Increase in Plasma Renin in Aggressive Mice Originates from Kidneys, Submaxillary and Other Salivary Glands, and Bites

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SUMMARY Aggressive behavior in mice caused a vast release of renin into the plasma. The present data support previous findings that the main sources were the submaxillary gland and kidney. In addition, unidentified salivary glands capable of releasing renin into the saliva were demonstrated by alpha-adrenergic stimulation. The role of these glands in generating plasma renin is unknown. Experiments were performed that strongly support the possibility that aggression-provoked salivary renin may be transferred by bites from one animal to another. (Hypertension 5: 180-184, 1983)

KEY WORDS • extrarenal renin • salivary renin

A FEW years ago it was discovered incidentally that aggressive behavior, including fighting, in male mice provoked a vast prolonged rise in plasma renin with only slight, if any, increase in blood pressure. The source of renin was found to be the submaxillary gland and the kidneys; however, other sources were also demonstrated but not identified.

The aim of the present work was to identify these extrarenal and extrasubmaxillary sources, and to investigate whether the extremely high renin concentrations in the saliva of aggressive mice (Pedersen EB, Poulsen K, unpublished data) could be transferred to another mouse by bites.

METHODS

Animals

Male albino mice of the Danish State Serum Institute strain, weighing 30 to 40 g, were individually housed in cages (internal size: 25 x 20 x 12 cm) for at least 3 weeks. Their submaxillary glands contained large amounts of renin. A combined submaxillary, sublingual sialoadenectomy was performed several weeks before the experiment. The animals were nephrectomized 2 to 24 hours before the experiment.

Aggressive Behavior

Grouping two mice that had previously been housed individually for at least 3 weeks but often longer created aggressive behavior. The grouping took place in a small cage as described by Valzelli for 20 to 30 minutes.

Blood

A slight cut in the tail enabled blood to be collected in EDTA-coated capillary tubes. Conscious animals were restrained by hand. This method did not stimulate renin levels, and measurements of blood samples from the tail and a. femoralis gave similar results. Use of indwelling venous catheters was not possible in fighting mice.

Stimulation of Salivation

In pentobarbital sodium (120 mg/kg) anesthetized animals, injections of pilocarpine (Mecobenzon, 1 mg/kg) and phenylephrine (Metaoxedrine, Mecobenzon, 17 mg/kg) were given intraperitoneally. Saliva was collected from the mouth with a constriction pipette.

Renin Concentration

By the antibody-trapping method, Angiotensin I (formed by renin's catalytic activity at a high constant renin substrate concentration) was trapped, protected, and measured by an antibody in a radioimmunological technique.

Renin Antibody

An antisera elicited in rabbits against pure submaxillary mouse renin was used as the antibody. It
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cross-reacted completely with mouse plasma and kidney renin and had a Ka value of \(2 \times 10^3\) liter/mole; it did not cross-react with cathepsin D from the mouse spleen.

Transfer of Salivary Renin to the Tissues

Artificial bites were performed with a towel clip that bit the animal 10 times at different places in the back. Before each bite the tip of the clip was dipped in saline, pure submaxillary mouse renin (8 Gu/ml), or renin-rich saliva (600 Gu/ml). Application of salivary renin to the mouth, nose, and nephrectomy wound was performed with a constriction pipette, with care being taken not to hurt the animal. Application to the trachea was performed with a small plastic tube on a syringe.

Results

Aggression-Provoked Increase in Plasma Renin in Normal Mice

When a normal isolated mouse was confronted with a mouse without submaxillary glands (fig. 1 a), an increase in plasma renin of one to two orders of magnitude was found in the normal mouse. The values reached were 234 \(\pm\) 192 Gu \(\times\) 10\(^{-3}\) (n = 13). The lack of renin-containing submaxillary glands of the opponent precluded transfer of renin and showed that the source was within the normal animal in study.

Role of the Submaxillary Gland

Submaxillary sialoadenectomy of the mouse and confrontation with the same kind of opponent (fig. 1 b) gave a lower increase (\(p < 0.01\)) in plasma renin of about one order of magnitude. The values reached were 33 \(\pm\) 10 Gu \(\times\) 10\(^{-3}\) (n = 9). This indicated that the submaxillary glands participated in the increase, but also that other organs (e.g., the kidneys) released renin.

