Importance of the adrenal cortex for development and maintenance of hypertension in spontaneously hypertensive rats

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Abstract. Adrenal regeneration following complete bilateral adrenalectomy in spontaneously hypertensive rats (SHR) was used to study the significance of glucocorticoid and mineralocorticoid hormones for the development and maintenance of hypertension. Pre-hypertensive (5 weeks of age) and hypertensive (10 and 16 weeks of age) male SHR underwent adrenalectomy (ADN) and were kept on 0.9% NaCl. The rats were ether stressed at various intervals to assess adrenal steroid production. Following ADN of all three age groups the development or maintenance of hypertension depended on the presence of adrenal regenerates. Animals without signs of adrenal regeneration remained or became normotensive. There was a significant correlation between plasma corticosterone levels following ether stress and blood pressure.

Aldosterone and corticosterone production of regenerates and of adrenal cortex of intact SHR was studied in vitro. Under basal condition and following ACTH stimulation both tissues produced similar amounts of corticosterone, however considerably less aldosterone was secreted by regenerates. Betamethasone substitution in adrenalectomized rats caused a dramatic increase of blood pressure which was attenuated by 1-propranolol. Aldosterone had no significant effect on blood pressure. It is concluded that glucocorticoids play a permissive role in the development of hypertension presumably via alteration of sympathetic neurotransmission.

The necessity of the adrenal gland for development and maintenance of hypertension in the spontaneously hypertensive rat (SHR) is still disputed. Aoki (1976) claimed that both the adrenal and thyroid glands are necessary for the development of arterial hypertension in SHR and that adrenalectomy in hypertensive animals results in a fall in blood pressure to normotensive levels (Aoki et al. 1973). In contrast, Baer et al. (1972) found no evidence that the adrenals involved in the pathogenesis of hypertension.

A role for adrenal steroids in essential hypertension is suspected but clear experimental evidence is still lacking. Studies of the steroid patterns in SHR compared to various control strains have yielded discrepant results (Melby & Dale 1979; Komanicky et al. 1982). Corticosterone levels in SHR have been reported to be higher, lower, or not to differ from those of WKY controls (Sowers et al. 1981; De Vito et al. 1981; Yamori et al. 1973). Adrenal response to ether stress is also controversial (Yamori et al. 1973; De Vito et al. 1981; McMurthy & Wexler 1981).

However, morphometric studies (Bartsch et al. 1978; Nickerson 1976) indicated an enhanced secretory activity of the adrenocortical cell presum-
ably due to chronic stimulation by ACTH. Moreover, Häusler et al. (1983a) demonstrated a significantly enhanced activity of the pituitary adrenocortical axis of SHR during the early development of hypertension.

In order to study the role of steroid hormones in hypertension in SHR and to circumvent the necessity of comparing different rat strains, we exploited the phenomenon of regeneration of accessory adrenocortical tissue following total bilateral adrenalectomy (Jaffe 1926; Gaunt 1933). The development and maintenance of arterial hypertension was compared in animals bearing regenerates with those lacking adrenocortical tissue. The production of corticosterone and aldosterone by regenerating adrenals was measured in vivo and in vitro. In addition, the effect of glucocorticoid and mineralocorticoid substitution on blood pressure was studied in animals lacking adrenocortical tissue.

Materials and Methods

Male SHR of different ages were purchased from Madörin AG (Füllinsdorf, Switzerland) and allowed to adapt to our animal quarters for at least 7 days. The rats were housed in groups of 3 to 5 under a 12 h light-dark regimen. Food and water were available ad libitum.

Blood pressure was measured and recorded with a W+W BP recorder 8008 (W+W Electronic AG, Basel, Switzerland), using the indirect tail cuff method for pretrained animals. Mean blood pressure is reported as the mean of two measurements.

Adrenal function and regeneration were monitored after stimulation of the hypothalamo-pituitary axis by exposing the rats for 1 min to an atmosphere saturated with ether between 8 and 11 a.m. Twenty minutes later, the animals were bled retroorbitally and 0.5–1 ml blood was collected in polystyrole tubes coated with EDTA. Plasma was quickly separated and stored at −20°C until assayed. Some animals were ether stressed repetitively at intervals to assess adrenal regeneration.

Corticosterone was measured directly in plasma by RIA using [125I]cortisol (NEN) as tracer and corticosterone (Sigma) as standard. Binding to corticosterone binding protein was inhibited by carrying out the assay at pH 3.0 in glycine buffer. The antiserum was raised in rabbits against cortisol coupled to bovine serum albumin using cortisol hemisuccinate. The antiserum gave a cross-reaction of 26% with corticosterone (cortisol: 100%) and 1.4% with betamethasone. Antibody-bound and free antigen were separated with dextran-charcoal. All samples from one experiment were measured in one assay.

