

Micropuncture Study of Sodium and Potassium Excretion in Rat Parotid Saliva: Role of Aldosterone (34311)

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(Introduced by Harry A. Waisman)

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Recent studies (1, 2) of salivary gland function have demonstrated that the rat parotid gland, like the parotids of man and dog (3, 4), produces saliva hypotonic to plasma. Micropuncture and microanalytical techniques have been used to demonstrate that salivary hypotonicity is produced in the striated ducts of the gland by reabsorption of sodium in excess of water from a plasma-like primary secretion (2). The cellular structure of the striated ducts is similar to the structure of the distal tubules of the kidney (5). Since previous studies have indicated that the major action of aldosterone is on the distal tubules of the mammalian nephron (6), we undertook the present study of the role of aldosterone on the function of the rat parotid gland. The effects of this hormone on the handling of sodium and potassium by the parotid in general and the striated ducts in particular were investigated before and after adrenalectomy. The ability of exogenous aldosterone to reestablish normal handling of sodium and potassium by the rat parotid in the adrenalectomized state was investigated.

Methods and Materials. Male, albino rats of the F.W. 49 and Sprague-Dawley strains, weighing 150–200 g, were used in these experiments. The animals were fasted for 24 hr prior to the experiments but were allowed free access to water. They were anesthetized with either Inactin (sodium 5-ethyl-5-methyl-propyl-2-thiobarbiturate) or sodium pentobarbital in doses of approximately 8.0 mg/100 g of body weight. The methods of surgical preparation of the animals, cannulation of the main ducts, and micropuncture of the intercalated and lobular ducts have been described in previous publications (1, 2).

The adrenals were removed 7–14 days

prior to the experiments through bilateral flank incisions under light ether anesthesia. Postoperatively, the drinking water was replaced with 0.9% NaCl solution, but the animals remained on standard rat pellet diet. The effectiveness of adrenalectomy was determined by the following criteria: (a) elevation of the serum potassium concentration above 5.0 meq/liter (mean: 6.35 ± 0.34 SD); (b) decrease in the ability of the rat to excrete a standard water load given by a nasogastric tube (7); (c) decrease in serum sodium concentration (mean: 132.5 ± 3.52 meq/liter). Aldosterone (*d*-isomer) was obtained from Sigma Chemical Company. The hormone was dissolved in a small volume of 95% ethanol and then in a larger volume of 5% glucose in water before injection into the animal. The effects of *d*-aldosterone were studied in 7 adrenalectomized rats. A solution containing 25 μ g of *d*-aldosterone in 1.0 ml of isotonic dextrose in water was prepared. The left jugular vein of the rat was cannulated and 2.5 μ g of aldosterone was injected intravenously 60 min prior to stimulation of the parotid with pilocarpine. By using a Harvard constant infusion pump, an intravenous infusion of 0.4 μ g of *d*-aldosterone/min was maintained throughout the experiment. Salivation was induced by the intravenous injection of pilocarpine (0.1 mg/100 g of body weight). Collections of saliva were made at progressively lower flow rates in preweighed polyethylene vessels under mineral oil. The methods of ultramicroanalysis of the samples for sodium and potassium have been described in previous reports (1, 2).

Results. 1. *Sodium and potassium concentrations in the parotid saliva of control and*

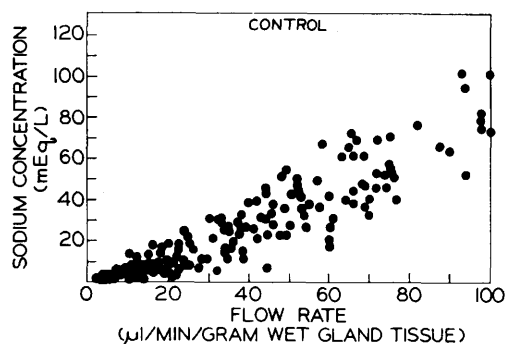


FIG. 1. Relationship of flow rate to sodium concentration in rat parotid saliva (15 rats) following stimulation with pilocarpine.

