Potassium Release Mediated by the Epinephrine α-Receptor in Rat Parotid Slices

PROPERTIES AND RELATION TO ENZYME SECRETION*

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SUMMARY

Epinephrine caused rapid release of intracellular K+ by acting on an α-adrenergic receptor in rat parotid gland slices. Continuous measurement by a K+-selective electrode served as an assay for the α-adrenergic response. The reaction reached a plateau 3 to 5 min after epinephrine addition when 30 to 50% of the K+ which had been in the slices was released. Isoproterenol, which is specific for β-adrenergic receptors and causes amylase secretion from the slices, did not induce K+ release. The concentration of epinephrine which caused a half-maximal K+ release was 15 μM, whereas the concentration which caused a half-maximal rate of amylase secretion in the same system was almost two orders of magnitude lower (0.2 μM).

The hypothesis that the α-adrenergic response is mediated by a reduction in the intracellular cyclic adenosine 3',5'-monophosphate (cAMP) level was tested. Preincubation with isoproterenol which activated the adenyl cyclase did not inhibit K+ release induced by subsequent addition of epinephrine. There was also no inhibition of K+ release when the slices were preincubated with N6-monobutyryl cAMP which acted as an efficient inducer of enzyme secretion in this system. Furthermore, the initial rise in the intracellular cAMP level caused by epinephrine was found to be the same whether or not the α-receptor was blocked by phentolamine. A subsequent more rapid decline of the cAMP level in absence of phentolamine seemed to be due to indirect effects of the α-receptor which causes extensive vacuolation in addition to K+ release. The experiments suggest that the α-adrenergic response is not mediated by changes in the concentration of cAMP in the cell. Electron microscopy showed that phenylephrine which activates the α-adrenergic receptor causes extensive vacuolation of the acinar gland cells. Isoproterenol and N6-monobutyryl cAMP cause fusion of the secretory granules with the cell membrane facing the lumen but no vacuolation. The findings substantiate and extend our previous evidence that both α- and β-adrenergic receptors reside in the same acinar cells. Injection of tyramine and phenylephrine into rats produced larger volumes of saliva than injection of isoproterenol. It is therefore suggested that K+ release mediated by the α-receptor is associated with water transport which sweeps the secretory protein from the acinar lumen into the gland ducts.

The presence in rat parotid gland of an α-adrenergic receptor (α-receptor) which mediates K+ release and a β-adrenergic receptor (β-receptor) which mediates enzyme secretion has been briefly reported in a previous publication (1). An increase in K+ permeability due to the action of catecholamines appears to occur in many tissues (2-5). Ellis and Beckett (2) found extensive release of K+ by the liver in vivo in response to epinephrine and showed that this reaction is inhibited by agents which block α-receptors. Although the above findings indicate that K+ release is mediated by an α-receptor, Friedman and Somlyo (6) reported also on a K+ release from rat liver which appeared to be mediated by a β-receptor.

Studies by Robison et al. (7) leave little doubt that the β-receptor is equivalent to or is part of the epinephrine-activated adenylate cyclase system. In contrast, little is known about the nature of the α-receptor and its mechanism of action. It has been suggested that the α-receptor may function by reducing the cyclic adenosine 3',5'-monophosphate (cAMP) level in the cell (8, 9). A detailed characterization of the α-adrenergic response in a slice system of a noncontractile tissue has apparently not been carried out before. The fact that in parotid gland slices epinephrine activates α- and β-receptors in the same cell, simultaneously and independently (1), also provides an opportunity to study the relationship between the two receptors. Whereas the preceding paper (10) dealt with enzyme secretion activated by the β-receptor, the present paper shows that K+ release is mediated by an α-receptor.

A procedure is described for the continuous monitoring of the α-adrenergic response with an ion-sensitive electrode. It is also shown that changes in the cAMP level in the slices neither inhibit nor mediate the α-adrenergic response. A subsequent paper (11) deals with Cr3+ and energy requirements for the α-adrenergic response.

