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Effects of ouabain and furosemide on saliva secretion induced by sympathomimetic agents in isolated, perfused rat submandibular glands

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Summary. The presence of 10^{-3} M ouabain or furosemide in the perfusate inhibited saliva secretion induced by either isoproterenol (10^{-5} M) or phenylephrine (10^{-5} M) from isolated rat submandibular glands and caused characteristic alterations in the electrolyte composition of saliva.

Recent studies in this laboratory have demonstrated that an isolated, perfused preparation of the rat submandibular gland secretes saliva in response to either parasympathomimetic[1,2] or sympathomimetic[3] agents. Both the volume and electrolyte composition of the secretion elicited by each of these agonists were similar, furthermore, to those observed following stimulation of the gland in situ[1,3,4]. It was also found that the presence of ouabain in the perfusion solution caused a 96% reduction in the volume of saliva secreted in response to acetylcholine and altered salivary Na^+ and K^+ concentrations[2]. Furosemide, on the other hand, caused a 75% reduction in saliva volumes and altered salivary Cl^- concentrations[2]. These findings were interpreted as indicative of the presence of both an ouabain-sensitive Na^+, K^+ ATPase and a furosemide-sensitive NaCl cotransport system in the salivary cells, which contribute to acetylcholine-induced fluid secretion in the rat submandibular gland[2]. The purpose of the present study was to compare the effects of these 2 transport inhibitors on saliva secretion induced in the isolated, perfused gland preparation by α and β-adrenergic receptor agonists, in order to establish whether the same ion transport systems also participate in the formation of saliva elicited by adrenergic agents.

Methods. Adult, male rats of the Sprague-Dawley strain were fed a standard diet and water ad libitum and were anesthetized with Na pentobarbital (6–8 mg/100 g b.wt, i.p.). The procedure for the isolation and perfusion of the submandibular gland was carried out as previously described[1-3]. The glands were prepared with the aid of a Leitz binocular dissecting microscope. After cannulation of the main excretory duct with a short plastic cannula (Clay Adams polyethylene tubing PE10), the arterial branch to the gland was identified and the main trunk of the external mandibular artery and its branches to other neck structures were identified and dissected. All branches except the glandular branch were cut between tight double ligatures and the main arterial trunk was cannulated with a plastic cannula (Clay Adams polyethylene tubing PE50), which was advanced to within a few mm of the glandular branch. Loose ligatures previously placed proximal to the glandular branch were then securely tied and the vessel divided. The gland was removed and placed in a perfusion bath kept at 37 °C. The gland was perfused at a rate of 3 ml min^{-1} with a Krebs Ringer bicarbonate solution (KRB) of the composition previously indicated[1-3]. The perfusate was infused by means of a peristaltic pump and was kept at 37 °C and continuously exposed to a 95% O_2–5% CO_2 mixture. Secretion was induced by administering either isoproterenol sulfate or phenylephrine hydrochloride by way of a 3-way valve connected to the perfusion line and infused at a rate of 0.3 min^{-1} from a Harvard Instruments Co. constant infusion pump, to obtain a final concentration of 10^{-5} M. Either ouabain (10^{-3} M) or furosemide (10^{-3} M) were added to the perfusate in parallel experiments. Saliva samples were collected in all experiments from the main duct cannula in pre-weighted microsample tubes. The volume of each sample was estimated gravimetrically and analyses for Na^+, K^+, Cl^- and Ca^{++} were performed as previously noted[1-3]. Electrolyte concentrations were expressed in relation to rates of flow (mg min^{-1} g^{-1}) as conventionally done in this type of study[1,3-5]. The latter were calculated from the sample volumes, the wet weight of the gland and the time of collection. The doses of secretagogue and of inhibitors which were used are those previously determined to cause maximum effects[1-3].

