THE THERMOLYTIC SALIVATION OF THE RAT AND THE KALLIKREIN-KIN SYSTEM

Jacques Damas  
Department of Human Physiology, Faculty of Medicine, Institute of Leon Fredericq, University of Liege, Liege, Belgium

SUMMARY

• Rat submandibular glands contain high amounts of tissue kallikrein which cleaves plasma kininogens to form bradykinin. A large release of tissue kallikrein can be experimentally obtained by a sympathetic stimulation. Parasympathetically evoked and reflex-induced salivary secretion is associated with a small release of kallikrein into the saliva, except in the case of heat stress. In rats exposed to heat, submandibular glands are stimulated by both parasympathetic and sympathetic nerves, saliva contains relatively high level of tissue kallikrein, plasma levels of this enzyme are increased and a large depletion of tissue kallikrein in submandibular glands occurs. Simultaneously, a swelling of the glands and an edema of the soft tissues surrounding them develop. The release of tissue kallikrein can participate to the thermoregulation of rats exposed to heat as the thermolytic flow of saliva is reduced and the hyperthermia is increased in kallikrein-deficient rats. The kallikrein-kinin system is not involved in the functional vasodilatation occurring in salivary glands during heat stress. This massive release of kallikrein during heat stress could be seen as an emergency response of the animals to the increase of body temperature. (Biomed Rev 1998; 9: 69-77)

INTRODUCTION

• In a hot environment, rats do not sweat or pant but they store substantial amounts of heat with a rapid rise of body temperature. Thereafter, they present two thermolytic responses. The first one is initiated when the body temperature reaches 38.5-39°C and causes heat loss by evaporating saliva that the animals spread on their fur and skin. The second thermolytic response is peripheral vasodilatation which is initiated at a body temperature of 39.2°C. It results in increased heat loss by radiation and conduction from vascularized nude surfaces, mostly from the tail (1,2). The thermolytic salivary secretion arises mainly from the submandibular glands (SMG). At an ambient temperature of 36°C, normal rats secrete only SMG saliva and at 40°C secrete three times more SMG saliva than parotid gland saliva (2, 3). On the other hand, compared with the parotid and sublingual glands, the rat SMG are very rich in tissue kallikrein (4). It was observed that in rats exposed to an ambient temperature of 55°C for 20 min, the salivary secretion contains relatively high amounts of kallikrein and is associated with an increase in the plasma levels of this enzyme (5). Other kinds of reflex-induced salivary secretion do not induce any significant release of kallikrein into the blood (5). These observations suggest that tissue kallikrein would be involved in the thermolytic salivary secretion.

Kallikreins in salivary glands

Kallikreins are serine proteases divided into two main groups, including tissue and plasma kallikreins. These enzymes release...
kinins from plasma kininogens which are glycoproteins synthesized by the liver. In mammals, there are usually high and low molecular weight kininogens. In rats, tissue kallikrein releases bradykinin from both kininogens while plasma kallikrein releases bradykinin mainly from high molecular weight kininogen. Rat plasma contains a third type of kininogen, T-kininogen, which is not vulnerable to kallikrein hydrolysis. Trypsin, however, in high concentrations, cleaves T-kininogen and releases Ile-Ser-bradykinin or T-kinin. The kinins have a number of physiological effects including vasodilatation, increase of vascular permeability, contraction or relaxation of smooth muscles, and pain. These effects are mediated via the stimulation of two subtypes of kinin receptors, B1 and B2, and are amplified or dependent on prostanoids and nitric oxide released by kinins from several types of cells (6).

Tissue kallikrein is a member of a multigene family of several closely related enzymes which show different patterns of tissue distribution and substrate specificity (6). The presence of tissue kallikrein in salivary glands has been known for a long time (7) and the SMG of the rat contain this enzyme, designated rK1, and several other members of the kallikrein gene family such as tonin (rK2) and a T-kininogenase (8). This latter enzyme has been previously identified as rK10 kallikrein, although recent data indicate that rK3, also present in low amount in SMG, specifically cleaves T-kininogen and releases T-kinin (9). In contrast with other members of the rat kallikrein gene family, true kallikrein (rK 1) mRNA levels in the SMG are androgen- and thyroid-hormone-independent (10).

