MOLECULAR PHARMACOLOGY, 18, 356–361

α-Adrenergic Regulation of Cholinergic Responses in Rat Parotid Acinar Cells

JAMES N. DAVIS, EDWARD OLENDER, WENDY MAURY AND ROBERT MCDANIEL

Neurology Research Laboratory, Durham Veterans Administration Medical Center, and Departments of Medicine and Pharmacology, Duke University, Durham, North Carolina 27705

Received February 20, 1980; Accepted June 30, 1980

SUMMARY

DAVIS, J. N., E. OLENDER, W. MAURY AND R. MCDANIEL. α-Adrenergic regulation of cholinergic responses in rat parotid acinar cells. *Mol. Pharmacol.* 18: 356–361 (1980).

α-Adrenergic or muscarinic cholinergic stimulation leads to a release of K^+ from dispersed parotid acinar cells. Cells exposed to (−)-epinephrine at physiological pH in Hepes medium and then washed become refractory or densensitized to subsequent α-adrenergic challenge with (−)-epinephrine and to cholinergic challenge with carbachol. This α-adrenergic-elicited attenuation of the cholinergic response appears to result from a decrease in Ca^{2+} entry during cholinergic challenge since A23187 incubation releases as much K^+ in desensitized cells as in washed controls. This desensitization occurs during (−)-epinephrine preincubation in the absence of Ca^{2+} and without K^+ release. It is prevented if phentolamine is present. The desensitization is rapid, being complete in 2 min. Desensitization is partly reversible by incubation in fresh buffer or in the presence of phentolamine; it is completely and rapidly reversed by exposure to a high-K^+ medium. Although the cholinergic response is attenuated after α-adrenergic stimulation, the binding of [^{3}H]quinuclidinyl benzilate is the same in these cells as in washed controls. These data suggest that the attenuated cholinergic response in α-adrenergic desensitized cells results from an alteration between cholinergic receptor occupation and Ca^{2+} entry. This type of α-adrenergic desensitization differs from a type described previously in these cells at lower pH in Hanks' medium. Although both types may take place at physiological pH levels, this α-adrenergic regulation of the cholinergic response is clearly an important cellular response to persistent stimulation. Since other tissues and presynaptic nerve terminals appears to have α-adrenergic and muscarinic cholinergic receptors, this regulation may be an important mechanism of adaptation to continued neuronal activity.

INTRODUCTION

Parotid acinar cells have proved a useful model for studying receptor-mediated cellular responses. A homogeneous dispersion of these cells can be prepared from fresh rat parotid glands. The cells release K^+ when exposed to α-adrenergic or muscarinic cholinergic agents (1, 2) or to several peptides including substance P (3, 4). The α-adrenergic (5) and muscarinic cholinergic (6) membrane receptors mediating K^+ release have been identified and characterized by radioligand binding techniques.

The work was supported by grants from the Veterans Administration (1680) and the NIH (NS 06233). Ed Olender was a postdoctoral fellow of the Duke Aging Center (AG 00029). The expert secretarial assistance of Ms. Nanci DeMarco is gratefully acknowledged. Portions of this work were presented at the International Pharmacology Congress in Paris, July 1978, and at the Society for Neuroscience Meeting, November 1979.

We previously described a desensitization of the α-adrenergic response of parotid acinar cells after persistent α-adrenergic stimulation (7). In those experiments cells were exposed to α-adrenergic agonist, epinephrine, and then washed and rechallenged with epinephrine. When rechallenged, cells preincubated with epinephrine released less K^+ than washed controls. Since concurrent reductions in α-adrenergic membrane receptor binding were noted in the desensitized cells and since cholinergic stimulation of the desensitized cells produced a full response we concluded that this α-adrenergic desensitization was mediated, at least in part, by a reduction in membrane receptors (7). Furthermore when the desensitized cells were exposed briefly to high concentrations of K^+, there was a restoration of α-adrenergic response and receptor binding.

We have subsequently noted that this α-adrenergic desensitization was taking place at pHs from 6.0 to 6.5 since the cell medium (Hanks') was only weakly buffered

0026-895X/80/060356-06$02.00/0

and the epinephrine used to desensitize the cells was in the hydrochloride form. Although α-adrenergic desensitization was consistently observed in cells exposed to epinephrine at lower pHs, we now report that cells incubated with epinephrine at physiological pHs exhibit a second type of α-adrenergic desensitization distinct from that previously described. The experiments described in this paper were undertaken to characterize this second type of α-adrenergic desensitization.

