Effects of Carbamylcholine and Isoproterenol on Electrolyte Fluxes in Perfused Main Duct of Submandibular Gland of Reserpinized Rat (42875)

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Abstract. **Administration of reserpine (RES) at a dosage of 0.5 mg/kg body wt, ip daily for 7 days was found to lower the dose of carbamylcholine and isoproterenol that alters sodium and potassium transport by cells of the main duct of rat submandibular gland. In the perfused main excretory duct of the submandibular gland of the RES rat, administration of carbamylcholine at a dosage of 1 μg/kg body wt, inhibited net efflux of sodium (17%) and administration of isoproterenol at a dosage of 2 μg/kg body wt increased net efflux of sodium (20%); these drugs, at the same dosages, did not induce significant change in electrolyte flux of normal rat. At a dosage of 5 μg/kg body wt, carbamylcholine decreased net influx of potassium (15%) in the RES rat but was without effect on normal rat. Isoproterenol at the dosage of 5 μg/kg body wt significantly inhibited net influx of potassium in both the RES rat and normal rat. The data suggested that the duct cells developed supersensitivity to sympathomimetic and parasympathomimetic stimulation after chronic RES treatment.** [P.S.E.B.M. 1989, Vol 190]

Chronic administration of reserpine (RES) has been shown to produce supersensitivity to catecholamines in heart and blood vessels and also in salivary glands (1–3). The site of changes may be postjunctional and possibly involve alterations of the adrenergic and cholinergic receptors (4, 5). Martinez *et al.* (2) found that administration of RES (0.5 mg/kg body wt/day) for 7 days produced supersensitivity of the submandibular gland, and salivary flow was evoked with a lower dose of carbamylcholine than that required to elicit flow from untreated glands. There are to date no observations on the effects of RES treatment on electrolyte transport across cells of the main duct of submandibular gland. Since the duct cells have an important role in regulation of the concentrations of electrolytes in the final saliva, it was of interest to investigate the effects of chronic administration of low doses of RES on the sensitivity of cells of the main duct of submandibular gland to isoproterenol (ISO) and carbamylcholine as measured by agonist-induced changes in sodium and potassium transport across the duct cells.

Materials and Methods

Experimental Animals. Male Long-Evans rats, 4–5 months old, weighing between 300 and 400 g were used. RES was administered daily in a dose of 0.5 mg/kg body wt ip for 7 days to some groups of rats. Experimental (RES) and control rats were maintained on rat lab chow and water *ad libitum* until 18 hr before the experiment when food but not water was removed. The last doses of RES were administered about 24 hr before the rats were used. The animals were anesthetized with pentobarbital sodium (50 mg/kg body wt ip); tracheas were cannulated to provide clear airways. One main excretory duct of the submandibular gland was cannulated at its oral opening by insertion of polyethylene tubing (PE 10) (pulled to a tip diameter of about 100 μm and ligated in place) (6–9). The other end of the cannula was connected to a 1-ml glass syringe which was mounted on a microperfusion pump (Harvard model 940) and set to deliver fluid at 990 nl/min.

Perfusion. The perfusion solution contained 144 m*M* sodium, 5 m*M* potassium, 122 m*M* chloride, and 27 m*M* HCO_3. Samples of perfusate were collected from the free end of the oral cannula. Three-microliter

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samples of perfusate were collected by disposable micropipette (Drummond Scientific Co.) for analysis of sodium and potassium by flame photometry (Instrument Labs model 143). There was no sodium contaminant in such micropipettes.

In some experiments, a trace amount of [^{3}H]methoxyinulin was added to the perfusion solution for the determination of water flux. The radioactivity of inulin was measured by liquid scintillation spectrometry. Then the inulin ratio was calculated as the ratio of the inulin activity in the collected perfusate to that in the initial perfusion solution (8–11). As in previous reports (8–11), the inulin ratio was used for the calculation of net electrolyte flux for the entire duct (neq/min/duct).