**Release of tissue kallikrein from the rat submandibular gland**

In rat SMG, tissue kallikrein (rK 1) is found in an active form on one hand in the secretory granules of the granular convoluted tubule associated with tonin (rK2), T-kininogenase (rK 10) and esterase B (rK7) and on the other hand in the secretory granules of striated ducts (11). The acinar cells do not contain significant amounts of this enzyme. In the absence of external stimulation, a continuous apical secretion of tissue kallikrein occurs from ductal cells through a constitutive secretory pathway. This secretion accumulates into glandular lumina (12). Simultaneously, kallikrein appears to be constitutively secreted from the basolateral surface of striated duct cells and released in close contact of fenestrated capillaries (13). This latter transfer could explain that kallikrein (rK 1) from rat SMG contributes to a large fraction of the amount of tissue kallikrein found in the circulating blood, as the removal of SMG greatly reduces serum rK 1 levels (14-16). Furthermore, the gene delivery of human tissue kallikrein in the rat SMG results in the presence of human tissue kallikrein in the systemic circulation (17).

The stimulation of the autonomic nerves supplying the SMG results in a release of kallikrein into the saliva. Parasympathetic nerve stimulation causes a copious flow of saliva that has a low concentration of rK 1 (18). In this case, the secretion of kallikrein would be performed by a constitutive vesicular transport (19). On the other hand, the sympathetic stimulation releases large amounts of tissue kallikrein by granule exocytosis but evokes a secretion of a low volume of saliva (18,19). However, under natural reflex conditions, sympathetic impulses are likely to be acting on cells that are under the influence of parasympathetic activity at the same time (20). Graded sympathetic stimulation on a background of parasympathetic excitation shows that the release of kallikrein occurs as a large surge of the enzyme in the saliva. This large secretion of kallikrein appears only at a high frequency of sympathetic stimulation and is suppressed by α- and p-adrenoceptor blockade (21). This very interesting observation would indicate that an important release of kallikrein takes place only in presence of a high sympathetic stimulation. However, this secretion of kallikrein soon diminishes, as a large depletion of the enzyme from the glands rapidly occurs during sympathetic excitation (22). Several observations suggest that during saliva secretion, a transfer of kallikrein from the SMG into the blood is possible. Kallikrein is mainly situated in preformed granules at the apical pole of tubule cells of the salivary glands. However, in cells of the granular convoluted tubules of mice, some granules containing kallikrein have been also found near the basolateral pole of the cells, sometimes very close to the basolateral membrane (13). Two studies (23,24) reported that parasympathetic nerve stimulation of the glands induced secretion of saliva with a low concentration of kallikrein but no change in the enzymatic level of the venous blood escaping from the gland. However, sympathetic nerve excitation induced a massive secretion of kallikrein into saliva and into the circulating blood. Kallikrein secretion was suppressed by an α-adrenergic blocker. These studies have been recently reexamined by Gar-rett and colleagues (16). They observed that a continuous sympathetic stimulation at relatively high frequency usually induces edema of SMG which could be explained by the very viscous first-formed saliva. Therefore, these authors developed a protocol of stimulation in bursts during one hour, and reported that, in nearly all cases, plasma levels of kallikrein increased by 46-48% after both parasympathetic and sympathetic excitation, although only sympathetic stimulation induced a massive release of kallikrein into the saliva (16). However, in one case, sympathetic excitation triggered the development of a marked glandular edema, a reduction of gland-associated kallikrein and a marked concomitant increase of kallikrein in the blood. Similar results were obtained when the duct of the glands was deliberately obstructed during sympathetic stimulation. No significant increase of tissue kallikrein in blood has been observed when glandular edema occurred during parasympathetic stimulation. These authors conclude that the salivary glands release small amounts of kallikrein into the blood when they are activated. The role of this release remains hypothetical because tissue...
The release of kallikrein by several drugs has been also studied. As the parasympathetic excitation of the glands does not induce a large secretion of kallikrein, the cholinomimetic drugs, acetylcholine and pilocarpine, are not potent releasers of this enzyme into the saliva (4,5,18,26). It is also the case for substance P (26), a cotransmitting peptide present in parasympathetic nerve fibres reaching the rat salivary glands (27). Similarly, the atropine-resistant secretion of SMG saliva induced by a parasympathetic stimulation does not contain high amounts of kallikrein (28). On the other hand, sympathomimetic agents, like epinephrine, norepinephrine, pheny lephrine and isoproterenol, release significant amounts of rK1 into the saliva (4,18,26) and into the perfusates of the SMG (29). Significantly more kallikrein is secreted by the cc-adrenergic agents than by the (3-adrenergic drug isoproterenol (4,18,26,29). The enzymatic activity of true tissue kallikrein rK1 is only slowly blocked by kallikrein-binding protein (30) which, like a 1 -proteinase inhibitor, belongs to the serpin family. Kallikrein-binding protein is present at relatively low concentration in rat plasma. On the other hand, rat cd-proteinase inhibitor slowly inhibits all known rat tissue kallikrein except rK1 (9). Thus the tissue kallikrein rK1 would be able to form some amounts of bradykinin before its inactivation (9). Indeed, a fraction of tissue kallikrein released from the salivary glands into the blood by a sympathetic stimulation has been found to be enzymatically active (24).

