β1-Adrenergic Regulation of Cyclic Nucleotide Levels and Potassium Fluxes in Rat Parotid Gland

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It is generally considered that both α- and β-adrenocceptors exist in rat parotid glands (1, 2). In our previous paper, the effect of adrenergic stimuli on spontaneous potassium fluxes was found to be distinctly different between α1- and β-adrenergic agonists, the former increases potassium efflux and the latter decreases potassium release (3, 4).

The present in vitro investigation was undertaken in an attempt to obtain further information as to the role of β1-adrenoceptor subtypes on isoproterenol-induced decrease in potassium release from rat parotid tissue.

Male Wistar rats (260–280 g) were used throughout the experiments. Parotid glands were removed under pentobarbital (40 mg/kg, i.p.) anesthesia, and then the parotid slices were prepared and incubated according to the procedures described by Ohshika et al. (5). Briefly, the slices were incubated for 30 min at 37°C in the Krebs-Ringer bicarbonate (KRB) medium gassed with a 95% O2-5% CO2 mixture (equilibration period). Then, they were incubated for 5 min with isoproterenol or selective β-adrenergic agonists (challenge). β-Adrenergic antagonists were added into the incubation medium 2 min before the addition of isoproterenol (pretreatment). At the end of the final incubation, 100 μl aliquots of the medium were removed, and the slices were homogenized together with the remaining medium. The homogenates were centrifuged at 1000×g for 10 min. The potassium concentrations in aliquots of the medium and the supernatants were determined by a flame photometer (Corning) using a lithium internal standard. The release of potassium was expressed as the percent of total potassium in the slices, using the formula described by Martinez et al. (6).

The cyclic AMP content of parotid tissue was measured after a 5 min incubation of the parotid slices in KRB medium with various β-adrenergic agonists. After the incubation, the tissue slices were immediately frozen with liquid nitrogen and stored until time for measurement. Cyclic AMP was assayed by radioimmunoassay (7) with a cyclic AMP assay kit (Yamasa Shoyu Co., Japan).

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

The following drugs were used: (-)-isoproterenol HCl (Sigma), (+)-propranolol HCl (Sigma), and butoxamine HCl (Burroughs-Wellcome). Dobutamine HCl was a gift from the Shionogi Pharmaceutical Co. (Japan), procaterol HCl was a gift from Otsuka Pharmaceutical Co. (Japan), and metoprolol HCl was a gift from Fujisawa Pharmaceutical Co. (Japan).

Table 1 shows the effects of various β-adrenoceptor agents on spontaneous potassium release (basal release) from the parotid slices. This release was significantly decreased by 10 μM isoproterenol and 10 μM dobutamine (a selective β1-adrenoceptor agonist) (P<0.01 vs. none), but not affected by 10 μM procaterol (a selective β2-adrenoceptor agonist). On the other hand, the decrease in potassium release induced by 10 μM isoproterenol was completely inhibited by pretreatment with 10 μM propranolol or 10 μM metoprolol (a selective β1-adrenoceptor antagonist) (P<0.01 vs. isoproterenol alone), but not affected by 10 μM butoxamine (a selective β2-adrenoceptor antagonist). In addition, no significant changes were found with each β-adrenoceptor antagonist alone.
Table 1. Effects of various β-adrenoceptor agents on the spontaneous potassium release from incubated rat parotid slices

<table>
<thead>
<tr>
<th>Drug concentration (μM)</th>
<th>Potassium release (% of total)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>5.53±0.82</td>
<td>8</td>
</tr>
<tr>
<td>Isoproterenol (10)</td>
<td>-0.76±0.64**</td>
<td>7</td>
</tr>
<tr>
<td>Dobutamine (10)</td>
<td>2.20±0.96**</td>
<td>5</td>
</tr>
<tr>
<td>Procaterol (10)</td>
<td>6.27±0.68</td>
<td>5</td>
</tr>
<tr>
<td>Propranolol (10)</td>
<td>5.20±0.35</td>
<td>5</td>
</tr>
<tr>
<td>Propranolol (10) + Isoproterenol (10)</td>
<td>4.44±0.92††</td>
<td>5</td>
</tr>
<tr>
<td>Metoprolol (10)</td>
<td>4.96±0.31</td>
<td>5</td>
</tr>
<tr>
<td>Metoprolol (10) + Isoproterenol (10)</td>
<td>4.38±0.74††</td>
<td>6</td>
</tr>
<tr>
<td>Butoxamine (10)</td>
<td>5.25±0.21</td>
<td>5</td>
</tr>
<tr>
<td>Butoxamine (10) + Isoproterenol (10)</td>
<td>0.18±0.09**</td>
<td>5</td>
</tr>
</tbody>
</table>

