Calcitonin gene-related peptide modulates adrenal hormones in conscious dogs

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Abstract. The effects of a small dose (2 pmol/kg) of human calcitonin gene-related peptide I on plasma renin activity and hormones, including, aldosterone, ACTH, cortisol, AVP and ANH, were investigated in 14 conscious dogs. In addition, we studied the effects of calcitonin gene-related peptide on aldosterone secretion when it is stimulated by angiotensin II and ACTH. An intravenous bolus injection of 2 pmol/kg of calcitonin gene-related peptide raised plasma renin activity (by 216%, p<0.05), ACTH (by 85%, p<0.05), AVP (by 89%, p<0.05), and ANH (by 36%, p<0.05). Despite the elevation of plasma renin activity, aldosterone was decreased (by 52%, p<0.05). Cortisol did not change significantly. Infusion of 1 pmol·kg⁻¹·min⁻¹ of angiotensin II produced an elevation of aldosterone (by 186%, p<0.01), which was completely inhibited by pretreatment with an injection of 2 pmol/kg of calcitonin gene-related peptide. On the other hand, aldosterone secretion stimulated by ACTH was not altered significantly by pretreatment with an injection of 2 pmol/kg of calcitonin gene-related peptide. These results suggest that calcitonin gene-related peptide inhibits aldosterone secretion, especially when aldosterone is stimulated by angiotensin II. In addition, calcitonin gene-related peptide may be involved as an endocrine modulator in the physiological control of other several hormones closely related to the hemodynamics.

Calcitonin gene-related peptide (CGRP) is a 37-amino acid polypeptide that results from the tissue-specific processing of the primary RNA transcript of the calcitonin gene (1). CGRP is detected in the systemic circulation (2,3), and the plasma level of CGRP increases during normal human pregnancy (4), in endotoxemia (5) or after sensory nerve injury (6).

CGRP has been reported to have a potent vasodilatory action on various isolated vascular tissue preparations (7-9). Intravenous administration of CGRP induced potent dose-related hypotensive and tachycardiac responses in rats and human subjects (10,11).

In addition to its potent vasoactive property, CGRP has an effect on hormonal regulation. CGRP immunoreactivity and specific binding sites for the peptide have been detected in the heart, the adrenal gland, the hypothalamus and the pituitary gland as well as in other organs (12,13). CGRP was shown to stimulate renin secretion both in normal human subjects and in rat renal juxtaglomerular cells (14). More recently CGRP has been found to exhibit stimulatory effects on ANH secretion in vitro in the isolated rat atria (15,16). More recently, we reported that CGRP had an inhibitory action on aldosterone secretion in isolated glomerulosa cells (17). However, possible interaction of CGRP with angiotensin II and ACTH on the adrenal gland in vivo received little attention. Therefore, this study was designed to examine the endocrine responses to intravenous administration of a small dose of CGRP with a specific focus on adrenal hormones, especially aldosterone, in conscious dogs.
Material and Methods

Surgery and animal preparation
Fourteen male mongrel dogs weighing between 12 and 18 kg were used in these experiments. Under pentobarbital anesthesia, catheters (Tygon, Akron, OH) were placed in the right iliac artery and vein as previously described (18,19). The catheters were inserted sc, externalized through the back between the scapula and secured. When the dogs woke up, they were placed in individual cages to allow free mobility. During the recovery and experimental period, all dogs were fed a diet (Oriental Yeast Co, Tokyo, Japan) containing 70 mmol of sodium and 60 mmol of potassium daily. Free access to tap water was permitted at all times. All studies were performed with dogs in the fasting morning stage, in a quiet room, following a training period of approximate 2-3 weeks after the operation. Each experiment was begun after a 30-min stabilization period.

Experimental protocol
A small dose of CGRP (2 pmol/kg), which had no significant effects on mean arterial pressure in hemodynamic studies, was employed in the experiments. The study was begun at 8.00 h in the fasting condition. Fourteen dogs were divided into two groups and were studied in the following manner: Group A (N=7) received iv injection of 3 ml of normal saline as a control, and group B (N=7) received iv injection of 2 pmol/kg of human CGRP II (Peptide Institute Inc, Osaka, Japan) dissolved in 3 ml of normal saline after a 30-min stabilization. Ten ml of blood was collected for determinations of plasma renin activity (PRA), plasma aldosterone concentration, ANH, AVP, cortisol, and serum electrolytes before and at 15 and 30 min after the bolus injection.

