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IRREVERSIBLE INHIBITION BY ACETALDEHYDE OF CHOLECYSTOKININ-INDUCED AMYLASE SECRETION FROM ISOLATED RAT PANCREATIC ACINI*

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Abstract—Acetaldehyde inhibited both amylase secretion induced by maximal concentrations (300 pM) of cholecystokinin octapeptide and the binding of radioiodinated cholecystokinin to receptors on isolated rat pancreatic acini. This inhibition was concentration dependent (10 mM to 1 M for amylase secretion and 100 mM to 1 M for binding). However, a correlation between the two inhibitory effects could not be obtained. Furthermore, the inhibitory effects were not reversible. Acetaldehyde did not alter the basal amylase secretion between 6 and 45 mM concentrations. However, 60, 100 and 300 mM acetaldehyde significantly decreased basal amylase secretion; no significant change in amylase secretion was observed at 600 mM and 1 M. Higher concentrations of acetaldehyde produced a 2- to 10-fold increase in basal amylase secretion. ^{51}Cr release from prelabeled acini revealed no significant cell membrane damage between 10 and 600 mM acetaldehyde. These data suggest that acetaldehyde inhibition of cholecystokinin-induced amylase secretion is intracellularly mediated.

Acetaldehyde, a major product of ethanol metabolism in liver and other tissues [1–3], has been incriminated in the short-term effects of ethanol and also in the development of ethanol dependence [4]. It also has been shown to affect a number of other biochemical and cellular processes [5–10]. However, reports on the effects of acetaldehyde on the pancreas, especially on the exocrine secretory function, are scarce. *In vivo* it is difficult to ascertain whether the effects of ethanol on the pancreas are due to ethanol or acetaldehyde. Although this controversy has been due mainly to the lack of conclusive demonstration of ethanol metabolism by the pancreas [11–13], the direct action of circulating acetaldehyde on the pancreas cannot be ruled out. The pancreas, thus, could be exposed to the toxic effects of both ethanol and acetaldehyde.

In an earlier study [14], employing isolated rat pancreatic acini, we demonstrated the concentration-dependent inhibition by ethanol of amylase secretion induced by the octapeptide of cholecystokinin (CCK_8) and also the binding of radioiodinated cholecystokinin$_{33}$ ($[^{125}I]CCK_{33}$) to its specific receptors on acini. The two inhibition curves were identical, indicating the participation of specific receptors in ethanol inhibition of $[^{125}I]CCK_{33}$ binding and CCK_8-stimulated amylase secretion. Since CCK receptor occupancy has been correlated with CCK-induced biological actions in acini [15, 16], the observed correlation between the inhibitory effects of ethanol suggested that inhibition of binding of the hormone was the primary cause of inhibition of the hormone-induced amylase secretion. In addition, these inhibitory effects of ethanol were reversible when ethanol-treated acini were successively washed and re-incubated in fresh *N*-2-hydroxyethyl-piperazine-*N*′-2-ethanesulfonic acid (HEPES)-buffered Ringer medium [14]. The present investigation on the action of acetaldehyde on isolated rat pancreatic acini was performed to determine whether acetaldehyde exhibited inhibitory effects similar to ethanol.

EXPERIMENTAL PROCEDURES

All materials employed to prepare isolated acini and to radioiodinate CCK_{33} were obtained from sources previously described for similar studies [17]. ^{51}Cr-sodium chromate (200–500 Ci/g chromium) was obtained from the New England Nuclear Corp., Boston, MA. CCK_8 was purchased from Research Plus, Bayonne, NJ. CCK_{33} was obtained from Dr. V. Mutt, Karolinska Institute, Stockholm, Sweden. Since the two peptides possess the same biological activity (marginally differing in their potencies) in binding and in releasing amylase from acini, CCK_{33} was used only for radioiodination and CCK_8 was

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used for amylase secretion and for the determination of nonspecific binding of tracer CCK_{33}. Krebs–Henseleit bicarbonate buffer (KHB) and HEPES-buffered Ringer (HR) containing essential amino acid supplement and 0.01% soybean trypsin inhibitor were prepared as previously described [17].

Isolated pancreatic acini were prepared by the enzymatic digestion of the pancreas from female Wistar rats (150 g) fasted 18 hr, as described by Williams *et al.* [17]. After 30 min of preincubation, the acini were stimulated with various concentrations of CCK_8 in both the absence and presence of acetaldehyde, and the amylase secreted into the medium after 30 min at 37° was measured by the method of Jung [18] employing procion yellow dye coupled to starch as substrate. α-Amylase (type 1A from porcine pancreas, Sigma Chemical Co., St. Louis, MO) was used to generate the standard curve. Protein was measured by the method of Lowry *et al.* [19] using bovine serum albumin as a standard.