METHODS

Materials. [^{3}H]Dihydroergocryptine (DHE)[1] and [^{3}H]quinuclidylbenzilate (QNB) were obtained from New England Nuclear. (−)-Epinephrine (E), carbamylcholine (carbachol), and hyaluronidase were obtained from Sigma Chemical Company and collagenase was from Worthington Biochemical. A23187 was the gift of Eli Lilly Company, while phentolamine was donated by Smith, Kline and French. All other materials were the finest grade commercially available.

Cell preparation and K^+ measurements. Cells were prepared as previously (5). Fresh parotid glands were minced and incubated with collagenase and hyaluronidase for 1 h with frequent mechanical agitation. At the end of the incubation the cells were filtered through nylon mesh and washed by gentle centrifugation. Cells were routinely kept at 37°C in an atmosphere of 95% oxygen, 5% CO_2. Two buffers were used. The first, called "Hanks'" medium in this paper, was 136.7 mM NaCl, 5.4 mM KCl, 0.81 mM $MgSO_4 \cdot 7H_2O$, 1.3 mM $CaCl_2$, 0.33 mM Na_2HPO_4, 0.44 mM KH_2PO_4, 5.6 mM dextrose, 4.2 mM $NaHCO_3$ adjusted to pH 7.4 at 37°C. The second buffer has been called "Hepes" medium in this paper and was similar to Hanks' medium except that the Na_2HPO_4, KH_2PO_4, and $NaHCO_3$ were replaced by 20 mM Hepes (Calbiochem). Hepes medium was also adjusted to pH 7.4 at 37°C. Cells were routinely dispersed in Hanks' medium with the Ca^{2+} and Mg^{2+} omitted (5) and then transfered to regular Hanks' or Hepes medium.

Potassium was measured by atomic absorption spectrometry. Cells were suspended in 10 vol of either Hanks' or Hepes oxygenated medium. Of this cell suspension 25 μl was pipetted into a microcentrifuge tube containing 25 μl of appropriate medium with or without drug. The tubes were gently mixed, incubated for 30 s at room temperature in air, and then centrifuged for 30 s in a Beckman microfuge. The supernatant (25 μl) was placed in 1.0 ml of an Acationox (Scientific Products) solution (5) and the concentration of K^+ was measured with a Perkin–Elmer atomic absorption spectrophotometer. Net K^+ release was calculated from the amount of K^+ in the supernatant of stimulated cells less the amount of K^+ in the supernatant of unstimulated cells. Total cellular K^+ was calculated from the K^+ content of 25 μl of an uncentrifuged cell suspension less the amount of K^+ in the supernatant of unstimulated cells. Total cellular K^+ contents determined in this way were no different than those determined using the supernatant from repeatedly freeze–thawed cells.

Desensitization experiments. Desensitization was produced by incubating cell suspensions with 100 μM (−)-epinephrine for 15 min in a 95% oxygen, 5% CO_2 atmosphere, while controls were incubated in buffer alone. Cells were then washed four times by centrifugation and resuspension in fresh, oxygenated buffer to remove the epinephrine. The washed cells were challenged with either carbachol or epinephrine by adding the drugs directly to the cell suspension for 30 s, centrifuging, and measuring the K^+ concentration in the supernatant. In some experiments phentolamine was added directly to the preincubation medium at various times to stop the epinephrine-induced desensitization. In other experiments washed, desensitized, and control cells were used in radioligand binding experiments to determine the number of receptor binding sites in these cells.

Radioligand binding experiments. Binding of [^{3}H]-DHE and [^{3}H]QNB to dispersed parotid cells was measured as previously described (5). Incubations in a final volume of 150 μl were carried out at 20°C for either 20 min ([^{3}H]DHE) or 45 min ([^{3}H]QNB) and consisted of approximately 10^6 cells in Hepes buffer. Incubations were stopped by addition of 2.0 ml of Hepes buffer, followed by rapid filtration through two Schleicher and Schuell glass fiber filters (No. 25) under vacuum and washing with 30 ml of room-temperature Hepes buffer. The filters were then placed in 10 ml of a toluene/Triton scintillation fluid and counted.

Specific binding was determined by measuring the total binding of the ligand to the cells and subtracting the binding observed in parallel incubations in which either 10 μM phentolamine ([^{3}H]DHE) or 1 μM atropine ([^{3}H]QNB) was present. In this paper the term receptor binding is used to denote the specific binding of one of these radioligands.

