MOLECULAR PHARMACOLOGY, 21:121-127

Rat Parotid Gland Protein Kinase Activation

Relationship to Enzyme Secretion

TERRY N. SPEARMAN[1] AND FRED R. BUTCHER[2]

Department of Biochemistry, West Virginia University School of Medicine, Morgantown, West Virginia 26506

Received June 5, 1981; Accepted August 10, 1981

SUMMARY

The degree of activation of rat parotid gland cyclic AMP-dependent protein kinase (EC 2.7.1.37) was measured in tissue minces *in vitro* in order to assess the involvement of this enzyme in the parotid stimulus-secretion coupling mechanism. Kinase activation, determined by the activity ratio method, was measurably increased by isoproterenol, a *beta*-adrenergic agonist and a potent stimulator of α-amylase (EC 3.2.1.1) secretion. Muscarinic cholinergic and *alpha*-adrenergic stimulation, less effective in releasing amylase, did not affect protein kinase activation. Kinase activation closely paralleled the cyclic AMP concentration when the concentration of isoproterenol was varied. Amylase release exhibited a similar isoproterenol dose-dependence, except that amylase release was measurably increased at an isoproterenol concentration slightly lower than that required to increase detectably the cyclic AMP concentration or kinase activation. Partial dissociation between cyclic AMP levels, kinase activation, and secretion was seen when submaximal *beta*-adrenergic stimulation was combined with submaximal and supramaximal cholinergic stimulation. These results suggest an involvement of cyclic AMP-dependent protein kinase in *beta*-adrenergic-stimulated amylase release, but show that the extent of secretion is not rigidly coupled to the extent of kinase activation as determined by the activity ratio method. Protein kinase activation may function in concert with other factors in the regulation of exocytosis in this tissue.

INTRODUCTION

Cyclic AMP is believed to mediate most or all of its effects by activation of cyclic AMP-dependent protein kinase (EC 2.7.1.37) (1). In the absence of cyclic AMP this enzyme exists as an inactive holoenzyme composed of two regulatory (*R*) subunits and two catalytic (*C*) subunits (2). In the presence of cyclic AMP, each *R* subunit binds two molecules of the nucleotide and the holoenzyme dissociates (2). The liberated active *C* subunit catalyzes the transfer of the terminal phosphate of ATP to susceptible target proteins (1).

The major mechanism of inducing exocytosis in the rat parotid gland is the elevation of the acinar cell cyclic AMP concentration via *beta*-adrenergic stimulation (3). Circumstantial evidence indicates that cyclic AMP regulates exocytosis by stimulating cyclic AMP-dependent protein kinase. This enzyme has been demonstrated in the parotid glands of several species (4–9), and dibutyryl cyclic AMP and isoproterenol-induced amylase secretion has been reported to be accompanied by an increase in protein phosphorylation (10). Tolbutamide, an inhibitor of rat parotid cyclic AMP-dependent protein kinase (5), has been reported to inhibit isoproterenol-stimulated amylase release (5).

Corbin and co-workers (11), Soderling *et al.* (12), and Corbin *et al.* (13) have developed a method for the determination of the effect of hormones on the cyclic AMP-dependent protein kinase of tissue slices *in vitro*. We have adapted their techniques to the rat parotid gland and have obtained data consistent with an involvement of this enzyme in *beta*-adrenergic amylase release.

MATERIALS AND METHODS

Tissue preparation and incubation. Male Sprague-Dawley rats were obtained from Charles River Breeding Laboratories (Wilmington, Mass.) and were maintained on standard laboratory chow until the day of the experiment. They were anesthetized with Nembutal and killed by exsanguination. The parotid glands were quickly removed and placed in KRH[3] at 37°. The glands were then

This work was supported in part by Grant AM-25249 from the National Institutes of Health.

[1] Recipient of a Cystic Fibrosis Foundation Postdoctoral Fellowship.

[2] Recipient of Research Career Development Award AM-00586 from the National Institutes of Health.

