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# Effects of Saliva from Chronically Reserpinized Rat on Na and K Transport in Perfused Main Excretory Duct of Submandibular Gland of Normal Rat (42559)

DUSIT JIRAKULSOMCHOK[1] AND CHARLOTTE A. SCHNEYER

*Department of Physiology and Biophysics, University of Alabama, Birmingham, Alabama 35294*

*Abstract.* Reserpine (RES) (0.5 mg/kg body wt, ip) was administered to rats for 7 days. On Day 8 saliva was evoked from these animals by intraperitoneal injection of pilocarpine nitrate (10 mg/kg body wt) and saliva from submandibular and parotid glands was collected separately. These collected salivas were used to perfuse through the main ducts of the submandibular glands of normal rats. After a control period of perfusion of the main duct with bicarbonate saline solution, parotid saliva from RES rats was perfused through the duct followed by regular perfusion. There was inhibition of Na absorption (22%) and K secretion (23%). Moreover, when submandibular saliva from treated rat was perfused through the main duct prior to regular perfusion, there was a decrease in Na absorption (31%) and K secretion (28%). In contrast, perfusion of the main duct with either parotid or submandibular saliva from normal rats caused no significant changes in Na and K transport. The present experiments confirm previous studies that there is some Na-inhibitory factor(s) present in saliva of the chronically RES-treated rat. 

Administration of RES (0.5 mg/kg body wt, ip, 7 days) to rats results in changes in morphology and secretory response of salivary glands (1–4), lung (5), and pancreas (6). In addition, the submandibular saliva from the chronically RES rat showed a cilioinhibitory effect when it was added to gills of freshwater mussels (2). Moreover, when submandibular and parotid salivas from the treated rat were retroperfused into a parotid gland of the normal rat, there was an inhibition of Na reabsorption and a reduction in flow rate (3). However, the procedure of retroperfusion of saliva into a parotid gland may introduce some problems. For example, retrograde perfusion may cause some degree of mechanical (pressure) damage to acinar and duct cells (3). Moreover, with regard to the increase in Na concentration in the final saliva of the normal gland after retroperfusion of the saliva from the chronically RES rat, the site and mechanism of this effect are uncertain; it may be due either to inhibition of Na reabsorption of the duct cells or to an alteration of Na secretion of the acinar cells or to both. Therefore, it seemed possible that clarification of the mechanism of these changes might be obtained by investigating the effects of submandibular and parotid salivas from the chronically RES rat on electrolyte transport in the perfused main duct of the submandibular gland of the normal rat. This procedure was selected since it has several advantages over retrograde perfusion. First, any mechanical damage to the duct cells can be avoided since saliva from the treated animal is slowly perfused through the main duct under free-flow conditions. Second, any alterations in electrolyte transport can be readily separated from changes to water transport or in electrolyte transport mechanisms. Third, the change can be related to ductal effects alone and the obscuring effect of damage to acinar cells can be avoided.

**Materials and Methods.** *Preparation of the chronically RES rat.* Male Long–Evans rats 3–4 months of age used in all experiments had free access to water and lab chow. The rats were given a daily dose of RES (0.5 mg/kg body wt, ip) for 7 days (1–4). Twenty-four hours after the last injection of the drug, the

[1] Present address: Department of Physiology, Khon Kaen University, Khon Kaen, Thailand.



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rats were anesthetized with sodium pentobarbital (50 mg/kg body wt, ip) and tracheotomized. One main excretory duct of the submandibular gland was cannulated at the oral opening by a short length of polyethylene tubing (PE 10) and one main excretory duct of the parotid gland was dissected and cut free for collection of saliva samples. Pilocarpine nitrate, at a dosage of 10 mg/kg body wt, ip, was given to rats to elicit saliva. Saliva secreted in the first 5 min was discarded and samples from parotid and submandibular glands were separately collected for 60 min in plastic tubes and stored at 4°C. These salivas were used within 3 hr after collection. The same procedures for saliva collection were used in untreated control rats.

*Procedure for duct microperfusion.* Animals were prepared for microperfusion of the main excretory duct as previously described (7–10). In brief, rats were anesthetized and tracheotomized. One main excretory duct of submandibular gland was cannulated at the oral opening with a short length of polyethylene tubing (PE 10) for collection of samples (perfusates). The ipsilateral hilar end of the submandibular duct was exposed, cut, and cannulated (3-mm depth) with a length of fine-tipped (70–100 $\mu$m) polyethylene tubing and ligated in place. The average length of the ducts was 27 ± 2.6 mm and 150 ± 14 $\mu$m ($n$ = 10) in diameter. This cannula was connected to a syringe containing perfusion medium. The syringe was mounted on an infusion pump (Harvard, Model 940) and adjusted to deliver solution at the rate of 1 $\mu$l/min.

The perfusion solution contained 145 m$M$ Na, 5 m$M$ K, 125 m$M$ Cl, and 25 m$M$ $HCO_3$. Trace amounts of [$^3$H]inulin were added as a marker of possible net water fluxes. For analysis of Na and K, 3 $\mu$l of perfusate was collected and analyzed by flame photometer (IL Model 143). Three microliters of perfusate was collected and counted for radioactivity of [$^3$H]inulin by a liquid scintillation counter.