The abbreviations used are: α-receptor, α-adrenergic receptor; β-receptor, β-adrenergic receptor; cAMP, cyclic adenosine 3',5'-monophosphate; butyryl cAMP, N6-monobutyryl cyclic adenosine 3',5'-monophosphate.
The extent of $K^+$ release varied by +5%.

The initial rate of epinephrine-induced $K^+$ release varied by ~8%.

Efflux due to activation of the $\alpha$-receptor and the influx due to $c$-AMP is an important factor in the regulation of $K^+$ release. In replicate experiments using the same batch of pooled slices, the rate served as the more dependable measure of the $\alpha$-adrenergic response. As will be shown in a subsequent paper (11), the rate was more reliable in the absence of $c$-AMP.

Slices were prepared as described in the preceding paper (10).

Epinephrine caused the release of 50% of the $K^+$ from the slices, when the system has reached a steady state. Usually, 5 min of incubation after hormone addition were required to reach the steady state. The tissue from four glands (about 80 mg of protein) used in a single experiment contained about 40 μeq $K^+$. During the first minutes of preincubation, 15 to 25% of the $K^+$ leaked out from the slices, whereas the remaining amount in absence of epinephrine was retained for at least 60 min. When epinephrine caused the release of 50% of the $K^+$ from the slices, the concentration of $K^+$ in the Krebs-Ringer bicarbonate medium increased from 5 mM to about 8 mM. Measurement of the rate of $K^+$ release is dependent on the rate of diffusion of the ion from the masses of tissue to the electrode. This may explain the empirical finding that the extent of $K^+$ release rather than the rate served as the more dependable measure of the $\alpha$-adrenergic response. As will be shown in a subsequent paper (11), the extent of $K^+$ release represents an equilibrium between the eflux due to activation of the $\alpha$-receptor and the influx due to action of the $Na^+\text{-}K^+$-activated ATPase.

In replicate experiments using the same batch of pooled slices, the initial rate of epinephrine-induced $K^+$ release varied by ±8%. The extent of $K^+$ release varied by ±5%.

**RESULTS**

**Kinetics of Epinephrine-induced Potassium Release**—Addition of epinephrine to the slices caused a release of $K^+$ which could readily be determined over the drift and noise of the system (Fig. 1A). The time lag between hormone addition and ion release was not more than 2 to 3 s, and a new steady state was reached within 3 to 5 min (Fig. 1B). Potassium release as depicted in Fig. 1D follows first order kinetics, whereas epinephrine-induced enzyme secretion in the same slices proceeds as a zero order reaction (10). The lag period between hormone addition and the beginning of potassium release appears to be of the same order of magnitude or less than that required for onset of enzyme secretion (19). With the existing methods, especially in the case of the assay of enzyme secretion, it is difficult to determine more accurately in nitrocellulose test tubes containing 5 ml of fresh medium.
FIG. 1. Time course of epinephrine-induced K⁺ release from rat parotid slices. Release of K⁺ was initiated by addition of epinephrine (arrow) to give a final concentration of 20 μM. A, recorder tracing in presence and absence of epinephrine and also in the absence of slices. The latter system measures the basal noise and drift of the instrumental setup. The drift prior to hormone addition and in the absence of slices was 0.02 mV per min. The net response for the first minute after hormone addition was 0.21 mV. The K⁺ release from the slices to the medium at 6 min was 33% of total. B, kinetics of K⁺ release as percentage of total in the slices. The inset on the lower right shows K⁺ release during the first minute after hormone addition. The points for the semilogarithmic curve were taken arbitrarily from the continuous curve on the upper left.