RESULTS

Parotid acinar cells desensitized by exposure to epinephrine in Hanks' medium were different from cells exposed to epinephrine in Hepes medium (Fig. 1). In agreement with our earlier study (7), cells desensitized in Hanks' medium demonstrated an attenuated response when rechallenged (in Hanks' medium) with epinephrine, but not when challenged with carbachol, a muscarinic cholinergic agonist. This α-adrenergic desensitization in Hanks' buffer was due to the presence of epinephrine in the incubation since desensitization did not occur in the presence of phentolamine (7) nor did it occur in medium buffered to pH 6.0 without epinephrine (data not shown). By contrast cells prepared at the same time but desensitized in Hepes medium had diminished responses when rechallenged with either carbachol or epinephrine. This second type of desensitization was not influenced by Hepes in the medium since cells exposed to epinephrine in Hanks' medium with the pH adjusted to 7.4 also showed diminished response to rechallenge with either carbachol or epinephrine.

[1] Abbreviations used: DHE, dihydroergocryptine; QNB, quinuclidylbenzilate; E, epinephrine; Hanks' medium, 136.7 mM NaCl, 5.4 mM KCl, 0.81 mM $MgSO_4 \cdot 7H_2O$, 1.3 mM $CaCl_2$, 0.3 mM Na_2HPO_4, 0.44 mM KH_2PO_4, 5.6 mM dextrose; 4.2 mM $NaHCO_3$, adjusted to pH 7.4 at 37°C; Hepes medium, similar to Hanks' medium except that Na_2HPO_4, KH_2PO_4, and $NaHCO_3$ are replaced by 20 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; EGTA, ethylene glycol bis(β-amino ethyl ether) *N,N'*-tetraacetic acid.

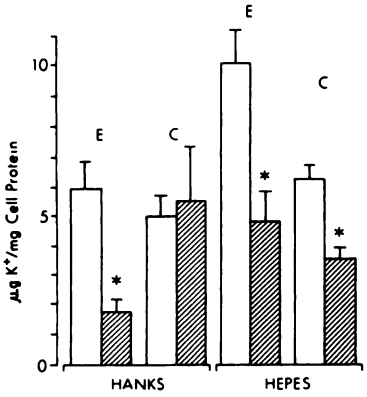

FIG. 1. *α-Adrenergic desensitization of parotid acinar cells in two different media*

Parotid acinar cells were preincubated with either Hanks' or Hepes medium with (▨) and without (□) 100 μM (−)-epinephrine, washed, and then rechallenged with either 1 mM (−)-epinephrine (E) or 1 mM carbachol (C) in either Hanks' or Hepes medium as described under Methods. The μg K^+ released per mg cell protein was measured 30 s after the rechallenge began. Statistical significance ($^*P < 0.01$) was calculated by first carrying out an analysis of variance (F test) followed by a Student's t test ($^*P < 0.005$ compared to buffer-preincubated controls). Each bar represents the mean of seven to eighteen determinations while each vertical line represents one SEM. This experiment was replicated twice with similar results.

α-Adrenergic and muscarinic cholinergic receptors appear to mediate release of K^+ in the parotid by causing the entry of Ca^{2+} into the cell (8, 9). In fact acinar cells release K^+ when they are exposed to A23187, an ionophore that facilitates Ca^{2+} entry into cells (9, 10). Although cells desensitized by exposure to epinephrine in Hepes medium demonstrated a diminished response to subsequent adrenergic or cholinergic challenge, they released as much K^+ when exposed to A23187 as washed controls (Fig. 2). The full response of the desensitized cells to A23187 suggests that the cell's ability to release K^+ remained undistrubed by the preincubation with epinephrine.

Exposure of acinar cells to epinephrine in Hepes medium clearly evoked a type of desensitization different from that seen when cells were exposed to epinephrine in Hanks' medium. In order to further characterize this second type of desensitization, cells were exposed to epinephrine in Hepes medium, washed, and then challenged with carbachol. Figure 3 demonstrates the effects of several drugs added during the epinephrine desensitization on the subsequent carbachol challenge. When EGTA was added, the epinephrine preincubation was carried out in Hepes medium without Ca^{2+}. Under these conditions no release of K^+ takes place during the preincubation. Even though no release of K^+ took place, the muscarinic response was still attenuated after the cells had been washed and were put in Hepes medium with Ca^{2+}. The EGTA was clearly removed by washing since washed control cells which had been exposed to EGTA released as much K^+ as control cells which had been incubated in regular Hepes medium (Fig. 3).