In addition, 10 dogs were divided into two groups and we studied the effects of CGRP on responses of aldosterone to angiotensin II (N=5) and ACTH (N=5). Continuous iv infusion of 1 pmol·kg\(^{-1}\)·min\(^{-1}\) angiotensin II (Peptide Institute Inc, Osaka, Japan) was administered in a volume of 0.15 ml/min over a period of 30 min using Harvard syringe infusion pump (Model 2620, Harvard apparatus). Ten ml of blood was taken for determinations of plasma aldosterone concentration, ANH, PRA and potassium from the iliac artery before and at 15 and 30 min after infusion. After a week, we examined the effects of pretreatment with 2 pmol/kg of CGRP on plasma aldosterone concentration and cortisol secretion stimulated by ACTH, using the same dogs.

In each experiment, mean arterial pressure and heart rate were measured directly via an iliac arterial catheter connected to a transducer (Nihon Kohden Instrument, Tokyo, Japan). These data were recorded and analyzed by Macintosh computer, using analog-digital instruments of MacLab™.

Measurements of hormones
PRA was determined by radioimmunoassay using kits from Dinabott Radioisotope Inst, Tokyo, Japan. The intra- and inter-assay coefficients of variation (CV) were 5.5-6.9 and 3.7-8.2%, respectively (20). ANH was measured by RIA using kits from Eiken Chemical Co, Ltd, Tokyo, Japan (21,22). Plasma aldosterone concentration and cortisol were measured by RIA using kits from Daiichi Radioisotope Lab, Ltd, Tokyo, Japan, as previously described (19,23). Plasma ACTH and AVP concentrations were measured by RIA using kits from Mitsubishi Yuka Bio-Clinical Lab, Inc, Tokyo, Japan. The intra- and inter-assay CV of ACTH were 3.8-6.7 and 5.7-7.5% (24) and those of AVP were 8.3-10.3 and 7.8-10.8% (25), respectively. Serum electrolytes were measured with a flame photometer.

Statistical analysis
All data were expressed as the mean±standard error of the mean. Two-way and one-way analysis of variance followed by Dunnet’s test and Student’s t-test for paired data were used for statistical analysis. A p-value of less than 0.05 was considered significant. All animal studies were conducted in accordance with institutional animal care guidelines.

Results
A significant reduction of plasma aldosterone concentration was observed at 30 min (190±34 to 91±31 pmol/l) but not at 15 min after CGRP injection (Table 1), whereas PRA increased significantly at 15 min (0.6±0.1 to 1.9±0.3 pmol of angiotensin I·ml\(^{-1}\)·h\(^{-1}\)). ANH was also raised significantly at 15 min (22±2 to 30±2 pmol/l, p<0.05), but not at 30 min (Table 1). AVP was significantly increased at 15 and 30 min after injection (by 89 and 103%, respectively).

ACTH was significantly raised at 15 and 30 min (by 85 and +111%, respectively), but cortisol was not changed by the CGRP injection (Table 1). No significant changes occurred in the serum levels of Na and K during the experiment.

Continuous infusion of 1 pmol·kg\(^{-1}\)·min\(^{-1}\) angiotensin II produced an elevation of plasma al-
Table 1.
Effects of a bolus injection of CGRP on PRA, aldosterone, ANH, ACTH, cortisol, and AVP in conscious dogs.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Before injection</th>
<th>15</th>
<th>30</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRA (pmol of angiotensin I · ml⁻¹· h⁻¹)</td>
<td>0.6±0.1</td>
<td>1.9±0.3&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>1.4±0.6</td>
</tr>
<tr>
<td>Aldosterone (pmol/l)</td>
<td>190±34</td>
<td>140±42</td>
<td>91±31&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>ANH (pmol/l)</td>
<td>22±2</td>
<td>30±2&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>26±1</td>
</tr>
<tr>
<td>ACTH (pmol/l)</td>
<td>6.1±0.8</td>
<td>11.3±1.7&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>12.9±1.6&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cortisol (nmol/l)</td>
<td>62±4</td>
<td>59±3</td>
<td>59±3</td>
</tr>
<tr>
<td>AVP (pmol/l)</td>
<td>2.8±0.5</td>
<td>5.3±0.8</td>
<td>5.7±1.1&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Data presented as mean±SEM. *p<0.05 vs pre-injection values, †p<0.05 vs control values.

Table 2.
Effects of CGRP on plasma renin activity and ANH during angiotensin II infusion.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Before injection</th>
<th>15</th>
<th>30</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANH (pmol/l)</td>
<td>27±3</td>
<td>25±5</td>
<td>25±5</td>
</tr>
<tr>
<td>PRA (pmol of angiotensin I·ml⁻¹·h⁻¹)</td>
<td>0.8±0.3</td>
<td>0.7±0.4</td>
<td>0.6±0.3</td>
</tr>
<tr>
<td>Angiotensin II</td>
<td>28±2</td>
<td>27±2</td>
<td>29±3</td>
</tr>
<tr>
<td>Angiotensin II + CGRP</td>
<td>0.6±0.2</td>
<td>0.7±0.2</td>
<td>0.5±0.2</td>
</tr>
</tbody>
</table>

Data presented as mean±SEM.

