The Chronically Reserpinized Rat as a Possible Model for Cystic Fibrosis. VI. Synergistic Effects of Isoproterenol on Ca^{++} and Protein in the Submaxillary Gland

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Summary

Elevated calcium and protein concentrations are a consistent abnormality in submaxillary saliva from patients with cystic fibrosis (CF) and from experimental animal models developed by the chronic administration of either isoproterenol (IPR) or reserpine. The possibility that the effects of the two drugs may be additive was investigated by assessing their combined effects on glandular and salivary Ca^{++} and protein in the rat submaxillary gland. Individually, their effects were also assessed in relation to the dose used. Results indicate that: (1) treatment for 7 days with 0.05, 0.50, and 5.0 mg/kg daily doses of reserpine caused, respectively, a 45%, 95%, and 120% increase in glandular Ca^{++} and a 9.3%, 16.5%, and 37.4% increase in glandular protein; (2) treatment for 7 days with a 5.0 mg/kg daily dose of isoproterenol caused a 138% increase in gland Ca^{++} and a 12.38% increase in gland protein. Treatment with a 5.0 mg/kg daily dose of this drug caused increases of 166% and 10.3% in gland Ca^{++} and protein; (3) in experiments involving a combination of the two drugs, isoproterenol was administered in a 5.0 mg/kg daily dose from days 1-7 and reserpine in a 0.5 mg/kg daily dose from days 4-10 of the treatment schedule. This procedure resulted in (a) a 217% increase in gland Ca^{++} and a 25.7% increase in gland protein; (b) a marked accumulation of a granular, basophilic material in acinar cells and the development of intraductal precipitates; (c) the secretion of turbid saliva with high Ca^{++} and protein concentrations after a secretory dose of isoproterenol (this type of stimulation also reduced the gland Ca^{++} (50%) and protein (20%) contents and produced vacuolization in the acinar cells); (d) the secretion of saliva with elevated Ca^{++} and protein in response to pilocarpine (these elevated concentrations were, however, one-fifth of those obtained after isoproterenol stimulation); (e) the secretion of smaller volumes of saliva after both types of stimulation. These findings indicate that both IPR and reserpine have a dose-related and significant effect on submaxillary gland Ca^{++} and protein and that their individual effects are synergistic. The implications of this synergism for the physiologic state of the submaxillary gland and for the secretory abnormality of cystic fibrosis are discussed.

Speculation

Chronic administration of either isoproterenol or of reserpine to rats results in significant morphologic and secretory changes in the salivary glands, resembling those seen in cystic fibrosis. An important effect of the two drugs involves Ca^{++} and protein, which are increased in the gland and saliva from the treated animals. Since a disturbance of Ca^{++} and protein (glycoprotein) secretion seems to be a fundamental and consistent finding in the submaxillary gland of CF patients, such drug effects on the rodent gland are of interest as they may contribute to our

understanding of possible mechanisms underlying the CF abnormality. This is particularly so since the two drugs seem to affect salivary Ca^{++} and protein through independent mechanisms, which raises the possibility that their effects may be synergistic under appropriate experimental conditions and result in an even greater disturbance of salivary Ca^{++} and protein than that produced by either drug alone.

The chronic administration of either isoproterenol or reserpine to rats has been shown to induce significant morphologic and secretory changes in the salivary glands, and both types of chronically treated animals have been proposed as experimental animal models for cystic fibrosis (15-17), a hereditary human disease that involves exocrine tissues and secretions (8). In the rat submaxillary gland, both drugs cause an increase in glandular and salivary Ca^{++} and protein contents (9, 10, 17, 18). Since a change in the protein (glycoprotein) and Ca^{++} metabolism of the submaxillary gland appears to be a fundamental and consistent disturbance in cystic fibrosis (2-4, 6, 12, 14), the effects of isoproterenol and of reserpine on the Ca^{++} and protein homeostasis of the rat submaxillary gland are of interest, particularly since the two drugs appear to induce changes through distinct and independent mechanisms. This raises the possibility, therefore, that their individual effects on protein and Ca^{++} metabolism may be additive or synergistic under appropriate experimental conditions. This, in turn, may contribute to our understanding of possible pathogenetic mechanisms operating in the Ca^{++} and protein disturbance of the submaxillary gland of the CF patient. In this investigation we have studied, therefore, the individual and combined effects of isoproterenol and of reserpine on the rat submaxillary gland. Glandular composition has been determined at rest and after stimulation with secretagogues in animals treated in a chronic fashion with: (1) three different daily doses of reserpine; (2) two different daily doses of isoproterenol; and (3) a combination of the two drugs in two different dosage schedules. In addition, the composition of saliva in the latter group of animals has been studied. Finally, the morphology of the gland in animals treated with a combination of the two drugs was studied in the resting (unstimulated) and post-stimulation states.

