Role of Kallikrein in the Hypotensive Effect of Captopril after Sympathetic Stimulation of the Rat Submandibular Gland

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SUMMARY. After cervical sympathetic nerve stimulation, the rat submandibular gland releases a significant amount of kallikrein into the circulation. To determine whether this glandular kallikrein has kininogenase activity (kinin-generating capabilities) in the peripheral circulation and whether it plays a role in blood pressure regulation, we studied the effect of captopril on blood pressure in 48 hour-nephrectomized rats with and without prior sympathetic stimulation of the submandibular gland. Administration of captopril 10 minutes after gland stimulation resulted in a mean blood pressure decrease (ΔBP) of 43 ± 8.3 mm Hg (P < 0.01), whereas the BP did not decrease significantly in the rats in which the gland was not stimulated (ΔBP = 3.3 ± 0.5; P > 0.05). To confirm that the effect of captopril was due to a blockage of kinin destruction generated by glandular kallikrein and not to the inhibition of angiotensin II formation, we determined the effect of captopril after gland stimulation in rats pretreated with either IgG from nonimmunized rabbits (normal-IgG), or IgG from rabbits immunized against kinins (antikinin-IgG) or kallikrein (antikallikrein-IgG). Normal-IgG did not significantly alter the hypotensive effect of captopril (ΔBP = 30 ± 7.7; P < 0.01), while pretreatment with antikinin or antikallikrein almost completely blocked its hypotensive effect (ΔBP = 5.8 ± 1.9 and 6.4 ± 0.4, respectively). In the latter two groups, the decrease in BP was significantly smaller (P < 0.001) than in the groups that were not pretreated or in the group pretreated with normal-IgG. These data suggest that, upon adrenergic stimulation of the submandibular gland, glandular kallikrein released into the vascular compartment has kininogenase activity in the peripheral circulation. The kinins released by glandular kallikrein induced hypotension when their breakdown was prevented by the kininase II inhibitor, captopril. These results suggest that kinins may be responsible in part for the antihypertensive effect of captopril in situations in which glandular kallikrein in blood is increased. (Circ Res 51: 385-390, 1982)

GLANDULAR KALLIKREINS are serine proteases which release the potent vasodilator peptides, kinins, from plasma substrate called kininogens (Frey et al., 1968). There are two main classes of kallikrein: plasma and glandular. The plasma kallikrein differs from the glandular kallikrein, not only in its biochemical and immunological characteristics, but, also, in its functions. The plasma kallikrein system participates in the blood-clotting and fibrinolysis mechanisms, whereas the glandular kallikrein system may participate in the local regulation of blood flow in organs rich in kallikrein (Ørstavik et al., 1982). Glandular kallikreins may also help to regulate arterial blood pressure, but direct evidence for such a role has not yet been presented (Carretero and Scicli, 1981; Ørstaffik, 1981). Glandular kallikreins are found in the salivary and sweat glands, pancreas, intestine, and kidney, and in the exocrine secretion of these organs. Recently, we and others (Nustad et al., 1979; Rabito et al., 1979; Johansen et al., 1981; Lawton et al., 1981; Rabito et al., 1982a) have also demonstrated the presence of glandular kallikrein in plasma.

When a kallikrein-rich organ like the rat submandibular gland is stimulated, significant amounts of this enzyme are released into the circulation (Ørstaffik, Johansen, Nustad, unpublished data; Rabito et al., 1982b). Although it has been assumed that this enzyme is rapidly inactivated by plasma protease inhibitors, recent reports suggest that some glandular kallikrein may circulate in the active form (Geiger et al., 1980; Johansen et al., 1981). Therefore, it is possible that submandibular gland kallikrein released into the circulation after sympathetic stimulation of the gland has kininogenase activity (kinin-generating capability) and that the kinins generated in the peripheral circulation and/or interstitial space play a role in blood pressure regulation in special circumstances.

To test this hypothesis, we have studied the blood pressure effect of the kininase II- or angiotensin I-converting enzyme inhibitor, captopril, on rats with and without prior sympathetic stimulation of the submandibular gland. To minimize the effect of captopril on the renin-angiotensin system, we nephrectomized all the rats 48 hours before the study. To investigate further whether the hypotensive effect of captopril after the sympathetic stimulation of the submandibular gland is mediated by the glandular kallikrein-
kinin system, we repeated these studies in rats pretreated with antibodies against either kinins or glandular kallikrein.