The inter-assay coefficient of variation was 12.0% and the intra-assay coefficient of variation was 10.1%. The minimum limit of detectability was 7–10 ng/ml plasma. Aldosterone was measured with a tritium labelled kit from International CIS. Cross-reactivity with corticosterone is 2.5 × 10−3%.

Blood pressure measurements and ether stress experiments were always performed on separate days to avoid mutual interactions. Adrenalectomy was performed under a light ether anaesthesia. Care was taken to remove both adrenals completely with surrounding fat, which was verified histologically. The adrenalectomized rats were placed on 0.9% NaCl as drinking water, whereas intact animals were kept on tapwater. For histological examinations, adrenals and regenerated adrenal tissue were quickly removed, fixed in Bouin's solution, processed and stained with haematoxylin-eosin.

To measure in vitro aldosterone and corticosterone production, adrenal cortical regenerates and intact adrenal cortex from age-matched control rats were quartered and each quarter pre-incubated with Krebs-Ringer solution, containing 11 mmol D-glucose and 3% BSA at 37°C with shaking under an atmosphere of 95% O2/5% CO2. After 30 min, one quarter from each preparation was left unstimulated as control and the remaining 3 quarters stimulated with ACTH1–24 (Synacthen®) and further incubated for 2 h. At the end of the incubation period, the medium was assayed for corticosterone and aldosterone and the tissue fragments weighed. Steroids produced were expressed as mg per mg tissue.

For glucocorticoid substitution, betamethasone-sodium phosphate (Celestone®, Schering Company, Kenilworth, New Jersey) was added to 0.9% NaCl to obtain dosages between 2–100 μg/kg/day: the higher 0.9% NaCl intake in treated animals was accounted for when calculating the dosage.

Aldosterone (Sigma) was dissolved in propyleneglycol and injected sc twice daily.

Statistical analysis of the results was carried out using Student's t-test (two tailed) as well as oneway analysis of variance, followed by Student-Newman-Keuls test.

Results

Fig. 1 and Table 1 show the behaviour of blood pressure and plasma corticosterone following ether stress in SHR adrenalectomized at the age of 5, 10 and 16 weeks, respectively. In animals which failed to develop adrenal regenerates, confirmed at autopsy and demonstrated by the plasma corticosterone values following ether stress, the blood pressure remained at or decreased to normotensive levels (125–140 mmHg) depending on age at adrenalectomy. In contrast, animals with low but
substantial levels of corticosterone developed or regained hypertension. In rats, 5 weeks old at adrenalectomy, which developed adrenocortical regenerates, the plasma corticosterone levels approached 50% of the controls within 6 weeks after removal of the adrenal glands. Interestingly, the resulting blood pressure in these animals was significantly higher than in the age-matched controls. With increasing age at adrenalectomy, both plasma corticosterone levels and blood pressure values tended to decrease in animals with regenerates. In animals adrenalectomized at 10 weeks of age, the blood pressure levels regained the hypertensive levels of those of the controls within 5 weeks. However, in animals operated at 16 weeks of age, the resulting arterial blood pressure was still significantly lower than that of controls 11 weeks after operation.

Table 1.
Plasma corticosterone (ng/ml) 20 min following ether stress in SHR adrenalectomized (ADN) at the age of 5, 10 and 16 weeks.

<table>
<thead>
<tr>
<th>ADN at 5 weeks of age</th>
<th>−1 week</th>
<th>+1 week</th>
<th>+3 weeks</th>
<th>+6 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact controls</td>
<td>482 ± 82</td>
<td>572 ± 105</td>
<td>450 ± 108</td>
<td>400 ± 53</td>
</tr>
<tr>
<td>Rats without regenerates*</td>
<td>−</td>
<td>12 ± 3</td>
<td>13 ± 4</td>
<td>16 ± 4</td>
</tr>
<tr>
<td>Rats with regenerates*</td>
<td>−</td>
<td>23 ± 5</td>
<td>156 ± 38</td>
<td>181 ± 31</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>ADN at 10 weeks of age</th>
<th>−1 week</th>
<th>+1 week</th>
<th>+5 weeks</th>
<th>+7 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact controls</td>
<td>533 ± 92</td>
<td>407 ± 102</td>
<td>391 ± 97</td>
<td>357 ± 114</td>
</tr>
<tr>
<td>Rats without regenerates*</td>
<td>−</td>
<td>9 ± 2</td>
<td>15 ± 2</td>
<td>12 ± 3</td>
</tr>
<tr>
<td>Rats with regenerates*</td>
<td>−</td>
<td>15 ± 19</td>
<td>129 ± 19</td>
<td>107 ± 24</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>ADN at 16 weeks of age</th>
<th>−1 week</th>
<th>+4 weeks</th>
<th>+7 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact controls</td>
<td>600 ± 73</td>
<td>584 ± 91</td>
<td>567 ± 87</td>
</tr>
<tr>
<td>Rats without regenerates*</td>
<td>−</td>
<td>8 ± 2</td>
<td>7 ± 3</td>
</tr>
<tr>
<td>Rats with regenerates*</td>
<td>−</td>
<td>34 ± 9</td>
<td>82 ± 23</td>
</tr>
</tbody>
</table>

