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Okadaic acid inhibits amylase exocytosis from parotid acini stimulated by cyclic AMP

Taishin Takuma and Tokuro Ichida

Department of Oral Biochemistry, School of Dentistry, Higashi Nippon Gakuen University, Tobetsu, Hokkaido 061-02, Japan

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To evaluate the role of protein phosphorylation in amylase exocytosis, we studied the effects of okadaic acid, a potent inhibitor of protein phosphatase types 1 and 2A, on amylase release and protein phosphorylation in rat parotid acini. Although okadaic acid by itself weakly stimulated amylase release, it did not potentiate amylase release stimulated by half-maximum doses of isoproterenol or cAMP, and markedly inhibited their maximum effects. Okadaic acid dose-dependently increased cAMP-independent phosphorylation of some proteins and enhanced cAMP-dependent phosphorylation of 21- and 26-kDa proteins. These results indicate that increase in protein phosphorylation does not necessarily enhance the exocytosis of amylase from parotid acini.

Okadaic acid; Amylase exocytosis; Protein phosphorylation; cAMP-dependent protein kinase; Protein phosphatase inhibitor

1. INTRODUCTION

Protein phosphorylation has been generally recognized to play a central role in the regulatory mechanism of diverse cellular functions [1,2]. Cyclic AMP-evoked amylase exocytosis from parotid acinar cells is also believed to be regulated by protein phosphorylation mediated by cAMP-dependent protein kinase [3–6]. However, previous studies using protein kinase inhibitors (H-8 and peptide fragments of heat-stable protein kinase inhibitor) suggested that protein phosphorylation was not directly involved in the exocytosis, since the inhibitors markedly reduced protein phosphorylation without decreasing amylase release [7,8].

The phosphorylation state of proteins is reversibly controlled by phosphorylation and dephosphorylation mediated by various protein kinases and phosphatases, respectively. Thus, the phosphorylation level is elevated not only by activation of protein kinases but also by inhibition of protein phosphatases. Recently, okadaic acid, a potent selective inhibitor of protein phosphatase types 1 and 2A, has become available for investigation of the physiological role of protein phosphorylation [9–17]. To evaluate the role of protein phosphorylation in amylase exocytosis, we have further examined the effect of okadaic acid on amylase release and protein phosphorylation in parotid acini. The results indicate that okadaic acid increases protein phosphorylation, but inhibits rather than potentiates amylase release stimulated by cAMP.

Correspondence address: T. Takuma, Department of Oral Biochemistry, School of Dentistry, Higashi Nippon Gakuen University, Tobetsu, Hokkaido 061-02, Japan

2. EXPERIMENTAL

Okadaic acid isolated from the marine sponge (*Halichondria okadai*) was a generous gift from Dr. Y. Tsukitani (Fujisawa Pharmaceutical Co., Tokyo). Other chemicals were the same as described previously [7,8].

Rat parotid acini were prepared by enzyme digestion, and amylase release from intact and saponin-permeabilized parotid cells was determined as described [18,19].

Protein phosphorylation in saponin-permeabilized parotid acini was performed as follows: acini were preincubated for 5 min at 37°C with 20 μg/ml saponin and ~0.2 mCi/ml [γ-^{32}P]ATP in calcium-free medium composed of 120 mM KCl, 40 mM Na-HEPES (pH 7.2), 2 mM $MgSO_4$, 1 mM EGTA, 10 μg/ml Phenol red, and 1 mg/ml bovine serum albumin and further incubated for 15 min after addition of okadaic acid and/or cAMP. After incubation, the medium was removed and cells were homogenized in 0.3 M sucrose, 10 mM EDTA, 5 mM EGTA, 10 mM K-phosphate (pH 6.8), and 1 μM okadaic acid in a Teflon-glass homogenizer. The homogenate was processed as described previously [7,8].

3. RESULTS

Fig. 1 shows that 1 μM okadaic acid itself slightly stimulated amylase release. The stimulation was not detectable at less than 100 nM, and was attenuated at 10 μM okadaic acid. Although the extent of stimulation was very small, the stimulation was reproducible (Figs. 2 and 3).

To examine whether or not okadaic acid potentiates cAMP-dependent amylase release, parotid cells were incubated with the maximum or half-maximum doses of isoproterenol in the presence or absence of 1 μM okadaic acid. As shown in Fig. 2, okadaic acid did not enhance amylase release stimulated by 0.01 μM isoproterenol, and markedly inhibited that evoked by 1 μM isoproterenol.

