Biochemical Pharmacology, Vol. 45, No. 7, pp. 1395–1401, 1993.
Printed in Great Britain.

0006-2952/93 $6.00 + 0.00

ADRENERGIC REGULATION OF RNA SYNTHESIS IN THE RAT PAROTID GLAND

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(*Received* 12 *October* 1992; *accepted* 19 *January* 1993)

Abstract—Adrenergic regulation of RNA synthesis by *in vivo* stimulated parotid glands and dispersed parotid lobules was studied by a combination of *in vivo* and *in vitro* methods. Following a single intraperitoneal injection of isoproterenol, [^{3}H]uridine incorporation into RNA was increased by 50% after the first hour. Amylase mRNA content was also elevated within 1 hr and was 2–3-fold higher than control values at 4 hr. An increase in the rate of total protein synthesis was detectable after 2 hr, and maximal rates were achieved 6 hr after isoproterenol administration. In dispersed parotid lobules, both isoproterenol and epinephrine stimulated [^{3}H]uridine incorporation and at optimal concentrations increased incorporation by almost 200%. Phenylephrine (10 μM) caused a slight increase of about 20% whereas methoxamine (10 μM) had no effect. Stimulation by epinephrine was reversed by propranolol, but not by either phentolamine or prazosin. The increase in RNA synthesis induced by isoproterenol or epinephrine was dose dependent and half-maximal stimulation required 5.0×10^{-8} M isoproterenol and 7.9×10^{-7} M epinephrine. Dibutyryl cyclic AMP also stimulated [^{3}H]uridine incorporation, whereas 8-bromo cyclic GMP, A23187 and phorbol myristate acetate had no effect. The importance of protein phosphorylation in mediating the observed stimulation was evaluated using protein kinase and phosphatase inhibitors. *N*-[2-(Methylamino)ethyl]-5-isoquinolinesulphonamide, an inhibitor of cyclic nucleotide-dependent protein kinases, substantially diminished the isoproterenol-induced stimulation. Okadaic acid treatment of lobules increased [^{3}H]uridine incorporation. Furthermore, okadaic acid synergistically potentiated the stimulatory effect of a suboptimal concentration of isoproterenol. The results demonstrate that activation of the β-adrenergic receptor induces the synthesis of certain RNA species in the parotid gland and that protein phosphorylation by a cyclic AMP-dependent protein kinase is a key event in the signal transduction pathway.

Protein secretion by the rat parotid gland is regulated by a variety of neurotransmitters including norepinephrine, acetylcholine, substance P and vasoactive intestinal peptide [1]. These agonists exert their effects primarily through two different signalling pathways. In one, receptor occupancy leads to activation of adenylyl cyclase, elevation of intracellular cyclic AMP and stimulation of cyclic AMP-dependent protein kinase [1–3]. The other is coupled to activation of a phosphoinositide-specific phospholipase C resulting in elevation of cytosolic free Ca^{2+} and protein kinase C activity [4]. It is generally accepted that the cyclic AMP-dependent pathway represents the principal mechanism for stimulating protein secretion by exocytosis while the phosphoinositide pathway is more important in controlling water and electrolyte secretion [1, 4].

Acute stimulation of parotid glands *in vivo* with isoproterenol, a β-adrenergic agonist, results in rapid depletion of secretory proteins such as amylase, followed by a more prolonged period of resynthesis [5, 6]. Lillie and Han [7] observed that protein synthesis in parotid glands was maximally stimulated 6 hr after intraperitoneal administration of isoproterenol. This temporal dissociation between receptor activation and stimulation of protein synthesis as well as more recent observations that the amylase mRNA content is elevated in parotid glands or cultured cells stimulated with isoproterenol or cyclic AMP [6, 8] suggests that transcriptional regulation may be involved.