By contrast the presence of the α-adrenergic antagonist, phentolamine, in the preincubation with epinephrine prevented the desensitization of the cholinergic response (Fig. 3). Thus it appears that epinephrine must interact with the α-adrenergic receptor to produce attenuation of the cholinergic response. Tetracaine, a local anesthetic, has been reported to inhibit α-adrenergically elicited K^+ release in parotid slices (9). Although tetracaine did not prevent epinephrine-induced desensitization of the cholinergic response (Fig. 3), it should be noted that in contrast to reports in slices tetracaine only partly blocked the release of K^+ by epinephrine in control dispersed cells (data not shown).

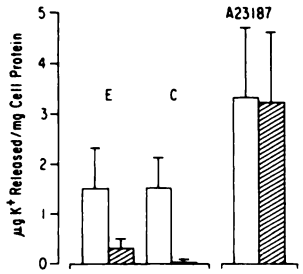

FIG. 2. *Comparison of K^+ release by the inonphore, A23187, with adrenergic and cholinergic stimulation in desensitized cells*

Parotid acinar cells were preincubated either with (▨) or without (□) 100 μM (−)-epinephrine in Hepes medium. After washing, the cells were incubated in Hepes buffer without Ca^{2+} but containing either 1 mM (−)-epinephrine (E), 1 mM carbachol (C), or 1 μg/ml A23187. Cells were incubated for 5 min and K^+ release was triggered by the addition of Ca^{2+}. K^+ release was measured 1 min later as described under Methods. Each bar represents the mean of five or six experiments carried out in triplicate while each vertical line represents one SEM. The attenuated response to carbachol in the desensitized cells was statistically significant compared to that of buffer-preincubated controls ($P < 0.05$, statistics as in Fig. 1). There was no statistically significant difference between the A23187-treated cells. It should be noted that the absolute release of K^+ in these experiments was consistently lower than when cholinergic or adrenergic drugs were added to Ca^{2+}-containing media.

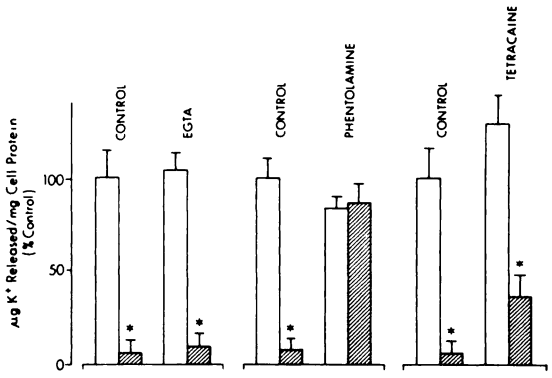

FIG. 3. *The effect of various treatments of α-adrenergic desensitization of parotid acinar cells*

Parotid acinar cells were preincubated with (▨) and without (□) 100 μM (−)-epinephrine in Hepes medium, washed, and then rechallenged with 1 mM carbachol. In each experiment, cells were preincubated with or without either EGTA, phentolamine, or tetracaine. Since the absolute amount of K^+ released in the control preparations varied (5.3 μg K^+/mg protein, tetracaine; 9.2, EGTA; 11.7, phentolamine), the data were normalized with the release of washed controls set to 100%. However, statistics were calculated on the absolute K^+ release in each experiment. Each bar represents the mean of at least three experiments carried out in triplicate while each vertical line represents one SEM normalized to a percentage. Statistical significance was calculated as in Fig. 1 ($^*P < 0.001$).

Since the presence of phentolamine blocked epinephrine-induced desensitization of the cholinergic response, phentolamine was used to study the time course of this α-adrenergic desensitization. Phentolamine was added at various times after the beginning of an epinephrine preincubation. The cells were preincubated for a total of 15 min, washed, and then challenged with carbachol. A zero time point was obtained by adding phentolamine before the epinephrine preincubation. The K^+ released by carbachol in these "zero time point" cells was no different than the K^+ released from cells incubated in Hepes medium alone. However, the attenuation of the carbachol response occurred very rapidly after the epinephrine addition (Fig. 4), being complete by 2 min of incubation. This rapid time course was fitted to a first-order kinetic model and a $t_{1/2}$ of 17 s (rate constant (k), 0.04 s^{-1}) calculated.