Protocol. After preparation of the animals, the perfusion was continuous until the end of the experiments. At least a 30-min period was allowed to stabilize the animal and samples collected during this period were discarded. Then three samples of perfusate were collected for analysis of sodium and potassium. These samples served as control. Then either carbamylcholine chloride (Sigma Chemical Co., St. Louis, MO) was administered with dosages successively larger (0.5 1, 5, 10, 20, and 40 μg/kg body wt) or isoproterenol hydrochloride (Winthrop-Stern, Inc.) was administered with dosages successively higher (2, 5, and 10 μg/kg body wt). Following a 30-min equilibration period for each dosage of a drug, three samples were collected for analysis of sodium and potassium.

Statistical significance was determined by the use of paired Student's t test. Difference was considered significant if P was less than 0.05.

Results

The inulin ratio was unchanged during control periods (0.98 ± 0.03, n = 12) and during experimental (administration of either carbamylcholine or ISO) periods (0.97 ± 0.03) in both normal and reserpinized rats. Therefore, net movement of water across the main duct of submandibular gland was negligibly small.

Effects of Carbamylcholine. *Normal rat.* During the control period the net efflux of sodium and net influx of potassium was 42.1 ± 2.8 and 32.9 ± 2.8 neq/min/duct, respectively (Fig. 1). After administration of carbamylcholine at dosages of 5 μg/kg body wt and higher, there was a significant decrease in net efflux of sodium, but there was no effect on sodium efflux at the dosages of 0.5 and 1 μg/kg body wt. Carbamylcholine had no effect on potassium influx at dosages of 0.5, 1, 5, 10, and 20 μg/kg body wt; only at a dosage of 40 μg/kg body wt was net potassium influx affected and an 18% inhibition from control value was observed.

Reserpinized rat. In contrast to effects on the normal rat, carbamylcholine, even at a dosage of 1 μg/kg body wt, caused a significant (17%) reduction of net efflux of sodium from that observed during the control period (Fig. 1). At dosages of 5, 10, and 20 μg/kg body wt, carbamylcholine induced a reduction in net efflux of sodium from that of the control period and the inhibitions were, respectively, 32, 37, and 40%; the inhibitions in the normal rat at these same dosages were, respectively, only 9, 10, and 20%. Only at a dosage of 40 μg/kg body wt did carbamylcholine cause the same inhibition of net sodium efflux (42%) in both normal and RES rats. The drug had no effect on influx of potassium at the dosages of 0.5 and 1 μg body wt, but in contrast to effects on normal rat, it decreased potassium efflux at dosages of 5 μg/kg body wt and higher (about 15% at all dosages) (Fig.1).

Effects of Isoproterenol. *Normal rat.* The administration of ISO at a dosage of 2 μg/kg body wt had no significant effect on net fluxes of sodium and potassium (Fig. 2). Subsequent addition of the drug at dosages of 5 and 10 μg/kg body wt caused significant increase in net efflux of sodium (26 and 42%, respectively) and decrease in net influx of potassium (13 and 32%, respectively when compared with values of the control period.

Reserpinized rat. Administration of ISO at a dosage of 2 μg/kg body wt resulted in increased net efflux of sodium (about 20% increase over control levels) (Fig. 2). At a higher dose (5 μg/kg body wt), the drug caused even a greater increase in net efflux of sodium, i.e., 34% increase from that of control, and at 10 μg/kg body wt the increase was 50%. At dosages of 5 and 10 μg/kg body wt, ISO caused significant inhibition of net influx of potassium (23 and 40%, respectively), but it was without effect at the dosage of 2 μg/kg body wt (Fig. 2).

Discussion

The present data demonstrate that chronic administration of RES alters the threshold dose of the main duct cells of submandibular gland to carbamylcholine and ISO stimulation and the main duct appears to be considerably more sensitive to both agonists. Thus, in the RES rat carbamylcholine (1 μg/kg body wt) inhibited net efflux of sodium and at the dosage of 5 μg/kg body wt inhibited net influx of potassium, whereas in normal rat the drug had no significant effects on net sodium and potassium transport at these dosages.