[3] The abbreviation used is: KRH, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid-buffered Krebs-Ringer solution supplemented with 6.1 mM glucose and 5 mM β-hydroxybutyrate.

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trimmed of extraneous tissue and finely minced with scissors. After two rinses in KRH they were blotted on filter paper and divided into aliquots of one-sixth gland each (approximately 15–25 mg of tissue). Subsequent procedures varied depending on the type of experiment to be performed.

Protein kinase activity ratios. Each aliquot of tissue was placed in 1 ml of KRH at 37° in 15-ml plastic scintillation vials. After a 30-min preincubation, additions were made as indicated in the legends to individual figures and tables. After an additional 5-min incubation, 1 ml of homogenizing solution [Norit SG Extra charcoal (J. T. Baker, Phillipsburg, N. J.), 20 mg/ml in 165 mM NaCl, 20 mM EDTA, and 1 mM 3-isobutyl-1-methyl xanthine (pH 8.0)] was added and the tissue was homogenized with a Polytron PT-10 (Brinkman Instruments, Westbury, N. Y.). The homogenates were immediately transferred to glass test tubes (10 × 75 mm) and centrifuged for 5 min at 10,000 × g_{max}. Duplicate 5- or 10-μl aliquots of the resulting supernatants were assayed for protein kinase activity both in the absence and the presence of 2 μM cyclic AMP. The activity ratio was calculated as activity minus cyclic AMP/activity plus cyclic AMP.

Amylase release. Each aliquot of tissue was placed in 1 ml of KRH in a 15-ml glass scintillation vial. Following a 15-min preincubation at 37°, a 50-μl sample of the medium was withdrawn and the test substances were added. After an incubation period of 30 min, another 50-μl sample of the medium was withdrawn. An additional 1 ml of KRH was added to each tissue sample and the samples were homogenized with a Polytron PT-10. A 50-μl sample of the homogenate was withdrawn and was, like the samples withdrawn previously, diluted to 500 μl with 20 mM phosphate buffer (pH 6.9) containing 6.7 mM NaCl and bovine serum albumin, 1 mg/ml. Duplicate aliquots of these diluted samples were subsequently assayed for amylase activity (14). The amount of amylase released into the medium during the 30-min incubation period is expressed as a percentage of the total amount of amylase present within the tissue immediately prior to the addition of the test substance. Variations of this procedure are noted in the legends to individual tables and figures.

Cyclic AMP levels. Each sample consisted of minced tissue equivalent to one-sixth of a gland suspended in 0.5 ml of KRH in a plastic test tube (17 × 100 mm). Test substances were added after a 15-min preincubation at 37°. Incubations were terminated at 5 min by the addition of 1.17 ml of absolute ethanol (70% final concentration) followed by immediate homogenization with a Polytron PT-10. The samples were held at −20° for 15 min and then centrifuged at 1000 × g for 15 min at 0°. The supernatants were decanted and the protein pellets were resuspended in 0.5 ml of 70% ethanol. The suspensions were heated for 5 min in a boiling water bath, held at −20° for 15 min, and centrifuged as before. The respective supernatants of the two centrifugation steps were combined and evaporated to dryness (Buchler Evapo-Mix, Buchler Instruments, Fort Lee, N. J.). The dried residues were dissolved in 200 μl of 25 mM Tris-HCl (pH 7.4), and duplicate or triplicate 20-μl aliquots were assayed for cyclic AMP (15). Protein pellets from the final centrifugation step were dissolved in 2 ml 0.2 N NaOH, and triplicate aliquots were assayed for protein by the method of Lowry *et al.* (16).