- **Effects of kinins on the salivary glands**

Injected into the common carotid artery, bradykinin in the dose range of 0.05-0.1 μg induces a pressure rise in the rat SMG. This effect would depend on the contraction of myoepithelial cells (31). Indeed, bradykinin by itself does not evoke secretion from the SMG and parotid glands of the rat (31). Administered into the carotid artery, bradykinin (25 to 250 ng/kg) also increases SMG blood flow. This vasodilator effect is suppressed by a bradykinin B2-receptor antagonist (32).

Beside the examination of the direct effects of bradykinin, the involvement of the kallikrein-kinin system in the physiological processes occurring in salivary glands has been also studied by several indirect means, using inhibitors of angiotensin converting enzyme (ACE) which inactivates bradykinin. bradykinin B2-receptor antagonists such as HOE 140 (32), antibodies to kinins or to kallikreins, or using kininogen-deficient rats (33). Administration of captopril or enaprilat, two inhibitors of ACE, increased blood flow in rat SMG (32,35). This effect did not appear in rats pretreated with anti-kinin antibodies (35) or with a B2-antagonist (32) and is thus explained by the enhancement of the vasodilator effect of bradykinin. Similarly, in rats pretreated with captopril, sympathetic stimulation of SMG evoked a decrease of the systemic blood pressure. This general hypotensive effect was suppressed by anti-kinin or anti-kallikrein antibodies (36), suggesting that the sympathetic stimulation releases tissue kallikrein from the gland. This release results in a formation of kinins in the circulating blood. As the inactivation of kinins is delayed by the ACE inhibitor, their vascular hypotensive effect become apparent. On the other hand, a bradykinin antagonist decreased basal blood flow in the salivary glands (32) and suppressed post-sympathetic vasodilatation (32). HOE 140, a potent bradykinin receptor antagonist, decreased salivary flow elicited by phenylephrine or isoproterenol, and did not modify the salivary flow induced by pilocarpine or substance P (26,37). Similarly, the flow of saliva evoked by isoproterenol or phenylephrine was reduced in kininogen-deficient rats (26,37). All these results suggest that the kallikrein-kinin system might participate in the control of blood flow and salivary secretion during sympathetic stimulation of the SMG.

**HEAT STRESS**

- **Kallikrein release**

In order to shed some light on the physiological mechanisms able to release tissue kallikrein, Berg etal (5) have examined the secretion of this enzyme into the saliva and into the blood during five different conditions. Salivation induced by aggression, by starvation following by drinking, by exposure to ether or by oral application of acid resulted in a small release of kallikrein into saliva but did not change the plasma levels of tissue kallikrein. In each case, the release of the enzyme into saliva was suppressed by atropine. The fifth condition was heat stress applied to anaesthetized rats (48°C, for more than 15 min) or to awake rats (55°C, for about 20 min). According to these authors, in both cases, the salivary secretion started when the body temperature reached 39°C and contained a larger amount of kallikrein than that observed after a parasympathetic stimulation. Heat exposure also induced a significant rise in the concentration of tissue kallikrein in plasma, from about 15 ng/ml to about 30-120 ng/ml. Heat stress was thus the only "natural" condition responsible of a kallikrein release into the blood. Surprisingly, atropine (0.1 ing/kg) abolished heat-induced salivation and simultaneously endocrine kallikrein secretion although the secretion of kallikrein was not modified by phentolamine, an α-adrenergic blocker. Berg et al (5) explained this inhibition by an interference of atropine with a central muscarinic pathway. However, another explanation could be given. These authors (5) used a rather high level of ambient temperature as stimulus and the end point
of heat exposure was a body temperature reaching 40.5°C. Atropine largely decreased heat tolerance (38) and, thus, by reducing the time of heat exposure, would have inhibited the release of kallikrein. On the other hand, Berg et al (5) proposed that the lack of effect of phentolamine depends on the involvement of an unidentified mediator like neuropeptide Y (NPY) or vasooactive intestinal polypeptide (VIP) which nevertheless are weak sialogogic agents in rats (39).

