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ANALYSIS OF PROTEIN SYNTHESIS IN RAT SALIVARY GLANDS AFTER CHRONIC TREATMENT WITH β-RECEPTOR AGONISTS AND PHOSPHODIESTERASE INHIBITORS

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Abstract—Chronic administration of the β-adrenergic receptor agonist isoproterenol (5 mg/200 g animal for 10 days) resulted in rat parotid and submandibular gland hypertrophy, and it induced synthesis of a series of proline-rich proteins (PRPs) and glycoproteins. Treated parotid glands additionally exhibit an increase in activity for the Golgi membrane enzyme UDP-galactose; *N*-acetylglucosamine 4β-galactosyltransferase. A series of β-receptor agonists and phosphodiesterase inhibitors were examined for their abilities to influence salivary gland protein biosynthesis in a fashion similar to that observed with chronic isoproterenol treatment. β_1/β_2-Adrenergic-receptor agonists exhibited the greatest effects on parotid gland hypertrophy and PRP biosynthesis. These β-agonists were also able to increase 4β-galactosyltransferase activity, but they did not promote the synthesis of a 220,000 dalton glycoprotein. Terbutaline (β_2-receptor agonist) induced parotid gland hypertophy but was only able to induce protein biosynthesis at higher drug concentrations. Finally, methoxyphenamine was unable to produce the observed changes in protein synthesis even at increased drug dosages. The phosphodiesterase inhibitors (theophylline and caffeine) were able to induce *de novo* PRP biosynthesis at drug doses of 20 mg/200 g animal. However, while causing mild gland hypertrophy, there was no observable change in 4β-galactosyltransferase activity with either phosphodiesterase inhibitor. This same regimen of β-receptor agonists was unable to induce submandibular gland hypertrophy, PRP or glycoprotein biosynthesis in the same animals. This was also true for the two phosphodiesterase inhibitors. Co-injection of a β_1 antagonist along with isoproterenol blocked the above protein changes in both the submandibular and parotid glands, suggesting that the stimulation of protein synthesis takes place by β_1-type receptors on the gland cell surfaces.

Administration of the catecholamine isoproterenol (ISO)‡ causes a variety of morphological and cytochemical changes in the parotid and submandibular glands in rats. Initially, ISO (β_1/β_2-adrenergic-receptor agonist) causes glandular secretion through interaction with the acinar cell surface β-adrenergic receptors which results in an increase in the levels of cyclic AMP [1–3]. Single injections of ISO result in increased DNA, RNA and protein synthesis [4]. Continued treatment of rats with ISO results in hypertrophy and hyperplasia of both the parotid and submandibular glands [5–7]. Morphological studies on enlarged glands suggest that alterations occur in the content of acinar cell secretory granules after prolonged treatment [8].

Biochemical analysis of the 100,000 *g* supernatant fraction of cells from ISO-treated parotid and submandibular glands has revealed the induced synthesis of a new series of proteins. These proteins, which contain 25–40% proline and high amounts of glycine and glutamic acid, are soluble in 10% trichloroacetic acid [9–11]. The parotid gland normally produces a single glycoprotein of 200,000 daltons, but upon chronic injection of ISO a second glycoprotein of 220,000 daltons is induced [12]. The submandibular gland normally produces two glycoproteins of 180,000 and 200,000 daltons [13, 14]. After chronic treatment with ISO, the submandibular gland produces a new glycoprotein of 158,000 daltons [11]. The parotid gland additionally shows a 6 to 10-fold increase in the Golgi enzyme marker UDP-galactose: *N*-acetylglucosamine 4β-galactosyltransferase (EC 2.4.1.22) [14].

In the course of our investigations of the effects of ISO on the salivary gland system in rats, it was observed that reductions in drug concentration resulted in the differential stimulation of these morphological and biochemical changes between the parotid and submandibular gland. At concentrations of 1 mg/200 g animal, the parotid gland exhibits hypertrophy and induction of the biochemical alterations listed above [12]. However, the submandibular gland is unable to respond to stimulation at this concentration as detailed by the *de novo* biosynthesis of PRPs, and the 158,000 dalton glycoprotein [12]. The same pattern of parotid gland

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‡ Abbreviations: ISO, isoproterenol; PRPs, proline-rich proteins; TCA, trichloroacetic acid; SDS, sodium dodecyl sulfate; MES, 2-[*N*-morpholine]ethanesulfonic acid; cAMP, 3′,5′-cyclic monophosphate; and UDPgalactose:2-acetamido-2-deoxy-D-glucosamine 4β-galactosyltransferase, UDPgalactose:*N*-acetylglucosamine 4β-galactosyltransferase or 4β-galactosyltransferase.

response and submandibular gland non-response has also been observed in ISO-treated neonatal rats [12].

