Reversible acetaldehyde inhibition of A23187-stimulated amylase secretion from isolated rat pancreatic acini

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The Ca^{2+} ionophore, A23187, stimulated amylase secretion from isolated rat pancreatic acini in a dose-dependent manner with a maximal effect at 6 μM. Acetaldehyde, a metabolite of ethanol, caused a reduction in the magnitude of ionophore-stimulated secretion with no evidence of competitive inhibition. Furthermore, 6 μM ionophore-stimulated amylase secretion was dose-dependently inhibited by acetaldehyde. This inhibitory effect of acetaldehyde, however, was reversible on washing and reincubating acetaldehyde-treated acini. These results suggest that acetaldehyde reversibly inhibits intracellular components mediating stimulated secretion and this inhibition requires a continuous chemical interaction between acetaldehyde and intracellular component(s) regulating stimulated enzyme secretion.

Acetaldehyde *Pancreatic acini* *Calcium ionophore* *Amylase secretion* *Reversibility*

1. INTRODUCTION

Ethanol and its metabolite, acetaldehyde, inhibit both the binding of radioiodinated cholecystokinin (CCK) to its specific receptors on isolated pancreatic acini and CCK-stimulated amylase secretion from acini [1,2]. A correlation between the inhibitory effects of ethanol on CCK binding and CCK-stimulated secretion was demonstrable [1]. A similar correlation between the inhibitory effects of acetaldehyde could not be elucidated since the half-maximal concentration of acetaldehyde for inhibition of binding was 6–7-fold higher than that for the inhibition of stimulated secretion [2]. Furthermore, acetaldehyde concentrations which dose-dependently inhibited CCK-stimulated secretion did not alter basal enzyme secretion from acini [2]. These observations suggest that acetaldehyde inhibition of stimulated secretion is (i) post-receptor mediated, and (ii) on a process regulating enzyme secretion mediated by CCK.

Enzyme secretion from pancreatic acini is mediated by either cyclic nucleotides (such as cyclic AMP) [3] or Ca^{2+} [4]. Since acetaldehyde inhibition of CCK-stimulated enzyme secretion is not receptor mediated and CCK action in acini is mediated through Ca^{2+} mobilization, it is possible that acetaldehyde interferes with Ca^{2+} metabolism regulating enzyme secretion. The Ca^{2+} ionophore, A23187, increases Ca^{2+} fluxes across the membrane and mimicks the actions of secretagogues, such as acetylcholine and CCK [5]. We therefore investigated the effect of acetaldehyde on stimulated secretion by A23187. Since acetaldehyde inhibition of CCK-stimulated amylase secretion from acini is irreversible [2], we also studied the reversibility of acetaldehyde effect on Ca^{2+} ionophore-induced amylase secretion from acini.

2. MATERIALS AND METHODS

Calcium ionophore A23187 was obtained from Calbiochem, La Jolla, CA. Krebs-Henseleit bicarbonate (KHB) and Hepes-buffered Ringer (HR) containing essential amino acid supplement and 0.01% soybean trypsin inhibitor were prepared as described [6]. Isolated pancreatic acini were

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prepared by the enzymatic digestion of the pancreas from female Wistar rats (200 g), fasted 18 h, as described by Williams et al. [6]. After 30 min preincubation, the acini were stimulated with varying concentrations of A23187 both in the absence and presence of acetaldehyde. Amylase secreted into the medium after 30 min at 37°C and the total amylase content in homogenized acini were measured by the method of Jung [7]. Protein was measured by the method of Lowry et al. [8] employing bovine serum albumin as a standard.

3. RESULTS

3.1. *Amylase secretion*

In rat pancreatic acini detectable amylase secretion was observed at 300 and 600 nM A23187. Significant increases in amylase secretion, however, occurred at higher concentrations. The stimulated response reached a peak at 6 μM and further increases in A23187 concentration resulted in a fall in amylase secretion (fig.1). In the presence of 45 mM acetaldehyde basal amylase secretion was not significantly altered, whereas A23187-stimulated amylase secretion was significantly reduced (fig.1). The dose-response curve for A23187, both in the absence and the presence of acetaldehyde, exhibited the characteristic biphasic shape with a peak at 6 μM. Acetaldehyde between 1 and 60 mM did not significantly alter the basal amylase secretion from acini. These concentrations, however, produced a dose-dependent inhibition of amylase secretion induced by 6 μM A23187 (fig.2). Although detectable inhibition was observed at 3 and 6 mM, the inhibition observed between 10 and 100 mM was significantly different from the control. The apparent half-maximal inhibitory concentration was 27 mM, however, we employed 45 mM acetaldehyde in reversibility studies in order to compare the reversibility of acetaldehyde effect on A23187 with that on CCK-stimulated amylase secretion from acini (45 mM acetaldehyde was employed in reversibility studies with CCK).

