CELL STRUCTURE AND FUNCTION 16: 405–409 (1991)

Roles of Potassium and Chloride Ions in cAMP-mediated Amylase Exocytosis from Rat Parotid Acini

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Key words: amylase secretion/cAMP-mediated exocytosis/saponin-permeabilized parotid acini/K^+ channel blocker/Cl^- channel blocker

ABSTRACT. **The roles of potassium and chloride ions in cAMP-mediated amylase exocytosis were studied using intact and saponin-permeabilized parotid acini. Cyclic AMP-evoked amylase release from saponin-permeabilized parotid acini decreased markedly when KCl in the incubation medium was isoosmotically replaced by K-glutamate, NaCl, Na-isothionate, or mannitol. Quinidine and barium, K^+ channel blockers, clearly inhibited amylase release from the permeabilized acini, but not from intact ones. The chloride channel blocker DPC (diphenylamine-2-carboxylate) also inhibited amylase release, while DIDS (4,4′-diisothiocyanostilben-2,2′-disulfonate) or bumetanide had little effect, if any, on the exocytosis. Hyperosmolarity with mannitol markedly reduced amylase release from permeabilized acini. These results suggest that potassium and chloride ions play important roles in cAMP-mediated amylase exocytosis, and that these ions act on secretory granules inside the acinar cells.**

Amylase release from the parotid gland has been extensively studied as a useful model of cAMP-mediated exocytosis (8), although the molecular mechanism of the exocytosis is mostly unknown, including the role of cAMP-dependent protein phosphorylation (15–18). Recent studies using saponin-permeabilized parotid acini showed that a very simple calcium-free KCl medium can maintain cAMP-evoked amylase release (16). This suggests that major ions, including Ca^{2+}, Na^+, HCO_3^-, and HPO_4^{2-}, are not necessary or that very low concentrations of them are sufficient for the exocytosis.

Meanwhile, the importance of potassium and chloride ions has been reported for protein secretion from permeabilized pancreatic acini (3), although Cl^- inhibits catecholamine secretion from adrenal medullary cells (11). Furthermore, osmotic lysis of the isolated zymogen granules in the presence of various ions and ionophores suggested the existence of K^+ and Cl^- ion channels on the granule membranes (4–7, 13). However, the roles of these ions or ion channels in exocytosis have yet to be established, since only limited research has been carried out concerning the effect of these ions on the exocytosis from permeabilized cells. Thus, we have evaluated the roles of K^+ and Cl^- in cAMP-mediated amylase release from parotid acini using various incubation media and ion channel blockers.

MATERIALS AND METHODS

Cyclic AMP, isoproterenol, and DIDS were obtained from Sigma (St Louis, MO, USA). Quinidine and DPC were from Wako (Osaka, Japan). Bumetanide was a gift from Dr. B.J. Baum (NIDR, NIH, USA). All other chemicals used were of the highest grade commercially available.

Rat parotid acini were prepared by enzyme digestion, and amylase release from intact acini was determined as described previously (14). Amylase release from ponin-permeabilized acini was measured as follows: acini were washed twice with Ca-free medium composed of 140 mM KCl (or other substitutes), 20 mM K- or Na-Hepes (pH 7.2), 1 mM EGTA, 2 mM $MgSO_4$ (or $MgCl_2$ especially for experiments with barium), 1 mg/ml bovine serum albumin, and 10 μg/ml phenol red and suspended in the same medium. One milliliter of cell suspension was transferred into a glass tube containing saponin (20 μg/10 μl), preincubated at 37°C for 5 min, and further incubated for 15 min after addition of 1 mM cAMP. After incubation, the medium was collected by filtration through glass-fiber paper. For measurement of total amylase activity, the acini were homogenized with the incubation medium in a Polytron homogenizer at speed 8 for 20 s, and then 0.2% Triton X-100 was added. The released amylase activity was given

Abbreviations: DPC, diphenylamine-2-carboxylate; DIDS, 4,4′-diisothiocyanostilben-2,2′-disulfonate.