Once cells were exposed to epinephrine in Hepes buffer, full restoration of carbachol responses was not observed even in washed cells incubated in fresh, drug-free buffer for up to 3 h. Although the absolute amount of carbachol desensitization varied from preparation to preparation, after washing and incubation in drug-free buffer, cells exposed to epinephrine demonstrated about 60% of the carbachol response seen in control washed, incubated cells. Immediately after washing, less than 10% of the control response was present on carbachol challenge. However, within 15 min, epinephrine-treated cells had approximately 60% of the response of control cells and no further change in responsiveness was seen even in prolonged incubations (data not shown).

Thus the resensitization response appeared to consist of at least a rapid and a slow component. To further study the rapid component, cells were incubated in epinephrine for 15 min and then phentolamine was added. Aliquots of cells were challenged with carbachol at various times after the phentolamine addition. The rapid phase of recovery of the carbachol response (Fig. 5) was

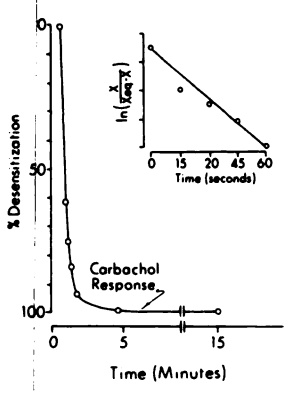

FIG. 4. *Time course of α-adrenergic desensitization of the cholinergic response in parotid acinar cells*

Parotid acinar cells were preincubated with 100 μM (−)-epinephrine in Hepes medium and phentolamine was added at various times as described under Results. After 15 min of incubation, cells were washed and challenged with 1 mM carbachol. K^+ release was measured and represented 6.9 μg K^+/mg protein in the controls and zero time points (phentolamine added before (−)-epinephrine). 100% desensitization was taken as the response at 15 min, 1.3 μg K^+/mg protein. Each point represents the mean of eight determinations and is expressed as percentage desensitization. The inset is a semilogarithmic plot of the data for the first minute of preincubation.

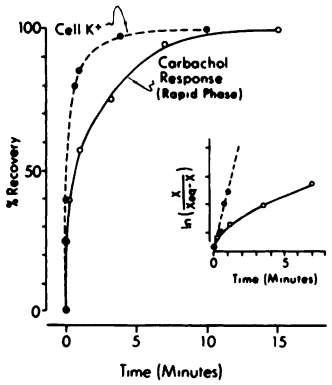

FIG. 5. *Time course for the rapid rate of recovery of carbachol-induced K^+ release after α-adrenergic desensitization*

Parotid acinar cells were preincubated with 100 μM (−)-epinephrine in Hepes medium for 15 min. 10 μM phentolamine was added to the incubation at zero time and the amount of intracellular K^+ (cell K^+, ●) and the amount of K^+ released by challenge with 1 mM carbachol (carbachol response, ○) measured. Each point represents the mean of eight determinations. The data were normalized to percentage recovery with the total cellular K^+ at 10 min being set to 100% recovery (dashed line). 100% recovery for carbachol release (solid line) was chosen as the amount of K^+ released by carbachol 15 min after phentolamine addition. It should be noted that the amount of K^+ released 15 min after the addition of phentolamine was only 60% of the K^+ released from buffer-preincubated controls and that percentage recovery in this case refers only to the rapid phase. Furthermore the data are presented as percentage recovery to allow comparison of the rates. 100% recovery of the total K^+ refers to 10.1 μg K^+/mg protein while 100% recovery of the rapid rate refers to 2.8 μg K^+/mg protein released by carbachol. The inset is a semilogarithmic plot of the data.

complete by 15 min with an approximate half-life of 2 min. This recovery process did not appear to follow simple first-order kinetics (Fig. 5) but was initially more rapid. Since the cellular K^+ was depleted by the exposure to epinephrine, K^+ was reaccumulated by the cells when phentolamine was added to the incubations. This reaccumulation of K^+ was complete in 2 min, roughly followed first-order kinetics (Fig. 5), and probably accounts for the very rapid component of the recovery phase. The rapid phase of recovery of the carbachol response was clearly slower than the rate of total cellular K^+ reaccumulation (Fig. 5).

