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Adrenergic Regulation of Cyclic Nucleotide Levels, Amylase Release, and Potassium Efflux in Rat Parotid Gland

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SUMMARY

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Norepinephrine and phenylephrine caused a rapid rise in the level of cyclic 3',5'-GMP in slices of rat parotid gland. The increase in the cyclic GMP level caused by these agents was blocked by phentolamine but not by propranolol or atropine. Isoproterenol increased the cyclic GMP level in the parotid slightly. The stimulatory effect of isoproterenol on the parotid cyclic GMP level was blocked by propranolol. Since *alpha* adrenergic agonists caused a much larger increase in parotid cyclic GMP levels than isoproterenol, the response was classified primarily as an *alpha* adrenergic response. *Alpha* adrenergic agonists and 8-bromo-cyclic GMP caused K^+ release from parotid slices. Isoproterenol and dibutyryl cyclic AMP also caused K^+ release from parotid slices, but were not nearly as effective as the *alpha* adrenergic agonists or 8-bromo-cyclic GMP. Increased cyclic GMP accumulation was not always associated with increased K^+ release from parotid slices. 1-Methyl-3-isobutylxanthine potentiated the effect of limiting concentrations of phenylephrine on cyclic GMP accumulation but did not potentiate the effect of the same limiting concentrations of phenylephrine on K^+ release. *Alpha* adrenergic agonists inhibited the stimulation by isoproterenol of cyclic AMP accumulation. Similarly, isoproterenol reduced the ability of phenylephrine to increase the parotid slice level of cyclic GMP. Amylase release caused by *alpha* adrenergic agonists and isoporterenol was less than additive, whereas the amount of K^+ release caused by these agonists was additive. Thus the lack of additivity between the actions of *alpha* and *beta* adrenergic agonists on cyclic nucleotide levels was not always accompanied by a similar lack of additivity at the level of the physiological response.

INTRODUCTION

It is now apparent that *alpha* and *beta* adrenergic agonists have specific effects on parotid gland physiology. *Beta* adrenergic agonists have been associated with increased cyclic 3',5'-AMP levels and increased release of amylase (1). Batzri *et al.* (2) reported that *alpha* adrenergic agonists caused a specific, massive K^+ efflux from rat parotid slices but did not affect cyclic AMP levels.

In other systems *alpha* adrenergic agonists reduced or prevented increases in

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cyclic AMP levels caused by *beta* adrenergic agonists (3–5). Schultz *et al.* (6) reported that norepinephrine and phenylephrine increased the levels of cyclic 3′,5′-GMP in the rat ductus deferens by an *alpha* adrenergic mechanism. We have previously reported that several adrenergic agonists increased cyclic GMP levels in mouse parotid *in vivo* (7).

Since it was not known whether increased cyclic GMP accumulation is an *alpha* adrenergic response unique to the ductus deferens or is a typical *alpha* adrenergic response observed in a variety of tissues, we extended these studies to the rat parotid. In addition, the effect of *alpha* adrenergic agonists on the level of cyclic GMP in parotid slices was correlated with alterations in the physiological responses exhibited by the rat parotid to *alpha* adrenergic agonists. Effects of adrenergic agonists on cyclic GMP levels similar to some of those reported here have been reported recently by Wojcik *et al.* (8), using slices of rabbit parotid glands.

METHODS

Parotid tissue slices from female Sprague-Dawley rats (CD strain, Charles River Breeding Laboratories) were prepared and incubated in Krebs-Ringer-bicarbonate buffer containing 5 mM β-hydroxybutyrate and 6 mM glucose as described previously (9). The amylase activity was assayed according to Bernfeld (10), except that the reaction was conducted at 37°. One unit of amylase activity was taken as the amount of enzyme which caused the formation of 1 mg of maltose in 5 min. During the early phases of this work some of the amylase release data were expressed as a percentage of the initial total tissue amylase content that was released into the Krebs-Ringer-bicarbonate incubation buffer.

