Isoproterenol Inhibition of Potassium Release from Rat Parotid Gland

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Abstract—The mechanism of isoproterenol-induced inhibition of potassium release from rat parotid slices has been determined. Spontaneous potassium release from the slices was significantly inhibited by isoproterenol at concentrations above $10^{-6}$ M. This isoproterenol effect was completely abolished in the presence of propranolol ($10^{-5}$ M) and ouabain ($10^{-3}$ M) and was abolished during Na$^+$-exclusion from the incubation medium. Isoproterenol caused an enhancement of the microsomal Na$^+$, K$^+$-ATPase activity at concentrations above $10^{-5}$ M, and this activity was inhibited by propranolol ($10^{-5}$ M). The stimulatory effect of isoproterenol on the Na$^+$, K$^+$-ATPase exhibited a strong correlation with the inhibition of potassium release on each dose of isoproterenol. Moreover, dibutyryl cyclic AMP at concentrations above $10^{-4}$ M inhibited potassium release in a dose-dependent manner and cyclic AMP caused an enhancement of the microsomal Na$^+$, K$^+$-ATPase activity. These results suggest that the inhibitory effect of isoproterenol on potassium release is clearly derived from the elevated Na$^+$, K$^+$-ATPase activity and that it may in part be mediated by cyclic AMP.

In the parotid glands, sympathetic secretory activity is mediated through the activation of both $\alpha$- and $\beta$-adrenoceptors (1-3). $\alpha$-Adrenergic agonists have been shown to cause a specific, passive potassium efflux from rat parotid slices, but do not affect cyclic AMP levels (4). $\beta$-Adrenergic agonists increase cyclic AMP levels and amylase release (5). In contrast to the action of the $\alpha$-agonists, the action of $\beta$-adrenergic stimulation on membrane transport has not been clarified in this tissue. Although a number of investigators have described the inhibitory effect of isoproterenol on potassium release in salivary gland cells (6-11), their reports do not refer to the correlation between the $\beta$-adrenergic action on potassium release and Na$^+$, K$^+$-ATPase activity in parotid cell membranes.

Na$^+$, K$^+$-ATPase is the biochemical manifestation of the Na$^+$ pump. The Na$^+$, K$^+$-ATPase enzyme regulates the active transport of Na$^+$ and K$^+$ across the cell membranes (12, 13). In addition, the Na$^+$ pump is involved, either directly or indirectly, in several physiological processes. These include the generation of the Na$^+$ and K$^+$ gradients across the cell membrane necessary for the maintenance of the cell membrane resting potential in parotid acinar cells (14) and the secretion of fluid in several epithelial tissues (15). It has already been documented that $\beta$-adrenergic stimulation can directly activate the Na$^+$, K$^+$-ATPase in other tissues such as skeletal muscle (16) and the synaptic vesicle membrane in cerebral cortex (17).

Our present study deals with the inhibitory mechanism of the $\beta$-adrenergic agonist isoproterenol on potassium release from the parotid gland.

Materials and Methods

Animals: Adult male rats of the Wistar strain (260-280 g) were used. Parotid glands were removed under pentobarbital (40 mg/ kg, i.p.) anesthesia and were then prepared for the following experiments.

Measurement of potassium release from parotid slices: The parotid gland was removed, placed in a small quantity of incubation
medium, and rapidly cut into slices of approximately 1 mm³. The slices were incubated at 37°C in Krebs-Ringer bicarbonate buffer (KRB) medium gassed with a 95% O₂-5% CO₂ mixture (equilibration period). Each slice was rapidly placed in a polyethylene tube containing 5 ml of the incubation medium for a 15 min pre-incubation. Following this, each slice was washed with the warm oxygenated medium and then placed in 5 ml of the fresh medium for the final incubation. The medium used for most of the experiments was a KRB medium with the following composition (in mM): NaCl, 118.46; KCl, 4.74; KH₂PO₄, 1.18; MgSO₄·7H₂O, 1.18; CaCl₂·2H₂O, 2.54; NaHCO₃, 24.87; D-glucose, 11.5; and adjusted to pH 7.4 by HCl. In one experiment (Table 2), Krebs-Ringer Tris (KRT) buffer with the following composition (in mM) was used for incubation: NaCl, 120; KCl, 5; CaCl₂·2H₂O, 1; MgCl₂·6H₂O, 1; Tris (hydroxymethyl) aminomethane, 20; and adjusted to pH 7.4 by Trizma HCl. In a Na⁺-free medium, NaCl was replaced by an equimolar amount of Trizma HCl. The time when the slices were placed in the final incubation medium was considered as zero time. Ouabain, isoproterenol, propranolol and dibutyryl cyclic AMP were added, alone or in combination, to the slice systems. The β-antagonist propranolol was added to the incubation medium 2 min before the addition of isoproterenol. Aliquots of the medium were subsequently taken at timed intervals (usually 2, 5 and 10 min) for analysis of their potassium concentration. At the end of the incubation period, the slices were homogenized in a Teflon homogenizer. The potassium concentration of aliquots of the medium removed in the course of the final incubation and of the homogenates of the slices was measured by a flame photometer (Corning) with a lithium internal standard. The potassium release was expressed as a percentage of the potassium contained in the slices by using the formula of Martinez et al. (6).