METHODS

EXPERIMENTAL ANIMALS AND SCHEDULES OF DRUG ADMINISTRATION

Male adult rats of the Sprague-Dawley strain were used. The animals were divided into the following groups: *group 1*, control animals which received no drugs but were housed in the same quarters for periods of time comparable to those used in the drug

treatments; *group 2*, animals treated with reserpine for 7 days in the following daily dosages: 0.05, 0.5, and 5.0 mg/kg; *group 3*, animals treated with isoproterenol for 7 days in daily doses of 5 mg/kg and 5 mg/rat; *group 4*, animals treated with a combination of reserpine and isoproterenol. In this group, the dose of reserpine was 0.5 mg/kg-day and the dose of isoproterenol was either 5 mg/kg-day or 5 mg/rat-day. Administration of isoproterenol was started on day 1 of the treatment schedule and continued for 7 days. Administration of reserpine was started on day 4 of the treatment schedule and also continued for 7 days. The two drugs were administered concomitantly, therefore, on days 4, 5, 6, and 7 of the treatment period. Drugs were injected intraperitoneally after the rats had been weighed. The initial weight of the animals at the start of the different drug treatments was between 175 and 225 g. The animals were housed in pairs and had free access to food and water during the treatment period.

GLAND HOMOGENATES

Wet tissue composition was determined in the resting (unstimulated) submaxillary glands of all groups of animals by homogenization of the freshly excised and weighed tissues in 10 cc ice-cold distilled water by using a Polytron homogenizer. The following analyses were performed on the gland homogenates: (1) Ca^{++} in a calcium analyzer from Corning Instruments (23); and (2) protein by the Lowry method (13). The percentage of water content and the dry tissue composition of the resting glands were also determined in animals treated with the 0.5-mg/kg dose of reserpine, with the two doses of isoproterenol (5 mg/kg and 5 mg/rat) and with the combination of the two drugs. For this purpose, the glands were excised, weighed, and placed in a drying oven for 48 hr at 90°. It was found that no additional water loss occurred after this time.

STIMULATION OF SALIVARY SECRETION

Since salivary secretions have been well characterized previously in rats treated with isoproterenol (9, 19) and with a 0.5-mg/kg dose of reserpine (17, 18), this investigation was concerned primarily with the composition of submaxillary saliva in animals treated with the combination of the two drugs. Salivary secretion was stimulated in these animals with either pilocarpine nitrate (1 mg/100 g body weight) or isoproterenol sulfate (10 mg/rat). The animals were anesthetized by intraperitoneal injection of sodium pentobarbital (8 mg/100 mg body weight) 24 hr after the last drug injection and both submaxillary glands were exposed through a neck incision. After tracheal intubation, the main excretory ducts were cannulated below the hilum with a short length of polyethylene tubing (Clay Adams PE 10). The tube was pulled over a microflame to obtain a tip diameter of 120–140 μm and was inserted 0.3 cm into the lumen. No ligatures were used in order to prevent obstruction of flow. Salivary secretion was elicited by intraperitoneal injection of the secretagogues and the saliva extruded in the first 3 min was discarded, as it represents fluid that was present in the duct system before stimulation. After this time, saliva samples were collected in preweighed plastic microsample tubes, timing the collection period in order to obtain from 50–100 μl for analyses. Collection of saliva proceeded in this fashion until no more fluid could be seen at the tip of the cannula. The collection tubes were reweighed when the experiment was completed to obtain a gravimetric estimation of the volumes of fluid in each sample. After secretion had terminated, the submaxillary glands were separated from the adjoining sublingual glands, excised, gently blotted, and weighed to the nearest milligram in a semimicro analytic balance. Rates of salivary flow were calculated in terms of wet gland weight. The glands were placed rapidly in 10 cc ice-cold distilled water and homogenized in a Polytron homogenizer. The homogenates and the saliva samples were analyzed for Ca^{++} and protein as previously indicated.

MORPHOLOGICAL STUDIES

Both resting and stimulated glands from animals treated with a combination of isoproterenol (5 mg/kg) and reserpine (0.5 mg/kg) were excised in some experiments and immediately placed in 10% buffered formalin. The tissues were transferred 24 hr later to 70% alcohol, embedded, cut, and stained with Alcian blue-periodic acid-Schiff stain or with toluidine blue.

