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AN ALPHA-ADRENERGIC RECEPTOR MECHANISM CONTROLLING POTASSIUM PERMEABILITY IN THE RAT LACRIMAL GLAND ACINAR CELL

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SUMMARY

1. Rat lacrimal gland slices, incubated in a balanced, buffered salt solution, were found to be physiologically stable for up to 2 hr with respect to O_2 consumption, extracellular space, and water and ion content.

2. The release of ^{86}Rb serves as a good substitute for ^{42}K in monitoring the movement of K through the cell membrane.

3. Adrenaline appears to increase membrane permeability to K as evidenced by an increase in the rate of ^{86}Rb efflux.

4. This response to adrenaline was blocked by phentolamine but not by propranolol and was mimicked by phenylephrine but not by isoprenaline.

5. The magnitude of the ^{86}Rb release indicates that it is being released, at least in part, from the lacrimal gland acinar cell.

6. It is concluded that the lacrimal gland acinar cell has an α-adrenergic receptor, activation of which leads to an increase in membrane permeability to K.

INTRODUCTION

The lacrimal gland is innervated by the autonomic nervous system which contains both parasympathetic and sympathetic components. While it is generally accepted that the activation of muscarinic receptors initiates the secretion of tears by the lacrimal gland (Botelho, 1964), there is still some controversy over the functional relevance of sympathetic innervation in this tissue.

Histochemical localization of adrenergic fibres in the monkey lacrimal gland (Ehinger, 1966) and electron microscopical localization of these nerve fibres in both monkey and man (Ruskell, 1969, 1975) have shown that adrenergic nerves are distributed in the interstitial spaces between acini and are associated with blood vessels; however, these fibres have not been seen to penetrate into the acini. Other studies on the rabbit lacrimal gland have shown that both exogenously added catecholamine (Goldstein, De Palau & Botelho, 1967; Botelho, Goldstein & Martinez, 1973) and sympathetic nerve stimulation (Botelho, Martinez, Pholpramool, Van Prooyen, Janssen & De Palau, 1976) modify stimulated lacrimal flow. These data are not incompatable with the hypothesis that the function of the lacrimal gland's sympathetic innervation is to change vascular resistance and thereby alter lacrimal output (Botelho *et al.* 1976).

We wondered if there were adrenergic receptors present on the acinar cell which might modify lacrimal flow but whose existence was previously masked by the effects of catecholamine on the vasculature. Therefore a series of *in vitro* experiments with rat exorbital lacrimal gland slices was conducted. First, the physiologic stability of rat exorbital lacrimal gland slices was characterized. With this preparation, the release of ^{86}Rb (as an index of K release) due to receptor activation was measured. The results indicate that activation of an α-adrenergic receptor located on the acinar cell leads to an increase in membrane permeability to K.

METHODS

Animals and preparation of lacrimal slices

Male Wistar rats weighing between 70 and 120 g were anaesthetized with sodium pentobarbitone (50 mg/kg I.P.) and the exorbital glands were removed bilaterally and decapsulated. Although the rat has both an infraorbital and exorbital lacrimal gland, there is no evidence to suggest any morphological or physiological difference between the two. On this basis, only the exorbital gland was used in these experiments due to its accessibility. Each gland was divided in half with a Stadie-Riggs microtome and incubated in a physiological salt solution of the following composition (mM): NaCl, 120; KCl, 6·2; $CaCl_2$, 3·1; $MgCl_2$, 1·6; dextrose, 10; Tris (hydroxymethyl) aminomethane, 20. The solution was buffered with HCl to a pH of 7·40 at 37 °C and continuously gassed with 100% O_2. This procedure provided four paired lacrimal slices per rat weighing approximately 25 mg each and with a thickness of approximately 0·5 mm.

O_2 uptake

O_2 consumption was measured with an oxygen electrode and standard polarographic techniques (Davies, 1962).