The bolus injection of ACTH induced increases in plasma aldosterone concentration (by 677 pmol/l, p<0.01) and cortisol (by 270 nmol/l, p<0.01). Pretreatment with CGRP induced a mild but not significant inhibition of this stimulated aldosterone secretion (Fig. 2). The increase in cortisol was not inhibited significantly by the CGRP injection (Fig. 3).

Hemodynamic data including mean arterial...
pressure and heart rate are presented in Table 3 and 4. The bolus injection of 2 pmol/kg of CGRP did not induce any significant changes in mean arterial pressure and heart rate. Continuous infusion of 1 pmol·kg⁻¹·min⁻¹ angiotensin II with or without pretreatment with CGRP did not change mean arterial pressure and heart rate significantly.

Discussion

The present study showed that bolus injection of CGRP increased PRA, ACTH, AVP and ANH. Plasma aldosterone fell, whereas cortisol remained unchanged. The rise in aldosterone in response to infused angiotensin II was blocked by CGRP, whereas the response to ACTH was not.

Regulation of aldosterone secretion is complex in terms of the number of secretagogues and the number of second messengers utilized by these secretagogues. Agents that stimulate aldosterone secretion can be divided into five subgroups, the angiotensins, potassium, pituitary factors, neurotransmitters, and others (26). Factors that inhibit aldosterone secretion are ANH, dopamine, ouabain-like natriuretic factor and somatostatin (26-28). In the present study, iv administered CGRP induced a decrease in aldosterone and increases in both aldosterone secretagogues and inhibitors.

ACTH and AVP increased in response to the bolus injection of CGRP. CGRP has been found to have several actions in the central nervous system and the pituitary gland (29-31). Brasilis et al. (32) showed that a hypotensive dose (5 pmol/kg) of CGRP increased arginine vasopressin in conscious sheep. A recent study has revealed an inhibitory effect of CGRP on growth hormone (30). Because the dose of 2 pmol/kg of CGRP employed in our study did not induce significant changes in mean arterial pressure and heart rate, the present result suggests that CGRP may have a direct action on the pituitary gland, but other factors such as neural reflex cannot be ruled out.

Table 3.
Changes in mean arterial pressure (mmHg) in response to CGRP alone and angiotensin II with or without pretreatment with CGRP in conscious dogs.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>0</th>
<th>1</th>
<th>5</th>
<th>15</th>
<th>30</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>92±3</td>
<td>89±4</td>
<td>89±4</td>
<td>90±3</td>
<td>88±3</td>
</tr>
<tr>
<td>CGRP</td>
<td>94±4</td>
<td>94±4</td>
<td>92±3</td>
<td>92±3</td>
<td>95±4</td>
</tr>
<tr>
<td>Angiotensin II</td>
<td>90±4</td>
<td>92±3</td>
<td>90±3</td>
<td>91±4</td>
<td>93±3</td>
</tr>
<tr>
<td>Angiotensin II + CGRP</td>
<td>89±4</td>
<td>92±4</td>
<td>90±4</td>
<td>88±3</td>
<td>92±3</td>
</tr>
</tbody>
</table>

Data presented as mean ± SEM

Table 4.
Changes in heart rate (beats/min) in response to CGRP alone and angiotensin II with or without pretreatment with CGRP in conscious dogs.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>0</th>
<th>1</th>
<th>5</th>
<th>15</th>
<th>30</th>
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<tr>
<td>Control</td>
<td>98±8</td>
<td>100±9</td>
<td>96±8</td>
<td>94±7</td>
<td>96±9</td>
</tr>
<tr>
<td>CGRP</td>
<td>90±9</td>
<td>92±10</td>
<td>93±9</td>
<td>95±8</td>
<td>94±7</td>
</tr>
<tr>
<td>Angiotensin II</td>
<td>89±8</td>
<td>92±8</td>
<td>86±6</td>
<td>93±7</td>
<td>87±7</td>
</tr>
<tr>
<td>Angiotensin II + CGRP</td>
<td>92±6</td>
<td>90±9</td>
<td>94±9</td>
<td>90±6</td>
<td>91±8</td>
</tr>
</tbody>
</table>

Data presented as mean ± SEM.

