The Chronically Reserpinized Rat as a Model for Cystic Fibrosis: Abnormal Cl^- Transport as the Basis for Reduced Salivary Fluid Secretion

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ABSTRACT. Saliva secretion induced by 10^{-6} M acetylcholine was reduced 74% in isolated, perfused submandibular glands of control rats when the gland was perfused with solutions containing either furosemide (10^{-3} M) or sulfate (instead of chloride) as the major anion. In regular (Cl^- -containing) perfusates without furosemide, saliva secretion was reduced 74% in isolated glands of rats treated with seven intraperitoneal doses of reserpine (0.5 mg/kg body weight). In the latter, addition of furosemide or replacement of perfusate Cl^- with SO_4^{2-} caused a further 35% drop in saliva volumes. Salivary Cl^- concentrations were lower in saliva from the treated animals and were reduced further by furosemide, which also reduced the Cl^- concentrations of control saliva. In submandibular acini isolated from control glands, acute exposure to ^{36}Cl (1 $\mu\text{Ci/ml}$) resulted in a rapid uptake of tracer so that a constant content of isotope (approximately 9.5 nM/mg protein) was attained in 4–5 min and maintained for 30 min. This basal uptake reached 8.4 nM/mg protein in acini isolated from glands of reserpine-treated rats and attainment of a steady state of tracer content was delayed and required 8–10 min. Exposure to acetylcholine reduced uptake and steady state tracer content by 35% in control acini, but had no effect in acini of reserpine-treated rats. Acetylcholine caused a rapid decrease (42% in 1 min) in ^{36}Cl content of control acini which were preloaded with tracer for 12 min, but only a 23% decrease in acini of reserpine-treated rats. Exposure to furosemide at zero time caused a 50% reduction in ^{36}Cl uptake in control acini and a 41% reduction in acini of the treated animals. Addition of the diuretic to acini preloaded with tracer caused a slower reduction in ^{36}Cl content of control acini (48% in 7 min) and a similarly slow and smaller reduction (26%) in acini of reserpine-treated rats. These findings indicate that: 1) Salivary fluid secretion requires the transepithelial movement of Cl^- in salivary acinar cells. This involves both entry of Cl^- , which occurs in part by a furosemide-sensitive transport system, and efflux of Cl^- . 2) Both steps of this mechanism are abnormal in salivary glands of reserpine-treated animals. Cl^- uptake is delayed and shows a somewhat reduced sensitivity to furosemide, while Cl^- efflux is inhibited. The resulting decrease in overall transepithelial Cl^- transport can explain, therefore, the reduced saliva secretion observed in reserpine-treated animals. 3) Since the reserpine-treated rat has been used as an animal model for cystic fibrosis, similar abnormalities in Cl^- transport could exist in sali-

vary cells of patients with this disease and, similarly, explain the reported disturbances in saliva secretion. (*Pediatr Res* 19:711–716, 1985)

Secretion of saliva occurs by a dual process involving the formation of an isotonic primary secretion by glandular acini and the subsequent modification of its electrolyte composition by ion absorption and secretion in the salivary ducts (1). The mechanism underlying the formation of saliva is only partially understood, but recent evidence showing that secretion is inhibited by furosemide, by ouabain, and by the absence of extracellular Na, K, and Cl (2–7) has suggested a model of the ionic mechanism underlying saliva secretion with the following elements: 1) Na and Cl are thought to enter the secretory cells by way of a furosemide-sensitive cotransport system localized in the basolateral cell membrane. This movement is secondarily driven by the Na gradient generated by Na, K, ATPase present in the same membrane. 2) The Cl which enters the cell is thought to subsequently diffuse out of the cell through the luminal (apical) membrane and to generate an electrochemical gradient which stimulates the passage of Na into the lumen across the tight junction. 3) The presence of both Na and Cl within the lumen would generate an osmotic gradient which induces the transepithelial transfer of water and, thus, the formation of an isotonic primary secretion (2, 4, 7). This model applies primarily to acinar cells, which are the major source of fluid in saliva (8), and includes as a major component the transepithelial (transacinar) movement of Cl. In support of this model, both furosemide-sensitive Cl^- uptake (entry) and secretagogue-sensitive Cl efflux have been recently shown in isolated salivary acini incubated *in vitro* (9).