Role of the Kidney

To investigate the role of the kidney, both the submaxillary glands and the kidneys were removed before fighting with the same kind of opponents as above. No increase was indicated (fig. 1 c). When compared with figure 1 b (\(p < 0.001\)), the role of the kidneys in the aggression-provoked renin release is shown.

Extrarenal Extrasubmaxillary Renin Release to Plasma

When, however, a mouse deprived of submaxillary glands, sublingual glands, and kidneys was confronted with a normal mouse (which had its incisors cut to avoid bites), more vigorous fighting evolved. In this case, plasma renin in the deprived mouse increased (\(p < 0.005\)) almost uniformly, indicating that extrarenal and extrasubmaxillary renin may have been released (fig. 1 d).

Stimulation of Renin Containing Saliva from Unidentified Salivary Glands

Since \(\alpha\)-adrenergic stimulation with phenylephrine provokes a vast renin release in the saliva,\(^1\) it was investigated whether the submaxillary gland was the sole source of this renin. When phenylephrine was given to animals without submaxillary (and sublingual) glands, saliva obtained after 40 to 60 minutes contained renin, that could be neutralized with an antibody against pure renin (fig. 2 b). The renin concentrations in the saliva were independent of the plasma renin concentrations, the latter being lower in animals who also lacked kidneys (fig. 2 a). These unidentified renin-containing salivary glands may be the unknown source in figure 1 d. Stimulation with pilocarpine provoked a much lower salivary renin concentration (fig. 2 c and d).

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**Figure 1.** Role of the glandular submaxillaris, kidney, and other organs in the increase of plasma renin in fighting mice. a. Increase in plasma renin (logarithmic scale) with time in normal mice that were fighting with mice without submaxillary glands. b. Increase in mice that have had the glandula submaxillaris and sublingualis removed and when fighting with mice without submaxillary glands. c. Plasma renin in mice without glandula submaxillaris and sublingualis, which were nephrectomized 2 to 24 hours before confrontation with mice without submaxillary glands. d. Increase in mice without glandula submaxillaris, glandula sublingualis, and kidneys, fighting with toothless normal mice. The symbol (\(\bullet\)) was used when the opponent was submaxillary and sublingually sialoadenectomized, (\(\bigcirc\)) was used when the opponent was additionally nephrectomized.
Role of Bites in the Transfer of Salivary Renin

To study the effect of bites on plasma renin, the salivadectomized opponent used in figure 1 was substituted with a normal intact mouse in the experiments shown in figure 3. When a normal mouse was fighting, its plasma renin could rise to about the same level whether the opponent was salivadectomized (fig. 1 a) or not (fig. 3 a). On average, however, the values were higher when the opponent was normal (p < 0.001). If the animal had undergone submaxillary salivadectomy the plasma renin rose to much higher values (p < 0.001) if the opponent was intact (fig. 3 b) than if it lacked the submaxillary glands (fig. 1 b). Similar results were found if the animal in question also was nephrectomized (compare fig. 3 c with figure 1 c, p < 0.05). Artificial bites of salivadectomized and nephrectomized animals were performed with a towel clip dipped in saline (fig. 3 d), pure submaxillary renin (fig. 3 e), and renin-rich saliva (fig. 3 f). The increase in plasma renin level supports the concept of transfer of renin from one animal to another through the biter.

Other Routes of Transfer of Renin

Other routes for transferring renin were investigated by applying phenylephrine-stimulated renin-rich saliva (600 GU x ml⁻¹) to the mouth (Fig. 4 a), trachea (fig. 4 b), nose (fig. 4 c), and the wound immediately after nephrectomy (fig. 4 d). Only through the nose was a considerable amount of renin absorbed in the blood. As expected, however, if 5 μl of saliva were applied directly on the spot of blood collection from the tail vein, high apparent renin values were seen (6-2600 x 10⁻¹ GU x ml⁻¹, n = 6). Application of pilocarpine-stimulated saliva (with a low renin concentration, 0.3 GU x ml⁻¹) had no effect (fig. 4 e).