Results. The effects of 10^{-3} M ouabain or furosemide on the volume of saliva secreted in response to either isoproterenol or phenylephrine are summarized in the table. These results indicate that ouabain reduced salivary fluid secretion 79% and 95%, respectively, when isoproterenol and phenylephrine were used to elicit salivation. The corresponding values for furosemide were 69% and 87%, respectively. Both inhibitors significantly inhibited, therefore, saliva secretion induced from the isolated gland preparation by stimulation of α- or β-adrenergic receptors, but the effect of ouabain was generally larger. As expected, the maximum rate of flow observed with the 2 secre-

tagogues was also significantly reduced by both transport inhibitors (table). The effects of ouabain on the monovalent cation concentrations of isoproterenol-induced saliva are shown in figure 1. It can be seen that the presence of the glycoside in the gland perfusate resulted in significantly elevated Na^+ concentrations and in reduced K^+ concentrations in saliva. Similar effects were observed when ouabain was added to the perfusate and the gland was stimulated with phenylephrine (not shown). At the reduced flow rates observed in the presence of the glycoside (table), Na^+ concentrations of saliva elicited by phenylephrine reached values of 70–95 mEq/l, compared to 20–30 mEq/l in the absence of ouabain. Similarly, K^+ concentrations dropped from approximately 70 mEq/l to 40 mEq/l when ouabain was added to the perfusate. Addition of ouabain to the perfusate also resulted in an increase in salivary Cl^- concentrations which paralleled that in Na^+ concentrations (not shown) and in a significant increase in the Ca^{++} concentration of isoproterenol-induced, but not of phenylephrine-induced, secretion. In isoproterenol-induced saliva, Ca^{++} concentrations increased from 4–6 mEq/l to 14–16 mEq/l.

The most noticeable effects of furosemide on salivary electrolytes were a significant reduction in Cl^- concentrations and a marked increase in Ca^{++} concentrations. These are illustrated in figure 2 for phenylephrine-stimulated secretion. Similar effects were noted in isoproterenol-stimulated saliva (not shown). The effects of furosemide on the Na^+ and K^+ concentrations of isoproterenol- and phenylephrine-induced saliva were small and somewhat variable (not shown). Thus, slightly reduced Na^+ and K^+ concentrations were observed in saliva elicited by isoproterenol stimulation, while no change in K^+ concentrations and an increase in the Na^+ concentration of a few samples were observed in phenylephrine-evoked secretion.

Discussion. The mechanisms underlying fluid secretion in the salivary glands are only partially understood, but it is generally accepted that they involve the transepithelial transport of ions and, by osmotic transfer, of water[5,6]. Studies with isolated, perfused preparations of the cat[7] and rat[2] submandibular glands have shown that fluid secretion elicited by cholinergic agents is inhibited by ouabain and by furosemide. Fluid is also secreted by the perfused[3] or in situ[4] rat submandibular gland following stimulation of α and β-adrenergic receptors, but the possible involvement of ouabain- and furosemide-sensitive ion transport systems in these responses has not been previously investigated. The results of this study indicate that both of these transport systems are also involved in isoproterenol- or phenylephrine-stimulated fluid secretion in the rat submandibular gland. As in the case of acetylcholine-induced secretion[2], the presence of ouabain or furosemide in the perfusate significantly inhibited fluid secretion when either of the 2 adrenergic agents were used to stimulate the isolated gland preparation. This suggests that the mechanism of fluid secretion is similar regardless of which of the 3 major autonomic receptors present in this gland is stimulated. As previously suggested for both the salivary glands[2,7] and other epithelial tissues[8], this mechanism seems to involve the entry of Na and Cl into salivary acinar cells by a furosemide-sensitive cotransport system localized in the basolateral cell membrane, a process which is favored by the Na^+ gradient generated by the Na^+, K^+ ATPase present in the same membrane (fig. 3). Chloride would diffuse out of the cell through the luminal membrane, creating an electrochemical gradient which favors the passage of Na^+ into the lumen (fig. 3). This, in turn, would generate an osmotic gradient for the transepithelial transfer of water. The inhibition of fluid secretion (induced by either type of autonomic agonist) when Na^+ is replaced in the perfusate[2,3] supports the view that Na^+ entry is a critical step in the secretory response. An active or secondarily active Cl^- transport in salivary cells is also supported by electrophysiological evidence suggesting that this ion is not in equilibrium across the acinar cell membrane, i.e., is not passively distributed across the barrier[6].

