Effects of Isoproterenol Pretreatment on Phosphatidylinositol Turnover in Rat Parotid Gland

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Abstract—The effects of isoproterenol pretreatment on phosphatidylinositol turnover in rat parotid slices were studied to elucidate the relationship between \( \beta \)- and \( \alpha_1 \)-adrenoceptors. \(^{32}\)P-Labeling of phosphatidylinositol in parotid slices was increased by an \( \alpha_1 \)- and \( \beta \)-agonist (epinephrine and norepinephrine) and \( \alpha_1 \)-agonists (methoxamine and phenylephrine), but not by an \( \alpha_2 \)-agonist (clonidine) and a \( \beta \)-agonist (isoproterenol). Prazosin inhibited the increase in phosphatidylinositol turnover elicited by epinephrine, but propranolol did not. These results indicate that the stimulation of phosphatidylinositol turnover elicited by adrenergic agonists is mediated by activation of \( \alpha_1 \)-adrenoceptors in the parotid glands. Isoproterenol pretreatment of the parotid slices caused a significant increase in \(^{32}\)P-labeling of phosphatidylinositol and a decrease in that of phosphatidic acid. The epinephrine- or phenylephrine-induced increase in \(^{32}\)P-labeling of phosphatidylinositol were further enhanced by the isoproterenol pretreatment of the slices. In the isoproterenol-treated membranes of the parotid glands, \(^{3}\)H]prazosin binding to \( \alpha_1 \)-receptors increased, but \(^{3}\)H]dihydroalprenolol binding to \( \beta \)-receptors did not. These findings indicate that the acceleration of phosphatidylinositol turnover induced by the isoproterenol pretreatment may be associated with an increase in \( \alpha_1 \)-adrenoceptor binding sites which might have appeared as a result of the isoproterenol pretreatment of the parotid slices.

The increase in phosphatidylinositol turnover is a well established phenomenon associated with the stimulation of several receptors, including \( \alpha_1 \)-adrenergic and muscarinic cholinergic receptors (1-6). Rat parotid glands have been excellent model systems for studying adrenergic control of defined biochemical and physiological processes during exocrine secretion. Our previous studies have demonstrated that incubation of rat parotid slices with isoproterenol causes an increase in \( \alpha_1 \)-adrenoceptor number in addition to a potentiation in K\(^+\) release (7, 8); a close relationship between \( \beta \)- and \( \alpha_1 \)-receptors has been suggested in the rat parotid gland.

The questions which should be clarified are: 1) Is the stimulation of phosphatidylinositol turnover in rat parotid glands mediated by activation of \( \alpha_1 \)-adrenoceptors; 2) Does isoproterenol stimulation of \( \beta \)-receptors specifically induce an increase in number of \( \alpha_1 \)-receptors; and 3) Does the increase in \( \alpha_1 \)-receptor number produced by \( \beta \)-receptor stimulation result in alterations of phosphatidylinositol turnover?

In the present study, the effects of isoproterenol pretreatment on phosphatidylinositol turnover in rat parotid slices were studied to elucidate the relationship between \( \beta \)- and \( \alpha_1 \)-adrenoceptors, that is, the questions mentioned above.

Materials and Methods
Male, Wistar strain rats (260-280 g; obtained from Japan Clea Co.) were used for the experiments. \((-\)Epinephrine bitartrate, \((-\)norepinephrine bitartrate, phenylephrine HCl, \((-\)isoproterenol HCl, yohimbine HCl and \((\pm)\)propranolol HCl were obtained from
Sigma Chemical Co. (St. Louis, MO). Methoxamine HCl, clonidine HCl, prazosin HCl and phentolamine mesylate were gifts from Nippon Shinyaku Co. (Kyoto, Japan), Japan C.H. Boehringer Co. (Tokyo, Japan), Taito-Pfizer Co. (Tokyo, Japan) and Nippon Ciba-Geigy Co. (Tokyo, Japan), respectively. 

[^32P]Orthophosphate (carrier-free), [^3H]-prazosin (specific activity of 23 Ci/mmol), [^3H]-dihydroalprenolol (specific activity of 51 Ci/mmol) and ACS II were obtained from Amersham Japan Co. (Tokyo, Japan).