Protein kinase assay. The reaction was carried out in a final volume of 50 μl and contained 50 mM morpholinopropanesulfonic acid-NaOH (pH 6.5), with or without 2 μM cyclic AMP, 20 mM NaF, 7 mM $MgCl_2$, and histone II AS (Lot 88C-8120; Sigma Chemical Company, St. Louis, Mo.), 1 mg/ml. The ingredients of the assay were mixed in the given order, the sample was added, and the reaction was initiated by adding 0.5 μCi of [γ-^{32}P]ATP (50 μM final concentration). The incubation was carried out for 5 min at 35°. The reaction was terminated and phosphohistone was separated from ATP by a modification of the phosphocellulose-paper absorption method of Witt and Roskoski (17). A portion of the reaction mixture (35 μl) was spotted on a 2-cm square of Whatman P81 chromatography paper (Whatman, Clifton, N. J.) and immersed in ice-cold 1% sodium pyrophosphate. The squares were washed for 90 min with three changes of the wash medium. They were given two 5-min washes with acetone, dried, and counted in 3.5 ml of ACS counting solution (Amersham, Arlington Heights, Il.) in a Packard 3255 liquid scintillation spectrometer.

Synthesis of [γ-^{32}P]ATP. Labeled ATP used in this study was synthesized from ADP and carrier-free ^{32}P phosphoric acid in an HCl-free aqueous solution (New England Nuclear Corporation, Boston, Mass.) by the method of Johnson and Walseth (18). Enzymes used in the synthesis were obtained from Boehringer Mannheim Biochemicals (Indianapolis, Ind.). Other reagents were obtained from Sigma Chemical Company. The ATP was purified on DEAE-Sephadex (Pharmacia Fine Chemicals, Piscataway, N. J.) by stepwise HCl elution as described (18). As 1-ml eluent fractions were collected they were immediately neutralized by the addition of 50 μl of 5 N NaOH and 100 μl of 0.1 M morpholinopropanesulfonic acid-NaOH (pH 7.6). Dilutions of the fractions were counted for radioactivity, and the most active ones were pooled, divided into 0.5-ml aliquots, and stored at −70°. [γ-^{32}P]ATP prepared by this method usually resulted in protein kinase assay blank values considerably lower than those given by most batches of [γ-^{32}P]ATP obtained from commercial suppliers. However, blank values did increase upon storage of the [γ-^{32}P]ATP, especially at temperatures above −70°.

Source of other materials. (±)-Propranolol HCl was from Ayerst Laboratories, New York. Phentolamine HCl (Regitine HCl) was a gift from Ciba Pharmaceutical Company, Summit, N. J. L-Isoproterenol HCl, L-phenylephrine HCl, atropine sulfate, carbamylcholine chloride, and most other biochemicals were obtained from Sigma Chemical Company.

RESULTS AND DISCUSSION

Preliminary experiments indicated that it was necessary to assay high dilutions of the crude parotid extract in order to achieve a linear relationship between protein concentration and protein kinase activity. This could be due to the presence, in parotid homogenates, of high levels of either ATPase activity, phosphoprotein phos-

phatase activity, or cyclic AMP-dependent protein kinase inhibitors. Preliminary experiments suggested that high ATPase activity was a contributing factor to the nonlinearity of the kinase activity seen at higher protein concentrations. The concentration of NaF employed in the kinase assay does not completely inhibit parotid ATPase, even at high dilutions of homogenate where kinase activity is linear with respect to protein concentration (not shown). Higher NaF concentrations inhibited the kinase reaction. This made it necessary to work with very low levels of enzyme activity. The conditions described under Materials and Methods were found to give both optimal activity and low assay blank values.

In order that the measured activity ratios accurately reflect intracellular protein kinase activation, it is necessary to ensure that post-homogenization shifts in the equilibrium between protein kinase holoenzyme and dissociated subunits do not occur. Hydrolysis and/or dilution of cyclic AMP upon tissue homogenization tends to shift the equilibrium toward holoenzyme formation; and, were preventive measures not undertaken, could lead to an underestimate of protein kinase activation *in situ*. Inaccuracy could also result from some of the protein kinase residing in a cellular compartment other than the one in which cyclic AMP increases. This kinase, inactive *in situ*, could be activated in the homogenate by cyclic AMP originating from a different cellular compartment. Overestimation of the degree of protein kinase activation *in situ* would result.

