PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 161, 479–483 (1979)

α- and β-Adrenergic Effects on Na, K, Cl, and HCO_3 Transport in Perfused Salivary Duct during Sympathetic Nerve Stimulation (40578)[1]

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Salivary glands generally produce a final saliva that has a lower Na concentration and a higher K concentration than that of the precursor fluid (1–4). Micropuncture studies indicate that the precursor fluid is modified in two distinct regions of the duct system of the gland, the sublobular ducts and the main excretory duct. The electrolyte transport processes at these two regions appear to be qualitatively similar (4–6). Several studies using luminally perfused main excretory duct of rat and rabbit, *in vivo* and *in vitro*, showed that ductal epithelial cells reabsorb Na and secrete K and HCO_3 by processes involving active transport (5, 7–11), whereas reabsorption of Cl involves passive transport processes (12–14). Regardless of the mechanisms involved in transport of Na, K, Cl, and HCO_3, it is evident that the concentration of these ions present in the final saliva is modified by the autonomic nervous system. Thus, it has been shown that direct stimulation of the autonomic innervation to the gland (15, 16) or the administration of either sympathomimetic (17, 18) or parasympathomimetic agents (11, 12, 17) can alter the net flux of electrolytes and transductal potential difference of the perfused main excretory duct of submaxillary gland. Very recently Schneyer (15) found that direct electrical stimulation of the sympathetic innervation to the duct does inhibit net fluxes of Na and K in the perfused main excretory duct of rat submaxillary gland. These effects are similar to those that have been observed with high doses of isoproterenol (17) but dissimilar to those produced with low doses of isoproterenol (18, 19). Furthermore, while Schneyer (15) reported that α-adrenergic receptors were primarily involved in mediating the electrical response of duct cells to sympathetic nerve stimulation, the separate roles of α- and β-adrenergic receptors on electrolyte flux were not determined. Therefore, the purpose of this work was twofold: (i) to determine the separate roles of α- and β-adrenergic receptors during stimulation of the sympathetic innervation, and (ii) to see if, from analysis of these data, the reason for the differences between effects of high (17) and low (18) doses of isoproterenol on electrolyte flux could be determined.

Materials and methods. Male Long–Evans rats, 4–5 months of age, were used as the experimental animals. They were fasted overnight but allowed water *ad libitum*. The rats were anesthetized with sodium pentobarbital (50 mg/kg body wt., i.p.) and tracheotomized. The carotid sheath was carefully dissected, and the cervical sympathetic trunk was isolated from the common carotid artery and vagus nerve for at least 1 cm. Bipolar platinum electrodes were mounted on a micromanipulator and the tips of the electrodes were placed under and around the sympathetic trunk. A strip of Parafilm was placed under the nerve and electrodes, so that the leakage of electric current to the surrounding tissues was minimized (15). The sympathetic nerve was stimulated by a stimulator (Grass Instruments Co., Model SD5) which delivered square-wave pulses (5 msec duration) at 4–5 V and 20 Hz. The appearance of saliva from the cut end of the main excretory duct of the parotid gland of the ipsilateral side was used as a criterion of successful stimulation of the sympathetic nerve.

The main excretory duct of one submaxillary gland was cannulated at its oral opening by insertion of a fine beveled end of polyethylene tubing (PE 10) approximately 3 cm long to a depth of about 3 mm. For cannulation of the hilar end of the duct, the submaxillary and sublingual gland complex was exposed and cleared. The sublingual gland and duct were separated from the submaxillary gland and duct to expose the hilar end

[1] This work was supported by NIH Research Grant DE 02110.

0037-9727/79/090479-05$01.00/0

of the submaxillary gland. An incision was made in the wall of the submaxillary duct close to the hilar end, the polyethylene tubing (PE 10), pulled to a tip diameter of 70 to 100 μm, was inserted and ligated in place. The other end of the cannula was connected to a 0.5-ml glass syringe. The syringe was mounted on a microperfusion pump (Harvard Model 940) and set to deliver fluid at the rate of 940 nl per minute. Samples of perfusate were collected from the free end of the oral cannula. Samples of perfusate were collected by 3-μl disposable micropipets (Drummond Scientific Co.) (to which were added 1.5 ml lithium solution) for analysis of Na and K by flame photometry (Instrumentation Laboratories, Inc., Model 143). Ten microliters of perfusate sample were collected by disposable micropipets and used for analysis of chloride by chloridometer (Buchler-Cotlove). HCO_3 was calculated as the residual anion concentration, i.e., $HCO_3 = Na + K - Cl$; this approximation has been shown to be feasible (17, 19, 20).

