Blood Kinins after Sympathetic Nerve Stimulation of the Rat Submandibular Gland

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SUMMARY  We studied whether stimulation of kallikrein release into the vascular compartment by sympathetic nerve stimulation of the submandibular gland resulted in an increase in the concentration of kinins in the venous effluent from the gland. Electric stimulation of a cervical sympathetic nerve was performed at 8 V, 10 Hz, 2 msec duration. At 1 minute after sympathetic stimulation, the blood flow to the gland increased from 66.1 ± 10.3 to 367.0 ± 66.0 μl/min (p < 0.01), and the kinin output in the venous effluent from the gland increased from 0.019 ± 0.013 to 14.4 ± 8.8 ng/min (p < 0.001), but kinin concentration in the arterial blood did not change (0.177 ± 0.037 before and 0.303 ± 0.137 ng/ml after stimulation; p > 0.05). We also studied whether arterial blood kinins were increased in 48-hour nephrectomized rats during the hypotension that results when captopril is administered after the gland has been sympathetically stimulated. Arterial blood kinins concentration were 0.19 ± 0.042 ng/ml in sham control rats (without sympathetic stimulation and captopril administration) and increased to 3.0 ± 0.8 ng/ml after sympathetic stimulation plus captopril administration (p < 0.05). Arterial blood kinin concentration in rats with sympathetic stimulation alone or captopril administration alone was similar to that of the sham control rats. Blood pressure in the rats that received sympathetic stimulation and captopril administration decreased from 99.8 ± 5.3 to 57.8 ± 5.8 mm Hg (p < 0.001). Blood kinins and blood pressure were inversely correlated (r = −0.84; p < 0.01). Blood pressure did not change in the sham control group, in the group that received sympathetic stimulation alone, or in the group that received captopril alone. Pretreatment with meclofenamate, a cyclooxygenase inhibitor, did not affect the hypotensive response to captopril administration after sympathetic stimulation. We conclude that stimulating the release of kallikrein from the submandibular gland results in a significant increase in kinin concentration in venous blood from the gland but does not significantly change kinin levels in arterial blood. In nephrectomized rats, inhibition of kininases with captopril after sympathetic stimulation of the submandibular gland significantly increased arterial blood kinins and decreased blood pressure. Prostaglandins are not involved in this hypotensive response. (Hypertension 5 (supp I): 1-101–1-106, 1983)

Key Words • kinins • kallikrein • submandibular gland • hypotension • captopril • converting enzyme inhibitor

Glandular kallikreins are serine proteases that release potent vasodilator peptides, kinins, from plasma kininogens.1 Glandular kallikreins are found in the salivary and sweat glands, pancreas, intestine, kidney, and in the exocrine secretions of these organs.2 We and others have recently demonstrated that immunoreactive glandular kallikrein is present in plasma.3,5 While the sources of glandular kallikrein in plasma are not fully delineated, we have found that a positive arteriovenous difference in immunoreactive glandular kallikrein exists in the submandibular gland of the rat, indicating that part of the glandular kallikrein present in plasma originates from this gland.5 Moreover, we have observed that sympathetic stimulation of the rat submandibular gland results in the release of substantial amounts of immunoreactive glandular kallikrein into the circulation.6 It has yet to be demonstrated, however, that release of glandular kallikrein increases the generation of kinins in blood. Furthermore, we have shown that, in nephrectomized rats after the submandibular gland has been sympathetically stimulated, administration of a converting enzyme inhibitor (CEI) causes the arterial
pressure to drop. Pretreatment with either kinin or kallikrein antibodies blocked this hypotensive response, suggesting that hypotension is due to the systemic vasodilator action of kinins generated within the circulatory system. In our previous study, however, we did not measure the levels of arterial blood kinins during the period of CEI-induced hypotension.

In the present study, we again performed sympathetic nerve stimulation (SNS) of the rat submandibular gland. To determine whether the release of kallikrein is accompanied by an increase in blood kinins, we measured kinin levels in arterial blood and in the venous effluent of the submandibular gland. We also studied whether kinins in arterial blood were increased after captopril (CEI) administration to rats that had undergone sympathetic stimulation of the submandibular gland. In addition, since kinins may stimulate prostaglandin synthesis, we investigated whether inhibition of cyclooxygenase alters the blood pressure response to CEI administration after the gland has been sympathetically stimulated.

**Methods**

We carried out all experiments on rats that had been fasted overnight, anesthetized with pentobarbital (50 mg/kg intraperitoneally), and placed on a heated table (38°C).

### Kinins in Venous Blood from the Submandibular Gland

In male Sprague-Dawley rats weighing 450 to 500g, a polyethylene catheter (PE 260) was inserted into the trachea to aid breathing. Catheters were implanted into the abdominal aorta and inferior vena cava (PE 10) through the femoral artery and vein, respectively. The right submandibular gland was exposed and heparin (1000 U/kg) was injected intravenously. All tributaries joining the submandibular gland vein were tied off and a catheter (PE 60) was positioned to collect only submandibular gland venous blood, as previously described. The main excretory duct of the right submandibular gland was catheterized and the right cervical sympathetic trunk was stimulated for 3 minutes, and 10 minutes later, captopril was administered intravenously (10 mg in 0.5 ml of saline). Then 10 minutes later, blood was drawn for measurement of kinins, hematocrit, and plasma renin activity.