adrenalectomized rats and effects of d-aldosterone. As shown in Figs. 1-4, the concentrations of sodium and potassium in the parotid saliva of control and adrenalectomized animals were related to the salivary flow rate. The correlation between salivary sodium concentration and flow rate is shown in Fig. 1. At flow rates less than $10 \mu\text{l}/\text{min}/\text{g}$ of wet gland tissue, minimal sodium concentrations (1-6 meq/liter) were observed. At progressively higher flow rates the salivary sodium concentration gradually increased. The same correlation between salivary sodium concentration and flow rate was observed in the parotid saliva of the adrenalectomized rats (Fig. 2) but the sodium concentrations were higher than those of the saliva of control rats

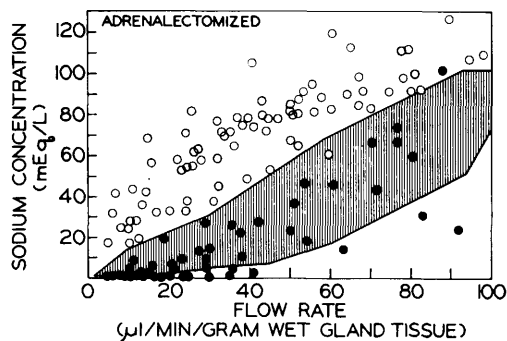


FIG. 2. Relationship of flow rate to sodium concentration in rat parotid saliva following stimulation with pilocarpine. (shaded area), range of control values taken from Fig. 1; (○), values from 10 rats 2 weeks after bilateral adrenalectomy; (●), values from 6 adrenalectomized rats following administration of *d*-aldosterone (see text).

at any given flow rate. There was little overlap between these two groups of measurements. Following administration of *d*-aldosterone to the adrenalectomized rats, the excretion of sodium in the parotid saliva as indicated by the relation of sodium concentration to flow rate shown in Fig. 2, became identical to that of the control rats.

The salivary potassium concentration was also related to the flow rate. This relationship, however, was the inverse of that for sodium as shown in Fig. 3. The potassium concentrations in the control rats were high at low flow rates and gradually decreased as the flow rates increased. In the adrenalectomized rats, as shown in Fig. 4, the salivary potassium concentrations were lower than those of the control animals at any observed flow rate. There was, however, significant

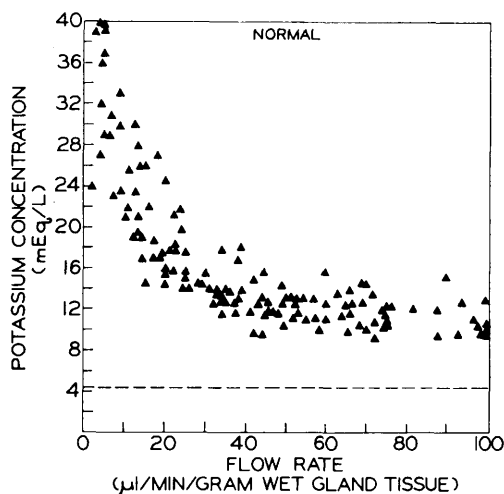


FIG. 3. Relationship of flow rate to sodium concentration in rat parotid saliva (15 rats) following stimulation with pilocarpine; (---), mean serum potassium concentration in these rats.

overlap between the two groups of measurements, especially at high flow rates. Following administration of *d*-aldosterone to the adrenalectomized rats, the excretion of potassium in the parotid saliva returned to control levels as indicated by the relationship of potassium concentration to flow rate shown in Fig. 4.

2. *Sodium and potassium concentrations in the fluid from different segments of the duct*

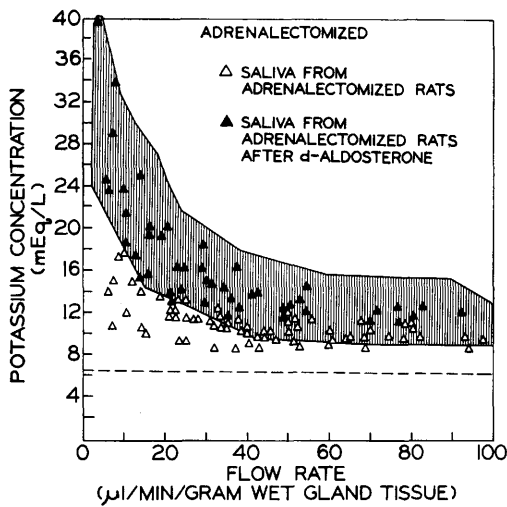


FIG. 4. Relationship of flow rate to potassium concentration in rat parotid saliva following stimulation with pilocarpine; (shaded area, range of control values taken from Fig. 3. (Δ), values from 10 rats 2 weeks after bilateral adrenalectomy; (\blacktriangle), values from 6 adrenalectomized rats following administration of *d*-aldosterone (see text); (---), mean serum potassium concentration in these rats.