CCK binding to acini was determined by employing biologically active $[^{125}I]CCK_{33}$ prepared by the method of Sankaran *et al.* [20]. Nonspecific binding was determined by incubating acini with tracer CCK_{33} in the presence of a large excess of unlabeled CCK_8 (10^{-6} M). Specific binding was calculated by subtracting nonspecific binding from the total binding. The effect of acetaldehyde on CCK binding to acini was determined by incubating a 1.5-ml suspension of acini with $[^{125}I]CCK_{33}$ (20 pM) in the presence of various acetaldehyde concentrations for 30 min at 37°. At the end of the incubation, the hormone bound to acini was separated from free hormone by microcentrifugation, and after two washes the radioactivity in the pellet was measured in a Searle gamma counter.

Determination of cell membrane damage. Acinar cell membrane damage was determined using a modification of the method of Sacks *et al.* [21]. The modified procedure elucidated in our previous study on ethanol effects [14] was employed to determine the effect of acetaldehyde on acinar cell membrane.

Reversibility of acetaldehyde effect. Two 8-ml acini suspensions were incubated with 300 pM CCK_8 in the absence and presence of 45 mM acetaldehyde. At 30 min (first incubation), 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 of suspension was resuspended in 4 ml HR after two washes and were restimulated with 300 pM CCK_8. After 30 min of 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 CCK_8 were the controls.

To study the reversibility of acetaldehyde inhibition of CCK binding, two sets of acini (in the absence and presence of 300 mM acetaldehyde) were incubated for 30 min, centrifuged, washed and resuspended in identical volumes of HR, and the incubation was continued for a further 30 min. At the end of the second incubation, acini were centrifuged, washed, resuspended in the same volume of HR and then pulsed with $[^{125}I]CCK_{33}$, and the ligand binding was carried out for 30 min at 37°. At the end, 2 0.5 ml aliquots were removed and the pellets were processed as in CCK binding studies for gamma counting. Nonspecific binding of CCK to acini was determined by adding 10^{-6} M CCK_8. B/F values calculated for the two conditions were compared with identical counterparts which did not go through the wash and reincubation procedures. Statistical significance was determined by Student's *t*-test.

RESULTS

Determination of cell damage. Acini prelabeled with ^{51}Cr spontaneously released 5% of the label at 30 min. Acetaldehyde between 10 and 600 mM concentrations did not alter the release of ^{51}Cr from acini, indicating no apparent damage to the cell membrane. ^{51}Cr release from acini induced by greater than 600 mM acetaldehyde was significant. In comparison, the label release from acini in the presence of 3 M acetaldehyde approached that of acini resuspended in distilled water (see Fig. 3).

Acetaldehyde on amylase secretion. In rat pancreatic acini, 30 pM CCK_8 caused a marked increase in amylase secretion; the stimulated response reached a peak at 300 pM (Fig. 1). Further increases in CCK_8 concentration resulted in a progressive fall in amylase secretion. In the presence of 45 mM acetaldehyde, basal amylase secretion was not altered significantly, whereas CCK_8-stimulated response was diminished markedly. The dose–response curve, in the presence or absence of acetaldehyde, exhibited the characteristic biphasic shape with a peak at 300 pM CCK_8. The reduction in the magnitude of amylase secretion in the presence of 45 mM acetaldehyde, without a parallel rightward shift in the dose–response curve, is suggestive of a noncompetitive type of inhibition.

Acetaldehyde concentrations between 6 and 60 mM did not alter significantly basal amylase secretion (amylase values in μg/mg acinar protein, mean ± S.D., at 0, 6, 10, 30, and 45 mM concentrations: 2.6 ± 0.6, 1.9 ± 0.6, 2.0 ± 0.5, 1.6 ± 0.4, and 1.8 ± 0.3; P values between 0 and respective acetaldehyde concentrations > 0.1).

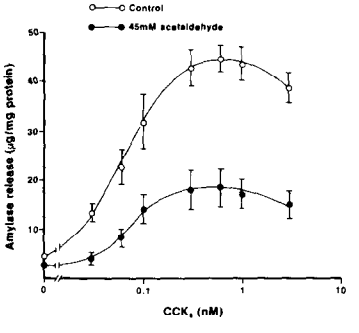

Fig. 1. Concentration dependence of CCK_8-stimulated amylase secretion from isolated rat pancreatic acini in the presence and absence of acetaldehyde. Amylase secretion from acini over 30 min at 37° is plotted as a function of CCK_8 concentration in the medium. Each point is the mean ± S.E. of five experiments.