Fig. 2 Induction of K⁺ release from rat parotid slices by various catecholamines. Catecholamines were added to the medium at zero time to give the following concentrations: epinephrine, 20 μM; norepinephrine, 20 μM; phenylephrine, 50 μM; isoproterenol, 20 μM and 1 μM. Isoproterenol at either concentration did not cause K⁺ release.

exactly the time lapse between addition of hormone and the release of intracellular molecules to the medium.

Catecholamine Specificity in the Induction of Potassium Release

To characterize the adrenergic receptor that mediates potassium release, the reaction was studied by using different catecholamines as inducers. Both epinephrine and norepinephrine effectively induced potassium release (Fig. 2). In contrast, isoproterenol did not cause potassium release even at concentrations which were much higher than those which readily produced enzyme secretion. As with other α-adrenergic systems (20), phenylephrine appeared less potent than epinephrine in causing potassium release. This specificity pattern of the catecholamines indicates that potassium release is controlled by an α-adrenergic receptor (21).

Surprisingly, and contrary to some preliminary findings (1), in many experiments phenylephrine also caused enzyme secretion although it activates β-receptors only to an insignificant extent (20). The following additional findings indicate that phenylephrine caused secretion indirectly by releasing endogenous catecholamines present at nerve endings in the slices (22, 23). Enzyme secretion caused by phenylephrine was blocked by propranolol which is known to inhibit the catecholamine-activated adenylate cyclase of rat parotid (16). However, phenylephrine completely failed to activate the adenyl cyclase when tested on a washed membrane preparation from this tissue (Table I). Amphetamine which has no direct action on α- or β-receptors but displaces catecholamines from nerve endings (20) produced enzyme secretion as efficiently as phenylephrine.

Attempts were made to prepare slices free of endogenous catecholamines by prior injection of reserpine into the animals (24). Unfortunately, such slices did not respond to catecholamines in a consistent manner. In spite of its indirect action on enzyme secretion, it seems likely that phenylephrine acted directly on the α-receptor. As shown below the concentration of hormones which produced a half-maximal α-adrenergic response is relatively high, and it is therefore unlikely that the small amounts of endogenous catecholamine displaced by phenylephrine would be sufficient to cause the K⁺ release.

Extent of Potassium Release and Initial Rates of Enzyme Secretion as Functions of Epinephrine Concentration—The curves shown in Fig. 3 demonstrate that the half-maximal extent of potassium release is obtained at a concentration of 15 μM epinephrine, whereas the half-maximal rate of enzyme secretion is already obtained at 0.2 μM hormone. The latter figure is similar to that which can be roughly estimated from earlier experiments which were carried out under less favorable conditions (25). Although not tested as extensively, norepinephrine had approximately the same affinity for both receptors as epinephrine.

Ultrastructural Characterization of the α- and β-Adrenergic Responses—Previous work (1) on rat parotid slices showed that epinephrine causes fusion of the secretory granules with the cell membrane, resulting in enzyme secretion. Epinephrine also causes formation of large vacuoles associated with K⁺ release.
that isoproterenol which selectively activates $\beta$-receptors does not cause formation of vacuoles which are associated with $\alpha$-receptor activity (Fig. 4). Butyryl cAMP also does not cause vacuole formation. Both isoproterenol and butyryl cAMP lead to the same ultrastructural changes in causing fusion of the secretory granules with the cell membrane facing the lumen. This finding indicates that the action of isoproterenol is confined to elevation of the cAMP concentration in the cell through stimulation of the adenylyl cyclase (7, 16). It is also shown in Fig. 4 that phenylephrine, which selectively activates $\alpha$-receptors, causes widespread vacuolation. The picture is identical to that observed with epinephrine in the presence of the $\beta$-receptor blocking agent propranolol (1). The fact that fusion of the secretory granules with the cell membrane caused by isoproterenol and vacuole formation caused by phenylephrine both occur in acinar cells strongly supports the previous conclusion that $\beta$- and $\alpha$-receptors operate in the same cell independently.