STATISTICAL ANALYSIS

In experiments involving the composition of gland homogenates, means and standard deviations of the mean were calculated for each of the parameters studied. Comparison between mean values was made by using Student's *t*-test of statistical significance. A *P* value of less than 0.05 was considered significant.

RESULTS

COMPOSITION OF UNSTIMULATED SUBMAXILLARY GLAND

The wet tissue composition of the unstimulated submaxillary gland of the different groups of animals is illustrated in Table 1. When compared to unstimulated control glands, the composition of the glands of drug-treated animals shows the following characteristics: (1) treatment with reserpine produces progressive increases in glandular Ca^{++} and protein contents, which are significant ($P < 0.005$) at all three dosages of reserpine used. The increases in Ca^{++} and protein content, furthermore, were significantly different from each other at each successively higher dose of reserpine, which implies that the effect of reserpine on glandular Ca^{++} and protein is dose-dependent; (2) treatment with isoproterenol results in a significant increase in Ca^{++} and protein contents of the gland when these values are compared with those of control glands ($P < 0.005$); (3) treatment with a combination of IPR and reserpine results in significant increases ($P < 0.005$) in glandular Ca^{++} and in protein content. Further comparison of the data illustrated in Table 1 indicates the following: (1) the

Table 1. Calcium and protein contents of unstimulated submaxillary gland (wet tissue)

Treatment	Daily dose, mg/kg	<i>n</i>	Ca^{++} , $\mu\text{g/g}$ wet wt	Protein, $\mu\text{g/g}$ wet wt
None (controls)		18	0.301 $\pm 0.030^1$	102.51 $\pm 6.87^1$
Reserpine	0.05	10	0.434 ± 0.050	112.06 ± 4.34
	0.50	36	0.586 ± 0.036	119.45 ± 5.54
	5.00	10	0.662 ± 0.030	140.80 ± 9.30
Isoproterenol	5.00	24	0.717 ± 0.023	115.20 ± 2.10
	5.00 ²	12	0.800 ± 0.034	113.00 ± 1.60
Isoproterenol + reserpine	5.00 +	22	0.954 ± 0.032	128.80 ± 1.50
	0.50			
	5.00 ² +	12	0.936 ± 0.074	139.76 ± 2.50
	0.50			

¹ Values represent standard deviations of the mean.

² This corresponds to a dose of isoproterenol of 5 mg/rat-day.

Table 2. Submaxillary gland weight, gland weight/body weight (GW/BW) ratios and percentage of water content (unstimulated glands)

Treatment	Daily dose	Gland weight, mg	GW/BW ($\times 100$)	% Water content
Control		208.6 $\pm 9.8^1$	83.89 ± 4.58	77.45 $\pm 0.42^1$
Reserpine	0.5 mg/kg	148.1 ± 7.0	89.36 ± 3.66	75.98 ± 0.34
Isoproterenol	5.0 mg/kg	289.7 ± 12.6	124.39 ± 5.35	74.39 ± 0.23
	5.0 mg/rat	323.9 ± 13.5	143.97 ± 7.01	73.53 ± 0.22
Isoproterenol + reserpine	5.0 mg/kg +	240.2 ± 6.3	146.31 ± 3.73	72.73 ± 0.53
	0.5 mg/kg			
	5.0 mg/rat +	234.7 ± 9.2	158.1 ± 9.4	71.35 ± 0.31
	0.5 mg/kg			

¹ Values represent standard deviations of the mean.

Ca^{++} content is significantly higher in the gland of rats treated with the IPR-reserpine combination than in those of animals treated with reserpine alone or with IPR alone. The higher dose of reserpine (5.0 mg/kg-day) produces an increase in the Ca^{++} content of the gland comparable to that induced by the 5 mg/kg-day dose of IPR, but the higher dose of IPR (5 mg/rat-day) results in a significantly higher glandular Ca^{++} content; (2) the increase in protein content is comparable after the two doses of IPR and the two lower doses of reserpine (0.05 and 0.5 mg/kg-day) but is significantly greater ($P < 0.005$) after the higher dose of reserpine (5.0 mg/kg-day) and after combined IPR-reserpine treatment, regardless of the dose of IPR used in the combined drug treatment. The glandular protein content in rats treated with a 0.5 mg/kg-day dose of reserpine plus a 5 mg/kg-day dose of IPR is significantly higher than that of the glands of rats treated with each individual dose alone. This indicates that, as in the case of Ca^{++} , the effect of the combined drugs on glandular protein is greater than the individual effect of each drug.