Salivary fluid secretion is reduced both in patients with cystic fibrosis (10–12) and in an experimental animal model for this disease developed by the chronic administration of reserpine to rats (13, 14). On the basis of the model of salivary fluid secretion described above, it can be speculated that this abnormality could result from a defective transepithelial transport of Cl in salivary cells. Abnormalities in either the diuretic-sensitive Cl uptake (*i.e.* the cotransport system) or in Cl efflux could explain the decreased fluid secretion. This possibility is of interest in view of recent reports of abnormal Cl transport (*i.e.* permeability) in the sweat gland (15) and the airways (16) of CF individuals. In this study, we have compared, therefore, some aspects of Cl transport in the submandibular salivary gland of normal rats and of the experimental animal model for cystic fibrosis. Two approaches were used for this purpose: 1) An isolated, perfused gland preparation was used to investigate overall fluid secretion in the

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presence of furosemide (a "loop" diuretic which inhibits cation-coupled Cl transport) and of fluids in which Cl was replaced with the more impermeant anion SO_4 . 2) A preparation of isolated acini was also used to measure ^{36}Cl fluxes in the absence and in the presence of furosemide and acetylcholine. Our purpose was to determine if an abnormality of Cl transport was present in the submandibular salivary gland of the animal model which could explain the reduced fluid secretion. Such an abnormality could be relevant in terms of the similar secretory disturbance observed in the salivary glands of patients with cystic fibrosis.

METHODS

Adult male rats of the Sprague Dawley strain were used. Animals weighing 175–225 g received seven daily intraperitoneal injections of reserpine (0.5 mg/kg body weight) as previously reported (13, 14). Other rats received no drug treatment and were used as controls. All animals were fed with a standard diet and received water *ad libitum*. For the experiments with the isolated, perfused gland preparation animals of each group were anesthetized with sodium pentobarbital (6–8 mg/100 g body weight, intraperitoneal) and the submandibular gland and its blood supply were dissected. The procedure for preparation of the gland for perfusion was as previously described (2–4). Briefly, the external carotid artery was cannulated with a plastic cannula made of Clay Adams PE tubing no. 90 to 2–3 mm distance from the branch supplying the gland. All other branches to different neck structures were ligated and cut, as was the main trunk below the glandular branch. The main excretory duct was also cannulated (PE tubing no. 10) for saliva collection. The gland was removed with the attached cannulae and placed in an incubator bath at 37° C and perfused at a rate of 3 ml/min with a Krebs Ringer solution of the following composition (in mM): NaCl, 118; KCl, 3; CaCl_2 , 2.7; MgSO_4 , 1.2; NaHCO_3 , 25; KH_2PO_4 , 1.2; glucose, 10; pH, 7.4. The perfusate was warmed to 37° C and constantly gassed with a 95% O_2 –5% CO_2 mixture. Delivery was by means of a Buchler peristaltic pump. Stimulation of secretion was accomplished by infusing acetylcholine chloride into the perfusion system at a rate of 0.3 ml/min by means of a constant infusion pump, to provide a final concentration of 10^{-6} M. Saliva samples were collected in preweighed plastic microsample tubes at timed intervals and their volume estimated by reweighing the tubes. Samples were analyzed for Na and K by flame photometry, for Cl with a Buchler chloride titrator, and for Ca^{++} with a Corning Instruments calcium analyzer as previously described (2–4). Concentrations were expressed in relation to flow rates, which were normalized on the basis of tissue weight as conventionally done in this type of study (1, 2). Comparisons between untreated and reserpine-treated rats were made in terms of salivary volumes, flow rates, and electrolyte concentrations. In some experiments the perfusate contained furosemide (10^{-3} M) or SO_4 replacing Cl as the major anion.