**Measurement of cyclic AMP content in parotid tissues:** Cyclic AMP content in parotid tissues was measured after a 5 min incubation of the parotid tissues in KRB medium containing isoproterenol. The β-antagonist propranolol was added to the incubation medium 2 min before the addition of isoproterenol. The tissues were immediately frozen with liquid nitrogen and stored until measured. Cyclic AMP was assayed by radioimmunoassay (18) with a cyclic AMP assay kit (Yamasa Shoyu, Co., Ciba, Japan).

**Measurement of Na⁺, K⁺-ATPase activity:** The parotid tissues were homogenized in a glass homogenizer with 10 vol. of ice-cold 0.25 M sucrose containing 2 mM EDTA. The medium for homogenization of the parotid tissues also contained 0.1% sodium deoxycholate. After centrifugation at 1000×g for 10 min at 4°C, the supernatant was separated and centrifuged at 10,000×g for another 10 min. The supernatant was then separated and centrifuged at 100,000×g for 30 min, and the microsomal pellet obtained was resuspended by gentle homogenization. Na⁺, K⁺-ATPase activity was determined by measuring the amount of Pi produced in 15 min at 37°C under the appropriate assay conditions. Each reaction tube contained a final vol. of 1.0 ml with the following composition (in mM): NaCl, 100; KCl, 10; MgCl₂, 4; Tris ATP, 4; Tris, 33; at pH 7.4 and 0.1-0.2 mg protein. After all additions except the enzyme were made, the reaction tubes were pre-incubated at 37°C for 5 min in a shaking water bath. The reaction was initiated with the enzyme and terminated after 15 min by the addition of 1 ml of 20% trichloroacetic acid. After vigorous mixing, the tubes were centrifuged for 10 min at 1000×g. The Pi in the upper phase was measured using the Fiske-Subbarow method (19). Na⁺, K⁺-ATPase activity (ouabain-sensitive) was determined by subtracting the amount of Pi produced in the presence of ouabain (10⁻³ M) from the amount of Pi produced in the absence of ouabain. All determinations were made in duplicate. The β-antagonist propranolol was added to the pre-incubation media 5 min before the addition of isoproterenol.

Protein determinations were performed by the method of Lowry et al. (20).

Data are presented as the mean±S.E.
Statistical analysis was performed using Student’s t-test (two-tailed).

Drugs used: (-)-Isoproterenol HCl (Sigma), (-)-propranolol HCl (Sigma), ouabain (Merck Japan, Co.), dibutyryl adenosine 3',5'-cyclic monophosphate monosodium salt (DB cAMP, Yamasa Shoyu, Co., Japan), and adenosine 3',5'-cyclic monophosphate, free acid (cAMP, Yamasa Shoyu, Co., Japan).

Results

Effect of isoproterenol on potassium release from the slices: Figure 1 shows the effect of various concentrations of isoproterenol on potassium release from the parotid slices. In the absence of drugs (no stimulation) in the KRB medium, the potassium releases at 5 and 10 min after the beginning of the incubation period were 5.53±0.82 and 8.60±0.05% of the total tissue potassium content, respectively. Potassium release from the slices was significantly inhibited by isoproterenol at concentrations above $10^{-6}$ M, although there was no effect at concentrations below $10^{-7}$ M (data not shown). The dose-response for the inhibitory effect of isoproterenol had an obvious transition between $10^{-4}$ and $10^{-5}$ M, and the order of ability was $10^{-6}>10^{-5}>10^{-4}$ M after the incubation for 5 min. The effect of isoproterenol ($10^{-5}$ M) was significantly abolished in the presence of propranolol ($10^{-5}$ M). However, propranolol alone ($10^{-5}$ M) had no effect on potassium release from the slices (data not shown).

Effect of isoproterenol on cyclic AMP levels of the tissues: The effect of isoproterenol on cyclic AMP levels is shown in Table 1. Five min after the addition of isoproterenol ($10^{-4}$–$10^{-6}$ M), the cyclic AMP content in the tissues was markedly elevated; after this, it exhibited a tendency to decline gradually. A significant increase in cyclic AMP level brought about by $10^{-5}$ M isoproterenol was obviously inhibited in the presence of propranolol ($10^{-5}$ M). In addition, this stimulatory effect was completely inhibited by $10^{-4}$ M propranolol, and propranolol alone did not affect cyclic AMP level at the concentration of $10^{-4}$ M (data not shown).