In many tissues, receptor-mediated regulation of gene transcription occurs via cyclic AMP-, Ca^{2+}- or protein kinase C-dependent mechanisms [9–11]. Little however is known about the role of adrenergic receptors in regulating gene transcription in the parotid acinar cell except for the fairly well documented β-adrenergic stimulation of RNA synthesis and increased expression of certain genes in parotid glands induced to proliferate by chronic stimulation with isoproterenol [12–14]. In this study, we have investigated the more short term effects of adrenergic agonists on total RNA synthesis with a view to evaluating the importance of transcriptional regulation in secretory protein biosynthesis. Using a dispersed parotid lobule system, we have also attempted to delineate the signalling mechanisms involved in such regulation. Our results indicate that activation of the β-adrenergic receptor results in a rapid increase in the rate of RNA synthesis in the parotid gland. The sequence of cellular events participating in the signalling pathway include elevation of cyclic AMP and cyclic AMP-dependent protein phosphorylation.

MATERIALS AND METHODS

[^{3}H]Uridine, [^{3}H]phenylalanine and a random primer labelling kit were purchased from Amersham International (Amersham, U.K.). Tissue solubilizer

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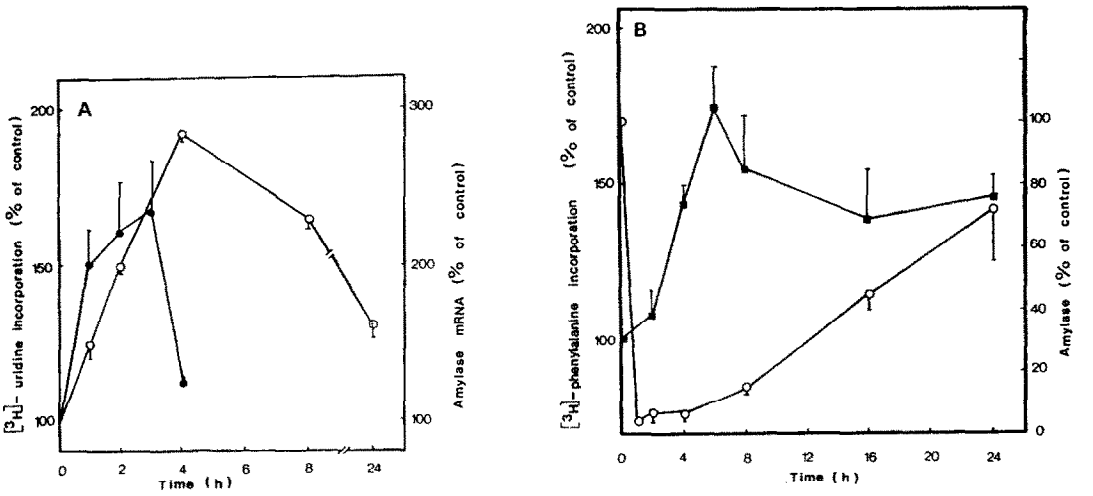

Fig. 1. Effect of *in vivo* isoproterenol stimulation on RNA synthesis, amylase mRNA, protein synthesis and amylase content of rat parotid glands. Rats starved for 24 hr were injected with 1 mg/100 g body weight of isoproterenol. At the times indicated, the glands were removed for determination of amylase and amylase mRNA content. For measurement of rates of [^{3}H]uridine incorporation into RNA and [^{3}H]phenylalanine incorporation into protein, rats were killed 15 min prior to the indicated time, and the tissue slices incubated for 1 hr in the presence of [^{3}H]uridine or [^{3}H]phenylalanine. All results are expressed as percentages of control values obtained for weight-matched rats injected with saline, and are the means ± SEM of a minimum of three determinations. Panel A: (●) [^{3}H]uridine incorporation into RNA, (○) amylase mRNA; panel B: (■) [^{3}H]phenylalanine incorporation into proteins, (○) amylase content.

(BTS-450) was from Beckman Instruments Inc. (CA, U.S.A.). Collagenase (type IV), trypsin inhibitor (type II-S), *N*-[2-(methylamino)ethyl]-5-isoquinolinesulphonamide (H-8*), okadaic acid and other biochemicals were from the Sigma Chemical Co. (St Louis, MO, U.S.A.).

