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Effects of Reserpine Treatment on β-Adrenergic/Adenylate Cyclase Modulated Secretion and Resynthesis by the Rat Submandibular Gland

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Summary. Chronic reserpine (adrenergic blocking) treatment causes a marked accumulation of secretory protein in the rat submandibular gland (SMG) but discharge of this material is delayed in response to isoproterenol stimulation. The purposes of this study were to investigate the effects of chronic reserpine treatment on 1) the number of β-adrenergic receptors, 2) the sensitivity of cell-surface-associated adenylate cyclase to various concentrations of isoproterenol, and 3) to correlate these data to morphologic studies of the secretion and resynthesis phases of the isoproterenol-induced secretory cycle in the rat SMG. Animals were injected with reserpine (0.5 μg/g b.w.) for 6 days. Plasma membrane fractions were prepared. The adenylate cyclase response to a series of isoproterenol concentrations, and the number of β-adrenergic receptors ([^{3}H]-alprenolol binding) were determined. Other animals were given a single dose of isoproterenol (0.8 mg/100 g b.w.) and the SMG was examined by light and electron microscopy at various times (30 min to 24 h) after treatment. Chronic reserpine treatment leads to a 2.5-fold increase in SMG β-adrenergic binding sites and a 50-fold increase in adenylate cyclase sensitivity to IPR stimulation when compared to controls. However, secretion and resynthesis of secretory product in response to IPR stimulation was greatly delayed in reserpinized rats.

Key words: Adenylate cyclase – β-adrenergic receptors – Salivary glands – Secretion

Introduction

Cystic fibrosis (CF) is the most common genetic disease of the Caucasian population, affecting about 1 in 2,000 live births. This autosomal recessive disease

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is characterized by a panexocrinopathy which leads to serious problems in digestion and respiration which ultimately result in the premature death of the afflicted individual. The underlying defect resulting in the panexocrinopathy of CF has not been identified as yet.

In its simplest terms the disease is characterized by disturbances in the protein and electrolyte concentrations of the exocrine secretions. The major salivary glands are affected by the disease with the submandibular glands being more dramatically involved than the parotid or sublingual glands. The submandibular glands may be bilaterally enlarged due to obstruction of the acini by abnormally viscous secretions. The increased viscosity of the submandibular gland secretions is due to elevated free calcium in the saliva (Blomfield et al. 1974) as well as increases in the concentration of sodium, chloride, amylase, alkaline and acid ribonuclease, lysozyme and total protein (Mandel et al. 1967). The sodium concentration is also elevated in minor salivary gland secretions but is normal in parotid saliva.

There is no true animal model of cystic fibrosis. However, the chronic administration of the adrenergic blocking agent reserpine to adult rats results in dramatic changes in the submandibular gland and other exocrine tissues which are analogous to many of the changes seen in cystic fibrosis (Martinez et al. 1975a, b, c; Perlmutter et al. 1978; Thompson et al. 1976). The acinar cells of the submandibular glands from reserpine treated rats show a marked accumulation of carbohydrate rich secretory protein when compared to normal controls. The submandibular glands also show decreased saliva flow rates and the saliva shows alterations in the Na^+ and Ca^{2+} content. In addition, serum and salivary factors which inhibit ciliary movement (ciliary dyskinesis factor) have been isolated from these animals. This information has suggested that the chronically reserpinized rat might be a good model in which to investigate the mechanisms of the panexocrineopathies seen in the human disease, cystic fibrosis. These and other observations have implicated defects in neurohumoral responsiveness of exocrine tissues in the pathogenesis of cystic fibrosis. Further, analogous morphologic changes are seen in human salivary glands in the general pathologic condition referred to as sialadenosis (Donath and Seifert 1975). As in CF, the morphologic and functional changes in sialadenosis seem to be related to defects in the stimulus/secretion coupling system (Donath et al. 1973; Donath and Seifert 1975).

A recent report by Simson et al. (1978) indicated that isoproterenol stimulated release of secretory protein by the submandibular gland from reserpine treated rats was reduced and delayed when compared to untreated controls. This observation is not consistent with current theories on the regulation of the β-adrenergic stimulated adenylate cyclase system (Glaubiger and Lefkowitz 1977). In this regard, depriving the cells of their adrenergic stimuli should lead to an increased number of adrenergic receptors at the cell surface and a consequent increased response (supersensitivity) of adenylate cyclase to adrenergic challenge.

Protein secretion from rodent salivary glands is regulated by a β-adrenergic response (Batzri and Selinger 1973; Bogart and Picarelli 1978; Cutler et al., in press). This response is elicited when a β-adrenergic agonist, such as isoproterenol, binds to a specific receptor at the cell surface. The receptor is coupled,

or becomes coupled, to the membrane associated enzyme adenylate cyclase (EC 4.6.1.1.) leading to the activation of the enzyme. Adenylate cyclase catalyzes the production of cyclic AMP from intracellular ATP. This elevated intracellular level of cyclic AMP, in turn, initiates a cascade of events culminating in protein secretion.

Since reserpine is as effective as sympathetic denervation in reducing the catecholamine content of the submandibular gland (Benmiloud and Euler 1963) an increased secretory response to isoproterenol challenge would be anticipated in reserpine treated animals. Therefore, the purposes of this study were to investigate the number of β-adrenergic receptors and the hormonal sensitivity of adenylate cyclase activity in reserpine treated rats and to correlate these data with morphologic studies of the secretion and resynthesis phases of the isoproterenol induced secretory cycle in order to compare the physiologic response with changes in the receptor/cyclase systems.

