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Membrane Effects Mediated by Alpha- and Beta-Adrenoceptors in Mouse Parotid Acinar Cells

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Summary. Epinephrine caused a dose dependent increase in the electrical potential difference across the mouse parotid acinar cell membrane (hyperpolarization). Norepinephrine, synephrine and acetylcholine also caused membrane hyperpolarization. This hyperpolarization was not abolished by ouabain (1 mM) and was enhanced in the absence of extracellular potassium. In contrast, the β-adrenoceptor agonist isoproterenol and its isomer metaproterenol caused membrane depolarization, this effect was blocked by propranolol (5 μM). In the presence of the α-adrenoceptor blocking agent phentolamine (10 μM), epinephrine stimulation also resulted in membrane depolarization while acetylcholine still hyperpolarized the cell membrane. The membrane effect of epinephrine (hyperpolarization) or isoproterenol (depolarization) was at least partly retained during superfusion of the parotid gland segments with Ca^{2+}-free solution containing EGTA (10^{-4} M) at a time when the resting membrane potential was reduced to 50% of its control value as a result of the extracellular Ca^{2+} deprivation.

It has recently been described that α- and β-adrenoceptors in addition to cholinergic receptors are present in parotid acinar cells (Batzri, Selinger, Schramm & Robinovitch, 1973). It has furthermore been shown that β-adrenoceptor activation results in amylase secretion and that this response is mediated by the activation of an adenylyl cyclase (Schramm & Naim, 1970; Batzri & Selinger, 1973). Activation of the α-adrenoceptor or the cholinergic receptor results in release of K^+ from parotid slices and it has been suggested that activation of these receptors results in salt and water secretion (Batzri *et al.*, 1973).

We have recently shown that epinephrine (10^{-5} M) causes a transient increase in the electrical potential difference across the mouse parotid acinar cell membrane (hyperpolarization) (Pedersen & Petersen, 1973). The results presented here show that there are two components in the membrane effect of epinephrine. One mediated by an α-receptor and another by β-receptor activation. These responses are not entirely dependent on the presence of

extracellular Ca^{2+}. The mechanism of action of the α-agonist is probably to increase the cell membrane K^+ permeability.

Materials and Methods

Parotid glands were removed from female mice. The parotid was secured to a perspex platform and placed in a perspex tissue bath (20 ml) through which a Krebs-Henseleit solution flowed at a constant rate (10 ml/min). The composition of the control Krebs-Henseleit solution was (mM): NaCl, 103; KCl, 4.7; $CaCl_2$, 2.56; $MgCl_2$, 1.13; $NaHCO_3$, 25; NaH_2PO_4, 1.15; D-glucose, 2.8; Na pyruvate, 4.9; Na fumarate, 2.7 and Na glutamate, 4.9. This solution was equilibrated with 95% O_2 and 5% CO_2 and warmed to 37 °C. In some experiments where the K^+ concentration was altered, corresponding alterations in Na^+ concentration ensured constant osmolarity.

Measurement of acinar cellular transmembrane electrical potentials was carried out with the help of K-citrate-filled microelectrodes as previously described (Pedersen & Petersen, 1973).

In some experiments the glands were superfused with solutions containing known amounts of epinephrine or other agonists. In other experiments a single dose of agonist was added directly to the tissue bath. In these experiments the concentration of stimulant in the extracellular fluid around the acinar cell under investigation could only be roughly estimated. This procedure, however, was generally preferred because it resulted in more rapid potential changes and therefore it was easier to obtain results from cells during shorter periods of cell impalements.

Results

Effect of Epinephrine and Norepinephrine

Fig. 1 shows a typical example of the time course of the membrane effect of epinephrine. Epinephrine invariably caused an increase in the intracellular negativity, but the effect was only transient (Pedersen & Petersen, 1973). The hyperpolarizing effect of epinephrine was clearly dose-dependent (Fig. 2).

