Contributions of Central Sympathetic Neural Activity to Furosemide-Induced Increases in Plasma Renin Activity and Noradrenaline

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SUMMARY
To evaluate the role of the central nervous system on the furosemide-induced increases in plasma noradrenaline (PNA), renin activity (PRA), and aldosterone concentration (PAC), central vasoactive sympathetic structures were inhibited by intravertebral artery infusion of colnidine. Intravertebral artery infusion of clonidine (0.06 μg/Kg/min) significantly reduced basal PNA, heart rate, and arterial pressure, while both PRA and PAC were increased. Intravenous infusion of the same dose of clonidine caused no significant changes in PNA, PRA, and PAC. Intravertebral artery infusion of clonidine (0.02 or 0.1 μg/Kg/min) significantly suppressed the furosemide-induced increases in PNA and heart rate, and induced a drop in arterial pressure. Although the furosemide-induced increase in PRA was suppressed by intravertebral artery infusion of clonidine, the furosemide-induced increase in PAC was not affected. These results suggest that the furosemide-induced increase in PNA may be mediated by the central sympathetic nervous system and that some of the furosemide-induced increase in PAC may be mediated by central sympathetic neural activation.

Additional Indexing Words:
Central nervous system Renin-angiotensin system Plasma noradrenaline Intravertebral artery infusion Clonidine

The renin-angiotensin system may contribute to the regulation of blood pressure via a direct action on blood vessels and fluid and electrolyte balance, and by indirect cardiovascular effects, mediated through the sympathetic nervous system.1-4 On the other hand, it has been demonstrated...
that renin release from the kidney may be stimulated by an increase in sympathetic neural activity.\(^5\)\(^{–}\)\(^7\) Recently, it has been reported that sodium and volume depletion, induced by low sodium intake and/or diuretics, increase plasma noradrenaline (PNA), plasma renin activity (PRA) and the aldosterone concentration (PAC).\(^8\)\(^{–}\)\(^10\) Therefore, the interaction between the renin-angiotensin system and the sympathetic nervous system may play a significant role in the regulation of blood pressure in the sodium and volume depleted state. It was previously reported that the furosemide-induced increase in PNA may be mediated by the renin-angiotensin system.\(^11\)

Clonidine is an \(\alpha\)-adrenergic agonist, known to reduce blood pressure and heart rate. Several investigators have postulated that the hypotensive effect of clonidine may be due to central inhibition of the sympathetic nervous system.\(^12\)\(^{–}\)\(^14\) The failure of clonidine to lower blood pressure in subjects with complete cervical spinal cord transections provides further evidence that the hypotensive effect of the drug is centrally mediated, with a probable site of action at the level of the brain stem.\(^15\) In this study, the role of the central sympathetic nervous system in the furosemide-induced increases in PRA and PNA was assessed by intravertebral artery infusion of clonidine.

**Materials and Methods**

Healthy mongrel dogs of both sexes, weighing from 7 to 19 Kg, were anesthetized with \(\alpha\)-chloralose (100 mg/Kg, i.v.). A catheter was inserted into the femoral artery for recording the arterial blood pressure with a Statham P37b pressure transducer and the femoral vein was cannulated for blood sampling and the injection of furosemide. The heart rate (HR) was obtained from the blood pressure tracings. A polyethylene cannula was inserted into one vertebral artery, keeping the flow in the artery, and it was used for the infusion of clonidine (Boehringer Ingelheim) into the vertebro-basilar circulation. Clonidine was diluted with 0.9% saline and infused at a rate of 0.02 or 0.1 µg/Kg/min for 40 min with a Harvard infusion pump. The infusion rate of the solution was 0.25 ml/min. Ten min after the onset of the clonidine infusion, four intravenous injections of furosemide (1.0 mg/Kg) were made at 10 min intervals. Blood samples were collected during the control period, 10, 25, and 40 min after the infusion of clonidine and during the recovery period (30 min after the cessation of the clonidine infusion). As a control experiment, clonidine (0.06 µg/Kg/min) was infused intravenously.