3.2. *Reversibility of acetaldehyde effect*

Reversibility of acetaldehyde inhibition of ionophore-stimulated amylase secretion was carried out by an identical procedure described for studying ethanol and acetaldehyde reversibility of CCK-stimulated amylase secretion [1,2]. Briefly, two 8 ml acini suspensions were incubated with 6 μM A23187 in the absence and presence of 45 mM acetaldehyde. At 30 min (first incubation)

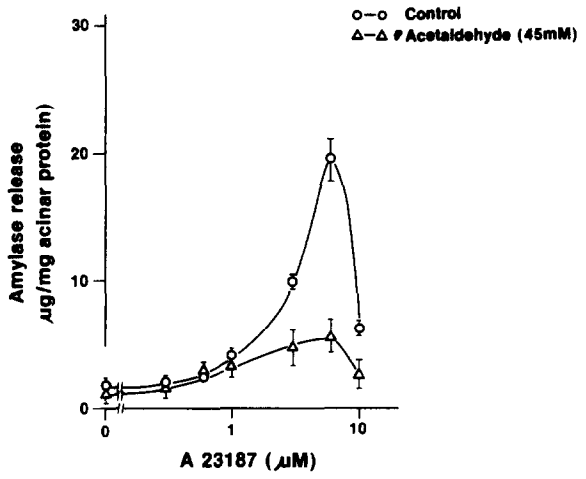

Fig.1. Effect of acetaldehyde on ionophore-stimulated amylase secretion. Isolated acini were incubated with increasing concentrations of the ionophore, A23187, in the absence and presence of 45 mM acetaldehyde for 30 min at 37°C. Values are means ± SE of 4 experiments.

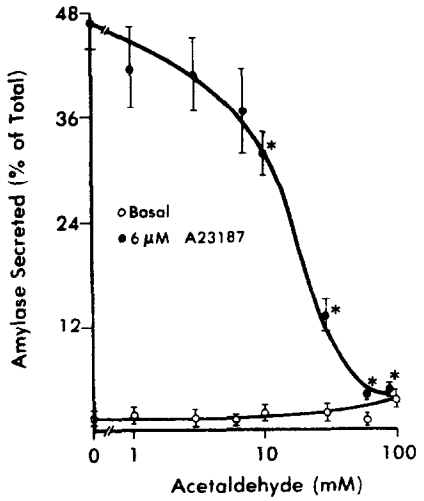

Fig.2. Effect of acetaldehyde on basal and 6 μM ionophore-stimulated amylase secretion. Amylase secreted over 30 min at 37°C is plotted as a function of acetaldehyde concentration in the medium. Values are means ± SE of 5 experiments.

Table 1

Reversibility of acetaldehyde inhibition

	No acetaldehyde	45 mM acetaldehyde	45 mM acetaldehyde, washed and reincubated
Basal amylase secretion (% of basal)	100	104 ± 6	100
6 μM A23187-induced amylase secretion (% over basal)	201 ± 11[a]	139 ± 12[b]	256 ± 35[a] (251 ± 22[a])

[a] Significance between stimulated and corresponding basal values ($p < 0.025$)

[b] Significance between ionophore-stimulated values in the absence and presence of acetaldehyde ($p < 0.025$). For details see sections 2 and 3

Values are means ± SE of 5 experiments.

two 1 ml aliquots from each set were removed for amylase assay. Each of the remaining 6 ml acini suspensions was centrifuged, washed, recentrifuged, and resuspended in 6 ml of HR. After reincubation for 30 min (second incubation), two 1 ml aliquots, as before, were saved for amylase assay. Each of the remaining 4 ml suspensions was resuspended in 4 ml HR after two washes and were restimulated with 6 μM A23187. After 30 min incubation (third incubation), a final set of 1 ml aliquots was removed for amylase assay. Identical incubations of acini with and without acetaldehyde and without A23187 were the controls. The results are summarized in table 1. Acetaldehyde inhibition of ionophore-stimulated amylase secretion was reversible on washing and reincubating acini in fresh HR. Amylase release on restimulation with 6 μM A23187 after washing and reincubation of acetaldehyde-treated acini was not significantly different from that of restimulated acini which had not been treated with acetaldehyde (value in parentheses).

4. DISCUSSION

Cytosolic Ca^{2+} plays a major role in mediating intracellular events induced by neural, hormonal or other external stimuli at the plasma membrane leading to a physiological response [9]. Pancreatic enzyme secretion is regulated by the secretagogues acetylcholine (ACh) and CCK that bind to the specific receptors on acini and then cause a rise in cytoplasmic calcium. There is abundant evidence for Ca^{2+} mediation in CCK and ACh-induced enzyme secretion from acini [10]. Here, we have demonstrated that acetaldehyde, in the concentrations which do not alter basal secretion, inhibits A23187-stimulated amylase secretion. The reduction in the magnitude of ionophore-stimulated amylase secretion by acetaldehyde, without a parallel rightward shift in the dose-response curve, indicates a noncompetitive type of antagonism. This inhibition by acetaldehyde is similar to that observed with CCK [2]. Acetaldehyde inhibition of A23187-stimulated enzyme secretion was reversible on washing and reincubating acini whereas the inhibition by acetaldehyde of CCK-stimulated enzyme secretion was not reversible [2]. A chemical interaction between acetaldehyde and A23187 in solution would also result in either total or partial inhibition of the ionophore-stimulated amylase secretion. This, however, is not the case since mixing of A23187 and acetaldehyde (at the concentrations utilized here) and evaporation of acetaldehyde by oxygenation prior to the addition of isolated acini results in stimulation of enzyme secretion comparable to that of acetaldehyde-untreated, ionophore-stimulated acini (unpublished). These observations suggest that (i) acetaldehyde reversibly interferes with component(s) mediating ionophore-stimulated amylase secretion, and (ii) for acetaldehyde to exert its inhibition of ionophore-stimulated secretion a continuous chemical interaction between the intracellular component(s), mediating ionophore-stimulated secretion, and acetaldehyde is essential.

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