Preparation of parotid lobules. Parotid lobules were prepared according to the method of Amsterdam *et al.* [15] with slight modifications. Briefly, the glands were removed from male Wistar rats (180–200 g) which had been starved for 24 hr, cleared of extraneous tissue and cut into small pieces. The pieces were then digested for 30 min at 37° in Hanks' balanced salt solution containing 10 mM HEPES-KOH, pH 7.4, 5 mM glucose, 2% (w/v) bovine serum albumin and 100 U/mL of collagenase. The medium was decanted and replaced with fresh collagenase containing medium and digestion continued for a further 30 min. The lobules were dispersed by pipetting, filtered and washed three times by centrifugation at about 100 *g*. The lobules were finally resuspended at a concentration of 0.15 mg of DNA/mL in Hanks' solution supplemented with 5 mM glucose, amino acids (basal medium Eagles), 2% (w/v) bovine serum albumin and 0.4 μM each of adenosine, guanosine and cytidine (referred to as supplemented Hanks' solution in all future references to it). The lobules were routinely preincubated for at least 1 hr prior to initiating any experiments. The viability of the preparation as ascertained by Trypan blue exclusion remained more than 90% for 4 hr.

[^{3}H]*Uridine incorporation.* Parotid lobules were incubated in supplemented Hanks' solution containing other additions as stated in the text or legends. [^{3}H]Uridine (2 μCi/mL, final concentration 0.4 μM) was added and incubation continued for another hour unless otherwise indicated. The lobules were pelleted by centrifugation at 10,000 *g* for 20 sec, and lysed in ice-cold buffer containing 10 mM Tris–HCl, pH 7.0, 1 mM EDTA, 1 mM uridine and 0.1% sodium dodecyl sulphate. A portion of the lysate was retained for estimation of DNA. Carrier RNA (0.5 mg/mL) was added to the remaining portion followed by an equal volume of cold 10% (w/v) trichloroacetic acid (TCA). After standing in ice for 1 hr, the samples were centrifuged and the pellets washed three times with cold 5% (w/v) TCA. The TCA-insoluble material was finally digested with BTS-450 tissue solubilizer and radioactivity determined by liquid scintillation counting in a toluene-based cocktail.

Uridine incorporation into *in vivo* stimulated rat parotid glands was determined by a combination of *in vivo* and *in vitro* methods. Rats which had been starved for 24 hr were injected intraperitoneally with 1 mg/100 g body weight of (±)-isoproterenol in saline. Control rats received saline alone. Fifteen minutes prior to the times indicated, the rats were killed and parotid gland slices prepared as described previously [16] in supplemented Hanks' solution. After preincubation for 5–10 min, the slices were incubated for 1 hr in supplemented Hanks' solution

* Abbreviations: H-8, *N*-[2-(methylamino)ethyl]-5-isoquinolinesulphonamide; TCA, trichloroacetic acid; PMA, phorbol 12-myristate 13-acetate; But$_2$cAMP, dibutyryl cyclic AMP; 8-Br-cGMP, 8-bromo cyclic GMP.

containing 1 μCi/mL of [^{3}H]uridine. The slices were homogenized in buffer containing 10 mM EDTA, pH 7.0, 1 mM uridine and 0.5 mg/mL of RNA, and radioactivity incorporated into the TCA-insoluble fraction was determined as described above.

Since it is difficult to determine the specific activity of the nuclear pool of UTP which serves as the immediate precursor for RNA synthesis, it is assumed throughout this study, that changes in the rate of incorporation of [^{3}H]uridine into TCA-insoluble material reflect changes in the rate of RNA synthesis.

Estimation of amylase mRNA. Total RNA was isolated from parotid glands by the guanidinium thiocyanate method of Chirgwin *et al.* [17]. The RNA was denatured, hybridized and amylase mRNA quantitated by standard procedures [18]. Constant amounts (1 or 2 μg) of the RNA samples were loaded onto nitrocellulose membranes using a slot blot manifold (Bio Rad). The membranes were fixed by baking at 80° for 2 hr, prehybridized and then hybridized for 16 hr with an amylase cDNA probe encoding 1176 bp of the pancreatic amylase gene [19]. The probe was labelled with [α-^{32}P]dCTP using a random primer labelling kit to a specific activity of 1×10^9 dpm/μg. The filters were washed and the radioactivity of each slot was determined either by autoradiography followed by densitometry or by liquid scintillation counting.

