Ca\(^{2+}\)/calmodulin-dependent cyclic nucleotide phosphodiesterase in cGMP metabolism in rabbit parotid acinar cells

Nakayasu SAIRENJI\(^1\), Keitaro SATO\(^2\) and Hiroshi SUGIYA\(^2, 3\)

Departments of \(^1\) Anesthesiology and \(^2\) Physiology, and \(^3\) Research Institute of Oral Science, Nihon University School of Dentistry at Matsudo, Matsudo, Chiba 271-8587, Japan

(Received 25 November 2005; and accepted 10 January 2006)

ABSTRACT

Muscarinic cholinergic receptor activation provokes cGMP formation in parotid acinar cells. We investigated the involvement of Ca\(^{2+}\)/calmodulin-dependent cyclic nucleotide phosphodiesterase (PDE1) in cGMP breakdown in rabbit parotid acinar cells. The muscarinic agonist carbachol stimulated cGMP formation in the cells. The carbachol-induced cGMP formation was enhanced in the presence of 8-methoxymethyl-3-isobutyl-1-methylxanthine (MM-IBMX), a PDE1 inhibitor. cGMP-PDE activity in rabbit parotid acinar cells was reduced by about 25% in the absence of Ca\(^{2+}\)/calmodulin or in the presence of MM-IBMX. Ca\(^{2+}\)/calmodulin-dependent cGMP-PDE in rabbit parotid acinar cells was purified using Calmodulin-Sepharose 4B and Mono Q ion-exchange column chromatography. Two dominant fractions with cGMP-PDE activity, referred to as the P-1 and P-2 fractions, were eluted from the Mono Q ion-exchange column. The \(K_m\) values for cGMP of PDE in the P-1 and P-2 fractions were 0.82 \(\mu\)M and 0.40 \(\mu\)M, respectively, which were much lower than that for cAMP. The \(EC_{50}\) for Ca\(^{2+}\) and calmodulin of PDEs in the P-1 and P-2 fractions were 458 nM and 426 nM, respectively, and 32 nM and 137 nM, respectively. Protein bands that cross-reacted with anti-PDE1A antibody were detected. These results suggest that Ca\(^{2+}\)/calmodulin-dependent PDE, PDE1A, is involved in cGMP breakdown in rabbit parotid acinar cells.

Cyclic guanosine-3’,5’-monophosphate (cGMP) is an intracellular signal that is involved in the action of various hormones, autacoids, neurotransmitters, and vasoactive substances (27). cGMP synthesis is catalyzed by soluble or particulate guanylyl cyclase, and the actions of cGMP are carried out via cGMP mediators such as cGMP-gated ion channels and cGMP-dependent protein kinases (19). To terminate intracellular cGMP signaling, cGMP is converted to the inactive 5’GMP by cyclic nucleotide phosphodiesterases (PDEs) (1, 22). PDEs have been classified into 11 families on the basis of substrate and inhibitor specificities, allosteric properties and amino acid sequences (12). In the PDE families, 8 families have been reported to hydrolyze cGMP; Ca\(^{2+}\)/calmodulin-dependent PDE (PDE1), cGMP-stimulated PDE (PDE2), cGMP-inhibited PDE (PDE3), cGMP-binding cGMP-specific PDE (PDE5), photoreceptor cGMP-PDE (PDE6), high-affinity cGMP specific and IBMX-insensitive PDE (PDE9), and the recently recognized PDE10 and PDE11 (12).