For the measurement of ^{36}Cl fluxes, submandibular acini were isolated from the submandibular gland of control and reserpine-treated rats by a procedure which involves enzymatic digestion of the tissue (9, 17). Glands from two to three rats of either group were excised, cut into small fragments, and incubated in a modified Krebs medium of the following composition (in mM): NaCl, 120; KCl, 4; MgSO_4 , 1.2; KH_2PO_4 , 1.2; HEPES, 15; glucose, 10. The medium also contained a basal amino acid mixture (BEM, Gibco Co.) and 2.7 U/mg tissue of purified collagenase (Worthington Chemical Co.) plus 10 U/mg of hyaluronidase (Sigma Chemical Co.). The fragments were incubated in this digestion (dispersion) solution at 37° C for 60 min in stoppered flasks placed in a shaker incubator bath. The flasks were gassed with 100% O_2 at the beginning of the digestion period and every 10 min thereafter, at which times the pH was monitored and adjusted to 7.4 if necessary. The tissue fragments were further dispersed at 45 and 55 min by passage through pipettes of different bores and the suspension was then centri-

fuged ($50 \times g$, 2 min). The enzyme-containing medium was removed and an equal volume of fresh medium containing 2% albumin was added to the pellet. The acini were then centrifuged, washed again with fresh medium, filtered through nylon mesh, and finally washed with medium of the same composition of the dispersion medium but containing 2.7 mM CaCl_2 . The acini were then placed in appropriate volumes of this medium and preincubated for 15 min at 37° C. One $\mu\text{Ci/ml}$ of ^{36}Cl was then added to the flasks and 200- μl samples of the suspensions were taken at timed intervals in triplicate. The acini were separated from the medium by suction filtration through nucleopore filters (25 mm diameter, 3 μM pore size) followed by a wash with 10 ml of ice-cold, isotope-free medium. The filters were placed in scintillation vials and the radioactivity remaining was counted by scintillation spectrometry. Aliquots of each suspension were taken and their protein content of the acini determined by the Lowry method (18). Intracellular concentrations of Na, K, and Cl were measured in similar aliquots of acinar suspensions by methods previously described (9). The uptake of ^{36}Cl into acini acutely exposed to the tracer was measured in the absence (basal uptake) and in the presence of either acetylcholine (10^{-6} M) or furosemide (10^{-3} M) added to the suspensions 15 min before the tracer. In other experiments, acini were preloaded with tracer for 12 min until a steady state of ^{36}Cl content was achieved and then acetylcholine or furosemide were added to the flasks. Samples were then processed as indicated above.

The viability of the acinar preparations was monitored by their ability to maintain constant intracellular ion concentrations and by their ability to release K^+ . In the latter case, acinar suspensions were incubated in the absence and in the presence of 10^{-5} M acetylcholine and samples were taken at timed intervals for a period of 10 min and rapidly centrifuged. The supernatants were separated and analyzed for K content by flame photometry. At the end of the incubation period the acini were sonicated and their K content measured in the same manner. Release of K was calculated from these values and expressed as a percentage of the K content of the acini according to the formula of Quissell (17). The effect of 10^{-5} M atropine on the acetylcholine-induced K^+ release was also studied by incubating acinar suspensions with the blocker for 10 min before addition of acetylcholine.

RESULTS

Fluid and electrolyte secretion by the isolated, perfused gland preparations. Secretion by the isolated, perfused submandibular gland of control rats has been previously characterized and shown to be similar to that observed from glands *in situ* (2, 6). Secretion in either of these preparations does not occur in the absence of stimulant (2, 3, 13). In the present experiments, the secretory response to acetylcholine was essentially the same as that previously observed in the isolated gland preparation and, as shown in Table 1, involved the secretion of almost 1.4 ml of saliva in a 60-min period at rates of flow greater than $300 \mu\text{l min}^{-1} \text{g}^{-1}$. As in previous studies, the flow of saliva decayed with time, an observation consistently made in this type of preparation (2, 5, 6). Addition of furosemide (10^{-3} M) or replacement of perfusate Cl^- with SO_4^{--} significantly reduced the secretory response to acetylcholine in control glands (Table 1). The effects of these two experimental manipulations were comparable and involved a 74% reduction in the volume of saliva secreted in 60 min (Table 1), with parallel reductions in the maximum flow rate attained (Table 1).