Volumes and flow rates of saliva secreted

Stimulant	Perfusate[a]	N[b]	Volume[c] mg (µl) in 60 min	Flow rate (maximum)[c] mg min^{-1} g^{-1}
Isoproterenol (10^{-5} M)	KRB	15	111 ± 16	9.6 ± 1.6
	KRB + ouabain	15	23 ± 4	2.1 ± 0.3
	KRB + furosemide	10	34 ± 6	4.1 ± 1.2
Phenylephrine (10^{-5} M)	KRB	12	582 ± 94	123 ± 14
	KRB + ouabain	8	25 ± 7	2.1 ± 0.7
	KRB + furosemide	10	75 ± 18	12 ± 5

[a] KRB = Krebs Ringer bicarbonate solution; ouabain and furosemide concentrations = 10^{-3} M/l; [b] N = number of experiments; [c] Values are means ± SE of the mean.

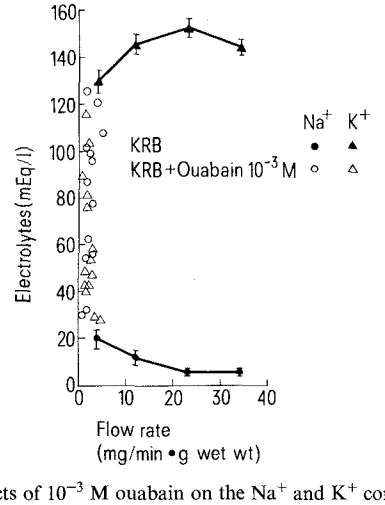

Figure 1. Effects of 10^{-3} M ouabain on the Na^+ and K^+ concentrations of saliva secreted by the isolated, perfused rat submandibular gland in response to isoproterenol (10^{-5} M). The gland was perfused with a Krebs-Ringer bicarbonate solution (KRB) containing the glycoside. The lines represent the normal concentration/flow relationship for salivary K^+ (solid triangles) and Na^+ (solid circles) in the absence of ouabain. Data shown are based on 15 experiments.

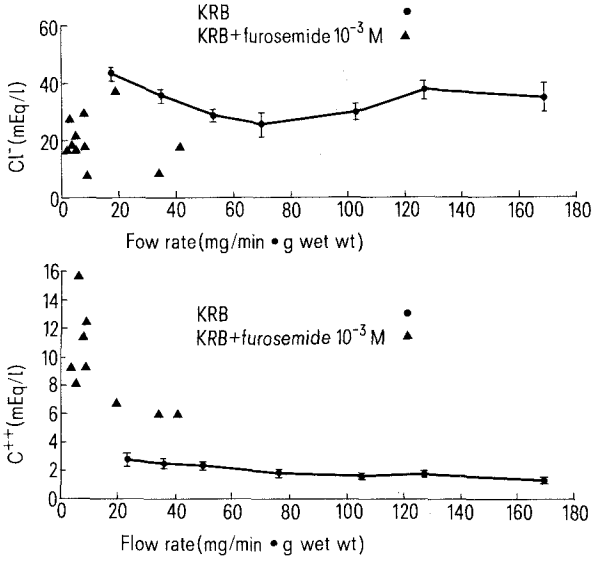

Figure 2. Effects of 10^{-3} M furosemide on the Cl^- (upper panel) and Ca^{++} (lower panel) concentrations of saliva secreted by the isolated, perfused rat submandibular gland in response to 10^{-5} M phenylephrine. Perfusion conditions were as in figure 1. The lines represent the normal concentration/flow relationship for these 2 ions in the absence of furosemide. Data shown are from 10–12 experiments.