In parotid glands of mouse, rat and rabbit, since secretagogues such as muscarinic agonists provoke cGMP formation, cGMP is considered to be involved in parotid functions as an intracellular messenger (3, 4, 16, 28). We previously demonstrated that cGMP production is coupled to nitric oxide (NO) generation in rabbit parotid acinar cells: muscarinic receptor activation resulted in Ca\(^{2+}\) mobilization, which activated Ca\(^{2+}\)/calmodulin-dependent NO syn-
MATERIALS AND METHODS

Materials. Collagenase A, bovine serum albumin (BSA) and Protease Inhibitors Cocktail (Complete™) were purchased from Roche (Basel, Switzerland). Trypsin (type III), trypsin inhibitor (type I-S), 3-isobutyl-1-methylxanthine (IBMX), Crotalus atrox snake venom and bovine brain PDE were obtained from Sigma (St. Louis, MO, USA). Calmodulin and 8-methoxymethyl-3-isobutyl-1-methylxanthine (MM-IBMX) were obtained from Wako (Osaka, Japan) and Calbiochem (San Diego, CA, USA), respectively. AG 50W-X4 resin (200–400 mesh, hydrogen form) and a Mono Q ion-exchange column were purchased from Bio-Rad (Hercules, CA, USA) and Pharmacia (Piscataway, NJ, USA), respectively. Centricon-30 and Calmodulin-Sepharose 4B were purchased from Amersham (Piscataway, NJ, USA). [3H] cGMP (277.5 GBq/mmol) and [8-3H]cAMP (1187.7 GBq/mmol) were obtained from PerkinElmer (Wellesley, MA, USA). An [32P]cGMP radioimmunoassay kit was purchased from Yamasa (Tokyo, Japan). Anti-PDE1 antibodies (rabbit, polyclonal) were purchased from FabGennix (Frisco, TX, USA).

Preparation of parotid acinar cells. Acinar cells of the parotid glands of male Japanese white rabbits (2–2.5 kg) were prepared as previously described (23). The parotid glands were removed and placed in a small volume with Krebs-Ringer-bicarbonate (KRB) solution with the following composition (in mM): NaCl, 116; KCl, 5.4; MgSO_4, 0.8; CaCl_2, 1.8; NaH_2PO_4, 0.96; NaHCO_3, 25; HEPES (pH 7.4), 5; and glucose, 11.1. The KRB solution was equilibrated with an atmosphere of 95% O_2/5% CO_2. After mincing with a razor, the glands were treated with KRB buffer with 0.5% BSA in the presence or absence of enzyme. First, the glands were incubated with trypsin (0.5 mg/mL) at 37°C for 10 min. After incubation, the trypsin-treated glands were removed by centrifugation at 200 g for 1 min. The glands were subsequently incubated in Ca^2+-Mg^2+-free KRB solution containing 1 mM EGTA, trypsin inhibitor (0.5 mg/mL) at 37°C for 5 min. After the solution was removed by centrifugation (200 g for 1 min), the glands were incubated in Ca^2+-Mg^2+-free KRB solution without trypsin inhibitor at 37°C for 5 min. After the solution was removed by centrifugation (200 g for 1 min), the glands were incubated in KRB solution with collagenase (1.5 mg/mL) at 37°C for 20 min. The suspension was passed through eight layers of nylon mesh to separate the dispersed cells from undigested connective tissue, and was gently put on KRB solution containing 4% BSA. After centrifugation (50 g for 5 min), the cells were suspended in the appropriate amounts of KRB solution containing 0.5% BSA and 0.02% trypsin inhibitor.

Determination of cellular cGMP. The dispersed acinar cells from one rabbit were suspended in 8 mL of KRB solution containing 0.5% BSA and 0.02% trypsin inhibitor and incubated at 37°C with the indicated agents. The cell suspension was separated to 4 parts (each 2 mL), and incubated with vehicle, MM-IBMX (0.5 mM), carbachol (10 μM), and MM-IBMX/carbachol. Carbachol was added after preincubation with vehicle or MM-IBMX for 10 min. At the indicated time, 200 μL of cell suspension was removed, mixed with 30 μL of 35% perchloric acid and put on ice for 30 min. Then, 60 μL of 17.5% potassium hydroxide was added to the mixture for neutralization, the mixture was centrifuged at 10,000 g for 5 min, and the supernatant was isolated. The cGMP concentration in the supernatant was measured using a radioimmunoassay kit (Yamasa).