Table 2 illustrates other characteristics of the resting (unstimulated) gland of the drug-treated rats. The percentage of water content of the gland was similar in all groups of animals. The average gland weight was significantly lower in reserpine-treated animals than in the other two groups of drug-treated animals. When the gland weight/body weight (GW/BW) ratios are compared, the higher dose of IPR (5.0 mg/rat-day) induces a significant increase in this ratio when compared to that in animals treated with reserpine alone or with the smaller dose of IPR (5.0 mg/kg-day). This ratio is also significantly higher in rats treated with a combination of IPR and reserpine, with the highest ratio observed in those rats treated with a 0.5 mg/kg-day dose of reserpine plus a 5.0 mg/rat-day dose of IPR. Although the GW/BW ratio is higher after treatment with the IPR-reserpine combination, the values for gland weight alone suggest that reserpine reduces the hypertrophy induced by IPR.

RESPONSE TO STIMULATION

The secretory response from the gland of animals treated with a combination of IPR (5.0 mg/kg-day) and reserpine (0.5 mg/kg-day) was assessed in terms of the volume and the composition of saliva secreted in response to stimulation with pilocarpine and

with isoproterenol. Results are shown in Figures 1-4 which illustrate the salivary Ca^{++} and protein concentrations in relation to the rate of salivary flow. Figure 1 shows the Ca^{++} concentrations of saliva obtained after pilocarpine stimulation. The shaded area in these figures represents the limits of normal concentrations in control saliva within ± 1 SD of the mean. Ca^{++} concentrations are higher than normal at low rates of salivary flow, but show some scatter at flow rates between 30 and 80 mg/min $\times$ g wet weight. Protein concentrations, on the other hand, are higher throughout the range of salivary flows obtained in these experiments (Fig. 2).

After stimulation with a secretory dose of IPR, saliva from the treated animals was grossly turbid and had the composition shown in Figures 3 and 4. A significant increase in the Ca^{++} and protein concentrations of saliva from the animals treated with the drug combination is illustrated in these figures. The highest

Fig. 1. Relationship between Ca^{++} concentration and rate of salivary flow in submaxillary saliva of rats treated with a combination of isoproterenol and reserpine. Salivary secretion was elicited by intraperitoneal injection of pilocarpine. The shaded area represents mean control values ± 1 SD from the mean. O: values obtained in the treated animals.

Fig. 2. Relationship between protein concentration in submaxillary saliva of rats treated with a combination of isoproterenol and reserpine obtained after stimulation with pilocarpine. Symbols and other pertinent data as in Figure 1.

Fig. 3. Ca^{++} concentration as a function of flow rate in submaxillary saliva of rats treated with a combination of isoproterenol and reserpine after stimulation with intraperitoneal isoproterenol. Symbols are the same as in Figure 1. Number of experiments: control rats = 22, treated rats = 18.

Ca^{++} concentrations were between 60 and 70 mg/100 ml and the highest protein concentrations were approximately 90 mg/ml.

The secretory response to pilocarpine and to IPR is further illustrated in Table 3. The total volume of saliva secreted and the maximum flow rate attained after either type of stimulation were reduced in the animals treated with the drug combination when compared with the corresponding values in untreated controls. However, the total protein secreted and the amount of protein secreted per g of wet weight were markedly elevated not only in comparison to control rats but also, in the case of IPR stimulation, to rats treated with reserpine alone (17).

The wet tissue composition was analyzed for the submaxillary gland of rats treated with a combination of IPR and reserpine after stimulation with the two secretagogues and is illustrated in Table 4. Of the two secretagogues, IPR resulted in a significant decrease in the Ca^{++} and protein contents of the submaxillary gland. This effect correlates with the high salivary concentrations of Ca^{++} and protein observed after this secretagogue, in contrast to the much smaller effect of pilocarpine on these two salivary components.

MORPHOLOGY

The effect of the combined IPR-reserpine treatment on the morphology of the resting submaxillary gland is shown in Figure 5. Acinar elements appeared completely filled with secretory material which, in contrast to that of reserpine-treated rats (16), appeared granulated. There was an increased granularity in the granular tubules and the presence of intraductal precipitates. Acinar glycoproteins showed an increased basophilia. After stimulation with pilocarpine, there was no change in the acinar elements and a slight reduction in the granularity of the tubules (Fig. 6). In contrast, IPR stimulation caused a marked depletion of the acinar cells, which appeared vacuolated. The granular tubules were also depleted of granules and some also appeared vacuolated. Secretory material was evident in the lumen of most ducts.