Other assays. Protein synthesis by parotid slices obtained from rats starved for 24 hr and then stimulated *in vivo* with isoproterenol was determined as described previously [16]. DNA content was assayed by the method of Burton [20] and RNA concentration estimated from E_{260}. Amylase activity was determined according to Bernfeld [21].

RESULTS

The results of preliminary experiments aimed at studying changes in the rate of RNA synthesis, amylase mRNA levels, rates of protein synthesis and amylase content of parotid glands from rats subjected to a single intraperitoneal injection of isoproterenol are shown in Fig. 1. A 50% increase in the rate of incorporation of [^{3}H]uridine into RNA was observed 1 hr after administration of isoproterenol. The elevated rates were sustained for up to 3 hr before declining to near control levels at 4 hr. In order to ascertain whether the increase in uridine incorporation was directed towards the synthesis of mRNAs coding for secretory proteins, we assayed for amylase mRNA since amylase, the principle protein of rat parotid saliva, accounts for almost 30% of the secreted proteins [22]. Slot blot analysis of the total RNA showed that during the initial 4 hr following isoproterenol stimulation, the parotid gland content of amylase mRNA rose steadily and reached a maximum of 183% above control at 4 hr. Amylase mRNA in the stimulated glands remained significantly higher than that of controls for up to 24 hr. The rate of protein synthesis as determined by [^{3}H]phenylalanine incorporation into total protein increased in parallel with the increase in amylase mRNA. As reported previously by Lillie and Han [7], the rate peaked 6 hr after isoproterenol stimulation and thereafter declined gradually. Amylase present in the gland was almost completely discharged within 1 hr of isoproterenol injection. Restitution of the cellular content of this enzyme was detectable at 4 hr and proceeded at an almost steady rate for up to 24 hr.

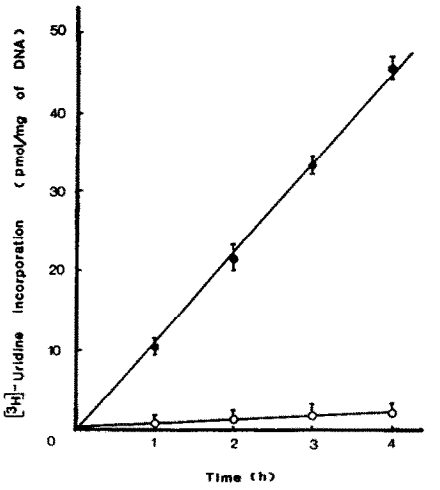

Fig. 2. Time course of [^{3}H]uridine incorporation by dispersed parotid lobules. Parotid lobules preincubated for 1 hr were incubated in supplemented Hanks' buffer containing 2 μCi/mL of [^{3}H]uridine. Radioactivity incorporated into the TCA-insoluble fraction was determined as described in Materials and Methods. Actinomycin D, when present, was added 10 min before the addition of [^{3}H]uridine. (●) Control, (○) 5 μg/mL actinomycin D. Results are the means ± SEM of three separate experiments.

Further characterization of the β-adrenergic regulation of RNA synthesis was conducted on dispersed parotid lobules since this system is more amenable to experimental manipulation. Freshly isolated lobules displayed relatively high and rather variable rates of incorporation of [^{3}H]uridine into RNA during the first hour of incubation. Subsequently, incorporation occurred at a linear rate of 7.83 ± 0.84 pmol/mg of DNA per hr (mean ± SEM for 12 different preparations) for up to 4 hr. Addition of actinomycin D, an inhibitor of RNA polymerase, inhibited uridine incorporation by more than 90% (Fig. 2). Because of the high initial rates observed, we adopted the practice of routinely preincubating the lobules for 1 hr prior to initiating any experiments.