Perfusion of submandibular glands of reserpine-treated rats with unmodified perfusates containing 10^{-6} M acetylcholine resulted in a markedly reduced secretory response, when compared to that of control glands under similar experimental conditions (Table 1). Thus, the volume of saliva secreted in 60 min was only 26% (*i.e.* a 74% reduction) of that secreted by control glands. This means that the glands of the treated animals secreted an amount of saliva comparable to that produced by control

Table 1. Secretory response to 10^{-6} M acetylcholine from the isolated, perfused submandibular gland of control and reserpine-treated rats

Perfusate	Type of animal*	n†	Volume of saliva (mg in 60 min)	Maximum flow rate (mg min ⁻¹ g ⁻¹)
Krebs (Cl)	C	16	1345 ± 109‡	321 ± 23
	R	11	385 ± 58	167 ± 39
Krebs + furosemide	C	16	372 ± 46	91 ± 6
	R	11	244 ± 12	36 ± 9
Krebs (SO ₄)	C	16	356 ± 48	67 ± 10
	R	17	251 ± 54	55 ± 11

* C, control; R, reserpine.

† Number of experiments.

‡ Means ± SD of the mean.

Fig. 1. Changes in salivary flow rates with time of perfusion in isolated, perfused submandibular glands of rats treated with reserpine. Secretion was stimulated with acetylcholine (10^{-6} M) and the glands were perfused with Cl⁻ containing solutions with and without furosemide or with solutions in which Cl⁻ was substituted by SO₄²⁻. Data shown represent the means ± SE of 11–16 experiments.

glands in the presence of furosemide or sulfate (Table 1). Addition of furosemide to the perfusate caused a further reduction in the volume of saliva secreted by the glands of reserpine-treated rats, which corresponded to 37% (Table 1). Similarly, replacement of Cl⁻ in the perfusate caused a further 35% reduction in saliva volumes in the glands of the treated animals (Table 1). The characteristics of the secretory response from the glands of reserpine-treated rats are further illustrated in Figure 1, which shows the changes in rate of salivary flow with time. As in control glands, salivary flows induced by acetylcholine decay with time and the effects of furosemide and of Cl replacement can be seen throughout the 60-min secretory period.

In control glands, addition of furosemide to the perfusate did not modify the Na⁺ concentrations of saliva (Fig. 2). Previous studies had also shown that furosemide lacked an effect on the monovalent cation concentrations of saliva from control glands (2, 7). Therefore, the results of these experiments confirm this observation. Salivary Na⁺ concentrations tended to be lower in saliva secreted by reserpine-treated rats in response to acetylcholine in the absence of furosemide (Fig. 2). Addition of the diuretic to the gland perfusate resulted in higher salivary Na⁺ concentrations at the reduced flow rates observed (Fig. 2). Furosemide significantly reduced salivary Cl⁻ concentrations in control glands (Fig. 3). This was previously shown in similar studies with the transport inhibitor (2, 7). A similar effect was observed in the glands of reserpine-treated rats (Fig. 3). It is interesting to

Fig. 2. Relationship between salivary Na⁺ concentrations and flow rates in saliva secreted by submandibular glands of control and reserpine-treated rats perfused with Cl⁻ containing solutions in the absence and in the presence of furosemide following stimulation with 10^{-6} M acetylcholine. Results from the same experiments of Figure 1. Fig. 3. Relationship between salivary Cl⁻ concentrations and flow rates in the same experiments depicted in Figure 2.

note, in addition, that Cl⁻ concentrations in saliva from the treated animals were generally lower than those of control secretion when the glands were perfused with unmodified perfusates (*i.e.* not containing furosemide) in the presence of acetylcholine (Fig. 3).

³⁶Cl fluxes in isolated acini. Submandibular acini isolated from the glands of control and of reserpine-treated rats maintained constant intracellular ion concentrations during incubations lasting for up to 75 min (Fig. 4). Similarly, both types of preparations released K⁺ when exposed to acetylcholine, but not in the absence of the secretagogue (Fig. 5). The effect of acetylcholine was blocked in both by previous exposure to atropine (Fig. 5). In acinar suspensions from control animals the pattern of K⁺ release observed in these experiments is similar to that previously reported (9, 17) and involves a rapid efflux of K (which reaches 40% of the K⁺ content in 1 min), followed by reuptake (Fig. 5).

Fig. 4. Intracellular ion (Na, K, Cl) concentrations in dispersed acini isolated from the submandibular glands of control and reserpine-treated rats and incubated for varying periods of time. Values shown represent the means $\pm$ SE of the mean in six experiments.