Although complete resensitization was not observed in cells incubated in drug-free buffer, desensitization was reversible. When epinephrine-treated cells were exposed for 5 min to a 50 mM K^+ Hepes buffer and then washed, the carbachol response was restored to the level seen in control, washed cells (Fig. 6). It should be noted that the total cellular K^+ of the desensitized cells was not affected by the exposure to a high-K^+ buffer.

Parotid acinar cells rapidly lost their response to carbachol during persistent stimulation by epinephrine. The attenuated cholinergic response appeared to result from a decreased entry of Ca^{2+} into the cells during exposure to carbachol. In order to determine if α-adrenergic stimulation altered the muscarinic cholinergic membrane receptor, the binding of [^{3}H]QNB was studied. [^{3}H]QNB appears to bind to muscarinic cholinergic receptors in a variety of tissues (11) including the rat parotid (6). The ligand bound saturably to dispersed parotid acinar cells (27 fmol [^{3}H]QNB bound/mg protein with a K_d of 6 ×

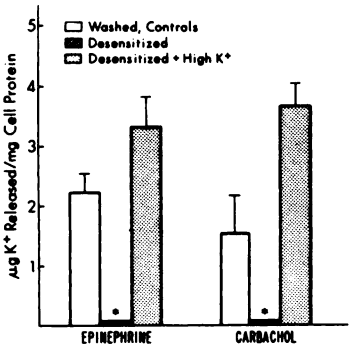

FIG. 6. *The effect of incubation in high K^+ buffer on α-adrenergic desensitization in parotid acinar cells*

Parotid acinar cells were preincubated with (■, ▩) or without (□) 100 μM (−)-epinephrine in Hepes buffer and then washed. Some of the desensitized cells were exposed to a high-K^+ buffer (▩) while the others were incubated in buffer alone. The cells were washed again and then challenged with either 1 mM carbachol or 1 mM (−)-epinephrine. Each bar represents the mean of eight determination, while each vertical line represents one SEM. Statistics were calculated as in Fig. 1 ($^*P < 0.01$ compared to buffer-preincubated controls).

10^{-10} M). Scatchard analysis of the binding yielded a straight line, suggesting that the ligand was interacting with a homogeneous population of sites in the dispersed cells. Perhaps most importantly the ability of a small series of cholinergic agents to compete with [^{3}H]QNB binding to these cells was the same (oxotremorine > acetylcholine > carbachol; QNB ≥ atropine) as the reported ability of these ligands to compete for [^{3}H]QNB binding to membranes from other tissues (11).

The binding of [^{3}H]QNB to dispersed parotid acinar cells was not affected by preincubation with epinephrine. The binding of [^{3}H]DHE, an α-adrenergic radioligand, was also studied in these cells. Although the binding was reduced to approximately one-half of the binding in washed control cells, this difference was not statistically significant (data not shown). It should be noted that the binding of [^{3}H]DHE to parotid cells is complex since specific binding is not saturable (7). In these experiments specific [^{3}H]DHE binding was approximately 10% of the total binding to cells, a problem common to the binding of ligands to intact cell preparations but not present with the [^{3}H]QNB, where specific binding to cells was 50% of total binding.

DISCUSSION

Putney (8) has proposed that α-adrenergic receptors and muscarinic cholinergic receptors share the same Ca^{2+} channels in parotid acinar cells. The experiments reported here agree well with this model of adrenergic and cholinergic function. Persistent stimulation of the α-adrenergic receptor in dispersed parotid cells leads to a desensitization of the cell response to subsequent challenge by both adrenergic and cholinergic agents. This A23187 experiment is crucial to our argument that the site of this "crossed" desensitization is at Ca^{2+} entry. While A23187 is generally thought of as specifically promoting Ca^{2+} entry, like all drugs it may have other actions. However, it seems likely that the ability of A23187 to promote K^+ release in desensitized cells indicates that the desensitization stems from a failure of receptor stimulation to increase intracellular Ca^{2+} levels in desensitized cells.

The desensitization is reversible since brief exposure to high-K^+ concentrations restores full responsiveness. The desensitization requires interaction with α-adrenergic receptors since the presence of an adrenergic antagonist, phentolamine, prevents desensitization. Finally although the cholinergic response is diminished in desensitized cells, there is no significant alteration in the numbers of [^{3}H]QNB binding sites, suggesting that the cholinergic membrane receptors are not affected by adrenergic stimulation. The most parsimonious explanation for these findings is that the adrenergic and cholinergic receptors share a common Ca^{2+} entry mechanism and that the diminished cholinergic response appears to result from some alteration between cholinergic receptor occupation and Ca^{2+} entry. This alteration could reside at the Ca^{2+} entry mechanism or at a transduction step linking receptor occupation to Ca^{2+} entry.

