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Renin-like activity of the rat submaxillary gland: characterization and the effect of several drugs and stimuli

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Summary

1. Renin-like activity was found in rat submaxillary glands.

2. This activity was destroyed by boiling, was non-dialyzable and showed an optimum at approximately 50° C.

3. Renin-like activity in the submaxillary gland was not diminished 24 h after nephrectomy but was considerably reduced after ligature of the submaxillary duct.

4. Renin-like activity in the submaxillary gland was reduced following food-deprivation, water-deprivation or hypovolemia.

5. Renin-like activity in the rat submaxillary gland was increased after isoproterenol administration but not following pilocarpine.

6. Renin-like activity in the rat submaxillary gland was increased considerably by administration of NaCl or KCl, as well as following adrenalectomy.

7. Chlorothiazide and ouabain increased submaxillary renin-like activity but diazoxide did not affect this activity.

Introduction

Renin-like activity has been found in several extra-renal tissues. Thus, such activity has been described in the placenta and uterus of the rabbit (Gross, Schaechtelin, Ziegler & Berger, 1964 and in the submaxillary gland of the mouse (Werle, Vogel & Goldel, 1957) and more recently in brain (Ganten, Minnich, Granger, Hayduck, Brecht, Barbeau, Boucher & Genest, 1971). However, the physiological role of extra-renal renin and the effects on it of various stimuli (hypovolemia, sodium deficiency or surplus) and drugs (e.g. diuretics) which affect kidney-renin have not been elucidated.

Hypovolemic drinking is selectively suppressed in the rat by ablation of the submaxillary gland but not the parotid gland (Gutman, Livneh & Pietrokovski, 1970), an effect resembling suppression of hypovolemic drinking following nephrectomy (Gutman & Benzakein, 1969).

In a preliminary study of rat salivary glands, renin-like activity was found in the submaxillary gland but not in the parotid or sublingual glands (Gutman, Livneh & Levy, 1971). Therefore, we have further investigated the renin-like activity in the rat submaxillary gland as well as the effect of sodium, potassium, adrenalectomy, hypovolemia, food and water deprivation and the administration of several drugs on this activity.

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Methods

All experiments were carried out in male rats of the Hebrew University strain (Sabra), wt 200–350 grams. All operative procedures were performed under ether anaesthesia, except collection of saliva which was carried out under pentobarbitone anaesthesia.

Operative procedures

1. *Adrenalectomy.* Bilateral adrenalectomy was performed through a midline incision in the back. Sham operated rats underwent the same procedure except for removal of the adrenals. Seven days following operation the submaxillary glands were removed.

2. *Nephrectomy.* Bilateral nephrectomy was performed through a midline incision in the abdomen. The submaxillary glands were removed 24 h later.

3. *Elimination of submaxillary saliva from the buccal mucosa.* The submaxillary ducts were isolated and cleaned through a midline cut in the neck. The ducts were ligated as close as possible to the floor of the mouth. Proximal to the ligature the ducts were cut and left free in the tissues of the neck (to avoid increased pressure within the gland if the duct remained closed). Three weeks later the submaxillary glands were removed both from these rats and from sham operated rats.

Collection of saliva

Saliva from the submaxillary glands was collected through polyethylene cannulae introduced into the submaxillary duct. Secretion of saliva was stimulated by subcutaneous injection of 1 mg isoprenaline. The saliva was collected into a test tube placed in ice.

Various stimuli

1. *Hypovolemia.* Hypovolemia was induced according to the method of Stricker (1966) by subcutaneous injection of 20 ml/kg of a 20% solution of polyethyleneglycol (M.W.=20,000, Carbowax 20) in 0·9% NaCl. The submaxillary glands were removed 18 h later.

2. *Water-deprivation.* Rats were allowed food *ad-libitum* but no water for 48 h, after which time the submaxillary glands were removed.

3. *Food-deprivation.* Rats were allowed water *ad-libitum* but no food for 48 h after which time the submaxillary glands were removed.

Extraction of the submaxillary glands

The salivary glands were removed from rats under ether anaesthesia and were immediately placed in cold water. The glands underwent three cycles of freezing and thawing and were then homogenized. After centrifugation at 16,000 *g* for 10 min, the supernatant was separated and served as the solution of enzyme (renin-like substance). Rat renin substrate (angiotensinogen) was prepared from plasma obtained from rats 24 h after bilateral nephrectomy. The substrate was prepared according to the method of Haas, Goldblatt, Gipson & Lavera (1966) as modified by Boucher, Menard & Genest (1967).

