Chronically Reserpinized Rat as a Model for Cystic Fibrosis: Na⁺ Transport Inhibitory Effect in Submaxillary Saliva

J. RICARDO MARTINEZ, A. M. MARTINEZ, LINDA GARRETT, AND PATTY KORMAN

Department of Child Health, University of Missouri, School of Medicine, Columbia, Missouri, USA

Summary

The retrograde perfusion assay in the rat parotid gland was used to investigate the effects of salivary secretions from control and reserpine-treated rats on Na⁺ reabsorption. Results demonstrated that submaxillary saliva from the treated animals caused a 69% inhibition of Na⁺ reabsorption, accompanied by a 28% reduction in the volume of saliva secreted, and a 22% reduction in flow rate. By contrast, submaxillary saliva from control rats caused a 6% inhibition of Na⁺ reabsorption, a 6% reduction in volume, and a 5% reduction in flow rate. Parotid saliva from reserpine-treated rats also inhibited Na⁺ reabsorption to the extent of 39% and caused a 38% reduction in volume and a 33% reduction in flow. Parotid saliva from control rats only inhibited Na⁺ reabsorption to the extent of 2.7% and caused a 4-6% reduction in salivary volumes and flow rates. The inhibition of Na⁺ reabsorption and the reduction in salivary volume and flow rates caused by submaxillary saliva of reserpine-treated rats were either abolished or significantly reduced when the saliva was previously heated to 100°C, frozen, and then thawed or kept in glass tubes at 4°C for 24 hr. These results indicate that saliva from reserpine-treated rats has comparable effects in this assay system to those of saliva from cystic fibrosis (CF) patients and further support its use as an animal model for this disease.

Speculation

A similar Na⁺ transport inhibitory effect to that produced by CF saliva in the parotid retroperfusion assay has been found in submaxillary saliva, and to a lesser extent in parotid saliva, of reserpine-treated rats. This suggests that the same inhibitory component or components are present in both types of secretion.

Elevations of the sodium or chloride concentrations in sweat (2) and in the secretions from the minor salivary glands (12) are a consistent finding in CF, an hereditary disease affecting the exocrine glands. Although a similar electrolyte abnormality in the secretions from the major salivary glands has not been always found in this disease, it has been reported that mixed saliva (4) or submaxillary and sublingual saliva (9) from patients with CF inhibited Na⁺ reabsorption when injected in a retrograde fashion into the parotid gland of normal rats. The nature of the substance responsible for this effect on salivary Na⁺ concentration is not known, but it could explain the elevated Na⁺ concentrations observed in some exocrine secretions of the CF patient.

Several lines of evidence have indicated that the chronic administration of reserpine to rats induces exocrine gland alterations that resemble those of CF (5, 6, 8, 10, 13). Submaxillary saliva from the treated rats had elevated Na⁺, Ca⁺⁺, protein, and carbohydrate concentrations (6) and, significantly, had a marked ciliolinhibitory effect comparable to that of CF fluids (1). In view of these observations, the effect of salivary secretions from reser-

pine-treated rats on the Na⁺ concentrations of rat parotid saliva was investigated using the retrograde perfusion assay. With this methodology, it has been demonstrated that submaxillary saliva and, to a lesser extent, parotid saliva of the reserpine-treated animals significantly increased the Na⁺ concentration of parotid saliva.

METHODS

Collections of saliva from both reserpine-treated rats and untreated controls were made immediately before the retrograde perfusion assay, in accordance with techniques previously described in detail (6, 13). Treated animals received the daily dose of reserpine of 0.5 mg/kg body wt previously used in this laboratory and had free access to water and to a standard pelleted diet. After exposure of either the submaxillary or parotid glands, the main ducts were cannulated with a short length of polyethylene tubing (Clay Adams PE 10) and the animals received an ip injection of pilocarpine nitrate (10 mg/kg body wt). Saliva secreted in the first 3 min was discarded and samples were subsequently collected at time intervals in preweighed plastic microsample tubes and stored at 4°C. Chemical analysis of these samples included the measurement of Na⁺, K⁺, Ca⁺⁺, and protein concentrations. At the end of the collection period, the glands were excised, gently blotted in tissue paper, and weighted to the nearest milligram in a top loading balance. The volume of saliva was estimated by reweighing the collection tubes and rates of flow were calculated in terms of gland weight. The same procedures for saliva collection were used in untreated control rats. Most experiments involved paired observations on the test substances from normal and reserpine-treated rats.