Isolation of cytosolic fraction and purification of PDE. Parotid acinar cells from 4 rabbits were homogenized with 10 mL of 20 mM Tris-HCl (pH 7.4) containing 2 mM MgCl_2, 1 mM dithiothreitol (DTT), 1 mM phenylmethylsulfonyl fluoride (PMSF), 1.3 mM benzamidine, and Protease Inhibitors Cocktail (Complete™) and centrifuged at 105,000 g for 60 min at 4°C. The supernatant was used as a cytosolic fraction.

For the purification of PDE, the cytosolic fraction was used. All the purification steps were carried out at 4°C. The supernatant was loaded onto a Calmodulin-Sepharose 4B column (0.46 × 10 cm) equilibrated with buffer A (20 mM Tris-HCl (pH 7.4) with trypsin (0.5 mg/mL) at 37°C for 10 min. After incubation, the trypsin-treated glands were removed by centrifugation at 200 g for 1 min. The glands were subsequently incubated in Ca^2+-Mg^2+-free KRB solution containing 1 mM EGTA, trypsin inhibitor (0.5 mg/mL) at 37°C for 5 min. After the solution was removed by centrifugation (200 g for 1 min), the glands were incubated in Ca^2+-Mg^2+-free KRB solution without trypsin inhibitor at 37°C for 5 min. After the solution was removed by centrifugation (200 g for 1 min), the glands were incubated in KRB solution with collagenase (1.5 mg/mL) at 37°C for 20 min. The suspension was passed through eight layers of nylon mesh to separate the dispersed cells from undigested connective tissue, and was gently put on KRB solution containing 4% BSA. After centrifugation (50 g for 5 min), the cells were suspended in the appropriate amounts of KRB solution containing 0.5% BSA and 0.02% trypsin inhibitor.
containing 2 mM MgCl₂, 1 mM DTT, 1 mM PMSF and 1.3 mM benzamidine). The column was washed with 45 mL of buffer A containing 0.25 mM CaCl₂, and the fractions containing cGMP-PDE activity were eluted with buffer A containing 0.5 mM EGTA. The cGMP-PDE fractions were collected, concentrated to about 10 mL by ultrafiltration using Centricon-30 (Amersham), and loaded at 1 mL/min onto a Mono Q ion-exchange column (1 × 10 cm) equilibrated with buffer A. The column was washed with 50 mL of buffer A, and cGMP-PDE was eluted with a 50 mL linear gradient of 0–500 mM NaCl in buffer A at a flow rate of 1 mL/min. Fractions (1 mL) were collected and assayed for cGMP-PDE activity.

**Assay of PDE activity.** cGMP-PDE activities were determined by the two-step method (9). The enzymatic reaction was performed in a total volume of 0.1 mL. The reaction mixture contained 50 mM Tris-HCl (pH 7.4), 5 mM MgCl₂ and substrate (0.5 μM [³H]cGMP; 200,000 dpm) and enzyme. The mixture was incubated at 30°C for 10 min, and then the reaction was terminated by heating at 90°C for 5 min. 5'-[³H]GMP formed by PDE was converted to [³H]guanosine by the action of nucleotidase (25 μL of 1 mg/mL snake venom at 30°C for 10 min). The reaction was terminated by the addition of 0.5 mL of water and denatured protein was removed by centrifugation (10,000 g, 3 min). Five hundred microliters of clear supernatant fluid was applied to a 0.5-mL column of AG 50W-X4 resin (200–400 mesh; hydrogen form). The reaction product, [³H]guanosine, was eluted with 1.5 mL of 3N ammonium hydroxide after the column was washed with 15 mL of water, and radioactivity was measured using a liquid scintillation counter. The PDE activity was assayed in the presence or absence of the indicated concentrations of 1 mM CaCl₂ with 0.4 μM calmodulin or 1 mM EGTA. When the concentrations of Ca²⁺ and calmodulin were changed, the Ca²⁺ concentrations in the reaction mixture were adjusted using fura-2 (8). When cAMP-PDE was assayed, 0.1 μM [³H]cAMP instead of [³H]cGMP was used as substrate, and [³H]adenosine converted from 5'-[³H]AMP formed by PDE was determined.