Dialysis of the enzyme-solution obtained from the submaxillary gland was performed against 100 volumes of distilled water for 24 h at 4° C.

Heat denaturation was carried out by placing the solution of renin-like material in a bath of boiling water for 20 minutes.

Assay of renin-like activity

Incubation with substrate was carried out according to the method of Boucher, Menard & Genest (1967) at pH 6·5, 37° C in a shaking bath and with Dowex 50WX2 (100–200 mesh) resin present. The reaction was stopped by immediate freezing.

For separation of the angiotensin formed, the whole content of the tube was added to a column containing 1 ml of the resin (Dowex 50WX2). The fluid was allowed to flow through the column. The column was then washed with 10 ml of 0·2 M ammonium acetate followed by 20 ml of acetic acid (10% v/v) and 30 ml of water. Angiotensin was then eluted from the column with 7 ml of 0·1 N diethylamine, followed by 7 ml of 0·2 N ammonia. The eluate was lyophilized and the dry material was dissolved in 0·9% NaCl immediately before testing. The assay was performed in nephrectomized rats under pentobarbitone anaesthesia, pretreated with 4 mg/kg subcutaneous pentapyrrolidinium bitartrate (Ansolysen, obtained from May & Baker). The blood pressure responses were compared to standard solutions of angiotensin II (valine 5-angiotensin II aspartic β-amide, obtained from CIBA).

Drugs

Chlorothiazide was injected subcutaneously, 100 mg/kg at 8 a.m. and 8 p.m. and a third dose of 100 mg/kg was given i.p. on the next morning. The submaxillary glands were removed 4 h later.

(±)-Isoprenaline, 1 mg/kg, was injected subcutaneously, three times over 36 hours. The submaxillary glands were removed 2 h after the last injection.

Pilocarpine was injected subcutaneously, 2 mg/rat at 8 a.m.; at 8 p.m. 1 mg/rat was injected i.p. and 1 mg subcutaneously. On the next morning 1 mg/rat was injected i.p. and the glands were removed 3 h later.

Diazoxide was given 100 mg/kg subcutaneously at 8 a.m., 200 mg/kg i.p. at 8 p.m. and on the next morning 200 mg/kg i.p. The submaxillary glands were removed four hours after the last injection.

Ouabain was injected i.p. twice daily, 0·5 mg/rat each time, for four days.

Results

Table 1 shows that incubation of the rat submaxillary homogenate without addition of substrate (angiotensinogen) produced almost no pressor material. When substrate was added to the gland homogenate and immediately passed through the resin column, without incubation, no appreciable pressor activity was present. Boiling the homogenate before incubation also abolished the pressor activity. Dialysis of the gland extract produced only a small decrease of the activity of the gland homogenate.

TABLE 1. *Renin-like activity in submaxillary gland homogenate under various conditions of incubation*

		μg angiotensin liberated/g gland
Exp. 1:	SM homogenate+renin-substrate from plasma+incubation*	49·6
	SM homogenate+renin-substrate from plasma, no incubation	3·0
	SM homogenate; no substrate+incubation	1·9
	Boiled homogenate+renin-substrate from plasma+incubation	2·2
Exp. 2:	SM homogenate+renin-substrate from plasma+incubation	44·8
	Dialyzed homogenate+renin-substrate from plasma+incubation	32·2

* Incubation for 2 h at 37° C.

Figure 1 shows that with a given amount of substrate, incubation of the submaxillary gland homogenate produced a linear increase of the pressor activity with time. The decreased rate after incubation for more than 2 h was presumably due to exhaustion of substrate.

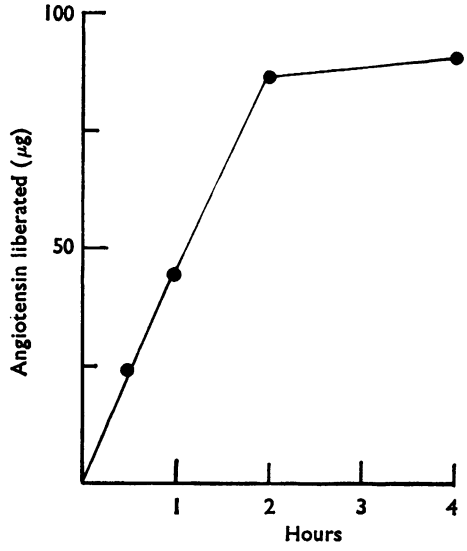

FIG. 1. Effect of duration of incubation on angiotensin liberated from renin-substrate by rat submaxillary homogenate. Incubation at 37° C.