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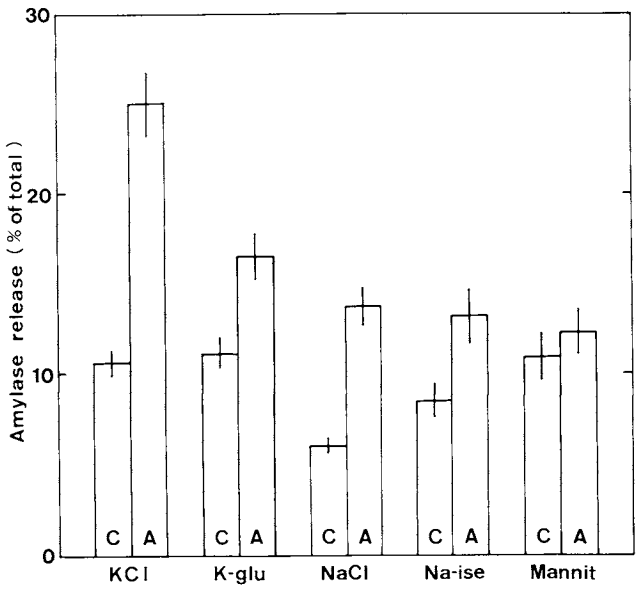

Fig. 1. Effects of K^+ or Cl^- depletion on cAMP-mediated amylase release from saponin-permeabilized parotid acini. KCl in the incubation medium was isoosmotically replaced by K-glutamate (K-glu), NaCl, Na-isothionate (Na-ise), and mannitol (Mannit). Parotid acini were preincubated at 37°C for 5 min in each medium containing 20 μg/ml saponin and were further incubated for 15 min after addition of 1 mM cAMP. C and A are control and cAMP, respectively. Data are shown as mean±SD (n=10).

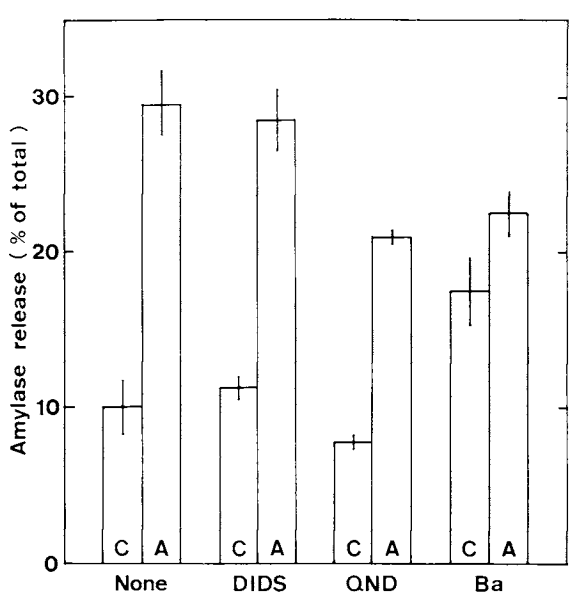

Fig. 2. Effects of K^+ and Cl^- transport inhibitors on amylase release from saponin-permeabilized parotid acini. Acini were preincubated for 5 min with 100 μM DIDS, 400 μM quinidine (QND), or 1 mM $BaCl_2$ (Ba) in Ca-free KCl medium containing 20 μg/ml saponin and were further incubated for 15 min with 1 mM cAMP. C and A are control and cAMP, respectively. Data are shown as mean±SD (n=6).

as the percentage of the total activity.

RESULTS

The effects of ion replacement on cAMP-mediated amylase release from saponin-permeabilized parotid acini are shown in Fig. 1. In these experiments, 140 mM KCl in the incubation medium was isoosmotically replaced by K-glutamate, NaCl, Na-isethionate, and mannitol. Amylase release was greatly reduced in all substitutes. In the sodium media (NaCl and Na-isethionate), basal release was also decreased. Amylase release was almost completely inhibited in mannitol medium.