Potassium release studies were conducted in vials containing 1.0 ml of Krebs-Ringer-bicarbonate buffer and the equivalent of one parotid gland. Parotid contains a very active K^+ uptake mechanism which can obscure effects of weak agonists or low agonist concentrations. In order to obviate this problem, 1.0 mM ouabain was added to the incubation vials. The K^+ released was determined with an Instrumentation Laboratory model 143 flame photometer (Boston).

Tissue slice incubations for the determination of cyclic AMP were stopped by adding trichloracetic acid to a final concentration of 5% (w/v). The samples were immediately homogenized with a Polytron PT-10 homogenizer (Brinkmann Instruments) and assayed as described previously (11).

Experiments for determination of cyclic GMP were stopped by adding perchloric acid to a final concentration of 0.5 N, followed immediately by homogenization with a Polytron homogenizer. Tritiated cyclic GMP (0.4 pmole), New England Nuclear) was added to determine recoveries after the purification procedure. Cyclic GMP in the neutralized acid extracts was purified on 5 × 30 mm columns of Dowex 1-X8 (formate). Before sample addition the columns were washed with 5 ml of a solution containing 6 N formic acid and 4 M ammonium formate, followed by a distilled water rinse to neutrality. The samples were applied, and the columns were rinsed with 10 ml of distilled water. A fraction containing cyclic AMP was eluted with 10 ml of 1 N formic acid, and cyclic GMP was eluted with 10 ml of 4 N formic acid. The cyclic GMP fractions were taken to dryness on a Buchler Evapo-Mix flash evaporator and redissolved in 50 mM sodium acetate, pH 6.2. Aliquots were taken to determine recoveries and cyclic GMP content by the radioimmunoassay method of Steiner *et al.* (12).

The total cyclic GMP assay volume was 150 μl. Samples of cyclic GMP or cyclic GMP standards, antigen (Collaborative Research, Waltham, Mass.), and antibody were dissolved separately in 50 mM sodium acetate, pH 6.2, and added in 50-μl aliquots. After the assays had stood overnight in the cold, the bound and unbound antigens were separated by adding 500 μl of charcoal (5.0 mg/ml of Norit, SG extra) suspended in cold 50 mM sodium acetate, pH 6.2, containing 0.5% (w/v) bovine serum albumin (11). Blank extracts of Krebs-Ringer-bicarbonate buffer were prepared for each experiment in the same way

as the tissue extracts. The blank extracts did not alter the standard curve for either cyclic GMP or cyclic AMP.

Some lots of Dowex 1-X8 (formate) contained materials which eluted with cyclic GMP and interfered with the immunoassay. Washing the columns with a solution of 6 N formic acid containing 4 M ammonium formate, before using them to purify cyclic GMP, removed this material. Treatment of the purified extracts with beef heart phosphodiesterase removed more than 95% of the cyclic GMP.

D(−)-Norepinephrine hydrochloride, D(−)-phenylephrine hydrochloride, (±)-isoproterenol, atropine, and dibutyryl cyclic AMP were products of Sigma Chemical Company. *l*- and *d*-Propranolol were gifts from Ayerst Laboratories. Phentolamine was obtained from Ciba. 1-Methyl-3-isobutylxanthine was a product of Aldrich Chemical Company.

RESULTS

Adrenergic regulation of cyclic AMP levels and amylase release. We previously reported that phenylephrine did not affect basal cyclic AMP levels in rat parotid tissue slices (13). In the present study, phenylephrine inhibited the ability of isoproterenol to increase parotid cyclic AMP levels (not shown). The inhibitory effect of phenylephrine was concentration-dependent, with maximal inhibition at 25 μM. The amount of amylase released when phenylephrine and isopreoterenol were added together was less than expected from the amount of amylase release caused by phenylephrine or isoproterenol alone; i.e., their action on amylase release was less than additive (Table 1). Stimulation of amylase release by methoxamine, another *alpha* adrenergic agonist, was not additive with that caused by isoproterenol but was additive with the amylase release caused by dibutyryl cyclic AMP (Table 1). Methoxamine also inhibited the increased cyclic AMP accumulation caused by isoproterenol (Table 1).

