Direct effect of ACTH on renin release in isolated perfused guinea-pig kidneys with adrenal glands

Hong-Yi Hu, Osamu Mokuda, Yoshikazu Sakamoto and Naokata Shimizu

Third Department of Internal Medicine, Teikyo University School of Medicine, Anesaki, Ichihara-City, Chiba 299-01, Japan


To investigate the direct effect of corticotropin (ACTH) on the renin-angiotensin-aldosterone system, isolated guinea-pig kidneys with adrenal glands were perfused with various doses of ACTH (0.1–1000 μg/l) and 0.3 mmol/l of dibutyryl cyclic AMP (cAMP) through each cannula inserted into the abdominal aorta and the inferior caval vein. Perfusion renin activity was increased in a dose-dependent manner by the addition of ACTH in a range of 0.1–1000 μg/l, and reached a plateau at 20 min with each dose. The perfusate cAMP level was dose-dependently increased with 10–1000 μg/l of ACTH. Perfusion renin activity was also markedly increased by the addition of dibutyryl cAMP. The same effects of ACTH on renin and cAMP secretions were observed in the kidney perfusion model from which the adrenal glands were excluded. Aldosterone secretion failed to respond to 0.1 μg/l of ACTH, and was increased by higher concentrations (1–1000 μg/l) in the same experiments. These results demonstrate that ACTH has a direct effect on renal renin release in a physiological concentration (0.1 μg/l), and that the action of ACTH is probably mediated by cAMP. The sensitivity of renin release to ACTH stimulation is no less than that of aldosterone secretion during ACTH infusion, so it is possible that ACTH is an important stimulator of the renin-angiotensin system.

Osamu Mokuda, Third Department of Internal Medicine, Teikyo University School of Medicine, Anesaki, Ichihara-City, Chiba 299-01, Japan

There are several reports indicating that pharmacological doses of ACTH increase renin release in rats (1–4). Marks et al. (1) observed that ACTH increased the juxtaglomerular cell granularity in the intact, hypophysectomized and adrenalectomized rat. Haugar-Klevene et al. (2–4) reported that ACTH caused renin release in both the intact and the aminoglutethimide-treated rat, and that the actions of ACTH on renin release were involved with an amplification of the sympathetic nerve system. In the studies on humans, however, the reports concerning the effects of ACTH on renin release are conflicting. The levels of plasma renin activity were unaltered in patients with Cushing’s disease, in spite of high levels of ACTH (5), and in normal subjects with ACTH infusion of 40 IU or 10 IU per day (6, 7). On the other hand, Oelkers et al. (8–10) showed transient rises in plasma renin activity as a result of the infusion of a physiological dose of ACTH in normals. Belkien et al. (11) studied the ACTH-infused patients with primary adrenocortical insufficiency and hypotuitarism, concluding that ACTH stimulated renal secretion of renin.

It is generally considered that renin release is influenced by systemic factors such as sympathetic nerve activity, sodium concentration, or blood volume in vivo (12, 13). Furthermore, glucocorticoid induces the rise in plasma renin substrate level (14). Changes in plasma renin activity caused by ACTH may be regarded as the result of another feedback mechanism. It is unclear whether the stimulatory effect of ACTH is direct or not, whether the renin responding to ACTH is derived from the adrenal gland or from the kidney, and whether the effect of ACTH occurs only at pharmacological dosages or not. In the present study, to investigate the direct effect of ACTH on the renin-angiotensin-aldosterone system without the systemic effects, isolated guinea-pig kidneys with and without adrenal glands were perfused.

Materials and methods

Male Hartley guinea pigs (240–260 g), fed with a standard chow and tap water ad libitum, were anesthetized with halothane (Hoechst, Germany). The methods of rat kidney perfusion (15–17) were modified in compliance with our experiment. Briefly, through a midline incision, the abdominal aorta was cannulated as the influent route and infused with perfusion medium at 6 ml/min. After the coeliac and the mesenteric arteries were ligated the inferior caval vein was cannulated as the effluent route. Both kidneys with adrenal glands were isolated on bloc and placed in an incubator at 37°C. The kidneys without adrenal glands were also perfused in the same procedures.