Fig. 5. Release of K^+ from submandibular acini of control and reserpine-treated rats in the absence of added test substances and in the presence of 10^{-5} M acetylcholine, with and without atropine (10^{-5} M). Values are the means $\pm$ SE from three experiments in each protocol.

The response was essentially similar in acini isolated from reserpine-treated rats (Fig. 5).

In control acini, acute exposure to ^{36}Cl resulted in a rapid accumulation (uptake) of tracer (Fig. 6). This basal uptake of ^{36}Cl was such that the ^{36}Cl content of the acini reached 9.5 nM/mg protein after 4–5 min of incubation and remained stable thereafter for 25–30 min (Fig. 6). In the presence of acetylcholine a steady state of ^{36}Cl content was also reached in 4–5 min but the steady state tracer content was only 6.2 nM/mg protein, which represents a 35% reduction from the steady state reached under basal conditions (Fig. 6). Basal ^{36}Cl uptake in acini from reserpine-treated animals (in terms of steady state tracer content) reached 8.4 nM/mg protein (Fig. 6). In addition, uptake was clearly slower and 8–10 min were required to reach the new steady state of ^{36}Cl content. In contrast to control acini, furthermore, acini of reserpine-treated rats failed to show a response to acetylcholine, which reduced ^{36}Cl uptake by only 2.2% (0.2 nM/mg protein) and only appeared to retard further the time required to achieve a constant tracer content (Fig. 6). Rate constants for ^{36}Cl uptake were calculated from the individual data points and the following values were obtained: control acini, basal uptake = 1.9 ± 0.1 ; reserpine, basal uptake = 1.5 ± 0.1 ; control acini, acetylcholine = 1.2 ± 0.1 ; reserpine, acetylcholine = 1.4 ± 0.1 .

Addition of acetylcholine to control acini preincubated with tracer for 12 min (when a new steady state of tracer content is achieved, as shown in Fig. 6) resulted in a rapid net efflux of tracer, as indicated by a 42% drop in isotope content from 9.5 to 5.6 nM/mg protein (*i.e.* 3.9 nM mg/protein reduction) over a 1-min period (Fig. 7). This net efflux of ^{36}Cl was reduced in acini of reserpine-treated rats and amounted to 2.0 nM/mg protein or 23%. Experiments with furosemide indicated that the inhibitor (10^{-3} M) reduced ^{36}Cl uptake in acini of control (50%) and

Fig. 6. Uptake of ^{36}Cl in isolated submandibular acini of control and reserpine-treated rats in the absence (basal) or presence of 10^{-5} M acetylcholine. Values are the means $\pm$ SE of five to 15 experiments.

Fig. 7. Effects of acetylcholine on the ^{36}Cl content of acini from control and reserpine-treated rats previously exposed to the tracer for 12 min. Values are the means $\pm$ SE of four experiments.

reserpine-treated rats (41%) (Table 2) and that it caused a slower net ^{36}Cl efflux from tracer-preloaded control acini (compared to that induced by acetylcholine). In control acini, net efflux in the presence of furosemide amounted to 4.8 nM/mg protein or 48% of the tracer content of the acini (Table 2) but it required 10 min, as opposed to 1 min for the acetylcholine-induced efflux (as shown in Fig. 7). This net efflux was reduced in acini of reserpine-treated rats and amounted to 2.2 nM/mg protein or 26% of the steady state isotope content of the acini (Table 2).

DISCUSSION

The results of this study confirm previous observations with *in situ* glands (13, 14) that salivary fluid secretion is significantly reduced in the submandibular gland of reserpine-treated rats. Use of an isolated, perfused gland preparation allowed us to investigate this secretory malfunction further. In control glands, addition of furosemide or replacement of Cl^- in the perfusate caused strikingly similar (*i.e.* 74%) inhibitions of saliva secretion. These observations suggest that entry of external Cl^- into the salivary cells is essential for secretion and that this entry occurs in part by way of a furosemide-sensitive transport system, as previously suggested (2, 4). A decreased secretion of saliva should be observed, therefore, if Cl^- entry into the salivary cells is reduced or prevented in some manner. This seems to be the case in the glands of reserpine-treated rats, in which saliva secretion in response to acetylcholine alone is reduced to the same extent