Methods

Reserpine Treatment. Thirty male, Sprague Dawley rats weighing 200–225 g were injected intraperitoneally with reserpine (Serpasil, CIBA Pharmaceutical Company, Summit, New Jersey 07901) at a dose of 0.5 mg/kg of body weight daily for a total of 7 days. Twenty control animals were injected with 0.85% saline on the same schedule as the reserpine treated animals while fifteen others were left unhandled. The day after the last reserpine treatment, ten of the control and twenty of the experimental animals received a single challenging injection of L-isoproterenol (0.8 mg/100 g B.W.). SMG tissue was removed from animals at various times (1–24 h) after isoproterenol treatment and examined by light and electron microscopy. The glands from the remaining animals were homogenized and an enriched plasma membrane fraction was prepared for adenylate cyclase and receptor binding assays.

Preparation of Enriched Plasma Membrane Fraction. Glands were minced and washed in cold 0.1 M phosphate buffer containing 0.25 M sucrose and 1 mM EGTA and 1 mM dithiothreitol (DTT). The minced tissue was homogenized in a teflon-glass homogenizer for 30 s in cold wash buffer (4° C) and the homogenate was centrifuged at 1,000 × *g* for 10 min in the cold to remove unbroken cells, nuclei and other debris. The supernate was centrifuged at 30,000 × *g* for 15 min at 4° C on a Beckman J-21 centrifuge and the resultant pellet was suspended in cold 10 mM tris (pH 7.6) containing 1 mM DTT. This suspension was placed on a sucrose gradient with steps of 38% and 42%. The gradient was centrifuged at 100,000 × *g* for 60 min on a Beckman L-50 ultracentrifuge. The material which was concentrated at the suspension-38% interface was harvested, diluted in 10 mM tris (pH 7.6) – 1 mM DTT buffer and then concentrated by centrifugation at 30,000 × *g* for 15 min. The resulting pellet has been assayed for structure, adenylate cyclase activity, 5′-nucleotidase activity and succinic dehydrogenase activity and the results were compared to similar assays performed on the 1,000 × *g* pellet and the 30,000 × *g* pellet prior to the sucrose gradient step. The material recovered from the gradient contained predominantly smooth surfaced membrane vesicles with no mitochondria when examined by electron microscopy, showed a 5–10 fold increase in the specific activity of adenylate cyclase, and 18–20 fold increase in the specific activity of 5′-nucleotidase, had no measurable succinic dehydrogenase activity and was low in glucose-6-phosphatase activity when compared to the post 30,000 × *g* supernate (Cutler et al. in press).

Protein Determination. All protein determinations were done using the procedure of Lowry et al. (1951).

Adenylate Cyclase (ac) Assay. Membrane fractions from control and reserpine treated animals were studied for enzyme responsiveness to varying concentrations of L-isoproterenol. An aliquot of the enriched membrane fraction (10–20 μg of protein) was incubated for 10 min in 100 μl of

assay mixture containing 25 mM Tris-HCl (pH 7.6), 5 mM $MgCl_2$. 1 mM cAMP, 1 mM dithiothreitol, 10 mM phosphocreatine, 50 units phosphocreatine kinase, 10^{-6} M GTP and 0.1 mM ATP (3×10^6 cpm α^{-32}P-ATP). The incubation was stopped by adding 100 µl of stopping solution (4 mM ATP, 1.4 cAMP and 2% dodecylsulfate) and then 20,000 cpm of ^{3}H-cAMP for estimation of recovery from chromatography on Dowex Ag 50WX4 and neutral alumina columns. Duplicate samples were counted for 10 min in 10 ml of Brays' solution in an isocap/300 liquid scintillation counter set with separate channels for ^{3}H and ^{32}P (Salomon et al. 1974; Cutler and Rodan 1976; Cutler et al. 1978; Cutler et al., in press). The results were calculated as picomoles of cyclic AMP/per mg./protein per minute. All experiments were run three times and the data reported are the mean of triplicate samples.

Receptor Binding Studies. To measure β-adrenergic binding sites, assays were initiated by adding 100–200 µg of membrane protein to an incubation media of 10 mM Tris (pH 7.6) containing 1 mM DTT and L-[^{3}H]-dihydroalprenolol (New England Nuclear, Boston, MA) with a final volume of 500 µl. All samples were run in duplicate. The reaction was run for 5 min at 37° C and was terminated by adding 6 ml of cold (4° C) 0.85% saline and immediate filtration of the sample through a Whatman GB/F filter. The filter was then washed with an additional 30 ml of saline, then placed in Brays' liquid scintillation fluid and counted in an isocap/300 liquid scintillation counter with an efficiency of between 40–60% on the ^{3}H channel. Duplicate tubes containing 10^{-5} M DL-propranolol were incubated and treated as above and run for each sample to determine non-specific binding. Only those counts which could be displaced by the DL-propranolol were considered specific (Mukherjee et al. 1975a, b; Cutler et al., in press). The number of binding sites and the dissociation constants (K_d) were determined by a computer assisted numerical curve fitting procedure which is based on an iterative search, which for a given expression $y = f(x)$, adjusts the parameters to minimize $\Sigma (Ye\text{-}Yc)^2$, where Ye is the experimental and Yc is the calculated value of the function (Hooke and Jeeves 1961).