Determination of extracellular space

The distributions of [^{14}C]inulin and [^{14}C]sucrose were determined by incubating slices for a predetermined length of time in the presence of the appropriate ^{14}C-compound, dipping the slice quickly in a large volume of non-radioactive medium, blotting and weighing. After weighing the tissue was placed in 5 ml. deionized water containing potassium cyanide (1 mg/ml.) and allowed to equilibrate overnight. Aliquots from the tissue dialysates were then counted for ^{14}C-content in a Beckman scintillation counter. The ^{14}C-space was expressed as the ratio of counts per minute per gram of tissue divided by counts per minute per millilitre of medium and thus represents an apparent volume of distribution with units of millilitres per gram.

Water and cation content

For experiments in which water and cation content were of interest, the lacrimal slice, after weighing, was placed in a tared fused-quartz crucible. The crucible was dried overnight at 105 °C and reweighed. This procedure allowed the tissue water content to be determined. The dried residues were then ashed at 550 °C for approximately 18 hr. The ash was dissolved in 0·1 N-HCl containing 10 mM-$SrCl_2$ and assayed for Na, K, Mg and Ca with a Perkin-Elmer Model 403 atomic absorption spectrophotometer.

Efflux of ^{42}K and ^{86}Rb

The measurement of nuclide efflux has been described previously (Putney, 1976). Briefly, lacrimal slices were prepared, placed in a small basket of polyethylene and nylon net, and incubated in medium containing nuclide (5-20 μc/ml.) for 30 min. The tissue was then transferred sequentially through a series of 2 min washes, each in 2 ml. incubation medium, for up to 40 min. After the final incubation, the tissue slice was quickly removed from the basket, blotted and weighed (total time approximately 15 sec). The slice was then placed in 2 ml. deionized water and the radioactivity in each incubation volume and the residual activity in the tissue were determined with a Beckman 300 gamma counter. These determinations allowed the data to be expressed as the first order rate coefficient for efflux computed as percent per minute (Putney, 1976).

Materials

The ^{14}C-materials were obtained from ICN Pharmaceuticals, Inc., and nuclides were purchased from New England Nuclear Corp., Boston, Mass. The specific activity of ^{42}K was 16 to 27 c/mole; ^{86}Rb was 400–800 c/mole. Carbachol, L-isoprenaline, propranolol, atropine, L-adrenaline, histamine, N^6-monobutyryl cyclic adenosine 3′,5′-monophosphate and 8-bromo cyclic guanosine 3′,5′-monophosphate were obtained from Sigma Chemical Co., St Louis, Mo. Sotalol was purchased from Regis Chemical Co., Morton Grove, Ill. The Sterling-Winthrop Research Institute, Rensselaer, N.Y. kindly donated phenylephrine HCl and D-adrenaline. Phentolamine HCl was a gift from the Ciba-Geigy Corp., Summit, N.J. Eledoison and Substance P were purchased from Peninsula Laboratories, San Carlos, Calif. Serotonin was obtained from Aldrich Chemical Co., Milwaukee, Wis.

Statistics

Summarized data are generally represented by the arithmetic mean; dispersions are given as 1 S.E. of the mean. For statistical comparisons between two groups, Student's t test for paired data was used when appropriate. The analysis of variance was used when three or more groups were compared. The critical probability for rejection of the null hypothesis was 0·05 throughout.

RESULTS

Stability of the in vitro preparation

O_2 consumption. There was no significant change in the rate of O_2 consumption by slices incubated for 2 hr; the pooled mean respiratory rate from four tissues was $14{\cdot}6 \pm 0{\cdot}8$ μl./g.min ($n = 28$). Regression analysis showed that the slope of the line relating O_2 consumption to time was not significantly different from zero.

Extracellular space. The apparent volumes of distribution of [^{14}C]inulin and [^{14}C]-sucrose were determined. Analysis of variance indicated that at all times tested there was no significant difference in the volume of distribution between the two carbohydrates. This analysis also showed that there was no significant change in the radiocarbon space between 30 and 120 min; thus these determinations were pooled to give a value for the extracellular space of $0{\cdot}277 \pm 0{\cdot}006$ ml./g ($n = 48$).

Water and cation content. Fig. 1 shows the cellular changes in cation and water content in lacrimal slices incubated for various times up to 120 min. Zero minute values were obtained by biopsy of the gland as soon as it was exposed in dissection.