Effects of K^+- and Cl^--transport inhibitors were then examined in KCl medium (Fig. 2). Potassium channel blockers (400 μM quinidine and 1 mM $BaCl_2$) (1) clearly inhibited amylase release, but 100 μM DIDS, an inhibitor of Cl^--HCO_3^- exchange, had no effect. The effects of these inhibitors were also examined in intact acini incubated in normal Hanks' medium (NaCl is the major component) in the presence or absence of 1 mM isoproterenol. As shown in Fig. 3, all inhibitors had no effect. In these experiments, barium increased basal amylase release in permeabilized acini, but not in intact ones. Recently, 10 mM barium was reported to increase amylase

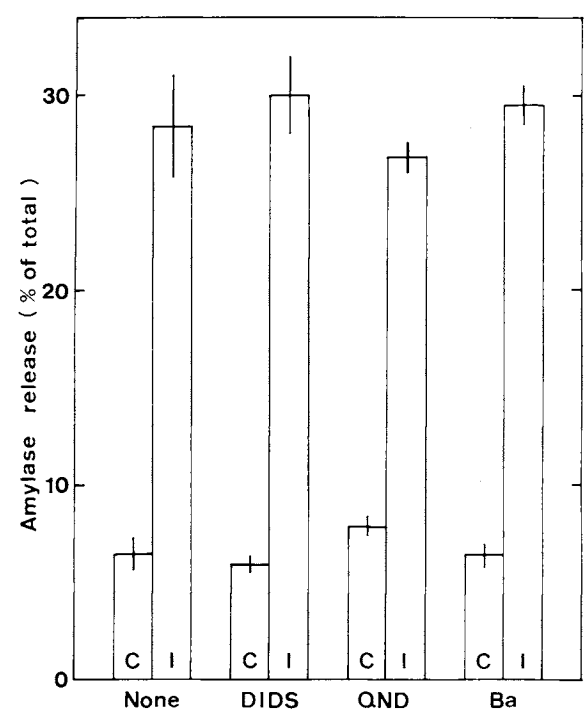

Fig. 3. Effects of K^+ and Cl^- transport inhibitors on amylase release from intact parotid acini. Acini were incubated as in Fig. 2 in normal Hanks' medium without saponin and amylase release was stimulated by 1 μM isoproterenol. C and I are control and isoproterenol, respectively. Data are shown as mean±SD (n=6).

release from intact parotid acini (2), suggesting that the high concentration of barium in the medium increases its intracellular level.

Although DIDS had no effect in either intact or permeabilized acini, the depletion of Cl^- from the medium inhibited amylase release even in intact acini stimulated by isoproterenol (Fig. 4). Amylase release was similarly decreased in Na-isethionate medium (14). Thus, the effect of DPC, a chloride channel blocker, on amylase release was examined (Fig. 5). At 1 mM DPC, amylase release was strongly inhibited, but the basal release was markedly increased in both intact and permeabilized acini, suggesting that DPC has some side effects at this concentration. In permeabilized acini, however, 100 μM DPC clearly inhibited amylase release without any increase in basal release which was actually slightly decreased. The inhibitory effect in intact acini was weaker than that in permeabilized ones.

Bumetanide, the most effective inhibitor of Na/K/2Cl cotransporter, had no effect on amylase release up to 100 μM, but strongly inhibited it at 1 mM; at which basal release was clearly increased in both intact and permeabilized acini (Fig. 6).

The effect of osmolarity on amylase release from permeabilized acini was examined (Fig. 7). Increase in osmolarity with mannitol markedly decreased cAMP-me-