In the present paper, we have employed a series of β_1/β_2 and β_2 adrenergic-receptor agonists, as well as phosphodiesterase inhibitors, to further examine changes in protein biosynthesis similar to those detected with chronic ISO treatment. Previous studies have shown other β-agonists to cause salivary gland hypertrophy and increase secretion of amylase and Ca^{+2} into saliva. Similar ISO drug concentrations of other β_1/β_2 receptor agonists (5 mg/200 g animal) and the phosphodiesterase inhibitors were able to induce synthesis of the PRPs to varying degrees, although terbutaline, a β_2 specific agonist, had no effect on parotid gland protein biosynthesis. We were unable to detect alterations in protein synthesis in the submandibular gland of the same animal with the β-receptor agonists other than ISO or phosphodiesterase inhibitors. This was contrary to the results obtained with high doses of the β_1/β_2 agonist isoproterenol, albeit similar to the response observed in ISO-treated neonatal rats or adult animals given low drug doses. These results suggest that substantial differences exist for the cell surface β-receptors between the two glands or that the effects of ISO on the submandibular gland require the participation of other factors *in vivo* (such as hormones) which may influence cAMP levels and subsequent gland response to chronic treatment.

MATERIALS AND METHODS

Chemicals. *d,l*-Isoproterenol, trichloroacetic acid, theophylline and caffeine were purchased from the Sigma Chemical Co. All reagents for polyacrylamide gel electrophoresis were of ultrapure quality and were obtained from Bio-Rad. UDP-[1-^{14}C]galactose (sp. act. 300 Ci/mmole) and [^{35}S]methionine (sp. act. 800 Ci/mmole) were from Amersham. Reticulocyte lysates for *in vitro* translation were obtained from BRL. All other chemicals were of analytical quality and obtained through commercial sources. Terbutaline sulfate and protokylol were gifts from Dr. W. J. Hudak of Merrell Dow Pharmaceuticals. Methoxyphenamine hydrochloride was obtained from Dr. P. W. O'Connell of the Upjohn Co., and metaproterenol sulfate was gift from Dr. S. Traudt of Dorsey Laboratories. Butoxamine and atenolol were gifts from Dr. C. Schneyer, Department of Physiology, University of Alabama at Birmingham.

Drug treatment. Male Wistar rats weighing 200 g received intraperitoneal injections of 5 mg of the various β-receptor agonists or phosphodiesterase inhibitors for 10 days and were fed *ad lib.* unless stated otherwise in the results.

Tissue preparation. Parotid and submandibular glands were removed from animals after they were anesthetized with sodium pentobarbital and killed by exsanguination. The soluble and insoluble membrane fractions were prepared by homogenization in 10 mM Tris/HCl buffer, pH 8.0, with a Dounce apparatus. The slurry was then centrifuged at 100,000 *g* for 1 hr. Protein assays were performed by a modification of the Lowry method, with bovine serum albumin as a standard [15]. For the purification of the salivary gland glycoproteins and PRPs, the soluble fraction was diluted with an equal volume of 20% TCA. The PRPs and parotid glycoproteins remain soluble in 10% TCA [1, 2]. The insoluble proteins were removed by centrifugation at 15,000 *g* for 15 min, and TCA was removed by dialysis against distilled water, followed by lyophilization, instead of diethyl ether extraction [16].

Polyacrylamide gel electrophoresis and protein staining. Protein samples were subjected to electrophoresis in 10% polyacrylamide gel using a modification of Laemmli's Tris/glycine system [17]. All gels were fixed and stained by a modification of the method of Fairbanks *et al.* [18]. Briefly, the staining of PRPs was shortened to 30 min in Fairbanks solution containing 10% acetic acid and 0.1% Coomassie brilliant blue R-250 followed by destaining over 24 hr in 10% acetic acid. Samples for gels were made up to 1 mg protein/ml of sample buffer. Thirty-five micrograms of protein per well was routinely used in subsequent gel electrophoresis.

Galactosyltransferase assay. The activity of UDP-galactose: *N*-acetylglucosamine 4β-galactosyltransferase was measured by the method of Carlson *et al.* [19] using 10 mM *N*-acetylglucosamine or 100 μg ovalbumin (Sigma) as an acceptor followed by high voltage paper electrophoresis as described previously or TCA precipitation onto glass fiber filters for ovalbumin [14].

Isolation of poly(A$^+$) RNA and in vitro *translation.* Total gland RNA was isolated by the method of Chirgwin *et al.* [20]. Immediately upon removal, 1 g of fresh tissue was homogenized in 4 M guanidine thiocyanate, pH 7.0, containing 1.0% sodium sarkosyl, 50 mM EDTA, 25 mM sodium citrate. Total RNA was subsequently recovered by an 18-hr centrifugation through 5.7 M $CsCl_2$ at 35,000 rpm in an SW 27 rotor. Poly(A$^+$) RNA was obtained from total RNA using the procedure of Aviv and Leder [21] on oligo dT cellulose. This RNA was subsequently translated *in vitro* using the method of Pelham and Jackson [22] with a reticulocyte lysate (nuclease treated) prepared by the Bethesda Research Laboratories. Reaction volumes used were 30 μl, containing 0.5 μg normal, β-agonist-treated or phosphodiesterase-treated parotid or submandibular RNA. Reaction mixtures were incubated for 90 min at 30°. Total incorporation of [^{35}S]methionine into protein was determined by 10% TCA precipitation on glass filters of 5 μl from *in vitro* translations. After termination of the reaction, samples were acetone precipitated and resuspended in SDS polyacrylamide gel sample buffer and boiled for 5 min before electrophoresis. Gels were prepared for X-ray fluorography by treating fixed and stained gels with En ^{3}Hance (Amersham) and drying of filter backing paper. Dried gels were exposed to Kodak X-Omat XRP film at −80°.