Schneyer and Thavornthon (11) observed that a low dose of ISO (13 μg/kg body wt) causes an increase in sodium efflux and a decrease in potassium influx. These effects may be due to activation of β_1 adrenoceptors (10). The present study confirms previous observations (11) and, furthermore, the data show that even a smaller dose of ISO (5 μg/kg body wt) gave qualitatively similar effects. Moreover, ISO enhanced net sodium efflux at a lower dose (2 μg/kg body wt) in the RES rat than in that of normal rat. Sensitization in salivary glands is reflected by the increased amount of

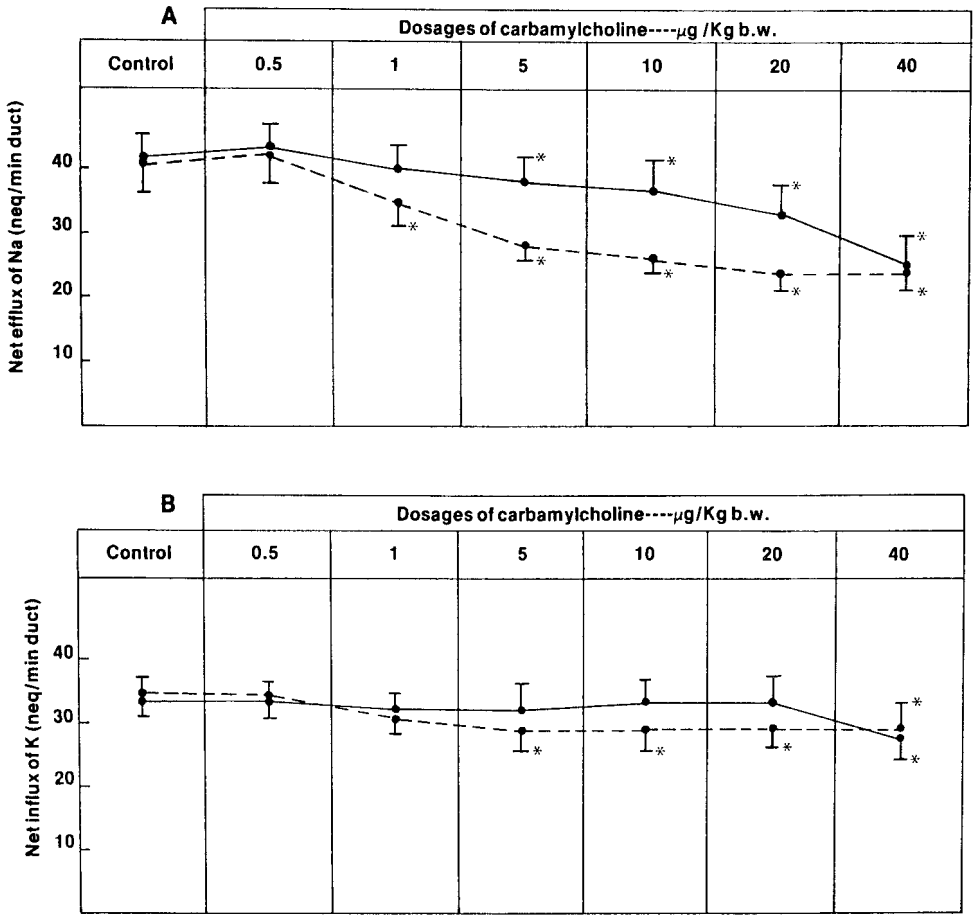

Figure 1. Effects of carbamylcholine on net transductal fluxes of sodium and potassium after the administration (ip) of the drug at different dosages successively. At least 30 min was allowed for equilibrium after each dose of the drug. Values are means ± SE. Nine rats were used in each group, three samples of perfusate were collected from each rat at each dosage of drug. ●——●, normal rat; ●- - -●, RES rat (chronic reserpine treatment for 7 days). * Indicates that values were significantly different from that of controls ($P < 0.05$).

