SECRETION OF ALDOSTERONE IN RESPONSE TO HISTAMINE IN HYPOPHYSECTOMIZED-NEPHRECTOMIZED DOGS

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SUMMARY

In hypophysectomized-nephrectomized dogs after intravenous injection of histamine, a marked increase was observed in the rate of secretion of aldosterone, although it was smaller than that in intact dogs. Hypophysectomy plus bilateral nephrectomy greatly impaired the secretion of corticosterone and cortisol in the dog in response to histamine. However, a small yet significant increase in corticosterone and cortisol secretion was observed in hypophysectomized-nephrectomized dogs after intravenous injection of histamine. Additional experiments showed that plasma concentrations of potassium and sodium in hypophysectomized-nephrectomized dogs remained unchanged after intravenous injection of histamine. These results suggest that histamine stimulates aldosterone secretion in the dog partly by a direct effect on the adrenal cortical cells, whereas the effect of histamine on corticosterone and cortisol secretion is mediated mainly, but not totally, by pituitary release of ACTH.

INTRODUCTION

In a previous study in the dog it was shown that secretion of adrenal corticosterone and cortisol in response to histamine was mediated largely by release of adrenocorticotrophin (ACTH) from the pituitary gland and could be attributed partly to a direct effect of histamine on the adrenal cortex or to some extra-pituitary factors (Hirose, Matsumoto, Aikawa & Suzuki, 1977). Evidence for a direct effect of histamine on the secretion of corticosterone and cortisol from the adrenal gland in the dog was recently obtained by experiments using isolated adrenal cells in vitro (Hirose, Matsumoto & Aikawa, 1978).

There is much evidence to support the view that, in the dog, the renin–angiotensin system, pituitary ACTH and changes in concentrations of potassium and sodium in the plasma are major regulators of aldosterone secretion under many different circumstances. However, less information is available about the factors to which the response in aldosterone secretion to the presence of histamine in the dog may be attributed. The purpose of the present study was to explore these factors.

MATERIALS AND METHODS

Animals and experimental procedure

Studies were conducted on 11 adult male mongrel dogs ranging in weight from 10.2 to 21.7 kg.

The left lumboadrenal veins of six dogs anaesthetized with sodium pentobarbitone (25 mg/kg, i.v.) were cannulated on the day before the experiment by the technique of Satake, Sugawara & Watanabe (1927) as modified by Suzuki, Yamashita & Mitamura (1959). The lumboadrenal vein was exposed through the lumbar route and small branches
of the vein were doubly ligated and cut off. Then a small cannula attached to a short rubber tube was inserted in the vein and fixed just lateral to the adrenal gland. The cannula and rubber tube were filled with heparin-0.9% saline solution and the free end of the rubber tube was clamped. A long silk thread was loosely passed around the lumboadrenal vein medial to the adrenal gland. On the day of the experiment, 2000 units heparin were injected i.v. to prevent blood clotting after the animals were re-anaesthetized with sodium pentobarbitone (25 mg/kg, i.v.). After primary collection of two samples of adrenal venous blood at 20 min intervals, the animals were injected i.v. for 60 s with 0.1 mg histamine dihydrochloride/kg dissolved in 0.9% saline solution. At the time the adrenal venous blood samples were collected, the silk thread which had been passed around the lumboadrenal vein was gently pulled and the clamp on the rubber tube was taken off so as to direct the adrenal venous blood flow towards the exterior through the cannula and rubber tube. Samples of adrenal venous blood were collected 5, 10, 20, 40, 60 and 120 min after the start of the histamine injection. The blood which was collected was replaced by i.v. injection of the same volume of 0.9% saline solution. At 130 min, 0.1 i.u. ACTH/kg was infused i.v. over 5 min and adrenal venous blood was sampled 10 and 20 min after the start of the infusion.

The hypophyses of five dogs, anaesthetized with sodium pentobarbitone (25 mg/kg, i.v.), were exposed by the transbuccal approach on the day before the experiment and then left lumboadrenal venous cannulation and right nephrectomy were performed. On the day of the experiment, after the animals were again anaesthetized with sodium pentobarbitone (25 mg/kg, i.v.), hypophysectomy was performed by aspiration of the pituitary gland, followed by left nephrectomy. The experiment was started approximately 3 h later. The time schedule and procedure of the experiments on intact dogs and those on hypophysectomized-nephrectomized dogs were almost the same.