The perfusion solution contained 144 m*M* Na, 5 m*M* K, 124 m*M* Cl, and 25 m*M* HCO_3 with a total osmolality of 287 mOsm as determined by freezing-point osmometer (Advanced Instruments). In some experiments a trace amount of [^{3}H]methoxyinulin was added into the perfusion solution for the determination of water flux across the transductal membrane of the main excretory duct. The perfused solution was collected by 3-μl disposable micropipets (Drummond Scientific Co.) and transferred into 10 ml of aqueous counting scintillant (Amersham/Searle Corp.) in a glass scintillation vial. Radioactive [^{3}H]methoxyinulin counts were measured by liquid scintillation counter (Nuclear Chicago). The unperfused medium was prepared and measurements made in the same way. Then, inulin ratio was calculated as the ratio of counts per minute per microliter of unperfused medium to counts per minute per microliter of perfused medium.

To separate the effects of α- and β-adrenergic responses, when sympathetic nerve stimulation was employed, the rats were divided into four groups: (i) with no adrenergic-blocking agent present, (ii) with the α-adrenergic antagonist phenoxybenzamine present, (iii) with the β-adrenergic antagonist propranolol present, or (iv) with both adrenergic-blocking agents present. Analysis of collected perfusates with or without drugs or nerve stimulation, in each case, served as the control for comparison with perfusates collected following the subsequent experimental manipulations. Either phenoxybenzamine or propranolol (kindly supplied by Smith, Kline, and French Labs and Ayerst Laboratories, Inc., respectively) was given i.p. at a dosage of 5 mg/kg body wt, and 25 min later, stimulation of the sympathetic nerve was initiated and then continued for 60 min.

Analysis of data. All data in text, table, and figures are expressed as means ± SE. Control data were compared with experimental data within the same animals by paired Student's *t* test (22). Values were considered to be statistically significant if *P* values were less than 0.05.

Results. Inulin ratio. The inulin ratio was 0.98 ± 0.003 ($n = 26$) in the control period and it was not significantly changed from the control value by either the administration of drugs (phenoxybenzamine and/or propranolol) and/or sympathetic nerve stimulation. Thus, the net movement of water across the perfused duct was negligibly small. The calculation of net transductal electrolyte fluxes could be done without considering the changes in volume of the perfusate (13, 15, 16).

Na, K, Cl, and HCO_3 net fluxes during sympathetic nerve stimulation. Direct stimulation of preganglionic fibers to the main excretory duct of rat submaxillary gland during perfusion of the duct with bicarbonate–saline solution resulted in marked changes in net transport of the ions studied, i.e., Na, K, Cl, and HCO_3 (Table I). Stimulation of the sympathetic nerve alone (both α- and β-adrenergic receptors were activated) markedly decreased ($P < 0.001$) the net efflux of Na (46% reduction) and the net influx of K (46% reduction), whereas the net efflux of Cl was significantly increased ($P < 0.001$) (44%). However, no significant change in net HCO_3 influx occurred with this condition of stimulation (Table I). Changes in transductal electrolyte fluxes were generally observed in the first sample of perfusate collected after the initiation of stimulation. The effects of sympathetic nerve stimulation on Na, K, and Cl

TABLE I. EFFECTS OF SYMPATHETIC NERVE STIMULATION ALONE OR IN THE PRESENCE OF PHENOXYBENZAMINE (PZB) AND/OR PROPRANOLOL (PPN) ON TRANSDUCTAL NET FLUXES OF Na, K, Cl, AND HCO_3[a]

Perfusion period	Net flux (neq/min × duct)			
	Na	K	Cl	HCO_3[b]
Control (8)—GRP I	−30.7 ± 3.2	26.8 ± 2.9	−10.5 ± 1.0	15.2 ± 1.5
Symp. nerve stim. (8)	−16.7 ± 2.1	15.5 ± 1.5	−15.0 ± 1.2	15.8 ± 0.9
	$P < 0.001$	$P < 0.001$	$P < 0.001$	NS
Control (8)—GRP II	−33.3 ± 3.2	28.5 ± 3.0	−10.0 ± 1.6	15.0 ± 1.2
After PBZ (8)	−27.9 ± 2.2	23.4 ± 3.1	−9.8 ± 1.4	14.4 ± 1.0
	$P < 0.001$	$P < 0.01$	NS	NS
After PBZ (8)	−27.9 ± 2.2	23.4 ± 3.1	−9.8 ± 1.4	14.4 ± 1.0
After PBZ + symp. nerve stim. (8)	−20.7 ± 2.8	14.2 ± 2.0	−16.8 ± 1.2	23.9 ± 1.4
	$P < 0.001$	$P < 0.01$	$P < 0.01$	$P < 0.01$
Control (5)—GRP III	−32.0 ± 1.9	25.3 ± 2.9	−11.0 ± 2.3	18.1 ± 1.4
After PPN (5)	−30.6 ± 2.0	24.4 ± 1.8	−10.7 ± 2.2	17.2 ± 1.5
	NS	NS	NS	NS
After PPN (5)	−30.6 ± 2.0	24.4 ± 1.8	−10.7 ± 2.2	17.2 ± 1.5
After PPN + symp. nerve stim. (5)	−18.6 ± 1.5	16.6 ± 2.1	−9.7 ± 2.2	12.2 ± 1.8
	$P < 0.01$	$P < 0.01$	NS	NS
Control (5)—GRP IV	−32.7 ± 1.9	27.3 ± 2.7	−10.4 ± 0.9	16.1 ± 1.2
After PBZ + PPN (5)	−26.1 ± 2.1	22.8 ± 2.1	−9.1 ± 1.0	13.2 ± 1.2
	$P < 0.05$	$P < 0.05$	NS	NS
After PBZ + PPN (5)	−26.1 ± 2.1	22.8 ± 2.1	−9.1 ± 1.0	13.2 ± 1.2
After PBZ + PPN + symp. nerve stim. (5)	−24.7 ± 2.3	20.5 ± 1.9	−10.1 ± 1.0	13.9 ± 0.8
	NS	NS	NS	NS