Adrenergic regulation of K^+ efflux. We have confirmed the findings of Batzri *et al.* (14), who reported that K^+ efflux from rat parotid slices caused by epinephrine was blocked by *alpha* adrenergic antagonists but not by *beta* adrenergic antagonists or atropine (not shown). The effect of phenylephrine and norepinephrine concentration on K^+ efflux and amylase release is shown

TABLE 1

Effects of phenylephrine and methoxamine with either isoproterenol or dibutyryl cyclic AMP on amylase release and cyclic AMP levels

Parotid slices from two rats were incubated for 45 min with the indicated additions for amylase release. Parotid slices from three rats were incubated with 0.2 μM isoproterenol added simultaneously with the indicated concentrations of phenylephrine and methoxamine for determination of cyclic AMP levels. The reactions were terminated after 5 min. The values are the means ± standard errors for the averages from duplicate incubations in three separate experiments. Basal amylase release was 6.5 ± 1.4%, and the basal value for cyclic AMP was 8.0 ± 2.3 pmoles/mg of protein for the three experiments.

Addition	Increase in amylase release			Increase in cyclic AMP				
	Control	Methoxamine, 20 μM	Phenylephrine 20 μM	Control	Methoxamine		Phenylephrine	
					5 μM	25 μM	5 μM	25 μM
	% basal			% basal	% basal		% basal	
None		110 ±10	190 ±30		9.0 ±7.0	1.0 ±4.0	10 ±5.0	7.0 ±3.0
Dibutyryl cyclic AMP, 0.5 mM	240 ±40	370 ±30	440 ±30					
Isoproterenol, 25 nM	290 ±10	290 ±20	310 ±10					
Isoproterenol, 0.2 μM				700 ±70	430 ±70	30 ±10	390 ±90.0	40 ±20

in Fig. 1. These results indicate that low norepinephrine concentrations markedly increased amylase release while causing only a small increase in K^+ efflux. In contrast, phenylephrine stimulated amylase release and K^+ efflux to the same extent.

Unlike Batzri *et al.* (2), we found that isoproterenol (Table 2) and dibutyryl cyclic AMP (Fig. 2) caused K^+ efflux from parotid slices. The amount of K^+ release caused by isoproterenol and dibutyryl cyclic AMP was much less than that caused by phenylephrine or norepinephrine (compare Fig. 1 and Table 2). The effect of isoproterenol on K^+ efflux was blocked by propranolol but not by phentolamine or atropine (Table 2). In studies not shown, the maximal effect of isoproterenol on K^+ efflux was observed by 5 min and at 10 μM isoproterenol.

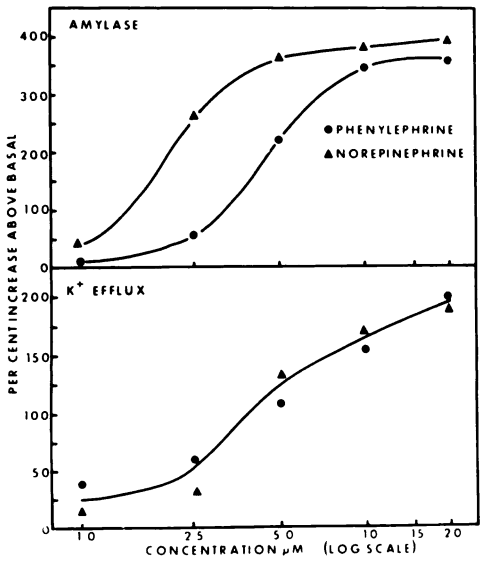

FIG. 1. *Effect of phenylephrine and norepinephrine concentrations on K^+ efflux and amylase release from rat parotid slices*

For each experiment parotid slices from four rats were incubated in 1.0 mM ouabain for 10 min before the addition of the amines. Following addition of the amines, aliquots of the incubation buffer were taken at 10 min for determination of K^+ efflux, and at 40 min for amylase determinations. The values plotted are the means of three separate experiments. The mean basal values from the three experiments for amylase release and K^+ efflux were 47 units and 24.3%, respectively.