The alteration of a physiological response during persistent stimulation is a well-recognized phenomenon and can result from a variety of mechanisms such as receptor down-regulation (7, 12), substrate depletion (13), changes in intracellular regulatory enzymes (14). Persistent α-adrenergic stimulation in parotid slices has been reported to produce desensitization of cyclic nucleotide responses (15–17) and of potassium release (18). Under the conditions used to study α-adrenergic desensitization in slices (18) the K^+ release mechanism appeared to be the site of the diminished response. Tissue slices are complicated systems containing many cell types. Substrates must often diffuse through tissue spaces to reach relevant cells or to be released from the tissue into the bath. In earlier studies, we used dispersed parotid acinar cells because they represented a reasonably homogeneous population of cells in suspension. It should be noted that these cells are prepared by incubation with proteolytic enzymes in a Ca^{2+}-free medium and thus they may not be strictly comparable to slices. Furthermore we have chosen not to use trypsin in our dispersion. In our hands trypsin incubation for short periods increases the ability of adrenergic and cholinergic agents to elicit K^+ release while incubation for longer periods causes the disappearance of membrane binding sites for [^{3}H]DHE and [^{3}H]QNB (Davis, Olender, and Strittmater, unpublished observation). Thus our studies of α-adrenergic desensitization may not be comparable to studies in trypsin-treated cell preparations.

Using these cells, we observed an α-adrenergic desensitization at lower pHs and attributed at least part of this loss of response to receptor down-regulation (7). In this paper we describe a second more rapid type of α-adrenergic desensitization in which both adrenergic and cholinergic desensitization result from adrenergic stimulation. The two types of desensitization share many features. Both are reversed by K^+ exposure, require α-adrenergic agonists, and occur in the absence of Ca^{2+}. Although both types of desensitization could occur at physiological pH, the high nonspecific binding of [^{3}H]-DHE to cells and the complex nature of that binding prevented a determination of whether α-adrenergic membrane receptor changes occurred in these experiments.

The presence of at least two different sites for α-adrenergic desensitization in parotid acinar cells is consistent with the observations of multiple mechanisms for desensitization in other tissues (19).

The pharmacological specificity of adrenergic agents in eliciting α-adrenergic responses differs from tissue to tissue. One explanation for this difference is that several subclasses of α-adrenergic receptor exist. At present most available data are consistent with two hypothetical subclasses, termed α_1 and α_2 (20, 21). α-Adrenergic receptors on salivary gland cells fit best into the α_2 subclass (21, 22). α_2 receptors are also present on nerves and have been termed presynaptic receptors. The α_2 receptors on parotid acinar cells are clearly postsynaptic since they are unaltered by sympathetic denervation (22). However α receptors in these parotid cells may share many properties with presynaptic α receptors particularly since muscarinic cholinergic and peptide receptors are also present presynaptically (23). It is intriguing to speculate that the adrenergic desensitization of the cholinergic response demonstrated in these experiments may also take place in nerve endings. This type of cross-desensitization could provide an explanation for the modulation of complex neuronal behaviors at a presynaptic level.