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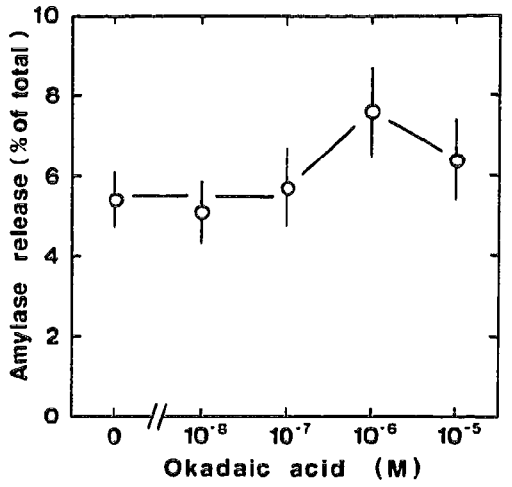

Fig. 1. Effect of okadaic acid on amylase release from parotid acinar cells. The cells were incubated at 37°C for 15 min with various concentrations of okadaic acid in normal Hanks' medium. Data shown are means ± SD ($n = 4$).

In intact cells, amylase release is decreased for various reasons, including inhibition of adenylate cyclase. Thus, similar experiments were carried out using saponin-permeabilized cells incubated in Ca^{2+}-free KCl medium containing cAMP. As seen in Fig. 3, okadaic acid inhibited rather than potentiated amylase release evoked by exogenous cAMP.

As in the case of stimulation, the inhibitory effect of okadaic acid on isoproterenol- or cAMP-induced

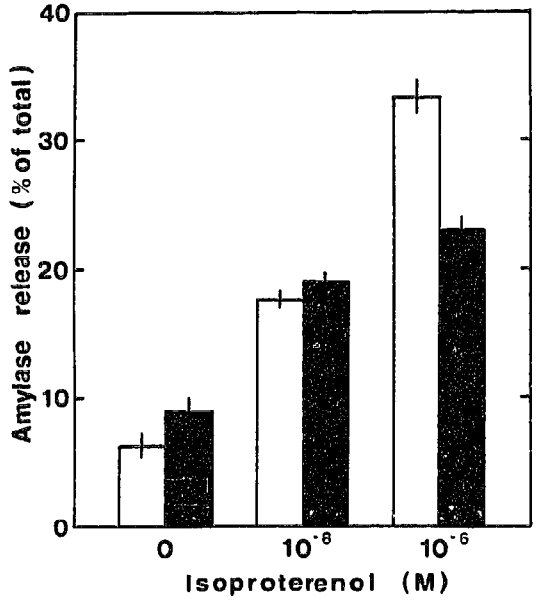

Fig. 2. Effect of okadaic acid on amylase release from parotid acini. Parotid acini were incubated for 15 min in normal Hanks' medium containing 0, 0.01, or 1 μM isoproterenol in the presence (solid bars) or absence (open bars) of 1 μM okadaic acid. Data shown are means ± SD ($n = 4$).

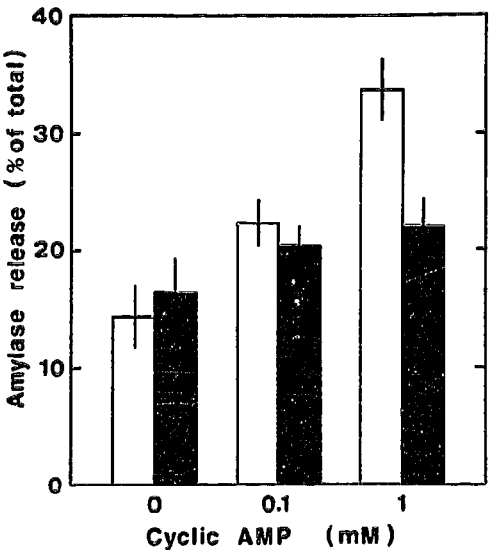

Fig. 3. Effect of okadaic acid on amylase release from saponin-permeabilized parotid acini. The acini were incubated for 15 min with 0, 0.1, or 1 mM cAMP in the presence (solid bars) or absence (open bars) of 1 μM okadaic acid in Ca-free medium containing 20 μg/ml saponin. Data shown are means ± SD ($n = 4$).

amylase release was not detectable at less than 100 nM, but was increased at higher concentrations of okadaic acid (Fig. 4).

Fig. 5 shows the effects of okadaic acid and cAMP on protein phosphorylation in the 15 000 × g pellet fraction of saponin-permeabilized cells, where cAMP-dependent protein phosphorylation was detectable [3,6,7]. Okadaic acid dose-dependently increased phosphorylation of some proteins, including 30-, 33-,