[a] Values are means ± SE. Either phenoxybenzamine or propranolol (5 mg/kg body wt) was injected ip. after a control perfusion period of 42 min. Sympathetic nerve was stimulated 42 min later and continued for an additional 52 min during the perfusion period. Net flux from the lumen is given as a negative sign. Symp. nerve stim. = sympathetic nerve stimulation. Control data were compared with experimental data within the same animals by paired Student's t test. Numbers in parentheses indicate number of animals used. NS = not significant. The Roman numerals in each case indicate a particular group (GRP) of animals.

[b] Values are calculated (HCO_3 = Na + K − Cl).

net fluxes were generally maintained as long as the nerve was stimulated.

Na, K, Cl, and HCO_3 net fluxes during sympathetic nerve stimulation in the presence of phenoxybenzamine. The administration of the α-adrenergic-blocking agent phenoxybenzamine altered the transductal fluxes of electrolytes. These effects could be observed within 15–20 min after injection of phenoxybenzamine. This drug alone thus caused an inhibition of net transductal fluxes of Na (16%) and K (18%) ($P < 0.05$). However, no effect of phenoxybenzamine on net fluxes of Cl and HCO_3 was observed (Table I).

Stimulation of the sympathetic nerve in the presence of phenoxybenzamine, i.e., β-adrenergically evoked response, caused a further decrease in net flux of Na of about 26% ($P < 0.001$), and of K of about 39% ($P < 0.01$). On the other hand, the net efflux of Cl was increased ($P < 0.01$) by approximately 70% and the net influx of HCO_3 was increased under these conditions of stimulation to about 65% (Table I).

Na, K, Cl, and HCO_3 net fluxes during sympathetic nerve stimulation in the presence of propranolol. No statistically significant difference from the control mean values of any electrolyte under study was effected by propranolol itself (Table I). However, α-adrenergic responses, i.e., stimulation of the sympathetic innervation to the duct following administration of propranolol, resulted in a marked inhibition of net fluxes of Na, with a 39% reduction from control values ($P < 0.01$). Net flux of K was altered also, and a 32% reduction from control values was recorded ($P < 0.01$). The transductal net fluxes

of Cl and HCO_3 were, however, not significantly affected by the stimulation of sympathetic nerve in the presence of propranolol (Table I).

Na, K, Cl, and HCO_3 net fluxes during sympathetic nerve stimulation in the presence of propranolol and phenoxybenzamine. To rule out the possibility that some receptors other than α- and β-adrenergic receptors might be activated during sympathetic nerve stimulation, both antagonists (propranolol and phenoxybenzamine) were administered together prior to nerve stimulation. The effects of sympathetic nerve stimulation on electrolyte fluxes were prevented when both adrenergic antagonists were present during the period of stimulation (Table I). As already pointed out, however, phenoxybenzamine itself had an inhibitory effect on Na and K flux. This inhibitory effect of phenoxybenzamine was also evident when propranolol was administered in conjunction with phenoxybenzamine. Therefore, since phenoxybenzamine had an effect dissociated from stimulation influences, it was necessary to consider this in ascertaining the net effect of both adrenergic antagonists on effects produced by sympathetic nerve stimulation.

