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cystic fibrosis
kallidin
parotid gland
prostaglandin E_1
sodium

Inhibition of Na^+ Reabsorption in the Rat Parotid Gland by Prostaglandin E_1 and Kallidin: Implications for Cystic Fibrosis

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Summary

Prostaglandin E_1 caused a dose-related inhibition of sodium reabsorption in the rat parotid gland when injected by retrograde perfusion into the glandular ducts. The extent of inhibition ranged from 11.7 ± 2.4% at a dose of 2.5 μg to 63.8 ± 8.9% at a dose of 31.2 μg. Both phospholipase A_2, an enzyme involved in prostaglandin synthesis, and arachidonic acid, a precursor of prostaglandins, also increased the Na^+ concentration of parotid saliva in a dose-dependent fashion. With phospholipase A_2 the inhibition ranged from 21.6 ± 4.4% at a dose of 3 μg to 73.5 ± 8.2% at a dose of 30 μg. With arachidonic acid, the degree of inhibition was 5.1 ± 3.0% at a 10^{-5} M dose and 57.7 ± 10.2% at a dose of 10^{-3} M. Lysine bradykinin (kallidin), a peptide present in salivary and other exocrine glands and their secretions, also caused a 30% inhibition of Na^+ reabsorption when retroperfused at a concentration of 12.5 μg, as did kallikrein (176 μg) and trypsin (33.3 μg). These results indicate that prostaglandins and kinins can inhibit Na^+ reabsorption in the rat parotid duct when present in the luminal side of the cells. Since they are normally present in exocrine glands and can presumably be secreted, they may have a role as luminal factors in the regulation of transductal transport of Na^+. The possibility that they may be increased in the exocrine secretions of patients with cystic fibrosis and that they may act as the so-called cystic fibrosis "factors" is also raised by the findings of this study.

Speculation

Prostaglandin E_1 and, to a lesser extent, lysine-bradykinin (kallidin) markedly inhibit Na^+ reabsorption in the rat parotid bioassay. These substances also enhance mucus secretion and it is speculated that they are present in abnormal amounts in the exocrine glands and secretions of patients with cystic fibrosis, where they alter both electrolyte and mucus secretion.

The ability of sweat and saliva from patients with cystic fibrosis to increase the sodium concentration of parotid saliva from normal rats when retroinjected into the glandular duct system has been interpreted as evidence that these fluids contain a "factor" that inhibits sodium reabsorption in the parotid ducts (9, 15). The presence of such factor in exocrine secretions of the affected individuals would provide an explanation of the increased sodium concentration observed in some but not all, exocrine fluids in cystic fibrosis. No evidence is available concerning the chemical nature of this factor, but the fact that it is present in two major exocrine secretions suggests that it is produced by the affected glands and secreted as a component of the secretion product. The factor could be either a normal component that is secreted in increased quantities or an abnormal product that is only present in tissues affected, either directly or indirectly, by the genetic defect.

In an attempt to investigate the first of these possibilities, we have studied the effects of several naturally occurring, biologically active substances present in exocrine glands on parotid saliva sodium in the rat. We used the retrograde perfusion assay system to study the effects of kallikrein and other proteases, since they are present in salivary and other exocrine glands (6, 13) and since they have a wide range of metabolic and vascular regulatory function (6). Similarly, the products of kallikrein action on glandular and blood proteins are peptides with intense biologic activity, including bradykinin and lysine-bradykinin (kallidin) (5, 7). In addition, prostaglandins are ubiquitous lipid derivatives with equally potent biologic actions, including effects on electrolyte transport (17). Prostaglandin synthesis is enhanced, furthermore, by kinins (16). Other substances involved in prostaglandin synthesis were also of interest to us, in view of their role in the generation of prostaglandins upon stimulation of glandular activity. These included phospholipase A_2, an enzyme with important actions on membrane lipids, and arachidonic acid, a precursor in prostaglandin synthesis. Interest in these substances as potential regulators of Na^+ reabsorption in both normal and diseased exocrine glands is strengthened by other evidence suggesting that they may be altered in cystic fibrosis patients. Thus, alterations in plasma esterases (14), prostaglandins (2), and trypsin (3) levels have been reported in cystic fibrosis. Also, salivary kallikrein content was found to be increased in saliva from patients with cystic fibrosis (8).

