Direct Stimulatory Effect of Histamine on Aldosterone Secretion of the Perfused Dog Adrenal Gland

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Abstract The left adrenal gland of the hypophysectomized-nephrectomized dog was perfused in situ with the blood from the femoral artery of the hypophysectomized-nephrectomized donor dog. After infusion of histamine (0.1 mg/min for 5 min) into the arterial inflow circuit, the rates of secretion of aldosterone, corticosterone and cortisol (ng/100 mg adrenal wt. each min) increased from the basal level of 0.66±0.21 (mean±S.E.M.) to 2.24±0.45 at 10 min, from 4±1 to 20±4 at 5 min and from 19±5 to 64±15 at 5 min, respectively. The maximal increments above the basal value after the infusion of histamine in the secretory rates of aldosterone, corticosterone and cortisol were 51, 16, and 13% that of the respective steroid after the infusion of ACTH (20 mIU/min for 5 min). The results indicate that histamine has a direct stimulatory effect on the secretion of aldosterone by the adrenal gland as well as on that of corticosterone and cortisol.

Previous studies have shown that the secretions of corticosterone and cortisol by the adrenal gland of the dog in response to intravenous (i.v.) injection of histamine are markedly reduced, but not totally eliminated, by hypophysectomy (HIROSE et al., 1977); direct administration of histamine to the isolated adrenal cells of the dog in vitro results in significant increases in production of corticosterone and cortisol (HIROSE et al., 1978, 1979). In hypophysectomized-nephrectomized dogs, an i.v. injection of histamine results in a marked increase in the secretion of adrenal aldosterone without any changes in plasma concentration of potassium and sodium (AIKAWA et al., 1979). The results suggest that histamine stimulates the secretion of aldosterone by the adrenal gland of the dog partly by a direct stimulatory effect on the adrenal cortex or by some unknown factors other than the renin-angiotensin system (kidney), pituitary factors, plasma potassium and plasma sodium. In the present work, the direct effect of histamine on the secretion of adrenal aldosterone, corticosterone and cortisol was examined by means of

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arterial perfusion of the adrenal gland of hypophysectomized-nephrectomized dog in situ.

MATERIALS AND METHODS

Animals and experimental procedure. Fourteen adult male mongrel dogs were used. A small dog (6.0-12.0 kg body wt.) to be used as the recipient was paired with a large one (10.2-15.7 kg body wt.) to be used as the donor in each experiment. The isolated left adrenal gland of the hypophysectomized-nephrectomized recipient dog was perfused in situ with the blood from the femoral artery of the hypophysectomized-nephrectomized donor dog according to a modification of the method of Hilton et al. (1958). On the day before the experiment, two dogs were anesthetized with pentobarbital sodium (25 mg/kg, i.v.). The hypophyses of both dogs were exposed by transbuccal approach. A cannula was placed in the left lumboadrenal vein of the recipient through the left retroperitoneal lumbar route by the technique of Satake et al. (1927) as modified by Suzuki et al. (1959). A long silk thread was passed loosely around the left lumboadrenal vein medial to the adrenal gland (Fig. 1; 10). The aorta of the recipient was gently lifted with the fingers so as to facilitate the isolation of the origin of the lumbar artery from the aorta through the left retroperitoneal lumbar route, and six lumbar arteries were ligated (Fig. 1; 1, 5, and 8). The left phrenicoabdominal artery lateral to the adrenal gland and the right phrenicoabdominal artery medial to the adrenal gland were also ligated (Fig. 1; 4 and 6). The right kidneys of the recipient and donor were removed through the right retroperitoneal lumbar route. On the day of the experiment, both dogs were re-anesthetized with pentobarbital sodium, hypophysectomized transbucally and nephrectomized on the left side through the retroperitoneal lumbar route. Left splanchnicotomy of the recipient was also performed. To

Fig. 1. Schema of the circulation of the left adrenal gland of the recipient dog. 1, 5 and 8, lumbar arteries; 2, celiac trunk; 3, superior mesenteric artery; 4, left phrenicoabdominal artery; 6, right phrenicoabdominal artery; 7, right renal artery; 9, left renal artery; 10, left lumboadrenal vein; 11, right renal vein; 12, left renal vein. Details of operative procedures were described in the text.

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isolate the left adrenal circulation of the recipient, the celiac trunk (2), superior mesenteric artery (3), aorta just below the renal arteries (7 and 9) and inferior vena cava just below the renal veins (11 and 12) were ligated as shown in Fig. 1. After 2,000 U heparin had been injected i. v. into both dogs to prevent blood clotting, the right renal artery of the recipient and the femoral artery of the donor were cannulated. The femoral artery cannula of the donor was connected to a long arm of a silicon T-tube which was covered with a water jacket and the renal artery cannula of the recipient was connected to a short arm of the T-tube equipped with a screwclamp. The water jacket was maintained at 39°C by circulating water from a constant temperature bath. A side arm of the T-tube was connected with a polyethylene tube to a Statham arterial pressure transducer.