The perfusion medium was Krebs-Ringer bicarbonate buffer (pH 7.4) containing 10 mmol/l glucose, 0.2% bovine serum albumin (fraction V, Boehringer, Ger-
many) and 4.6% dextran (mean MW 70000, Pharmacia AB, Sweden), saturated with 95% O₂ + 5% CO₂ gas. After the stabilization perfusion for 15 min and the basal perfusion for 5 min, 1–24ACTH (Organon, The Netherlands) at various doses (0.1, 1, 10, 100 and 1000 µg/l) or dibutyryl cAMP (Sigma, USA) at 0.3 mmol/l was infused for 30 min. The perfusion pressure was maintained at 40–50 mmHg throughout the experiments.

The effluent perfusates were collected every 5 min. For the determination of perfusate renin activity, the samples were incubated with the nephrectomized rabbit serum as renin substrate at 37°C for 60 min. The produced angiotensin I, cAMP, aldosterone and cortisol were measured using radioimmunoassay kits from Dainabot, Yamasa, Shionogi and Elken (Japan), respectively. Student’s t-test was used for the statistical analysis.

Results

The metabolic integrity of the guinea-pig adrenal gland was confirmed by the observation of ACTH-induced cortisol secretion. Total output of cortisol during ACTH infusion for 30 min was increased from 18.9 ± 2.6 µg (mean ± SEM, N = 4) at 0 µg/l ACTH to 29.0 ± 3.2 µg at 0.1 µg/l (p < 0.05).

Perfusate renin activity was significantly increased in a dose-dependent manner by the addition of ACTH in a range of 0.1–1000 µg/l (Fig. 1). Renin activity reached a peak level of 6.6 ± 1.4 µg l⁻¹ h⁻¹ at 20 min from 0.5 ± 0.1 µg l⁻¹ h⁻¹ at 0 min by the addition of 1000 µg/l ACTH (p < 0.01). Total output of renin activity at 0.1 µg/l ACTH for 30 min (294 ± 37 ng/h) was significantly higher than that at 0 µg/l ACTH (89 ± 27 ng/h) (p < 0.05).

Perfusate cAMP level was elevated by the addition of ACTH in a range of 10–1000 µg/l in the same experiment (Fig. 2): 0.3 mmol/l of dibutyryl cAMP markedly stimulates renin release (Fig. 3). Perfusate renin activity was increased from 0.5 ± 0.1 µg l⁻¹ h⁻¹ at 0 min to 5.1 ± 1.2 µg l⁻¹ h⁻¹ at 20 min (p < 0.01).

Aldosterone secretion was increased in a dose-dependent manner by ACTH except in the case of 0.1 µg/l (Fig. 4). Total output of aldosterone for 30 min was increased from 76 ± 7 ng at 0 µg/l ACTH to 117 ± 13 ng at 1 µg/l ACTH (p < 0.05).

The effects of 100 µg/l ACTH on renin, cAMP and aldosterone secretions from the perfused kidneys without adrenal glands are given in Table 1. These results make clear that ACTH has its effect mainly on the kidney-derived renin.
Fig. 4. Effect of ACTH on aldosterone secretion. ACTH increased aldosterone secretion dose-dependently since 1 μg/l. The symbols are the same as those in Fig. 1.

Table 1. Effects of ACTH on renin, cAMP and aldosterone secretions from the kidney-perfusion preparation from which the adrenal glands were removed. ACTH at 100 μg/l significantly stimulated renin and cAMP produced by the kidney. The results are given as mean ± SEM (N=4).