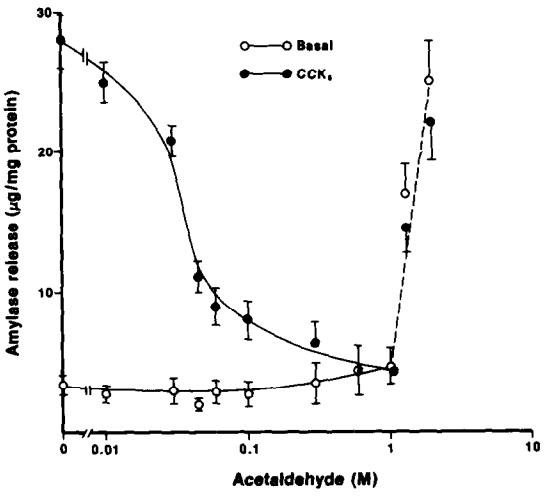

Fig. 2. Effect of acetaldehyde on basal and 300 pM CCK_8-induced amylase secretion. Basal and stimulated amylase secretion over 30 min at 37° is plotted as a function of acetaldehyde concentration in the medium. The broken line represents enhanced amylase secretion, in the absence or presence of 300 pM CCK_8, due mainly to acinar cell membrane damage. Each point is the mean ± S.E. of six experiments.

Acetaldehyde, however, at 60, 100 and 300 mM caused a significant decrease in amylase secretion compared to control (amylase values 1.4 ± 0.5, 1.0 ± 0.4, 1.5 ± 0.2; $P < 0.05$); 600 mM and 1 M did not have a significant effect (1.8 ± 0.1, 2.9 ± 1.2). Higher concentrations (1.3 M and 2 M) produced a 2- to 10-fold increase in amylase secretion (5.4 ± 1.3, 20.8 ± 1.2; $P < 0.02$). Identical concentrations of acetaldehyde, up to 1 M, produced a dose-dependent inhibition of 300 pM CCK_8-induced enzyme release from acini (Fig. 2). This inhibition was half-maximal at 45 mM and maximal at 1 M. Further increases in acetaldehyde produced a profound increase in amylase secretion which was comparable to that of the basal release in the presence of identical concentrations of acetaldehyde (Fig. 2).

Acetaldehyde on binding of CCK to acini. CCK binding to the acinar cell membrane correlates with CCK-induced amylase secretion from the acini [15, 16]. In the present study, increasing concentrations of acetaldehyde (between 100 mM and 1 M) caused a progressive fall in the binding of [^{125}I]CCK_{33} to its specific receptors on acini (Fig. 3). There was no significant change in the binding of [^{125}I]CCK_{33} in the presence of 6–60 mM acetaldehyde. The apparent acetaldehyde concentration for half-maximal inhibition of [^{125}I]CCK_{33} binding was 300 mM.

Reversibility of acetaldehyde inhibition. Acetaldehyde (45 mM) inhibited 300 pM CCK_8-induced amylase secretion from acini by 50% after 30 min of incubation. At the end of the second incubation, the acetaldehyde control and basal amylase values were comparable to the first 30-min amylase levels. CCK_8 plus acetaldehyde-treated acini, however, compared to CCK_8-stimulated acini, showed greater than 50% inhibition in amylase secretion. This is further documented by the third incubation were acetaldehyde-treated acini still exhibited 50% inhibition in amylase secretion, compared to CCK_8-treated acini, despite restimulation with 300 pM CCK_8. Similar inhibition data were obtained in reversibility studies on acetaldehyde inhibition of [^{125}I]CCK_{33}. Acini treated with 300 mM acetaldehyde and incubated for 30 min, compared to untreated acini, showed 50% inhibition of binding. Furthermore, acini treated with 300 mM acetaldehyde and incubated for 30 min, washed and reincubated with [^{125}I]CCK_{33} and with no acetaldehyde added, exhibited 50% inhibition in binding tracer CCK_{33} (Table 1).

DISCUSSION

The specific binding of radioiodinated CCK_{33} to receptors on acini and the correlation between CCK binding and CCK-induced biological functions in