Samples of adrenal venous blood were centrifuged immediately after collection and the plasma was analysed for aldosterone, corticosterone and cortisol. The rates of secretion of aldosterone, corticosterone and cortisol were calculated by multiplying the concentrations of the steroids in the adrenal venous plasma (ng/ml) by the adrenal plasma flow (ml kg\(^{-1}\) min\(^{-1}\)). In hypophysectomized-nephrectomized dogs, the completeness of hypophysectomy was carefully checked by gross inspection after the removal of the brain at the end of the experiment.

To establish the effect of histamine on peripheral plasma concentrations of potassium and sodium, additional experiments were carried out on six adult male mongrel dogs weighing between 7.1 and 14.8 kg. Right nephrectomy was performed on the day before the experiment and left nephrectomy and hypophysectomy were carried out on the day of the experiment after the animals were anaesthetized with sodium pentobarbitone (25 mg/kg, i.v.). Samples of the peripheral blood were obtained by jugular venepuncture before and 5, 10, 20, 40, 60 and 120 min after injection of 0.1 mg histamine dihydrochloride/kg. The blood that was collected was replaced by i.v. injection of the same volume of 0.9% saline solution. The blood samples were immediately centrifuged and the plasma was analysed for potassium, sodium and chloride concentrations.

The data were subjected to statistical analysis using Student’s \(t\)-test for ‘between-group’ comparisons and Student’s paired \(t\)-test for ‘within-group’ comparisons.

**Analytical technique**

Aldosterone, in samples of adrenal venous plasma, was, after solvent extraction and preliminary purification by column chromatography, purified by paper chromatography and measured by radioimmunoassay using the slightly modified method of Mayes, Furuyama, Kem & Nugent (1970).

Plasma (0.5 ml) was added to tubes containing 4000 counts of [1,2-\(^3\)H]aldosterone (New England Nuclear Corp., Boston, Massachusetts, U.S.A.; 57 Ci/mmol) which had been...
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purified by paper chromatography in the solvent system n-hexane : benzene : methanol : water (10 : 90 : 50 : 25, by vol.). After mixing, the plasma was extracted with 4 ml dichloromethane. Dichloromethane extract (2 or 3 ml) was applied to an 8 × 30 mm column of silica gel, which was washed with 5 ml dichloromethane : methanol (98 : 2, v/v) and eluted with 5 ml dichloromethane : methanol (90 : 10, v/v). The eluate was dried with a stream of nitrogen at 40 °C, dissolved with dichloromethane, applied to the paper and then, after 5 h of equilibration, it was developed with n-hexane : benzene : methanol : water (10 : 90 : 50 : 25, by vol.) for 9 h at 30 °C. The paper chromatogram was scanned for radioactivity with a strip paper scanner (Aloka, model PCS 101) to locate the [1,2-3H]aldosterone. A section of paper (4 cm) containing aldosterone was cut into pieces which were then eluted with 2 ml methanol in a glass vial. A portion (0.05 or 0.2 ml) of the eluate was dried under a stream of nitrogen at 40 °C and used for radioimmunoassay. Another portion of the eluate was transferred to a scintillation counting vial for determination of recovery.

The radioimmunoassay was performed using an aldosterone radioimmunoassay kit from Sorin Biomedica (Saluggia, Italy) and a Packard Tri-Carb liquid scintillation spectrometer (model 3330) with Bray's scintillation fluid. d-Aldosterone (Sigma Co., St Louis, Missouri, U.S.A.), used as standard, was dissolved before use. Aldosterone assay of serial diluted purified samples of adrenal venous plasma produced a curve parallel to the standard curve. There was no difference between the bindings of tracer in the absence and in the presence of dried residue of 0.2 ml methanol. Graded amounts of the standard (25–400 pg) added to the sample were assayed and the recoveries were 91–104%. The sensitivity of the assay was defined as the amount of aldosterone required to inhibit binding of the tracer in the absence of unlabelled aldosterone by 5% and was 11 ± 1.7 pg (mean ± s.d., n = 4). Two sample pools were assayed to determine the intra- and interassay variability. The intra-assay coefficients of variation were 13.4 and 14.7% (n = 5). The interassay coefficients of variation were 14.9 and 13.3% (n = 4). The antiserum supplied in the kit did not cross-react (<0.01%) with corticosterone, cortisol, deoxycorticosterone, dehydroepiandrosterone, progesterone or cortisone.