The retrograde perfusion assay was performed in the parotid gland of normal rats. The gland on one side was used as the test gland for the retrograde injection of the different test substances, whereas the contralateral gland was used as a control. Both main parotid ducts were cannulated and the cannula in the retroperfused side was attached to a syringe containing the material to be tested. All of the test substances were diluted 1:4 in normal saline. The volume of diluted test substance injected by retrograde perfusion was calculated on the basis of the observations of Taylor *et al.* (9). They found that the greatest inhibitory effect occurred when the ratio of milliliters injected per gram dry weight of gland was greater than 1.8. Thus, a 1.8-2.2 ratio was used in these experiments. The diluted test fluid was retro-injected slowly and left in contact with the glandular duct system for 90 sec. Pilocarpine nitrate was then injected ip (10 mg/kg body wt) and the cannula in the retroperfused side was then cut to the same length (3-4 cm) as that on the control side. Salivary secretion started in 1-1½ min and the fluid secreted during the first 3 min was discarded. Samples were then collected at timed intervals for up to 2½ hr in preweighed plastic microsample tubes, stored at 4°C and analyzed for Na⁺, K⁺, Ca⁺⁺, and protein. The volume of each

sample was estimated gravimetrically and the glands on both sides were removed at the end of the collection period and weighed. Temperature of the rats was kept at 37°C by the use of a heated operating table.

The rate of Na⁺ reabsorption (RNa) was calculated in each experiment at comparable flow rates for both the retroperfused and control glands as described by Mangos *et al.* (4). This assumes a constant Na⁺ concentration in primary saliva at the different flow rates. The percent inhibition of Na⁺ reabsorption was calculated by the formula

$$\% \text{ inhibition} = 100 - \left(100 \times \frac{\text{RNa perfused gland}}{\text{RNa control gland}} \right)$$

The total volume of saliva secreted by both the retroperfused and control glands during comparable time periods was measured and the percent inhibition, if present, was also calculated in each experiment. Maximum flow rates were recorded for each of the two glands in each experiment and the reduction observed in the retroperfused side was also calculated as % inhibition when present.

The sodium concentration was measured in an Instrumentation Laboratory flame photometer with lithium internal standard. Ca⁺⁺ concentrations were measured in a Corning Instruments calcium analyzer. Protein was measured by the Lowry method and amylase concentrations by the Harleco reagent method. In this method, an amylase unit is defined as the amount of enzyme that will hydrolyze 10 mg starch in 30 min.

Fig. 1. Relationship between Na⁺ concentration and flow rate in parotid saliva from control glands (dark circles) and from glands retroperfused with submaxillary saliva from reserpine treated rats (open circles). See text for details.

Fig. 2. Relationship between Na⁺ concentration and flow rate in parotid saliva from control glands (dark circles) and from glands retroperfused with submaxillary saliva of normal rats (triangles). See text for details.

Table 1. Inhibition of Na⁺ Reabsorption

Test Substance	N	% Inhibition
Submaxillary saliva, control rat	12	6.6 ± 0.6
Submaxillary saliva, reserpine-treated rat	12	69.3 ± 2.6
Parotid saliva, control rat	6	2.7 ± 0.3
Parotid saliva, reserpine-treated rat	10	39.6 ± 9.2

Table 2. Inhibition of salivary volume and flow rate¹

Test substance	Volume % inhibition	Flow rate % inhibition
Submaxillary saliva, control rat	6.7 ± 2.4	5.3 ± 0.7
Submaxillary saliva, reserpine-treated rat	28.7 ± 3.5	22.0 ± 2.4
Parotid saliva, control rat	5.3 ± 1.6	4.2 ± 0.9
Parotid saliva, reserpine-treated rat	38.4 ± 12.2	33.2 ± 10.3

¹ Number of experiments is the same as in Table 1.