The ability of different adrenergic agonists to modulate RNA synthesis by parotid lobules incubated in their continuous presence is shown in Fig. 3. At a concentration of 10 μM, both isoproterenol and epinephrine doubled the rate of [^{3}H]uridine incorporation within 1 hr. Consistent with our earlier observations on *in vivo* stimulated parotid glands, incorporation remained elevated for up to 3 hr. Unlike isoproterenol and epinephrine, the α-adrenergic agonist phenylephrine induced only a slight 20% elevation in [^{3}H]uridine incorporation. Since phenylephrine at high concentrations can also activate the β-adrenergic receptor, the effect of

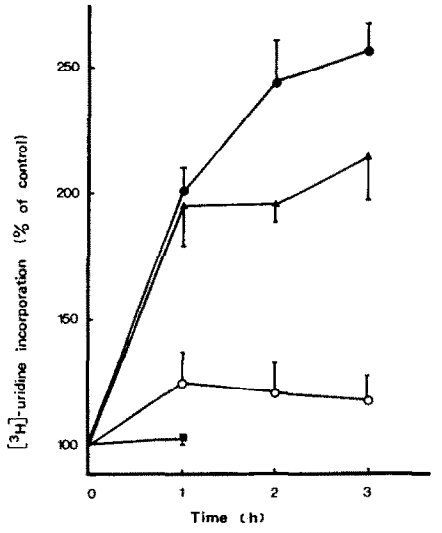

Fig. 3. Effect of different adrenergic agonists on [^{3}H]-uridine incorporation. [^{3}H]Uridine incorporation by parotid lobules incubated in the continuous presence of different adrenergic agonists was determined at hourly intervals. Results are the means ± SEM of three separate experiments. (●) 10 μM isoproterenol, (▲) 10 μM epinephrine, (○) 10 μM phenylephrine and (■) 10 μM methoxamine.

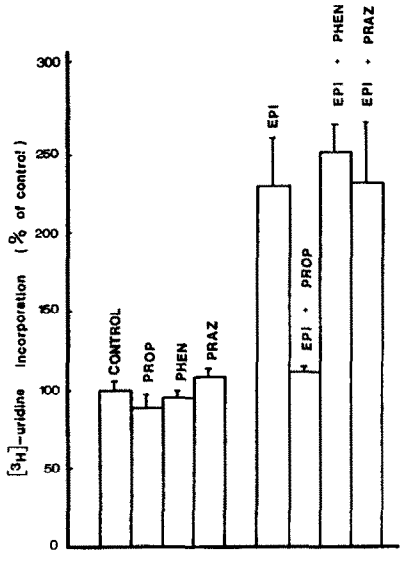

Fig. 4. Effects of adrenergic receptor antagonists on epinephrine-stimulated [^{3}H]uridine incorporation. Lobules were incubated in the presence of epinephrine (10 μM) or adrenergic receptor antagonists (10 μM for all except prazosin which was present at 1 μM) for 1 hr before addition of [^{3}H]uridine. When the antagonist and epinephrine were both present, the antagonist was added before epinephrine. Results are the means ± SEM of six determinations and are expressed as % of controls. Abbreviations: PROP, propranolol; PHEN, phentolamine; PRAZ, prazosin; EPI, epinephrine.

methoxamine, an α_1-selective adrenergic agonist, was also determined. Lobules incubated with 10 μM methoxamine for 1 hr failed to show any difference in the rate of RNA synthesis. Confirmation that the stimulation of RNA synthesis was predominantly a β-receptor-regulated event was obtained from studies using adrenergic receptor antagonists. Addition of the β-adrenergic antagonist propranolol completely abolished the stimulation by epinephrine (Fig. 4) or phenylephrine (results not shown). However, the α-receptor antagonists phentolamine and prazosin failed to reverse the epinephrine-stimulated [^{3}H]-uridine incorporation. The antagonists themselves had no significant effect on uridine incorporation at the concentrations used.

Stimulation of RNA synthesis by isoproterenol and epinephrine was dose dependent (Fig. 5). In accordance with their well-established differences in affinity for the β-adrenergic receptor, sensitivity to stimulation by isoproterenol was more than one order of magnitude greater than by epinephrine. Thus, the concentrations of isoproterenol and epinephrine required for half-maximal stimulation were 5.0×10^{-8} and 7.9×10^{-7} M, respectively. The maximal responses elicited by the two agonists were of a similar magnitude, with uridine incorporation being 2.5–3-fold greater than the rates observed in unstimulated lobules. Activation of the β-adrenergic receptor also increased the amylase mRNA content of parotid lobules. After 2.5 hr of stimulation with 10 μM isoproterenol, the amylase mRNA of lobules was 238 ± 4% (mean ± SEM, N = 8) of controls (100%).