Procedures developed by Corbin and co-workers (11) utilize EDTA and 3-isobutyl-1-methyl xanthine to control cyclic AMP hydrolysis. Reassociation of protein kinase subunits is controlled by regulation of the salt concentration of the homogenate (11–13, 19). The concentration of salt used is dictated by the protein kinase isozyme content of the tissue of interest. A 0.5 M concentration of NaCl prevents Type II protein kinase subunit reassociation and is therefore used for tissues in which this isozyme predominates (11). This salt concentration alone dissociates Type I protein kinase holoenzyme (13). However, dissociated Type I protein kinase subunits reassemble much less readily than do Type II subunits, and the activity ratio of Type I-containing tissues is sufficiently stable in the absence of added salt (13). An intermediate salt concentration, 150 mM, has been used for tissues containing both Type I and Type II kinase (19). This concentration was used in the present study because rat parotid gland contains both types of cyclic AMP-dependent protein kinase.[4] In order to obviate the need for the difficult and time-consuming task of separating the minced tissue from the Krebs-Ringer incubation medium, homogenizing medium was made to contain 165 mM NaCl, 20 mM EDTA, and 1 mM 3-isobutyl-1-methyl xanthine. The addition of 1 ml of homogenizing medium to tissue in 1 ml of Krebs-Ringer followed by homogenization produced a homogenate of the desired composition.

Palmer *et al.* (20) have recently demonstrated that the procedures commonly used to extract tissue for activity ratio determinations do not prevent post-homogenization dissociation of protein kinase holoenzyme. Thus, extracts prepared from hormonally stimulated, but not control, tissues were able to dissociate exogenous protein kinase (20). However, the inclusion of charcoal in the tissue homogenization medium was able to prevent this effect. These authors conclude that cyclic AMP is the agent responsible for the dissociation of exogenous protein kinase. This conclusion raises the possibility that hormonally induced increases in the protein kinase activity ratio determined without charcoal in the homogenizing medium may reflect the cyclic AMP concentration during extraction rather than protein kinase activation occurring within the cell (20).

In view of this question, we compared the effect of 2μM isoproterenol on the protein kinase activity ratio of parotid tissue homogenized in the absence and presence of charcoal (10 mg/ml). Charcoal had little effect on the activity ratio of unstimulated tissue, but stimulated tissue gave a lower ratio with charcoal present than in its absence (Fig. 1). Isoproterenol thus resulted in approximately a 2.3-fold elevation of the activity ratio in the absence of charcoal as compared with approximately a 1.4-fold elevation in its presence. These findings suggest that post-homogenization dissociation of protein kinase is a potential problem in rat parotid gland and can lead to an overestimate of protein kinase activation *in situ*. These findings also constitute evidence for compartmentalization of either cyclic AMP or protein kinase in rat parotid gland. The observation that a portion of the protein kinase can be dissociated by cyclic AMP after homogenization implies that some of the kinase was not exposed to cyclic AMP *in situ*. As the activity ratios obtained in the presence of charcoal are believed to

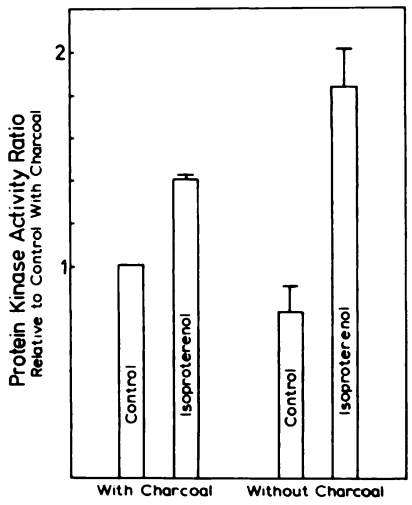

FIG. 1. *Effect of charcoal on basal and stimulated cyclic AMP-dependent protein kinase activity ratios*

Experimental conditions were as described under Materials and Methods except that charcoal was omitted from the homogenizing medium for the indicated samples. Results are shown as the means ± standard error from three independent experiments, in which each treatment was performed three times. In order to compensate for differences in the basal activity ratios among the three experiments, in each experiment the measured ratios were divided by the ratio obtained with control tissue homogenized in the presence of charcoal.