* The presence or absence of regenerates was confirmed at autopsy. Each group consists of 8–10 animals. Values represent mean ± se.
At autopsy, adrenocortical tissue was found in almost all animals with detectable levels of corticosterone following stress, i.e. in approximately 50% of the adrenalectomized SHR. In most cases only one regenerate was found. The size of the regenerate ranged from a diameter of 0.2 mm to that of normal adrenals. Histologically, the tissue consisted of an irregular fasciculata-like arrangement with a barely developed zona glomerulosa; a zona reticularis was not found.

Fig. 2 shows that there is a significant correlation \( r = 0.856, P < 0.001 \) between blood pressure and corticosterone output following ether stress in 15 weeks old SHR, 5 weeks after adrenalectomy at an age of 10 weeks.

The ability of the adrenal cortex of intact SHR and adrenocortical regenerates to synthesize and release corticosterone and aldosterone in vitro under basal conditions and following ACTH-stimulation is presented in Fig. 3. Under basal conditions, both control tissue and regenerates released about equal amounts of corticosterone into the medium. Following stimulation with 250 ng ACTH1-24, an 8-fold increase of corticosterone was observed in both control and regenerate adrenal cortex. On the contrary, considerably less aldosterone is secreted by regenerates, compared to control cortex both under basal conditions and especially following ACTH stimulation, confirming our histological data (see above).

In order to elucidate the role of glucocorticoid and mineralocorticoid hormones on blood pressure, betamethasone and aldosterone substitution was studied in SHR, adrenalectomized at 10 weeks of age, which showed no adrenal regenerates and had a blood pressure in the normotensive range.

Betamethasone treatment in a dose of 20 \( \mu \text{g/kg/day} \) caused a significant \( (P < 0.01) \) rapid increase of blood pressure within 12 h (Fig. 4A). This effect of betamethasone was dose-dependent and reversible after cessation of treatment (Fig. 4B).

In contrast, aldosterone substitution in doses of 30 and 150 \( \mu \text{g} \) given in 2 doses at 9 a.m. and 5 p.m sc failed to influence blood pressure significantly (Table 2). Aldosterone blood levels determined 6 h following the last injection were in the same range or considerably higher than the concentration in hypertensive SHR bearing adrenal cortical regenerates (Table 2).

To study a possible modulation of sympathetic nervous system activity by glucocorticoids, betamethasone substitution was studied under concomitant beta-blockade with L-propranolol (Fig. 5). L-propranolol given twice daily in a dose of 2 mg/kg sc prevented the betamethasone induced increase in blood pressure 17 h after combined treatment \( (P < 0.01) \). However, 42 h after start of treatment, the attenuation of blood increase was no more significant.
In vitro corticosterone (B) and aldosterone (A) production of regenerated cortical tissue and of cortices of age-matched controls under basal conditions and following stimulation with 250 ng ACTH$_{1-24}$. Each group consists of 7–9 quarters of regenerates or control cortex. Values represent mean ± se. *P < 0.05, **P < 0.01, ***P < 0.001 vs basal secretion.

Effect of betamethasone provided in 0.9% NaCl on blood pressure in SHR adrenalectomized 3 weeks previously at the age of 10 weeks. Prior to betamethasone treatment the animals did not show any signs of adrenal cortical regeneration. Panel A: Increase of blood pressure during administration of 20 µg/kg/day betamethasone. Panel B: Dose-response relationship between blood pressure and various doses of betamethasone (a = 100, b = 50, c = 20, d = 2 µg/kg/day). † Some values of this group were above the limits of measurement (250 mmHg). Each group consisted of 6–8 animals.

Values represent mean ± se. P < 0.01 vs blood pressure at the beginning of the experiment.
Influence of 1-propranolol on betamethasone-induced rise of blood pressure. Betamethasone was given in 0.9% NaCl in a dose 20 µg/kg/day. 1-propranolol was given sc in a dose of 2 mg/kg twice daily at 9 a.m. and 5 p.m. Blood pressure was recorded 1 h after administration of 1-propranolol. Controls, Betamethasone, Propranolol, Propranolol + betamethasone. *P < 0.01 vs controls, **P < 0.01 vs betamethasone.