Adrenergic regulation of cyclic GMP levels. Increased cyclic GMP levels were detected at 15 sec, and the increase was maximal by 1 min after addition of either phenylephrine or norepinephrine (Fig. 3). Phenylephrine and norepinephrine caused only marginal increases in parotid cyclic GMP levels at 1 μM, and a 100–150% increase was observed at 20 μM agonist (Fig. 3). The increased level of cyclic GMP caused by either phenylephrine or norepinephrine was blocked by phentolamine but not by propranolol or atropine (Table 3).

We observed that injection of isoproterenol and of catecholamine analogues increased the levels of cyclic GMP *in vivo* in mouse parotid glands (7).

The data in Table 4 indicate that isoproterenol also increased cyclic GMP levels in rat parotid slices, and that the increase was blocked by *l*-propranolol but not by *d*-propranolol. The increased cyclic GMP level caused by isoproterenol was much less than that observed with the *alpha* adrenergic agonists. Isoproterenol significantly reduced the increase in the level of cyclic GMP caused by phenylephrine (Table 4).

Effect of exogenous cyclic nucleotides on K^+ efflux. Exogenous cyclic nucleotides were tested for an effect on K^+ efflux and amylase release from parotid slices. Exog-

TABLE 2

Effect of isoproterenol on K^+ efflux from rat parotid slices in the presence and absence of propranolol, phentolamine, and atropine

Parotid tissue slices from four rats were incubated for 10 min with 1 mM ouabain and the blocking agents before the addition of isoproterenol. Aliquots for determination of K^+ efflux were taken 10 min after the addition of isoproterenol. The values are the means ± standard errors from four separate experiments. The mean basal efflux for the four experiments was 22.4 ± 2.3% of the total tissue K^+ content. Propranolol, phentolamine, and atropine alone had no effect on basal K^+ efflux.

Additions	Increase in K^+ efflux
	% basal
Isoproterenol, 10 μM	73.0 ± 12.0
+Propranolol, 10 μM	14.0 ± 2.0
+Phentolamine, 10 μM	69.0 ± 11.0
+Atropine, 10 μM	73.0 ± 10.0

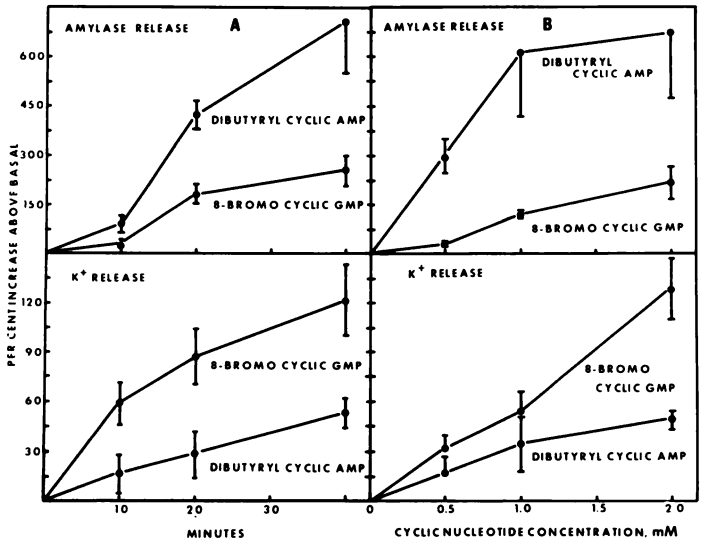

FIG. 2. *Effect of N^6,$O^{2'}$-dibutyryl cyclic AMP and 8-bromo-cyclic GMP on amylase release and K^+ efflux*