Effect of dibutyryl cyclic AMP on potassium release from the slices: The effect of the

| Addition     | Concentration (M) | Cyclic AMP (pmol/mg protein) (n) |
|--------------|-------------------|----------------------------------|
| None         |                   | 6.37±0.96 (6)                    |
| Isoproterenol| $10^{-4}$         | 231.58±23.66** (5)               |
|              | $10^{-5}$         | 227.32±13.87** (5)               |
|              | $10^{-6}$         | 131.41±4.85** (5)                |
| Isoproterenol| $10^{-5}$+|    | 72.74±18.77** (4)               |
|              | Propranolol       |                                  |

Cyclic AMP level was determined 5 min after the addition of isoproterenol. Values are the mean±S.E. **P<0.01, vs. no stimulation; **P<0.01, vs. $10^{-5}$ M isoproterenol.
dibutyryl derivative of cyclic AMP on potassium release from the parotid slices is shown in Fig. 2. Potassium release from the slices was significantly inhibited by dibutyryl cyclic AMP at concentrations above $10^{-4}$ M, and it was without effect at concentrations below $10^{-5}$ M.

Effect of ouabain on isoproterenol-inhibited potassium release: As shown in Fig. 3, $10^{-3}$ M ouabain caused an increase in potassium release from 5.53±0.82% (no stimulation) to 7.63±0.27% after incubation for 5 min. The inhibitory effect of isoproterenol ($10^{-5}$ M) was completely abolished in the presence of $10^{-3}$ M ouabain throughout the potassium release experiments (2, 5 and 10 min).

Effect of Na⁺-free medium on isoproterenol-inhibited potassium release: After a 15 min pre-incubation with Na⁺-free KRT medium (see Materials and Methods), the slices were exposed to $10^{-5}$ M isoproterenol for 5 min under the same conditions. This effect is shown in Table 2, which also shows that isoproterenol-induced inhibition of potassium release was significantly abolished. Indeed, when the slices were

![Fig. 2. Effect of dibutyryl cyclic AMP on potassium release from the parotid slices. X. No stimulation; •, $10^{-3}$ M dibutyryl cyclic AMP; ■, $10^{-4}$ M dibutyryl cyclic AMP; ▲, $10^{-5}$ M dibutyryl cyclic AMP. Values are the mean±S.E. of 7 control experiments (no stimulation) and of at least 5 experiments in the other groups. **P<0.01, *P<0.05, vs. no stimulation.](image)

![Fig. 3. Effect of ouabain on isoproterenol-inhibited potassium release from the parotid slices. X. No stimulation; ●, $10^{-5}$ M isoproterenol; ■, $10^{-3}$ M ouabain, ▲, $10^{-5}$ M isoproterenol plus $10^{-3}$ M ouabain. Values are the mean±S.E. of 7 control experiments (no stimulation) and of at least 5 experiments in the other groups. **P<0.01, *P<0.05, vs. no stimulation. ¶¶P<0.01, vs. $10^{-5}$ M isoproterenol alone.](image)

**Table 2.** Effect of Na⁺-free medium on the inhibition of potassium release from parotid slices by isoproterenol

| Addition     | K⁺ release ( % of total) | K⁺ release ( % of total) |
|--------------|--------------------------|--------------------------|
|              | with Na⁺          | (n)          | without Na⁺   | (n)          |
| None         | 4.81±0.43         | 8            | 10.58±1.37¶¶ | 6            |
| Isoproterenol| 0.91±0.39**       | 6            | 11.47±1.67¶¶  | 6            |

Isoproterenol ($10^{-6}$ M) was added at zero time to the incubation medium. K⁺ release was determined 5 min after the addition of isoproterenol. Values are the mean±S.E. **P<0.01 (isoproterenol added vs. none added). ¶¶P<0.01 (without Na⁺ vs. with Na⁺).
incubated with Na\(^+\)-free medium, the potassium released in the absence of isoproterenol elevated to about 2-fold that of the basal release with normal medium.

**Effects of isoproterenol and cyclic AMP on the microsomal Na\(^+\), K\(^+\)-ATPase activity:** A comparison of the effects of isoproterenol and cyclic AMP on the Na\(^+\), K\(^+\)-ATPase activity of the parotid microsomes is shown in Table 3. Isoproterenol significantly stimulated the Na\(^+\), K\(^+\)-ATPase activity of the microsomes at concentrations above 10\(^{-5}\) M. Isoproterenol-induced activation of the enzyme activity was obviously inhibited in the presence of 10\(^{-5}\) M propranolol. Propranolol alone did not affect Na\(^+\), K\(^+\)-ATPase activity at the concentration of 10\(^{-5}\) M (data not shown). Cyclic AMP also significantly stimulated the Na\(^+\), K\(^+\)-ATPase activity of the microsomes at concentrations above 10\(^{-5}\) M.