Perfusion experiment and blood sampling. The perfusion experiment started 3 hr after the end of hypophysectomy and nephrectomy. The aorta between the celiac trunk and superior mesenteric artery of the recipient was clamped with hemostatic forceps when the blood from the femoral artery of the donor began to circulate the isolated left adrenal gland of the recipient. The silk thread which had been passed around the left lumboadrenal vein was pulled to direct the adrenal venous flow toward the exterior. The adrenal venous effluent was collected and not allowed to return to the donor. The rate of adrenal perfusion was adjusted with the screwclamp attached to the short arm of the T-tube. Blood lost from the donor was replaced by i. v. infusion of Ringer’s solution containing heparin (2 U/ml). Approximately 35 min after the start of the perfusion, 0.5 mg histamine dihydrochloride (Wako Pure Chemical Industries) dissolved in Ringer’s solution was infused into the arterial inflow circuit over 5 min. Samples of about 3 ml adrenal venous blood were collected 5 min before, and 2.5, 5, 10, 20, and 30 min after the start of infusion of histamine. Thirty-five minutes after the infusion of histamine, 100 mIU ACTH (Armour Pharmaceutical Co., U.S.A.) was infused into the arterial inflow circuit over 5 min. Samples of adrenal venous blood were collected 5 and 10 min after the onset of infusion of ACTH. Completeness of hypophysectomy was checked by inspection of the brain after removal and the left adrenal gland of the recipient was weighed at the end of the experiment.

Fluorescein test. To ascertain that the perfusion system of the adrenal gland was isolated from the systemic circulation of the recipient, 10 mg fluorescein (Chroma Gesellschaft Schmid & Co., Germany) dissolved in 1 ml 0.1 M-NaOH was administered into the jugular vein of the recipient at the end of four experiments. The blood from the jugular vein of the recipient was collected 1, 2, 3, 4, and 5 min after the infusion of fluorescein. Samples of adrenal venous blood were collected before and 0–5 min after the injection of fluorescein. The venous blood samples were centrifuged and the plasma was diluted on 1: 200 (v/v) with 0.05 M NaOH. The fluorescence intensities of the diluted plasma samples were measured by a Hitachi 512 spectrofluorophotometer (extinction 490 nm; emission 510 nm) and expressed as the percentages of that of the plasma sample collected from the jugular
vein of the recipient at 1 min after the injection of fluorescein.

**Biochemical analysis.** The adrenal venous blood was collected in prechilled tubes and centrifuged. After solvent extraction and preliminary purification by column chromatography, aldosterone in samples of adrenal venous plasma was purified by paper chromatography and measured by radioimmunoassay using the slightly modified method of Mayes et al. (1970). All the procedures used have been described in detail previously (Aikawa et al., 1979). Analyses of corticosterone and cortisol in samples of adrenal venous plasma were performed by the fluorometric method subsequent to thin-layer chromatography (Hirose, 1977). The rates of secretion of aldosterone, corticosterone and cortisol were calculated by multiplying the concentration of each steroid in the adrenal venous plasma (ng/ml) by the adrenal plasma flow (ml/100 mg adrenal wt. each minute). Concentrations of potassium, sodium, and chloride of the adrenal venous plasma were determined using a Photovolt PVA-4 electrolyte analyzer.

**Statistical analysis.** The data were subjected to statistical analysis using Student’s paired t-test.

**RESULTS**

To minimize the vasodilative effect of histamine, the rate of adrenal blood flow was adjusted with the screwclamp attached to the inlet of the arterial inflow circuit. The rates of adrenal blood flow 5 min before, and 2.5, 5, 10, 20, and 30 min after the start of infusion of histamine were 0.26±0.05 (mean±S.E.M.), 0.27±0.05, 0.23±0.05, 0.23±0.05, 0.22±0.04, and 0.25±0.04 ml/100 mg adrenal wt. each minute (n=7), respectively. No significant increases in the rate of adrenal blood flow were observed after the onset of infusion of histamine (P>0.05).

The rates of secretion of aldosterone, corticosterone and cortisol of the perfused adrenal gland are presented in Table 1. The secretory rate of aldosterone increased markedly 5 and 10 min after the onset of infusion of histamine and returned to the basal level after 30 min. The secretory rate of aldosterone increased markedly after the infusion of ACTH and the maximal increment above the basal after the injection of histamine was 51% that after the infusion of ACTH. The secretory rates of corticosterone and cortisol also increased significantly soon after the onset of injection of histamine and returned to the basal level after 20 min. The maximal increments above the basal after the infusion of histamine in the secretory rates of corticosterone and cortisol were only 16 and 13% that of the respective steroid after the infusion of ACTH.