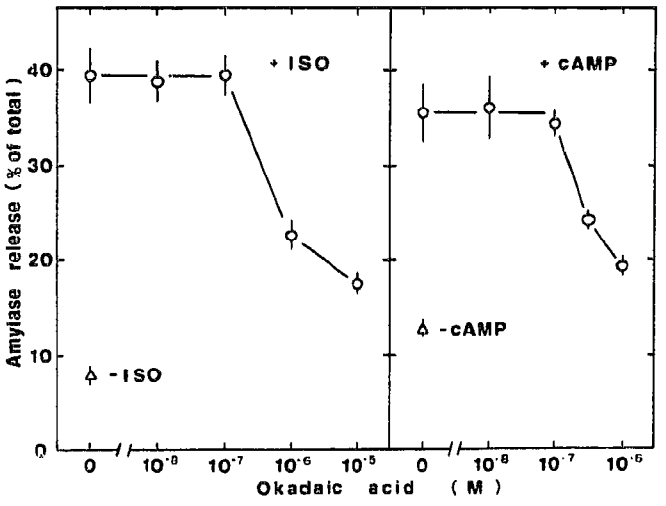

Fig. 4. Effect of okadaic acid on amylase release from intact or saponin-permeabilized parotid cells. The cells were incubated for 15 min with various concentrations of okadaic acid in either normal Hanks' medium (left; intact cells, ± 1 μM isoproterenol) or in Ca-free medium containing 20 μg/ml saponin (right; ± 1 mM cAMP). Data shown are means ± SD ($n = 4$).

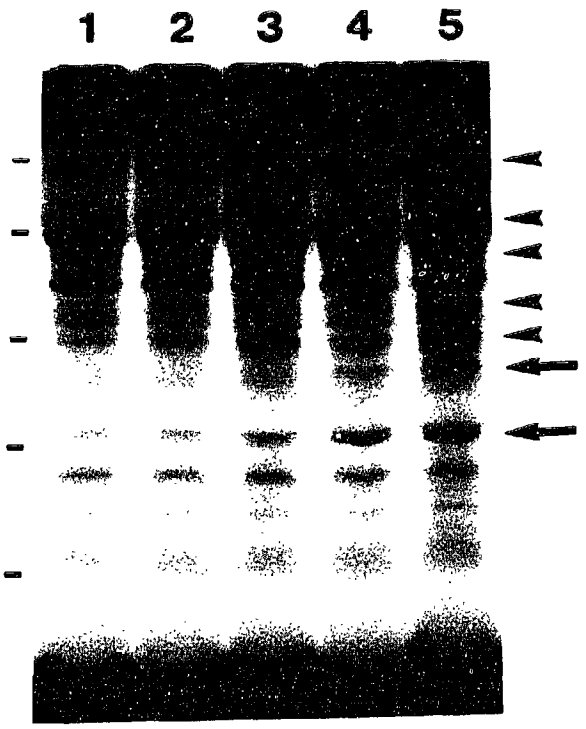

Fig. 5. Effects of okadaic acid and cAMP on protein phosphorylation in saponin-permeabilized parotid cells. Cells were preincubated at 37°C for 5 min with ~0.2 mCi/ml [γ-^{32}P]ATP in Ca-free medium containing 20 μg/ml saponin, and further incubated for 15 min after addition of okadaic acid and/or cAMP. Lanes 1, 2, 3, 4 and 5 are: control, 100 nM okadaic acid, 1 μM okadaic acid, 1 mM cAMP, and 1 μM okadaic acid plus 1 mM cAMP, respectively. Arrows indicate 21- and 26-kDa proteins; arrowheads = proteins phosphorylated by okadaic acid; bars on the left = the positions of molecular markers (from the top, 67, 43, 30, 20.1 and 14.4 kDa).

39-, 48- and 67-kDa proteins, and enhanced cAMP-dependent phosphorylation of 21- and 26-kDa proteins. Okadaic acid by itself seemed to increase phosphorylation of 21- and 26-kDa proteins slightly.

4. DISCUSSION

It has been increasingly reported that okadaic acid affects various cells and induces a wide variety of effects [9–17]. Especially okadaic acid was shown to have cAMP-like effects on smooth muscle and platelets: 1 μM okadaic acid inhibited muscle contraction and platelet aggregation induced by norepinephrine and thrombin, respectively [17]. Furthermore, okadaic acid enhanced and prolonged the effect of isoproterenol on Ca^{2+}-dependent K^+-channel activity in tracheal myocytes, and directly potentiated the channel activity induced by the catalytic subunit of cAMP-dependent protein kinase [16].

Unexpectedly, however, okadaic acid alone slightly increased amylase release, but markedly inhibited rather than potentiated amylase release stimulated by isoproterenol or cAMP, although protein phosphorylation was clearly enhanced by okadaic acid. These findings appear to support the view that cAMP-dependent protein phosphorylation is not obligatory for the exocytosis of amylase from parotid acini [7,8]. As can be seen in Fig. 5, the effect of okadaic acid on protein phosphorylation was nonspecific and was completely different from that of cAMP. Thus, it is also possible that the nonspecific increase in protein phosphorylation stimulates amylase release to a small extent, but greatly reduces the maximum level of amylase release.

In the present study, okadaic acid did neither mimic nor potentiate the effect of cAMP on amylase exocytosis. These results suggest that the exocytosis is regulated by highly specific or intricate ways, even if protein phosphorylation is involved in the process. Protein phosphatases may play an important role in the process by preventing unnecessary and excessive protein phosphorylation.

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