POTENTIATION OF ISOPROTERENOL-INDUCED SALIVATION BY LABETALOL

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Abstract—The authors previously reported that low doses of labetalol potentiated isoproterenol (ISO)-induced salivation in mice. The present study was carried out to ascertain the potentiating effect of labetalol in in vitro experiments using rat parotid tissue slices and their isolated cells. ISO-induced amylase release was enhanced by low concentrations of labetalol (less than 10^{-6} M), while it was inhibited by high doses (more than 10^{-5} M). This potentiating effect of labetalol did not occur in the Ca-free medium used for incubation or in experiments using the dispersed parotid cells or the parotid tissues which were obtained from 6-hydroxydopamine- or reserpine-treated rats. The effect of ISO on cyclic AMP accumulation in parotid tissue was completely blocked by the addition of labetalol in concentrations which were sufficient to increase the ISO-induced amylase release. Labetalol also inhibited the ISO-induced reduction of potassium release in parotid tissue. From these results, it is concluded that increases in the secretion of amylase and potassium from salivary glands due to the stimulation of glandular \( \alpha \)-adrenoceptors by endogenous norepinephrine may play an important role in the potentiating effect of labetalol on ISO-induced salivation in mice.

Labetalol is an antihypertensive agent which has a combined \( \alpha \)- and \( \beta \)-adrenoceptor blocking action in various tissues (1). It is well known that an increase of salivation is elicited by stimulation of sympathetic nerves or by administration of \( \alpha \)- and \( \beta \)-(adrenoceptor) agonists (2). An intravenous administration of labetalol in mice, however, resulted in increased salivation despite a prediction that due to its blocking action on both \( \alpha \)- and \( \beta \)-adrenoceptors, it would cause the dry mouth which has previously been described as a side effect of labetalol (3). Furthermore, a small dose (1 mg/kg) of labetalol surprisingly enhanced isoproterenol (ISO)-induced salivation, but the same dose completely blocked the salivary effects of epinephrine and norepinephrine (4).

It has been reported that the amylase concentration in saliva is not affected by an increased volume of saliva (5) and that it is closely correlated with the flow rate of saliva (6). These results suggest that whenever the release of amylase is increased by secretagogues or nerve stimulations, some dilution mechanisms may be activated to keep the concentration of amylase in saliva at a constant level and may contribute to an increase of salivary volume. Recently, we reported that ISO-induced amylase release from rat parotid slices was potentiated in the presence of \( \alpha \)-agonists (7). From the results of experiments using rat anococcygeus muscle, Doggrell and Paton (8) suggested
trypsin and was incubated for 10 min at 37°C under pure oxygen. The composition of the dispersion solution was as follows (mM): NaCl, 103; KCl, 4.7; CaCl₂, 2.56; MgCl₂, 1.13; beta-hydroxybutylate, 5.0; sodium pyruvate, 4.9; sodium fumarate, 2.7; sodium glutamate, 4.9; and Tris (hydroxymethyl) aminomethane, 20.0; and the pH was adjusted to 7.4.

After the cell suspension had been centrifuged at 200×g for 5 min, the resulting pellet was resuspended and then was incubated in 10 ml of a Ca- and Mg-free dispersion solution to which was added collagenase (240 units/ml), hyaluronidase (1 mg/ml), and soybean trypsin inhibitor (0.1 mg/ml). Five ml of filtered cell suspension was added to the same volume of a dispersion solution containing 20 mg/ml bovine serum albumin (BSA) and 0.1 mg/ml trypsin inhibitor, after which the mixture was centrifuged. The pellet was then washed three times by resuspensions and centrifugations. Finally, it was suspended in a dispersion solution with BSA (10 mg/ml) and adjusted to an approximate concentration of 1×10⁶ cells/ml. The cell viability value as checked by the trypan blue exclusion test was 95±0.7% (mean±S.E.).