Discussion

The cause of the elevated plasma renin concentration in aggressive mice was found to be multifactorial. We attempted to remove the sources one by one. This strategy was, however, seriously hampered by the fact that not all the sources were satisfactorily identified. Another problem was that operated animals showed less tendency to aggressive behavior, especially after nephrectomy. This made the very stimulus, the effect of which we were studying, variable. Dexamethasone treatment restored to some extent the aggression, in accordance with the findings by Leshner et al.⁶ that the ACTH level is a critical parameter in the control of aggressiveness. Furthermore, when two normals are fighting, they are not only better fighters, they are also more capable of protecting themselves. The resulting fight was therefore less fierce, than when the opponent was a weaker relatively nonaggressive mouse, in accordance with the findings of others.⁶ This means that the relative contribution of the different sources may vary in the different experiments and the magnitude remains an open question.

It seems justified to conclude that the increased plasma renin within a normal fighting mouse, originates from its own organs, since the increase is independent of the kind of opponent. It is also likely that most of the renin originates from the submaxillary gland, as previously described.¹ The previously favored conclusion, that the kidneys also participate in the increase,¹ is still valid although the contribution seems considerably weaker after the demonstration of an extrarenal and extrasubmaxillary source.² Removal of the kidneys abolishes the increase in plasma renin, but also the aggressiveness. Intensifying the fight with a normal toothless mouse restored part of the increase in plasma renin in the nephrectomized animals, thereby favoring extrarenal organs.

The α-adrenergic stimulation of renin-rich saliva from animals without submaxillary glands, sublingual glands, and kidneys indicates that sources other than submaxillary gland and the kidneys may liberate renin.
The role of bites for the increase in plasma renin in aggressive mice is shown in (a) the increase in plasma renin in normal mice fighting with other normal mice, (b) the increase in mice without glandula submaxillaris and glandula sublingualis fighting with normal mice, and (c) the increase in mice, without glandula submaxillaris and glandula sublingualis, which were nephrectomized before confrontation with normal mice. In the last columns no opponents were present, but mice without glandula submaxillaris, glandula sublingualis, and kidneys were given 10 artificial bites (d) with saline, (e) with pure submaxillary renin, 8 GU/ml, and (f) with saliva, 600 GU/ml.

Transfer of salivary renin through other routes is shown when mouse saliva with a high renin concentration (15 μl, phenylephrine stimulated, 600 GU/ml) was applied to the (a) mouth, (b) trachea, (c) nose, and (d) nephrectomy wound. The recipient was in all cases a submaxillary and sublingual sialoadenectomized and nephrectomized mouse and no aggression took place. In (e) mouse saliva with a low renin concentration (15 μl, pilocarpine stimulated, 0.3 GU/ml) was applied. The symbols being the same as in (a) through (d).
Concentration in the saliva sometimes being higher than the plasma concentrations indicates active secretion. The \(\alpha\)-adrenergic stimulation mimics to some extent aggression-provoked secretion of renin to saliva and blood from the submaxillary gland (unpublished data), and the unknown salivary glands may react similarly. These unidentified salivary glands may therefore be the extrarenal, extrasubmaxillary, and extrasublingual source for which we have been looking. They may also be the source of the basal renin values seen in animals without kidneys and submaxillary glands.

That salivary renin may be transferred from one animal to another through bites is strongly supported, especially in the experiments in which a normal mouse attacked the weak sialoadenectomized and nephrectomized animal. The transfer of salivary renin between normal intact mice during aggressive behavior is possible as described above, but less likely. The transfer of salivary renin from the unidentified salivary glands is unlikely because these glands secrete about four orders of magnitude less renin than the submaxillary glands.

The vast renin concentration in aggression-provoked saliva, which may be mimicked by \(\alpha\)-adrenergic stimulation,\(^5\) reaches values of 6000 \(\text{GU} \times \text{ml}^{-1}\) (Pedersen EB, Poulsen K, unpublished data). Such high concentrations are a hazard to all physiological studies because of the risk of contamination. The animal may even contaminate itself by licking the blood sampling wound in the tail. However, measurements of blood samples obtained from the tail and \(a.\) femoralis gave similar results,\(^1\) and the almost uniform pattern of the individual plasma values reduces the likelihood of contamination in the present data, although it is not ruled out.

The aggression-provoked renin fulfills all the criteria so far studied for being active renin, identical with normal mouse plasma renin and pure submaxillary mouse renin.\(^7\) The present data do not rule out the possibility that other organs participate in the aggression-provoked renin release. Vascular renin activity may be a likely source of the persistent renin in nephrectomized animals\(^8\) belonging to species not so rich in salivary renin as the mouse. It is still unknown how the mouse controls its blood pressure with these high renin values.\(^1\)

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