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; Ørstavik, 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 (Ørstavik, 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 (Brandtzæg 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; Ørstad 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 excretory 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 arterial catheter by a strain gauge transducer (Statham) connected to a Sanborn polygraph. The submandibular gland was stimulated electrically for 1 minute through the sympathetic cervical nerve with a Grass SD9 stimulator (8 V, 10 Hz, 2 msec duration). Submandibular gland stimulation was confirmed by observing saliva secretion in the catheter placed in the salivary duct. Captopril was administered in a bolus dose (10 mg/rat) through the femoral vein catheter. Blood for plasma renin activity (PRA) was drawn at the end of the experiment by heart puncture in groups 1 and 2 and from the femoral vein in groups 3, 4, and 5 prior to an intravenous injection of exogenous kallikrein. PRA was measured by a modification of the radioimmunoassay of Haber et al. (1969) as previously described (Carretero et al., 1973). PRA was expressed as nanograms of angiotensin I generated per ml of plasma per 1 hour of incubation.

The following five experimental groups were studied:

1. Control (six rats): In this group, the cervical sympathetic chain was dissected but the submandibular gland was not stimulated before captopril was administered.

2. Sympathetic cervical nerve stimulation (seven rats): In this group, the sympathetic cervical nerve was stimulated for 1 minute. Captopril was administered 10 minutes after sympathetic stimulation of the submandibular gland ended.

3. Normal-IgG group (six rats): This group was pretreated with IgG fraction (125 mg/rat) obtained from serum of nonimmunized rabbits. The IgG were dissolved in 2 ml of PBS and injected intravenously for 2 minutes. The submandibular gland was stimulated 5 minutes after IgG administration. Captopril was administered 10 minutes after sympathetic stimulation of the submandibular gland ended.

4. Antikinin-IgG group (five rats): This group was treated like group 3 except that the rats were injected with antikinin-IgG (100 mg/rat) instead of normal-IgG. The IgG was dissolved in 2 ml of PBS.

5. Antikallikrein-IgG group (five rats): This group was treated like group 3 except that the rats were injected with antikallikrein-IgG (100 mg/rat) instead of normal-IgG.

To determine whether the antibodies used effectively blocked the vasodepressor effect of kallikrein in groups 3, 4, and 5, we tested the blood pressure response to an intravenous injection of 2 μg submandibular gland kallikrein at the end of the experiment, 35 minutes after the administration of captopril.

Statistical Analysis

Significant differences within the group were analyzed by Freedmen's nonparametric two-way analysis of variance. Significant delta differences in BP among the groups were analyzed by the Kruskal-Wallis nonparametric one-way analysis of variance (Conover, 1971). All results are given as mean ± SEM.

Results

Captopril administered to the 48 hour-nephrectomized rats in which the submandibular gland was not stimulated (group 1) did not lower blood pressure. However, it significantly lowered 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 the 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 antikinins 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-
TABLE 1
Mean Blood Pressure (mm Hg) before and after Captopril Administration

<table>
<thead>
<tr>
<th>Group</th>
<th>Pretreatment</th>
<th>Gland stimulation</th>
<th>Before 1 minute</th>
<th>After 1 minute</th>
<th>After 10 minutes</th>
<th>Maximum effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>None</td>
<td>-</td>
<td>108 ± 4.6</td>
<td>105 ± 4.6</td>
<td>106 ± 4.2</td>
<td>129 ± 7.9</td>
</tr>
<tr>
<td>2</td>
<td>None</td>
<td>+</td>
<td>107 ± 8.2</td>
<td>113 ± 5.9</td>
<td>88 ± 11.3</td>
<td>79 ± 10.0</td>
</tr>
<tr>
<td>3</td>
<td>Normal-lgG</td>
<td>+</td>
<td>107 ± 8.2</td>
<td>85 ± 7.9 *</td>
<td>78 ± 11.8 *</td>
<td>76 ± 11.4 *</td>
</tr>
<tr>
<td>4</td>
<td>Antikinin-lgG</td>
<td>+</td>
<td>116 ± 5.3</td>
<td>113 ± 5.9</td>
<td>111 ± 6.9 *</td>
<td>124 ± 7.6 *</td>
</tr>
<tr>
<td>5</td>
<td>Antikallikrein-lgG</td>
<td>+</td>
<td>129 ± 7.9</td>
<td>123 ± 7.5 *</td>
<td>122 ± 7.7 *</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.