Electron Microscopy. Tissue was fixed by both perfusion and immersion procedures. Tissue from 2 animals at each time point was minced into 1 mm cubes and fixed by immersion in 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4) for 1 h. In some cases, animals were perfused with the same fixative and then the tissue was fixed by immersion for 1 h. The tissue was then washed in 0.1 M cacodylate buffer followed by post-fixation in 1% cacodylate buffered osmium tetroxide. After post-fixation the tissue was stained en bloc in 0.25% aqueous uranyl acetate and then dehydrated in a graded series of acetones and embedded in Spurr low viscosity resin (Spurr 1969). Thick (1 µ) sections were cut for light microscopic analysis and orientation purposes. Thin sections (60–90 mµ) were cut on an LKB Ultratome III, stained with lead citrate (Venable and Coggeshall 1965) and examined on a Zeiss EM 10 electron microscope (Cutler 1977; Cutler et al. 1978; Cutler and Rodan 1976).

Results

Adenylate Cyclase. The results of a typical experiment are shown in Fig. 1. The basal adenylate cyclase activity in SMG membranes was not affected by chronic reserpine treatment. Similarly, activation of the adenylate cyclase by 10^{-6} M GTP was unaffected by reserpine treatment. However, adenylate cyclase from SMG membranes from reserpine treated rats was sensitive to much lower concentrations of isoproterenol than control membranes. There was a left shift in the dose response curve for adenylate cyclase activation in the SMG membranes from the reserpine treated animals with an apparent Km of 10^{-7} M isoproterenol. Control SMG membranes had an apparent K_m of 5×10^{-6} M isoproterenol. In addition, the SMG membranes from reserpine treated animals showed a maximal stimulation of adenylate cyclase by isoproterenol that was 25% to 35% higher than that of control SMG membranes.

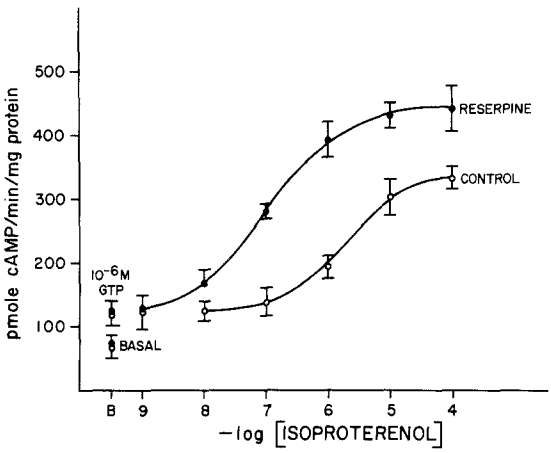


Fig. 1. Activation of SMG adenylate cyclase in membranes from control (○) and reserpine-treated (●) animals. Basal and GTP stimulated adenylate cyclase activity was unaffected by reserpine treatment. Reserpine treatment caused a left shift in the apparent K_m for isoproterenol stimulation of SMG adenylate cyclase when compared to control enzyme [(K_m reserpine) $= 10^{-7}$ M; K_m (control) $= 5 \times 10^{-6}$ M]. Maximal stimulation of SMG adenylate cyclase from reserpine treated animals was increased about 30–35% when compared to the control levels. The experiment was run three times and each point represents the mean of triplicate samples ± SEM

Table 1

	Number of [^{3}H] DHA bindings sites	K_d
Control	418 ± 66 f moles/mg protein	2.95×10^{-9}
Reserpine	1,136 ± 82 f moles/mg protein	1.75×10^{-9}

The number of β-adrenergic bindings sites was determined by assessing the binding of [^{3}H] dihydroalprenolol (DHA) to enriched plasma membrane fractions obtained from the submandibular glands from control and reserpine treated rats. Specific binding was defined as only that [^{3}H] DHA which could be displaced from the membrane by 10^{-5} M DL-propranolol. The number of binding sites and the dissociation constants (K_d) were determined by a computer assisted numerical curve fitting procedure (Hooke and Jeeves 1961) and by Scatchard analysis

β-Adrenergic Binding Sites. The number of β-adrenergic binding sites and the apparent dissociation constants (K_d) for SMG membranes from control and reserpine treated animals are shown in Table 1. The membranes from reserpine treated animals had approximately 2.5 fold more β-adrenergic binding sites than membranes from control glands. In a typical experiment there were approximately 418 f moles of [^{3}H]-dihydroalprenolol bound/mg protein of control membranes while SMG membranes from reserpine treated animals bound 1,136 f moles of [^{3}H]-dihydroalprenolol/mg protein. The dissociation constant for the control membranes was 2.95×10^{-9} while that of the membranes from reserpine treated animals was 1.75×10^{-9}. These small differences were not statistically significant.