Both PRA and PAC were measured by radioimmunoassay, using a Dainabot RIA kit. PNA was measured by the radioenzymatic method of Henry et al.\(^16\) Blood samples were collected in cold tubes containing EDTA
(1.0 mg/ml), centrifuged at 4°C, and the plasma was kept frozen at −20°C until the assay.

A paired Student's t test was used for within-group comparisons and a non-paired t test was used for between-group comparisons. A p value of at least 0.05 was taken as the level of statistical significance. The results were shown as the mean±SEM.

Results

1. Effects of furosemide on PRA, PAC, and PNA.
When furosemide (1.0 mg/Kg) was injected intravenously PNA was

Table I. Effects of Furosemide (1.0 mg/Kg, i.v., 4 times every 10 min) on Mean Blood Pressure (MBP), Heart Rate (HR), Plasma Renin Activity (PRA), Aldosterone Concentration (PAC), and Noradrenaline (PNA) in 5 α-Chloralose Anesthetized Dogs

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>MBP mmHg ±SEM</th>
<th>HR beats/min ±SEM</th>
<th>PRA ng/ml/hr ±SEM</th>
<th>PAC ng/dl ±SEM</th>
<th>PNA ng/ml ±SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>-10</td>
<td>114.4 ± 7.0</td>
<td>117.6 ± 12.1</td>
<td>5.5 ± 1.1</td>
<td>13.1 ± 4.2</td>
<td>0.26 ± 0.26</td>
</tr>
<tr>
<td>0</td>
<td>113.7 ± 6.2</td>
<td>117.8 ± 14.1</td>
<td>5.6 ± 1.1</td>
<td>13.2 ± 4.7</td>
<td>0.28 ± 0.04</td>
</tr>
<tr>
<td>15</td>
<td>119.0 ± 6.2**</td>
<td>130.4 ± 13.6*</td>
<td>12.5 ± 2.5**</td>
<td>20.8 ± 6.6</td>
<td>0.45 ± 0.05**</td>
</tr>
<tr>
<td>30</td>
<td>118.0 ± 5.8**</td>
<td>144.0 ± 14.8*</td>
<td>14.3 ± 3.5*</td>
<td>31.1 ± 10.8*</td>
<td>0.64 ± 0.06**</td>
</tr>
<tr>
<td>60</td>
<td>116.2 ± 5.3</td>
<td>155.2 ± 13.1**</td>
<td>11.7 ± 1.5**</td>
<td>42.2 ± 13.2**</td>
<td>0.64 ± 0.07**</td>
</tr>
</tbody>
</table>

mean±SEM * p<0.05 ** p<0.01 compared to control values.

Table II. Effects of Intravertebral Artery and Intravenous Infusion of Clonidine (0.06 μg/Kg/min) on Mean Blood Pressure (MBP) and Heart Rate (HR) in α-Chloralose Anesthetized Dogs

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>MBP mmHg ±SEM</th>
<th>HR beats/min ±SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>-10.2±2.3**</td>
<td>-10.7±2.6**</td>
</tr>
<tr>
<td>25</td>
<td>-14.3±2.3**</td>
<td>-19.4±2.3**</td>
</tr>
<tr>
<td>40</td>
<td>-14.8±2.8**</td>
<td>-20.0±3.8**</td>
</tr>
<tr>
<td>70 (recovery)</td>
<td>-8.0±4.6*</td>
<td>-7.1±6.8</td>
</tr>
</tbody>
</table>

mean±SEM * p<0.05 ** p<0.01
significantly increased from the control value of 0.28±0.04 ng/ml to 0.64±0.06 ng/ml (p<0.01). Both PRA and PAC also increased from 5.6±1.1 ng/ml/hr to 14.3±3.5 ng/ml/hr (p<0.05) and from 13.2±4.7 ng/dl to 31.1±10.8 ng/dl (p<0.05), respectively. During furosemide administration, HR increased markedly and a slight, but significant elevation in mean blood pressure (MBP) was observed. These changes are summarized in Table I.