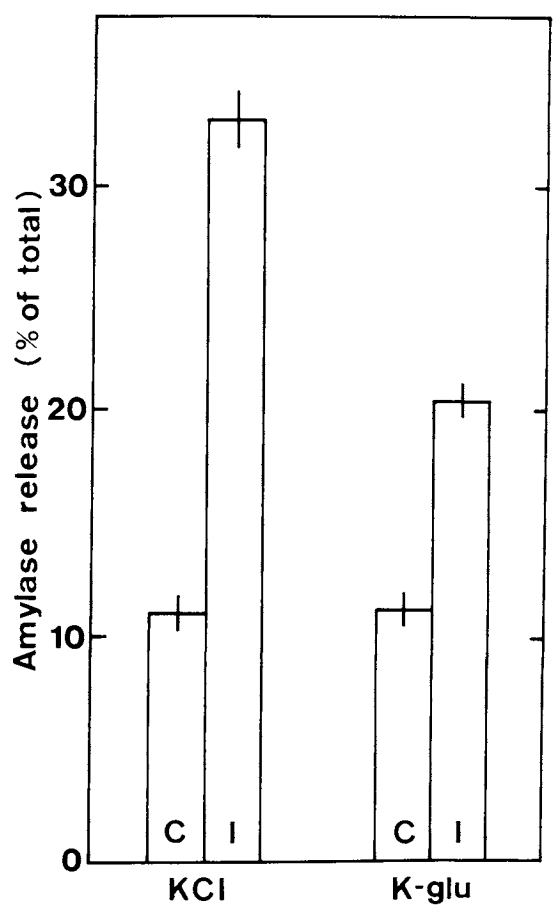

Fig. 4. Effect of Cl^- depletion on amylase release from intact parotid acini. Acini were incubated for 15 min in Ca-free KCl or K-glutamate (K-glu) medium in the presence or absence of 1 μM isoproterenol. Data are shown as mean±SD (n=6).

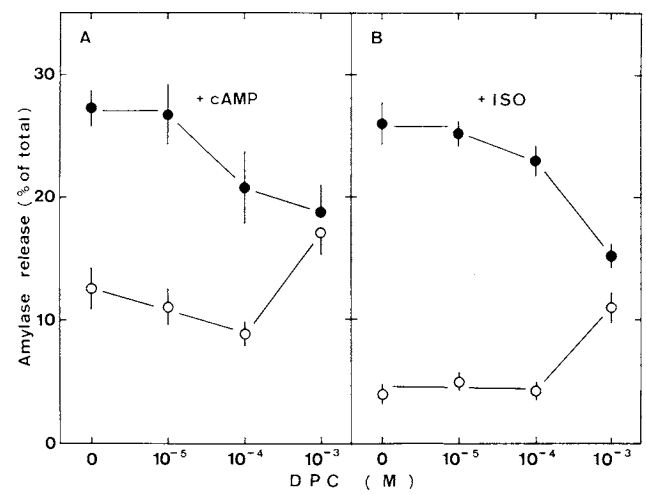

Fig. 5. Effect of DPC on amylase release from intact and saponin-permeabilized parotid acini. Acini were preincubated for 5 min with various concentrations of DPC in Ca-free KCl medium containing 20 μg/ml saponin (A) or normal Hanks' medium (B) and were further incubated for 15 min in the presence or absence of 1 mM cAMP (A) or 1 μM isoproterenol (B). Data are shown as mean±SD (n=6).

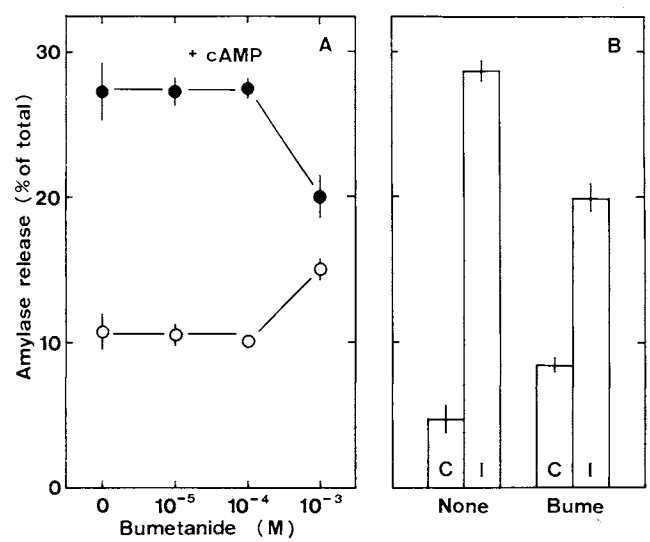

Fig. 6. Effect of bumetanide on amylase release from intact and saponin-permeabilized parotid acini. Acini were preincubated for 5 min with various concentrations of bumetanide in Ca-free KCl medium containing 20 μg/ml saponin (A) or with 1 mM bumetanide (Bume) in normal Hanks' medium (B) and were further incubated for 15 min in the presence or absence of 1 mM cAMP (A) or 1 μM isoproterenol (B). Data are shown as mean±SD (n=3–6).