saliva secreted in response to moderate doses and by induction of secretion at doses less than are usually required to evoke secretion (12). Present data thus suggest that supersensitivity to sympathomimetic and parasympathomimetic drugs develops in salivary duct of submandibular gland after RES treatment, since the transport of sodium and potassium is affected at doses that do not affect transport in normal ducts. The increase in sensitivity to catecholamines of RES rats has been previously observed in heart (3) and smooth muscles of the blood vessels (1), as well as in whole salivary glands (2). The site of change is probably postjunctional and may involve the alteration of adrenergic receptors (4, 5, 13). Carbamylcholine has also been observed to induce secretory responses in salivary glands of both acute (14) and chronically reserpinized rats (2) at doses lower than those required to elicit flow in normal rats. This effect may be due to an increase in the number of cholinergic receptors (15). Reserpine also has been shown to alter cell permeability and metabolism by a direct toxic effect on target cells (4, 5, 13). Thus, the present study shows that supersensitivity of a specific glandular component, the duct cells, to sympathomimetic and parasympathomimetic can be induced by chronic administration of RES. Moreover, the data support previous observations (6, 7) that show that administration of a parasympathomimetic drug, carbachol, causes a decrease in net sodium efflux, net potassium influx, and transductal PD. These effects are associated with a decrease in sodium conductance of the luminal membrane (16). It may be mentioned that effects of nerve and agonist stimulation may not be comparable since Schneyer (9) found that direct stimulation of the parasympathetic innervation to the gland resulted in a decrease in transductal PD, without any significant effects on net sodium and potassium fluxes. The discrepancy between the effects of direct nerve stimulation and parasympathomimetic drug administration may be partially due to involvement of the sympathetic pathway in the parasympathomimetic effects.

Saliva evoked by carbamylcholine from the RES rat contains higher concentrations of sodium and potassium than that of normal rat. It is not likely that

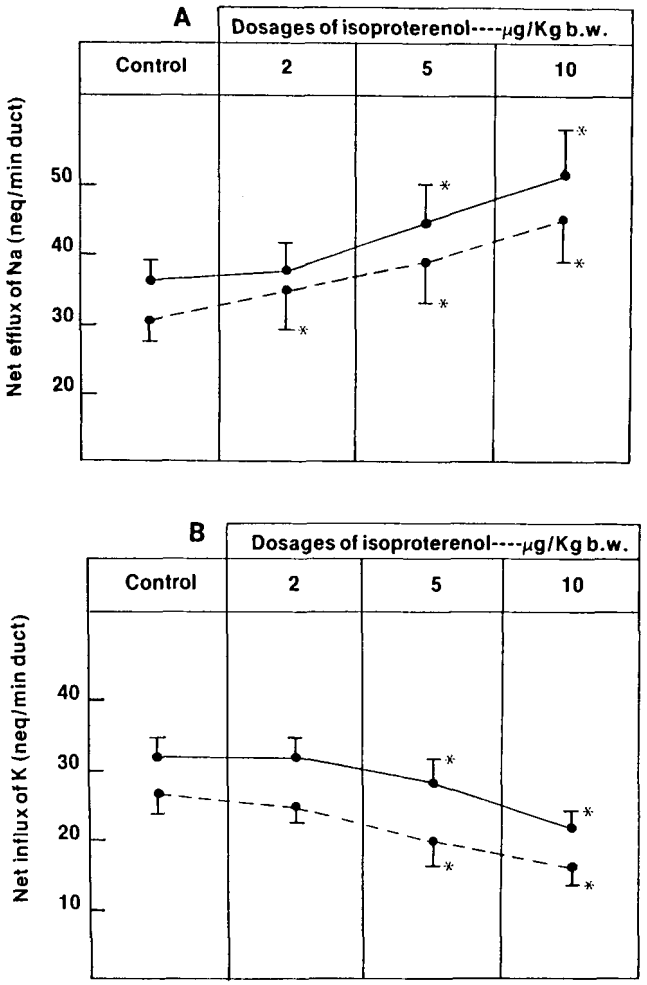

Figure 2. Effect of isoproterenol on net transductal fluxes of sodium and potassium after the administration (ip) of the drug at different dosages successively. Protocol, symbols, and number of animals are the same as in Figure I.

these changes stem from a "water leak" in the salivary duct, since the present data show that there was no significant water loss in the main excretory duct of submandibular gland of either normal or RES rat. However, the possibility that water loss may occur in smaller ducts cannot be excluded. In view of the RES-induced changes in cell permeability (13), such effects may also contribute to the observed changes. Although present data do not provide evidence for change in composition of the primary fluid that is secreted by the acinar cells (2, 17), it is possible that such changes may modify final saliva. Only micropuncture studies to obtain the primary fluid for analysis of its composition will give a definite answer.

Supersensitivity of duct cells to sympathomimetic and parasympathomimetic drugs was induced by chronic administration of RES. Chronic administration of RES did not qualitatively alter the transductal fluxes of sodium and potassium.

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