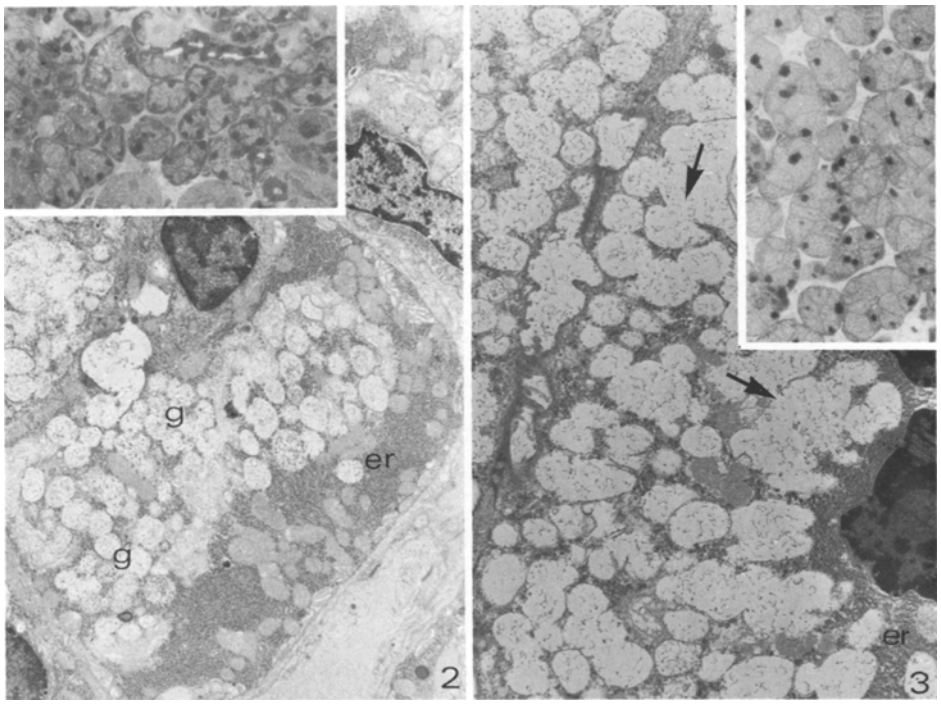


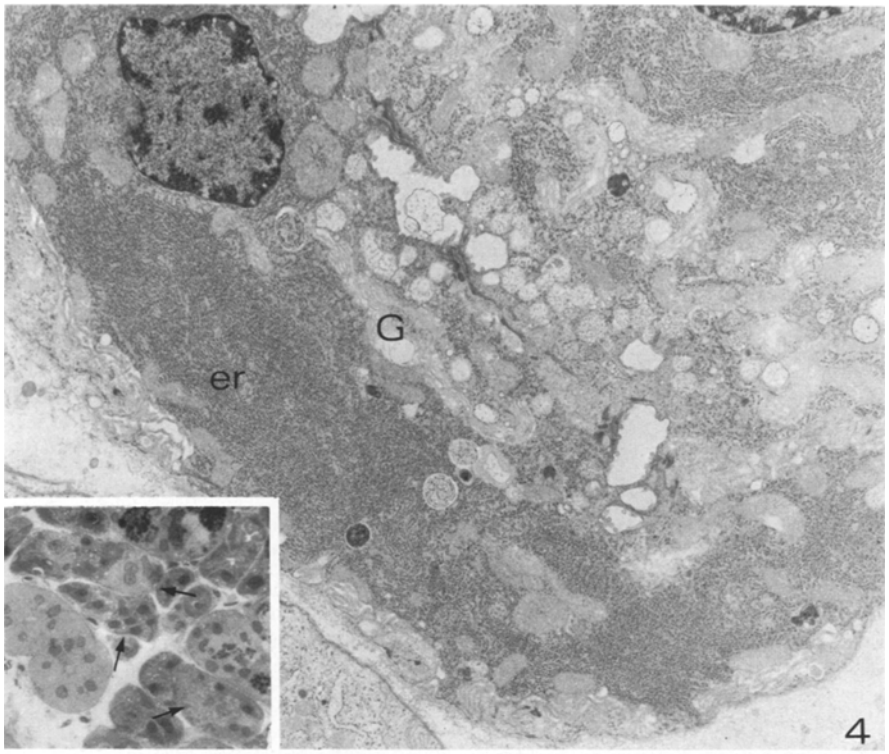
Fig. 2. Electron micrograph showing acinar cells from the SMG of a control animal (0 time). The typical secretory granules (*g*) and distribution of endoplasmic reticulum (*er*) can be seen (× 5,250). Inset: light micrograph showing typical SMG acinar structures (× 190)

Fig. 3. Electron micrograph showing acinar cells from the SMG of a reserpine treated animal (0 time). The secretory granules are coalesced (*arrow*) and their matrix seems dilute compared to the control granules. There is a reduced amount of endoplasmic reticulum (*er*) in the cells (× 8,000). Inset: light micrograph showing enlarged acini filled with secretory granules. Note the differences between these acini and those in Fig. 2 (× 190)

Morphologic Studies of Secretion and Resynthesis. The acinar cells of the SMG from reserpine treated rats were filled with large coalesced secretory granules and had little rough endoplasmic reticulum. The cytoplasm of the acinar cells thus looked foamy and the cells lacked substantial amounts of endoplasmic reticulum (e.r.). The comparison between glands from control and reserpine treated animals is illustrated in Figs. 2 and 3.

Fig. 4. Electron micrograph of SMG acinar cells from a control animal, 1 h after isoproterenol challenge. Only a few secretory granules (*g*) can be seen and the endoplasmic reticulum (*er*) and Golgi apparatus (*G*) are prominent. Exocytosis is about complete at this time (× 6,700). Inset: light micrograph showing collapsed acinar structures (arrows) which are devoid of secretory granules. The excretory ducts and secretory ducts appear prominent because of the collapsed acini (× 190)