**Immunoblot analysis.** The fractions containing cGMP-PDE activity eluted from a Calmodulin-Sepharose 4B column were concentrated by ultrafiltration using Centricon-30 (Amersham) and used as a sample for immunoblot analysis. The sample (100 μg protein) was resolved by 10% SDS-polyacrylamide gel electrophoresis. The proteins on the gel were transferred to a nitrocellulose membrane (12 V, overnight). The membrane was blocked at room temperature for 50 min in Tris-buffered saline with 3% gelatin, washed briefly with Tris-buffered saline, and probed for 2 h with rabbit anti-PDE1 antibodies (0.2 μg/mL). Blots were washed three times with Tris-buffered saline containing 0.05% Tween 20, and probed for 90 min with anti-rabbit IgG. Immunoreactivity was determined with the ECL chemiluminescence reaction (Amersham).

**Protein determination.** Protein concentrations were determined by the method previously described (2) with BSA as the standard.

**Statistical analysis.** Statistical differences were determined using Student’s t test. P values below 0.05 were regarded as statistically significant, and are indicated by asterisks (*).

**RESULTS**

**Effect of a PDE1 inhibitor on carbachol-induced cGMP formation in parotid acinar cells**

In rabbit parotid acinar cells, muscarinic receptor activation has been demonstrated to stimulate cGMP formation (16). Thus, we first studied the effect of MM-IBMX, an inhibitor of PDE1, on cGMP formation induced by muscarinic receptor activation. As Fig. 1A shows, the muscarinic agonist carbachol (10 μM) induced cGMP formation in a time-dependent manner in the absence of the inhibitor. When acinar cells were pretreated with MM-IBMX (0.5 mM) for 10 min and then stimulated, carbachol-induced cGMP formation was clearly enhanced. Fig. 1B shows the concentration-dependence of cGMP formation on MM-IBMX (0.1 to 1 mM) when cells were stimulated by carbachol (10 μM) for 1 min. These observations suggest that PDE1 is involved in the breakdown of cGMP formed by carbachol stimulation.

**PDE1 activity in parotid acinar cells**

We next investigated the effect of Ca²⁺/calmodulin and MM-IBMX on the cGMP-PDE activity in the cytosolic fraction. In the presence of CaCl₂ and calmodulin, MM-IBMX (100 μM) inhibited cGMP-PDE activity to 26.1% of the control level (Table 1). In the presence of EGTA (1 mM) and absence of CaCl₂ and calmodulin, cGMP-PDE activity in the presence of 1 mM CaCl₂ and 0.4 μM calmodulin was markedly reduced to 27.7% of the control level, but MM-IBMX showed no further effect on the ac-
in rabbit parotid acinar cells, PDE1 in the cytosolic fraction of rabbit parotid acinar cells was partially purified using calmodulin-affinity and Mono Q ion-exchange column chromatography. Figure 2 shows the elution profiles of PDE1 activities from the calmodulin-affinity column (Fig. 2A) and the Mono Q ion-exchange column (Fig. 2B). Two dominant cGMP-PDE activity peaks and several minor peaks were eluted from the Mono Q ion-exchange column. The dominant peaks are referred to as the P-1 and P-2 fractions according to their order of elution from the column, and the characteristics of the PDEs in the fractions were examined. The specific activities of cGMP-PDEs in the P-1 and P-2 fractions were 42 ± 3 pmol/min/mg protein and 51 ± 8 pmol/min/mg protein, respectively.