An analysis of variance showed the following for Na, K and Mg. The initial values (0 min) were significantly different from later ones. However, at 15 min the tissue appears to have reached a new equilibrium (i.e. there is no significant change in the content of these cations from 15 to 60 min). The 120 min value for these cations was significantly different than the values from 15 to 60 min. There was no significant change in Ca during the times tested. The water content did not change significantly from 15 to 120 min and the pooled mean for thirty measurements of water content was $81{\cdot}8 \pm 0{\cdot}3$ %.

Table 1 gives the apparent intracellular concentration of Na and K. These data were obtained by dividing the intracellular content of these ions (total tissue content of each ion minus their content in the extracellular space) in μmole/g by the value for intracellular water, 0·541 ml./g [total tissue water (0·818 ml./g) minus the extracellular space (0·277 ml./g)]. Also shown are their respective intracellular/extracellular concentration ratios (I/E). For Mg and Ca, Table 1 lists the cellular content

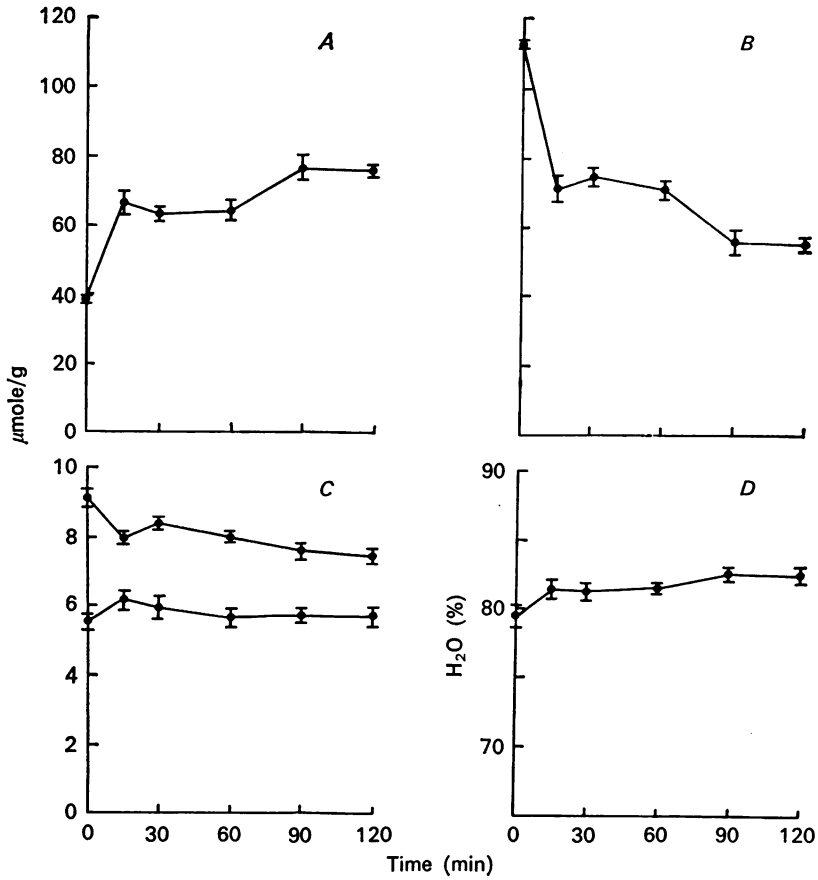

Fig. 1. Na (*A*), K (*B*), Mg (upper curve, *C*), Ca (lower curve, *C*) and water content (*D*) of lacrimal slices at various times of incubation. The values at time zero were obtained from samples taken directly from anaesthetized animals. Each point is the mean of six paired experiments. Dispersions represent ± 1 S.E. of the mean.