Table 2. Effects of furosemide on ^{36}Cl content of submandibular acini of control and reserpine-treated rats

Type of acini	Basal	Steady state ^{36}Cl content (nM/mg protein)					
		Furosemide at zero time	Difference with basal	% reduction	Furosemide at 12 min	Difference with basal	% reduction
Control	9.9 $\pm$ 0.7*	4.9 $\pm$ 0.8	5.0	50	5.1 $\pm$ 0.2	4.8	48
Reserpine	8.4 $\pm$ 0.5	4.9 $\pm$ 0.4	3.6	41	6.2 $\pm$ 0.5	2.2	26

* Means $\pm$ SD of the mean.

as it is in control glands exposed to furosemide or to Cl^- -free perfusates. This suggests that Cl^- entry into salivary cells is abnormal in the glands of the treated animals. The apparently reduced sensitivity to furosemide in these glands suggests that abnormal Cl^- entry may be due, at least in part, to abnormalities in the diuretic-sensitive cotransport system (2, 4). It is not immediately apparent from the results with the isolated, perfused gland preparation if this represents a reduction in the number of cotransport sites (*i.e.* carriers) or an alteration in the kinetics of transport by a normal number of carriers. The studies with isolated acini, however, indicate that although Cl uptake was of smaller magnitude in acini from reserpine-treated rats, the most important alteration seemed to be a delay in the time required to reach a steady state of isotope content. This suggests that it is the kinetics of transport which may be altered in salivary cells of the treated animals. The somewhat reduced sensitivity of ^{36}Cl uptake to furosemide in these acini would support the view that the diuretic-sensitive cotransport system is involved in the delayed Cl^- entry.

In addition to the apparent defect in Cl uptake, salivary cells of reserpine-treated animals showed a reduced efflux of ^{36}Cl (Fig. 7). This efflux can be considered as a net flux (*i.e.* the difference between unidirectional fluxes) and was rapidly enhanced by acetylcholine in control acini but significantly less in those of reserpine-treated animals. The enhancement of net Cl efflux induced by acetylcholine in tracer preloaded acini can explain the 35% reduction in tracer uptake observed when control acini were acutely exposed to this secretagogue at zero time (Fig. 6). This stimulant-induced reduction in ^{36}Cl uptake was not observed, however, in acini from reserpine-treated rats (Fig. 6), which also suggests that net Cl efflux did not occur as efficiently in these cells as in control acini (*i.e.* uptake was not reduced by the rapid efflux induced by acetylcholine). Cl efflux can likely occur in salivary cells by several mechanisms including passive movement through Cl^- channels in the membrane. In other epithelia, a primary effect of secretagogues seems to be the opening of these channels (22) and it is interesting to speculate that the same is true in salivary acini and that these channels are altered following reserpine administration, thus explaining the reduced net efflux of Cl observed in our experiments.

The mechanism by which chronic reserpine administration alters Cl transport in salivary acini is not clear from our observations. Little is known about the nature or the regulation of either the diuretic-sensitive cotransport system or the proposed Cl channels in salivary cells. The role of electrical potential changes following stimulation or of cellular mediators such as Ca^{++} and the cyclic nucleotides in the activation of these transport systems needs to be further investigated in normal salivary acinar cells before the reserpine effect can be further elucidated. What our results suggest is that there is a delayed Cl entry and a reduced Cl efflux in salivary acini of reserpine-treated rats. These two effects are likely to result in a reduced overall transacinar Cl transport and, thus, in reduced fluid secretion, as shown in previous studies (13, 14). The lower Cl concentrations in the saliva of reserpine-treated animals (Fig. 3) suggest that less Cl is available for secretion in the salivary acini.