[4] K. Ku and F. R. Butcher, manuscript in preparation.

reflect more accurately the intracellular situation, charcoal was used in all subsequent experiments.

In order to evaluate the efficiency with which the adopted procedures stabilize the activity ratios, extracts were prepared from control and isoproterenol-stimulated tissue and assayed at three different times following tissue homogenization. Both control and stimulated tissue yielded activity ratios that were constant for up to 80 min (Fig. 2). However, it should be noted that 30 min are required to process a reasonable number of samples for assay. The possibility that shifts in the equilibrium between protein kinase holoenzyme and its subunits occur instantaneously upon tissue homogenization has therefore not been excluded.

Basal activity ratios were found to vary somewhat from experiment to experiment. However, isoproterenol elevated the ratio to a similar extent regardless of the basal level. The experiment-to-experiment variability in the measured ratios tended to obscure the effect of isoproterenol when results from more than one experiment were combined. Therefore, results from individual experiments were calculated as relative to control (stimulated activity ratio divided by control activity ratio), and these figures were averaged when combining results from different experiments. In Fig. 2, where the actual activity ratios of both control and stimulated tissue are of interest, results of a single typical experiment are shown.

Isoproterenol was found to increase the protein kinase activity ratio with a time course similar to that previously reported for isoproterenol-induced cyclic AMP accumulation (21). Activity ratios were maximally elevated at 2 min after the addition of isoproterenol, remained high at 5 min, and declined to near-basal levels by 20 min (Fig. 3). The cyclic AMP concentration has previously been

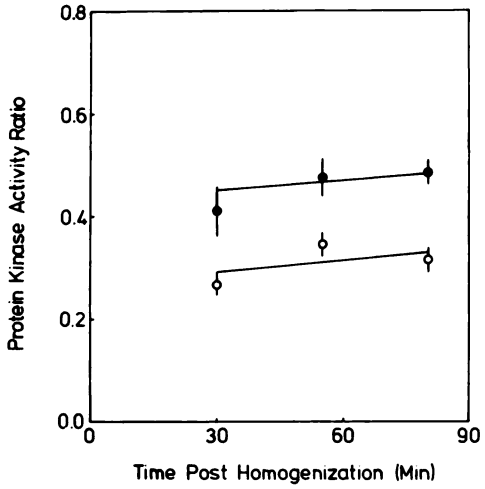

FIG. 2. *Time dependence of measured basal and stimulated cyclic AMP-dependent protein kinase activity ratios*

Tissue was prepared, incubated with (●) or without (○) 2 μM isoproterenol, and extracts were prepared as described under Materials and Methods. The protein kinase activity ratios of the extracts were determined as described under Materials and Methods at the indicated times (expressed as minutes elapsed between the homogenation of the first sample and the initiation of the protein kinase assay). Results shown are the means ± standard error from a single typical experiment in which each treatment was performed in triplicate.

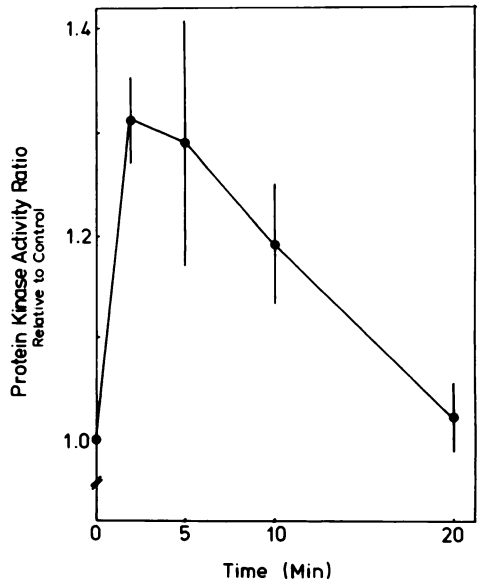

FIG. 3. *Time course of isoproterenol-induced cyclic AMP-dependent protein kinase activation*