Amylase release from dispersed parotid cells: The dispersed cells were incubated for 30 min with 1% BSA solution in a dispersion solution containing ISO as the secretagogue. Labetalol was added 10 min prior to the addition of ISO. At the end of incubation, the cells were separated from the incubation medium by centrifugation at 1,000×g for 30 sec. Then they were frozen and thawed three times. Amylase activities in the medium and cells were determined by the method of Searcy et al. (13). The amount of amylase released from the cells was expressed as the percent of the initial content of the enzyme in the cells (7).

Statistical analysis: The Student’s t-test was used for a statistical comparison of the mean values.

Drugs used: The following drugs were used: (-)-isoproterenol HCl (Sigma), (-)-epinephrine bitartrate (Sigma), (±)-propranolol HCl (Sigma), phentolamine HCl (®Regitin, CIBA), 6-hydroxydopamine HCl (Sigma), and reserpine solution for injection (®Apoplon, Daiichiseiyaku). Labetalol HCl was a gift from the Shinnihonjitsugyo Co. (Tokyo).

RESULTS
Effects of labetalol on ISO-induced amylase release: The basal release of amylase from rat parotid slices was 0.3±0.0% of the total amylase in the tissue during a 10 min incubation period and was unaffected by the addition of labetalol (10⁻⁶ to 10⁻⁴ M) (data not shown). The effect of labetalol in concentrations of 10⁻⁸ to 10⁻⁴ M was investigated on amylase release induced by 10⁻⁵ M ISO (6.7±0.5%) (Fig. 1). While labetalol in doses over 3×10⁻⁵ M decreased ISO-induced amylase release in a dose-dependent manner, the same drug in concentrations of less than 10⁻⁶ M potentiated the action of ISO. Enzyme release by ISO was increased by 33% in the presence of 10⁻⁶ M labetalol (P<0.01).

Fig. 1. Effect of labetalol on isoproterenol (ISO)-induced amylase release in rat parotid tissue. The broken lines show the range of amylase release induced by 10⁻⁶ M ISO (6.7±0.5%) during a 10 min incubation period. Each value represents the mean±S.E. of 4 to 6 experiments. Labetalol (LAB) was added into the incubation medium 10 min prior to ISO.
Some experiments were carried out on isolated parotid cells to determine the site of action of labetalol for exerting its potentiating effect. Labetalol (10^{-8} and 10^{-7} M) failed to enhance the increase of amylase release induced by 10^{-6} M ISO, and 10^{-6} M labetalol markedly reduced the increase (Fig. 2). These concentrations of labetalol per se had no effect on the basal release of amylase.

Pretreatment with 6-HDA and reserpine:

To investigate the role of endogenous catecholamines in the effect of labetalol, parotid slices from rats given 6-HDA (10 mg/kg) or reserpine (5 mg/kg) were used. As shown in Table 1, there was no significant difference in the amount of amylase released by 10^{-5} M ISO between the control and 6-HDA-treated groups. The potentiating effect of labetalol completely disappeared, however, in the 6-HDA-treated group. Although the effect of ISO itself was decreased by reserpine treatment, the disappearance of the potentiating effect of labetalol was observed as in the 6-HDA-treated group.

Ca deprivation from the incubation medium: To examine the requirements of extracellular Ca for the potentiating effect of labetalol, the tissue slices were incubated in Ca-free medium for 10 min. As shown in

![Fig. 2. Amylase release from isolated rat parotid cells. Dispersed parotid cells were incubated for 30 min with isoproterenol (ISO) and/or labetalol (LAB). Each value indicates the percent release of total amylase in the cells. Solid columns indicate the amount of amylase released in the presence of labetalol alone. Experimental details are given in Methods.](image)

![Fig. 3. Amylase release from parotid slices in normal (open column) and Ca-free medium (hatched column) during a 10 min incubation period. ISO: 10^{-5} M isoproterenol and LAB: 10^{-6} M labetalol. **P<0.01 compared with the corresponding control.](image)

### Table 1. Amylase release from rat parotid slices from 6-hydroxydopamine- or reserpine-treated animals. Experimental details are given in Methods. Each value represents the mean±S.E. (n: 6 to 10 experiments). **P<0.01 compared with ISO alone.