MATERIALS AND METHODS

Adult male Sprague-Dawley rats were used. The animals weighed between 200 and 275 g and were allowed free access to water and to a standard pelleted diet. For the retrograde perfusion of the parotid gland, the rats were anesthetized with sodium pentobarbital (7 mg/100 g body weight), the trachea was cannulated with a plastic tube (Clay-Adams polyethylene tubing PE 240) and the parotid ducts were dissected in the cheek and cannulated with a short length of polyethylene tubing (Clay-Adams PE 10). The ductal cannula on one side was connected with a 0.5-ml plastic syringe containing the substance to be tested. Approximately 125 μl of the solution containing the test substance were then injected in a retrograde fashion into the glandular duct system. This volume was based on previous observations (11, 15) suggesting that the greatest inhibitory effect occurred when the ratio of volume injected per gram dry weight of gland was greater than 1.8. The test solution was allowed to remain in contact with the glandular epithelium from 1½ to 2 min and then the animal was given an IP injection of pilocarpine nitrate in a dose of 10 mg/kg body weight. With this type of stimulation, salivary secretion begins in a few minutes. However, the saliva secreted in the first 3 to 4 min was discarded since it represents fluid contained within the duct system before stimulation. Saliva samples were subsequently collected at timed intervals in preweighed, plastic microsample tubes for a period of 90 min. The volume of saliva secreted in each sample was estimated gravimetrically by reweighing the collection tubes. At the end of the experiment both parotid glands were removed and weighed in a top-loading balance. Rates of salivary flow were expressed mg/min g gland weight. The sodium concentration in the collected samples was measured in an Instrumentation Laboratories flame photometer with an inter-

nal lithium standard. The amount of sodium reabsorbed by the retroperfused gland and by the contralateral control gland were calculated by assuming a sodium concentration in the primary secretion of 140 mEq/liter and by using the following formula:

$$RNA^+ = PsNa^+ \times F.R. - FsNa^+ \times FR$$

in which RNa^+ = amount of sodium reabsorbed; $PsNa^+$ = sodium concentration in the primary saliva; $FsNa^+$ = sodium concentration in final saliva; and F.R. = rate of salivary flow. The comparison of sodium reabsorption rates in the retroperfused and control glands was made by determining the percent inhibition evident in the retroperfused gland by using the formula:

$$\% \text{ inhibition} = \left(100 - \frac{RNa^+R}{RNa^+C}\right) \times 100$$

in which RNa^+R = amount of sodium reabsorbed in retroperfused gland and RNa^+C = amount of sodium reabsorbed in control gland.

Pilocarpine was selected as the secretagogue in these experiments for the following reasons: (1) it induces a well-characterized response from the rat parotid gland (9, 11, 15); (2) the Na^+ excretion pattern in the saliva secreted under these conditions of stimulation shows a wide range of concentrations (11, 15). Na^+ reabsorption at low rates of flow is intense and this facilitates the observation and interpretation of potential inhibitory effects; and (3) pilocarpine was used in all previous studies of Na^+ transport inhibition in this bioassay system (9, 11, 15).