**Tissue slices preparation and incubation conditions:** Rat parotid glands were removed under pentobarbital (40 mg/kg, i.p.) anesthesia. The parotid slices were prepared in Krebs-Ringer bicarbonate (KRB) buffer (mM concentrations: NaCl, 118.46; KCl, 4.74; KH2PO4, 1.18; MgSO4 7H2O, 1.18; CaCl2, 2H2O, 2.54; NaHCO3, 24.87; D-glucose, 11.5, pH 7.4). The parotid glands were placed in a small quantity of the KRB buffer and rapidly cut into slices of approximately 1 mm³. Parotid slices (approximately 40 mg) were preincubated in 5 ml of the KRB buffer containing 20 μCi/ml of [^32P]phosphate at 37 °C for 60 min under an atmosphere of 95% O2/5% CO2. Following the preincubation, the parotid slices were washed twice with the KRB buffer, divided into several portions, and incubated with 5 ml of KRB buffer containing adrenergic agonists for 10 min (1st incubation). The slices were then washed with the KRB buffer for 10 min (washing period) and then reincubated for 10 min with adrenergic agonists (2nd incubation). In antagonist experiments, parotid slices were incubated with antagonists for 5 min prior to the addition of epinephrine (1st incubation). Experiments were terminated by removal of the incubation buffer, washed with the KRB buffer and immediately frozen with liquid nitrogen. Then 5 ml of CHCl3/MeOH (2:1, v/v) was added, followed by homogenization with a Kinematica Polytron (Speed 5, 20 sec).

**Lipid extraction and separation of individual lipids:** The lipids from the parotid slices were extracted by the method of Folch et al. (9). Individual phospholipids were separated by two-dimensional thin-layer chromatography with silica gel G (Merck, Darmstadt, West Germany). The solvent system used for the first dimension was CHCl3/MeOH/28% NH3OH/H2O (140:60:6:4, v/v/v/v). The plates were then developed in the second dimension using CHCl3/MeOH/petroleum ether/acetic acid (80:40:60:10, v/v/v/v). By using these solvent systems, phosphatidic acid, phosphatidylinositol, phosphatidyl-ethanolamine and phosphatidylcholine were clearly separated from other phospholipids. Phospholipids were visualized on the plates by exposure to I2 vapors. The areas containing individual lipids were scraped into scintillation vials, and the radioactivity was counted using 8 ml of ACS II with a liquid scintillation spectrometer (Beckman LS-9000). All data are based on duplicate samples for various conditions in each experiment.

**Preparation of crude membranes and [^3H]-prazosin or [^3H]-dihydroalprenolol binding experiments:** Rat parotid slices were incubated in KRB buffer for 10 min with isoproterenol (1st incubation), and were then washed with KRB buffer for 10 min (washing period). The slices were homogenized with a Kinematica Polytron (speed 5, 20 sec) in 10 vol. of ice-cold 50 mM Tris-HCl buffer (pH 7.4). The homogenate was centrifuged twice at 45,000×g for 20 min, after which the supernatants were discarded. The final pellet was suspended in 10 vol. of the same buffer. [^3H]-Prazosin or [^3H]-dihydroalprenolol binding experiments were carried out by the method of Karliner et al. (10) or Bylund and Snyder (11). Reaction mixtures composed of 0.4 ml of the crude membrane fraction (0.8-1.0 mg protein), 0.05 ml of [^3H]-prazosin (0.09-2.70 nM) together with 0.05 ml of unlabeled phentolamine solution (10⁻⁶ M) or 50 mM Tris-HCl buffer (pH 7.4) were incubated at 25°C for 30 min. Bound [^3H]-prazosin was separated by filtration on Whatman GF/C fiberglass filters, and the radioactivity was determined by a Beckman LS-9000 liquid scintillation spectrometer. Specific binding was defined as the excess over the blank containing 10⁻⁶ M phentolamine. The Kd and Bmax values were estimated from Scatchard (12) analysis. For experiments of [^3H]-dihydroalprenolol binding, [^3H]-dihydroalprenolol (0.50-20.32 nM) and
propranolol (10^{-5} M) were used as the binding ligand and displacer, respectively.

Other analytical methods: Protein was determined by the method of Lowry et al. (13). Statistical differences between control and test values were analyzed by Student’s t-test.