Fig. 5. Electron micrograph of SMG acinar cells from a reserpine treated animal, 1 h after isoproterenol challenge. The cells are filled with fused secretory granules (*g*) which form pools of secretory material within the cells. The endoplasmic reticulum (*er*) is compressed to the base of the cells and has a swirled configuration. Very little secretion has taken place (× 5,250). Inset: light micrograph showing the vacuolated appearing cytoplasm of the acinar cells (× 190)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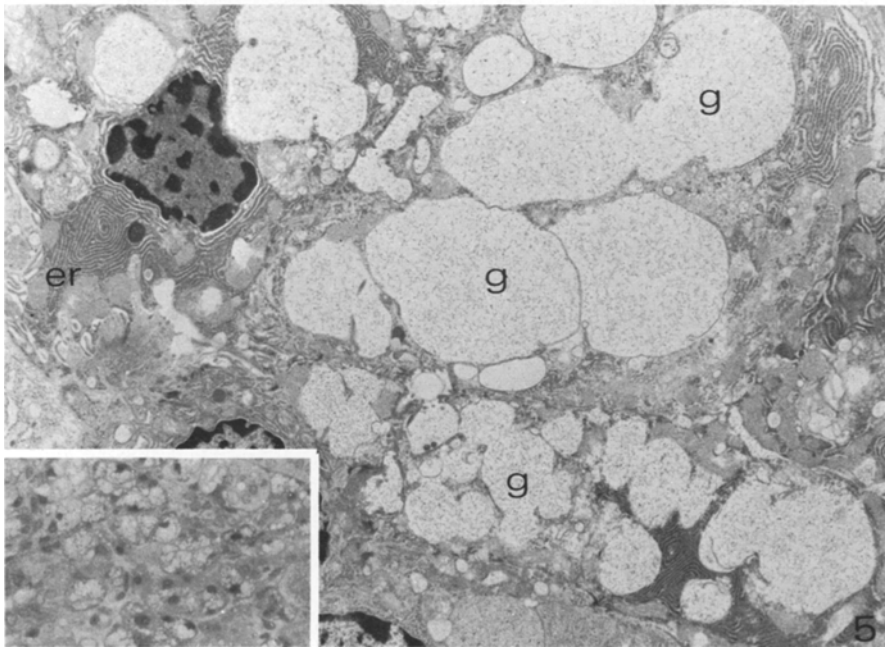
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One hour after challenge with isoproterenol (IPR) the glands from control animals were essentially devoid of acinar secretory material. The acinar cells were cuboidal in shape and their cytoplasm was filled with endoplasmic reticulum, a prominent Golgi apparatus, a few mitochondria and occasional lysosomes (Fig. 4). In contrast, the acinar cells from the glands of the reserpine treated animals appeared vacuolated and full of secretory material 1 h after IPR challenge. The cells were filled with large pools of secretory material which appeared to have been formed by the fusion of secretory granules. Swirls of rough e.r. were also visible in the cytoplasm as were occasional mitochondria and lysosomes (Fig. 5).

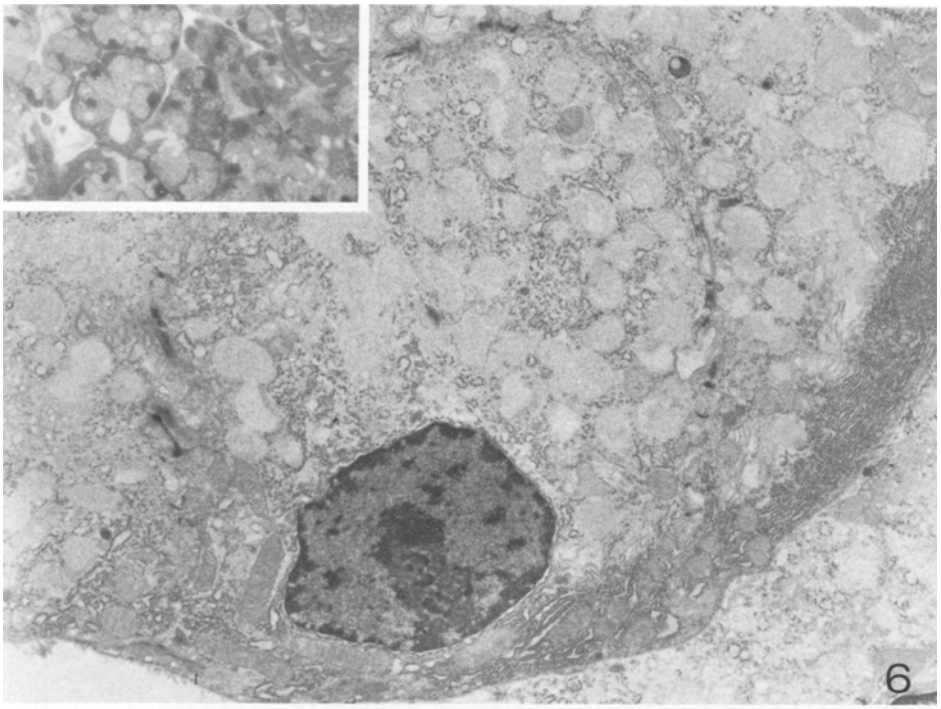
The process of resynthesis following IPR induced secretion in the normal rat salivary gland has been well documented (Lillie and Han 1974; Bogart 1975; Radley 1969; Takahama and Barka 1967). This process involves development of increased amounts of rough e.r. followed by the appearance of condensing vacuoles and secretory granules. This resynthesis process is generally complete by 8 h following IPR treatment. Figure 6 shows SMG tissue from control animals 8 h after IPR challenge. The acinar cells have restored their secretory material and look essentially the same as the normal cells seen in Fig. 1. The acinar cells from the SMG of the reserpine treated animals are filled with irregular arrays of moderately dilated rough e.r. and large, convoluted Golgi zones. Several condensing vacuoles and lipid droplets were also visible (Fig. 7).

Control tissue was not examined further as the resynthesis process was complete. At 12 h after IPR treatment in the cytoplasm of the acinar cells was somewhat more organized than it was at 8 h. Rough e.r. dominated the cytoplasm but there did not appear to be as many profiles of e.r. per unit volume of the cytoplasm as was observed in control cells at a comparable stage of resynthesis. The Golgi apparatus was still prominent and a few secretory granules were apparent. However, numerous condensing vacuoles, some lysosomes and lipid droplets were also seen in the cytoplasm (Fig. 8).