RESULTS

Submaxillary saliva collected from reserpine-treated rats caused a significant increase in the Na⁺ concentration of parotid saliva from control rats when retroperfused into the glandular duct system. This effect is illustrated in Figure 1, which shows the composite results from 12 such experiments where this test substance was used. The typical relationship between the Na⁺ concentration of parotid saliva and rate of salivary flow for the nonretroperfused contralateral gland is also illustrated in Figure 1. By contrast, retrograde perfusion of saline-diluted submaxillary saliva from control rats did not significantly modify the Na⁺ concentration of parotid saliva (Fig. 2). The mean percent inhibition of Na⁺ reabsorption calculated in each of these two groups of experiments is depicted in Table 1, which shows that at flow rates between 30–60 mg/min g wet wt, submaxillary saliva from reserpine-treated rats caused a significantly larger inhibition than saliva from control animals. This table also illustrates the effects of parotid saliva from control and treated animals. Parotid saliva of reserpine-treated rats caused a 39.6% inhibition, whereas that from control rats caused a 2.7% inhibition of Na⁺ reabsorption.

Figure 1 also illustrates that the maximum flow rate was reduced in the retroperfused parotid gland when diluted submaxillary saliva from reserpine-treated rats was used as the test substance. This was accompanied by a significant reduction in the total volume of saliva secreted by the retroperfused gland. The mean values for these effects are illustrated in Table 2 for 12 experiments. Volume was inhibited 28.7% and flow rate 22%. Submaxillary saliva from control rats caused a slight inhibition in the secretory response from the retroperfused gland (Table 2) that was significantly smaller than that caused by saliva from the treated animals. Parotid saliva from reserpine-treated rats had a mean effect on volume and flow rate which was actually greater than those of submaxillary saliva. However, the effect was also quite variable, as reflected in the large SD, and the difference was only of borderline significance. Parotid saliva from control rats caused a small inhibition of volume and flow rate (Table 2).

Saliva from the reserpine-treated animals did not affect either the Ca⁺⁺ or the amylase concentration in the saliva secreted by the retroperfused gland.

In the original study of the Na⁺ transport inhibitory effect of CF saliva, Mangos *et al.* (4) indicated that the inhibitory factor was destroyed by heating saliva to 100°C and by freezing-thawing of the saliva. Storage of the saliva at 4°C for 24 hr caused a decrease in the activity by 20–40%. Freshly collected submaxillary saliva from reserpine-treated rats was, therefore, subjected to these various treatments and then used in the retrograde perfusion assay. The results, which are summarized in Table 3, indicate that the effects of this test substance are markedly reduced by heating,

Table 3. *Modification of the inhibitory effects of saliva from reserpine-treated rats*

Test substance	Treatment	N	Na ⁺ reabsorption % inhibition	Volume % inhibition	Flow rate % inhibition
Submaxillary saliva	Heating 100°C	5	9.8 ± 1.0	2.2 ± 0.4	0.00
	Freezing-thawing	4	2.7 ± 0.5	16.9 ± 2.4	14.9 ± 1.4
	Glass container, 4°C for 24 hr	4	18.6 ± 1.8	10.6 ± 2.4	16.3 ± 0.4

freezing, and storage. The activity responsible for the inhibition of Na⁺ reabsorption was greatly reduced by freezing-thawing, and by heating this test substance to 100°C. Storage in glass tubes at 4°C for 24 hr also caused a reduction in this activity, although it was not as pronounced as that induced by the other two procedures. A certain degree of inhibition of salivary volumes and flow rates persisted in saliva that was freeze-thawed or stored at 4°C for 24 hr. Inhibition of these two secretory parameters was almost nonexistent, however, when the saliva was heated to 100°C (Table 3).

DISCUSSION

The results of these experiments demonstrate that submaxillary saliva from reserpine-treated rats has a marked inhibitory effect on Na⁺ reabsorption in the parotid gland of normal rats when injected in a retrograde fashion into the glandular duct system. This effect is similar to that described for submaxillary saliva of CF patients (9), but of larger magnitude when calculated as the percent inhibition of Na⁺ reabsorption. If one uses the values for Na⁺ reabsorption presented by Mangos *et al.* (4) in the original description of the Na⁺ transport inhibitory effect of CF saliva, however, a 68% inhibition by CF mixed saliva can be calculated, a value that is similar to the one obtained with submaxillary saliva of the reserpine-treated rat.