The substances to be tested were prepared and used in the following manner: (1) Kallikrein, prepared by Sigma Chemical Company (St. Louis, MO) from porcine pancreas. The preparation contains 5 to 15 units per mg of protein and a stock solution containing 8.8 mg protein/ml was prepared in saline. The solution used for retrograde perfusion was prepared from this stock solution (6 μl in 375 μl saline) and contained 17.6 μg protein or 100.3 mU. The other solution used for retrograde perfusion (see "Results") contained 10 times this amount of kallikrein. (2) Lysine-bradykinin (kallidin) was purchased from Vega Chemical Company Tuscon, AZ or from Sigma Chemical Company. A stock solution containing 1 mg/ml saline was diluted for retrograde perfusion in such a way to provide perfusates containing either 12.5 or 25 μg of kallidin in the usual volume used (125 μl). (3) Trypsin was obtained from Sigma Chemical Company. This preparation is doubly crystallized from bovine pancreas, dialyzed, and lyophilized and is essentially salt free. The activity of this preparation is 10,000 to 13,000 BTEE units/mg protein. A stock solution containing 1 mg/ml saline was diluted to obtain enzyme concentrations of 3.33 μg (33.33 mU) or 33.3 μg in 125 μl of retroperfusion fluid. (4) Aprotonin (trypsin inhibitor) was obtained from Sigma Chemical Company. This inhibitor is obtained from bovine lung and the commercial preparation contains 19.8 TIU/ml in 10-ml sterile solution of 0.9% NaCl and 0.9% benzyl alcohol. The solution for retrograde perfusion was prepared by dissolving 5 μl of the stock solution into 500 μl of saline. Only 125 μl of this diluted solution were retroinjected in each instance. (5) Phospholipase A_2 is prepared by Sigma Chemical Company from porcine pancreas and is available in 3.2 M $(NH4)_2$ SO_4, pH 5.5. The activity of the enzyme is 740 units/mg protein. The solution for retrograde perfusion was prepared by dissolving this stock solution in saline to provide the total amounts of enzyme indicated in "Results." (6) Prostaglandin E_1 was obtained from the Upjohn Company Kalamazoo, MI or from Sigma Chemical Company. A stock solution containing 1 mg/ml was prepared in 95% ethanol. Solutions for the retrograde perfusion assays were prepared from this stock solution by dissolving appropriate amounts in saline so as to provide the amounts of PGE_1 indicated in "Results." (7) Arachidonic acid (sodium salt) was obtained from Sigma. It was prepared from porcine liver and a stock solution containing 10 mg/ml saline was prepared and diluted to the desired concentration for the retrograde perfusion assays. (8) Ouabain was purchased from Sigma and prepared in saline to the desired concentration. (9) Amiloride hydrochloride was a gift from Merck, Sharp and Dohme Laboratories, West Point, PA. A 10^{-3} M stock solution was prepared and appropriate dilutions were made for the assay. (10) Ethacrynic acid was purchased as the commercially available product from Merck, Sharp and Dohme Laboratories. This preparation contains 50 mg of ethacrynic acid plus mannitol (62.5 mg) as a preservative. The stock solution was diluted to the desired concentration for the assay.

Saline was used as the vehicle for the various test substances since it was also used in previous investigations concerned with the effects of human and rat saliva (9, 11, 15). Most or all of the solution that is retroperfused into the gland is removed in the first few minutes of the secretory response by the increasing secretion of saliva. In some expriments, the vehicle alone was retroperfused into the glandular duct system. These included the injection of either saline or of a solution containing 5 to 50 μl of alcohol in saline.

RESULTS

Retroperfusion of the parotid gland with saline alone in six experiments caused a 2.7 ± 0.8% inhibition of Na^+ reabsorption. Solutions containing alcohol in saline caused a 3.5 ± 0.9% increase in Na^+ concentration of parotid saliva. It was concluded from these observations that neither procedure caused an inhibition of Na^+ transport in this bioassay system.

Two of the substances tested were found to have a pronounced effect on the Na^+ concentrations of parotid saliva. Both phospholipase A_2 and prostaglandin E_1 caused a significant increase in salivary Na^+ when injected in a retrograde fashion into the glandular ducts. Figure 1 shows the results of experiments performed with phospholipase A_2. Figure 1 illustrates the typical relationship between salivary Na^+ concentration and rate of salivary flow. In control, nonperfused parotid glands this relationship involves a low Na^+ concentration at low rates of flow, with increasing ion concentrations as the flow rate becomes more rapid. Such a pattern of Na^+ excretion has been interpreted as an indication that Na^+ reabsorption in the glandular ducts proceeds to a greater extent when the saliva flows slowly through the ducts. In the present experiments, retroinjection of a solution containing 30 μg of phospholipase A_2 prevented the normal drop in Na^+ concentration observed at low rates of flow and resulted in salivary Na^+ of between 80 and 120 mEg/liter. In six experiments performed with this substance, the mean percent inhibition of Na^+ reabsorption was found to be 73.5 ± 8.2%.