2. Effects of clonidine on MBP, HR, PRA, PAC, and PNA.

The effects of intravertebral artery and intravenous infusions of clonidine on MBP and HR are shown in Table II. In α-chloralose anesthetized dogs, an intravertebral artery infusion of clonidine (0.06 μg/Kg/min, for 40 min) reduced MBP (−14.8±2.8 mmHg, p<0.01) and HR (−20.0±3.8 beats/min, p<0.01). Intravenous infusions of the same dose of clonidine did not affect MBP and HR. As shown in Fig. 1, intravertebral artery infusions of clonidine (0.06 μg/Kg/min, for 40 min) produced a significant decrease in PNA (−29.6±6.4%, p<0.01). By contrast, PRA and PAC were increased by intravertebral artery infusions of clonidine. However, intravenous infusions of clonidine did not cause any significant changes in PNA, PRA or PAC.

![Fig. 1. Effects of intravertebral artery and intravenous infusion of clonidine (0.06 μg/Kg/min) for 40 min on plasma noradrenaline (PNA), renin activity (PRA), and aldosterone concentration (PAC).](image1)

![Fig. 2. Effects of intravertebral artery infusion of clonidine on the furosemide-induced increase in plasma noradrenaline (PNA).](image2)
3. Effects of intravertebral artery infusion of clonidine on the furosemide-induced increase in PRA, PAC, and PNA.

The furosemide-induced increase in PNA was significantly inhibited by intravertebral artery infusions of clonidine (Fig. 2). In a dose of 0.02 μg/Kg/min, intravertebral artery infusions of clonidine suppressed the furosemide-induced increase in PNA from the control value of 149.4±21.1% to 31.1±10.5% 40 min after the infusion. Furthermore, clonidine (0.1 μg/Kg/min) infusions via the vertebral artery completely suppressed the furosemide-induced increase in PNA. The effects of intravertebral artery infusions of clonidine on the furosemide-induced increase in PRA and PAC are shown in Fig. 3. The furosemide-induced increase in PRA was significantly inhibited by intravertebral artery infusions of 0.02 and 0.1 μg/Kg/min of clonidine at 25 and 40 min after the onset of the infusion. However, intravertebral artery infusions of clonidine did not suppress the furosemide-induced increase in PAC.

![Fig. 3. Effects of intravertebral artery infusion of clonidine on the furosemide-induced increase in plasma renin activity (PRA) and aldosterone concentration (PAC).](image)

![Fig. 4. Effects of intravertebral artery infusion of clonidine on mean blood pressure (MBP) and heart rate (HR) during furosemide administration.](image)
in PAC in doses of 0.02 and 0.1 μg/Kg/min. Although furosemide administration produced significant increases in MBP and HR, the combined use of furosemide and clonidine in doses of 0.02 and 0.1 μg/Kg/min changed MBP by -3.5 mmHg and -12.4 mmHg, and HR by 3.5 beats/min and -32.6 beats/min, respectively, 40 min after the infusion of clonidine (Fig. 4).

**DISCUSSION**

The regulation of blood pressure may involve many factors, including the renin-angiotensin system and the sympathetic nervous system. However, none of these factors is sufficient to explain the pathogenesis of hypertension. Various investigators demonstrated that angiotensin II may augment the sympathetic neural activity by affecting the central and peripheral sympathetic nervous system. Recently, some investigators have reported that PNA and PRA are increased by sodium restriction and/or the use of diuretics. The present study was performed to evaluate the role of central sympathetic neural activity on the furosemide-induced activation of the renin-angiotensin system and the sympathetic nervous system.

In this study, the administration of furosemide produced a significant increase in PNA, PRA and PAC. In addition, a slight elevation in blood pressure was observed, despite the furosemide-induced diuresis and natriuresis. We previously reported that the furosemide-induced increase in PNA was dose-dependently inhibited by intravenous infusions of an angiotensin II antagonist, Sar1-, Ile8-angiotensin II, indicating a significant role of the renin-angiotensin system on the increased sympathetic neural activity.