In their experiments on heat exposure, Damas and Bourdon (26) used also awake and anaesthetized rats but milder stimuli (36°C or 40°C for one hour) allowing a longer time for a release of kallikrein. In anaesthetized rats, the salivation started when the body temperature reached 41.7-41.9°C and the saliva contained levels of kallikrein in the same range as that obtained with parasympathicomimetic agents. In awake rats, previous results indicate that the salivary process starts when the body temperature goes over 39°C (2) whereas in anaesthetized animals, the salivation is absent until rectal temperature attains at least 40°C (40). This difference in the threshold of body temperature inducing salivary secretion suggests that anaesthesia depresses the thermolytic salivary response. In awake rats, the saliva was not collected but the kallikrein content of the SMG was measured after heat exposure (26). After one hour, this content was reduced by 43% in rats exposed to 36°C as well as in those exposed to 40°C. This reduction was abolished by the pretreatment of the animals with a combination of an a- and P-adrenergic antagonist and was not inhibited by atropine or by a- or P-adrenergic antagonist used alone (26). Indeed, kallikrein secretion from SMG induced by a sympathetic stimulation has been previously reported to be inhibited by a simultaneous a and P-adrenergic blockade in the rat (21) as well as in the cat (41) or in the guinea-pig (42). The involvement of a sympathetic cotransmitter, NPY, is thus ruled out. Otherwise, all VIP-containing nerve fibres reach the salivary glands of rats via parasympathetic pathways (43). The heat-induced depletion of kallikrein in SMG thus depends on a sympathetic stimulation. This depletion, however, was associated with a decrease of the plasma levels of kininogens by about 30%, which suggests that the kallikrein release triggered the formation of kinins (26).

- **Heat-induced salivary secretion and the kallikrein-kinin system**

Heat-induced salivary secretion in awake rats starts when rectal temperature of the rats reaches about 39°C (1,2,44). Desalivated rats cannot adequately regulate body temperature in a hot environment. Rats whose parotid glands were ligated can control body temperature and survive during heat exposure as well as normal rats, but rats whose SMG and sublingual glands are excised rapidly die from hyperthermia (2,3,45). Male rats evaporate more salivary water than female rats when the ambient temperature is below 44°C (46). By the comparison of water evaporation of normal rats and of desalivated rats exposed to heat, it was estimated that the salivary secretion in male rats reach about 3 ing/gr per hour at 3 6°C of ambient temperature and 6-10ng/g per hour at 40°C(46,47). Studies using the cannulation of SMG duct to directly measure the flow of saliva indicated that the salivary flow of one SMG in rats exposed to 40°C attains 20 to 3 5 ul/min (44-48). The thermolytic salivary secretion is thus considerable. On the other hand, the salivary flow in the heat is reduced by sympathetic denervation of the glands and nearly abolished by parasympathetic denervation (48) and thus depends on a simultaneous sympathetic and parasympathetic excitation of the SMG.

In the heat, rats become so greatly dehydrated that this dehydration can be measured by the reduction of body weight. This reduction shows a rapid initial loss gradually slowing and reaching a rather constant hourly rate of loss after the second hour of heat exposure (49). This reduction of body weight can attain 2.5 to 3% per hour. In this weight loss, urination, defecation and oxidative metabolism play only a trivial role. Indeed, urination and defecation can be largely reduced during heat exposure by handling the animals several times before the experiment; during this procedure, usually rats empty their bladder and rectum (50). On the other hand, the decrease of body weight by oxidative metabolism during one hour of heat exposure is quite small (50). In the heat, the diminution of body weight thus arises mainly from a water loss (49,50). The importance of the salivary secretion in the total water loss can be estimated by the comparison between the water loss measured in normal and desalivated rats (46,47,50). This comparison indicates that the reduction of body water induced by salivation amounts to 50-70% of the total evaporative water loss. Moreover, the reduction of body weight attributable to water evaporation in the heat starts when the rectal temperature goes over 39°C thus at the level of body temperature which initiates the thermolytic salivary secretion (51). We have thus taken the changes of body weight as an indirect measure of the salivary secretion of rats exposed to heat (50-52). However, the heat tolerance and the associated body weight loss of rats of several strains have been previously compared and major differences between strains have been observed (52).