Adrenergic agonists exert their wide ranging effects on cellular metabolism by generating a variety

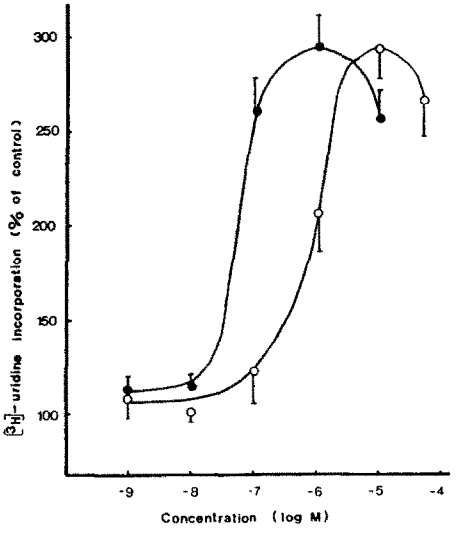

Fig. 5. Dose-response to isoproterenol and epinephrine. Parotid lobules were incubated in the presence of varying concentrations of either isoproterenol (●) or epinephrine (○). One hour after addition of the agonist, 2 μCi/mL of [^{3}H]uridine was added and incubation continued for an additional hour. Radioactivity incorporated into RNA was determined. Results are the means ± SEM of six determinations.

Table 1. Effects of cyclic nucleotides, A23187 and PMA on [^{3}H]uridine incorporation by parotid lobules

Additions	[^{3}H]Uridine incorporation (% of control)
But$_2$cAMP (1 mM)	227 ± 14 (9)*
8-Br-cGMP (1 mM)	104 ± 4 (9)
A23187 (1 μM)	101 ± 8 (9)
PMA (100 μM)	107 ± 7 (6)

Parotid lobules were incubated in supplemented Hanks' solution containing additions as indicated above. [^{3}H]-Uridine was added simultaneously with A23187 or 1 hr after addition of But$_2$cAMP, 8-Br-cGMP or PMA, and incorporation into RNA determined as described in Materials and Methods.

Results are the means ± SEM of the number of determinations indicated in parentheses and are expressed as a % of paired controls.

* $P < 0.01$ for difference from control.

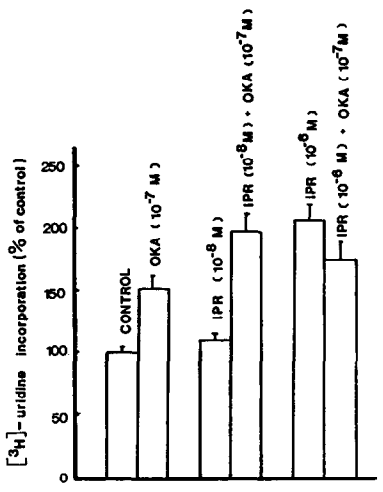

Fig. 6. Effect of okadaic acid on isoproterenol-stimulated [^{3}H]uridine incorporation. Parotid lobules were incubated under standard conditions and [^{3}H]uridine incorporation determined 1 hr after addition of okadaic acid or isoproterenol. When present together with isoproterenol, the okadaic acid was added 10 min prior to isoproterenol. Results are expressed as a percentage of paired controls and are the means ± SEM of six determinations. Abbreviations: OKA, okadaic acid; IPR, isoproterenol.

of second messengers including cyclic AMP, Ca^{2+} and diacylglycerol. Further studies were therefore conducted in order to assess the importance of each of these in the signalling pathway. Dibutyryl cyclic AMP (But$_2$cAMP), a membrane-permeable analogue of cyclic AMP, stimulated [^{3}H]uridine incorporation (Table 1). The magnitude of the response elicited was similar to that observed with optimal β-receptor activation. 8-Bromo cyclic GMP (8-Br-cGMP) failed to have any effect. The calcium ionophore A23187 and the protein kinase activator phorbol 12-myristate 13-acetate (PMA) also had no significant effect when tested at the concentrations indicated.