**Results**

Changes in phospholipid turnover by \( \alpha \)-or \( \beta \)-agonist stimulations in parotid slices: Figure 1 shows changes in phospholipid labelings produced by the addition of 10^{-5} M epinephrine to rat parotid slices prelabeled with ^{32}P. A marked increase in the radiactivity of phosphatidic acid was found within 1 min after the addition of epinephrine. Its radiactivity reached a peak within 2 to 5 min. However, change in phosphatidylinositol labeling occurred slightly later than phosphatidic acid labeling, and its radiactivity increased continuously over the 20 min of incubation. The ^{32}P-labelings in phosphatidic acid and phosphatidylinositol increased by 48 and 56% after 10 min of incubation, respectively. The changes in phosphatidic acid and phosphatidylinositol were not found in the absence of epinephrine (data not shown). No significant changes in the labelings of phosphatidylethanolamine and phosphatidylcholine were detected by the epinephrine stimulation.

To examine the relative potency of several adrenergic agonists in stimulating the phospholipid turnover, we compared the effects of the adrenergic agonists at the concentration of 10^{-5} M (Table 1). Changes in the ^{32}P-labelings were measured 10 min after the additions of adrenergic agonists. Epinephrine

![Fig. 1. Effect of epinephrine on the phospholipid turnover of rat parotid slices labeled with ^{32}P.](image)

**Table 1.** Effects of various adrenergic agonists on phospholipid turnover of rat parotid slices labeled with ^{32}P

| Additions       | PA               | PI               | PE               | PC               |
|-----------------|------------------|------------------|------------------|------------------|
| None            | 1.63±0.07        | 5.75±0.58        | 1.06±0.14        | 4.21±0.47        |
| Epinephrine     | 2.50±0.18*       | 8.85±0.87*       | 1.02±0.45(96)    | 4.67±0.88(111)   |
| Norepinephrine  | 2.68±0.15*       | 8.19±0.51*       | 0.87±0.09(82)    | 4.09±0.29(97)    |
| Methoxamine     | 3.25±0.08**      | 7.80±0.40**      | 1.14±0.13(108)   | 5.36±0.66(127)   |
| Phenylephrine   | 2.98±0.16**      | 7.94±0.44**      | 1.04±0.05(98)    | 5.07±0.56(120)   |
| Clonidine       | 1.60±0.15(98)    | 4.94±0.78(86)    | 0.88±0.10(83)    | 4.35±0.48(103)   |
| Isoproterenol   | 1.45±0.08*       | 4.66±0.67(81)    | 1.08±0.11(102)   | 5.36±0.18(127)   |

All drugs were used at a concentration of 10^{-5} M. The ^{32}P-labelings into phospholipids were measured 10 min after the addition of various adrenergic agonists. Each value represents the mean±S.E. of 4 to 8 experiments. Percentile stimulation from the control (no additions) is shown in parentheses. The abbreviations used are: PA, phosphatidic acid; PI, phosphatidylinositol; PE, phosphatidylethanolamine; PC, phosphatidylcholine. **P<0.01, *P<0.05, as compared to the control (no additions).
and norepinephrine (mixed α₁- and α₂-agonist) and pure α₁-agonists such as methoxamine and phenylephrine had a potent stimulatory effect on the ³²P-labeling of phosphatidic acid and phosphatidylinositol. The α₂-agonist, clonidine, was not effective, and the β-agonist, isoproterenol, slightly decreased the ³²P-phosphatidic acid labeling. However, clonidine and isoproterenol had no effect on ³²P-phosphatidylinositol labeling. None of the adrenergic agonists examined had any effect on ³²P-phosphatidylethanolamine and ³²P-phosphatidylcholine labelings at the concentrations of 10⁻⁵ M.

Effects of adrenergic antagonists on epinephrine-induced phosphatidylinositol turnover: In order to examine the role of α₁-adrenoceptor in the stimulation of phosphatidylinositol turnover induced by epinephrine, we studied whether the stimulated phosphatidylinositol turnover by epinephrine is reversed by α₁-antagonists (Fig. 2). The results are presented as the percentage of maximal inhibition in the radioactivity (i.e., 10⁻⁶ M prazosin = 100%). Prazosin (α₁-antagonist) and phentolamine (mixed α₁- and α₂-agonist) inhibited the phosphatidylinositol labeling stimulated by epinephrine in a concentration-dependent manner, while yohimbine (α₂-antagonist) was less inhibitory. Propranolol (β-agonist) had no inhibitory effect on the action of epinephrine.