system of the rat parotid before and after adrenalectomy. A diagram of the different segments of the duct system of the rat parotid is shown in Fig. 5. The gland is composed of clusters of acini which drain into the intercalated ducts and then into the striated ducts which are located within the lobules of the gland and connect with the lobular ducts. These structures form the lobar ducts which in turn lead into the main duct of the gland.

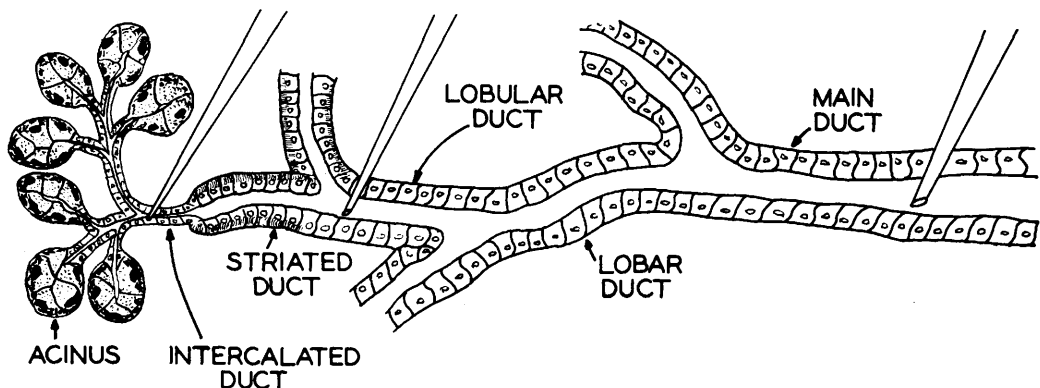


FIG. 5. Diagram showing the different parts of the duct system of the rat parotid gland and the sites of fluid collections.

The main duct conveys the saliva to the mouth. Since the striated ducts were not accessible to micropuncture, the intercalated and lobular ducts were punctured and duct fluid samples were collected. Final saliva was obtained from the end of the main duct following cannulation with a fine polyethylene tube. The sites of collection of fluid samples are indicated in Fig. 5. The sodium and potassium concentration of fluid from different segments of the pilocarpine-stimulated rat parotid, before and after adrenalectomy, are shown in Fig. 6. In the nonadrenalectomized

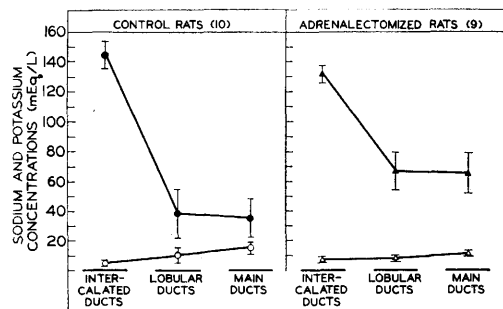


FIG. 6. Mean concentrations of sodium and potassium \pm SD (1 standard deviation) in the fluid obtained from the different parts of the duct system of the parotid glands of 10 control and 9 adrenalectomized rats (for flow rates: see text).

rats, during salivary flow rates of 29.1 ± 4.8 μ l/min/g of wet gland tissue, the fluid from the acini-intercalated ducts had sodium and potassium concentrations of 141.4 ± 8.5 and 4.8 ± 0.9 meq/liter, respectively. The fluid from the lobular and main ducts had low