TABLE 1. Mean cation contents in rat exorbital gland slices. Values shown for Na, K, and Mg were obtained from eighteen determinations between 15 and 60 min. The Ca value was obtained by averaging all determinations (0–120 min, $n = 36$). Extracellular values are those for the incubation medium. Intracellular values are per millilitre cell water assuming total tissue water is 0·818 ml./g and extracellular space is 0·277 ml./g. Cellular values for Mg and Ca are similarly derived but expressed per g of cells rather than ml. cell water (i.e., solids are included). *I/E* values for Na and K are ratios of intracellular to extracellular concentration

	Na	K	Mg	Ca
Content (μmole/g)	64·5 ± 1·5	72·3 ± 1·8	8·06 ± 0·10	5·78 ± 0·11
Extracellular (μmole/ml.)	120·0	6·2	1·6	3·1
Intracellular (Na, K, μmole/ml.)	57·9	130·5	—	—
Cellular (Mg, Ca μmole/g cells)	—	—	10·53	6·81
I/E	0·48	21·0	—	—

per gram of cells rather than per millilitre intracellular water, because it is likely that only a small fraction of these metals is present in free solution intracellularly.

Validation of ^{86}Rb substitution for ^{42}K

It has been shown previously that carbachol stimulates the release of ^{86}Rb from rat lacrimal slices (Putney, Parod & Marier, 1977). Fig. 2 shows the response to carbachol of lacrimal slices loaded with ^{86}Rb or ^{42}K. A two-way analysis of variance showed that there was a significant difference in the rate of efflux between the two nuclides both in the presence and absence of agonist. The rate of ^{86}Rb efflux was significantly slower than that of ^{42}K, however, the percent increase in membrane permeability initiated by the addition of carbachol (30–40 min) was significantly greater for ^{86}Rb.

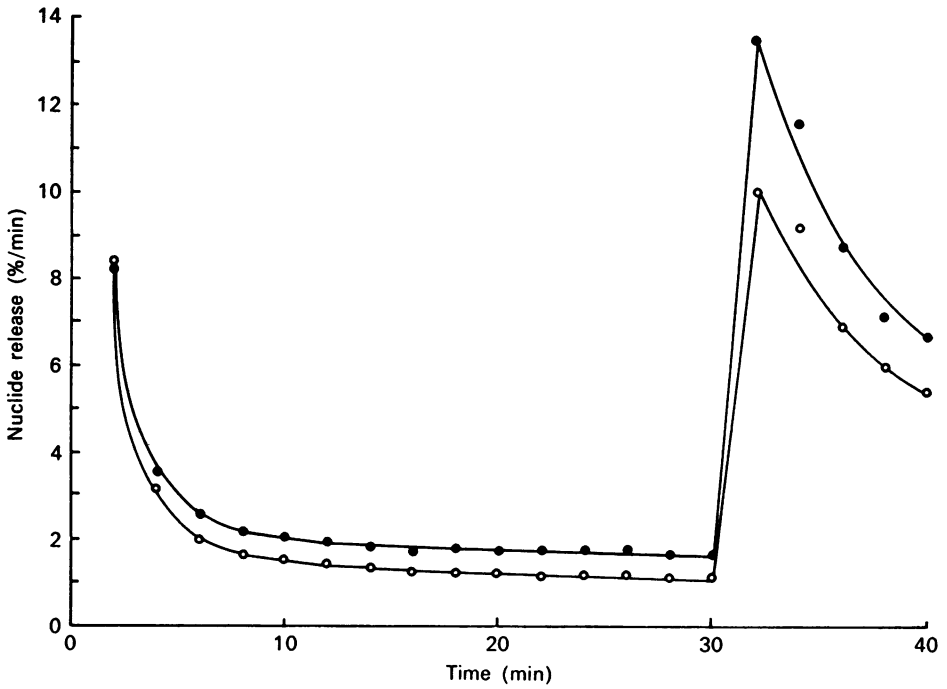

Fig. 2. Release of ^{86}Rb (○—○) and ^{42}K (●—●) from rat lacrimal slices due to carbachol. Slices were exposed to isotope for 30 min and 'washed out' for another 40 min. Carbachol (10^{-5} M) was added from 30 to 40 min. Each point is the mean of six paired experiments. S.E.S averaged less than 10% of the means.