In mammalian cells, most if not all effects of cyclic AMP are mediated through activation of cyclic AMP-dependent protein kinases. We therefore examined the effect of H-8, a protein kinase inhibitor which preferentially inhibits cyclic nucleotide-dependent kinases [23], on β-receptor-mediated induction of RNA synthesis. Incubation of lobules in medium containing 200 μM H-8 resulted in a marked lowering of the basal rate of [^{3}H]uridine incorporation (Table 2). When added 10 min prior to the addition of isoproterenol, H-8 almost completely reversed the isoproterenol-induced stimulation.

To confirm the participation of protein phosphorylation in the regulatory mechanisms governing gene transcription, the effects of the protein phosphatase inhibitor okadaic acid were also studied. Incubation of lobules with 100 nM okadaic acid resulted in a persistent elevation of RNA synthesis during a 3 hr incubation (results not shown). When added together with 10^{-8} M isoproterenol, RNA synthesis was synergistically potentiated (Fig. 6). However, if lobules were maximally stimulated with isoproterenol, okadaic acid caused no further elevation in [^{3}H]uridine incorporation.

DISCUSSION

The rat parotid gland displays high rates of protein

Table 2. Inhibition of isoproterenol-stimulated [^{3}H]uridine incorporation by H-8

Additions	[^{3}H]Uridine incorporation (% of control)
No additions	100 ± 3
H-8 (200 μM)	61 ± 2*
Isoproterenol (10 μM)	311 ± 55
Isoproterenol (10 μM) + H-8 (200 μM)	130 ± 9†

[^{3}H]Uridine incorporation by parotid lobules was determined 1 hr after addition of isoproterenol or H-8. When present together, H-8 was added 10 min before addition of isoproterenol.

Results are the means ± SEM of six determinations.

* $P < 0.001$ for difference from control.

† $P < 0.05$ for difference from isoproterenol-treated lobules.

synthesis, most of which is directed towards the synthesis of secretory proteins. The cellular control mechanism regulating the expression of the genes encoding the secretory proteins have not yet been delineated. Amylase represents the principal protein in rat parotid saliva accounting for almost 30% of the secretory protein [22] and about 15–20% of the total protein synthesized by the gland [24]. We therefore initially examined the effects of β-adrenergic stimulation on amylase gene expression in the *in vivo* stimulated parotid gland. An increase in the rate of [^{3}H]uridine incorporation into RNA was observed within 1 hr of isoproterenol administration indicating that induction of gene transcription is an early event following β-receptor activation. This increase is directed at least in part towards the accelerated transcription of the amylase gene as shown by the concurrent elevation in cellular amylase mRNA. In similar studies, Kim *et al.* [6] reported only a small (15%) increase in amylase mRNA of isoproterenol-stimulated glands after 6 hr, although at the same time point, they observed a 3–4-fold increase in amylase biosynthesis. The promoter region of the parotid amylase gene has been cloned and sequenced [25]. A possible cyclic AMP response element with a sequence TGACAGCA can be identified at position 180 on the sequence. Moreover, transcription of the amylase gene is induced by But_2cAMP or forskolin treatment of rat parotid–hepatoma hybrid cells [8]. The regulatory mechanisms governing amylase biosynthesis are therefore likely to encompass β-adrenergic receptor-mediated activation of amylase gene transcription via a cyclic AMP-dependent mechanism. Such a model is not only compatible with the observed elevation of amylase mRNA but also accommodates data obtained in this as well as other studies indicating that maximal stimulation of protein and amylase biosynthesis occurs several hours after activation of the β-adrenergic receptor.