### Group 1

Kinins were measured in venous blood from the submandibular gland before and 1 minute after sympathetic stimulation of the gland (n = 8).

### Group 2

Kinins were measured in arterial blood before and 1 minute after sympathetic stimulation of the submandibular gland (n = 6). This study was not done in Group 1 because the venous blood from the gland was diverted to measure venous blood kinins. The procedures were the same as for Group 1 except that the submandibular gland vein was not cannulated. Saline (1 ml) was given intravenously after the first blood collection to replace volume loss.

### Kinins in Arterial Blood after Sympathetic Nerve Stimulation and Captopril Administration

Male Wistar rats weighing 350-450 g were used in these experiments. Bilateral nephrectomy was performed 48 hours before the experiments to eliminate any effect that administration of captopril might have had by blocking the renin-angiotensin system. Catheters (PE 10) were placed in the inferior vena cava for injections and in the left and right femoral arteries for blood pressure monitoring and blood withdrawal, respectively. All catheters were flushed with 0.2 ml of a heparin solution (100 U/ml) in 5.0% dextrose. Blood pressure was recorded with a Statham P23 Gb pressure transducer connected to the arterial catheter and to a Brush 440 carrier preamplifier and recorder apparatus.

The main excretory duct of the right submandibular gland was catheterized and the right cervical sympathetic trunk was isolated, as described above. An initial blood sample (0.9 ml) was drawn to measure hematocrit. An equal volume of blood from 48-hour nephrectomized rats was immediately given intravenously to restore blood volume. Thirty minutes later the rats were treated in one of the following four ways.

### Group 3

Converting enzyme inhibition (CEI) was applied after sympathetic nerve stimulation (n = 10). The cervical sympathetic nerve was stimulated for 3 minutes, and 10 minutes later, captopril was administered intravenously (10 mg in 0.5 ml of saline). Then 10 minutes later, blood was drawn for measurement of kinins, hematocrit, and plasma renin activity.

### Group 4

This sham control group (n = 6) underwent the same procedures in Group 3 except that the current was not applied to the electrode on the cervical nerve, and the rats received 0.5 ml of saline instead of captopril.

### Group 5

This group received sympathetic stimulation only (n = 6). The procedures were the same as for Group 3, except that saline (0.5 ml) was given instead of captopril after the gland was stimulated.

### Group 6

This group received captopril administration alone without sympathetic stimulation (n = 7). The procedure was the same as in Group 3, except that no current was applied to the electrodes.
Role of Prostaglandins in the Hypotensive Effects of Captopril Administration after Cervical Sympathetic Nerve Stimulation

Rats were prepared as in Group 3 and divided into two groups.

Group 7

These rats were pretreated with meclofenamate (n = 9). Thirty minutes before the cervical nerve was stimulated, the rats received a bolus injection (0.25 ml) of a solution of meclofenamate in 5% dextrose (6 mg/kg body weight), followed by continuous infusion of the meclofenamate solution (6 mg/kg/hr; 0.8 ml/hr). This dose had previously been shown to inhibit prostaglandin synthesis in rats. The gland was then stimulated, and captopril was administered as described for Group 3.

Group 8

The procedures were the same as those in Group 7 except that the rats received 5% dextrose without meclofenamate (n = 9).

Analytical Techniques

Blood for kinins (1 ml) was collected into preweighed siliconized flasks containing 5 ml of 100% ethanol. The volumes of blood samples for kinin determinations were calculated gravimetrically without correcting for the blood's specific gravity. Blood kinins were measured by radioimmunoassay (RIA) as previously described.

PRA was measured by a modification of the method of Haber et al. and Carretero et al. Hematocrit was determined in a Readacrit centrifuge (Clay-Adams, Parsippany, New Jersey) according to the manufacturer’s recommendations.

Statistical analysis was performed by randomized complete block analysis of variance, by Scheffe’s multiple comparison test, and by Student’s t test as appropriate. A p < 0.05 was considered significant. Data are expressed as the arithmetic mean ± one standard error of the mean (SEM).

Results

Kinin Output

In Group 1, the concentration of kinins in venous blood from the submandibular gland increased more than 100-fold, from 0.26 ± 0.14 before to 31.9 ± 18.1 ng/ml after sympathetic stimulation (p < 0.001). The total output of kinins (kinin concentration times glandular blood flow) increased more than 700-fold after sympathetic stimulation, and these changes coincided with a more than fivefold increase in glandular blood flow (fig. 1). In Group 2, the concentration of arterial blood kinins before and at 1 minute after stimulation of the cervical sympathetic nerve was 0.177 ± 0.037 and 0.303 ± 0.137 ng/ml, respectively (p > 0.05).

Kinin Output

Arterial blood kinins in Group 3, which received captopril after sympathetic nerve stimulation, was 10 to 20 times higher than in sham control rats (Group 4), in rats that had sympathetic stimulation only (Group 5), or in rats that received captopril only (Group 6). These data are shown in figure 2. In Group 3, arterial hematocrit increased more than 10% while it remained unchanged in Groups 4, 5, and 6 (fig. 3).