Effect of adrenaline

The addition of 10^{-5} M-adrenaline to the incubation medium markedly increased the rate of efflux of ^{86}Rb (Fig. 3). After approximately 10 min, the efflux of ^{86}Rb fell to a lesser, but still significantly elevated, rate of release. This response is quantitatively and temporally similar to the response of this tissue to 10^{-5} M-carbachol (Putney *et al.* 1977). The response to adrenaline was blocked by 10^{-4} M-phentolamine but was not affected by 10^{-4} M-propranolol.

The following agonists were also tested for effect on ^{86}Rb release and failed to produce a significant response (data not shown): histamine (10^{-5} M), serotonin (10^{-5} M), eledoisin (10^{-6} M), Substance P (10^{-6} M), N^6-monobutyryl cyclic adenosine 3′,5′-monophosphate (10^{-3} M) and 8-bromocyclic guanosine 3′,5′-monophosphate (10^{-3} M).

Further support for the existence of an α-receptor in the lacrimal gland which

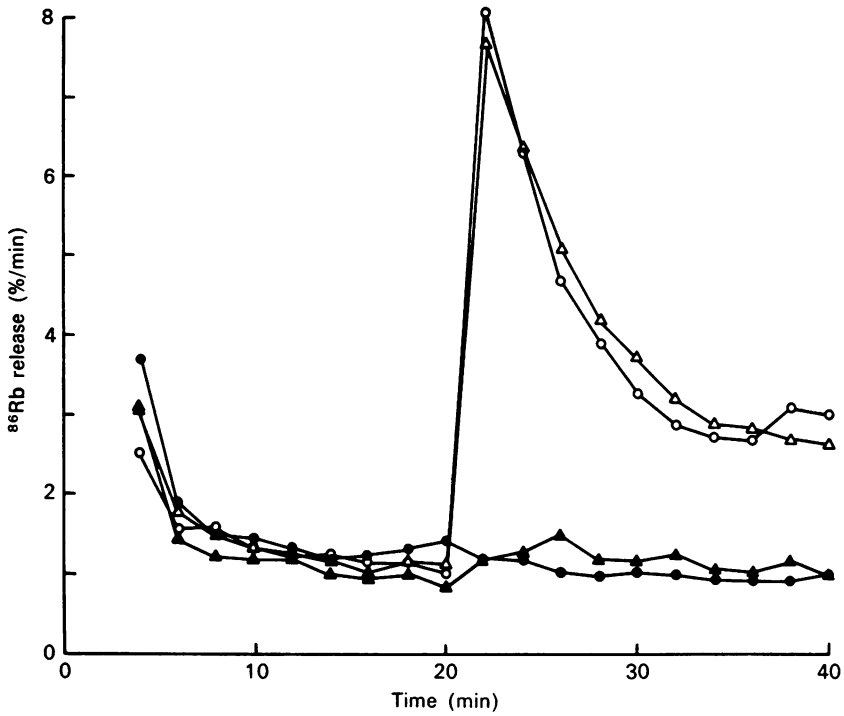

Fig. 3. Release of ^{86}Rb due to adrenaline. ●—●, control; ○—○, 10^{-5} M adrenaline 20–40 min; ▲—▲, 10^{-4} M-phentolamine 14–40 min, 10^{-5} M adrenaline 20–40 min; △—△, 10^{-4} M-propranolol 14–40 min, 10^{-5} M-adrenaline 20–40 min. Each point is the mean of three separate experiments, i.e. four slices from each of three rats were subjected to the four protocols. S.E.s averaged less than 10% of the means.

mediates K release is shown in Fig. 4. When 10^{-5} M-phenylephrine (an α-agonist) was added to the incubation media, it elicited the release of ^{86}Rb and this response was almost completely blocked by 10^{-4} M-phentolamine (not shown). Even in the presence of a tenfold excess of phentolamine (10^{-4} M), approximately 8% of the response remained to either adrenaline or phenylephrine. When the concentration of phentolamine was lowered from 10^{-4} to 10^{-6} M, the response to 10^{-5} M-adrenaline was blocked to approximately the same degree (i.e. 13% of the response remained). Fig. 4 also shows that isoprenaline caused a small but significant increase in membrane permeability to ^{86}Rb (note difference in ordinate scale with Fig. 3). However, this response to isoprenaline could not be blocked by either of the classical β-antagonists propranolol (10^{-4} M) or sotalol (10^{-4} M) nor by phentolamine (10^{-4} M) or atropine (10^{-4} M) (data not shown), and in another experiment (not shown) the less active