Effect of Isoproterenol and Butyryl cAMP on the $\alpha$-Adrenergic Response—Since it had been proposed (8, 9) that the $\alpha$-adrenergic response is mediated through a reduction in the cAMP level in the cell, experiments were performed to test this hypothesis. Slices were preincubated with isoproterenol which activates the adenylate cyclase (cf. Table I) and causes enzyme secretion. Subsequent addition of epinephrine produced $K^+$ release to an extent even larger than that obtained without preincubation with isoproterenol. The same results were obtained when epinephrine was added at a sub saturating concentration which, on a molar basis, was 10 times lower than the isoproterenol concentration (Fig. 5). In another experiment the level of cyclic nucleotide in the cell was increased independently of the adenylate cyclase activity by addition of butyryl cAMP. Although preincubation with butyryl cAMP (10) caused efficient enzyme secretion, it had no significant effect on the $K^+$ release produced upon subsequent addition of epinephrine (Fig. 6).

Effect of $\alpha$- and $\beta$-Adrenergic Agents on the Adenyl Cyclase System—Specificity of various catecholamines as activators of the adenyl cyclase was studied on a washed membrane preparation. Epinephrine, norepinephrine, and isoproterenol maximally activate the adenylate cyclase at a concentration of 100 $\mu$M. Phenylephrine which is known as a specific $\alpha$-adrenergic agent does not activate the adenylate cyclase (Table I). Epinephrine and norepinephrine which are also potent $\alpha$-adrenergic agents stimulate the adenylate cyclase to the same extent as isoproterenol which has no $\alpha$-adrenergic activity.

The effects of activation of the $\alpha$- and $\beta$-receptors on the cAMP level in the cell was also studied in a slice system. Fig. 7 demonstrates that epinephrine caused the same initial elevation in the intracellular level of cAMP in the presence and absence of phenolamine which blocks the $\alpha$-receptor. Only after longer incubation periods was the level of cAMP higher in the presence of the $\alpha$-adrenergic blocking agent. Since cAMP is the direct product of the reaction catalyzed by the adenylate cyclase, an inhibition of this enzyme through activation of the $\alpha$-adrenergic receptor seems unlikely.

Omission of $K^+$ and Supplements from the Slicing Medium and Its Effect on $K^+$ Release—It is shown in Fig. 8 that the epinephrine-induced $K^+$ release is not affected by omission of $K^+$ from the medium. It is also demonstrated that inosine, adenine, $\beta$-hydroxybutyrate, and nicotinamide have little, if any, effect on the $K^+$ release caused by epinephrine. This is in contrast to the hormone-induced enzyme secretion which is accelerated by the above supplements, apparently by causing an increase in the intracellular ATP concentration.

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**Table I**

**Activation of rat parotid adenylyl cyclase by catecholamines**

Reaction mixtures contained 70 $\mu$g of membrane protein in Experiment I and 100 $\mu$g of protein in Experiment II. Incubation in both experiments was 15 min at 37°. Preparation of membrane fraction (II) which contains the adenylate cyclase activity and assay of the enzyme were as previously described (16).

<table>
<thead>
<tr>
<th>Enzyme activators</th>
<th>Adenylyl cyclase specific activity</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>mmoles/mg protein/min</td>
</tr>
<tr>
<td>None</td>
<td></td>
</tr>
<tr>
<td>Epinephrine</td>
<td></td>
</tr>
<tr>
<td>0.01</td>
<td>0.47</td>
</tr>
<tr>
<td>0.1</td>
<td>0.47</td>
</tr>
<tr>
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<td>0.50</td>
</tr>
<tr>
<td>Isoproterenol</td>
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<tr>
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<td>0.44</td>
</tr>
<tr>
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<tr>
<td>Phenylephrine</td>
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<td>0.01</td>
<td>0.00</td>
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<tr>
<td>0.03</td>
<td>0.00</td>
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<tr>
<td>0.1</td>
<td>0.00</td>
</tr>
<tr>
<td>NaF, 10.0</td>
<td>1.43</td>
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</tbody>
</table>

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**Fig. 3.** Dependence of $K^+$ release and amylase secretion on epinephrine concentration. To preserve the low concentrations of hormone in the experiment of amylase secretion, all of the systems contained 0.5 mM dithioerythritol.