**Methods**

Sodium pentobarbital (Nembutal) was obtained from Abbott Laboratories, heparin from Nyco, and captopril from Squibb and Sons, Inc.

Rat submandibular gland kallikrein was purified as previously described (Brantzaeg et al., 1976). The specific activity of this kallikrein preparation was 330 amidolytic units per mg of protein (Amundsen et al., 1979). Antisera to kinins and rat salivary gland kallikrein were prepared in rabbits as previously described (Carretero et al., 1976; Østavik and Inagami, 1982). The respective immunoglobulin fractions (IgG) were partially purified by ammonium sulfate precipitation (33% saturation, 18 hours, 4°C). After centrifugation, the immunoglobulin fractions were extensively dialyzed against phosphate-buffered saline (PBS; 0.01 M Na phosphate, pH 7.4, 0.14 M NaCl, 18 hours, 4°C). IgG from nonimmunized rabbits was prepared similarly. The three immunoglobulin fractions (normal-IgG, antikinin-IgG, and antikallikrein-IgG) were adjusted to have similar protein concentrations per milliliter of PBS.

**Experimental Procedure**

Male Wistar rats (350-400 g body weight) were anesthetized with ether and bilaterally nephrectomized through a flank incision. Forty-eight hours later, they were anesthetized with sodium pentobarbital (65 mg/kg body weight, intraperitoneally) and kept on a heating table (38°C) during surgery and the experiment. The rats were tracheotomized, and a polyethylene tube (P.E. 260) was placed into the trachea to facilitate breathing. Catheters (P.E. 10) were implanted into the abdominal aorta and inferior vena cava through the femoral artery and vein, respectively. The cervical sympathetic chain was dissected, and the main efferent submandibular gland duct was cannulated as described previously (Østavik and Gautvik, 1977). After surgery, the rats were given heparin (500 I.U., iv). Mean blood pressure was recorded continuously through the femoral catheter by a strain gauge transducer (Statham) connected to a Sanborn polygraph. The submandibular gland was stimulated 5 minutes after IgG administration of captopril (Table 1). In group 1, in which the submandibular gland was unstimulated, a significant decrease in blood pressure produced by captopril was observed (Table 1). In groups 4 and 5, pretreatment with antikinin or antikallikrein IgGs before sympathetic stimulation of the gland blocked the decrease in blood pressure produced by captopril by 81 and 79%, respectively, as compared to the response in the normal IgG-pretreated group (Table 1). Nevertheless, these decreases were still statistically significant (P < 0.05). Figure 1 shows the delta blood pressure for the five groups studied. The decrease in blood pressure (P < 0.01) in group 2 in which the gland was stimulated 10 minutes before captopril administration (Table 1). Sympathetic stimulation of the gland by itself did not alter the blood pressure significantly (122 ± 6.4 mm Hg and 119 ± 6.0 before and after stimulation, respectively). In group 3, in which the rats were pretreated with normal-IgG before sympathetic stimulation of the gland, a significant decrease in blood pressure (P < 0.05) was observed after captopril was administered (Table 1). In groups 4 and 5, pretreatment with antikinin or antikallikrein IgGs before sympathetic stimulation of the gland blocked the decrease in blood pressure produced by captopril by 81 and 79%, respectively, as compared to the response in the normal IgG-pretreated group (Table 1). Nevertheless, these decreases were still statistically significant (P < 0.05). Figure 1 shows the delta blood pressure for the five groups studied. The decrease in blood pressure produced by captopril in the rats in which the submandibular gland was unstimulated (group 1), or in the rats pretreated with antikinin or antikallikrein IgGs, in which the gland was stimulated (groups 4 and 5, respectively), was significantly smaller (P < 0.001) than that observed in the stimulated nonpretreated group (group 2) or in the group pretreated with normal IgG (group 3). The differences between groups 2 and 3 were not significant. Figure 2 shows some representative blood pressure recordings of the effect of captopril in the different groups studied. Administration of the immunoglobulin frac-
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a.