It has been shown that the cardiovascular effects of clonidine are due to a centrally mediated decrease in sympathetic neural activity. In the present study, intravenous infusions of clonidine (0.06 μg/Kg/min) did not cause any significant changes in PRA, PAC, and PNA. The MBP and HR were also unaffected by intravenous infusion of clonidine. On the other hand, intravertebral artery infusion of the same dose of clonidine produced a significant decrease in PNA, and lowered MBP and HR. These results provide additional support for the concept that the clonidine-induced reductions in blood pressure and HR may be due to a central sympatholytic action. However, both PRA and PAC were significantly increased by intravertebral artery infusion of clonidine. It has been considered that the administration of clonidine was associated with a reduction in PRA, which appeared to be caused by decreased sympathetic neural activity. However, Wing et al reported that PRA was significantly increased by a single oral dose of clonidine (300 μg/day) in normotensive subjects and that the
elevation in PRA may be secondary to the fall in blood pressure. Nolan and Reid\textsuperscript{23} have reported that clonidine may have two effects on renin release, i.e., an inhibitory effect, which results from decreased sympathetic neural activity and a stimulatory effect, due to renal vasoconstriction. Thus, the observed increase in PRA in this study may be secondary to the fall in blood pressure, which activates renal baroreceptor reflexes and stimulates renin release from the kidney.

The intravertebral artery infusion of a higher dose of clonidine (0.1 µg/Kg/min) produced a more significant suppression of the furosemide-induced increase in PNA than a lower dose level (0.02 µg/Kg/min). The increase in PRA after furosemide administration was also inhibited by intravertebral artery infusion of clonidine. These observations support the concept that clonidine reduces the degree of stimulated renin release at a central site.\textsuperscript{24} However, other stimuli, such as a fall of perfusion pressure in the kidney (which stimulates renin release), may override the inhibitory effects of clonidine on renin release, since there was no significant difference between the suppression of PRA by a dose of 0.02 µg/Kg/min and 0.1 µg/Kg/min of clonidine.

On the other hand, the furosemide-induced increase in PAC was unaffected by intravertebral artery infusion of clonidine. Weber et al\textsuperscript{20} reported that chronic oral administration of clonidine to hypertensive patients reduced PRA and urinary aldosterone excretion, suggesting that the reduction in aldosterone excretion resulted from the clonidine-induced inhibition of renin release. However, Boyar et al\textsuperscript{25} recently reported that plasma aldosterone and cortisol levels were not affected by clonidine therapy in hypertensive adolescents, despite a significant reduction in PRA and PNA. Although the major effect on aldosterone secretion is possibly mediated through the renin-angiotensin system, the present results suggest that the regulation of aldosterone secretion during the combined use of furosemide and clonidine may be mediated by other mechanisms. It is not clear whether this is a direct action of clonidine on the adrenal cortex, or a consequence of its hypotensive action, or a result of a hormonal mechanism such as adrenocorticotropic hormone or dopamine.

The furosemide-induced increase in MBP and HR was suppressed significantly by intravertebral artery infusion of clonidine. The fall in MBP and HR was more marked with a dose of 0.1 µg/Kg/min than with a dose of 0.02 µg/Kg/min of clonidine when compared to the clonidine-induced decrease in PNA. These changes in PNA, MBP, and HR indicate that intravertebral artery infusion of clonidine may suppress central sympathetic neural activity and result in a decrease in MBP and HR. Furthermore, the clonidine-induced decrease in PRA may partially contribute to the fall in MBP.
in the combined use of furosemide and clonidine. Since it has been shown that the centrally mediated cardiovascular effects of angiotensin II are significantly suppressed by intravertebral artery infusion of clonidine,26) the present results indicate that the central actions of angiotensin II may contribute to the furosemide-induced increase in PNA, and that the stimulation of renin release by furosemide may be partially mediated through the activation of the sympathetic nervous system in the brain.

REFERENCES

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