The thermal tolerance of kininogen-deficient Brown Norway rats was compared to that of normal Brown Norway rats during heat exposure to 36°C or 40°C (50). In an ambient temperature of 36°C, the reduction of body weight of kininogen-deficient rats was smaller than that of normal rats whereas the rectal temperature of both types of rats rose to about the same level of 40°C. The lack of higher hyperthermia in kininogen-deficient rats suggests that, at this level of ambient temperature, the salivary response of normal rats is disproportionate to the heat stress. During exposure to 40°C, the body weight loss was similar in all the animals during the first hour but from the second hour it was
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significantly smaller in kininogen-deficient rats. Simultaneously, during the first hour, the increase of body temperature was more rapid in kininogen-deficient animals. These results would indicate that the kallikrein-kinin system participate to the thermolytic salivary secretion.

Wistar rats are more tolerant to heat than Brown Norway rats (50) and were used to study the influence of several drugs and of desalivation on the changes of body temperature and the reduction of body weight induced by heat exposure. After removal of the submaxillary glands, the weight loss of the animals in an ambient temperature of 36°C was reduced by 42% and in an ambient temperature of 40°C by 58% (50). These results confirm that the changes in body weight are an indirect measure of the thermolytic salivation. Atropine reduced the body weight loss at 36°C ambient temperature by 38% and by 43% at 40°C and simultaneously increased the body temperature and reduced heat tolerance (50, 51). Hexamethonium, a ganglion blocking drug, had the same effect (51). These observations confirm that the salivary secretion is necessary for thermoregulation in rats and is mainly controlled by the parasympathetic system. Substance P (27) through the stimulation of the NK1 receptor, induces salivation in rats (52, 54). RP 67580, a NK1 receptor antagonist, reduced the weight loss of Wistar rats exposed to 36°C, which suggests that SP takes part in the initiation of the salivary secretion (52). In kininogen-deficient Brown Norway rats treated with atropine and RP 67580, the changes of body weight at 36°C ambient temperature were suppressed (52). This latter suppression could indicate that in the absence of a functional kallikrein-kinin system, the salivary secretion induced by the sympathetic stimulation in the heat is inoperative. In Wistar rats exposed to 36°C, HOE 140 reduced the body weight loss (50, 51), whereas this B2 antagonist as well as trasylol, a tissue kallikrein inhibitor, failed to significantly modify the body weight loss of Wistar rats exposed to 40°C (50, 51). These results are to put in parallel with the observations made with the kininogen-deficient rats. They showed that the kallikrein-kinin system play a significant role in heat-induced salivary secretion only for the lesser degrees of heat stress.

• Vasodilatation and edema in submandibular glands during heat exposure

The blood content in the SMG was taken as an index of the vascular changes occurring during heat exposure. This content was measured using 51Cr-labelled red cells (51). After one hour of heat exposure, the blood content of the glands increased and reached up to 170% in Wistar rats exposed to 40°C. This change indicates that a vasodilatation had occurred in the glands. A similar increase was observed in SMG of kininogen-deficient Brown Norway rats (51). The vasodilatation was suppressed in normal rats by atropine or by N2-nitro-L-arginine (NOARG), a nitric oxide-synthase inhibitor, but was not affected by HOE 140 (51). The vasodilatation would thus depend on a cholinergic autonomic nerve stimulation presumably associated with a release of nitric oxide (NO) by acetylcholine. The kallikrein-kinin system takes no part in this vascular change.