Differential estimations of corticosterone and cortisol in samples of adrenal venous plasma were performed by the method described previously (Hirose, 1977). Adrenal venous plasma (0.1–0.5 ml) was extracted with 5 ml dichloromethane. After being dried under a stream of nitrogen at 40 °C and dissolved with ethanol, the extract was applied to a silica gel thin-layer plate (20 × 20 cm). The chromatogram was developed in a chloroform : methanol (94 : 6, v/v) system. After drying, areas of corticosterone and cortisol were separately scraped off. Each fraction was eluted twice with 5 ml ethyl acetate and was dried again in a stream of nitrogen. The quantity of corticosterone and cortisol in each fraction was measured by the fluorometric method of Silber, Busch & Oslapas (1958), as modified by Hirose (1977); 2 ml concentrated sulphuric acid : ethanol (65 : 35, v/v) were added to each fraction and the fluorescence intensity was determined after 1 h by a Farrand fluorometer.

Plasma concentrations of potassium, sodium and chloride were determined using a Technicon Stat/Ion.

RESULTS

The rates of secretion of aldosterone, corticosterone and cortisol before and after injection of histamine and after infusion of ACTH in intact and hypophysectomized-nephrectomized dogs are presented in Table 1.

The rate of secretion of aldosterone in intact dogs increased significantly after injection of histamine as well as after infusion of ACTH. The rate of secretion of aldosterone in hypophysectomized-nephrectomized dogs before injection of histamine was low, but it increased significantly after the injection. The maximal aldosterone secretion in
Table 1. Effects of histamine on rates of secretion of aldosterone, corticosterone and cortisol (ng kg⁻¹ min⁻¹) from the adrenal gland of intact and hypophysectomized-nephrectomized dogs (values are means ± S.E.M.; d.f. in parentheses)

<table>
<thead>
<tr>
<th></th>
<th>Intact dogs (n = 6)</th>
<th>Hypophysectomized-nephrectomized dogs (n = 5)</th>
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<tbody>
<tr>
<td></td>
<td>Aldosterone</td>
<td>Corticosterone</td>
</tr>
<tr>
<td>Before injection of histamine</td>
<td>0.68 ± 0.21 (5)</td>
<td>12 ± 5 (5)</td>
</tr>
<tr>
<td>Time after injection of histamine (min)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>3.71 ± 0.72 (5)**</td>
<td>161 ± 16 (5)***</td>
</tr>
<tr>
<td>10</td>
<td>3.26 ± 0.48 (5)**</td>
<td>201 ± 14 (5)***</td>
</tr>
<tr>
<td>20</td>
<td>1.65 ± 0.38 (5)</td>
<td>195 ± 16 (5)***</td>
</tr>
<tr>
<td>40</td>
<td>1.29 ± 0.43 (5)</td>
<td>32 ± 15 (5)</td>
</tr>
<tr>
<td>60</td>
<td>1.46 ± 0.40 (5)</td>
<td>8 ± 3 (5)</td>
</tr>
<tr>
<td>120</td>
<td>1.52 ± 0.52 (5)</td>
<td>10 ± 5 (5)</td>
</tr>
<tr>
<td>Time after start of ACTH infusion (min)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>4.32 ± 0.35 (5)**</td>
<td>233 ± 23 (5)***</td>
</tr>
<tr>
<td>20</td>
<td>4.41 ± 0.76 (5)**</td>
<td>207 ± 16 (5)***</td>
</tr>
</tbody>
</table>

*P < 0.05; **P < 0.01; ***P < 0.001 (Student's paired t-test): compared with the rate of secretion before injection of histamine or infusion of ACTH.
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hypophysectomized-nephrectomized dogs after injection of histamine, observed at 10 min, was more than half of the corresponding value in intact dogs, although it was significantly smaller ($P<0.05$) than the latter. The response of aldosterone secretion to ACTH in hypophysectomized-nephrectomized dogs was almost the same as that in intact dogs.

Corticosterone and cortisol secretion increased significantly after injection of histamine in intact and hypophysectomized-nephrectomized dogs. The secretory responses in the latter dogs were, however, significantly smaller ($P<0.001$) than those in intact dogs. The maximal secretion of corticosterone and cortisol in hypophysectomized-nephrectomized dogs after injection of histamine was less than 20% of that in intact dogs. Secretion of corticosterone and cortisol in response to ACTH in hypophysectomized-nephrectomized dogs was not significantly different from that in intact dogs.