For each experiment parotid slices from four rats were used. Aliquots for determination of K^+ efflux and amylase release were taken at the indicated times. The aliquots for determination of amylase and K^+ efflux were all taken at 40 min for experiment B. The concentrations of both dibutyryl cyclic AMP and 8-bromo-cyclic GMP were 2.0 mM for the time course study. The values are plotted as the means ± standard errors of the averages of duplicate incubations from three separate experiments. The mean basal values for amylase release at 10, 20, and 40 min were 27, 37, and 53 units, respectively. The mean basal values at 10, 20, and 40 min for K^+ efflux were 13.5%, 16.3%, and 22.7%, respectively. The mean basal values for amylase release and K^+ efflux were 47 units and 24.5%, respectively, for experiment B.

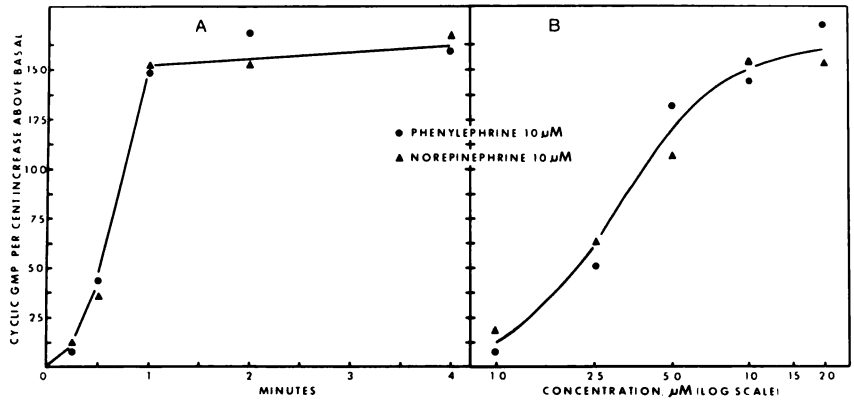

FIG. 3. *Effect of phenylephrine and norepinephrine on parotid slice levels of cyclic GMP*

For each experiment in A and B, parotid slices from four rats were incubated for the indicated times with the amines and then homogenized in 0.5 N perchloric acid. The values are plotted as the means of the averages of duplicate incubations from two separate experiments. Mean basal values for cyclic GMP in experiment A at zero time and 4 min were 365 ± 78 and 312 ± 125 fmoles/mg of protein, respectively. In experiment B the incubations were terminated at 2 min. The mean basal value for cyclic GMP in experiment B was 439 ± 151 fmoles/mg of protein.

enous cyclic GMP or cyclic AMP from 1 pM to 2 mM had no effect on K^+ efflux or amylase release (not shown). On the other hand, both dibutyryl cyclic AMP and 8-bromo-cyclic GMP enhanced K^+ efflux and amylase release. The time courses for the effects on K^+ and amylase release were similar for both cyclic nucleotides (Fig. 2).

TABLE 3

Effects of phenylephrine and norepinephrine on cyclic GMP levels in rat parotid slices in the presence and absence of l-propranolol, d-propranolol, phentolamine, and atropine

Parotid slices from four rats were incubated in the presence and absence of the blocking agents for 10 min before the addition of either norepinephrine or phenylephrine. The tissues were homogenized 2 min after the addition of the amines. The experiments with norepinephrine were a different set from those with phenylephrine. The mean basal value for cyclic GMP in the six sets of experiments was 330 ± 66 fmoles/mg of protein.