The fluorescence intensities of venous plasma samples, collected from the jugular vein of the recipient dog at 1, 2, 3, 4, and 5 min after the injection of fluorescein were 100, 75.9±6.4 (mean±S.E.M.), 66.1±6.7, 61.3±11.9, and 48.2±11.2% (n=4) respectively. The fluorescence intensities of plasma samples of adrenal venous effluent, collected before and 0–5 min after the injection of fluorescein were...
Table 1. Effect of histamine on the secretory rates of aldosterone, corticosterone and cortisol in seven perfused adrenal glands of hypophysectomized-nephrectomized dogs.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Aldosterone (ng/100 mg adrenal wt. each min)</th>
<th>Corticosterone</th>
<th>Cortisol</th>
</tr>
</thead>
<tbody>
<tr>
<td>-5</td>
<td>0.66±0.21</td>
<td>4±1</td>
<td>19±5</td>
</tr>
<tr>
<td>0</td>
<td>Start of injection of histamine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.5</td>
<td>1.12±0.24</td>
<td>14±3***</td>
<td>63±10***</td>
</tr>
<tr>
<td>5</td>
<td>1.91±0.34**</td>
<td>20±4***</td>
<td>64±15**</td>
</tr>
<tr>
<td>10</td>
<td>2.24±0.45**</td>
<td>10±3*</td>
<td>27±6</td>
</tr>
<tr>
<td>20</td>
<td>1.42±0.42</td>
<td>4±1</td>
<td>20±6</td>
</tr>
<tr>
<td>30</td>
<td>0.69±0.17</td>
<td>4±1</td>
<td>14±3</td>
</tr>
<tr>
<td>35</td>
<td>Start of infusion of ACTH</td>
<td></td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>3.64±0.69***</td>
<td>105±17***</td>
<td>355±65***</td>
</tr>
<tr>
<td>45</td>
<td>3.79±0.67***</td>
<td>95±16***</td>
<td>323±64***</td>
</tr>
</tbody>
</table>

Values are means±S.E.M. (*) P<0.05, ** P<0.02, *** P<0.01; compared with the secretory rate before injection of histamine or that before infusion of ACTH). Adrenal weight is 578±43 mg (mean±S.E.M.).

The concentrations of potassium, sodium, and chloride in the plasma of venous effluent of five perfused adrenal glands before infusion of histamine were 3.9±0.1 (mean±S.E.M.), 138±2, and 112±2 mEq/liter respectively. After the infusion of histamine, they were between 3.9±0.1 and 3.7±0.2, between 138±3 and 133±2, and between 116±1 and 112±2 mEq/liter respectively. No significant changes in levels of potassium, sodium and chloride in the plasma were observed after the injection of histamine (P>0.05).

**DISCUSSION**

L’Age et al. (1970) observed that an increase in adrenal blood flow in response to i. v. infusion of histamine in conscious dogs, whose histamine-induced pituitary ACTH release was prevented by pretreatment with dexamethasone, did not significantly elevate adrenal secretion of cortisol. However, in our previous studies using hypophysectomized or hypophysectomized-nephrectomized dog, i. v. administration of histamine caused significant increases in the rates of adrenal secretion of corticosterone and cortisol (Hirose et al., 1977; Aikawa et al., 1979). This discrepancy seemed to be due to the fact that histamine phosphate was infused at the rate of 24 μg/kg over 20 min in L’Age’s study, while histamine dihydrochloride was injected at the rate of 0.1 mg/kg for 1 min in our experiment. Previous studies using dispersed dog adrenal cells have shown that a high dose of histamine has a small yet significant direct stimulatory effect on the adrenal production of cortico-
sterone and cortisol (Hirose et al., 1978, 1979). This was clearly confirmed by the present study using the isolated perfused adrenal gland of the dog.

An infusion of histamine into the arterial inflow circuit of the perfusion system resulted in a marked increase in the secretion of aldosterone. This result agrees with that in in-vivo study using hypophysectomized-nephrectomized dogs (AIKAWA et al., 1979). The possibility that some unknown factors released from extra-adrenal tissues of the recipient by histamine might enter the perfusion system and stimulate the adrenal cortex can be excluded by the finding that fluorescein administered to the systemic circulation of the recipient did not appear in the adrenal venous effluent. Changes in concentrations of potassium and sodium in the plasma of the donor after nephrectomy may also affect the secretion of aldosterone (McCaa et al., 1973). However, no significant changes of concentrations of potassium and sodium in the plasma were observed after the infusion of histamine. Thus, major factors regulating the adrenal secretion of aldosterone, i.e., hypophysial factors, renin-angiotensin system (kidney), concentrations of potassium and sodium in the plasma, and some unknown factors of extra-adrenal, -renal, and -hypophysial origins were completely eliminated in this perfusion system. The direct effect on the adrenal cortex in stimulating aldosterone secretion was verified in the present study.

Recently, induction of anaphylactic shock, which is known to release a large amount of histamine into the blood stream (0.01–1 µg/ml plasma, Csaba et al., 1963), has been shown to increase markedly the adrenal secretion of aldosterone and slightly but significantly that of corticosterone and cortisol in hypophysectomized-nephrectomized dogs (AIKAWA et al., 1981). The plasma histamine level during anaphylactic shock is probably comparable to that after i.v. administration of histamine dihydrochloride in our in-vivo study (0.1 mg/kg for 1 min, AIKAWA et al., 1979). Therefore, both in-vivo studies and the present work suggest that endogenous histamine liberated into the blood stream may have a direct stimulatory effect on the secretion of aldosterone, corticosterone and cortisol by the adrenal gland. It still remains for future inquiry whether or not histamine liberated from the mast cell which can be found in the adrenal cortex plays a physiological role.

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