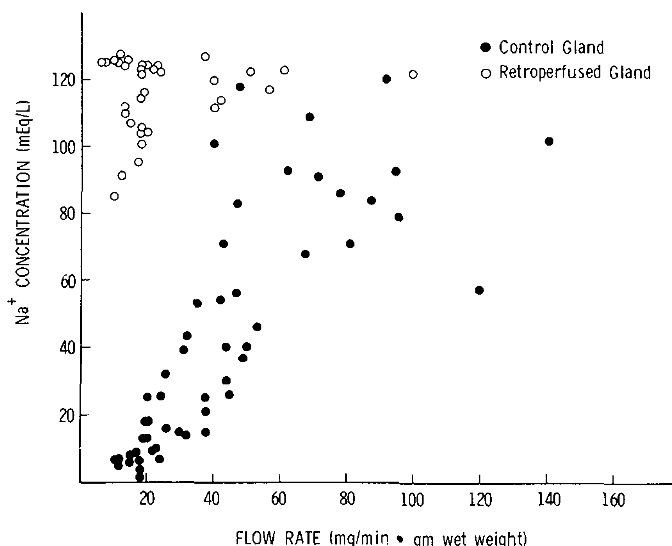

Fig. 1. *Relationship between salivary* Na^+ *concentrations and flow rates in parotid saliva collected from control (nonretroperfused) glands* (●) *and from glands retroperfused with a solution containing 30* μg *of phospholipase* A_2 (○).

Similar effects were observed when a solution containing 31.25 μg of prostaglandin E_1 was retroperfused into the parotid gland (Fig. 2). In this case, the mean inhibition of Na^+ reabsorption observed in six experiments was 63.8 ± 8.9%. In the case of this substance, a clear dose-response relationship could be observed (Fig. 3), in such a way that percent inhibition of Na^+ reabsorption increased with progressively larger doses of prostaglandin E_1.

The effects of the other substances tested in this study are summarized in table 1. Both kallikrein and trypsin caused approximately 30% inhibition of Na^+ reabsorption in this bioassay system, when used in doses of, respectively, 176 μg and 333.3 units. When the total amount of these substances was reduced to one-tenth of the above mentioned doses, the inhibition of Na^+ reabsorption was somewhat reduced in the case of trypsin (to 24.3%) and significantly reduced in the case of kallikrein (to 9.6%). Kallidin (lysine-bradykinin) also caused a 30% inhibition, but this effect was somewhat reduced when the total amount of the peptide retroinjected into the gland was increased 2-fold (Table 1).

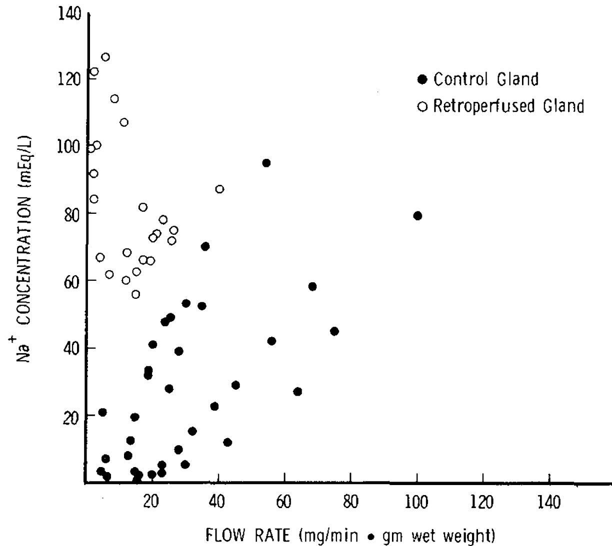

Fig. 2. Effects of retrograde perfusion with a solution containing 31.25 μg of prostaglandin E_1 on the Na^+ concentration of rat parotid saliva. ● represent values in saliva obtained from contralateral control glands and ○ those from retroperfused glands.