Estimation of corticosteroids in rat plasma. Serum was obtained from control and isoproterenol, metaproterenol- or methoxyphenamine-treated (10 days) animals in order to assay levels of free and protein bound cortisol and corticosterone [23]. After extraction with spectral grade methylene chloride, fluorescence of samples was obtained using a Gilson Spectral-glo Fluorometer with a 530 nm emitted wavelength setting following excitation at 470 nm.

The standard employed was hydrocortisone, purchased from the Sigma Chemical Co.

Measurement of adenosine 3′:5′ cyclic monophosphate levels. Analysis of cyclic AMP levels was performed by the method of Gilman [24] using a kit assay system obtained from Boehringer Mannheim. The assay was performed on fresh gland preparations prepared 10 min after drug treatment as described by Grand and Schay [3].

RESULTS

Observation of gland hypertrophy. Rats treated with ISO at a concentration of 5 mg/200 g animal for 10 days showed marked salivary gland hypertrophy (Table 1). The parotid gland had undergone a 7- to 8-fold increase in size, while the submandibular gland response was on the order of 3- to 4-fold increase in wet weight over control animals. Comparison of the changes in the parotid gland induced with ISO treatment with those observed with a series of other β adrenergic-receptor agonists revealed a varying degree to which these compounds affected gland hypertrophy. Under conditions of drug doses of 5 mg/200 g animal, gland hypertrophy was greatest after treatment with protokylol (β_1/β_2 adrenergic-receptor agonist) followed by metaproterenol ($\beta_2 > \beta_1$-agonist). A 2-fold increase in wet weight was obtained with terbutaline (β_2-agonist) and methoxyphenamine (β_1/β_2-agonist). Increasing the drug dose to 15 mg resulted in a 3-fold increase in parotid weight with terbutaline, but a decrease in hypertrophy was observed with methoxyphenamine compared with 5 mg doses (Table 1). Animals treated with phosphodiesterase inhibitors at concentrations between 5 and 20 mg/day obtained a 2- to 3-fold increase in wet weight.

An analysis of gland hypertrophy in the submandibular glands from the same animals revealed at most a 1- to 2-fold increase in wet weight with injection of β-receptor agonists other than ISO. The greatest increase was again observed with protokylol and metaproterenol. A minimal amount of hypertrophy was obtained with the phosphodiesterase inhibitors (Table 1). Since it is assumed that the effects of ISO and, therefore, other β-receptor agonists exert their influence on the cell by the stimulation of adenylate cyclase and subsequently increasing cAMP levels, we attempted to use a combination of β-receptor agonist and phosphodiesterase inhibitors together to stimulate submandibular gland hypertrophy. Using theophylline in combination with methoxyphenamine or metaproterenol, there was no observation of a synergistic effect on submandibular gland weight. This was also true for parotid gland hypertrophy under the same experimental conditions.

Biosynthesis of the proline-rich proteins and glycoproteins. It has been well established that ISO causes the synthesis of a new series of proteins in both the parotid and submandibular glands [9, 10, 12, 14]. After isolation of the 100,000 *g* cell supernatant fraction, this material was subsequently extracted with 10% TCA to isolate the proline-rich proteins. After treatment of animals with 5 mg ISO for 10 days, a series of proteins was isolated with molecular weights ranging from 17,000 to 35,000 daltons (Fig. 1A, lane 3). There was also the synthesis of a new glycoprotein of 220,000 daltons in addition to a 200,000 dalton glycoprotein present in the control parotid (Fig. 1A, lane 2). When the parotid gland was analyzed for the induction of PRP biosynthesis, it was evident that the appearance of these protein changes paralleled the degree of gland hypertrophy. Protokylol and metaproterenol showed the greatest degree of induction of these proteins (Fig. 1B, lanes 4 and 6). Terbutaline was intermediate in its effects on PRP synthesis in that it would appear from the gels that two of the PRPs were induced (Fig. 1B, lane 5). Methoxyphenamine showed no PRP induction

Table 1. Rat parotid and submandibular gland hypertrophy in response to chronic treatment with β-receptor agonists and phosphodiesterase inhibitors