Tissue was prepared and preincubated as described under Materials and Methods. Tissue was incubated with 2 μM isoproterenol for the indicated times and extracted, and activity ratios were determined as described under Materials and Methods. Results shown are the means ± standard error of three independent experiments in which each treatment was performed in triplicate.

reported to remain elevated 20 min after the addition of 15 μM isoproterenol (21). However, with 2 μM isoproterenol, as used in the present study, the cyclic AMP concentration returns to near-basal levels within 20 min.[5]

The neurotransmitter specificity of protein kinase activation was found to be the same as that previously reported for cyclic AMP accumulation (21, 22). The elevation of the activity ratio produced by the *beta*-adrenergic agonist isoproterenol was prevented by preincubating the tissue slices with the *beta*-adrenergic antagonist propranolol (Fig. 4). The *alpha*-adrenergic agonist phenylephrine and the cholinergic agonist carbamylcholine were without effect on the activity ratios. Preincubation of tissue with the *alpha*-adrenergic antagonist phentolamine or the cholinergic antagonist atropine did not affect the ability of isoproterenol to elevate the activity ratio.

Although *alpha*-adrenergic and cholinergic agonists produce limited amylase release, they are much less effective than are *beta*-adrenergic agonists (21, 22). Our findings are consistent with the involvement of cyclic AMP-dependent protein kinase in *beta*-adrenergic agonist-induced amylase release, but they indicate that *alpha*-adrenergic and cholinergic agonists must release amylase through a different mechanism.

In order to explore more fully the relationship between *beta*-adrenergic agonist-elevated cyclic AMP concentrations, protein kinase activation, and amylase release, isoproterenol dose-response curves were determined for each of the three parameters (Fig. 5). Isoproterenol maximally stimulated all three at a concentration of 2 μM. Stimulation of amylase release was apparent at doses of

[5] T. N. Spearman, unpublished observation.

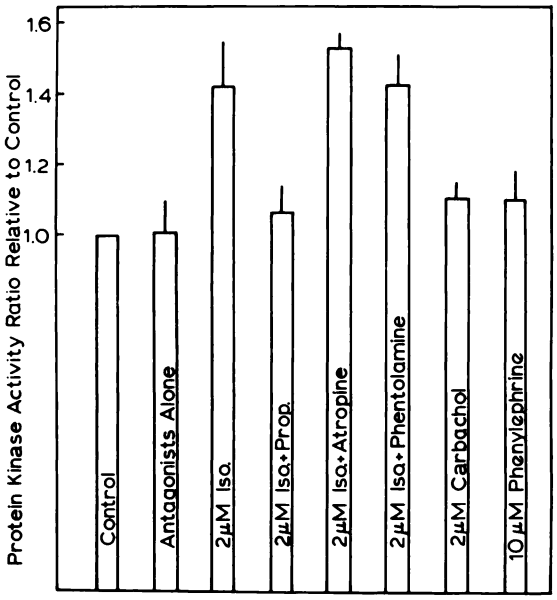

FIG. 4. *Neurotransmitter specificity of cyclic AMP-dependent protein kinase activation*

Tissue was prepared as described under Materials and Methods. Neurotransmitter antagonists were added after the tissue had been preincubated for 15 min. Propranolol (*Prop.*), phentolamine, and atropine were used at a final concentration of 5 μM. After an additional 15-min preincubation, agonists were added as indicated. The tissue was then incubated and extracted, and protein kinase activity ratios were determined as described under Materials and Methods. Results shown are the means ± standard error of three independent experiments in which each treatment was performed in triplicate. *Iso.*, Isoproterenol.