Parotid saliva from the treated animals was also found to cause a 39% inhibition of Na⁺ reabsorption in the retrograde assay system. Taylor *et al.* (9) found that parotid saliva from CF patients showed no inhibitory effect in this test system. The discrepancies in the extent of inhibition caused by submaxillary and parotid secretions of reserpine-treated rats and of CF patients are likely due to the inherent limitations of the retrograde perfusion assay. The selection of a particular flow rate for the calculation of Na⁺ reabsorption can significantly influence the values obtained for percent inhibition, and calculations based on flow rates above 80 mg/min g or below 30 mg/min g can result in an over or understimulation, respectively, of RNa values. Although our results on percent inhibition were calculated at an acceptable range of parotid flow rates, the selection of points in the lower portion of this acceptable range of flow rates, where the greatest differences in Na⁺ concentration are usually observed, can result in higher calculated values for percent inhibition.

The method of calculating Na⁺ reabsorption in this assay system assumes that the Na⁺ concentration of "primary" (acinar) parotid fluid does not change upon stimulation with secretagogues. Although this has been shown to be the case in the normal gland (3), the same may not be true of the retroperfused gland and there may be variations in the effects of different test substances on parotid acinar fluid. The calculation of percent inhibition of Na⁺ reabsorption may be, therefore, unreliable in the retroperfused gland. In fact, a great deal of caution is required, in our view, in the use of this assay system, because the injection of fluid under pressure into the parotid duct system most likely causes some degree of mechanical damage to both acinar and duct cells. Thus, although the lack of effect of saliva from control rats can be construed as an indication of the reliability of the assay, the variations in the inhibition of volume of saliva secreted (see Table 2) caused by the different test substances suggest that acinar secretion may be affected appreciably. Variations were also found in the effect of the same sample of submaxillary saliva from reserpine-treated rats when it was assayed simultaneously on the

parotid glands of two separate control rats. This was also observed in the previous study involving CF saliva (9).

The inhibition of volume and of flow rate are, in fact, a consistent observation in this assay. Because most salivary fluid is thought to originate in acinar cells (7), this suggests that inhibitory test substances also affect the secretory process at this level of the glandular epithelium. This process is not entirely understood at the present time, but is thought to involve the active pumping of Na⁺ (7). In the salivary ducts, reabsorption of Na⁺ probably involves a passive influx of this ion across the luminal membrane of duct cells, followed by its active pumping across the basolateral membrane (11). A test substance that inhibits both salivary volume and Na⁺ reabsorption could conceivably affect the Na⁺ pump mechanism at both the acinar and ductal levels of the glandular epithelium. The similarity of the effects of ouabain (4), CF saliva (9), and saliva from reserpine-treated rats (this study) also supports a mechanism involving the active Na⁺ transport component of the ductal reabsorptive process. However, Mangos *et al.* (4) found no effect of CF saliva on the *in vitro* activity of membrane ATPase preparations from beef parotid, human erythrocytes, and rat kidney. Although it is possible that the effects of inhibitory test materials from both human and rat can be different *in vivo*, the exact mechanism of the inhibition of Na⁺ reabsorption remains unknown. The finding that the inhibitory activity in saliva from both reserpine-treated rats and CF patients is sensitive to heating, freezing, and storage suggests, on the other hand, that the same type of inhibitory component is present in both fluids. These findings support the use of the reserpine-treated rat as a model for the exocrine gland disturbance of CF.

CONCLUSION

Submaxillary saliva from reserpine-treated rats, but not from control rats, was found to cause a significant inhibition of Na⁺ reabsorption when used in the parotid retroperfusion assay system. This effect was accompanied by significant reductions in the volume of saliva secreted and in the rate of flow. Parotid saliva from the treated animals also inhibited Na⁺ reabsorption, but to a lesser extent than submaxillary saliva. It also reduced the volume and the flow rate of parotid saliva. The inhibitory activity of submaxillary saliva from the treated animals was, like that of CF saliva, either reduced or abolished by heating, freezing, and storage. These results demonstrate that a similar Na⁺ transport inhibitory effect observed in saliva from CF patients is present in salivary secretions of the proposed animal model for this disease.

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