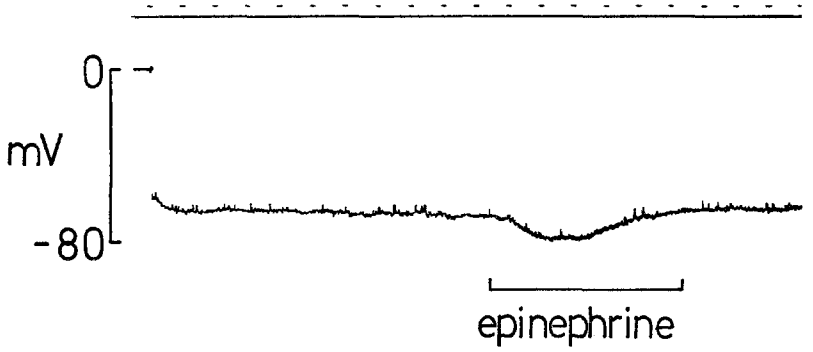

Fig. 1. Parotid acinar cell. Membrane potential measurement. The effect of superfusion with a solution containing 10^{-5} M epinephrine. Cell impalement shown in left part of the trace. In the time marker trace pulses occur at minute intervals

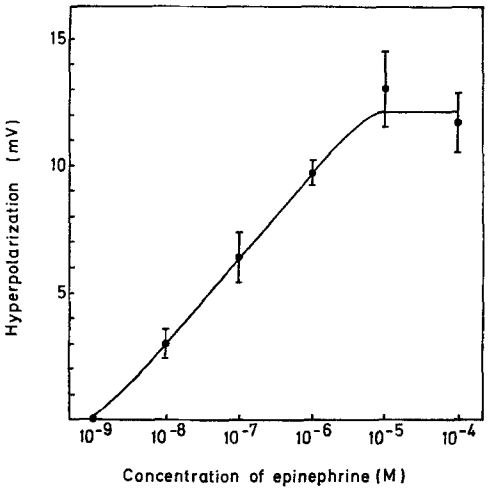

Fig. 2. Dose-response relationship for the hyperpolarizing effect of epinephrine. Each point represents the mean value ± SE of at least 10 measurements of the type shown in Fig. 1

It could be detected at concentrations as low as 10^{-8} M and was maximal at 10^{-5} M. Norepinephrine (10^{-5} M) had a similar hyperpolarizing effect and the mean value of the maximal increase in membrane potential was 13.3 ± 3.3 mV (SE) which is very close to the value of 12.0 ± 1.5 mV obtained with epinephrine stimulation (Pedersen & Petersen, 1973). In experiments where a single dose of epinephrine (0.5 μmole) was added to the tissue bath (20 ml) the mean value of the maximal increase in cell membrane potential was 8.6 ± 0.7 mV.

The Two Components in the Epinephrine Response

In the presence of the α-receptor blocking agent, phentolamine (10^{-5} M), epinephrine had an effect strikingly different from that seen during superfusion of the gland segment with a control Krebs-Henseleit solution. In the presence of phentolamine, epinephrine stimulation (0.5 μmole) always caused a reduction in cell membrane potential (Fig. 3). The mean value of this epinephrine-induced depolarization (measured at the time of maximal depolarization) was 7.3 ± 1.4 mV. The effect of phentolamine was not due to a general change of membrane properties. The resting membrane potential was unaffected by the presence of phentolamine and the effect of ACh was the same in the presence of phentolamine (Fig. 3) as in the absence of blocking agent (Pedersen & Petersen, 1973) namely to cause hyperpolarization.

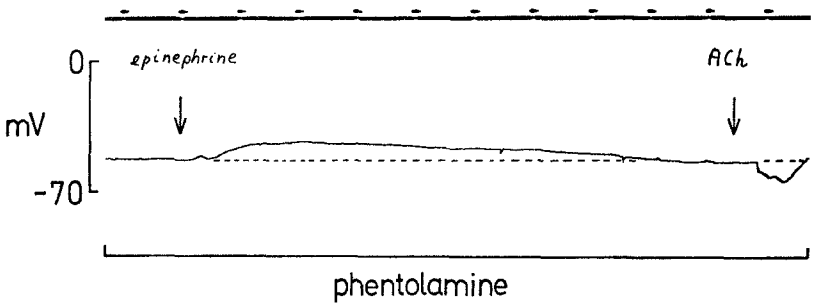

Fig. 3. The effect of addition of a single dose of epinephrine (0.5 μmole) to the tissue bath (20 ml) on the membrane potential in the presence of phentolamine (10^{-5} M). ACh was added in a dose of 0.05 μmole

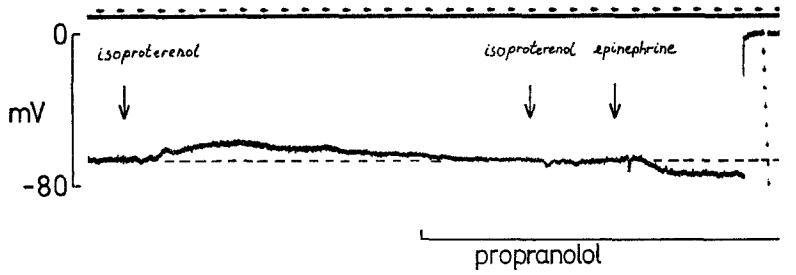

Fig. 4. The effect of single doses of isoproterenol (0.5 μmole) in the absence and presence of propranolol (5×10^{-6} M). Epinephrine was added in a dose of 0.5 μmole