Effect of isoproterenol pretreatment on phosphatidylinositol turnover in parotid slices labeled with ³²P: The effects of isoproterenol pretreatment on the phospholipids of rat parotid slices labeled with ³²P were studied. As shown in Fig. 3, ³²P-labeling of phosphatidic acid was significantly decreased and that of phosphatidylinositol was markedly increased by the 10⁻⁵ M isoproterenol pretreatment. The pretreatment with 10⁻⁵ M isoproterenol had no effects on ³²P-labeling of phosphatidylethanolamine and phosphatidylcholine. Furthermore, when the parotid slices were pretreated with isoproterenol in the presence of 10⁻⁵ M propranolol, there were observed no effects on the ³²P-phospholipid turnovers (data not shown).

The effects of various adrenergic agonists

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**Fig. 2.** Inhibitory effects of adrenergic antagonists on epinephrine-induced phosphatidylinositol turnover. Epinephrine was used at a concentration of 10⁻⁵ M. Antagonists were added 5 min before the addition of epinephrine. The ³²P-labeling into phosphatidylinositol was determined at 10 min. Each point represents the mean of 3 to 4 experiments. The abbreviations used are: PI, phosphatidylinositol; PRZ, prazosin; PHE, phentolamine; YOH, yohimbine; PRO, propranolol.

**Fig. 3.** Effect of isoproterenol pretreatment on the phospholipid turnover of rat parotid slices labeled with ³²P. ³²P-Labeled parotid slices were pre-incubated with (stippled bars) or without (open bars) 10⁻⁵ M isoproterenol for 10 min. The slices were washed with Krebs-Ringer bicarbonate buffer for 10 min. The radioactivities of ³²P-phospholipids were then determined. Each of the values represents the mean±S.E. of 6 to 7 experiments. The abbreviations used are: PA, phosphatidic acid; PI, phosphatidylinositol; PE, phosphatidylethanolamine; PC, phosphatidylcholine. **P<0.01, *P<0.05, as compared to control values (vehicle-treated).
on the $^{32}$P-phospholipid labelings in the isoproterenol-pretreated parotid slices were then studied. As shown in Table 2, epinephrine, norepinephrine or phenylephrine-induced labeling of phosphatidylinositol were further enhanced by the pretreatment with isoproterenol. This potentiating effect by the isoproterenol pretreatment was abolished in the presence of $10^{-5}$ M propranolol (data not shown).

### Table 2. Effects of various adrenergic agonists on $^{32}$P-phospholipid labelings in isoproterenol pretreated rat parotid slices

| Pretreatment | Change in radioactivity (c.p.m x 10$^{-2}$/10 mg tissue) |
|--------------|--------------------------------------------------------|
|              | Vehicle | Epinephrine | Norepinephrine | Phenylephrine |
| None         |         |             |                |               |
| Phosphaticid acid | 1.67±0.06 | 2.45±0.11** | 2.57±0.09** | 2.72±0.18** |
| Phosphatidylinositol | 5.95±0.48 | 8.92±0.78** | 6.98±0.55** | 7.24±0.61** |
| Phosphatidylethanolamine | 1.16±0.09 | 1.17±0.23 | 0.87±0.09 | 1.16±0.12 |
| Phosphatidylcholine | 4.39±0.41 | 4.78±0.53 | 4.29±0.36 | 5.11±0.42 |
| Isoproterenol |         |             |                |               |
| Phosphaticid acid | 1.17±0.09* | 2.43±0.27** | 2.79±0.18** | 2.46±0.38** |
| Phosphatidylinositol | 8.19±0.17* | 12.21±1.96** | 9.25±0.08** | 10.11±1.51** |
| Phosphatidylethanolamine | 1.11±0.11 | 1.48±0.26 | 1.27±0.29 | 1.38±0.45 |
| Phosphatidylcholine | 4.34±0.26 | 4.43±0.57 | 4.73±0.41 | 4.96±0.43 |

$^{32}$P-Labeled parotid slices were preincubated with or without $10^{-5}$ M isoproterenol for 10 min (1st incubation). The slices were then washed with Krebs-Ringer bicarbonate buffer for 10 min and then reincubated with $10^{-5}$ M adrenergic agonists for 10 min (2nd incubation). The radioactivity of $^{32}$P-phospholipids was determined. Each of the values represents the mean±S.E. of 4 to 6 experiments. **P<0.01, as compared to the vehicle in the respective pretreatment group, *P<0.01, as compared to the respective agonist alone in the no pretreatment group.