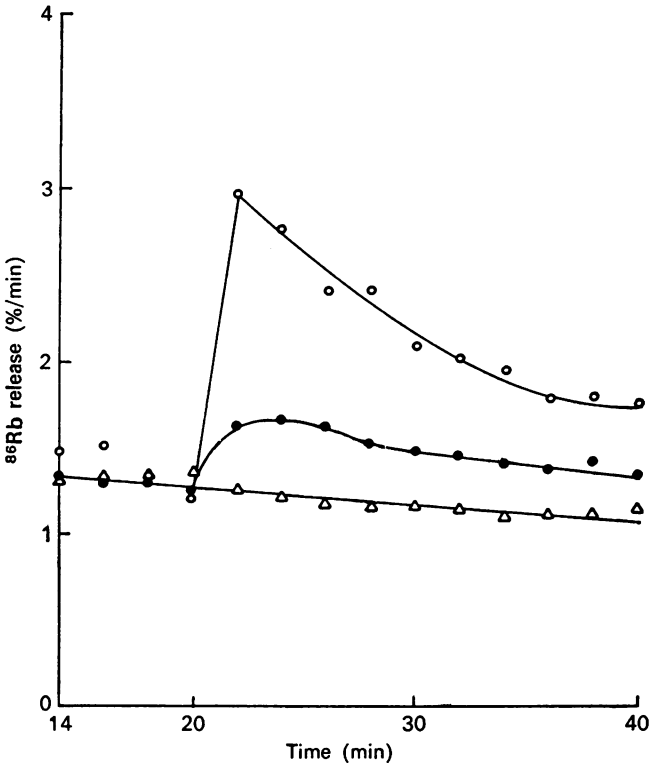

Fig. 4. Release of ^{86}Rb from lacrimal slices. △—△, control (n = 9); ●—●, 10^{-5} M-isoprenaline 20–40 min (n = 20); ○—○, 10^{-5} M-phenylephrine 20–40 min (n = 13). S.E. averaged less than 5% of the means.

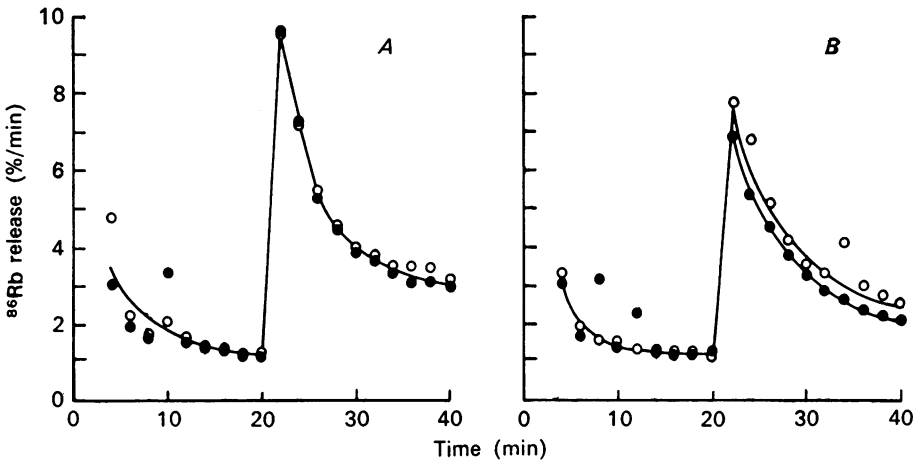

Fig. 5. Release of ^{86}Rb. (*A*): ○—○, 10^{-5} M-carbachol 20–40 min, ●—●, 10^{-6} M-phentolamine 14–40 min, 10^{-5} M carbachol 20–40 min. (*B*): ○—○, 10^{-5} M-adrenaline 20–40 min; ●—●, 10^{-6} M-atropine 14–40 min, 10^{-5} M-adrenaline 20–40 min. Each point is the mean of three paired experiments. S.E.s averaged less than 10% of the means.