The finding of abnormal Cl^- transport in the submandibular salivary gland of reserpine-treated rats is significant for two reasons: 1) The reserpine-treated rat has been used as an experimental animal model for cystic fibrosis on the basis of widespread

exocrine gland disturbances resembling those of the human disease (13, 19, 20). Recent evidence has indicated that Cl^- permeability is abnormal in the sweat gland (15) and airways (16) of patients with cystic fibrosis. Since the animal model shows functional disturbances in the salivary glands which are similar to those seen in cystic fibrosis, the results of the present study suggest, therefore, that defective Cl^- transport may be a generalized disturbance in exocrine (epithelial) tissues in this disease. 2) Transepithelial Cl^- transport by mechanisms involving diuretic-sensitive, cation-coupled transport systems and the opening of membrane conductance channels has been described in several epithelial tissues (21, 22). In secretory epithelia, Cl^- transport is an important component of the ionic mechanism underlying fluid secretion, while in absorptive epithelia it is likely to be of importance in regulating the electrolyte composition of the secretory products. If a defect in Cl transport similar to that found in this study is present in salivary and other exocrine glands of the cystic fibrosis patient, this could explain abnormalities in fluid secretion and electrolyte composition. In tissues secreting large amounts of mucus, these abnormalities could lead to altered concentrations and physicochemical behavior of the mucus and result in the obstructive phenomena which are common in cystic fibrosis.

REFERENCES

- Young JA, van Lennep EW 1979 Transport in salivary and salt glands. In: Giebisch G (ed) Membrane Transport in Biology, Vol 4. Springer, New York, pp 563-674
- Martinez JR, Cassity N 1983 Effect of transport inhibitors on secretion by perfused rat submandibular gland. *Am J Physiol* 245:G711-G716
- Martinez JR, Cassity N 1983 Salivary secretion induced from isolated, perfused rat submandibular glands by sympathomimetic agents. *Arch Oral Biol* 28:1101-1108
- Martinez JR, Cassity N 1984 Effects of ouabain and furosemide on saliva secretion induced by sympathomimetic agents in isolated, perfused rat submandibular glands. *Experientia* 40:557-559
- Case RM, Hunter M, Novak I, Young JA 1984 The anionic basis of fluid secretion by the rabbit mandibular salivary gland. *J Physiol (Lond)* 349:619-630
- Martinez JR, Cassity N 1984 Effect of extracellular K^+ on saliva secretion by isolated, perfused rat submandibular glands. *Arch Oral Biol* 29:729-734
- Martinez JR, Cassity N 1985 Cl^- requirement for saliva secretion in isolated, perfused rat submandibular glands. *Am J Physiol* (in press)
- Martinez JR, Bylund DB, Cassity N 1982 Progressive secretory dysfunction in the rat submandibular gland after excretory duct ligation. *Arch Oral Biol* 27:443-450
- Martinez JR, Cassity N 1985 ^{36}Cl fluxes in dispersed rat submandibular acini: effects of acetylcholine and transport inhibitors. *Pflügers Arch* 403:50-54
- Chauncey HH, Levine DM, Kass G, Shwachman H, Henriques BL, Kulczycki LL 1962 Composition of human saliva: parotid gland secretory rate and electrolyte concentrations in children with cystic fibrosis. *Arch Oral Biol* 7:707-713
- Chernick WS, Barbero GJ 1963 Studies on human tracheobronchial and submaxillary secretions in normal and pathophysiological conditions. *Ann NY Acad Sci* 106:698-708
- Mandel ID, Kutscher A, Denning CR, Thompson RH, Zegarelli EV 1967 Salivary studies in cystic fibrosis. *Am J Dis Child* 113:431-438
- Martinez JR, Adshear PC, Quissell DO, Barbero GJ 1975 The chronically reserpine-treated rat as a possible model for cystic fibrosis: II. Composition and cilioinhibitory effects of saliva. *Pediatr Res* 9:470-475
- Martinez JR, Quissell DO, Wood DL, Giles M 1975 Abnormal secretory response to parasympathomimetic and sympathomimetic stimulation from the submaxillary gland of rats treated with reserpine. *J Pharmacol Exp Ther* 194:384-395
- Quinton PM 1983 Chloride impermeability in cystic fibrosis. *Nature* 301:421-422
- Knowles MR, Stutts MT, Spock A, Fischer N, Gatzky JT, Boucher RC 1983 Abnormal ion permeation through cystic fibrosis respiratory epithelium.