The β-receptor activating agent isoproterenol or its isomer metaproterenol had the same effect on the membrane potential as epinephrine in the presence of phentolamine. The mean value of the isoproterenol-induced (0.5 μmole) depolarization was 13.3 ± 1.3 mV, whereas metaproterenol (0.5 μmole) caused a mean depolarization of 6.7 ± 1.5 mV. The depolarizing effect of isoproterenol was abolished in the presence of the β-receptor blocking agent propranolol (5×10^{-6} M) whereas epinephrine still caused hyperpolarization in this condition (Fig. 4). Interestingly the hyperpolarizing effect of epinephrine was sustained in the presence of propranolol (Fig. 4) but only transient in the absence of blocking agent (Fig. 1). The α-receptor activating agent, synephrine (50 μmole), (isomer of phenylephrine), in the few cases when it was tried, always caused hyperpolarization although the effect was relatively small (2 to 6 mV).

The Importance of Extracellular Ca^{2+}

Prolonged (1 hr) superfusion of the gland segments with a Ca^{2+}-free solution resulted in a reduction of the resting acinar cell membrane potential (Table 1). This phenomenon has been reported for other glandular tissues:

Table 1. Effect of epinephrine and metaproterenol in Ca^{2+}-free solution [a]

Solution	Resting potential (mV)	Epinephrine-induced (10^{-5} M) hyperpolarization (mV)	Metaproterenol-induced (0.5 μmole) depolarization (mV)
Control	-67.6 ± 1.3	12.0 ± 1.5	6.7 ± 1.5
Ca-free	-37.2 ± 3.1	12.9 ± 1.6	7.2 ± 2.2
Ca-free + EGTA	-32.6 ± 4.3	13.5 ± 4.5	

[a] Mean ± SE (n = 5 to 25).

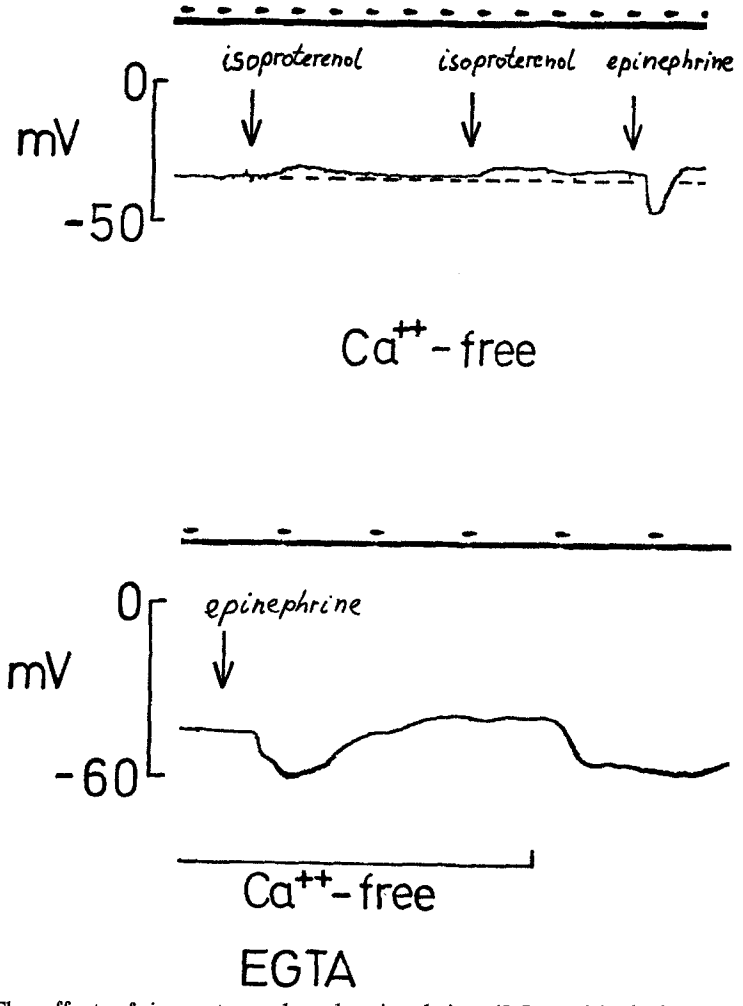

Fig. 5. The effect of isoproterenol and epinephrine (0.5 μmole) during exposure to Ca^{2+}-free solutions. Upper trace was obtained 2½ hr after start of exposure to the Ca^{2+}-free solution. Lower trace was obtained 1 hr after start of exposure to a Ca^{2+}-free solution containing EGTA (10^{-4} M). The effect of readmission of the Ca^{2+} containing control solution is also shown