Treatment	Wet wt (g) Parotid	Submandibular
Control	0.31 ± 0.05	0.42 ± 0.09
ISO (5 mg)*	2.33 ± 0.25	1.42 ± 0.15
Caffeine (5)	0.81 ± 0.18	0.73 ± 0.17
Caffeine (10)	0.65 ± 0.17	0.67 ± 0.12
Caffeine (20)	0.79 ± 0.09	0.88 ± 0.11
Theophylline (5)	0.84 ± 0.11	0.74 ± 0.13
Theophylline (10)	0.92 ± 0.11	0.81 ± 0.23
Methoxyphenamine (5)	0.69 ± 0.09	0.63 ± 0.11
Methoxyphenamine (15)	0.55 ± 0.07	0.56 ± 0.03
Methoxyphenamine/Theophylline (5/5)	0.95 ± 0.11	0.84 ± 0.15
Protokylol (2)	1.69 ± 0.10	0.52 ± 0.18
Protokylol (5)	2.12 ± 0.03	0.96 ± 0.13
Terbutaline (5)	0.67 ± 0.40	0.72 ± 0.13
Terbutaline (15)	1.01 ± 0.08	0.62 ± 0.14
Metaproterenol (5)	1.96 ± 0.15	1.06 ± 0.14
Metaproterenol/Theophylline (5/5)	2.02 ± 0.18	0.98 ± 0.09

Each value is the average of four animals for the parotid and submandibular gland.

* Drug dose per 200 g weight of rat. Daily injections were carried out for 10 days.