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>Dose (mg/kg)</th>
<th>ISO 10 μM</th>
<th>ISO 10 μM plus labetalol 10 μM</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td></td>
<td>7.8±0.9</td>
<td>14.1±1.4**</td>
</tr>
<tr>
<td>6-Hydroxydopamine</td>
<td>10</td>
<td>8.0±0.8</td>
<td>6.9±0.1</td>
</tr>
<tr>
<td>Reserpine</td>
<td>5</td>
<td>4.0±0.6</td>
<td>3.7±0.7</td>
</tr>
</tbody>
</table>
Fig. 3. neither basal nor ISO-induced amylase release was affected by removal of Ca from the incubation medium, while under the same conditions, enhanced amylase release by labetalol (14.0±1.6%) was reversed to the value obtained with ISO alone (6.9±0.7%).

ISO-induced accumulation of cyclic AMP: Two min after the addition of 10^{-5} M ISO, the content of cyclic AMP in the slices was elevated to about 22-fold the basal value (21.00±1.80 pmol/mg tissue) (Fig. 4). This effect of ISO was inhibited by 10^{-6} M labetalol, resulting in a reduction in the content to 3.06±0.21 pmol/mg tissue.

Effect of labetalol on potassium release: The time course of potassium release from the rat parotid slices is shown in Fig. 5. The basal release increased for 5 min from the start of incubation and then reached a plateau (2.4±0.9%). Potassium release in the presence of 10^{-5} M labetalol remained nearly the basal value at 5 min, but became slightly higher than the basal value. Epinephrine (10^{-5} M) markedly increased potassium release, but ISO (10^{-5} M) significantly reduced the spontaneous release.

As shown in Table 2, a tendency to increase the release of potassium in the presence of labetalol was observed with decreasing concentrations of the drug from 10^{-4} to 10^{-7} M. The inhibitory effects of

![Fig. 4](image_url) Effect of labetalol on isoproterenol-induced accumulation of cyclic AMP in parotid tissue after a 2 min incubation period. Each value indicates the mean±S.E. (n=5). ISO: 10^{-5} M isoproterenol and LAB: 10^{-6} M labetalol.

![Fig. 5](image_url) Time course of potassium release in the presence of 10^{-6} epinephrine (△), isoproterenol (○), or labetalol (×) and in the absence of drug (●). Each point indicates the mean value of 3 to 7 experiments.

| Table 2. Effect of labetalol on potassium release from parotid slices. Experimental details are given in Methods. *P<0.05 compared with None. |
|----------------|-----------------|-----------------|-----------------|
| Concentration of labetalol | Number of experiments | Incubation time 5 min | Incubation time 10 min |
| None | 7 | 2.4±0.9 | 3.6±1.1 |
| Labetalol | 100 μM | 6 | 3.6±0.9 | 5.6±1.9 |
| | 10 μM | 10 | 3.2±0.9 | 5.8±1.9 |
| | 1 μM | 7 | 3.3±0.8 | 7.0±0.9* |
| | 0.1 μM | 8 | 4.5±0.9 | 7.8±1.2* |
Fig. 6. Potassium release from rat parotid slices in the presence of adrenoceptor agonists and adrenoceptor antagonists. Slices were incubated for 5 min with 10^{-5} M epinephrine or ISO in the presence of 10^{-5} M labetalol (solid column), propranolol (hatched column), and phentolamine (stippled column). Experimental details are given in Methods. *P<0.05 and **P<0.01 compared with the corresponding control.

10^{-6} M labetalol on the action of epinephrine and ISO were equivalent to the inhibitory effects of the same molar concentration of phentolamine and propranolol, respectively (Fig. 6).

