Isoproterenol-induced stimulation of sodium absorption in perfused salivary duct

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IN THE SALIVARY GLAND, the electrolyte composition of the final secretion usually differs markedly from that of acinar, or "primary," fluid, particularly in concentrations of sodium, potassium, and total osmolalities. In regard to these parameters, primary fluid is generally quite similar to serum (5, 7, 9, 22), while final saliva usually shows relatively high [K] and low [Na] and total osmolality (1, 17-19). The transformation of primary fluid to final saliva occurs in the salivary duct system, principally in the striated segment but also in the excretory duct. The characteristics of this transformation have been primarily studied in excretory duct, however, since only in that segment has it been possible to obtain micropuncture samples (5, 7, 9, 22) or to perfuse the lumen (4, 15, 20). From work on rat submaxillary main excretory duct, it seems, so far, that this segment may serve as a suitable model to investigate ductal function generally. However, some results currently appear to be anomalous. Thus, in the intact animal, saliva evoked from the submaxillary main excretory duct is, on the other hand, stimulated by isoproterenol, rather than inhibited, when sodium is omitted from the perfusion medium (6). Since the stimulatory action of isoproterenol on secretion of K by the duct system seems to require a prior lowering of [Na] in the luminal fluid, it is surprising that isoproterenol should produce an inhibitory effect on sodium absorption. Hence, this investigation was undertaken to delineate further the effects of isoproterenol on ductal transport, particularly of sodium. For this work, perfused main excretory duct of rat submaxillary gland was used as the test system. However, microgram doses of isoproterenol were used in these experiments, rather than the milligram amounts employed previously, since with high doses the toxic effects might obscure physiologically important responses (12). It has been found that microgram doses of isoproterenol cause a stimulatory, rather than an inhibitory, effect on sodium exchange in the perfused excretory duct. This effect could be suppressed by prior administration of propranolol.

METHODS

Long-Evans male rats, weighing approximately 390 g, were anesthetized with sodium pentobarbital (50 mg/kg body wt) and then tracheotomized. In each experiment, the main excretory duct of one submaxillary gland was cannulated at its oral opening by insertion of fine polyethylene tubing (Clay-Adams PE-10) to a depth of approximately 3 mm. The duct was also cannulated at its hilar end, close to the gland, using PE-10 tubing drawn to a tip diameter of approximately 120 μ. The submaxillary duct was perfused in situ, from hilar to oral end, by the use of a Harvard (model 940) syringe pump set to deliver fluid from a 0.5-ml glass syringe at a rate of approximately 900 nl/min. Samples of perfusate were collected in short lengths of Clay-Adams PE-50 tubing loosely attached over the free end of the oral cannula. Samples of unperturbed medium were also obtained after an experiment was terminated by attachment of PE-50 tubing to PE-10 tubing leading from the perfusion syringe. Samples, 3-μl vol, were transferred by micropipette to 2.0 ml lithium solution for analysis of Na and K by flame photometry (Instrumentation Laboratory, Inc. model 143), or to 2.0 ml H2O for colorimetric analysis of inulin (11). In some experiments, inulin-methoxy-3H was used. Radioactive counts were measured in 3-μl samples added to 0.2 ml of 0.2 m NaCl in glass scintillation vial and made to W&ml by 10.220.33.1 on April 30, 2017 http://ajplegacy.physiology.org/ Downloaded from

The solution used for perfusion contained, in millimoles per liter: Na, 145; K, 5; Cl, 110; SO4, 11; PO4, 10 (pH 7.3).
Mannitol was added to give a final osmolality of 290 mOsm/liter. Osmolality was checked by a freezing point osmometer (Advanced Instruments, Inc.). Inulin was included in the perfusion medium, at a level of 9 mg/ml or, as methoxy-inulin-3H (New England Nuclear Corp.), at a level of approximately 10⁻² μg/μl.