By the use of epinephrine in combination with specific adrenergic blockers, it was possible to show that fusion of the granules to the plasma membrane is caused by activation of the $\beta$-receptor and that vacuole formation is associated with activation of the $\alpha$-receptor. Electron micrographs indicated that the vacuoles in the cells originate from the Golgi vesicles (1, 26). These observations are further substantiated in the present studies. It is shown...
FIG. 4. Ultrastructural changes in rat parotid slices caused by isoproterenol butyryl cAMP and phenylephrine. A, control slices incubated for 10 min in absence of hormone. Groups of cells situated around a common lumen (L) are seen. The lumen appears empty and small, about the size of a secretory granule (g). B, B1, B2, slices incubated for 10 min in presence of 20 μM isoproterenol. B, the lumen (L) is filled with secretory material and is already quite enlarged as compared to A. The lumen appears to have grown through fusion with the secretory granules (fg). B1, B2, detail of lumens fused with secretory granules. fg1 and fg2 are secretory granules fused in sequence (18) to the lumen. The desmosomes (d) delineate the cell borders which form the common lumen. C, slices incubated for 20 min with 1 mM butyryl cAMP. The structural changes resemble those obtained with isoproterenol. Enlarged lumens filled with secretory material are seen. D, slices incubated for 10 min in presence of 20 μM phenylephrine. In contrast to isoproterenol and butyryl cAMP, phenylephrine causes appearance of numerous vacuoles (V) which distort the shape and structure of the cells. The right side of the micrograph shows extensive vacuolation which appears to have destroyed the major cellular structures. A few constricted lumens can be observed. Incubation of slices and processing for electron microscopy are given under “Methods.”
In Vivo Stimulation of Rat Parotid Gland by Phenethylamines—

In search of the physiological role of the α-adrenergic response in the rat parotid, the glands were stimulated by intraperitoneal injection of various phenethylamines into anesthetized rats. The secreted saliva was collected by cannulating the duct leaving the parotid gland, and the flow rate and protein content were determined. Stimulation by the β-adrenergic agent isoproterenol resulted in secretion of highly concentrated protein in a relatively small volume of saliva. On the other hand, stimulation with an α-adrenergic agent, phenylephrine, caused a much higher flow rate of saliva with a low protein concentration. Tyramine, which is known to displace endogenous catecholamines from nerve terminals (20) gave rise to a somewhat higher flow rate and a lower protein concentration than that obtained by isoproterenol (Table II). It can be tentatively concluded from these experiments that stimulation of the α-adrenergic receptor results in secretion of a larger volume of saliva.

**TABLE II**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Flow (mg saliva/gland/15 min)</th>
<th>Protein concentration (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isoproterenol, 1 mg</td>
<td>11.4 ± 1.3</td>
<td>192 ± 6.4</td>
</tr>
<tr>
<td>Tyramine, 10 mg</td>
<td>14.2 ± 2.5</td>
<td>131 ± 7.7</td>
</tr>
<tr>
<td>Phenylephrine, 2 mg</td>
<td>37.5 ± 8.7</td>
<td>31.5 ± 3.3</td>
</tr>
</tbody>
</table>