REFERENCES

1. Mangos, J. A., N. R. McSherry, T. Barker, S. N. Arvanitakis and V. Wagner. Dispersed rat parotid acinar cells. II. Characterization of adrenergic receptors. *Am. J. Physiol.* **229:** 560–565 (1975).
2. Mangos, J. A., N. R. McSherry and T. Barker. Dispersed rat parotid acinar cells. III. Characterization of cholinergic receptors. *Am. J. Physiol.* **229:** 566–569 (1975).
3. Rudich, L., and F. R. Butcher. Effect of substance P and eledoisin on K^+ efflux, amylase release and cyclic nucleotide levels in slices of rat parotid gland. *Biochim. Biophys. Acta* **444:** 740–711 (1976).
4. Friedman, Z. Y., and Z. Selinger. A transient release of potassium mediated by the action of substance P on rat parotid slices. *J. Physiol. (Lond.)* **278:** 461–469 (1978).
5. Strittmater, W. J., J. N. Davis and R. J. Lefkowitz. α-Adrenergic receptors in rat parotid cells. I. Correlation of [^{3}H]-dihydroergocryptine binding and catecholamine-stimulated potassium efflux. *J. Biol. Chem.* **252:** 5472–5477 (1977).
6. Talamo, B. R., S. C. Adler and D. R. Burt. Parasympathetic denervation decreases muscarinic receptor binding in rat parotid. *Life Sci.* **24:** 1573–1580 (1979).
7. Strittmater, W. J., J. N. Davis and R. J. Lefkowitz. α-Adrenergic receptors in rat parotid cells. II. Desensitization of receptor binding sites and potassium release. *J. Biol. Chem.* **252:** 5478–5482 (1977).
8. Putney, J. W., Jr. Muscarinic, α-adrenergic and peptide receptors regulate the same calcium influx sites in the parotid gland. *J. Physiol. (Lond.)* **268:** 139–149 (1977).
9. Putney, J. W., Jr. Stimulus-permeability coupling: Role of calcium in the receptor regulation of membrane permeability. *Pharmacol. Rev.* **30:** 209–244 (1979).
10. Selinger, Z., S. Eimerl and M. Schramm. A calcium ionophore simulating the action of epinephrine on the α-adrenergic receptor. *Proc. Natl. Acad. Sci. USA* **71:** 128–131 (1974).
11. Yammamura, H. I., and S. H. Snyder. Muscarinic cholinergic receptor binding in the longitudinal muscle of the guniea pig ileum with [^{3}H] quinuclidinyl benzilate. *Mol. Pharmacol.* **10:** 861–867 (1974).
12. Wagner, H. R., and J. N. Davis. β-Adrenergic receptor regulation by agonists and membrane depolarization in rat brain slices. *Proc. Natl. Acad. Sci. USA* **76:** 2057–2061 (1979).
13. Mukherjee, C., and R. J. Lefkowitz. Regulation of beta-adrenergic receptors in isolated frog erythrocyte plasma membranes. *Mol. Pharmacol.* **13:** 291–303 (1977).
14. Oleshansky, M. A., and N. H. Neff. On the mechanism of tolerance to isoproterenol-induced accumulation of cAMP in rat pineal *in vivo*. *Life Sci.* **17:** 1429–1432 (1975).
15. Harper, J. F., and G. Brooker. Amylase secretion from the rat parotid: Refractoriness to muscarinic and adrenergic agonists. *Mol. Pharmacol.* **14:** 1031–1045 (1978).
16. Oron, Y., J. Kellogg and J. Larner. Alpha adrenergic and cholinergic-muscarinic regulation of adenosine cyclic 3′,5′-monophosphate levels in the rat parotid. *Mol. Pharmacol.* **14:** 1018–1030 (1978).
17. Harper, J. F., and G. Brooker. Refractoriness to muscarinic and adrenergic agonists in the rat parotid: Responses of adenosine and guanosine cyclic 3′,5′-monophosphate. *Mol. Pharmacol.* **13:** 1048–1059 (1977).
18. Putney, J. W., Jr. Role of Ca^{++} in the fade of the K^+ release response in the rat parotid gland. *J. Physiol.* **281:** 383–394 (1978).
19. Johnson, G. L., B. B. Wolfe, T. K. Harden, P. B. Molinoff and J. P. Perkins. Role of β-adrenergic receptors in catecholamine-induced desensitization of adenylate cyclase in human astrostoma cells. *J. Biol. Chem.* **253:** 1472–1480 (1978).
20. Berthelsen, S., and W. A. Pettinger. A functional basis for classification of α-adrenergic receptors. *Life Sci.* **21:** 596–606 (1977).
21. Wood, C. L., C. D. Arnett, W. R. Clarke, B. S. Tsai and R. J. Lefkowitz. Subclassification of alpha-adrenergic receptors by direct binding studies. *Biochem. Pharmacol.* **28:** 1277–1282 (1979).
22. Arnett, C. D., and J. N. Davis. Denervation-induced changes in alpha and beta adrenergic receptors of the rat submandibular gland. *J. Pharmacol. Exp. Ther.* **211:** 394–400 (1979).
23. Lokhandwala, M. F. Presynaptic receptor systems on cardiac sympathetic nerves. *Life Sci.* **24:** 1823–1832 (1979).

Send reprint requests to: Dr. James N. Davis, Neurology Research Laboratory, Durham Veterans Administration Medical Center, Durham, N.C. 27705.