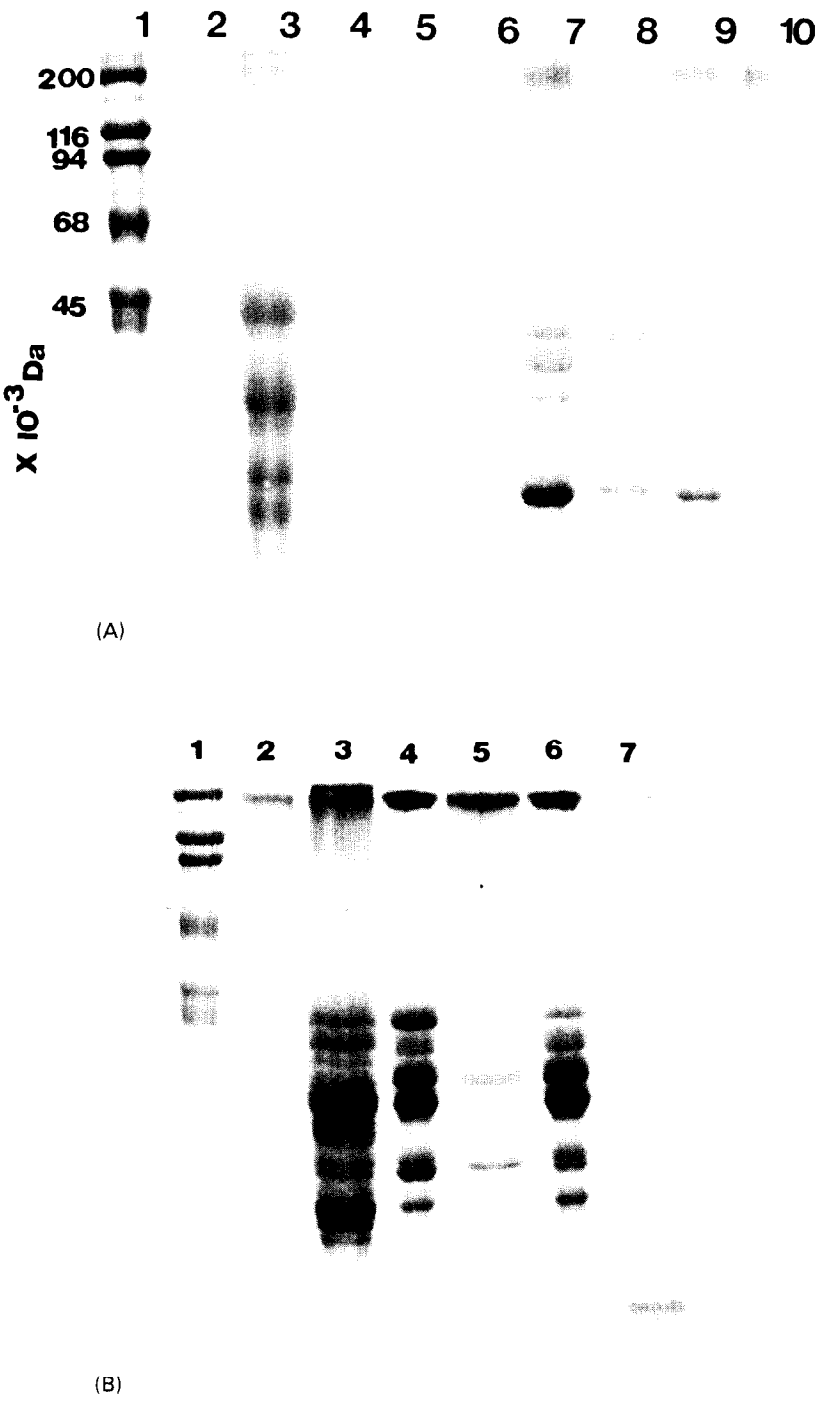

Fig. 1. 10% Polyacrylamide gel of TCA-soluble proline-rich proteins from treated parotid glands. (A) Lane 1, molecular weight standards: myosin, 200,000 daltons; β-galactosidase, 116,000; phosphorylase B, 94,000; bovine serum albumin, 68,000; and ovalbumin, 45,000; Lane 2, control TCA-soluble parotid gland; Lane 3, 5 mg ISO parotid gland; Lane 4, 5 mg methoxyphenamine parotid, Lane 5, 5 mg caffeine; Lane 6, 10 mg caffeine; Lane 7, 20 mg caffeine; Lane 8, 5 mg theophylline; Lane 9, 10 mg theophylline; Lane 10, 5 mg methoxyphenamine, 5 mg theophylline. (B) Lane 1, molecular weight standards; Lane 2, control parotid; Lane 3, 5 mg ISO; Lane 4, 5 mg protokylol; Lane 5, 5 mg terbutaline; Lane 6, 5 mg metaproterenol; and Lane 7, 15 mg methoxyphenamine. All wells were electrophoresed with 35 μg of protein in SDS gel sample buffer.

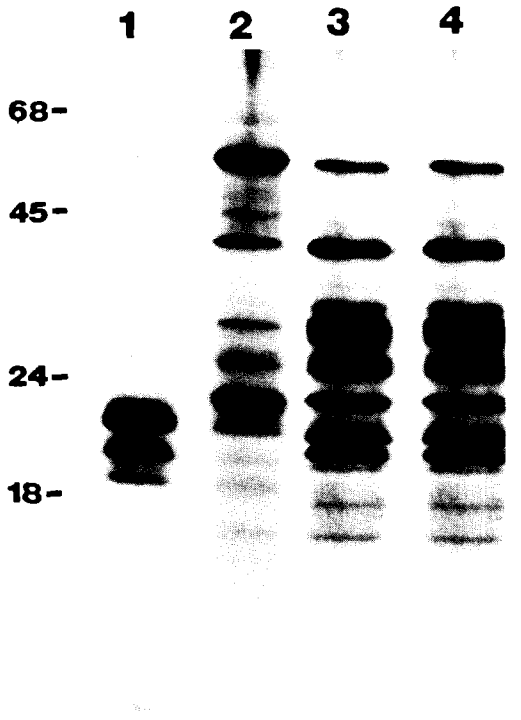

Fig. 2. X-ray fluorogram of *in vitro* translations from parotid gland poly(A^+) RNA. Lane 1, no added RNA; Lane 2, control parotid RNA; Lane 3, ISO-treated parotid RNA; and Lane 4, 10 mg theophylline-treated parotid RNA. *In vitro* translations were labeled with [^{35}S]methionine. Samples contained 30,000 cpm per well of total *in vitro* translated RNA.

detectable by SDS gel electrophoresis, while a higher drug dosage only served to increase the background protein contamination (Fig. 1A, lane 4; Fig. 1B, lane 7). At lower doses of terbutaline the two major bands induced in Fig. 1B (lane 5) were not apparent. Therefore, the high doses likely result in non-specific interaction with β_1 receptors on the cell surface to cause some protein synthesis. Larger drug doses had no effect on further induction for any of the drugs used as evidenced by the gel profile of TCA-soluble proteins isolated from the parotid gland treated with methoxyphenamine at a concentration of 15 mg/200 g animal (Fig. 1B, lane 7). In no case other than with ISO was the 220,000 dalton glycoprotein present.

Using caffeine and theophylline, PRP biosynthesis can be elicited through raising the intracellular concentration of cAMP by preventing the destruction of the compound by the enzyme cAMP phosphodiesterase. This was accomplished, however, only at concentrations of 20 mg for caffeine and 10 mg for theophylline (Fig. 1A, lanes 7 and 9). The differences in protein band intensities appear to be due to differences in *in vivo* translational and post-translational processing since isolation of the RNA and subsequent *in vitro* translation show the same level of mRNA for the proteins to be present from ISO-treated parotid glands and those receiving 10 mg doses of the phosphodiesterase inhibitor theophylline (Fig. 2). It is not known if ISO or other treatments alter the levels of proteolytic enzymes involved in processing of the PRPs. The same results were obtained for translations of RNA isolated from β_1/β_2-agonist-treated glands (data not shown). Translation of mRNA isolated from terbutaline- and methoxyphenamine-treated animals did not show induction of PRP mRNAs but increased levels of the normally present PRPs.