Addition	Increase in cyclic GMP		
	None[a]	10 μM norepinephrine[b]	10 μM phenylephrine[b]
		% basal	
None		150 ± 25.0	160 ± 30.0
d-Propranolol, 5 μM	1.0 ± 10.0	150 ± 25.0	180 ± 30.0
l-Propranolol, 5 μM	4.0 ± 20.0	160 ± 20.0	190 ± 30.0
Atropine, 10 μM	1.0 ± 20.0	140 ± 10.0	160 ± 5.0
Phentolamine, 10 μM	−3.0 ± 15.0	−1.0 ± 15.0	20.0 ± 15.0

[a] Means of six separate experiments.

[b] Means of three separate experiments.

TABLE 4

Effect of isoproterenol on cyclic GMP levels in rat parotid slices

Parotid slices from three rats were incubated with the blocking agents for 10 min before the addition of isoproterenol. In experiment 2 isoproterenol and phenylephrine were added simultaneously. Tissues were homogenized 2 min after the addition of isoproterenol. The values are given as the means ± standard errors for five separate experiments for experiment 1, and three experiments in experiment 2. The mean basal values for cyclic GMP were 382 ± 66 and 393 ± 8 fmoles/mg of protein in experiments 1 and 2, respectively.

Additions	Increase in cyclic GMP		
	Expt. 1	Expt. 2	
		Control	Isoproterenol, 20 μM
	% basal	*% basal*	
Isoproterenol, 10 μM	32 ± 10[a]		
+*d*-Propranolol, 5 μM	29 ± 10.0[a]		
+*l*-Propranolol, 5 μM	2 ± 10		
None			38 ± 10
Phenylephrine		173 ± 37	87 ± 32

[a] $p < 0.05$ (two-tailed Student's *t*-test).

Dibutyryl cyclic AMP caused more amylase release than did 8-bromo-cyclic GMP, while the latter caused more K^+ release than did dibutyryl cyclic AMP. 8-Bromo-GMP, sodium butyrate, or sodium butyrate plus 5'-AMP at concentrations equal to those used for the studies presented in Fig. 2A had no effect on amylase release or K^+ efflux (not shown).

1-Methyl-3-isobutylxanthine and effect of phenylephrine on K^+ efflux and cyclic GMP levels. If cyclic GMP mediates the effects of *alpha* adrenergic agonists on K^+ efflux, one would predict that agents which enhance the effect of rate-limiting concentrations of phenylephrine on cyclic GMP accumulation should also enhance the effect of rate-limiting concentrations of phenylephrine on K^+ efflux. The results in Table 5 show that 200 μM MIX[2] enhanced the effect of phenylephrine on cyclic GMP accumulation in parotid slices but had no effect on the stimulation of K^+ efflux by the same limiting concentrations of phenylephrine.

[2] The abbreviation used is: MIX, 1-methyl-3-isobutylxanthine.

TABLE 5

Effect of 1-methyl-3-isobutylxanthine on stimulation of K^+ efflux and cyclic GMP accumulation in rat parotid slices by phenylephrine

Parotid slices from three rats were used for K^+ efflux experiments, and from four rats, for cyclic GMP experiments. Cyclic GMP content was determined at 2 min, and K^+ efflux, at 8 min, after the addition of phenylephrine and MIX. The values are given as the averages from two separate paired experiments with the indicated ranges. The mean basal values for cyclic GMP was 364 ± 55 fmoles/mg of protein, and for K^+ efflux, 8.5 ± 2.2%.

Phenylephrine	Increase in K^+ efflux		Increase in cyclic GMP	
	Control	MIX, 200 μM	Control	MIX, 200 μM
μM	% basal		% basal	
0		−5 ± 24		36 ± 11
2.5	342 ± 11.1	317 ± 25	57 ± 22	125 ± 45
5.0	525 ± 47	640 ± 32	129 ± 39	238 ± 68

DISCUSSION

The findings of Batzri *et al.* (2), with parotid slices, and of Mangos *et al.* (15), with isolated parotid acinar cells, have established that stimulation of massive K^+ efflux by catecholamines is primarily an *alpha* adrenergic response. In the present studies we have confirmed their conclusions and have extended them to demonstrate that *alpha* adrenergic agonist effects on parotid tissue are also associated with elevated parotid cyclic GMP levels. Increased accumulation of cyclic GMP caused by *alpha* adrenergic agonists has also been reported for the rat ductus deferens (6) and rabbit parotid slices (8).