DISCUSSION

In our previous study (4), we found that labetalol given to mice unexpectedly potentiated ISO-induced salivation. Potentiation of salivation by 1 mg/kg labetalol was observed in mice which were given ISO in a dose range of from 2 to 20 mg/kg, but not in doses smaller than 2 mg/kg. On the contrary, salivation induced by epinephrine and norepinephrine was inhibited by labetalol. The occurrence of these phenomena might be explained in part by two results obtained in the present study, i.e., potentiation of ISO-induced amylase release (Fig. 1) and antagonism of the inhibitory effect of ISO on potassium release (Fig. 6).

As shown in Fig. 1, low concentrations of labetalol (10^{-7}–10^{-5} M) enhanced the amylase release induced by 10^{-5} M ISO in rat parotid tissue, while higher doses of labetalol (10^{-5}–10^{-4} M) markedly inhibited the effect of ISO. These results were consistent with the findings on salivation obtained in mice (4). It has been reported that two \( \alpha \)-agonists, norepinephrine and phenylephrine, elicit amylase release from parotid tissue (7, 14); and it has also been suggested that the release of endogenous norepinephrine might cause amylase release from this tissue (15). If labetalol could elicit a release of endogenous norepinephrine in rat parotid tissue, as shown in rat anococcygeus muscle by Doggrell and Paton (8), the potentiating action of labetalol may be the result of a similar mechanism. This hypothesis may be supported by the following three findings: First, labetalol failed to potentiate ISO-induced amylase release in parotid tissue from rats which were pretreated with sufficient doses of 6-HDA (16) or reserpine (17) to deplete endogenous norepinephrine in salivary glands (Table 1). Second, the potentiating effect of labetalol disappeared with deprivation of Ca from the incubation medium (Fig. 3). The potentiating effect of the \( \alpha \)-agonists on ISO-induced amylase release did not require the presence of extracellular Ca (7) as is also true of ISO-induced amylase release itself (Fig. 3). Although the release of catecholamines by mechanisms other than exocytosis may be independent of Ca ions (18), from the present results, it seems likely that labetalol-induced release of endogenous norepinephrine requires extracellular Ca. Third, labetalol could not induce an increase in ISO-induced amylase release in the dispersed parotid cells (Fig. 2). Although the retention of nerve terminals in enzymatically dispersed parotid cell preparations cannot be neglected (19), the amount of these remaining nerve endings practically seems to be too small to result in the labetalol-induced potentiation as compared with that of the parotid tissue.

The concentration of labetalol (10^{-6} M) sufficient for inhibition of \( \beta \)-adrenoceptors in
the tissue, which extremely suppressed the effect of ISO on cyclic AMP level (Fig. 4), caused an increase of ISO-induced amylase release (Fig. 1). The occurrence of the potentiating effect of labetalol was observed at the drug concentrations less than $10^{-6}$ M (Fig. 1). These results suggest that the potentiating effect of labetalol is probably resulted from the stimulation of $\alpha$-adrenoceptors, but not $\beta$-adrenoceptors, by release of endogenous norepinephrine. Recently, it was suggested that the mobilization of intracellular Ca may play an important role for this enhancement of ISO-induced amylase release by $\alpha$-adrenoceptor stimulation (7, 20).

In addition to the effect of labetalol on amylase release, its effects on potassium release appear to contribute to the enhancement of ISO-induced salivation in mice. In vitro experiments, an increase of potassium release from tissue of the glands has been referred to as stimulated water secretion (21). In the present study, labetalol reversed the ISO-induced reduction of potassium release on one side and labetalol itself slightly increased the electrolyte release on the other (Figs. 5, 6). These effects of the drug probably resulted in an increase of the net potassium release from the salivary glands. The effect of labetalol on spontaneous potassium release obviously occurred at its lower concentration (Table 2). This result suggests the contribution of endogenous norepinephrine to labetalol-induced potassium release as is the case with amylase release.

From our results, it is concluded that the potentiation of ISO-induced salivation by labetalol may be due in part to the increase of amylase and potassium release from the tissues of the salivary glands.

REFERENCES

14) Leslie, B.A., Putney, J.W., Jr. and Sherman, J.M.: $\alpha$-Adrenergic, $\beta$-adrenergic and cholinergic mechanisms for amylase secretion by