**DISCUSSION**

The present and recently published experiments (1) clearly show that the epinephrine-induced K⁺ release in rat parotid slices is due to activation of an α-adrenergic receptor. Norepineph-
rine, epinephrine, and phenylephrine which are capable of activating \(\alpha\)-receptors cause \(K^+\) release, whereas isoproterenol which is specific for \(\beta\)-receptors (20) is inactive even at high concentrations. Phenolamine which selectively inhibits \(\alpha\)-receptors completely blocks the epinephrine-induced \(K^+\) release (1, 11), whereas propranolol which specifically blocks \(\beta\)-receptors (20) has no effect. The same exocrine parotid cells also contain a \(\beta\)-receptor which activates enzyme secretion via the adenylate cyclase system (16). Since the relationship between the activities of \(\alpha\)- and \(\beta\)-receptors seems of importance for catecholamine responsive tissues, we attempted to study both activities in the same system under various conditions. As is shown in an accompanying paper (10), activation of the \(\alpha\)-receptor which results in \(K^+\)-release had little effect on the initial rate of enzyme secretion which was controlled by the \(\beta\)-receptor. Also, blocking the \(\beta\)-receptor with propranolol seemed not to affect the rate and extent of \(K^+\)-release (1). The present work furthermore shows that during short incubation periods the increase in intracellular cAMP levels caused by epinephrine is not significantly affected by inhibition of the \(\alpha\)-receptor. During this early period a large proportion of the total \(K^+\) has already been expelled from the cell through the action of the \(\alpha\)-receptor. It is therefore suggested that the \(\alpha\)-receptor does not exert its primary effect by decreasing the cAMP level in the cell. The faster decline in the cAMP level in the absence of phenolamine may well be an indirect result of the large \(K^+\) loss and vacuole formation resulting from activation of the \(\alpha\)-receptor.

Additional experiments indicate that the action of the \(\alpha\)-receptor in causing \(K^+\) release is not inhibited by increased levels of cAMP in the cell. Thus it is shown that prior incubation with isoproterenol which activates the adenylate cyclase even caused enhancement of \(K^+\) release upon subsequent addition of epinephrine. Similarly, butyryl cAMP which penetrates into the cell and efficiently induces enzyme secretion does not inhibit the \(K^+\) release caused by epinephrine. All of the above observations lead to the notable conclusion that \(\alpha\)- and \(\beta\)-receptors residing in the same cell can respond simultaneously and independently of each other. In light of these findings, the previous interpretation (8, 9), i.e. the \(\alpha\)-receptor operates mainly as an antagonist of the \(\beta\)-receptor by reducing the cAMP level in the cell, should be reconsidered.

It needs to be emphasized that, although the effect of the \(\alpha\)-receptor on the \(\beta\)-adrenergic response is probably indirect, it can nevertheless have extreme consequences. Thus it was found that the rate of amylase secretion rapidly declines and the process stops prematurely when the \(\alpha\)-receptor is activated simultaneously with the \(\beta\)-receptor (10). Even more extreme is the case of epinephrine-induced insulin secretion where the process seems almost completely inhibited unless the \(\alpha\)-receptor is blocked (8). It would therefore be of great interest to learn whether in the intact gland the rate of enzyme flow from the lumen of the acinus into the duct might depend on water secretion. Therefore, to secure a higher rate of enzyme secretion in the intact gland the \(\alpha\)-adrenergic response would also have to be increased. The dual action of one hormone on two independent receptors in the same gland cell may not be limited to the catecholamines. It is well known that two different receptors for acetylcholine are recognized, the nicotinic and the muscarinic (28). It would therefore be of great interest to learn whether both receptors have a function in exocrine pancreas, in the adrenal medulla, and in other glands in which acetylcholine is the inducer of secretion.

Addendum—The finding that the \(\alpha\)-adrenergic receptor requires a higher catecholamine concentration than the \(\beta\)-adrenergic receptor is not unique for the rat parotid gland. The same relationship between the two catecholamine receptors has recently been reported for frog skin epithelium (29). In this system a low epinephrine concentration activates the \(\beta\)-receptor to cause \(Na^+\) transport, whereas a high epinephrine concentration activates the \(\alpha\)-receptor to bring about an inhibition of \(Na^+\) transport.

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