Figure 2 shows that the production of the pressor activity by the submaxillary gland is temperature-dependent with an optimum at approximately 50° C.

The submaxillary gland of the rat, therefore, contains an enzymatic activity (non-dialyzable, heat denatured, temperature dependent) which produces during *in vitro* incubation with rat renin-substrate a pressor substance adsorbed and released from Dowex 50X2 resin under the same conditions as angiotensin II.

Table 2 shows that the renin-like activity in the rat submaxillary gland can be acutely reduced by several procedures: hypovolemia, water deprivation and food deprivation.

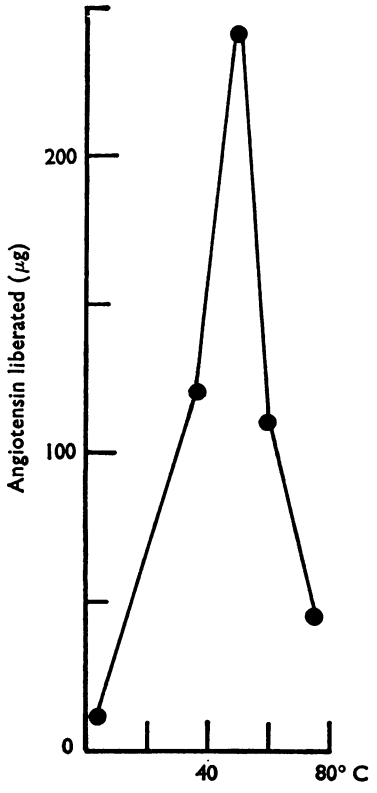

FIG. 2. Effect of temperature of incubation on angiotensin liberated from renin-substrate by rat submaxillary homogenate.

TABLE 2. *Renin-like activity in submaxillary gland under various stimuli*

	μg Angiotensin/g gland*	*P*
Control (n=12)	7·1±0·7	
Hypovolemia (n=10)	4·9±0·5	<0·05
Water deprivation (n=12)	4·3±0·3	<0·01
Food deprivation (n=10)	4·2±0·4	<0·01

* μg Angiotensin liberated during 2 h incubation at 38° C per gram of wet weight of gland. n=Number of experiments. Data given as mean±S.E.M.

Figure 3 shows that replacement of drinking water by 0·9% NaCl for varying times produced related increases in the renin-like activity in the submaxillary gland of the rat.

Figure 4 shows that administration of KCl to rats increased renin-like activity in the gland significantly above that of rats given NaCl.

The effects of sodium and potassium on renin-like activity in the submaxillary gland are, therefore, opposite to that on kidney renin: both sodium excess and potassium excess decrease renin release from the kidney and sodium excess reduces also the renin content of the kidney (Gross, 1967; Vander, 1970). This may suggest different function(s) of submaxillary renin-like activity from that of kidney renin.

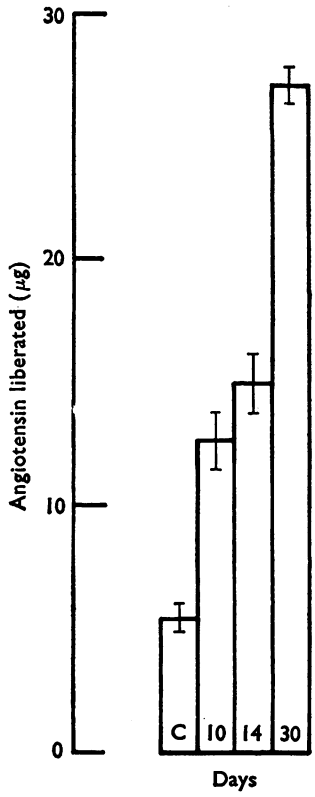

FIG. 3. Effect of NaCl on rat submaxillary renin-like activity. 0·9% NaCl was given as drinking fluid for the duration indicated at bottom of columns. Renin-like activity expressed as angiotensin liberated per gram tissue wet weight during incubation at 37° C for 2 h with renin-substrate. Vertical bars: S.E.M. Control, $n=11$; NaCl: 10 days, $n=5$; 14 days, $n=11$; 30 days, $n=12$.