- Science 221:1067-1070
17. Quissell DO 1980 Secretory response of dispersed rat submandibular cells: I. Potassium release. *Am J Physiol* 238:C90-C98
 18. Lowry OH, Rosebrough NF, Farrar AL, Randall RJ 1951 Protein measurement with the Folin phenol reagent. *J Biol Chem* 193:265-268
 19. Perlmutter J, Martinez JR 1978 The chronically reserpinized rat as a possible model for cystic fibrosis: VII. Alterations in the secretory response to secretin and to cholecystokinin from the pancreas *in vivo*. *Pediatr Res* 12:188-194
 20. Mawhinney TP, Feather MS, Martinez JR, Barbero GJ 1979 The chronically reserpinized rat as an animal model for cystic fibrosis: acute effect of isoproterenol and pilocarpine upon pulmonary lavage fluid. *Pediatr Res* 13:760-763
 21. Frizzell RA, Fields M, Schultz SG 1979 Sodium-coupled chloride transport by epithelial tissues. *Am J Physiol* 236:F1-F8
 22. Welsh M 1983 Inhibition of chloride secretion by furosemide in canine tracheal epithelium. *J Memb Biol* 71:219-226

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Defective Regulation of Immune Responses in Croup Due to Parainfluenza Virus

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ABSTRACT. In order to determine if defects in regulation of immune responses play a role in the pathogenesis of croup, we studied 37 infants and children with either croup or upper respiratory illness alone due to parainfluenza virus (PV). PV-specific IgE responses were determined by an enzyme-linked immunosorbent assay, cell-mediated immune responses to PV antigen were studied by *in vitro* lymphocyte transformation assays, and suppressor cell function was determined by addition of histamine to lymphocyte transformation assays. In comparison to patients with upper respiratory illness alone, patients with croup had increased production of PV-specific IgE antibody, increased lymphoproliferative responses to PV antigen, and diminished histamine-induced suppression of lymphocyte transformation responses to PV. These results suggest that a defect in suppressor function exists among croup patients. Similar defects have been demonstrated in bronchiolitis and atopic diseases, providing an immunologic link between the three illnesses. (*Pediatr Res* 19: 716-720, 1985)

Abbreviations

URI, Upper respiratory tract infection
 PV, Parainfluenza virus
 LTF, Lymphocyte transformation
 PHA, Phytohemagglutinin
 Δ cpm, Change in counts per minute
 NPS, Nasopharyngeal secretions
 RSV, respiratory syncytial virus

Croup is a common respiratory illness of childhood, yet fairly little is known about its pathogenesis. The agents that most commonly cause croup are the parainfluenza viruses (1), yet infection with these agents in most individuals results in minor upper URI alone (2, 3). It is conceivable therefore that host factors play a role in the pathogenesis of croup.

It has recently been demonstrated that patients with both croup and wheezing due to PV manifest greater PV-specific IgE antibody production in NPS than patients with URI alone due to PV (4). IgE synthesis in rodents (5) and, presumably, in humans (6, 7) is regulated by T-lymphocytes whose suppressive effect on antibody synthesis is specific for the IgE isotype. We undertook the current study with the hope of detecting evidence of a defect in immunosuppression among patients with croup due to PV. In this regard, we studied LTF responses to PV antigen in blood samples obtained from patients with croup and with URI alone due to PV. In addition, we investigated possible differences in histamine-induced suppression of lymphoproliferative responses (8) among patients with croup or URI alone due to PV.

MATERIALS AND METHODS

Study population and specimen collection. The study group consisted of 37 infants and children ranging in age from 3 months to 7 yr recruited primarily from the acute disease clinic of Children's Hospital at the time of initial presentation with respiratory illness. Parents were advised of the goals and risks of the study, and signed statements of informed consent were obtained. A few patients were either recruited at the time of hospitalization for croup or were followed as part of an ongoing study of respiratory illness in childhood. All patients were studied during the autumn of 1983. Twenty-seven patients were classified as having croup on the basis of the presence of inspiratory stridor and a barking cough. Seventeen of the patients had a history of a previous episode of croup. While the diagnosis of croup was made on a clinical basis, lateral neck radiographs were frequently ordered by house officers as part of routine care of these patients and, when obtained, uniformly were read by radiologists as demonstrating diffuse narrowing of the subglottic area consistent with croup. The median age of croup patient was 14 months

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