Unlike Batzri *et al.* (2), we detected a slight effect of the *beta* adrenergic agonist isoproterenol and of dibutyryl cyclic AMP on K^+ efflux. This effect on K^+ efflux was apparently specific for the *beta* agonist properties of isoproterenol, since it was blocked by propranolol and not by phentolamine or atropine. The reason why we observed a small stimulatory effect of isoproterenol on K^+ efflux and Batzri *et al.* did not (2) was probably the presence of 1.0 mM ouabain in all our studies on K^+ efflux. The amount of K^+ released at low agonist concentration was greatly reduced if the reuptake of K^+ was not blocked by ouabain (not shown). A small stimulatory effect of isoproterenol and dibutyryl cyclic AMP on K^+ release was suggested by the data in Figs. 5 and 6 of the paper by Batzri *et al.* (2), since the amount of K^+ release caused by *alpha* agonist was greater in the presence of isoproterenol or dibutyryl cyclic AMP.

There are several reports that *alpha* adrenergic agonists inhibit increases in the levels of cyclic AMP caused by other agonists in a variety of target cell types (3–5). In some instances it has been proposed that the reduction of cyclic AMP accumulation caused by *alpha* agonists is responsible for the metabolic responses attributed to the *alpha* agonists (3). If *alpha* adrenergic agonist activity in the parotid is associated with an inhibitory effect on cyclic AMP accumulation, the increased parotid levels of cyclic AMP caused by the *beta* adrenergic agonist isoproterenol should be inhibited by the *alpha* adrenergic agonist phenylephrine. This was the observed result. In addition, the amylase release caused by phenylephrine and isoproterenol was less than additive. It might appear that the amylase release caused by phenylephrine and isoproterenol was less than additive because phenylephrine inhibited the increased cyclic AMP accumulation caused by isoproterenol. However, the correlation between reduced cyclic AMP levels and reduced amylase release may be more apparent than real, since we previously reported that it was possible to completely prevent detectable increases in parotid cyclic AMP levels caused by isoproterenol without a concurrent inhibition of amylase release (13). The idea that not all metabolic effects of catecholamines are correlated with changes in cyclic AMP levels is best illustrated by our previous finding that stimulation of gluconeogenesis in

isolated rat hepatocytes by catecholamines was not correlated with increases in levels of cyclic AMP (16).

Increased K^+ release from parotid slices caused by *alpha* adrenergic agonists might be associated with increased levels of cyclic GMP. Such an idea is supported by the following observations: (a) the time courses and concentration curves for the effects of *alpha* adrenergic agonists on K^+ efflux and parotid cyclic GMP levels were similar, (b) the action of *alpha* adrenergic agonists on cyclic GMP levels and K^+ efflux was blocked specifically by phentolamine, (c) exogenous 8-bromo-cyclic GMP was more effective than dibutyryl cyclic AMP on K^+ efflux, and (d) isoproterenol, which had only a slight effect on K^+ efflux, also caused only a very small increase in parotid cyclic GMP. The argument that cyclic GMP mediates the effect of *alpha* adrenergic agonists on K^+ efflux from the parotid was seriously weakened by our observations that MIX enhanced the stimulatory effects of limiting phenylephrine concentrations on cyclic GMP accumulation while having no effect on the stimulation of K^+ efflux by phenylephrine.

Observations (a), (b), and (d) above only indirectly support a role for cyclic GMP in regulating K^+ release. The finding that high concentrations of exogenous 8-bromocyclic GMP caused a moderate degree of K^+ efflux is also only suggestive of a role for cyclic GMP in K^+ efflux, since 8-bromocyclic GMP could have caused K^+ efflux by indirect effects on parotid gland metabolism. In view of the data obtained in the experiments with MIX and the indirect nature of observations (a)–(d) above, we feel that cyclic GMP does not directly mediate the effects of *alpha* adrenergic agonists on K^+ efflux.