pancreatic acinar cells (Matthews & Petersen, 1973), pancreatic islet cells (Dean & Matthews, 1970), adrenal chromaffin cells (Douglas, Kanno & Sampson, 1967), and submaxillary acinar cells (Petersen, Poulsen & Thorn, 1967). The effect of epinephrine on the parotid acinar cell membrane was retained in the absence of extracellular Ca^{2+} as was the effect of β-agonists (Fig. 5). In some experiments, EGTA (ethylene glycol bis (β-amino-ethyl ether)-N,N'-tetra-acetic acid) (10^{-4} M) was added to the Ca^{2+}-free superfusion solution. Exposure of the tissue to this EGTA-containing solution for 1 hr did not inhibit the epinephrine-induced membrane hyperpolarization (Fig. 5). Table 1 summarizes the results obtained with Ca-free solutions. Although the amplitude of the epinephrine-induced potential change was little influenced by removal of extracellular Ca^{2+}, there was a shortening in the duration of the membrane potential change (Fig. 5).

As seen in Fig. 5, reintroduction of Ca^{2+} caused an immediate increase in the membrane potential. This hyperpolarization was not dependent on an immediately preceding epinephrine stimulation, but was also observed in the presence of phentolamine (10^{-5} M) plus atropine (1.4×10^{-6} M). Shift of the superfusion solution from Ca^{2+}-free + EGTA to control caused a hyperpolarization of 12.8 ± 2.1 mV whereas in the presence of the combination of atropine and phentolamine this same shift of superfusion fluid caused a hyperpolarization of 9.3 ± 2.6 mV.

The Mechanism of the Epinephrine-Induced Hyperpolarization

During exposure to a K^+-free solution the mean hyperpolarization induced by epinephrine (10^{-5} M) was greatly enhanced (Fig. 6) while at an extracellular K^+ concentration of 20 mM the epinephrine-induced hyperpolarization was severely reduced. In the presence of ouabain (1 mM) the hyperpolarizing effect of epinephrine was retained. Table 2 summarizes the results obtained.

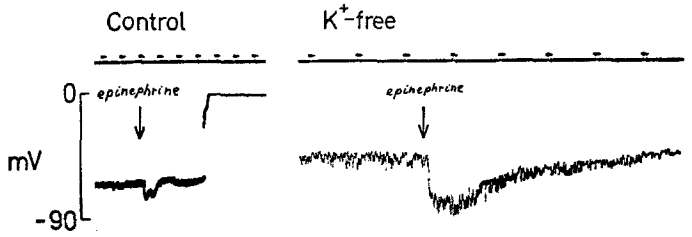

Fig. 6. The effect of epinephrine (0.5 µmole) during superfusion with control solution and in the absence of extracellular K^+. Both records were obtained from the same parotid gland. Same voltage calibration and zero line in both records

Table 2. Effect of epinephrine in solutions with varying $[K^+]_0$ and in the presence of ouabain (1 mM) [a]

Solution	Resting potential (mV)	Epinephrine-induced (10^{-5} M) hyperpolarization (mV)	Epinephrine-induced (0.5 μmole) hyperpolarization (mV)
Control	−67.6±1.3	12.0±1.5	8.6±0.7
K^+-free	−56.3±3.1	23.8±7.4	
$[K^+]_0$=20 mM	−44.8±1.5	4.5±0.7	
Ouabain	−42.5±4.9		12.0±2.0

[a] Mean±SE (n=4 to 25).

Discussion

With the help of drugs that block specifically certain actions of catecholamines and drugs which only elicit part of the effects caused by epinephrine, and the use of the α- and β-receptor classification of Ahlquist (Furchgott, 1967, 1970) it is possible to give an internally, entirely consistent description of the action of epinephrine on the parotid acinar cell membrane potential. Two distinct components are apparent: (1) a hyperpolarizing effect caused by epinephrine, norepinephrine and the α-agonist synephrine, which is uninfluenced by β-blockade (propranolol) but abolished by the α-blocking agent phentolamine; (2) a depolarizing effect caused by isoproterenol and metaproterenol, agonists which excite specifically β-receptors, which is abolished by propranolol; a depolarization which in the presence of the α-blocking agent phentolamine is also evoked by epinephrine stimulation. It is apparent that activation of the cholinergic receptor has the same effect as excitation of the epinephrine α-receptor.