By 15 h after IPR treatment many of the acinar cells in the SMG of the reserpine treated animals had attained a morphology similar to that seen at the start of the experiment. However, many cells had not yet fully resynthesized their secretory material. The non synchronous nature of the resynthesis process was readily observed (Fig. 9). Eighteen hours after IPR injection the acinar structures from the SMG of reserpine treated animals were similar in morphology to the acini of the glands of the reserpine treated animals before IPR administration (Fig. 10).

Fig. 6. Electron micrograph of SMG acinar cells from a control animal, 8 h after isoproterenol challenge. The cells have resynthesized the secretory material and have restored their normal architecture. The cells look essentially as they did at 0 time (×5,250). Inset: light micrograph showing recovered acini (compare to Fig. 2) (×190)

Fig. 7. Electron micrograph of SMG acinar cells from a reserpine treated animal 8 h after isoproterenol challenge. The cytoplasm of the cells contain numerous strands of mildly dilated endoplasmic reticulum (*er*), large Golgi zones (*G*), many condensing vacuoles (*CV*), a few secretory granules (*g*) and an occasional lipid droplet (*li*) (×6,700)



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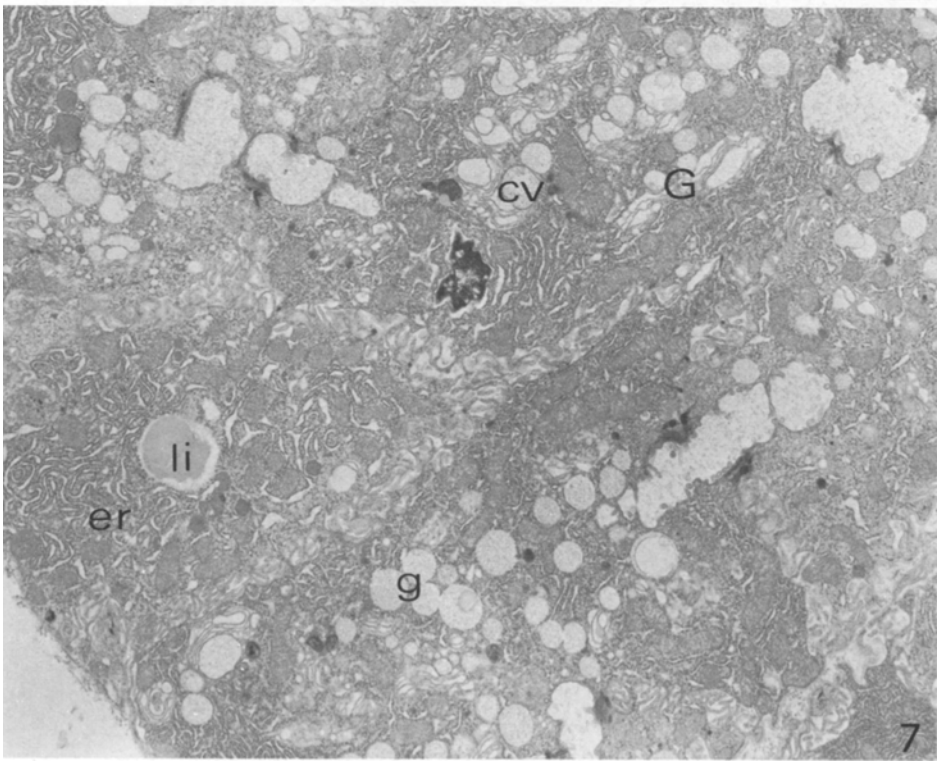

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Discussion

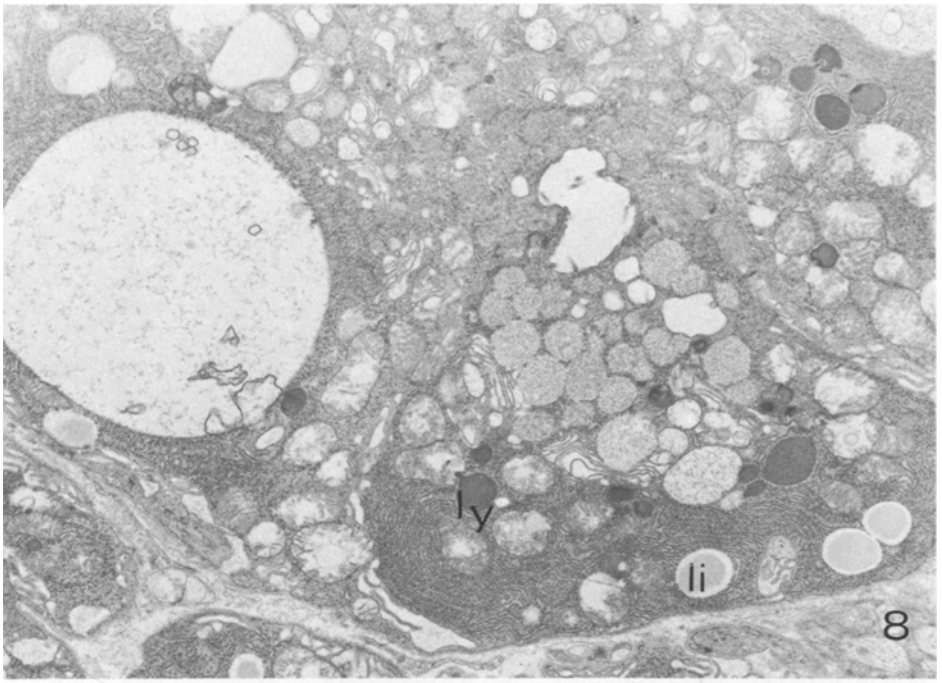
The results of this study indicate that chemical sympathectomy of the adult male rat by chronic administration reserpine leads to significant changes in the β-adrenergic regulated adenylate cyclase system within the submandibular gland. While SMG basal adenylate cyclase activity was not altered by chronic reserpine treatment the sensitivity of the enzyme to isoproterenol stimulation was increased 50 fold by such treatment. Further maximal levels of cyclase activation were about 30% higher in membranes from reserpine treated glands as compared to control membranes. This increased sensitivity of the membrane associated adenylate cyclase was correlated with an significant increase in the number of cell surface β-adrenergic binding sites. There were no significant changes in the affinity of the receptors for β-adrenergic agonists noted. Thus, the super-sensitivity of the adenylate cyclase system observed in membranes from glands from reserpine treated animals appear to be due to the increased number of β-adrenergic receptors being able to interact with the more of the available adenylate cyclase.