Isoproterenol hydrochloride (Winthrop-Stearns, Inc.) was freshly prepared for each experiment as a 0.2% solution of the racemic compound and injected intraperitoneally in a single dose of 5 μg (12.9 ± 0.3 μg/kg body wt, as the mean dose ± se, for the entire series of rats). The adrenergic drug was given after an initial hour of perfusion so that at least 10 samples of perfusate could be collected in the absence of the drug for analysis of Na, K, and inulin. Samples were then collected for an additional hour of perfusion, starting 10-20 min after the isoproterenol was injected. When propranolol (Inderal, Ayerst Laboratories) was given, this was injected (100 μg/rat, ip) after the initial control period of perfusion and was followed after 30 min by injection of the isoproterenol. The second period of sample collection was begun 10-20 min after administration of isoproterenol. It was known from previous work (15) that ion transport in the submaxillary main duct remains stable for as long as 5 hr of perfusion.

Net fluxes of electrolytes were determined from changes in concentration in the perfusate during perfusion at 900 nl/min, and the inulin ratios (OD of perfusate/OD of medium before perfusion). Net flux of water was also calculated from the inulin ratios and the perfusion rate. Net fluxes were determined for the whole duct (nEq/min X duct, or nl/min X duct) as in previous work (15, 16, 20) with this system. Changes in net flux that occurred in responses to isoproterenol were compared within an animal group, rather than between groups, since fluxes during the control period of perfusion and after administration of isoproterenol were both measured in each rat.

RESULTS

During a perfusion period of approximately 1 hr before administration of any autonomic drug, net flux of Na across the submaxillary main excretory duct averaged −28.3 nEq/min X duct (negative sign indicates efflux from lumen), while the average accompanying net flux of K was 19.9 nEq/min X duct in 14 rats. The inulin ratio (OD of perfusate/OD of medium before perfusion) was in each case close to 1.00. Mean values for the net fluxes are given in Table 1, while individual data from a single representative experiment are shown in Fig. 1.

Within 10 min after injection of 5 μg (12.9 ± 0.3 μg/kg body wt) isoproterenol hydrochloride, net efflux of Na from the lumen showed a sharp increase, while net influx of K began to decline. Inulin ratio usually showed no appreciable change. These effects are shown by data from a single experiment in Fig. 1 and as means from 14 experiments in Table 1.

It is of interest to note that the enhancement of net Na flux resulting from a single injection of isoproterenol ordinarily lasted for well over 1 hr; however, the inhibition of K flux generally showed signs of reversal within 1 hr. The means in Table 1 were calculated from averages that include all of the data from each experiment before and after injection of isoproterenol. The differences between means (Table 1) may in some cases slightly underestimate the maximal effect of the agent because of equilibratory changes in the early part of the control period and later partial reversal of the isoproterenol-induced inhibition of K flux (Fig. 1). However, the mean increase in net flux of Na after isoproterenol was nonetheless quite appreciable, amounting to approximately 40% of the control, and highly significant statistically (Table 1). The decrease in net flux of K, which followed injection of isoproterenol, was less appreciable (avg = 27%), but also

<table>
<thead>
<tr>
<th>Perfusion period</th>
<th>Sodium</th>
<th>Potassium</th>
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<tbody>
<tr>
<td>Control</td>
<td>−28.3</td>
<td>19.9</td>
</tr>
<tr>
<td>After isoproterenol</td>
<td>−40.5</td>
<td>14.5</td>
</tr>
<tr>
<td>Difference</td>
<td>−11.9 ± 1.7</td>
<td>−5.6 ± 0.9</td>
</tr>
<tr>
<td><em>P</em></td>
<td>&lt;.001</td>
<td>&lt;.001</td>
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Values are means, or means ± se, from 14 rats. Isoproterenol was injected in a single dose after a control period of perfusion (900 nl/min) of approximately 1 hr; perfusion was then continued at least for 1 additional hr, 0-6 samples of perfusate were collected during each period. The direction of the net flux is indicated by its sign (negative, from the lumen).
for parasympathetic control, at least of ductal function in salivary glands of man. Furthermore, evidence could not be found, from recent work with submaxillary and sublingual, as well as parotid, glands of man, Knauf and Frönter (3) have found that in all three main gland pairs, [Na] of cholinergic-evoked saliva approaches a level similar to that in serum, as a result, when flow rate is increased toward a maximum. Furthermore, evidence could not be found, from effects on the transmural PD in the main excretory ducts of human glands, that pilocarpine has any direct action on the epithelial cells of this duct segment (4). Hence, there seems to be no basis at present for assuming any important role for parasympathetic control, at least of ductal function in salivary glands of man.