These findings have important implications for the discussion of the cellular control mechanisms governing the salivary secretory processes. It is generally recognized that parasympathetic nerve stimulation mainly evokes salt and water secretion whereas sympathetic nerve stimulation evokes a smaller fluid secretion with a substantial enzyme content (Schneyer, Young & Schneyer, 1972). Recent data seem to indicate that α-adrenoceptor activation causes a higher salivary flow rate than β-receptor activation which on the other hand causes a much stronger protein secretion (Batzri *et al.*, 1973). It is therefore tempting to suggest that the processes underlying the membrane hyperpolarization are involved in the initiation of transcellular salt and water transport, whereas the depolarization is a sign of membrane events which ultimately cause extrusion of protein from the acinar cells.

The effect of acetylcholine on the salivary acinar cell membrane has been extensively investigated (Petersen, 1971, 1972; Nishiyama & Petersen, 1974) and it has been concluded that it acts by increasing the membrane permeability to K^+ and Na^+ causing a K^+ efflux and a Na^+ influx, the latter being essential in starting the secretory machinery. It has been shown that epinephrine stimulation results in a loss of intracellular K^+ (Petersen, 1970; Batzri *et al.*, 1973) and this is mediated by stimulation of α-receptors rather than β-receptors (Batzri *et al.*, 1973). This result in combination with the present finding that the epinephrine-induced hyperpolarization is enhanced by reducing the extracellular K^+ concentration and uninfluenced by ouabain, in a concentration (1 mM) that abolishes electrogenic sodium pump hyperpolarization in the same gland and the pancreas (Pedersen & Petersen, 1973; Petersen, 1973*a*), suggests that the mechanism of action of adrenergic α-agonists on salivary acinar cells is similar to that of acetylcholine.

It is interesting to note that β-receptor activation, which causes massive protein secretion by means of exocytosis from the parotid (Batzri & Selinger, 1973), is associated with a membrane depolarization, since stimulation of pancreatic acinar cells by acetylcholine and pancreozymin, causing protein secretion by the same mechanism of exocytosis (Palade, 1959), is also associated with a cell membrane depolarization (Dean & Matthews, 1972) caused by an enhanced cell membrane permeability (Petersen, 1973*b*). There is strong evidence indicating that stimulation of the epinephrine β-receptor of the parotid activates an adenylyl cyclase causing an increase in the rate of formation of intracellular cyclic AMP, stimulating exocytosis (Schramm & Naim, 1970; Batzri & Selinger, 1973). The depolarization we have observed following β-receptor activation could have been induced by an intracellular cyclic AMP accumulation, or a change in the cell membrane associated with the neurotransmitter-receptor interaction leading to the activation of the adenylyl cyclase might involve an increase in the permeability to one or more ions.

The enzyme secretory mechanism in the parotid is apparently independent of the presence of extracellular Ca^{2+} (Batzri & Selinger, 1973) and our result that isoproterenol-induced depolarization occurred normally during exposure of the tissue to a Ca^{2+}-free solution is in agreement with this. On the other hand Selinger, Batzri, Eimerl and Schramm (1973) describe the necessity for extracellular Ca^{2+} in the epinephrine-induced K^+ release. The results presented in this report indicate that epinephrine can increase the membrane permeability to K^+ in the absence of extracellular Ca^{2+}, but since we study transient responses to short pulses of stimulation it cannot

be excluded that Ca^{2+} is critically involved in the maintenance of the response to a prolonged stimulus. Our finding that the duration of the epinephrine-induced hyperpolarization is shortened during exposure to Ca^{2+}-free solutions may be related to this. Selinger, Eimerl, Savion and Schramm (1974) have suggested that the primary action of α-agonists is to cause Ca^{2+} entry to the cytoplasm and that the enhanced intracellular Ca^{2+} concentration is mediating the increase in K^+ permeability. Romero and Whittam (1971) have shown that intracellular Ca^{2+} can control Na^+ and K^+ permeability of the red cell membrane. Selinger *et al.* (1974) have shown that the Ca^{2+} ionophore A 23187 can simulate the action of epinephrine on the parotid K^+ release. Our finding that reintroduction of Ca^{2+} to a Ca^{2+}-free solution causes a hyperpolarization very similar to that induced by epinephrine could possibly be explained by this mechanism.

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