Examination of the submandibular gland from the same animals showed that the biochemical changes in the soluble fraction from cell lysates were similar to the results obtained for gland hypertrophy. Although the ISO-treated submandibular gland showed the induced synthesis of PRP when examined by SDS-polyacrylamide gel electrophoresis, the treatment of these glands with the other β-receptor agonists had no effect on *de novo* protein synthesis (Fig. 3). This was also in evidence when RNA was isolated from treated submandibular glands and translated *in vitro* to show that there was no induction of mRNA specific for PRPs (data not shown). The protein patterns that appear in Fig. 3 for the submandibular gland treated with other β-agonists were similar to that found for the untreated control, (lane 2), although there are differences in band intensity on the stained gel. This may represent changes in *in vivo* translation rates of specific mRNA species. The same results were obtained for the appearance of the ISO-induced submandibular gland glycoprotein. The submandibular gland produced two glycoproteins of 180,000 and 200,000 daltons, but with ISO treatment a new glycoprotein was present at 158,000 daltons. Treatment with the other β-adrenergic receptor agonists and phosphodiesterase inhibitors failed to induce the 158,000 dalton glycoprotein.

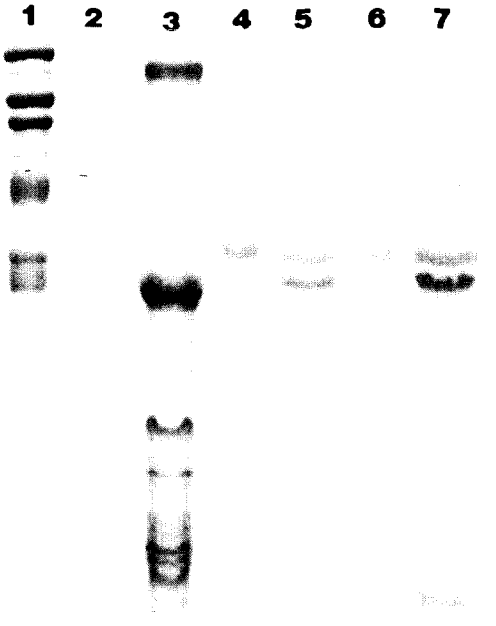

Fig. 3. 10% Polyacrylamide gel of TCA-soluble proline-rich proteins from treated submandibular glands. Lane 1, molecular weight standards; Lane 2, control TCA-soluble submandibular gland extract; Lane 3, 5 mg ISO-treated; Lane 4, 5 mg protokylol; Lane 5, 5 mg terbutaline; Lane 6, 5 mg metaproterenol; and Lane 7, 15 mg methoxyphenamine.

Table 2. Analysis of 4β-galactosyltransferase activity in parotid gland membranes

Treatment	Specific activity	
	Acceptor	
	10 mM GlcNAc*	Ovalbumin†
Control	0.28 ± 0.04	21.11 ± 0.32
ISO (5 mg)‡	1.68 ± 0.10	136.17 ± 1.12
Caffeine (5)	0.21 ± 0.02	30.10 ± 0.93
Caffeine (10)	0.27 ± 0.02	30.03 ± 1.03
Caffeine (20)	0.31 ± 0.03	26.54 ± 0.55
Theophylline (5)	0.34 ± 0.01	27.17 ± 1.32
Theophylline (10)	0.30 ± 0.01	26.44 ± 0.78
Methoxyphenamine (5 mg)	0.29 ± 0.03	64.34 ± 1.40
Methoxyphenamine/Theophylline (5/5)	0.57 ± 0.15	79.42 ± 1.25
Protokylol (5)	1.46 ± 0.27	127.00 ± 2.03
Terbutaline (5)	0.31 ± 0.05	20.31 ± 0.57
Metaproterenol (5)	1.83 ± 0.25	125.57 ± 1.42

Each value is the average of three experimental determinations.
* Values are expressed as specific activity (nmoles galactose/min/mg protein).
† One hundred milligrams per reaction of substrate; activity is expressed as nmoles/hr.
‡ Drug dose per 200 g animal for a 10-day injection regimen.

Activity of the 4β-galactosyltransferase. The final biochemical change induced by ISO treatment of parotid glands was an observed increase in the Golgi enzyme UDP-galactose:*N*-acetylglucosamine 4β-galactosyltransferase [15]. As shown in Table 2, the β-receptor agonists had differing effects on the ability to induce 4β-galactosyltransferase activity. This ability to affect expression followed the same time kinetics as ISO inducation (data not shown). The response was again strongest when protokylol or metaproterenol was used. Methoxyphenamine, terbutaline and the phosphodiesterase inhibitors were unable to cause an increase in 4β-galactosyltransferase activity.

β-Adrenergic receptor stimulation of cAMP levels. The level of cAMP was measured in the parotid and submandibular glands of animals treated with a single dose of the β-adrenergic agonists or phosphodiesterase inhibitors. The glands were removed 10 min after β-agonist stimulation since it has been shown previously that by this time cAMP accumulation is maximum for the salivary glands [1, 3]. At the same time Mehancho and Carlson [11] have shown that a single injection of ISO is sufficient to induce PRP synthesis in parotid and submandibular glands. As shown in Table 3, the β_1/β_2 receptor agonists (i.e. ISO and protokylol) stimulated intracellular concentrations of cAMP greater than 10-fold over control glands. Metaproterenol, which has a preference for β_2 receptors over β_1, was also able to drastically increase cAMP in parotid glands. At lower drug concentrations (0.5 mg) there was no accumulation of cAMP observed (data not shown). Terbutaline, a β_2-adrenergic receptor agonist, was unable to stimulate adenylate cyclase as was the case with methoxyphenamine. The use of phosphodiesterase inhibitors resulted in increased levels of cAMP with the highest dose of caffeine shown in concentrations comparable with those obtained with ISO treatment (Table 3).