Another control for the possible origin of the renin-like activity in the submaxillary gland was to study the fate of this activity following nephrectomy, since bilateral nephrectomy eliminates the main source of renin in the body. Twenty-four hours after nephrectomy the renin-like activity in the rat submaxillary was $93{\cdot}9 \pm 8{\cdot}3\%$ of that in sham operated rats ($n=13$); this value was not significantly different from 100%. On the other hand, ligature of the submaxillary duct resulted in a decrease of renin-like activity in the ligated glands to $31{\cdot}9 \pm 10\%$ of the control glands ($n=4$).

Adrenalectomy caused a significant increase of submaxillary renin-like activity. The mean activity was $7{\cdot}9 \pm 0{\cdot}6$ in sham operated rats ($n=10$) compared with $13{\cdot}8 \pm 1{\cdot}7$ μg angiotensin liberated/g gland during 2 h of incubation in adrenalectomized rats ($n=12$). This effect was similar to that seen after adrenalectomy on the kidney renin. A possible explanation for the increased submaxillary renin could be that the renin-like substance in the submaxillary gland originated from the kidney. However, since submaxillary renin-like activity was unchanged after nephrectomy, an alternative explanation for the increased renin-like activity in the submaxillary gland following adrenalectomy is that it is the result of the hyperkalaemia which accompanies adrenalectomy.

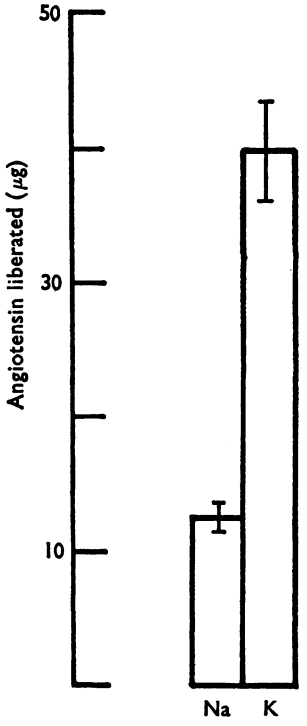

FIG. 4. Effect of KCl on rat submaxillary renin-like activity. KCl was administered subcutaneously, 2 g/kg twice daily for ten days. Control animals were given equivalent amounts of NaCl. Renin-like activity expressed as in Fig. 3. NaCl, $n=5$; KCl, $n=5$.

The effects of some drugs on renin-like activity in the submaxillary gland were also studied. Thus, isoprenaline, which stimulates salivary secretion, caused an appreciable increase of renin-like activity, whereas pilocarpine, a muscarinic stimulant of the salivary gland did not significantly augment renin-like activity in the gland (Table 3), although it produced a profuse salivation at this dosage.

The diuretic drug, chlorothiazide, which increases kidney renin secretion, caused a large increase in submaxillary renin-like activity in the rat (Table 3). Diazoxide, a compound resembling chlorothiazide in chemical composition, but lacking diuretic action, had no effect on submaxillary renin-like activity. It is of interest that diazoxide shares the hypotensive action of chlorothiazide and increases kidney renin secretion, but lacks effect on submaxillary renin-like activity (Table 3).

TABLE 3. *Effect of drugs on renin-like activity in rat submaxillary gland*

	μg Angiotensin/g gland*	*P*
Control ($n=37$)	8·9±0·7	
Isoprenaline ($n=10$)	26·2±2·2	<0·001
Pilocarpine ($n=10$)	11·3±0·9	n.s.
Chlorothiazide ($n=10$)	21·4±2·4	<0·001
Diazoxide ($n=7$)	11·3±0·8	n.s.
Ouabain ($n=10$)	27·8±1·9	<0·001

* Same conditions as in Table 2. n.s.=difference from control statistically not significant. Results expressed as in Table 2.

Ouabain, a cardiac glycoside which inhibits active sodium transport, increased significantly renin-like activity in the submaxillary gland (Table 3).

Saliva collected from the submaxillary duct of the rat also contained renin-like activity, as seen in Table 4. Incubation of saliva with renin-substrate obtained from rat plasma resulted in the production of a pressor substance resembling angiotensin. Incubation of saliva without substrate or mixing saliva with substrate but without incubation produced no pressor activity. Boiling of saliva before incubation abolished the activity while dialysis of the saliva against water for 24 h did not eliminate the renin-like activity.

TABLE 4. *Renin-like activity in submaxillary saliva of the rat*

	ng angiotensin liberated/ml saliva
Saliva+renin-substrate from plasma+incubation*	842
Saliva+renin-substrate from plasma, no incubation	5
Saliva; no substrate+incubation	15
Boiled saliva+substrate+incubation	8
Dialyzed saliva+substrate+incubation	706

* Incubation at 37° C for 18 h.