DISCUSSION

The Ca^{++} concentrations of submaxillary saliva (3, 6, 14) have been found to be elevated consistently in patients with cystic fibrosis. Increasing the Ca^{++} levels in submaxillary saliva

from normal individuals changes its appearance and electrophoretic pattern to that of CF saliva, and certain types of salivary glycoproteins, when present in high concentrations, combined with Ca^{++} to form a reversible precipitate (12). Small molecular weight glycoproteins in saliva are aggregated by additional Ca^{++} (4) and elevated Ca^{++} levels in submaxillary saliva of CF patients may be responsible for the formation of hydroxyapatite crystals (2). Increased Ca^{++} concentrations have also been found in the submaxillary gland and saliva of experimental animal models developed by the chronic administration of either isoproterenol or of reserpine to rats (9, 15, 17, 18).

Fig. 4. Protein concentration as a function of flow rate in submaxillary saliva of rats treated with a combination of isoproterenol and reserpine after stimulation with intraperitoneal isoproterenol. Symbols and other data as in Figure 3.

Table 3. Secretory response and protein output¹

Secretagogue	Type of animal	Maximum flow rate, mg/min × g	Total volume of saliva secreted, ml	Total protein secreted, µg	Protein secreted/mg wet wt
Pilocarpine	Control	140	1,024 ± 96 ²	996 ± 74 ²	3.9 ± 0.3 ²
	IPR-RES	88	335 ± 25	1,699 ± 109	6.7 ± 0.5
IPR	Control	36	188 ± 18	2,635 ± 178	14.9 ± 1.3
	IPR-RES	21	131 ± 7	8,608 ± 424	34.16 ± 4.24

¹ IPR: isoproterenol; RES: reserpine.

² Values represent standard deviations of the mean.

Table 4. Calcium and protein contents of stimulated submaxillary gland (wet tissue)

Treatment	Stimulation	n	Ca ⁺⁺	Protein
PR (5.0 mg/kg) + reserpine (0.5 mg/ kg)	Pilocarpine	30	0.937 ±0.014 ¹	127.2 ±2.8 ¹
	Isoproterenol	26	0.458 ±0.019	104.1 ±4.1

¹ Values represent standard deviations of the mean.

Fig. 5. Morphology of the resulting (unstimulated) submaxillary gland of rats treated with a combination of isoproterenol and reserpine. Note the mucous acini (A) filled with a finely granular secretion and the presence of some granules in convoluted granular tubules (GT). Alcian blue-periodic acid-Schiff stain, $\times 250$.

On the basis of this evidence, the results of the present experiments are significant in that they show that the effects of IPR and of reserpine on submaxillary gland Ca⁺⁺ are dose-dependent and, furthermore, synergistic. The extremely high Ca⁺⁺ concentrations obtained after combined IPR-reserpine treatment have important implications in some aspects of the secretory physiology of the submaxillary gland. First, it appears from previous and from the present findings that procedures which increase glandular Ca⁺⁺ consistently reduce the secretory response to secretagogues (18, 19), as evidenced by decreased flow rates and total salivary volumes. The mechanism underlying this effect can not be ascertained on the basis of presently available evidence, but may be related to Ca⁺⁺ effects on membrane permeability and, therefore, on the ion fluxes that occur upon stimulation. Secondly, since Ca⁺⁺ has been shown to affect the transductal transport of monovalent ions in the rat submaxillary gland (20), the elevated Ca⁺⁺ concentrations found in the saliva of the treated animals can alter this transport and result in abnormal salivary cation concentrations. Finally, by enhanced binding to salivary mucoproteins, the increased Ca⁺⁺ produced by the combined drug treatment can alter their physicochemical properties and, consequently, their release from the secretory cells and their subsequent behavior within the salivary ducts. Recently, Ca⁺⁺ has been shown to modify an intestinal epithelial mucin, rendering it less hydrated and soluble and more granular (11). A similar effect on submaxillary glycoproteins could explain the increased granularity of acinar cells and the formation of intraductal precipitates that were observed in the present experiments. Pretreatment with reserpine has been shown to cause the forma-

tion of an aggregate of soluble submaxillary glycoproteins and to alter their interaction in the presence of Ca⁺⁺ (21).