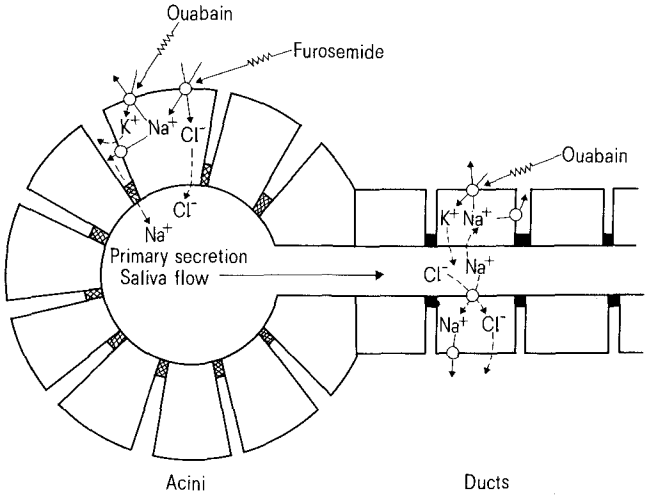

Figure 3. Proposed mechanisms of fluid formation in salivary acinar cells following stimulation with sympathomimetic agents. This mechanism seems to involve ouabain and furosemide-sensitive ion transport systems and the generation of an electrochemical gradient by diffusion of Cl^- across the luminal cell membrane, which favors the passage of Na^+ into the lumen. The presence of Na^+ and Cl^- in the lumen generates an osmotic gradient which causes water movement. An ouabain-sensitive pump is present in duct cells and modulates salivary Na^+ and K^+ concentrations.

Acinar cells are considered as the major source of salivary fluid[5] and effects of the transport inhibitors used in this study on saliva volumes can be ascribed primarily to actions on these cells. Salivary electrolytes, on the other hand, are for the most part the result of the activity of duct cells, which modify the plasma-like primary secretion produced by acinar cells, particularly when the flow is slow[5].

The presence of ouabain in the perfusate modified salivary Na^+ and K^+ concentrations, most likely as a result of inhibition of a Na^+, K^+ pump which is believed to be present in salivary ducts (fig. 3) and to be a major element in the transductal reabsorption[5] of Na^+ and secretion of K^+. The effect of furosemide on salivary electrolytes is consistent with its known action on anion transport[8] and is similar to its effect on the ion composition of saliva secreted in response to acetylcholine[2]. However, studies using the perfused rat submandibular duct[9] indicated that chloride was transported passively and that furosemide had no effect on net transport. The possibility arises, therefore, that furosemide may inhibit chloride secretion in acinar cells, a view that would be consistent with its effect on the NaCl cotransport system presumably present in these cells (fig. 3). Thus, in contrast to ouabain which inhibits the Na^+, K^+ pump in both acinar and duct cells causing, respectively, reduced fluid secretion and altered salivary Na^+ and K^+ concentrations, furosemide probably reduces fluid and salivary Cl^- concentrations by an effect on acinar cells (fig. 3). As expected, the reduced Cl^- concentration observed after furosemide was compensated by an increase in a 'residual anion' concentration calculated as $Na^+ + K^+ + Ca^{++} - Cl^-$. This is mostly HCO_3^- in rat submandibular saliva[5]. The markedly increased salivary Ca^{++} concentrations observed in the presence of furosemide with either isoproterenol or phenylephrine stimulation, do not seem to be the result of decreased volumes of secretion, since a similar effect was not observed with ouabain, at least when phenylephrine was used to stimulate secretion, despite a marked reduction in salivary volume. This effect requires further investigation since the handling of Ca^{++} by salivary glands is a complex process involving several interrelated mechanisms[5].

The presence of similar ionic mechanisms for salivary fluid secretion when cholinergic or adrenergic receptors are stimulated likely represents an integrated physiological mechanism to insure an adequate interaction of the secretory pathways for the 2 major fractions of saliva and the production of an adequate vehicle for the 'wash-out' of macromolecular components of saliva with important digestive and protective functions.

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Blood and tissue distribution of cyclosporin A after a single oral dose in the rat[1,2]

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Summary. After a single oral dose of cyclosporin A (82 mg/kg) in rats, tissue (kidneys liver and brain) and blood levels reached maximum values (approximately 80 µg/g and 3.5 µg/ml) between 3 and 7 h after drug administration. Drug elimination continued for at least 5 days. The 24-h urine and bile elimination was 2% for each.

Cyclosporin A (Cy-A), a novel type of immunosuppressive agent[3], is found to be of increasing clinical usefulness in the inhibition of graft rejection in organ and bone marrow transplantations[4-7]. (For a recent review on the in vivo studies with Cy-A, see White[8]). The drug molecule is an undecapeptide of fungal origin[9] with a T-cell associated immunosuppressive activity[10]. The clinical utility of Cy-A is limited mainly by its dose-related nephrotoxicity[11,12].

Although, after short or long term administration of Cy-A, nephrotoxocity as estimated by increased serum creatinine levels has been correlated with cyclosporin concentrations[11,13,14], detailed knowledge of the tissue distribution and elimination