Discussion

Our results clearly demonstrate that functioning adrenocortical tissue is essential for the development and maintenance of hypertension in SHR.

Substitution with glucocorticoid but not mineralcorticoid could replace the adrenal cortex in restoring arterial hypertension following adrenalectomy.

Adrenocortical regenerates presumably originate from pre-existing small accessory cortical islands scattered around the periadrenal and perirenal areas and occasionally in the retroperitoneum caudal to the kidneys as well as in the genital region (Bachmann 1954). The existence of 'accessory cortical corpuscles' has been long known and can hinder a successful adrenalectomy (Jaffe 1926; Gaunt 1933). Our studies show that, in experiments involving adrenalectomized rats, the animals should be controlled for circulating adrenocortical steroids. Measurements of basal levels of corticosterone are insufficient since the hormone levels are often below the threshold of detectability in animals with regenerates. The regeneration phenomenon might explain some of the discrepant findings mentioned in the introduction.

The low levels of aldosterone found in our animals following ether stress can be correlated with the narrow zona glomerulosa of the regenerates. This finding was further confirmed by our in vitro studies: very little aldosterone was produced following stimulation of quartered regenerates.
with ACTH_{1-24}, a potent short-term stimulus of glucocorticoids and aldosterone (Ganong et al. 1966).

The low aldosterone production in vitro is not unexpected, since the animals were kept on 0.9% NaCl. On the other hand, in the adrenal regeneration hypertension following adrenal enucleation 18-OH-DOC and DOC are elevated during certain stages of the development of hypertension despite the animals being kept on sodium chloride (Grekin et al. 1972).

Reports on peripheral aldosterone levels in SHR are conflicting but the general opinion is that aldosterone levels are low in established hypertension (Freeman et al. 1975; Willis & Bauer 1978).

The correlation between blood pressure and corticosterone levels found after ether stress and the low concentration of aldosterone found in vitro experiments strongly suggest that glucocorticoid rather than mineralocorticoid activity is of importance for the development and maintenance of hypertension. This assumption is further supported by our substitution experiments. Aldosterone did not cause a significant rise in blood pressure in SHR adrenalectomized at 10 weeks of age, even in supraphysiological concentrations. In contrast, Kenyon et al. (1981) reported that infusion of aldosterone in young, pre-hypertensive SHR maintained on sodium chloride resulted in hypertension within a week. The reason for the discrepancy between their results and ours is unclear and might result from the different age of the animals examined and thus possibly differing susceptibility towards aldosterone.

Interestingly, in another hypertensive animal model – namely the salt-sensitive hypertension prone rats – glucocorticoids were found to be indispensable for the development of hypertension (Iwai et al. 1969).

The sympathetic nervous system is known to be critically involved in the development of hypertension in SHR (Folkow & Hallbäck 1977). The rapid, primary effect of betamethasone treatment on restoration of hypertensive blood pressure levels and its attenuation by L-propranolol suggest that glucocorticoids alter sympathetic neurotransmission in SHR probably by modulation of a beta-receptor modulated process. Adrenocorticoids are known to induce important alterations in beta-adrenergic actions. Glucocorticoids enhance catecholamine-stimulated inotropism (Kauman 1972) and modulate beta-adrenergic receptor density (Wolfe et al. 1976; Guellaen et al. 1978; Foster & Harden 1980). Recently, it was shown that glucocorticoids may regulate the ability of beta-adrenergic receptors to form a 'high affinity' state (Davies & Lefkowitz 1981).

The low levels of glucocorticoids required to restore the elevated blood pressure levels in adrenalectomized rats without adrenal regenerates suggest that glucocorticoids play a crucial, permissive role in the development and maintenance of hypertension in SHR. It is of interest that chronic betamethasone treatment caused a dose-dependent rise in blood pressure in intact SHR and normotensive Wistar-Kyoto rats and had no effect in Wistar rats (Häusler et al. 1983b).

Beside their above-described effect on the sympathetic nervous system, glucocorticoids may also act on other systems, possibly involved in the pathogenesis of hypertension. E.g. glucocorticoids may increase the activity of angiotensin converting enzyme (Mendelsohn et al. 1982) apart from the well-known effect on angiotensinogen (Reid et al. 1973). In our hypertensive adrenalectomized SHR with proven regenerates, however, plasma renin activity was lower than in intact SHR up to 6 months after adrenalectomy (Siegl et al., unpublished observation).

In summary, our results strongly suggest that glucocorticoids are essential for the maintenance of high blood pressure in SHR and that the regeneration of accessory adrenocortical tissue following total bilateral adrenalectomy is a useful model for studying the pathogenetic mechanisms involved in arterial hypertension in SHR and probably in other animal hypertension models.

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References


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