**Table 3. Comparison of the effects of isoproterenol and cyclic AMP on Na\(^+\), K\(^+\)-ATPase of parotid microsomes**

| Drug               | Concentration (M) | Na\(^+\), K\(^+\)-ATPase | (n) |
|-------------------|-------------------|---------------------------|-----|
| Isoproterenol     | 10\(^{-4}\)       | 118.73± 5.72\(^{*}\)     | 4   |
|                   | 10\(^{-5}\)       | 144.89±10.28\(^{**}\)    | 5   |
|                   | 10\(^{-6}\)       | 109.51± 7.22              | 4   |
| Isoproterenol     | 10\(^{-5}\) +     | 113.52±11.25\(^{\dagger}\) | 4   |
| Propranolol       | 10\(^{-5}\)       |                           |     |
| 3',5'-cyclic AMP  | 10\(^{-4}\)       | 138.28± 8.37\(^{**}\)    | 4   |
|                   | 10\(^{-5}\)       | 119.57± 5.48\(^{*}\)     | 4   |

Typical absolute values (ouabain sensitive) for Na\(^+\), K\(^+\)-ATPase activity of the control: 4.05±0.57 (n=5) μmole P\(_i\)/mg protein/hr. The results are presented as the percentage of increase in activity (control mean value=100%). Values are the mean ± S.E. \(^{*}\)P<0.01, \(^{**}\)P<0.05, vs. controls. \(^{\dagger}\)P<0.05, vs. 10\(^{-5}\) M isoproterenol.

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As regards to the apparent inhibition of potassium release induced by isoproterenol, our results seem to suggest an increase in potassium influx into parotid acinar cells induced by activation of Na\(^+\), K\(^+\)-ATPase, although the inhibitory effect of isoproterenol on potassium release may have influenced passive potassium efflux as well. The fact that isoproterenol increases net uptake of potassium has been demonstrated in avian erythrocytes in vitro (24, 25). It appears, therefore, that the β-adrenoceptor and Na\(^+\)-pump may work in identical directions on the transmembrane shift of potassium (6), and β-adrenoceptor stimulation may directly activate the Na\(^+\), K\(^+\)-pump, thus resulting in K\(^+\) uptake (10).
concentration of K+ ions (26, 27). At high external K+ concentrations (above 10 mM), isoproterenol strongly activates the uni-directional efflux of potassium from turkey erythrocytes (26), and at low external K+ concentrations (below 0.1 mM), isoproterenol strongly inhibits the passive efflux of potassium from pigeon erythrocytes (27), as also observed in our experiment using normal K+ medium (Fig. 1 and Table 2). It remains unclear at present whether external K+ concentrations alter the effect of isoproterenol from activation to inhibition.

On the other hand, it is also well known that isoproterenol causes cyclic AMP accumulation (21, 28–30), and it is widely accepted that cyclic AMP is a major regulator of β-adrenergic action in this tissue. Figure 1 and Table 1 show that an equimolar amount of isoproterenol caused the inhibition of potassium release and the accumulation of cyclic AMP, the latter effect nearly corresponding with the peak time of inhibited potassium release. When dibutyryl cyclic AMP, a derivative of cyclic AMP that penetrates the cell membrane, is added to the medium, a weak inhibition of potassium release is observed (Fig. 2). The difference in potency between the dibutyryl cyclic AMP and isoproterenol is apparently the result of differences in the rates of increase of cyclic AMP levels which occur within the cells. In addition, isoproterenol obviously activated the Na+, K+-ATPase of the microsomes. This effect correlated with intracellular accumulation of cyclic AMP at each individual dose and its activation was inhibited in the presence of the β-antagonist propranolol (Table 1 and 3). Our results indicate, at least qualitatively, that the inhibitory effect on potassium release induced by isoproterenol may be mediated by increased intracellular accumulation of cyclic AMP. We would like to emphasize one point of interest. Both the activation of Na+, K+-ATPase and the inhibition of potassium release by isoproterenol may be mediated by a common sequence of events: binding of isoproterenol to β-adrenoceptors, activation of adenylyl cyclase, accumulation of cyclic AMP, and activation of Na+, K+-ATPase(s).

Our results suggest that the inhibitory effect of isoproterenol on potassium release is clearly brought about by its interaction with β-adrenoceptors and induced by activation of Na+, K+-ATPase, and this activation may be, in part, mediated via intracellular accumulation of cyclic AMP.

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