(+) stereoisomer of adrenaline also slightly increased membrane permeability to ^{86}Rb. The failure of phentolamine completely to inhibit the response to α-stimulation and the tissue's response to isoprenaline and d-adrenaline suggest that these agonists are capable of causing a small release of K by an unknown mechanism.

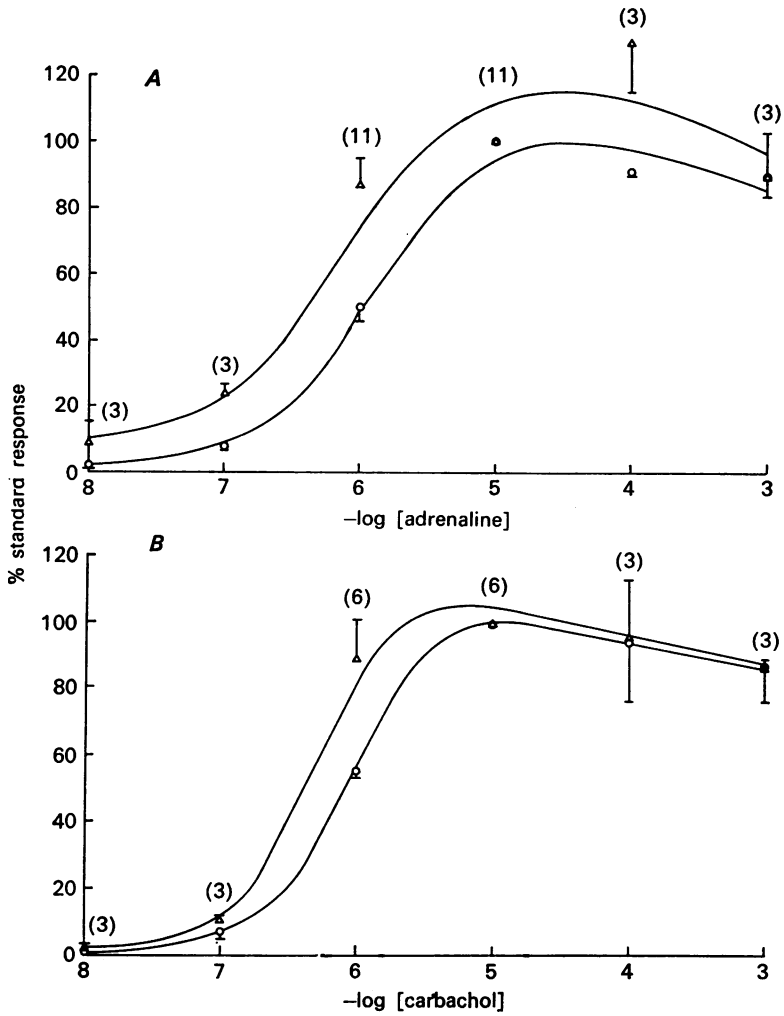

Fig. 6. Dose–response curves for net ^{86}Rb release due to adrenaline (*A*) and carbachol (*B*). In both cases the ○ represents the net ^{86}Rb release during the early phase (usually 2 min after the addition of agonist); the △ represents the net ^{86}Rb release during the late phase (average of net release 14–18 min after the addition of agonist). Responses are expressed as % of standard response (obtained with 10^{-5} M-agonist). Numbers in parenthesis give the number of experimental determinations. Dispersions represent ± 1 S.E. of the mean.

It has been suggested that the addition of an agonist to a tissue preparation may produce a response indirectly by releasing endogenous stores of neurotransmitter (Schramm, 1968). Fig. 5 shows that this is clearly not the case in the lacrimal gland. In Fig. 5*A*, 10^{-6} M-phentolamine, a concentration of antagonist that will eliminate

the α-stimulated K release initiated by adrenaline, did not affect the increased ^{86}Rb release elicited by a maximal concentration of carbachol. In Fig. 5*B*, the release of ^{86}Rb to a maximal concentration of adrenaline (10^{-5} M) was not blocked by 10^{-6} M-atropine. This concentration of atropine will totally block the tissue's response to a maximal concentration of carbachol (data not shown).