Concentrations of potassium, sodium and chloride in the plasma of six hypophysectomized-nephrectomized dogs before injection of histamine were $4.5 \pm 0.2$ (S.E.M.), $144 \pm 2$ and $106 \pm 1$ mequiv./l respectively. After injection of histamine these concentrations were between $4.3 \pm 0.3$ and $4.7 \pm 0.3$ mequiv./l, between $144 \pm 2$ and $146 \pm 2$ mequiv./l, and between $106 \pm 2$ and $107 \pm 2$ mequiv./l respectively; no significant changes in plasma levels of potassium, sodium or chloride were observed ($P>0.05$).

DISCUSSION

A number of studies have shown that systemic administration of histamine produces a marked increase in adrenocortical secretion in the dog (Suzuki, Hirai, Yoshio, Kurouji & Yamashita, 1963; Papp, Stark, Acu & Varga, 1964; Asano, 1966; Katsuki, Ito, Watanabe, Iino, Yuji & Kondo, 1967; Tanigawa, 1967; Narita, 1971; Yamashita, Shimizu, Mieno & Kawao, 1973; Hirose, Matsumoto & Suzuki, 1976; Hirose et al. 1977). In the study by Hirose et al. (1977), significant increases in corticosterone and cortisol secretion in hypophysectomized dogs in response to histamine were observed, although they were small and of short duration. These data have been confirmed by the present study; small yet significant increases in corticosterone and cortisol secretion were observed in hypophysectomized-nephrectomized dogs after histamine injection. Recently, a direct effect of histamine on the secretion of corticosterone and cortisol from the adrenal gland in the dog was verified by experiments using isolated adrenal cells in vitro (Hirose et al. 1978). Since responses in the secretion of corticosterone and cortisol to histamine in hypophysectomized dogs and hypophysectomized-nephrectomized dogs have been found to be much smaller than those in intact dogs, it is suggested that adrenal corticosterone and cortisol secretion in intact dogs in response to histamine might be due mainly to ACTH and in only a small part to a direct effect of histamine on the adrenal cortex.

Since in intact dogs, a prolonged hypotension induced by histamine would increase the concentrations of angiotensin II and ACTH in the circulation which are capable of stimulating aldosterone release by the adrenal cortex, histamine would stimulate the secretion of aldosterone via the renin–angiotensin system and/or pituitary ACTH release. In the present study, however, a marked increase in aldosterone secretion in response to histamine was observed in hypophysectomized-nephrectomized dogs as well as in intact dogs. These data indicate that an increase in aldosterone secretion in the dog after i.v. injection of histamine might, at least in part, be due to factors other than ACTH and the renin–angiotensin system. It has been well established in the dog that an increase in the plasma level of potassium or a decrease in the plasma concentration of sodium induces an increase in aldosterone secretion (Davis, Urquhart & Higgins, 1963). In the present study, however, no significant changes in plasma levels of potassium and sodium were observed in hypophysectomized-nephrectomized dogs after i.v. injection of histamine. Thus, in hypophysectomized-nephrectomized dogs the response in the secretion of aldosterone to histamine might be due to a direct effect of histamine on the adrenal cortex or to some unknown factor.

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A direct stimulatory effect of histamine on the secretion of aldosterone from the adrenal gland was recently verified in our laboratory by experiments of direct arterial perfusion of the adrenal gland of hypophysectomized-nephrectomized dogs in situ (T. Aikawa, T. Hirose, I. Matsumoto & T. Suzuki, unpublished observations).

From these observations it is suggested that aldosterone secretion in the dog in response to histamine might, at least in part, be due to a direct effect of histamine on the adrenal cortical cells.

In the present study a marked increase in the secretion of aldosterone and small increases in the secretion of corticosterone and cortisol were observed in hypophysectomized-nephrectomized dogs after the injection of histamine. Since it has been established in the dog that a low dose of ACTH produces marked increases in the secretion of adrenal corticosterone and 17-hydroxycorticosteroids but it is less effective on the secretion of aldosterone (Mulrow, Ganong, Cera & Kulfian, 1962), it may be concluded that the direct effect of histamine on adrenal aldosterone, corticosterone and cortisol secretions in hypophysectomized-nephrectomized dogs is not one that mimics ACTH.

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REFERENCES