*Protocol.* After preparation of the animal, a 30-min period was allowed to stabilize the rat before collection of samples. Three samples were collected for analysis of Na and K. After collection of control samples, the perfusion was switched from regular perfusion fluid to the saliva collected from either control or RES rat and perfused at the rate of 2 $\mu$l/min for 5 min. Then the regular perfusion was resumed for at least 20 min, and the perfusate collected during this period was discarded. Another set of samples was collected by the same procedure as were the control samples. Net transductal flux of electrolyte was calculated for the entire duct as previously described (8, 9).

*Analysis of data.* All data in the text and Table I are presented as means ± SE. Control data were compared with experimental data within the same animals by paired Student's $t$ test. Values were considered to be statistically significant if $P$ values were less than 0.05.

**Results.** Table I shows the effects of perfusion of parotid and submandibular saliva from normal and chronically RES-treated rats prior to normal perfusion of bicarbonate saline solution. During the control period, Na absorption and K secretion were 17.5 ± 1.7 and 19.7 ± 1.8 neq/min duct, respectively. After perfusion of the duct with parotid saliva from the

TABLE I. EFFECTS OF PERFUSION OF PAROTID AND SUBMANDIBULAR SALIVAS FROM CHRONICALLY RES RAT PRIOR TO NORMAL PERFUSION OF BICARBONATE SALIVA SOLUTION ON Na AND K TRANSPORT

| Perfusion period | Na absorption neq/min duct | K secretion meq/min duct |
|---|---|---|
| Control (6) group I | 17.5 ± 1.7 | 19.7 ± 1.8 |
| PA saliva from normal rat | 16.2 ± 1.5 | 18.4 ± 1.6 |
| Control (6) group II | 18.3 ± 1.7 | 19.8 ± 1.7 |
| PA saliva from RES rat | 14.3 ± 1.6[a] | 15.2 ± 1.4[a] |
| Control (6) group III | 16.7 ± 2.1 | 18.4 ± 1.8 |
| SM saliva from normal rat | 17.2 ± 1.9 | 16.1 ± 2.0 |
| Control (8) group IV | 19.7 ± 2.0 | 18.2 ± 2.2 |
| SM saliva from RES rat | 13.6 ± 2.2[a] | 13.1 ± 2.1[a] |

*Note.* Values are means ± SE. After the control period, collected saliva from either the normal or the RES rat was perfused at the rate of 2 $\mu$l/min for 5 min. Then a regular perfusion of bicarbonate saline solution was resumed. A 20-min period elapsed before another set of samples was collected. The number in parentheses indicates number of animals used. PA, parotid; SM, submandibular. Paired Student's t-test was used.

[a] Significantly different from control ($P < 0.05$).

normal rat, there was no significant change from control (group I). However, in group II the absorption of Na and secretion of K decreased significantly (by 22 and 23%, respectively; $P < 0.05$) after perfusion of the duct with parotid saliva from the RES-treated rat (Table I). In group III, perfusion with submandibular saliva from the normal rat did not alter electrolyte fluxes in any parameter measured. However, pretreatment of the duct with submandibular saliva from the RES rat (group IV) resulted in significant reduction in Na absorption (31%) and K secretion (28%; $P < 0.01$). Whether the inhibitory effects observed were reversible or irreversible was not determined in the present experiments; this awaits further investigation.

The inulin ratio (ratio of counts per min per 3 $\mu$l of unperfused medium to counts per min per 3 $\mu$l of collected perfusate) was 0.98 $\pm$ 0.005 in the control period and it was not significantly changed from the control value by perfusion with saliva from either the normal or the RES rat.

**Discussion.** A high Na concentration in submandibular saliva from the chronically RES rat is observed (4). When either parotid or submandibular saliva from the chronically RES rat was retroperfused into the duct of the parotid gland of the rat, in each case it resulted in inhibition of Na absorption (3). The present data confirm previous observations (3) which suggest that some Na-inhibitory factor(s) is present in saliva from the RES rat. Thus, when either parotid or submandibular saliva from the chronically RES rat was perfused through the main duct of the submandibular gland prior to the regular perfusion of bicarbonate saline solution, Na absorption and K secretion were decreased (Table I). Therefore, the results indicate that the Na-inhibitory factor acts on duct cells and causes reduction in Na absorption and K secretion. The elevation in Na concentration and reduction in K concentration in the collected perfusate cannot be attributed to changes in water transport since there is no significant change in inulin ratio. Moreover, it is very unlikely that the procedure of perfusion of collected salivas used in this experiment may cause any mechanical damage to duct cells since they were perfused under free-flow conditions and in a normal physiological range.

The chemistry and structure of the inhibitory factor are not known. However, this factor(s) is sensitive to heating, freezing, and storage (3, 11, 12).

In the salivary ducts, Na absorption involves Na conductance and an electroneutral $Na^+$:$H^+$ exchange. K secretion is suggested to be a $K^+$:$H^+$ antiport, coupling between Na absorption and K secretion. These processes are associated with $Na^+$–$K^+$-ATPase in the basolateral membrane (8, 10). The mechanism of action of the Na-inhibitory factor on the duct cells is not entirely understood at the present but it may be due to inhibition of the active Na pump since retroperfusion of the saliva from the chronically RES rat also reduces saliva flow (3). However, changes in ductal permeability to Na and K due to the effect of the inhibitors cannot be excluded.

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