Discussion

The presence of renin-like activity in tissues other than kidney has been reported by various authors (Carretero, Bujak & Houle, 1971; Ganten *et al.*, 1971; Gross *et al.*, 1964; Skeggs, Lentz, Kahn, Dorer & Levine, 1969). Among these tissues, is the submaxillary gland of the mouse (Werle *et al.*, 1957). Recently, Carretero *et al.* (1971) have shown that the various extra-renal renin-like enzymes catalyze the production of the same final product: angiotensin. Koch & Unger (1969) have also demonstrated that the final product of incubation of rat submaxillary homogenate with rat renin-substrate is angiotensin.

However, whereas the role of kidney renin in sodium balance is well established (Brown, Lever & Robertson, 1967), no functional significance of extra-renal renin has been clearly demonstrated. One possibility is that extra-renal renin merely reflects adsorption of circulating renin in the various tissues. Several experiments reported here refute this possibility for the rat submaxillary gland: Administration of saline decreases both content and secretion of kidney renin whereas submaxillary renin-like activity increased (see Fig. 3). Nephrectomy did not affect submaxillary renin-like activity at all, whereas plasma renin decreased to undetectable levels a few hours after nephrectomy. Ligature of the submaxillary duct, which does not interfere with circulation in the gland, decreased significantly submaxillary renin-like activity.

Takeda, Debusk & Grollman (1969) have reported renin-like activity in plasma from the submaxillary vein of the mouse. These authors found a decrease in submaxillary vein renin activity following administration of isoprenaline for several days or after combined treatment with desoxycorticosterone plus saline (1% NaCl) as drinking fluid. The different findings in the present experiments may be explained by species differences and by administration of isoprenaline for a short duration. In addition, submaxillary gland *tissue* was studied for renin-like activity,

rather than *blood* flowing out of the gland. Takeda *et al.* (1969) also reported a fall of systemic blood renin activity in the male mouse after ablation of the submaxillary glands. In preliminary studies in the rat, no significant change in plasma renin activity after ablation of the submaxillary glands was found (unpublished observations). The role or function of mouse and rat submaxillary renin-like activity may, therefore, be different.

Some of the experiments reported here demonstrate the specificity of stimuli which affect submaxillary renin-like activity. Thus, e.g. isoprenaline induced a considerable increase in submaxillary renin-like activity whereas pilocarpine, which also stimulated secretion of saliva, did not increase renin-like activity in the gland. Chlorothiazide and ouabain increased submaxillary renin-like activity. In the kidney both of these drugs decrease active sodium transport and increase potassium excretion. They may affect salivary secretion processes in a similar way. No single common mechanism can be suggested at present for the effect of the various compounds which increase submaxillary renin-like activity.

It is noteworthy that the submaxillary gland is of major importance in the rat for functions other than digestion, as, for example: heat regulation, which involves loss of considerable volumes of saliva, smeared on the fur (Hainsworth & Stricker, 1971) or water intake induced by hypovolemia (Gutman & Benzakein, 1969). Interestingly, both tissues which contain renin activity, the kidney and the submaxillary gland, are essential for thirst induced by hypovolemia (Gutman & Benzakein, 1969; Gutman *et al.*, 1971) and the renin-angiotensin system has been demonstrated to be a thirst-inducing stimulus (Fitzsimons & Simons, 1969).

The significance of the presence of renin-like activity in *saliva* from the submaxillary gland is at present obscure. It may represent 'overflow' or escape of renin-like enzyme into the lumen of ducts or acini with no functional meaning at all.

Saliva secretion bears some similarity to urine production in the nephron in that an initially isotonic fluid, with sodium as the major cation, is transformed at some stage along the nephron into a hypotonic, hyponatraemic fluid. Interestingly, the renin secreting apparatus in the kidney resides precisely at that site along the nephron where hypotonic-hypotraemic fluid is present (i.e. at the early distal convoluted tubule). The saliva in the excretory duct resembles the composition of distal convoluted tubule fluid; a point already made by Brown, Lever & Robertson (1967). It is intriguing to consider the possibility that the renin-secreting mechanism in the submaxillary gland may be regulated by the composition of saliva and may be involved either in an autoregulation function (as in the kidney) and/or as regulator of sodium and/or potassium transport in the salivary gland. However, at the present stage this is more of the nature of speculation awaiting much experimental data.

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