During heat exposure, the submaxillary glands of Wistar rats swelled and their weight increased as a function of the ambient temperature. The weight was significantly increased by 30% after one hour at 40°C and simultaneously, a local extravasation of labelled albumin occurred in the glands (51, 52). The increase of weight was not modified by atropine which slightly reduced the local accumulation of albumin (52). Indeed, atropine suppressed the functional vasodilatation and thus could reduce protein extravasation. RP 675 80 had no effect on these changes. Though, SP has a sialogogic effect in rats, it does not modify the vascular permeability in salivary glands (52, 55). On the other hand, the edema of the glands was significantly reduced by hexamethonium, by HOE 140 or by NOARG (51). In kininogen-deficient rats, no significant modification of the weight of the SMG was observed (51). Thus, the edema of the glands would depend on a autonomic nerve excitation of the glands, on the formation of kinins and on a release of NO. As atropine and RP 675 80 did not modify the glandular edema, this swelling would be induced by a sympathetic stimulation (16). This stimulation would release tissue kallikrein, as indicated by the kallikrein depletion in the glands (26). Tissue kallikrein then would trigger the formation of bradykinin which in turn could induce the formation of NO, as demonstrated in several tissues (56). It should be pointed out that a swelling of the salivary glands has been reported on several occasions during reflex-induced secretion (57-60) and is thus not a peculiar phenomenon associated with heat stress. Moreover, the swelling of the glands was associated with the development of a large edema of the soft tissues surrounding the SMG (51, 52), which contained a low concentration of proteins although the permeability to proteins of the blood vessels was increased as judged by the local extravasation of albumin labelled with 125I or with Evans blue (51, 52). In normal rats, the edema of the soft tissues was reduced by hexamethionium or by prazozine, an oc-adrenoceptor antagonist, but was increased by atropine (51, 52). The edema would thus result from the sympathetic stimulation of the glands and its development seemed to be facilitated by the release of tissue kallikrein, as the edema was inhibited by trasylol (51) and ap-eared in kininogen-deficient rats only after pretreatment of the animals with atropine (51, 52). The potentiating influence of atropine does not seem to depend on the suppression of the presynaptic autoinhibitory control of the release of parasympathetic cotransmitters (61, 62). Indeed, the inhibition of the effects of SP by RP 67580 did not modify the edema (52), and other parasympathetic cotransmitters, like VIP and calcitonin gene-related protein, have no edema-promoting effect (63, 64). The potentiating effect of atropine could be explained by the virtual suppression of salivation which would result in an...
increased excitation of the glands by sympathetic nerve fibres. The increase of the vascular permeability in the soft tissues surrounding the glands was induced by the formation of bradykinin. Indeed, there was no extravasation of proteins in kininogen-deficient rats even after atropine (52). Moreover, this increase of vascular permeability was suppressed by trasylool or by HOE 140 (51). The increase of vascular permeability was mediated by the release of prostanoids, NO and SP, as the extravasation of labelled albumin was inhibited by ketoprofen (51), a cyclooxygenase inhibitor, by NOARG (51) and by RP 67580 (52).

• Other changes induced by heat stress

In the heat, as dehydration developed, plasma volume decreased (47). Simultaneously, a reduction of skin water occurred and after a delay, a water deficit in muscles was apparent (47). The decrease in skin water was similar in normal and kininogen-deficient rats exposed to 40°C (50) whereas, the reduction of muscle water was significantly smaller in kininogen-deficient animals (50). That difference suggests that the water transfer from tissues to the environment was less extensive in the deficient rats and indirectly confirms the lower loss of water in these animals. Heat exposure induces a vasodilation of cutaneous vessels. In rats, this vasodilatation occurs in tail and in feet but not in the ears (65). This vasodilatation participates to the thermoregulation allowing heat loss by radiation, conduction and convection. Following amputation of the tail, rats regulate their body temperature 0.5-1.5 °C higher than do intact rats, and increase evaporative cooling by spreading saliva (66). This vascular response of the skin vessels can be explained by an inhibition of constrictor tone (67). However, a release of tissue kallikrein from the salivary glands into the blood might result in an active vasodilatation following bradykinin formation (36). The hyperthermia developed more rapidly in kininogen-deficient rats (50). Moreover, HOE 140 increased body temperature of normal rats treated with atropine and exposed to 36°C (50). In these latter animals, HOE 140 did not modify the salivary secretion which was already greatly reduced by atropine. These observations suggest thus that the formation of bradykinin induced by the release of tissue kallikrein might aid heat exchange in the skin. Although a vasodilatation occurred in blood vessels of the skin and the SMG, it was reported that the systemic blood pressure of the animals exposed to 55°C increased when the environment was heated and decreased when cooling began (5). Recently, heat stress has been shown to induce the synthesis of kinin-B 1-receptor in the cardiovascular system of rats, 6 h after its application (67). In rats subjected to heat (34°C) for several weeks, a marked hypertrophy of the SMG developed (68). This hypertrophy began after 15 hrs of heat exposure, reached maximum after 4 days, and progressively vanished. The glands returned to their normal weight after 6 weeks, although heat exposure went on (69). This process did not affect sublingual glands (68). The hypertrophy was largely prevented by parasympathetic denervation and suppressed by complete denervation of the SMG (68).