The results of the current studies on the number of β-adrenergic receptors are consistent with a recent report by Arnett and Davis (1979) on changes in β-adrenergic receptors in the denervated rat submandibular gland. These investigators found that the number of β-adrenergic receptors rose from about 370 f moles of [^{3}H]-dihydroalprenolol bound/mg of membrane protein in control glands to about 830 f moles bound/mg of membrane protein in denervated submandibular gland. The compatibility of this study with the current data in regard to the number of receptors and the magnitude of the increase is consistent with the finding of Benmiloud and Euler (1963) that reserpine treatment was as effective as denervation in reducing the catacholamine content of the rat SMG. The results reported here are also consistent with, but more dramatic than, the data reported by Pointon and Banerjee (1979). These investigators found only a modest elevation of β-adrenergic receptors (about 35%) in the SMG following chemical sympathectomy with reserpine or 6-hydroxydopamine. These investigators also found substantially fewer receptors in the SMG than reported here or by Arnett and Davis (1979). These differences can be attributed to the purity of the particulate fractions evaluated. Pointon and Banerjee (1979) evaluated a crude particulate fraction while in this study and that of Arnett and Davis a more highly enriched plasma membrane fraction was used.

In all of these studies it was found that sympathectomy (chemical or surgical) lead to an increased number of β-adrenergic binding sites with virtually no

Fig. 8. Electron micrograph of SMG acinar cells from a reserpine-treated animal 12 h after isoproterenol challenge. Some of the cells are beginning to show signs of recovery and resynthesis of secretory material while other cells show large pools of material in their cytoplasm. Lipid droplets (*li*) and lysosomes (*ly*) are seen in most cells (×6,700)

Fig. 9. Electron micrograph of SMG acinar cells from a reserpine treated animal 15 h after isoproterenol challenge. Many acinar cells have recovered from the hormonal stimulus while other cells still are in the process of recovery. This observation emphasizes the asynchronous nature of the recovery process (×6,700)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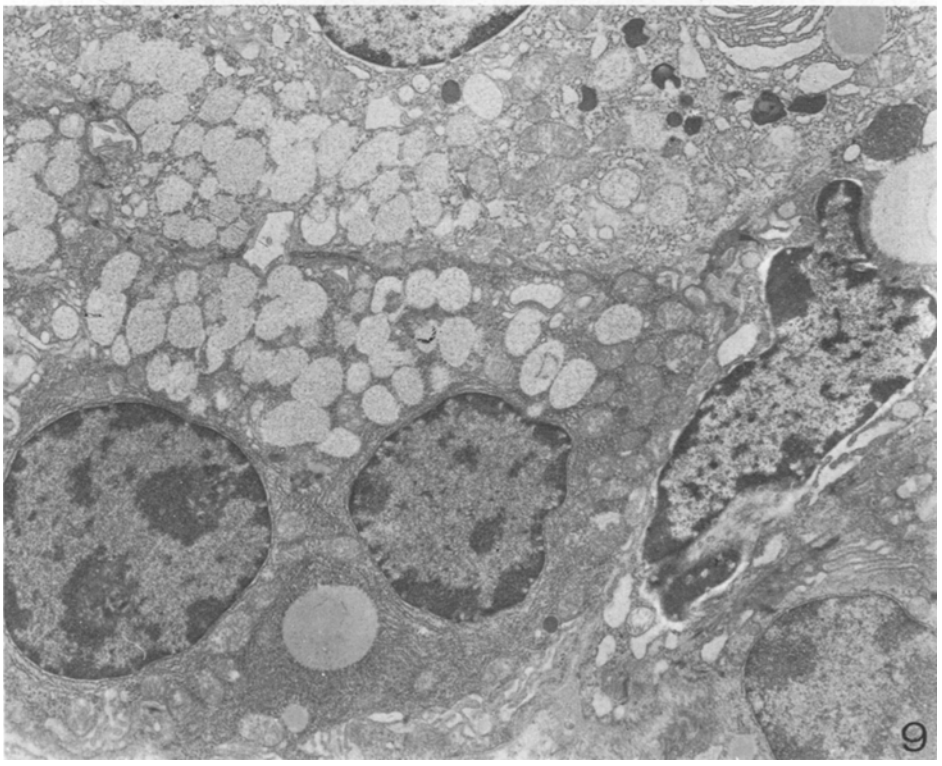
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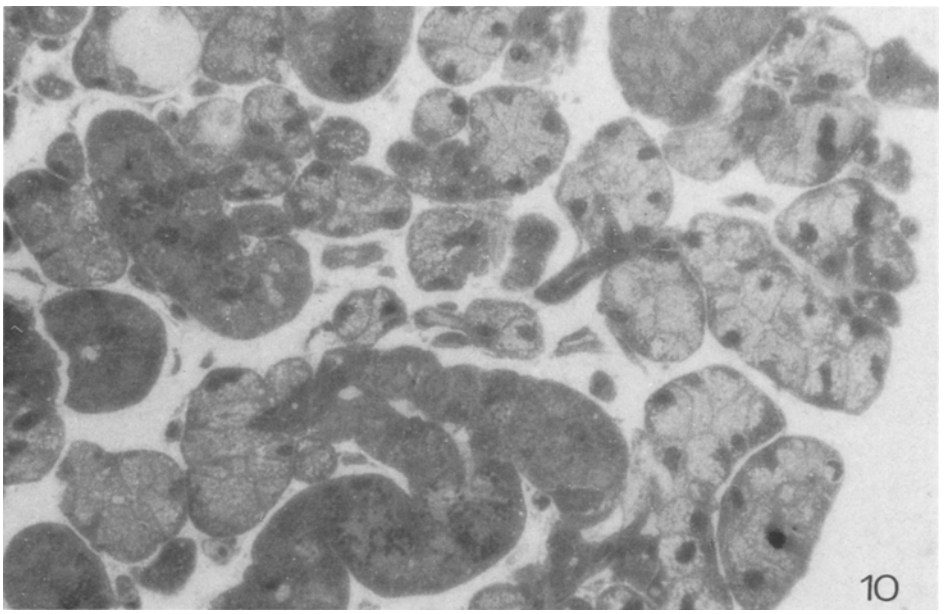