In the case of submandibular gland levels of cAMP, isoproterenol caused the greatest change in concentration. The β_1/β_2 receptor agonist protokylol produced the largest increase of the other β-agonists used. β_2-Adrenergic-receptor agonists metaproterenol ($\beta_2 > \beta_1$) and terbutaline were unable to increase cAMP concentration in these glands. The phosphodiesterase inhibitor caffeine produced cAMP levels comparable with those of ISO for the submandibular gland (Table 3). However, caffeine,

Table 3. *In vivo* levels of cAMP in rat salivary glands

Treatment	cAMP (pmoles/mg protein)	
	Parotid†	Submandibular
Control	3.3 ± 0.1	2.8 ± 0.7
ISO (5 mg)	41.2 ± 1.2	17.1 ± 3.1
Caffeine (20 mg)	48.8 ± 0.9	14.2 ± 2.6
Protokylol (5 mg)	40 0 ± 1.5	8.0 ± 1.7
Metaproterenol (5 mg)	35.1 ± 2.6	7.7 ± 1.1
Terbutaline (5 mg)	4.9 ± 2.6	2.8 ± 1.1
Methoxyphenamine (5 mg)	4.2 ± 0.6	3.0 ± 0.5

Each value is the average of four determinations performed on two treated animals per drug dose.

as well as the other β-receptor agonists, was unable to produce the induced biosynthesis of the PRPs and glycoprotein observed with ISO treatment.

DISCUSSION

The morphological and biochemical effects of isoproterenol on the rat salivary gland system have been well established. Responses to ISO vary in degree with the gland studied. The parotid gland shows the most pronounced sensitivity as judged by the increase in hypertrophy and hyperplasia, followed by the submandibular gland. The sublingual gland does not appear to be affected by ISO treatment [7]. Evidence has been presented in the present study indicating that the parotid gland response to a variety of β-receptor agonists and phosphodiesterase inhibitors was more sensitive to stimulation than the submandibular gland. To our surprise, the inability of the submandibular gland to demonstrate the induced PRP synthesis, with the above drug regimens, was similar to earlier observations from this laboratory under conditions of low ISO dosages or using young animals. While confirming past observations of parotid gland hypertrophy these agonists were able to induce some of the biochemical changes which have only been documented with ISO treatment until now.

Binding studies using β-adrenergic-receptor agonists have suggested that stimulation of parotid gland hypertrophy and amylase release by ISO treatment is evoked by interaction with cell surface β_1-type receptors [25, 26]. In the present report, the inability of terbutaline, a β_2-adrenergic-receptor agonist [2], to induce *de novo* synthesis of parotid gland PRP synthesis and 4β-galactosyltransferase activity reaffirms this conclusion by others [25, 26, 27]. The co-administration of a β_2-antagonist (butoxamine) with ISO did not effect PRP synthesis. However, co-administration of a β_1-antagonist (atenolol) eliminated the *de novo* biochemical changes observed with ISO alone (Fig. 4). The parotid and submandibular glands must contain a majority of β_1 receptors on their cell surface. The other β-receptor agonists employed must also be able to interact with salivary gland adrenergic receptors to stimulate adenylate cyclase activity [27]. The ability of metaproterenol ($\beta_2 > \beta_1$) to induce parotid gland hypertrophy and PRP biosynthesis was due to the ability of this compound to utilize the β_1-adrenergic receptor, albeit at a lower affinity than the β_2-adrenergic receptor. At lower drug concentrations we were unable to observe parotid gland stimulation. The absence of activity displayed with methoxyphenamine is likely due to the indirect interaction of the drug with β-adrenergic receptors [28].

Catecholamine binding to β-adrenergic receptors stimulates adenylate cyclase activity causing an increase in intracellular levels of cAMP. The differences in the abilities of various β-agonists to induce PRP synthesis and 4β-galactosyltransferase activity may be related to the differential sensitivity