Discussion. The present studies, by using direct sympathetic nerve stimulation, show that both α- and β-adrenergic receptors are present in the duct cells, and that both receptors play important roles in the regulation of net electrolyte fluxes. Thus, activation of β-adrenergic receptors decreases net fluxes of Na and K but enhances net fluxes of Cl and HCO_3; activation of α-adrenergic receptors inhibits net Na and K fluxes while no changes in net fluxes of Cl and HCO_3 are observed. The effects of β-adrenergic agonists on transductal electrolyte transport have previously been reported. Schneyer and Thavornthon (18) showed that very low doses of isoproterenol (13 μg/kg body wt) enhanced Na reabsorption but inhibited K secretion. These effects were blocked by prior administration of propranolol (250 μg/kg body wt). Martin and Young (17) used high doses of isoproterenol (40–50 mg/kg body wt) and found that net Na reabsorption and net K secretion were both inhibited. Therefore, Martin *et al.* (21) suggested that the microgram doses of isoproterenol evoke a relatively pure β-response and result in an increase in Na reabsorption, while high doses of isoproterenol predominantly stimulate α-receptors and inhibit Na reabsorption. Surprisingly, the results of the present study do not agree with this hypothesis at all. When the sympathetic innervation to the duct was directly stimulated in the presence of selective adrenergic-blocking agents, it was clear that neither α- nor β-adrenergically-evoked responses enhanced net transductal flux of Na. Therefore, the hypothesis held by Martin *et al.* (21) is not confirmed by present data, and the effect of the low doses of isoproterenol on the enhancement of net Na flux is still in question.

The present data thus confirm Schneyer's findings (15) that electrical stimulation of the sympathetic innervation to the duct cells does inhibit Na and K fluxes. In addition, as already indicated, the present data also show a distinct difference in effects of α- and β-adrenergically evoked responses on net Cl and HCO_3 fluxes. These results appear to be similar to a previous study of Martin and Young (17) in which isoproterenol was used. They reported that this agent increased net efflux of Cl and net influx of HCO_3, but, as already stated, decreased net fluxes of Na and K. Previous observations (11, 14) suggested that passive Cl reabsorption is secondary to active Na reabsorption. However, the present studies show that the stimulation of β-adrenergic receptors inhibit net Na efflux but enhance net Cl efflux; therefore, Cl reabsorption is not tightly coupled with Na reabsorption.

Although Garrett (23) has reported that sympathetic nerve fibers have never been shown near the main duct of any salivary gland, the previous study (15) and present data strongly indicate that the main excretory duct of rat submaxillary gland does have a sympathetic innervation, and furthermore, the present work shows in addition that both α- and β-receptors are present in the duct cells.

The mechanism of actions of autonomic nerve stimulation or autonomic drugs on electrolyte transport across ductal epithelial cells is not completely understood but it has already been shown that these effects are probably not primarily related to vascular changes (17). However, while the mechanisms involved in adrenergic regulation of transductal

electrolyte flux are not yet fully delineated, with regard to cholinergic regulation of such fluxes, there is some evidence pointing to the mechanisms involved. For example, Knauf *et al.* (24) found that carbachol, when applied at the interstitial surface of duct cells in an *in vitro* system (rabbit salivary duct) inhibited Na transport by lowering the Na conductance of the luminal surface of duct cells. However, even in the case of cholinergic regulation, the mechanisms for regulation of electrolyte fluxes are also not fully delineated since, for example, in turtle bladder the cholinergic agent, mecholyl, inhibits Na transport, and this effect has been suggested to be one that primarily involves decreased active Na transport rather than decreased Na conductance (25).

Summary. Effects of stimulation of α- and β-adrenergic receptors on transport of Na, K, and Cl by ductal epithelial cells were studied in luminally perfused main excretory duct of rat submaxillary gland with isotonic bicarbonate–saline solution. Either phenoxybenzamine or propranolol (5 mg/kg body wt) was given i.p. 25 min prior to the stimulation of the sympathetic innervation to the gland. Stimulation of the sympathetic nerve in the presence of phenoxybenzamine decreased net Na and K fluxes by 26 and 39%, respectively, while net efflux of Cl was increased by 70% and net influx of HCO_3 about 65%. Stimulation of the sympathetic nerve in the presence of propranolol caused a decrease of 39% in net efflux of Na and a decrease of 32% in net influx of K; no changes in net flux of Cl and HCO_3 were observed. Stimulation of the sympathetic nerve modifies electrolyte transport by duct cells, and present work shows that a reduction in net fluxes of Na and K can be induced by either α- or β-adrenergic stimulation. There was, however, a distinct difference between effects of α- and β-adrenergic receptors on net fluxes of Cl and HCO_3. Thus, activation of β-adrenergic receptors enhanced net Cl efflux, and net HCO_3 influx, whereas no changes in net flux of Cl and HCO_3 were oberved with α-adrenergically evoked responses.

We should like to thank Smith, Kline, and French Labs for supplying the phenoxybenzamine and Ayerst Laboratories, Inc. for supplying the propranolol.

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Received November 9, 1978. P.S.E.B.M. 1979, Vol. 161.