Glandular Kallikrein in BP Regulation

TABLE 1
Mean Blood Pressure (mm Hg) before and after Captopril Administration

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>Group 1 (n = 6)</th>
<th>Group 2 (n = 7)</th>
<th>Group 3 (n = 5)</th>
<th>Group 4 (n = 5)</th>
<th>Group 5 (n = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gland stimulation</td>
<td>None</td>
<td>None</td>
<td>Normal-IgG</td>
<td>Antikinin-IgG</td>
<td>Antikallikrein-IgG</td>
</tr>
<tr>
<td>Before</td>
<td>108 ± 4.6</td>
<td>122 ± 7.3</td>
<td>107 ± 8.2</td>
<td>116 ± 5.3</td>
<td>129 ± 7.9</td>
</tr>
<tr>
<td>1 minute</td>
<td>105 ± 4.6</td>
<td>85 ± 9.0†</td>
<td>85 ± 7.9*</td>
<td>113 ± 5.9</td>
<td>123 ± 7.5*</td>
</tr>
<tr>
<td>10 minutes</td>
<td>106 ± 4.2</td>
<td>88 ± 11.3†</td>
<td>78 ± 11.8*</td>
<td>111 ± 6.9*</td>
<td>124 ± 7.6*</td>
</tr>
<tr>
<td>Maximum effect</td>
<td>104 ± 4.1</td>
<td>77 ± 10.0†</td>
<td>76 ± 11.4*</td>
<td>110 ± 7.1*</td>
<td>122 ± 7.7*</td>
</tr>
</tbody>
</table>

* P < 0.05; †P < 0.01 (before vs. after captopril). Maximum effect indicates the greatest decrease in blood pressure within the first 10 minutes after captopril.

tions or sympathetic cervical nerve stimulation did not by themselves have any significant lasting effect on blood pressure. Figure 3 shows that pretreatment of the rats with antikallikrein or antikinin antibodies almost completely blocked the hypotensive effect of injected glandular kallikrein when it was tested at the end of the experiments.

PRA was undetectable (<0.1 ng/ml per hr) in all the rats with the exception of three rats in which PRA was 0.27, 0.14, and 0.10 ng/ml per hr.

Discussion

Vasoconstrictor and vasodilator humoral systems are important components of the many complex mechanisms that control blood pressure homeostasis. The renin-angiotensin system, a vasopressor system, has been shown to play an important role in regulating blood pressure. On the other hand, the role of the glandular kallikrein-kinin system, a vasodepressor system, is not as well established. While it has been suggested that this system participates in regulating blood pressure, a major role for this system has been considered unlikely because the plasma, particularly that of the rat, has a significant amount of protease inhibitors (Werle and Schmal, 1968; Hojima et al., 1977). However, recent reports in both human subjects and in rats indicate that some glandular kalli-

kine circulates in the active form (Geiger et al., 1980; Johansen et al., 1981). Furthermore, it has been reported that blood kinins are probably formed by glandular rather than by plasma kallikrein (Scicli et al., 1982). Therefore, it is possible that glandular
kallikrein released into the circulation has kininogenase activity and that the kinins generated in the peripheral circulation play a role as systemic vasodilators.

In the present study, we investigated the possibilities that glandular kallikrein released into the circulation by sympathetic stimulation of the submandibular gland may have kininogenase activity and that the kinins generated may have a systemic vasodilator effect. We used the angiotensin I-converting enzyme or kininase II inhibitor, captopril. To minimize the effect of captopril on the conversion of angiotensin I to II, we nephrectomized all the rats 48 hours before its administration; at the time of the experiments, PRA was undetectable or very low. The submandibular gland of the rat was selected because it contains more kallikrein than any other organ known, and upon sympathetic stimulation, releases into the circulation a large amount of kallikrein still showing some enzyme activity (Ør stavik, Johansen, Nustad, unpublished data; Rabito et al., 1982b). Thus, the results observed in this study may be unique for this organ and this species. In addition, nephrectomy may have also maximized the effect of the glandular kallikrein-kinin system, since plasma kininogen (kallikrein substrate) increases in the nephrectomized rat (Werle et al., 1968). If the reaction between glandular kallikrein and kininogen occurs normally under non-saturating conditions (first order kinetics), then the rise in plasma kininogen concentration caused by nephrectomy may have released more kinins than would be expected in nonnephrectomized rats. In addition, nephrectomy has been reported to increase immunoreactive glandular kallikrein in plasma which could result in further formation of kinins in the peripheral circulation (Lawton et al., 1981; Rabito et al., 1982a). Nevertheless, this immunoreactive glandular kallikrein in plasma appears to be mainly in the inactive form (Lawton et al., 1981).