<table>
<thead>
<tr>
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<th>Basal level (at 0 min)</th>
<th>100 μg/l ACTH infusion (at 20 min)</th>
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</thead>
<tbody>
<tr>
<td>Perfusate renin activity (μg·l⁻¹·h⁻¹)</td>
<td>0.8 ± 0.3</td>
<td>10.4 ± 3.3*</td>
</tr>
<tr>
<td>Perfusate cAMP (nmol/l)</td>
<td>0.4 ± 0.2</td>
<td>4.2 ± 0.9*</td>
</tr>
<tr>
<td>Perfusate aldosterone (ng/l)</td>
<td>185 ± 64</td>
<td>92 ± 32</td>
</tr>
</tbody>
</table>

Mean ± SEM (N=4). *p < 0.01.

Discussion

The present study showed the direct effect of ACTH, especially at the physiological concentration (0.1 μg/l), on renin release in the isolated perfused guinea-pig kidneys, both with and without adrenal glands. The results suggest that ACTH may be one of the physiological stimulators on the renin-angiotensin-aldosterone system in guinea pigs. Moreover, 0.1 μg/l of ACTH provoked renin release without the aldosterone response. This result implied that the stimulatory effect of ACTH on renin release was more potent than on aldosterone secretion. The direct effect of ACTH on renin can be inferred to aldosterone secretion as an indirect effect, since aldosterone secretion is dominantly regulated by the renin–angiotensin system in vivo (11, 13). It has been demonstrated that the ACTH-induced aldosterone secretion is blunted by captopril, an inhibitor of the angiotensin-converting enzyme (8, 18).

The results of previous in vivo reports of the effect of ACTH on renin release were different, probably due to the respective protocol of the study, i.e., the differences in the administered dosages and durations of ACTH (6–11). Reports by Oelkers et al. (8–10) showed that, in normal subjects infused with 5–10 IU of ACTH per day, plasma renin activity started to rise after 6–8 h of infusion, reached a maximum after 24 h and then tended to decline. Baba et al. (5) reported that each level of basal plasma renin activity and aldosterone in four patients with a high level of ACTH and adrenal hyperplasia was not higher than in the normal. The reponses of plasma renin activity to furosemide (1 mg/kg iv) were not changed except for the lowered aldosterone response. In explaining the discrepancy in renin response to ACTH, it may be postulated that renin release is more sensitive to an acute stimulus of ACTH than to a chronic one. The sodium volume expansion via the ACTH-aldosterone system may to some extent influence ACTH-induced renin release in vivo. And the difference in ACTH-induced renin release between animal species could not be neglected.

Renin has been identified in the adrenal gland. The main site of origin of adrenal renin is the zona glomerulosa cells (19–23). Nakamaru et al. (14) emphasized that the adrenal renin-angiotensin system had an important role in the regulation of aldosterone secretion. The possibility cannot be ruled out that ACTH causes renin release from the adrenal gland. Belkien et al. (11) carried out the ACTH-infusion test in patients with adrenalec- tomy, Addison disease and hypopituitarism, and came to the conclusion that ACTH raised plasma renin activity by stimulating the renal secretion of active-form renin. Our results on the perfused kidneys without adrenal glands show that renin responding to ACTH has its origin mainly in the kidney, supporting Balkien et al.’s report.

As the intracellular messenger of ACTH on renin release, cAMP is considered most (4, 24, 25). The potent effect of cAMP on renin release has been demonstrated in rats, dogs and human kidney slices (4, 25, 26). In the present study, ACTH stimulated cAMP release together with renin release, and dibutyryl cAMP produced a similar stimulation of renin release. These results show that cAMP mediates the stimulatory action of ACTH on renin in guinea pigs. At ACTH concentrations below 10 μg/l, cAMP release was not changed, whereas renin release was increased significantly. It is possible that some other signals besides cAMP are involved in the action of ACTH, such as calcium or cGMP (18). Since only cAMP in perfusate is monitored, the measurement of intracellular cAMP may be necessary in further studies.

In summary, ACTH has a direct effect on renal renin release in guinea pigs, and this action is probably mediated by the cAMP messenger system. The sensitivity of renin release to ACTH is slightly greater than that of aldosterone secretion. These observations suggest that ACTH has an important role to play in the regulation of the renin-angiotensin-aldosterone system.
References