The $K_m$ value for cGMP was determined by a Lineweaver-Burk plot analysis (Fig. 3). The $K_m$ values of the PDEs in the P-1 and P-2 fractions were 0.82 μM and 0.40 μM, respectively. Regarding the affinity for cAMP, the $K_m$ values of the PDEs in the P-1 and P-2 fractions were 13 μM and 25 μM, respectively (data not shown).

Figure 4 summarizes the Ca$^{2+}$- and calmodulin-dependency of PDEs in the P-1 and P-2 fractions, respectively. Omission of Ca$^{2+}$ or calmodulin from the reaction mixture essentially abolished the cGMP-PDE activities in both fractions. Although the range

Table 1. Effect of PDE inhibitors on cGMP-PDE activity in rabbit parotid acinar cells

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Concentration (μM)</th>
<th>Ca$^{2+}$/calmodulin (+) (%)</th>
<th>Ca$^{2+}$/calmodulin (-)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>100</td>
<td>27.7 ± 0.9</td>
<td></td>
</tr>
<tr>
<td>MM-IBMX</td>
<td>50</td>
<td>37.6 ± 0.4</td>
<td>28.4 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>26.1 ± 1.3</td>
<td>25.2 ± 0.5</td>
</tr>
<tr>
<td>IBMX</td>
<td>100</td>
<td>4.9 ± 0.5</td>
<td>3.3 ± 1.0</td>
</tr>
</tbody>
</table>

cGMP-PDE activity in the presence of 1 mM CaCl$_2$ and 0.4 μM calmodulin [Ca$^{2+}$/calmodulin (+)] was taken as 100%. When the activity was assayed in the absence of CaCl$_2$ and calmodulin [Ca$^{2+}$/calmodulin (-)], 1 mM EGTA was added to the reaction mixture. Results are means ± SE from 5 experiments.

Characterization of cGMP-PDE in rabbit parotid acinar cells

To characterize the Ca$^{2+}$/calmodulin-dependent PDE in rabbit parotid acinar cells, PDE1 in the cytosolic fraction of rabbit parotid acinar cells was partially purified using calmodulin-affinity and Mono Q ion-exchange column chromatography. Figure 2 shows the elution profiles of PDE1 activities from the calmodulin-affinity column (Fig. 2A) and the Mono Q ion-exchange column (Fig. 2B). Two dominant cGMP-PDE activity peaks and several minor peaks were eluted from the Mono Q ion-exchange column. The dominant peaks are referred to as the P-1 and P-2 fractions according to their order of elution from the column, and the characteristics of the PDEs in the fractions were examined. The specific activities of cGMP-PDEs in the P-1 and P-2 fractions were 423 pmol/min/mg protein and 518 pmol/min/mg protein, respectively.

The $K_m$ value for cGMP was determined by a Lineweaver-Burk plot analysis (Fig. 3). The $K_m$ values of the PDEs in the P-1 and P-2 fractions were 0.82 μM and 0.40 μM, respectively. Regarding the affinity for cAMP, the $K_m$ values of the PDEs in the P-1 and P-2 fractions were 13 μM and 25 μM, respectively (data not shown).

Figure 4 summarizes the Ca$^{2+}$- and calmodulin-dependency of PDEs in the P-1 and P-2 fractions, respectively. Omission of Ca$^{2+}$ or calmodulin from the reaction mixture essentially abolished the cGMP-PDE activities in both fractions. Although the range

Fig. 1 Effect of MM-IBMX on carbachol-induced cGMP formation in rabbit parotid acinar cells. A, Time-course of cGMP levels. After preincubation with (squares) or without (circles) MM-IBMX (0.5 mM) for 10 min, rabbit parotid acinar cells were stimulated with 10 μM carbachol (closed circles and closed squares) or vehicle (open circles and open squares) at 0 min; B, Dose-dependent effect of MM-IBMX on carbachol-induced cGMP formation. After preincubation in the presence of various concentrations of MM-IBMX for 10 min, cells were stimulated with carbachol (10 μM) for 1 min. The increase in cGMP formation in the presence of MM-IBMX is shown as fold of the control value (in the absence of MM-IBMX). Results are means ± SE from 4 experiments. *The values in the presence of MM-IBMX were compared to that in the absence of MM-IBMX.
of Ca\(^{2+}\)-dependency for the cGMP-PDE activity in the P-2 fraction was wider than that in the P-1 fraction, the EC\(_{50}\) values for Ca\(^{2+}\) of the cGMP-PDEs in the P-1 and P-2 fractions were the same, 458 nM and 426 nM, respectively (Fig. 4A). The EC\(_{50}\) values for calmodulin of the enzymes in the P-1 and P-2 fractions were 32 nM and 137 nM, respectively (Fig. 4B).