### Table 3. Influence of isoproterenol treatment on $K_d$ and $B_{max}$ of specific binding of $[^3H]$prazosin and $[^3H]$dihydroalprenolol to parotid membranes

| Ligand | Treatment | $K_d$ (nM) | $B_{max}$ (fmol/mg protein) |
|--------|-----------|------------|----------------------------|
| $[^3H]$Prazosin | Vehicle | 0.39±0.04 (4) | 29.77±1.08 (4) |
|         | Isoproterenol | 0.45±0.07 (4) | 39.70±3.02 (4)** |
| $[^3H]$Dihydroalprenolol | Vehicle | 4.20±0.11 (4) | 333.86±10.12 (4) |
|         | Isoproterenol | 4.95±0.47 (4) | 341.56±15.98 (4) |

Parotid slices were preincubated with or without $10^{-6}$ M isoproterenol for 10 min (1st incubation). The slices were washed with Krebs-Ringer bicarbonate buffer for 10 min. The crude membranes were then prepared from the intact (vehicle)- and the isoproterenol-treated parotid slices. $[^3H]$Prazosin and $[^3H]$dihydroalprenolol (final concentration: 0.09-2.70 and 0.50-20.32 nM, respectively) were used for the binding assay. The $K_d$ and $B_{max}$ values were estimated by Scatchard analysis. Each of the values show the mean±S.E. with the number of experiments in parentheses. **P<0.01, as compared to the control (vehicle treated).
value of \([3H]\)prazosin binding by the pre-
treatment of \(10^{-5}\) M isoproterenol was
found, but its \(K_d\) value was not changed.
However, neither \(K_d\) nor \(B_{\text{max}}\) in \([3H]\)-
dihydroalprenolol binding was affected by
the isoproterenol pretreatment. In addition,
when the crude membranes were pretreated
with \(10^{-5}\) M isoproterenol in the presence of
\(10^{-5}\) M propranolol, the \([3H]\)prazosin
binding was not significantly affected (data
not shown).

Discussion

The function of parotid glands, secretory
activity, is principally mediated by \(\alpha\) and \(\beta\)-
adrenergic and cholinergic agonists. Amylase
secretion is primarily controlled by \(\beta\)-
adrenoceptors through the adenylate cyclase-
cyclic AMP systems (14). However, release
of water and electrolytes is mediated
principally via \(\alpha\)-adrenergic and cholinergic
receptors (15, 16). \(\alpha\)-Adrenoceptors have
recently been subdivided into \(\alpha_1\) - and \(\alpha_2\)-
receptors on the basis of their different
responsiveness to adrenergic agonists and
antagonists (17-19). It is suggested that
stimulation of \(\alpha_1\)-adrenoceptors causes \(K^+\)
release and elevates cytosolic \(Ca^{2+}\) ions,
while stimulation of \(\alpha_2\)-adrenoceptors
induces nonspecific inhibition of adenylate
cyclase (20-22). We have recently
demonstrated the \(\alpha_1\)-adrenergic regulation of \(K^+\)
release from these slices (7, 8). The present
study demonstrated an \(\alpha_1\)-adrenoceptor-
mediated stimulation of phosphatidylinositol
and phosphatidic acid labelings in rat
parotid slices, as has been found in many
other tissues (4-6).

We found that epinephrine and phenyle-
phrine stimulated \(^{32}\text{P}\)-incorporation into
phosphatidylinositol (Table 1). This response
was inhibited by the \(\alpha_1\)-selective antagonist
prazosin (Fig. 2). These hormone-stimulated
labelings of phosphatidylinositol in rat
parotid slices primarily represents an
enhanced turnover of the molecule. Since
the stimulatory effect of epinephrine was not
antagonized by the \(\beta\)-antagonist propranolol
(Fig. 2), the effect of epinephrine was not
mediated through \(\beta\)-adrenoceptors. These
results suggest that the stimulation of
phosphatidylinositol turnover in the rat parotid
glands elicited by adrenergic agonists is
mediated by activation of \(\alpha_1\)-adrenoceptors.
In addition, since the phosphatidylinositol
turnover of these glands is stimulated at the
used doses of secretagogues (2, 3), the
activation of this turnover cannot be a toxic
effect. The enhanced incorporation of \(^{32}\text{P}\)
into phosphatidylinositol and phosphatidic
acid in secretory glands has been observed
in many laboratories using a variety of
regulatory agents (23, 24). After the addition
of epinephrine to rat parotid slices, the
incorporation of \(^{32}\text{P}\) into phosphatidylinositol
was significantly increased before detectable
changes in phosphatidylinositol were
observed in the present study (Fig. 1).
Similar rapid increase in the radioactivity of
phosphatidic acid has been observed in iris
muscle after acetylcholine (25) and in isolated
stomach smooth muscle cells after carbamyl-
choline exposure (26). Of particular interest
in this connection is the recent finding that
phosphatidic acid behaves as a \(Ca^{2+}\)
ionophore, and it was suggested that
phosphatidic acid may play some important
roles in the activation of \(Ca^{2+}\) influx into
smooth muscle cells (26, 27). However, the
exact role for phosphatidylinositol turnover
in this process remains obscure.