TISSUE KALLIKREITAHD THE SALIVARY GLANDS

• In the absence of external stimuli, a basal secretion of tissue kallikrein from the salivary glands constitutively occurs into the duct (9) and into the interstitial spaces (10). The role of this basal secretion is unknown, though it has been suggested that kallikrein can exercise an activity on the luminal side of the ductal cells (70). Parasympathetic stimulation of the glands gives rise to a large flow of saliva with a low level of kallikrein which would be constitutively secreted (19). During reflex-induced salivation, a small secretion of kallikrein into saliva is observed (5). The amounts of enzyme found in the saliva are in the range of that released by a parasympathetic stimulation of the glands. These reflex-induced releases are not associated with a significant transfer of kallikrein from the gland into the blood (5), although a small increase in the plasma levels of tissue kallikrein can be obtained after one hour of a parasympathetic stimulation (16). These parasympathetically-evoked secretions of kallikrein might play a role similar to that of the basal secretion. Indeed, the salivary flow during heat exposure depends on a simultaneous parasympathetic and sympathetic excitation of the glands (47,67). High level of sympathetic stimulation induces large and transient secretion of kallikrein (21,22). However, as in the kidneys which release kallikrein into the urine (6), in salivary glands, tissue kallikrein is mostly an exocrine constituent of saliva (16).

Heat stress induces a partial depletion of kallikrein in the submaxillary glands, a consumption of plasma kininogens (26) and a swelling of the glands (51); this swelling could facilitate the leakage of tissue kallikrein into the blood (16). The kininogen consumption would indicate that a formation of bradykinin takes place during heat exposure. The results obtained in kininogen-deficient rats or in rats treated with HOE 140 at 36°C ambient temperature suggest that the release of kallikrein is mainly involved in increasing the salivary flow (50). However, at this temperature, the swelling of the glands and the edema of the soft tissues are small (51,52). In contrast, at 40°C, the kallikrein-kinin system seem to play a major role in the initial facilitation of heat exchange in the skin (50). At this higher temperature, the flow of saliva is not initially reduced in kininogen-deficient rats, but the swelling of the glands and of the surrounding soft tissues largely develops in nonnal animals (50,51) suggesting a very high stimulation of the glands. The exposure of rats to 55°C, although relatively short, induce several cases of heat shock. In the heat-exhausted animals, plasma levels of kallikrein are higher (5). However, the experiments with rats exposed to heat are far from the situation of the animals in their natural environment. The stimulus is either very high, 55°C
The functional vasodilatation occurring in salivary glands during heat stress is mediated by acetylcholine and NO and does not depend on a kinin formation (51). Similarly, the kallikrein-kinin system is not involved in the atropine-resistant vasodilatation following stimulation of the parasympathetic nerve fibres (61,64,71).

CONCLUSION

- Tissue kallikrein of rat salivary glands would play two different roles. During basal secretion and salivation triggered by a direct parasympathetic stimulation or reflexively induced by gustation, mastication and aggression, kallikrein would be released in low amounts mainly in the salivary ducts. To induce a massive release of kallikrein from the salivary glands both in to the saliva and the blood, a high sympathetic excitation of the glands must be performed. This kind of stimulation is obtained with heat stress. During heat exposure, tissue kallikrein and the associated formation of kinins may contribute to the thermoregulatory processes. In further studies, it would be intriguing to examine whether the heat shock proteins (72) could be involved in the modulation of the kallikrein-kinin system in the SMG.

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Tissue kallikrein of rat salivary glands would play two different roles. During basal secretion and salivation triggered by a direct parasympathetic stimulation or reflexively induced by gustation, mastication and aggression, kallikrein would be released in low amounts mainly in the salivary ducts. To induce a massive release of kallikrein from the salivary glands both in to the saliva and the blood, a high sympathetic excitation of the glands must be performed. This kind of stimulation is obtained with heat stress. During heat exposure, tissue kallikrein and the associated formation of kinins may contribute to the thermoregulatory processes. In further studies, it would be intriguing to examine whether the heat shock proteins (72) could be involved in the modulation of the kallikrein-kinin system in the SMG.

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