Fig. 3.
Effects of CGRP on stimulation of cortisol secretion by ACTH. ○ = ACTH (25 units), ● = ACTH (25 units) with CGRP (2 pmol/kg) pretreatment. Data presented as mean±SEM ** p<0.01 vs pre-injection values.
Despite the increase in ACTH and AVP, aldosterone was decreased, suggesting CGRP decreased aldosterone secretion directly or indirectly. Pretreatment with 2 pmol/kg of CGRP partially but not significantly blunted aldosterone secretion at 30 min after ACTH injection. Aldosterone levels at this time were variable in five dogs. We could not clarify the cause of the variabilities of aldosterone at 30 min after ACTH and CGRP injections. Effects of CGRP on aldosterone secretion stimulated by ACTH remain to be elucidated and further studies are needed.

Plasma cortisol did not rise in spite of a rise in ACTH with CGRP given alone (Table 1). However, CGRP did not inhibit the plasma cortisol response to injection of ACTH. These observations suggest that there may be a dose-response relationship of the effect of CGRP on ACTH-stimulated cortisol secretion, i.e. at lower doses of ACTH CGRP inhibits the cortisol response, but at high ACTH concentrations CGRP has minimal effects. Further studies using low doses of ACTH are needed.

This is the first study which demonstrated inhibitory effects of a small dose of CGRP on both basal and angiotensin II-stimulated aldosterone secretion. Aldosterone secretion is inhibited by several substances including ANH, dopamine and others (26-28). In the present study these variables, including PRA, ANH and potassium, did not change significantly during angiotensin II infusion with or without CGRP pretreatment. Our latest study has clearly demonstrated that CGRP inhibits aldosterone secretion in isolated adrenal glomerulosa cells (17). These results strongly suggest that a small dose of CGRP directly inhibits aldosterone secretion also in conscious dogs, especially when aldosterone is stimulated by angiotensin II. These findings agree well with our in vitro study (17). However, Itabashi et al. (33) failed to demonstrate the inhibitory action of CGRP on aldosterone secretion, because they used a much larger dose (1 nmol·kg⁻¹·min⁻¹) of CGRP, which induced a marked hypotension and a large increase in PRA. In addition, the discrepancy between the Itabashi study and ours may be due to the responses in ACTH. In Itabashi's study, there might have been a great rise in ACTH, probably owing to the marked fall in blood pressure. This elevated ACTH could have stimulated aldosterone synthesis. By contrast, in the present study, since blood pressure levels were not changed after the bolus injection of CGRP, the elevation of ACTH might be less than in their study. Furthermore, the data from the present study in which CGRP did not significantly inhibit aldosterone production after an injection of ACTH, would support the explanation.

Aldosterone secretion is regulated by multiple factors, including 1. cAMP formation, 2. regulation of cytosolic free calcium and protein kinase C, 3. cGMP formation, 4. ion channel modification, and 5. modification of the membrane potential. The mechanisms and physiological role of this inhibitory action of CGRP on aldosterone secretion are still unknown. Lastly, in our previous study (17), cAMP did not show any significant changes after CGRP administration. Therefore, it is suggested that a transduction mechanism other than that described above may play a role in the responses of aldosterone to CGRP. The transduction mechanism utilized by angiotensin II for increasing aldosterone secretion is regulation of cytosolic free calcium and protein kinase C activity through the phosphatidylinositol system. In the stimulation of aldosterone by angiotensin II, the increase in cytosolic calcium is dose-dependent and is well correlated with aldosterone production (26). Recently, Kline et al. (34) reported that CGRP relaxed rat tale artery helical strips in vitro in an intracellular calcium-dependent manner. Taken together, our findings suggest that CGRP inhibits aldosterone secretion by reducing the mobilization of calcium in the glomerulosa cells. However, recently Kurtz et al. (35) reported that cytosolic free calcium was not altered by CGRP in the mesangium cells. Further study is needed to clarify the mechanism of this inhibitory action of CGRP.

The present study showed that a small dose of CGRP raised PRA significantly. Kurtz et al. (14) reported that CGRP stimulated renin release in vivo in normal human volunteers and in vitro in isolated rat renal juxtaglomerular cells. The present data are in good accordance with their results.

In addition to these hormonal changes, ANH secretion is also stimulated by a small dose of CGRP. Recently, CGRP has been found to stimulate ANH secretion in vitro (15,16). In this regard, CGRP may induce ANH secretion without hemodynamic changes.

To summarize our data: an iv bolus of 2 pmol/kg of CGRP inhibited both basal and angiotensin II-stimulated aldosterone secretion significantly and increased PRA, ANH, AVP and ACTH, which
might be involved in the regulation of hemodynamics indirectly.

In conclusion, CGRP may participate in the regulation of adrenal hormones as well as in the control of vascular tones.

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References


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