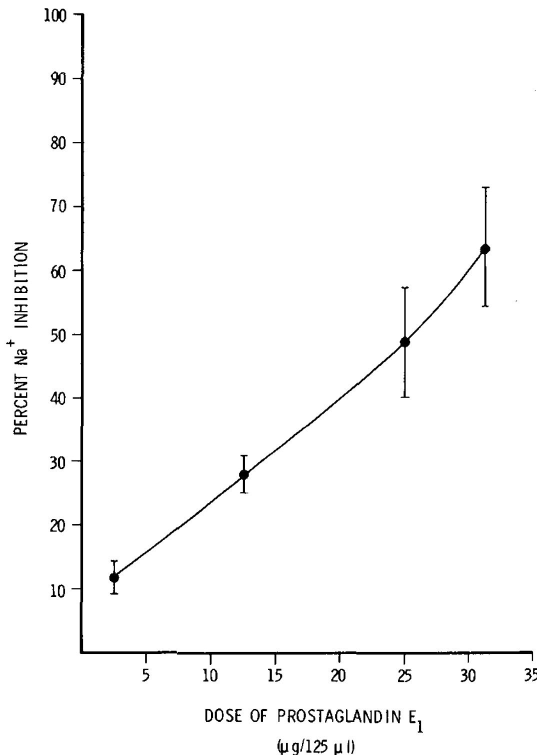

Fig. 3. Percent inhibition of Na^+ reabsorption caused in the rat parotid gland by varying doses of prostaglandin E_1 injected by retrograde perfusion into the glandular duct system. *Vertical bars* represent ± S.D.

Table 1. *Inhibition of Na^+ reabsorption*

Drug	Dose	*n*	Percent Na^+ inhibition
Kallikrein	17.6 μg	8	9.6 ± 2.9
	176 μg	7	31.9 ± 2.4
Kallidin	12.5 μg	8	30.6 ± 7.6
	25 μg	6	23.3 ± 4.6
Aprotonin	25 mU	4	19.8 ± 4.3
Trypsin	33.3 units	6	24.3 ± 5.3
	333.3 units	7	32.1 ± 4.3
Arachidonic acid	10^{-5} M	5	5.1 ± 3.0
	10^{-3} M	5	57.7 ± 10.2
Phospholipase A_2	3 μg	5	21.6 ± 4.4

Table 2. *Effect of drugs*

Test substance	Na^+ reabsorption % inhibition	Flow rate % inhibition
Amiloride 5 × 10^{-6} M	20.1 ± 7.7	28.4 ± 5.1
10^{-5} M	34.0 ± 11.2	
Ouabain 10^{-5} M	37.0 ± 9.1	18.3 ± 6.5
Ethacrynic acid 5 × 10^{-6} M	24.4 ± 5.5	16.6 ± 6.1

Arachidonic acid was found to have almost no effect on salivary Na^+ concentrations when retroperfused into the glandular duct system in a concentration of 10^{-5} M (Table 1). When retroperfused at a concentration of 10^{-3} M, however, this substance caused a 57.7 ± 10.2% inhibition of Na^+ reabsorption in the bioassay system. Reducing the amount of phospholipase A_2 which was retroperfused to one-tenth of the amount used in the experiments illustrated in Figure 1 resulted in a smaller inhibition of Na^+ reabsorption (Table 1). However, this lower concentration of enzyme still caused a 21.6% inhibition of Na^+ reabsorption.

By comparison, the effect of drugs known to inhibit Na^+ transport in epithelial tissues was also assessed in a separate series of experiments. The results obtained with ouabain, ethacrynic acid, and amiloride are shown in Table 2. These drugs caused a 24 to 35% inhibition of Na^+ reabsorption when retroperfused in concentrations of 5 × 10^{-6} M or 10^{-5} M.