Prior intraperitoneal administration of propranolol (Inderal) at a dose of 100 μg/rat decreased the effects of isoproterenol on fluxes of Na and K, as shown by data in Fig. 2 and Table 2. The data in Fig. 2 are from a single rat, while in Table 2 means are given from 5 rats. As shown by these data, propranolol produced complete suppression of the enhancing effect of isoproterenol on net flux of Na and reduced the K-flux inhibition, which then became statistically insignificant. Net influx of water, which was small (about 3% of perfusate flow) during the control period, was inappreciably changed after administration of isoproterenol and propranolol.

DISCUSSION

In early work on parotid gland of man, Thaysen, Thorn, and Schwartz (19) observed that the salivary concentration of sodium shows a dependence on the rate of flow of saliva, and hence on the degree of glandular stimulation, while salivary [K] is largely independent of stimulus intensity. With sodium, specifically, it was found that the concentration rises to a plateau as the intensity of cholinergic stimulation and flow rate are increased toward a maximum. To account for this, Thaysen et al. proposed that at the site of primary fluid formation Na and K are transferred at a rate that is directly proportional to the rate of fluid transfer, while in the ducts, Na, but not K, is partially reabsorbed. It was suggested (19) that the reabsorptive process for Na is of limited capacity (low T_m), and it was implicitly assumed that this T_m is unaffected by the degree of stimulation. In more recent work with submaxillary and sublingual, as well as parotid, glands of man, Knauf and Frönter (3) have found that in all three main gland pairs, [Na] of cholinergically-evoked saliva approaches a level similar to that in serum, as a result, when flow rate is increased toward a maximum. Furthermore, evidence could not be found, from effects on the transmural PD in the main excretory ducts of human glands, that pilocarpine has any direct action on the epithelial cells of this duct segment (4). Hence, there seems to be no basis at present for assuming any important role for parasympathetic control, at least of ductal function in salivary glands of man.

In submaxillary gland of rat, the likelihood of neural regulation of ductal transport of electrolytes is greater. In saliva from that gland, for example, [Na] hardly rises when flow rate is increased by increasing the intensity of cholinergic stimulation from values that are about half of maximal flow to near-maximal rates (2, 14, 21). In fact, Young and Martin (21) have calculated the limiting concentration of Na in carbachol-evoked rat submaxillary saliva to be approximately 70–80 mEq/liter. In saliva from adrenergically stimulated submaxillary gland, [Na] also is relatively low (13, 21), and not only shows no rise as stimulus intensity is increased but is, in fact, decreased (21). [K] in adrenergically stimulated saliva is strikingly high regardless of flow rate (13, 21). Yet the primary secretion in each of these conditions does not appear to differ in its composition of Na or K but only in its rate of formation (21). As Young and Martin have pointed out, these and other features with regard to the behavior of salivary [Na] and [K] do not accord well with the view that maximums for ductal transport of ions are unvarying in rat submaxillary gland. Martin and Young (6) have, in fact, recently shown that carbachol and isoproterenol can modify the rate of transport in the submaxillary main excretory duct. They have reported (6) that when carbachol in microgram dose or isoproterenol in milligram dose is injected intraperitoneally, during perfusion of the lumen of the main excretory duct, absorption of Na and secretion of K are decreased. Only when Na is omitted from the perfusion medium does a stimulatory effect on secretion of K appear.