**PDE1 isoform in rabbit parotid acinar cells**

The PDE1 subfamily consists of three different gene products, PDE1A, PDE1B and PDE1C (1, 5). To identify the PDE1 isoform in rabbit parotid acinar cells, immunoblot analysis was performed using antibodies against PDE1 isoforms. For this immunoblot analysis, the fraction eluted from a Calmodulin-Sepharose 4B column was concentrated and then analyzed, because we were not able to collect sufficient concentrations of protein for this analysis from the fractions eluted from a Mono Q ion-exchange column. No protein band immunoreacted with anti-PDE1B or anti-PDE1C antibodies was detected (data not shown), but, as shown in Fig. 5, approximately 61, 60 and 59 kDa protein bands that immunoreacted with anti-PDE1A antibody were detected in the fraction eluted from the calmodulin-affinity column; these bands were smaller than the 63 kDa bovine brain PDE (21). These observations suggest that PDE1A isoforms are expressed in rabbit parotid acinar cells.

**DISCUSSION**

Here we demonstrated that a Ca\(^{2+}\)/calmodulin-dependent PDE, PDE1, is involved in regulating cGMP concentrations in rabbit parotid acinar cells. The PDE1 subfamily consists of three different gene products, PDE1A, PDE1B and PDE1C, with different properties, such as their regulatory properties, substrate affinities, activation constants for calmodulin and molecular weights (1, 5, 12). The heterogeneity of PDE1 isoforms is further increased by alternative splicing, creating divergent N- and C-termini (1, 5, 12). PDE1 family members have been reported to have Km values for cGMP in the range of 0.33–5.2 μM (12). The Km values for cAMP of the PDE1A and PDE1B isoforms are generally higher than that of PDE1C. For example, PDE1A2 and PDE1B1 in bovine brain have Km values of 35 and 12 μM for cAMP, respectively (11, 20). Bovine PDE1A2 and PDE1B1 expressed in COS-7 cells have Km values of 113 and 24 μM for cAMP, respectively (30). On the other hand, rat PDE1C2 and mouse PDE1C1 and PDE1C4/5 expressed in COS-7 cells have Km values of 1.2, 3.5 and 1.1 μM for cAMP, respectively (29, 30). We demonstrated that the PDE1 enzymes in the P-1 and P-2 fractions had Km values of 13 μM and 25 μM for cAMP, respectively, and 0.82 and 0.40 μM for cGMP, respectively. Therefore, the enzymes in the P-1 and P-2 fractions are considered likely to be PDE1A or PDE1B. The 61, 60 and 59 kDa protein bands that were immunoreactive with anti-PDE1A antibody, but not anti-PDE1B or anti-PDE1C antibodies, were detected in the fraction with cGMP-PDE activity. The molecular masses of PDE1A in various tissues are 58–68 kDa (5). Therefore, the results of immunoblot analysis suggest that PDE1A isoforms are expressed in rabbit parotid acinar cells. The PDE1 enzymes in the P-1 and P-2 fractions appear to be alternative splicing variants of PDE1A.