DISCUSSION

The results of these experiments demonstrate that prostaglandin E_1, and to a lesser extent lysine-bradykinin, can inhibit Na^+ reabsorption in the rat parotid gland when present in the luminal side of the duct epithelium. It appears, therefore, that certain naturally occurring substances which are normally present in exocrine glands and can presumably be secreted in their products can influence the extent of Na^+ reabsorption in glandular ducts. The significance of this observation is 2-fold: (1) from the physiologic point of view, it provides experimental evidence for the potential role of luminal factors in the regulation of the transductal transport of Na^+; and (2) from the pathophysiologic point of view, it suggests that increased concentrations of these luminal factors, as may presumably occur in diseased glands, could cause alterations in this transport, and consequently, increase the Na^+ concentrations of final secretions in salivary and perhaps other exocrine glands.

Transepithelial Na^+ transport in the rat parotid, which can establish large concentration gradients across the duct epithelium,

has also been shown to be inhibited by fluids obtained from cystic fibrosis patients (9, 15) and from an animal model for this disease induced by the administration of reserpine to rats (11). The chemical identity of this inhibitory factor is not known, but a ciliary dyskinesia factor present in these fluids is thought to be a polypeptide of low molecular weight which binds to IgG (1). Polylysine, a basic polyamino acid, has also been shown to inhibit Na^+ reabsorption in the rat parotid bioassay (9). The inference from these observations is that a positively charged peptide, which presumably originates in one or more exocrine glands, or which arises at an unknown site but circulates in the blood and is secreted in some exocrine gland products (*i.e.*, processed for secretion in these tissues), is present in the fluids from cystic fibrosis patients and from the animal model and is responsible for the increased Na^+ concentrations observed in these fluids, as a result of an inhibitory effect on ductal Na^+ reabsorption.

The substances tested in these experiments are normally present in salivary and other exocrine glands, where they fulfill a number of physiologic functions. Preliminary experiments in our laboratory have shown, furthermore, that kinins and prostaglandins increase the release of mucous glycoproteins from the isolated rat trachea (12). Both the kinins and the prostaglandins which they generate seem to have, therefore, specific biologic activities in several areas of particular interest in cystic fibrosis, *i.e.*, ion transport, mucus secretion, membrane function, and neurohumoral regulation.

The mechanism by which kallidin and prostaglandin E_1 inhibit Na^+ reabsorption in the rat parotid gland was not investigated in these experiments. Sodium reabsorption in salivary ducts is a complex process that may involve both a passive influx across the luminal cell membrane and an active pumping mechanism localized in the contraluminal cell membrane. The fact that the test substances exerted an effect from the luminal side could be construed to imply that they primarily affect the apical membrane mechanism. This is also supported by the findings with amiloride, a diuretic agent that inhibits passive Na^+ influx. However, ouabain has been also found to affect Na^+ reabsorption in salivary glands when present in the luminal side of the duct epithelium (Table 2; 10), although it presumably inhibits a serosal pump. More refined techniques such as duct microperfusion would be necessary to eludcidate further the mechanism by which the test substances described in this report or the fluids from cystic fibrosis patients and from the animal model inhibit Na^+ transport in the rat parotid gland. We have previously outlined the limitations of the retrograde perfusion technique (11).

The results of the experiments with lysine-bradykinin do not support the hypothesis proposed by Dann and Blau (4). On the basis of reported deficiencies in arginine esterases in cystic fibrosis, these authors proposed that there was defective formation of kinins in this disease. They further speculated that kinin E may be the factor that triggers Na^+ reabsorption in the ducts. However, our results indicate that kallidin (the kinin formed in salivary and other exocrine glands) inhibits Na^+ reabsorption in the rat parotid bioassay. Thus, kinin formation may be increased, rather than decreased, in cystic fibrosis through a different mechanism.

The potential role of the prostaglandins in mediating both the electrolyte abnormality and the mucus hypersecretion in cystic fibrosis should be further investigated, particularly in terms of whether they are present in increased amounts in sweat and other exocrine secretions of the affected individuals.

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