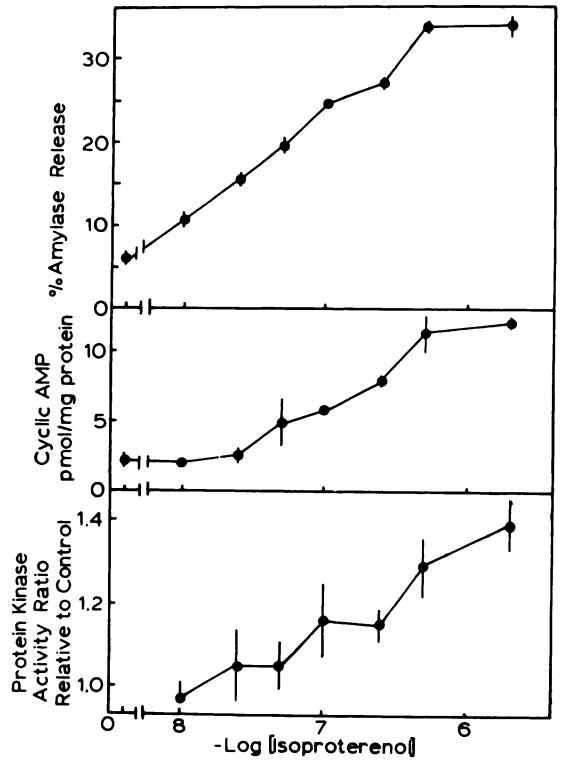

FIG. 5. *Isoproterenol dose response of amylase release, cyclic AMP accumulation, and cyclic AMP-dependent protein kinase activation*

Experimental conditions were as described under Materials and Methods. Amylase release and cyclic AMP levels were each determined in individual single experiments. Results are shown as the means ± standard error of triplicate treatments. Protein kinase activity ratios are shown as the means ± standard error of three independent experiments in which each treatment was performed in triplicate.

isoproterenol lower than those required to increase noticeably cyclic AMP accumulation or protein kinase activation. This dissociation between the cyclic AMP concentration and amylase release at low concentrations of isoproterenol has been previously reported and discussed (21). Several explanations are possible. Amylase release may be sensitive to the cyclic AMP concentration in a particular limited region of the cell. Low levels of stimulation may increase the cyclic AMP at this particular location without detectably increasing the total cell cyclic AMP concentration. Transfer of cyclic AMP from an ineffective to an effective cellular compartment may occur with no net change in the total cyclic AMP level. It may also be argued that, since low concentrations of isoproterenol stimulate amylase release without measurably affecting the cyclic AMP concentration or protein kinase activation, isoproterenol must release amylase through an alternate mechanism. However, the ability of exogenous cyclic AMP derivatives (6) and cholera toxin[6] to induce amylase release from parotid slices *in vitro* constitute strong evidence for cyclic AMP as a regulator of amylase release.

It has recently been reported that combined *beta*-adrenergic and cholinergic stimulation of rat parotid slices *in vitro* resulted in a larger release of amylase than either secretagogue alone (23). We have confirmed this observation and extended it to show that the agumentation is dependent on the concentration of the cholinergic agonist (Table 1). A dose of carbamylcholine submaximal with respect to amylase release (0.5 μM) augmented the effect of a submaximal dose of isoproterenol (0.1 μM). However, a supramaximal carbamylcholine dose (10 μM) partially inhibited the effect of 0.1 μM isoproterenol on amylase release. Although 0.5 μM carbamycholine augmented 0.1 μM isoproterenol-stimulated amylase release, neither

[6] T. N. Spearman and F. R. Butcher, manuscript in preparation.

TABLE 1

Effects of combined beta-adrenergic and cholinergic stimulation on rat parotid amylase release, cyclic AMP levels, and protein kinase ratios

Experimental conditions were as described under Materials and Methods. Amylase release results are shown as the means ± standard error of three independent experiments in which each experiment was repeated three times. Cyclic AMP levels are shown as the means ± standard error of three independent experiments in which each treatment was repeated four times. Protein kinase activity ratios are the means ± standard error of nine independent experiments in which each treatment was repeated three or four times.