The release of potassium was determined 5 min after the addition of various drugs. In the combination experiments, propranolol, metoprolol or butoxamine was added 2 min before the addition of isoproterenol. The values represent the mean±S.E. **P<0.01 vs. None (spontaneous release), ††P<0.01 vs. isoproterenol alone.

The effects of various β-adrenoceptor agonists on cyclic AMP accumulation are shown in Fig. 1. Five min after the addition of 10 nM isoproterenol and 10 nM dobutamine, the content of cyclic AMP in the tissues were markedly elevated (163.20±18.73 and 41.88±8.51 pmol/mg protein, respectively) (P<0.01 vs. none), and these effects almost corresponded with the peak time of decreased potassium release. No significant changes were obtained with 10 nM procaterol (6.98±0.89 pmol/mg protein). The basal cyclic AMP level at the same time was 5.43±0.92 pmol/mg protein.

β-Adrenoceptors can be divided into two subtypes: one present mainly in the heart and fat tissue (β₁), and the other present in the lung and blood vessels (β₂) (8, 9). However, the adrenoceptors have not yet been isolated or characterized biochemically, though the recent development of high affinity selective β₁-adrenoceptor blocking agents as well as selective β₁- and β₂-receptor agonists has facilitated more specific analysis of the β-receptors present in various tissues, including the parotid gland. Studies employing receptor selective drugs, in vitro, indicate that parotid amylase release in rats (10, 11) is mediated mainly via activation of β₁-adrenoceptors. Au et al. (12) have also shown that β-adrenergic receptors can be identified in the rat parotid gland and that these binding sites display a β₁ character.

In our present study, dobutamine was more effective than procaterol with regard to the decrease in potassium release from
parotid slices. Dobutamine is a highly potent $\beta_1$ selective agonist in the papillary muscle of the cat (13), and procaterol is a highly potent $\beta_2$ selective agonist in the soleus muscle and cardiovascular system of the cat (14). In the present study, we have also shown that the decrease in potassium release induced by isoproterenol from parotid slices was completely blocked by the selective $\beta_1$ antagonist metoprolol as well as the nonselective $\beta$ antagonist propranolol, but the selective $\beta_2$ antagonist butoxamine had no effect. The present data support the theory that $\beta$-adrenoceptors in rat parotid glands are mainly of the $\beta_1$ subtype, and they are similar to those present in the heart and adipose tissues. Our results suggest that $\beta_1$-adrenoceptors play an important role in the $\beta$-adrenergic stimulation, causing the decrease in the spontaneous potassium release from rat parotid slices.

On the other hand, the selective $\beta_1$ agonist dobutamine and isoproterenol caused a significant increase in the cyclic AMP level, but the selective $\beta_2$ agonist procaterol had no effect. Although we did not investigate if the intracellular cyclic AMP directly stimulates the membrane Na+, K+-ATPase of the parotid acinar cells, the fact that the dibutyryl derivative of cyclic AMP caused a significant decrease in potassium release from the slices (15) may support the hypothesis of $\beta_1$-adrenoceptor mediated suppression of the spontaneous potassium release. It is reported that ouabain, an inhibitor of Na+, K+-ATPase, increases the spontaneous potassium release in the rat submaxillary gland (6). In experiments using pigeon erythrocytes, isoproterenol-induced potassium uptake was enhanced by a inhibition of cyclic AMP phosphodiesterase, and dibutyryl cyclic AMP caused an increase in the potassium uptake in the cells (16).

Based on these findings, it was concluded that $\beta_1$-adrenergic stimulation induced the decrease in potassium release from the parotid slices, which is accompanied with the elevation of total intracellular content of cyclic AMP.

References
14 Yamashita, S., Takai, M. and Yabuuchi, Y.: Actions of procaterol (OPC-2009), a new $\beta_2$-