Blood pressure in rats that received cervical nerve stimulation only or CEI administration only did not differ from that of control rats that received sham stimulation and saline. In contrast, administration of CEI after cervical nerve stimulation resulted in a blood pressure fall of more than 40 mm Hg (fig. 4). Plasma renin activity was less than 0.15 ng/ml/hr in all groups.
Role of Prostaglandins in the Hypotensive Effects of Captopril after Cervical Sympathetic Nerve Stimulation

Pretreatment with meclofenamate did not alter the hypotensive response to CEI administration after cervical nerve stimulation (fig. 5).

Discussion

In the present study we have demonstrated that the output of kinins from the submandibular gland of the rat is greatly augmented at 1 minute after the gland has been sympathetically stimulated, while arterial blood kinins remains unchanged. These results clearly indicate that large amounts of kinins are produced within the vascular and, presumably, the interstitial compartment of the gland. This is the first time, to our knowledge, that endogenous production of kinins within the vascular territory of an organ rich in glandular kallikrein has been documented using a specific RIA for kinins. Although the kinins measured in the submandibular venous blood could have been generated within the glandular interstitium or the vein itself, either possibility is consistent with the release of glandular kallikrein in an active form following sympathetic stimulation. It is possible that kinins generated within the gland by locally released kallikrein could be responsible, at least in part, for the hyperemic response that occurs after sympathetic stimulation or CEI. Thus, kallikrein may be acting as an autacoid inducing changes at the site of its release.

Although the above data indicate that kinins are generated locally by the release of glandular kallikrein, we did not detect any change in the level of arterial kinins after the submandibular gland was sympathetically stimulated. This suggests that kininases present in both plasma and pulmonary tissue can rapidly destroy kinins. In previous work with 48-hour nephrectomized rats, we found that administration of a converting enzyme inhibitor, captopril, after sympathetic cervical nerve stimulation, resulted in hypotension. Because pretreatment with antibodies against glandular kallikrein or against kinins blocked the vasodepressor response, we proposed that the concentration of kinins in arterial blood was raised by inhibiting kininases, which thereby caused hypotension. In the present study, we have demonstrated that the concentration of arterial blood kinins increased at least tenfold in the group treated with CEI after cervical nerve stimulation (Group 3). Moreover, 10 minutes after captopril administration in this group, blood kinins and blood pressure were inversely correlated (r = −0.84; p < 0.01).

We also found that in Group 3 the hematocrit was significantly increased. This suggests that high levels of circulating kinins have increased capillary permeability leading to a loss in circulatory volume. This loss of plasma volume must have occurred very rapidly since the hematocrit was found elevated ten minutes after the CEI was administered. Similar effects have previously been observed after the exogenous administration of high doses of kinins or glandular kallikrein.
The present results, coupled with those of our earlier study, indicate that part of the kallikrein released into the systemic circulation from sympathetically stimulated submandibular glands remains active for 10 to 20 minutes or more.

The increase in hematocrit observed in rats treated with the CEI after sympathetic stimulation indicates that some plasma components may have leaked out of the vascular compartment into the interstitial compartment through an altered capillary barrier. It could be that under these circumstances, some kallikrein passes into the interstitial compartment. Thus, in addition to its action within the vascular compartment, kallikrein could release kinins in the interstitial compartment.

The fall in blood pressure induced by the CEI after cervical nerve stimulation was not affected by pretreatment with the cyclooxygenase inhibitor, meclofenamate. These results suggest that prostaglandins are not involved in this hypotensive response. Although the hemodynamic action of kinins has been linked to increased prostaglandin synthesis, most evidence has been obtained in species other than the rat. In the rat the augmentation of the hypotensive action of kinins by captopril does not appear to be dependent on increased prostaglandin synthesis. Hence, most of the hypotensive response is probably directly due to kinins.

The results of the present study may be unique to the rat. The rat submandibular gland contains more kallikrein than any other tissue studied. Further, we do not yet know whether species other than the rat are capable of releasing submandibular gland kallikrein into the circulation. In addition, the effects observed in nephrectomized rats may not be identical to those that can be elicited in intact rats. This is because nephrectomized rats have more kininogen in plasma than intact rats. If the reaction between glandular kallikrein and kininogen occurs following first order kinetics, the amount of kinins released by a given concentration of kallikrein would be higher in nephrectomized rats than in intact rats. Thus, nephrectomy may have potentiated the effects of the glandular kallikrein-kinin system.

The results of the present study suggest that tissues rich in glandular kallikrein could release the enzyme into the circulation. Kinins, released by glandular kallikrein, may then influence local blood flow. Normally, kinins are metabolized by kininases so they do not increase in arterial blood. When the steady state between kinin formation and destruction is altered by a kininase inhibitor such as captopril, kinins increase in arterial blood and may affect vascular resistance. Therefore, it is possible that, in situations in which the release of glandular kallikrein into the plasma is increased, part of the vasodepressor activity of converting enzyme inhibitors is partially due to increased circulating kinins.

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