Additions	Amylase Release % of total	Cyclic AMP pmoles/mg protein	Protein Kinase Activity Ratio fold basal
none	9.1 ± 3.2	2.58 ± 0.34	1.000
0.1 μM isoproterenol	37.0 ± 4.4	6.89 ± 1.17	1.095 ± .018
0.5 μM carbamylcholine	15.9 ± 3.1	2.65 ± 0.06	1.030 ± .049
10.0 μM carbamylcholine	17.9 ± 3.1	2.24 ± 0.35	1.041 ± .023
0.1 μM isoproterenol + 0.5 μM carbamylcholine	47.1 ± 1.7	5.66 ± 0.55	1.075 ± .027
0.1 μM isoproterenol + 10.0 μ carbamylcholine	29.0 ± 0.6	5.21 ± 0.52	1.183 ± .052

cyclic AMP levels nor protein kinase activity ratios were increased over those seen with 0.1 μM isoproterenol alone. With the high dose of carbamylcholine, protein kinase activation was increased even though cyclic AMP levels were slightly decreased, as was amylase release. The increase in protein kinase activation seen when 10 μM carbamylcholine was added with 0.1 μM isoproterenol which was not accompanied by an increased cyclic AMP concentration may be the result of a transfer of cyclic AMP from a cellular compartment inaccessible to protein kinase to one containing kinase. However, it is clear from this experiment that increased protein kinase activation is not sufficient to increase amylase release in all circumstances and that increased amylase release is not always accompanied by an increase in the protein kinase activity ratio.

The dissociation between the cyclic AMP concentration, protein kinase activation, and amylase release seen in Table 1 may be the result of alterations in the level of another factor believed to be involved in regulating amylase release, i.e., calcium. Isoproterenol is ineffective in stimulating amylase release from parotid slices that have been depleted of intracellular calcium (24), and *beta*-adrenergic agonists stimulate $^{45}Ca^{2+}$ efflux from parotid slices previously preloaded with this tracer (24). The amylase release mechanism may therefore require optimal levels of both calcium and cyclic AMP to operate at maximal efficiency. Carbamylcholine has been shown to increase calcium influx in parotid slices and to mobilize intracellular calcium stores (3). The increased amylase release seen with 0.5 μM carbamylcholine plus 0.1 μM isoproterenol as compared with 0.1 μM isoproterenol alone may therefore be due to carbamylcholine's increasing the intracellular calcium concentration to one closer to optimal for the amylase release mechanism. The inhibition of 0.1 μM isoproterenol-stimulated amylase release by 10 μM carbamylcholine in spite of increased protein kinase activation may indicate that the calcium dependence of the amylase release mechanism is biphasic, with high concentrations of calcium inhibitory to release.

Although protein kinase activation may not be the sole regulator of amylase secretion from the rat parotid gland, the results of the present study are consistent with kinase activation as an important factor in the *beta*-adrenergic release mechanism. However, this and similar protein kinase activity ratio studies are limited in that kinase activity is measured outside the cell with the use of substrates other than the physiologically relevant ones. Protein kinase activity *in situ* can be assessed by labeling the ATP pool of tissue minces with $^{32}P_i$ and assessing protein phosphorylation by electrophoresis and autoradiography (25, 26). A recent report by Jahn *et al.* (25) and experiments recently performed in this laboratory (26) using these methods indicate that *beta*-adrenergic stimulation of rat parotid minces results in an altered phosphorylation state of a limited number of specific phosphoproteins. The identity of these phosphoproteins is currently under investigation.

While this study was in progress we learned of another study demonstrating isoproterenol-induced elevations in the rat parotid cyclic AMP-dependent protein kinase activity ratio (27). Elevations of larger amplitude were observed than in the present study, perhaps due to the fact that tissue was homogenized without charcoal or to the use of a different histone fraction as substrate (27) in the protein kinase assay.

ACKNOWLEDGMENTS

Kevin Hurley, Linda Percy and Rafael Olivas provided technical assistance during the course of this investigation.

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Send reprint requests to: Dr. Fred R. Butcher, Department of Biochemistry, West Virginia School of Medicine, Morgantown, W. Va. 26506.