula and was found to average 30 ± 3 mmHg. After an initial equilibration period of 30 min, three 10-min samples were collected, and then the perfusion medium was substituted with one in which the chemicals to be tested were dissolved to the required concentration, and three more 10-min samples were collected. Two experiments were performed. In the first experiment, captopril, saralasin, or losartan was added to the perfusion medium in concentration ranging from 10–9 to 10–4 M. In the second experiment, perfusion medium contained 10–6 M captopril, 10–6 M saralasin, or 10–7 M losartan, and 10 mM K+ was added after the first three sample collections. This concentration of K+ is the maximally effective one in eliciting aldosterone secretion (6).

Hormone assays. Aldosterone and corticosterone were extracted from perfusion media and purified by HPLC (15). Their concentrations were measured by RIA with the following commercial kits: ALDOCTK2 (sensitivity, 5 pg/ml; intra-
and interassay variations, 5.8 and 7.5%, respectively), and CTRX-RIA (sensitivity, 50 pg/ml; intra- and interassay variations, 6.6 and 8.2, respectively).

Statistics. For each rat, the rate of hormone output was calculated as the average of the three 10-min collection periods before (control value) and after addition of the chemicals to the perfusion medium (experimental value). In the first experiment, for each experimental point five rats were perfused, control and experimental values were averaged, and their statistical significance was assessed. The data were graphically expressed as the means ± SE of the percent change from the group control value. Baseline (control) values from sodium-restricted and normally fed rats were averaged and expressed as picomoles per 10 minutes. In the second experiment, for each experimental point six rats were perfused, and data were expressed as the means ± SE of the average secretion rate (pmol/10 min) before and after 10 mM K[+] addition. The statistical comparison of the results was done by ANOVA, followed by Duncan's multiple range test. A value of P < 0.05 was considered significant.

RESULTS

Pilot experiments showed that, during the 60 min of sample collection, the basal rate of hormone production remained satisfactorily constant in both normally fed and sodium-restricted rats (Fig. 1). Sodium restriction raised aldosterone output by perfused rat adrenal (−80%), without affecting corticosterone release (Fig. 2).

Captopril, saralasin, or losartan did not evoke significant changes in aldosterone production by perfused adrenal of normally fed rats (Fig. 3). In contrast, they markedly lowered aldosterone output in sodium-restricted animals in a concentration-dependent manner with a maximally effective concentration ranging from 10⁻⁷ to 10⁻⁶ M (Fig. 3). Corticosterone production was not affected in either group of rats (data not shown).

K⁺ (10 mM) increased aldosterone production in both normally fed and sodium-restricted rats (three- and fivefold rise, respectively, Fig. 4). The presence of captopril (10⁻⁶ M), saralasin (10⁻⁶ M), or losartan (10⁻⁷ M) in the perfusion medium did not significantly affect either the basal rate of aldosterone production or aldosterone response to 10 mM K⁺ in normally fed animals (Fig. 4A). Conversely, in sodium-restricted rats, they lowered the basal rate of aldosterone secretion (by 48–62%) and aldosterone response to 10 mM K⁺ (from a five- to about a threefold rise, Fig. 4B).

DISCUSSION

Many lines of in vitro evidence (obtained by the use of capsular zona glomerulosa strips, adrenal slices, or zona glomerulosa cell cultures) suggest that adrenal RAS controls aldosterone secretion. ACE inhibitors were found to lower either basal (20) or K⁺- and ACTH-stimulated aldosterone output by rat zona glomerulosa (2, 14, 17, 18, 21) and basal aldosterone yield by cultured bovine (9) and human adrenal tissue (5), as well as by K⁺-stimulated aldosterone release by human adrenocortical NCI-H295 cell line (8). A selective AT₁ receptor antagonist was reported to block both basal and agonist-stimulated aldosterone secretion from cultured bovine zona glomerulosa cells (7) and K⁺-enhanced aldosterone production from the NCI-H295 cell line (8).

Our study, although partly confirming these observations, casts serious doubts on the possibility that adrenal RAS in vivo plays a major role in the regulation of aldosterone secretion under basal conditions. In fact, both ACE inhibition by captopril and ANG II receptor blockade by saralasin or losartan were ineffective on basal or K⁺-stimulated aldosterone secretion from in situ perfused adrenals in rats kept on a normal diet. This discrepancy stresses that marked differences occur in the adrenal cortex physiology between in vivo and in vitro conditions. In fact, when the structural integrity of the entire adrenal gland is preserved, several complex paracrine interactions between cortex...
and medulla are operative (for review, see Ref. 16), which conceivably may obscure under basal conditions the stimulatory effect of adrenal RAS on the zona glomerulosa secretory activity.

Conversely, our investigation strongly suggests that adrenal RAS may be involved in enhancing aldosterone secretion under pathophysiological conditions leading to prolonged stimulation of zona glomerulosa, such as those elicited by sodium intake restriction. Sodium restriction was found to increase adrenal renin mRNA

Fig. 3. Effect of captopril (A), saralasin (B), and losartan (C) on basal aldosterone output by in situ perfused adrenals of normally fed and sodium-restricted rats. Values are percentage changes (means ± SE; n = 5) from respective baseline value (B). *P < 0.05 and **P < 0.01 from baseline.

Fig. 4. Effects of captopril (10^{-6} M), saralasin (10^{-6} M), and losartan (10^{-7} M) on 10 mM K^+ stimulated aldosterone output by in situ perfused adrenals of normally fed (A) and sodium-restricted (B) rats. Aldosterone responses to 10 mM K^+ in absence of renin-angiotensin system inhibitors are also shown. Bars are means ± SE; n = 6. *P < 0.01 from respective control value; **P < 0.01 from the baseline control value.
and protein (1, 3). Hence, our observations could suggest that, only when overexpressed, adrenal RAS plays a role in enhancing aldosterone secretion. This contention appears to be in keeping with the fact that the transgenic rat strain TGR(mREN2)27, which overexpresses the murine Ren-2d gene in adrenal glands, secretes elevated amounts of aldosterone (for review, see Refs. 4, 10). Further studies are underway to see whether captopril and the ANG II receptor antagonists see Refs. 4, 10). Further studies are underway to see whether captopril and the ANG II receptor antagonists

**REFERENCES**