sodium concentrations (35.2 ± 6.6 and 33.1 ± 12.1 meq/liter respectively). The potassium concentrations were 9.9 ± 4.8 in the fluid from the lobular ducts and 15.4 ± 3.8 meq/liter in the fluid from the main duct. In the adrenalectomized rats, during salivary flow rates of 31.1 ± 7.2 $\mu\text{l}/\text{min}/\text{g}$ of wet gland tissue, the sodium and potassium concentrations of the fluid from the intercalated ducts were 131.31 ± 5.72 and 6.86 ± 0.82 meq/liter, respectively. These values are not significantly different from the concentrations of these ions in the plasma of the adrenalectomized rats which were 132.51 ± 3.52 and 6.35 ± 0.34 meq/liter for sodium and potassium, respectively. In the fluid from the lobular ducts of the adrenalectomized rats, the sodium concentration was 66.46 ± 12.72 meq/liter which is higher than the sodium concentration of the fluid from the lobular ducts of the control rats ($p > 0.001$). The fluid from the main ducts of the adrenalectomized rats had sodium concentration of 65.25 ± 13.48 meq/liter, not different from that of the lobular duct fluid. The potassium concentration of the duct fluid of the adrenalectomized rats was 7.74 ± 1.19 in the lobular ducts and 10.91 ± 0.87 meq/liter in the main duct, significantly lower than the potassium concentrations of the duct fluid from the corresponding ducts of the control rats.

Discussion. The present study demonstrates that aldosterone is actively involved in the regulation of the ionic composition of the rat parotid saliva. In the past, studies of the effects of adrenal steroids on salivary gland function were limited to measurements of the Na and K concentrations and Na/K ratios in final saliva. The use of micropuncture and microanalytical techniques has made possible the investigation of the effects of aldosterone on the different parts of the duct system of the rat parotid gland. The process of production of the primary secretory fluid in the acini-intercalated ducts of the gland was not significantly affected by adrenalectomy. The salivary flow rates per gram of gland tissue were identical to those of the control animals. On the other hand, the composition of the primary fluid obtained from the intercalated ducts was al-

tered. Sodium concentrations were lower and the potassium concentrations were higher than in the control rats. This change could have resulted from an effect of adrenalectomy on the secretory process but more probably it was associated with the altered sodium and potassium concentrations in the plasma of the adrenalectomized animals. Comparison of the sodium and potassium concentrations in the primary fluid of control and adrenalectomized rats to those of the plasma of the corresponding animals demonstrated that the primary fluid appears to be equilibrated to plasma as far as sodium and potassium concentrations are concerned. It was also demonstrated (unpublished experiments) that elevation of the plasma sodium concentrations in rats by infusion of hypertonic saline results in increased sodium concentrations in the primary fluid of the parotid gland. Thus, it was concluded that the changes in the composition of the primary fluid in the parotid of adrenalectomized animals were associated with similar changes in plasma, to which the primary fluid appears to be in equilibrium for sodium and potassium concentrations.

In a previous study, it was demonstrated that the major site of sodium reabsorption in the rat parotid is the striated duct of the gland (2). Since the striated ducts can not be reached by present micropuncture techniques, the next order of ducts (lobular ducts) were micropunctured and the changes in the sodium and potassium concentrations of the duct fluid were interpreted as the results of the transport activity of the striated ducts. Comparison between control and adrenalectomized rats reveals that in the latter there was a significant increase in the sodium concentration and a decrease in the potassium concentration of the fluid obtained from the lobular ducts at comparable flow rates. The change in the sodium concentration could have resulted from either a decrease in the reabsorption of sodium or an increase in the permeability of the striated ducts to water resulting in increased water reabsorption. The observation, however, of identical flow rates in the control and adrenalectomized rats and the decreased potassium concentrations in the lobular duct fluid of the adrenalectomized

rats are against the possibility of increased permeability to water. Thus, it appears that a marked decrease in the reabsorption of sodium occurs in the striated ducts of the rat parotid following adrenalectomy. Since the remaining excretory ducts (lobular, lobar, and main) of the rat parotid seem to have a minimal effect on the handling of sodium and potassium, adrenalectomy had little effect on their function. Some decrease in the potassium secretion was observed leading to a decrease in the potassium concentration of the final saliva.

Administration of aldosterone to the adrenalectomized rats resulted in complete restoration of the handling of sodium and potassium. Since the major changes in the handling of these ions after adrenalectomy were observed in the striated ducts, it is concluded that these ducts are the site of action of aldosterone in the parotid gland. It appears that aldosterone affects both the reabsorption of sodium and the secretion of potassium. Whether the effect on the secretion of potassium is primary or secondary to that on the reabsorption of sodium is not clear. The localization of the site of action of aldosterone in the striated ducts is also in agreement with the previously described morphological simi-

larity of the striated duct cells to the cells of the distal tubule, the major site of action of aldosterone in the kidney

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