The main site of sodium absorption and potassium secretion is not, however, the excretory duct but the more proximally located striated duct segment. In that segment [Na] must be relatively high since precursor fluid is of recent arrival. Thus, it seems inconsistent that isoproterenol, for example, which produces saliva with strikingly high [K], should cause inhibition of ductal Na transport, since inhibition of K transport must also then be expected. Inhibition of ductal transport of Na by isoproterenol also does not seem in accord with the observation that [Na] is generally low in isoproterenol-evoked saliva and changes reciprocally with flow rate (13, 21).

The data from the present investigation show that with a single dose of isoproterenol in the microgram range (1/2,000 that used by Martin and Young (6)) there is enhancement, rather than inhibition, of sodium absorption from main excretory duct, even when the perfusion medium contains sodium salts in isotonic concentrations. At the same time, there is inhibition of K secretion. It does not seem likely, with these two opposing actions on transport of Na and of K, that vascular effects of the isoproterenol can account for the findings to any significant extent. Moreover, a secretory effect has, in preliminary experiments, been observed in intact submaxillary after intraperitoneal injection of a single dose of 13 μg/kg, and even lower doses (1 μg/kg) are reported (10) to produce some secretion when the isoproterenol is given intravenously.

At present, only a tentative scheme can be offered to account for characteristics of the final saliva evoked by isoproterenol. Those characteristics include, most notably, a low rate of flow, high [K] and osmolality, and low [Na]. First, as suggested by Young and Martin (21) it seems likely that

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**Table 2. Transductal net fluxes of Na and K before and after injection of propranolol (Inderal) and isoproterenol**

<table>
<thead>
<tr>
<th>Perfusion Period</th>
<th>Net Flux</th>
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<tr>
<td></td>
<td>Sodium</td>
<td>Potassium</td>
</tr>
<tr>
<td></td>
<td>nEq/min × duct</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>−26.8</td>
<td>18.4</td>
</tr>
<tr>
<td>After propranolol + isoproterenol</td>
<td>−21.4</td>
<td>15.0</td>
</tr>
<tr>
<td>Difference</td>
<td>2.4 ± 1.5</td>
<td>−3.4 ± 1.8</td>
</tr>
<tr>
<td>P</td>
<td>&gt;.01</td>
<td>&gt;.01</td>
</tr>
</tbody>
</table>

Values are means, or means ± se, from 5 rats. Propranolol (100 μg) was injected ip after a control perfusion period of 1 hr. Isoproterenol (12.9 μg/kg) was given ip 20 min later and perfusion continued for an additional hour. Net flux from the lumen is given in a negative sign.

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the low salivary flow primarily reflects a low rate of primary fluid formation, when compared to the rate evoked by cholinergic stimulation, although an increase in ductal permeability to water and to small molecules (8) may play some role. In primary fluid, the concentrations of Na and K are little affected by stimulation (7, 21). Thus, when primary fluid enters the striated ducts, its composition, at least with regard to [Na] and [K], resembles that of serum (5, 7, 9, 21, 22). In the striated ducts, Na is absorbed from the luminal fluid, while K is secreted. It is possible that striated ducts are less sensitive to the inhibitory action of isotroproterenol than the more distally located excretory ducts. Hence, in the striated ducts the stimulatory action of the cholinergic stimulation, although an increase in ductal permeability to water and to small molecules (8) may play some role. In primary fluid, the concentrations of Na and K are little affected by stimulation (7, 21). Thus, when primary fluid enters the striated ducts, its composition, at least with regard to [Na] and [K], resembles that of serum (5, 7, 9, 21, 22). In the striated ducts, Na is absorbed from the luminal fluid, while K is secreted. It is possible that striated ducts are less sensitive to the inhibitory action of isotroproterenol than the more distally located excretory ducts.

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Enhancement of K secretion is continued in the excretory ducts, since [Na] is already low in the luminal fluid. Such a sequence of events seems to accord well, at least qualitatively, with the low flow of isotroproterenol-evoked saliva, the low salivary [Na], and the high [K] and osmolality.

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