With the increase in the rate of ^{86}Rb release as an index of receptor activation, Fig. 6 depicts the lacrimal gland's response to varying concentrations of adrenaline and carbachol. As stated previously, it appears that the release of ^{86}Rb due to adrenaline is biphasic in nature, consisting of a large increase in the rate of release followed by a lower, more sustained rate of release (Fig. 3). Figs. 6*A* and 6*B* show the dose–response curves for both of these phases of ^{86}Rb release.

DISCUSSION

Slices of the rat lacrimal gland, incubated under the conditions described in Methods, appear to be physiologically stable *in vitro*. Relatively constant values for respiration, extracellular space and water and cation content (Fig. 1) are obtained for up to 2 hr of incubation.

The mechanisms by which cells maintain a high intracellular K concentration are generally thought to be the result of both active pumping (i.e. Na-K ATPase) and passive membrane permeability. There is good evidence in a number of tissues (Bonting, 1970) and in reconstituted membrane transport systems (Hilden & Hokin, 1975) that Rb can replace K in activating the Na-K ATPase, and Fig. 2 serves to demonstrate that Rb can also substitute for K in passive movement through the membrane. Although the lacrimal cell membrane appears to handle ^{86}Rb and ^{42}K similarly, this is no guarantee that Rb can substitute for K in other cellular functions. Thus, we thought we could best simulate physiological conditions by adding only tracer amounts of ^{86}Rb to media containing normal concentrations of K.

With this technique, we obtained evidence suggesting that an α-adrenergic receptor in the rat lacrimal gland mediates an increase in membrane K permeability. First of all, the response to adrenaline (Fig. 3) is inhibited by phentolamine but is not affected by propranolol. Secondly, the tissue responds to phenylephrine (Fig. 4) and this response is also blocked by phentolamine (not shown). Thirdly, while isoprenaline does cause a slight but significant increase in membrane permeability to K (Fig. 4), this response is not sensitive to any of the classical receptor blocking drugs (not shown) and appears to be mediated through an unknown mechanism.

In this series of experiments we have demonstrated that the rat lacrimal gland responds to α-adrenergic activation with an increase in membrane permeability to K. This finding is not without precedent; α-adrenergic stimulation is known to release protein and K in other exocrine tissues (Batzri, Selinger, Schramm & Robinovitch, 1973; Martinez, Quissell & Giles, 1976; Petersen, Gray & Hall, 1977; Leslie, Putney & Sherman, 1976). Our work now provides evidence that there is an α-receptor located on the rat lacrimal gland acinar cell. There are two lines of evidence for this contention.

The release of K due to α-adrenergic stimulation is certainly not in itself conclusive evidence that the receptor is located on the acinar cell. The K response could be due

to activation of an α-receptor located on other cell types present in the lacrimal gland (i.e., ductal, myoepithelial or endothelial cells). However, the magnitude of the K release seen with adrenaline argues against this possibility. Our own observations (unpublished) and those of Herzog, Sies & Miller (1976) suggest that at least 80% of the rat lacrimal gland cell mass consists of acinar cells. When adrenaline was added between 20 and 40 min (Fig. 3), 60% of the ^{86}Rb remaining in the tissue was released. No combination of ductal, myoepithelial or endothelial cells (approximately 20% of tissue mass) can account for this massive turnover of ^{86}Rb.

Studies on the cytochemical localization of endogenous peroxidase in the lacrimal gland (Herzog & Miller, 1972) indicate that this enzyme is located primarily in acinar cells, although on rare occasions its reaction product can be seen in intercalated duct cells. We have recently found (unpublished observations) that adrenaline, phenylephrine, but not isoprenaline will stimulate the release of peroxidase from rat lacrimal slices.

In conclusion, we have demonstrated that in the rat lacrimal gland there is an α-receptor located on the acinar cell which can mediate the release of K. The relevance of these data as they apply to lacrimal flow has yet to be ascertained; however, theories on the role of sympathetic innervation in lacrimal secretion must now deal with the possibility that catecholamines may modify lacrimal flow by a direct action of the acinar cell.

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