Due to the limitations posed by *in vivo* studies, further characterization of the β-adrenergic stimulation of uridine incorporation was conducted on dispersed parotid lobules. In these studies, observed changes in [^{3}H]uridine incorporation were assumed to reflect corresponding changes in RNA biosynthesis, since it is technically difficult to determine the specific activity of nuclear UTP which serves as the immediate precursor for RNA synthesis. The parallel changes seen in [^{3}H]uridine incorporation and amylase mRNA synthesis in isoproterenol-stimulated cells suggest that this assumption holds true. The rapid induction of RNA synthesis in adrenergically stimulated lobules was due primarily to β-adrenergic receptor activation. Thus, the increase in RNA synthesis resulting from epinephrine stimulation was completely abolished by blockade of the β-receptor but not the α-receptor. Moreover, the α_1-receptor specific agonist methoxamine, failed to have any effect on uridine incorporation and the slight stimulation induced by phenylephrine was reversed by propranolol. The β-adrenergic receptor therefore plays a pre-eminent role not only in controlling protein secretion by the parotid gland [1] but also in regulating the transcription of cellular genes including the amylase gene. This concerted action on the pathways regulating protein secretion and protein synthesis may provide a convenient mechanism whereby synthesis of secretory proteins such as amylase may be coordinated with its secretion.

It is generally accepted that the signal transduction pathway associated with β-receptor activation includes elevation of cyclic AMP, binding of cyclic AMP to the regulatory subunit of protein kinase A and dissociation of the catalytic and regulatory subunits. The activated catalytic subunit then translocates into the nucleus, where it is believed to phosphorylate a cyclic AMP response element binding protein and increase its ability to activate transcription [10]. Several lines of evidence suggest that such a cyclic AMP-dependent protein phosphorylation may be important in the mechanism by which β-adrenergic agonists stimulate RNA synthesis in the parotid gland. Thus, the membrane-permeable cyclic AMP analogue But_2cAMP was almost as effective as isoproterenol or epinephrine in stimulating [^{3}H]uridine incorporation. 8-Br-cGMP, A23187 and PMA which have been commonly used to study the involvement of cyclic GMP, Ca^{2+} and protein kinase C, respectively, failed to have any significant effect. Addition of the protein kinase inhibitor H-8 not only lowered the basal rate of RNA synthesis but also reversed the stimulation induced by isoproterenol. Inhibition by H-8 has often been considered as evidence for the participation of cyclic AMP-dependent protein kinases [26, 27] since this isoquinoline derivative shows higher specificity for cyclic nucleotide-dependent protein kinases [23]. However, at the relatively high concentrations used in whole cell studies H-8 may also inhibit other protein kinases [23, 28]. Takuma [29] studied the effects of H-8 on the rat parotid cyclic AMP-dependent protein kinase and showed that 200 μM H-8 almost completely inhibits this enzyme *in vitro*. At the same concentration, H-8 also inhibits isoproterenol-induced phosphorylation of some cellular proteins. Surprisingly however, H-8 failed to have any effect on amylase secretion in response to either isoproterenol or cyclic AMP leading this worker to suggest that protein phosphorylation by cyclic AMP-dependent protein kinase may not be directly involved in the activation of exocytosis.

Confirmatory evidence that protein phosphorylation is indeed important in regulating gene transcription in the parotid gland was obtained from our studies using okadaic acid. This marine toxin is a potent and specific inhibitor of protein phosphatases Type 1 and 2A, and has been widely used in recent years to evaluate the role of protein phosphorylation in various regulatory processes [30]. When rat parotid cells were treated with okadaic acid, Takuma and Ichida [31] observed that cyclic AMP-dependent protein phosphorylation was increased in a dose-dependent manner. In the present study, 100 nM okadaic acid not only mimicked the effects of β-adrenergic receptor activation in stimulating [^{3}H]-uridine incorporation but also synergistically potentiated the stimulatory effect of a suboptimal concentration of isoproterenol. These observations are consistent with a scheme in which okadaic acid modulates the phosphorylation status of a regulatory

protein that participates in the β-receptor linked signalling pathway.

The data presented in this study provide convincing evidence that stimulation of gene transcription following β-adrenergic receptor activation involves cyclic AMP-dependent protein phosphorylation. Further studies are however necessary to determine the exact mechanism of transcriptional activation and to identify the various cellular genes that are induced.

Acknowledgements—We thank Dr Raymond J. MacDonald for providing the plasmid pcXP100 containing a fragment of the rat pancreatic amylase gene. We also thank Ms Lim Soh Chan for technical assistance provided. This research was funded by a grant RP 87/350 from the National University of Singapore.

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