These effects of Ca⁺⁺ on glandular secretory activity and composition can be relevant to the pathogenesis of the secretory abnormalities observed in the submaxillary gland of CF patients. An elevated Ca⁺⁺ content in this gland could explain the secretion of excess Ca⁺⁺ in saliva (3, 6, 14), the reduced salivary volumes reported in some studies (6, 22), and the progressive obstruction of ducts by the formation of insoluble precipitates (8). Increased Ca⁺⁺ concentrations can also have implications in

Fig. 6. Morphology of the submaxillary gland in rats treated with a combination of isoproterenol and reserpine 90 min after a pilocarpine secretory stimulus. The mucous acini (A) are still filled with finely granular material and there are increased amounts of intraductal material (ID). Granularity in the granular ducts (GT) is somewhat decreased. Alcian blue-periodic acid-Schiff stain, $\times 250$.

Fig. 7. Morphology of the submaxillary gland of rats treated with a combination of isoproterenol and reserpine 90 min after stimulation with isoproterenol. The mucous acini (A) are depleted of secretory material. There is abundant intraductal material (ID) and granules are evident in the granular tubules (GT).

the production of cilioinhibitory effects by secretions from the CF patient (1, 5) and from the experimental animal models (1, 17).

In addition to the effects on Ca^{++} , our results demonstrate that IPR and reserpine also increase glandular protein, the latter in a dose-dependent fashion similar to its effect on glandular Ca^{++} . They also indicate that the effect is synergistic, as in the case of Ca^{++} , when the two drugs are administered in the manner described in these experiments. IPR has been previously shown to increase protein synthesis in the submaxillary gland (10), and reserpine directly stimulates the accumulation of trichloroacetic acid-soluble glycoproteins in this tissue (22). In addition to these effects on the amount of glandular protein, treatment with the IPR-reserpine combination results in qualitative alterations of submaxillary glycoproteins, as suggested by the increased basophilia of acinar cells. An increased number of sialic acid residues in the carbohydrate components of submaxillary glycoproteins could account for the increased basophilia and generate additional binding sites for the increased Ca^{++} . This effect is most likely the result of a specific action of reserpine, which has been shown to increase the sialic acid content of the submaxillary gland (22) and to cause structural changes in submaxillary glycoproteins (21). The reserpine effect on protein accumulation probably involves, in addition, an inhibition of secretory activity and, therefore, of protein release, secondary to depletion of sympathetic neurotransmitter (18). The inhibition of the secretory activity is supported by the finding that reserpine partially prevented the glandular enlargement induced by IPR administration (Table 2).

As a corollary of the marked effects on glandular composition, salivary Ca^{++} and protein are markedly elevated in the animals treated with the IPR-reserpine combination, particularly after a secretory dose of IPR. In addition, stimulation with this secretagogue produced marked changes in the morphologic appearance of the gland and caused a parallel decrease in glandular Ca^{++} and protein (Table 4). These findings suggest that most of the increase in glandular protein induced by the combined drug treatment involved secretory proteins, although IPR alone also enhances the synthesis of structural protein, and that most of the increased Ca^{++} was bound to the secretory protein. In general, the results of the present experiments provide the basis for further analysis of the role of Ca^{++} and of its interaction with secretory macromolecules in the pathogenesis of secretory abnormalities such as those seen in the submaxillary gland of CF patients.

CONCLUSION

The effects of isoproterenol and of reserpine on Ca^{++} and protein in the rat submaxillary gland were investigated in experiments involving the chronic administration of graded doses of the two drugs, either alone or in combination. Results indicate that, individually, both drugs produce a dose-dependent increase in glandular Ca^{++} and that reserpine also causes a dose-dependent increase in glandular protein. When the two drugs are administered in combination, furthermore, their individual effects become synergistic and the increase in glandular Ca^{++} and protein are greater than those produced by either drug alone. These changes in glandular composition are accompanied by marked changes in glandular morphology, including the accumulation of a basophilic, granular material in acinar cells and the formation of intraductal precipitates. Submaxillary saliva from rats treated with the drug combination has markedly elevated Ca^{++} and protein concentrations, particularly after IPR stimulation, and is reduced in volume. These results indicate that combined administration of IPR and reserpine induces both quantitative and qualitative changes in submaxillary glycoproteins which, together with a marked drug-induced increase in glandular Ca^{++} , results in alterations in the glandular secretory

response and in the physiocochemical behavior of secretory macromolecules. It is speculated that a similar combination of pathophysiologic events, either in whole or in part, may explain the secretory disturbance in the submaxillary gland of CF patients.

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