In the unstimulated rat submandibular gland, a positive venous-arterial difference in glandular kallikrein has been observed (Rabito et al., 1982a), indicating that, even under basal conditions, the gland releases kallikrein into the circulation which is in part still enzymatically active (Ør stavik, Johansen, Nustad, unpublished data). Furthermore, we have observed that, in the unstimulated gland, inhibition of converting enzyme or kinase II significantly increased the gland blood flow that was mediated by kinins (Ør stavik et al., 1982). However, in the present study, captopril did not have a hypotensive effect when administered to nephrectomized rats in which no sympathetic stimulation of the submandibular gland was performed (group 1). These results suggest that, in the unstimulated gland, the released kallikrein acts mainly as an autacoid, since much of its action takes place at the site of its release. In contrast, 10 minutes after sympathetic stimulation of the gland, captopril produced a marked decrease in the blood pressure of the nephrectomized rats (Fig. 1). This decrease was not blocked by pretreatment with normal-IgG but was almost completely blocked by antikinin and antikallikrein IgGs, indicating that part of the glandular kallikreins released after sympathetic stimulation of the gland remain active in the peripheral circulation for at least 10 to 30 minutes. Thus, it may be that when glandular kallikrein is released into the circulation in small amounts, it functions as an autacoid regulating local blood flow, and when released in large amounts, such as after sympathetic submandibular gland stimulation, it functions as a vasodilator hormone, decreasing systemic blood pressure.

It could be argued that in the nephrectomized rats the blood pressure effect of captopril after gland stimulation is due to the release of submandibular gland renin. However, this is unlikely since, in contrast to the mouse, the Wistar rat submandibular gland has very little or no renin (Dej long et al., 1972). Although the rat submandibular gland is very rich in tonin, an enzyme that generates angiotensin II directly from angiotensinogen (Boucher et al., 1974), tonin is not inhibited by converting enzyme inhibitors (Boucher et al., 1974). Another possibility is that the hypotensive effect of captopril was due to the inhibition of the arterial wall renin-angiotensin system (Swales, 1980). However, this effect was observed only after stimulation of the gland, and presently there is no reason to assume that sympathetic cervical stimulation affects arterial wall renin. Further, it is also unlikely that the effect of captopril on subman-
dibular gland renin or tonin or on arterial wall renin systems would be blocked by both kinin and kalli-
krin antibodies. The antikinin- and antikallikrein-IgG almost completely blocked the vasodepressor effect of exog-}

enously injected kallikrein (Fig. 3). The antikinin-IgG used in this study has also been shown to block the vasodepressor effect of bradykinin without altering the response to nitroprusside, angiotensin II, or nor-
epinephrine (Carretero et al., 1981) indicating that their effect in vivo is specific. The possibility that the antikallikrein IgG blocked plasma kallikrein instead of glandular kallikrein is also unlikely since anti-
bodies to glandular kallikrein do not cross-react with plasma kallikrein (Nustad et al., 1974). Whereas it may be argued that the effect the antikinin- and antikallikrein-IgG was due to plasma volume expan-
sion as a consequence of administering large amounts of protein (≥ 130 mg), this is not likely since a similar amount of normal-IgG did not prevent the hypoten-
sive response to captopril (Table 1). Thus, it is rea-
sonable to conclude that the hypotensive effect of captopril in this situation was due to an increase in peripheral kinins generated by glandular kallikrein which was released from the submandibular gland during sympathetic stimulation.

The results of the present study may also help to explain the antihypertensive mechanism of angioten-
sin-converting enzyme inhibitors. It is possible that in circumstances in which significant amounts of gland-
ular kallikrein are released into the circulation, some of the antihypertensive effects of angiotensin-con-
verting enzyme inhibitors are mediated by the inhibi-
tion of kinin destruction. The effect of kinins on blood pressure after captopril administration may, in part, be mediated by prostaglandins, since kinins have been shown to stimulate prostaglandin release and since an increase in PGE2 and its metabolites has been reported after captopril administration (Terragno et al., 1972; Abe et al., 1980; Swartz et al., 1980). Fur-
thermore, in essential hypertensive patients, pretreat-
ment with indomethacin, a prostaglandin synthetase inhibitor, partially blocked the antihypertensive effect of captopril (Cratz et al., 1979; Abe et al., 1980).

In conclusion, the present study suggests that gland-
ular kallikrein released into the circulation by organs rich in this enzyme has kininogenase activity in the peripheral circulation. The released kinins induce hypertension when the steady state between their formation and destruction is altered by the adminis-
tration of the kininase II inhibitor, captopril. The present results may further suggest that the antihy-
pertensive effect of captopril may in part be due to a blockade of kinin destruction, particularly in situa-
tions where the concentration of glandular kallikrein in plasma is increased.

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