Fig. 10. Light micrograph of SMG from reserpine treated animal 18 h after isoproterenol challenge shows glandular tissue now appears similar to 0 time SMG from reserpine treated animal. The acini are filled with secretory granules (compare to Fig. 3) (×300)

significant change in the apparent affinity constants. All of the studies reported dissociation constants that are consistent with each other.

The results of the morphologic study clearly indicate that both the secretion and resynthesis phases of the isoproterenol induced secretory cycle in the rat SMG were delayed in reserpine treated animals as compared to secretion from glands from control animals. The delay in the secretion phase of the cycle is in agreement with the report by Simson and coworkers (1978). These observed delays in release and restoration of secretory product are not consistent with current theories on the regulation of secretion and resynthesis of exportable proteins by salivary glands or with the data on the β-adrenergic receptor/adenylate cyclase system reported earlier in this study.

Protein secretion from the SMG acinar cells is mediated by the interaction of β-adrenergic agonists with specific receptors at the secretory cell surface. The hormone-receptor interaction leads to stimulation of cell surface associated adenylate cyclase which catalyzes the hydrolysis of ATP to cyclic AMP and pyrophosphate. Cyclic AMP, in turn, initiates a cascade of intracellular events which culminate in the exocytosis and resynthesis of secretory material. In the reserpine treated (chemically sympathectomized) rat the plasma membrane of the SMG acinar cells has an increased number of β-adrenergic receptors and demonstrates a supersensitivity of the adenylate cyclase system. Therefore, an accelerated secretion and resynthesis cycle would be anticipated since, in general, supersensitivity of such systems is correlated with an exaggerated physiologic response. Quite the contrary situation has been observed in this study. It is possible that the extremely highly levels of cyclic AMP generated because

of the increased sensitivity of adenylate cyclase are in some way inhibitory to secretion and resynthesis. Since cyclic AMP appears to be a second messenger for a variety of hormones in various cells it is possible that each hormone signal is rendered specific by the actual level of intracellular cyclic AMP. A large change in the amount of signal molecule could cause the observed aberrations in secretion and recovery. Martinez has measured cyclic AMP in the SMG following IPR stimulation and found cAMP levels substantially increased in the SMG of reserpine treated animals as compared to control glands (personal communication). A similar observation with regard to cyclic AMP accumulation in SMG cells from surgically denervated glands has been reported by Arnett and Davis (1979).

The dose of reserpine used in these studies is quite high. It is possible that at such doses reserpine may have a direct effect on the acinar cells which is independent of the drugs' adrenergic blocking properties. It has been shown that many of the long term effects of reserpine on monamine containing neurons are due, to a large extent, to an irreversible binding of small amounts of the drug to specific sites on amine storage granules (Giachetti and Shore 1978). This bound reserpine apparently affects the granule membrane associated Mg^{2+} ATP uptake system thus blocking certain membrane fusion steps. It is possible that non-specific reserpine binding occurs on SMG granule or other membranes and in some analogous manner retards membrane fusion steps important in both secretion, resynthesis and packaging of secretory material.

In summary, chronic reserpine treatment of the adult male rat leads to a supersensitivity of SMG cell surface associated adenylate cyclase. This supersensitivity is parallelled by a significant rise in the number of plasma membrane associated β-adrenergic receptors. These changes in the β-adrenergic receptor-adenylate cyclase system do not lead to the predicted acceleration of secretion and resynthesis but rather are accompanied by an incongruous delay in these processes. The mechanism underlying this dichotomy between the physiologic response and the changes at the cell surface may be important in evaluating the similarities to cystic fibrosis observed in the submandibular glands of reserpine treated rats.

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