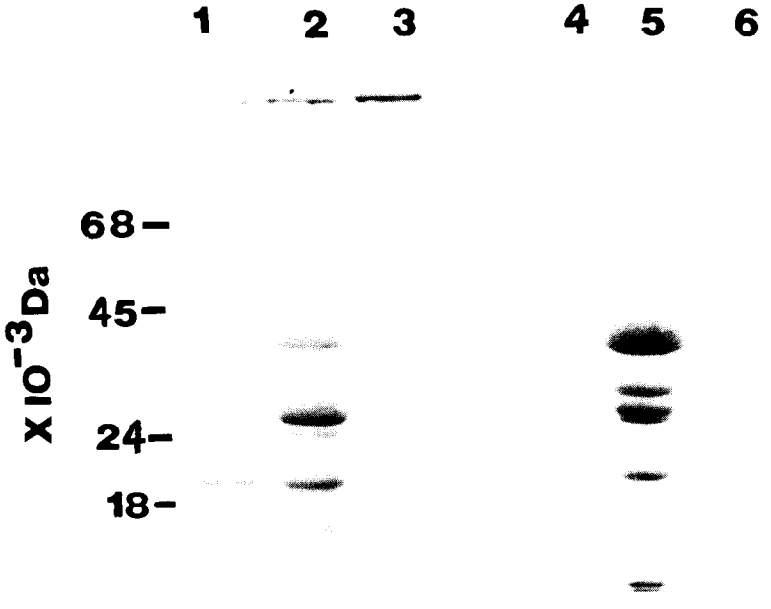

Fig. 4. 12% SDS polyacrylamide gel of TCA extracts from isopreterenol-treated parotid and submandibular glands co-treated with β-adrenergic-receptor antagonists. Lane 1, untreated parotid gland; Lane 2, isoproterenol-treated parotid gland co-treated with the β_2-antagonist butoxamine; Lane 3, isoproterenol-treated parotid gland co-treated with the β_1-antagonist atenolol; Lane 4, control submandibular gland; Lane 5, isoproterenol-treated submandibular gland co-treated with the β_2-antagonist butoxamine; Lane 6, isoproterenol-treated submandibular gland co-treated with the β_1-antagonist atenolol. Each animal was treated with a daily injection of the β-antagonist (5 mg/200 g animal) followed 30 min later with 5 mg/200 g animal of isoproterenol for 10 days. The absence of the submandibular gland glycoproteins is due to complete destaining of the gel.

to cAMP levels of the regulatory controls on gene expression. The induction of the PRP genes would therefore require the lowest accumulation of cAMP or increased cyclase activity. Single injections of phosphodiesterase inhibitors or β-adrenergic-receptor agonist do not support this type of mechanism in explaining the differences in *de novo* biosynthesis in the parotid gland (see Table 3). An examination of cAMP levels under chronic treatment did not reveal any permanent increases in parotid glands (data not shown). It is of interest to note that the separate induction of PRPs and proline-rich glycoprotein has been observed in the parotid gland of rats fed a high tannin diet although no measurements of cAMP levels were reported [29]. Similarly, there was no submandibular gland response.

The difference in β-adrenergic receptor stimulation of the submendibular gland may result from the number or type of β-receptors on the cell surface. Thulin [30] has suggested that the submandibular gland also contains β_1-type receptors on the cell surface after observing an inability to increase saliva production from duct cannulations with treatments of the β_2-agonist salbutamol, as compared with ISO stimulation. The employment of β-antagonists along with ISO in the above experiments substantiates this claim since β_1-antagonists were able to block *de novo* protein synthesis in the parotid and submandibular gland. However, the inability of β_1/β_2-adrenergic-receptor agonists as well as phosphodiesterase inhibitors, at concentrations equal to or greater than ISO, to induce submandibular gland protein synthesis suggests the involvement of additional factors in gland responsiveness. It may be that the differences in cAMP levels are sufficient in this gland to be responsible for the on or off expression of the PRP genes and 158,000 dalton glycoprotein. Such fine tuning, however, was not reflected in the parotid gland where this same drug regimen only induced the biosynthesis of the PRPs while ISO induced the synthesis of PRPs, 4β-galactosyltransferase and the 220,000 dalton glycoprotein.

Treatment of both rats and mice with hydrocortisone and thyroxine are known to lead to precocious development of neonatal salivary glands as well as to induce parotid and submandibular gland hypertrophy in the adult animal [31, 32]. Isoproterenol and the other β-agonists used in this study were tested for the ability to increase plasma cortisol and corticosterone levels. The drugs tested resulted in a 2-fold increase in these hormones, after a 10-day drug regimen, over control animals (15.3 mg/100 ml plasma for controls vs 28.9 to 31.3 for treated animals). We did not test the effects of the various drug treatments on the levels of thyroxine although they may play a role in submandibular gland response to β-agonist stimulation and subsequent induction of PRP biosynthesis when treated with ISO (unpublished data). Indeed, it has been reported that thyroxine levels influence the level of cell surface β-adrenergic receptors on rat myocarduum [33]. Recently, it has been shown that hyperthyroidism leads to an increased sensitivity to ISO treatment and β-receptor numbers in the rat submandibular gland [34]. The non-specificity of β-receptor agonists for target tissues under study makes identifying the interaction and isolation of other cellular factors from other organs difficult. Clearly further investigation of salivary gland β-receptor types, surface numbers and coupling to adenylate cyclase is required to resolve differences in gland sensitivity to chronic β-adrenergic agonist stimulation.

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