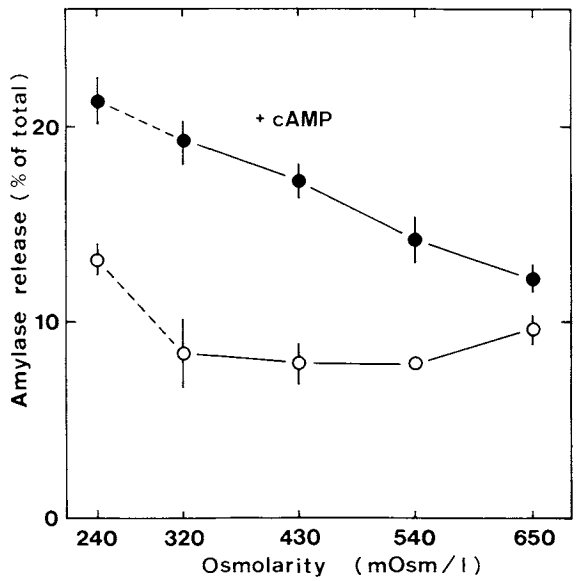

Fig. 7. Effect of osmolarity on cAMP-mediated amylase release from saponin-permeabilized parotid acini. Parotid acini were preincubated for 5 min in 1 ml of Ca-free medium containing 140 mM KCl and 20 μg/ml saponin and were further incubated for 15 min after addition of 2 mM cAMP in 1 ml Ca-free KCl medium containing various concentrations of mannitol. For hypoosmotic medium, KCl concentration in the latter medium was reduced. Data are shown as mean $\pm$SD (n=6).

diated amylase release. However, decrease in the osmolarity by dilution of the medium did not augment amylase release because of the steep increase in basal release.

DISCUSSION

The present study has demonstrated that K^+ and Cl^- play important roles in cAMP-mediated amylase exocytosis from rat parotid acini. Namely, 1) depletion of K^+ or Cl^- from incubation medium markedly decreased amylase release, and 2) K^+ and Cl^- channel blockers clearly inhibited amylase release. In addition, these blockers were more effective in saponin-permeabilized acini than in intact ones, suggesting that the blockers act on channels on the secretory granule membranes (6, 7).

Most findings in this study are consistent with those of the exocrine pancreas reported previouly (3), but the effects of DIDS and DPC are not; DIDS inhibited protein release from pancreatic acini stimulated by carbachol, but did not inhibit cAMP-mediated amylase release from parotid acini. In contrast, the chloride channel blocker DPC clearly inhibited amylase release from permeabilized parotid acini, whereas its related phenylanthranilic acid derivatives, including 5-nitro-2-(3-phenylpropylamino) benzoic acid (NPPB), had no effect in the pancreas. These findings suggest that the parotid gland and pancreas have heterogenous sensitivities to various Cl^- channel blockers. Recently, DPC and NPPB were found to inhibit adrenocorticotropic hormone (ACTH) secretion from mouse pituitary tumor cells (9).

Although 1 mM bumetanide strongly inhibited amylase release, Na/K/2Cl cotransporter is unlikely to be involved in amylase exocytosis, since 1) bumetanide concentration required for the inhibition is extraordinarily high as compared with that for the inhibition of cotansporters in other cells (12), 2) the basal release is increased at this concentration, and 3) Na^+ is not necessary for amylase release.

If K^+ and Cl^- channels on the secretory granule membrane play crucial roles in the exocytosis, osmotic swelling of the granule is very likely to be involved in the process. Indeed,hyperosmolarity inhibited exocytosis in various cells including pancreatic (3) and parotid (Fig. 7) acini. However, the role of osmotic swelling in the process of exocytosis is still controversial; i.e., the swelling promotes fusion of the plasma membrane and secretory granules or widens the exocytic pore for the extrusion of the granule contents (10, 19). Further studies are necessary for resolving these matters.

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(Received for publication, July 30, 1991
and in revised form, August 23, 1991)