The PDE1 enzymes in the P-1 and P-2 fractions had EC\(_{50}\) values of 458 nM and 426 nM for Ca\(^{2+}\) respectively, and EC\(_{50}\) values of 32 nM and 137 nM for calmodulin, respectively. These results strongly suggest that Ca\(^{2+}\) and calmodulin are regulators of
PDE activation. However, because the total amounts of calmodulin do not change rapidly in the cells, it is most likely that changes in \([\text{Ca}^{2+}]_i\) primarily contribute to the regulation of PDE1 activation. In 1321N1 human astrocytoma cells, PDE1 is considered to be activated by the entry of Ca\(^{2+}\) into the cells from extracellular sites (6, 7, 13, 14, 26). In parotid acinar cells, it is well known that muscarinic receptor activation provokes an increase in \([\text{Ca}^{2+}]_i\) (15, 17, 23, 24). This Ca\(^{2+}\) mobilization consists of Ca\(^{2+}\) release from intracellular Ca\(^{2+}\) pools mediated by inositol 1,4,5-trisphosphate derived from the receptor-activated hydrolysis of inositol phospholipids, and Ca\(^{2+}\) entry from extracellular sites (25). Depletion of the intracellular Ca\(^{2+}\) pools triggers Ca\(^{2+}\) en-
try, which is termed capacitative Ca\(^{2+}\) entry (18). In our results, carbachol-induced cGMP formation was enhanced in the presence of the PDE1 inhibitor MM-IBMX, but the control levels of cGMP were not affected. Therefore, it is conceivable that PDE1 activity is regulated by the increase in [Ca\(^{2+}\)], provoked by muscarinic receptor activation.

We previously demonstrated that cGMP formation induced by muscarinic receptor activation is coupled to Ca\(^{2+}\)/calmodulin-dependent NO synthase activation in rabbit parotid acinar cells (16, 23). Therefore, the increase in [Ca\(^{2+}\)], may trigger the synthesis and breakdown of cGMP simultaneously, which is supported by our observation that the carbachol-induced cGMP formation was clearly enhanced in the presence of a PDE1 inhibitor. However, the EC\(_{50}\) values for Ca\(^{2+}\) and calmodulin of NO synthase in the rabbit parotid gland are 356 nM and 5.2 nM, respectively (23), which are lower than that of cGMP-PDE in rabbit parotid acinar cells. Therefore, it is likely that Ca\(^{2+}\) activates NO synthase faster than cGMP-PDE.

Although the PDE1 enzymes in the P-1 and P-2 fractions had the same EC\(_{50}\) for Ca\(^{2+}\), the activities in the P-1 and P-2 fractions were regulated in the range of 75–1100 nM and 150–870 nM Ca\(^{2+}\), respectively. In parotid acinar cells, muscarinic receptor activation induces a rapid, transient and a subsequently sustained increase in [Ca\(^{2+}\)], which are lower than that of cGMP-PDE. The difference of the Ca\(^{2+}\) isoforms, PDE1A isoforms, whose activities are regulated by physiological concentrations of Ca\(^{2+}\)/calmodulin-dependent PDE1. In conclusion, Ca\(^{2+}\)/calmodulin-dependent PDE isoforms, PDE1A isoforms, whose activities are regulated by physiological concentrations of Ca\(^{2+}\), are present in rabbit parotid acinar cells, and are involved in regulating cGMP levels in the cells stimulated by muscarinic cholinergic receptor activation.

Acknowledgments

We thank Prof. K. Shibutani for helpful advice and discussions. This study was supported in part by a Grant-in Aid for 2003-Multidisciplinary Research Project from MEXT, and Grant-in Aid for Scientific Research from JSPS (#16390534).

REFERENCES


16. Michikawa H, Mitsui Y, Fujita-Yoshigaki J, Hara-Yokoyama M, Furuyama S and Sugiya H (1998) cGMP production is not affected. Therefore, it is conceivable that PDE1


27. Michikawa H, Mitsui Y, Fujita-Yoshigaki J, Hara-Yokoyama M, Furuyama S and Sugiya H (1998) cGMP production is not affected. Therefore, it is conceivable that PDE1