Goldberg *et al.* (17) and George *et al.* (18) suggested that there is a reciprocal relationship between cyclic AMP levels and cyclic GMP levels within a given tissue. Specifically, some authors have observed that increased cyclic GMP levels caused by cholinergic agonists were inhibited by *beta* adrenergic agonists (19, 20). Conversely, the ability of isoproterenol to increase cyclic AMP levels was antagonized by cholinergic agonists (19, 20). In the present study isoproterenol (*beta* adrenergic component) lowered the increased cyclic GMP accumulation caused by phenylephrine (*alpha* adrenergic component). In contrast, norepinephrine, which has both *alpha* and *beta* adrenergic agonist activities, increased cyclic GMP just as effectively as phenylephrine. Moreover, increased cyclic GMP accumulation caused by norepinephrine was not enhanced by propranolol, which blocked the increase in cyclic AMP caused by norepinephrine (13). Schultz *et al.* (6) also reported that a reciprocal relationship between cyclic AMP and cyclic GMP levels does not always exist.

Although isoproterenol reduced the ability of phenylephrine to increase the parotid cyclic GMP level, the K^+ release caused by phenylephrine and isoproterenol was additive (not shown). Batzri *et al.* (2) also observed that isoproterenol did not reduce the ability of *alpha* adrenergic agonists to stimulate K^+ efflux. It appears that the effects of *alpha* and *beta* adrenergic agonists on cyclic nucleotide levels and amylase release are less than additive, whereas the effects on K^+ efflux are additive. In order to explain these differences it will be necessary to learn more about other possible factors which modulate the action of *alpha* and *beta* adrenergic agonists on parotid gland physiology.

The *alpha* adrenergic agonist phenylephrine increased amylase release in addition to increasing K^+ efflux (Fig. 1) (13). We have previously reported that part of the effect of phenylephrine on amylase release was blocked by 10 μM phentolamine (13). In the present study this level of phentolamine completely blocked the stimulation of cyclic GMP accumulation by phenylephrine. The stimulatory effect of phenylephrine on cyclic GMP levels and part of the stimulatory effect of phenylephrine on amylase release (13) could be classified as *alpha* adrenergic effects. This suggests that cyclic GMP might play a role in amylase release, a conclusion also supported by our observation that 8-bromocyclic GMP increased amylase release as well as K^+ efflux. However, the considerations that cyclic GMP might increase amy-

lase release indirectly through effects on other aspects of parotid physiology, or that endogenous 8-bromo-cyclic GMP might increase amylase release by mimicking cyclic AMP, are both possibilities which should not be overlooked. This point is particularly reinforced by the disparity between the effects of 8-bromo-cyclic GMP on K^+ efflux and the effects of MIX on the stimulation of cyclic GMP accumulation and K^+ efflux (compare Fig. 2 with Table 5).

The possibility that cyclic GMP might be involved in various secretory processes has only recently begun to receive attention. Kuo *et al.* (21) reported that acetylcholine increased cyclic GMP levels in isolated pancreatic islets. This observation is particularly important, since cholinergic stimulation is also associated with increased insulin release (22). Kaliner *et al.* (23) reported that exogenous 8-bromo-cyclic GMP mimicked the stimulatory actions of carbachol and phenylephrine on histamine release from lung. Acetylcholine caused enhanced lysosomal enzyme release from human neutrophils and increased cyclic GMP levels (24). The effect of acetylcholine on lysosomal enzyme release was also mimicked by exogenous 8-bromo-cyclic GMP (24). Although these data are very suggestive, any conclusions at this time about the role of cyclic GMP in exocytosis are premature and must await results from additional experimental approaches.

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