The overall pathway of phosphatidylinositol
turnover is known as the phosphatidylinositol-
resynthesis cycle. The first step of this cycle
is initiated by a \(Ca^{2+}\)-dependent phos-
pholipase C-type phosphodiesterase(s) that
hydrolyzes inositol phospholipids to produce
diacetylglcerol and inositol phosphate (28,
29). In rat parotid glands, phosphatidylinositol
turnover is mediated by activation of \(\alpha_1\-
adrenoceptors, as shown in Table 1 and Fig.
2. Accordingly, the effect of isoproterenol
pretreatment on the phospholipids of rat
parotid slices labeled with \(^{32}\text{P}\) were studied.
The \(^{32}\text{P}\) labeling of phosphatidic acid was
decreased and \(^{32}\text{P}\) labeling of phosphatidyl
inositol was increased by \(10^{-5}\) M isopro-
terenol pretreatment. The pretreatment with
\(10^{-5}\) M isoproterenol had no effects on
the \(^{32}\text{P}\) labelings of phosphatidylethanolamine
and phosphatidylcholine (Fig. 3). These
results indicate that the pretreatment with
isoproterenol causes the acceleration of
phosphatidylinositol turnover. In addition,
the effects of various adrenergic agonists on $^{32}$P-phospholipid turnover in the isoproterenol pretreated parotid slices were studied. Epinephrine, norepinephrine (mixed $\alpha_1$- and $\alpha_2$-agonist) or phenylephrine (pure $\alpha_1$-agonist)-induced labelings of phosphatidylinositol were further enhanced by the isoproterenol pretreatment (Table 2). It appears, therefore, that the isoproterenol pretreatment-induced enhancement of phosphatidylinositol turnover may be indirectly related to an increase of $\alpha_1$-adrenoceptor binding sites.

It has been reported that the pretreatment with isoproterenol in rat parotid slices causes a supersensitivity of amylase secretory response to the $\beta$-agonists accompanying the changes in $\beta$-adrenoceptor (30). In our present study, the $B_{\max}$ value of $[^3H]$prazosin binding to rat parotid crude membranes was significantly increased, but no change of $[^3H]$dihydroalprenolol binding was observed after the same pretreatment (Table 3). Although these differences of the results between our experiments and the other can not be elucidated, our findings on the effect of isoproterenol pretreatment in the $[^3H]$-dihydroalprenolol binding was observed after the same pretreatment (Table 3).

Although these differences of the results between our experiments and the other can not be elucidated, our findings on the effect of isoproterenol pretreatment in the $[^3H]$-dihydroalprenolol binding are compatible with the observations of our previous experiments, because the $\beta$-adrenoceptor effects of isoproterenol on K$^+$ release (7, 8) and cyclic AMP accumulation (31) completely disappeared in the slices after the same isoproterenol pretreatment. It is possible that the isoproterenol pretreatment may increase $\alpha_1$-adrenoceptor binding sites in cell membranes of rat parotid glands by specifically stimulating $\beta$-adrenoceptors. Therefore, the potentiating effect of $\alpha_1$-agonists on phosphatidylinositol turnover may be a direct and/or indirect interaction between $\beta$- and $\alpha_1$-adrenoceptor molecules in the membranes. It has been reported that the treatment with isoproterenol in rat ventricular myocytes stimulates a compartment of phosphodiesterase activity in response to $\alpha_1$-agonists (32). In this respect, the enhancement of phosphatidylinositol turnover by the isoproterenol pretreatment may be a trigger potentiating phosphodiesterase activity. The present study clearly demonstrated that phosphatidylinositol turnover is enhanced by the isoproterenol pretreatment which results in an increase of $\alpha_1$-adrenoceptors. However, the detailed mechanism concerning the isoproterenol-induced increase of $\alpha_1$-adrenoceptors still remains to be clarified.

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