Mechanisms 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 kallikrein 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 kininogena-
see activity and that the kinins generated in the pe-
ripheral circulation play a role as systemic vasodila-
tors.

In the present study, we investigated the possibili-
ties that glandular kallikrein released into the circu-
lation by sympathetic stimulation of the submandib-
ular gland may have kininogenase activity and that
the kinins generated may have a systemic vasodila-
tor effect. We used the angiotensin I-converting enzy-
me 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 submandib-
ular gland of the rat was selected because it contains
more kallikrein than any other organ known, and
upon sympathetic stimulation, releases into the cir-
culation a large amount of kallikrein still showing
some enzyme activity (Ørstadvik, 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 kal-
lkrein-kinin system, since plasma kininogen (kalli-
lkrein 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 gland-
dular 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 kalli-
krein has been observed (Rabito et al., 1982a), indi-
cating that, even under basal conditions, the gland
releases kallikrein into the circulation which is in part
still enzymatically active (Ørstadvik, Johansen, Nustad,
unpublished data). Furthermore, we have observed
that, in the unstimulated gland, inhibition of convert-
ing enzyme or kinase II significantly increased the
gland blood flow that was mediated by kinins (Ørsta-
vik et al., 1982). However, in the present study, cap-
topril did not have a hypotensive effect when admin-
istered to nephrectomized rats in which no sympa-
thetic 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-lgG but
was almost completely blocked by antikinin and an-
tikkallikrein 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 circu-
lation in small amounts, it functions as an autacoid
regulating local blood flow, and when released in
large amounts, such as after sympathetic submandi-
bular 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 con-
trast to the mouse, the Wistar rat submandibular
gland has very little or no renin (Delong et al., 1972).
Although the rat submandibular gland is very rich in
tonin, an enzyme that generates angiotensin I 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 inhi-
bition 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-

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**Figure 3. Mean blood pressure response to an intravenous injec-
tion of 2 μg of kallikrein in the groups pretreated with normal-lgG,
antikallikrein-lgG, and antikinin-lgG. The asterisks indicate P <
0.001 between normal-lgG and the two antibody-treated groups.
The response of blood pressure to kallikrein was studied at the
eend of the experiment, approximately 35 minutes after administra-
tion of captopril.
dribular gland renin or tonin or on arterial wall renin systems would be blocked by both kinin and kallikrein antibodies.

The antikinin- and antikallikrein-IgG almost completely blocked the vasodepressor effect of exogenously 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 nitrupressus, angiotensin II, or noradrenaline (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 antibodies 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 expansion 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 hypotensive response to captopril (Table 1). Thus, it is reasonable 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 angiotensin-converting enzyme inhibitors. It is possible that in circumstances in which significant amounts of glandular kallikrein are released into the circulation, some of the antihypertensive effects of angiotensin-converting enzyme inhibitors are mediated by the inhibition 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). Furthermore, in essential hypertensive patients, pretreatment with indomethacin, a prostaglandin synthetase inhibitor, partially blocked the antihypertensive effect of captopril (Critz et al., 1979; Abe et al., 1980).

In conclusion, the present study suggests that glandular 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 administration of the kininase II inhibitor, captopril. The present results may further suggest that the antihypertensive effect of captopril may in part be due to a blockade of kinin destruction, particularly in situations where the concentration of glandular kallikrein in plasma is increased.


INDEX TERMS: Kinins • Converting enzyme • Kininase II • Blood pressure • Angiotensin-converting enzyme inhibitor • Submandibular gland


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