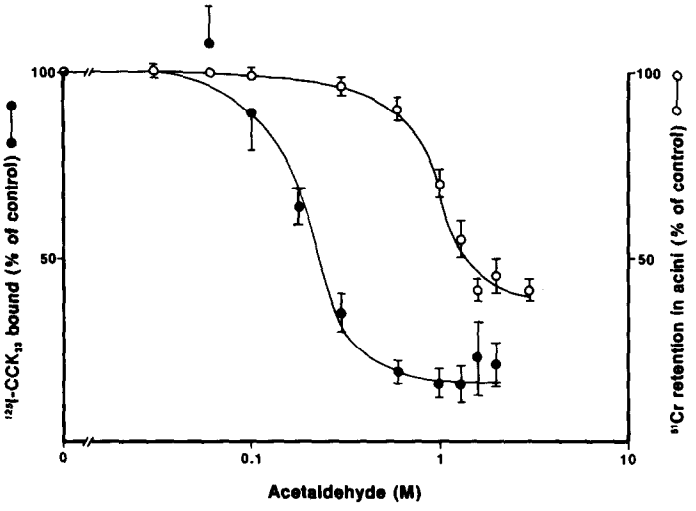

Fig. 3. Effect of acetaldehyde on [^{125}I]CCK_{33} binding and ^{51}Cr retention. Effect of increasing concentrations of acetaldehyde on the specific binding of [^{125}I]CCK_{33} to acinar receptors and ^{51}Cr retained by the cell is represented. Each point is the mean ± S.E. of six experiments.

Table 1. Irreversibility of acetaldehyde inhibition*

	No acetaldehyde	Acetaldehyde	Sig†	Acetaldehyde and wash	Sig‡
Basal amylase secretion (% of basal)	100	94 ± 6	$P > 0.5$	99 ± 3	$P > 0.5$
300 pM CCK_8-induced amylase secretion (% over basal)	547 ± 60	241 ± 24	$P < 0.002$	264 ± 57	$P > 0.5$
$^{125}I]CCK_{33}$ specifically bound (% of maximum)	100	53 ± 7	$P < 0.002$	26 ± 7	$P < 0.002$

* Amylase secretion was studied in the presence of 45 mM acetaldehyde. CCK binding was performed in the presence of 300 mM acetaldehyde. Values are mean ± S.E. of six experiments. For details, see experimental procedures.

† Between acetaldehyde and no acetaldehyde.

‡ Between acetaldehyde and wash, and acetaldehyde.

acini have already been reported [16]. In our earlier paper [14], we provided evidence for the inhibition by ethanol of both CCK binding and CCK-induced amylase secretion from acini and the correlation between the two inhibitory effects. Acetaldehyde, in the present study, inhibited enzyme secretion induced by maximal concentrations of CCK. This inhibition by acetaldehyde does not correlate with the inhibition of radioiodinated CCK binding to specific CCK receptors on acini, since the half-maximal concentration of acetaldehyde for inhibition of CCK binding is 6- to 7-fold higher than that for inhibition of stimulated enzyme secretion (300 mM/45 mM). Since CCK_{33} and CCK_8 have similar potencies in their reaction with acini, we compared the effect of acetaldehyde on CCK_{33} radioligand binding to the effect of acetaldehyde on CCK_8-induced amylase secretion. The lack of correlation suggests that acetaldehyde inhibition of CCK_8-stimulated enzyme release from acini is not due to inhibition of CCK binding to receptors on acini.

Basal amylase secretion was not altered by 6–45 mM acetaldehyde, whereas a significant reduction in basal release was observed at 60, 100 and 300 mM. Although 600 mM and 1 M acetaldehyde did not significantly affect amylase secretion, compared to the control, the 2- to 10-fold increase in basal amylase secretion observed at 1.3 and 2 M is suggestive of acinar cell membrane damage leading to leakage of enzymes from the acini. The fact that acetaldehyde concentrations (6–45 mM), that do not significantly alter basal secretion, cause dose-dependent inhibition of CCK_8-stimulated amylase secretion indicated that acetaldehyde inhibition of stimulated secretion is (1) post-receptor mediated, and (2) on a process mediating enzyme release that is regulated by CCK.

The inhibitory effects of acetaldehyde on both binding and stimulated enzyme release were not reversible. This nonreversibility of inhibitory effects of acetaldehyde was not due to cell membrane damage. ^{51}Cr release from prelabeled cells (conversely ^{51}Cr retained by the cell) is an indicator of the viability of cells since significant release of ^{51}Cr into the incubation medium occurs in cells with damage to their membranes. No significant cell membrane damage was observed when acini were treated with acetaldehyde up to 600 mM; however, there was a significant release of ^{51}Cr at 1 M acetaldehyde. This indicated acinar cell membrane damage with resultant leakage of amylase from either stimulated or unstimulated acini.

Acetaldehyde has been shown to react with amino groups, to interact with tissue thiol groups, condense with catecholamines and to covalently bind proteins [6, 22–24]. Both CCK_{33} and CCK_8 contain methionine and functional free amino groups. In addition, CCK_{33} at its amino terminal possesses amino acid residues (lysine and arginine) which have reactive amino groups. It is conceivable that a chemical reaction in solution between acetaldehyde and CCK_{33} or CCK_8 could render these peptides biologically less effective in their binding and enzyme secretory processes in acini. If such were the mechanism of acetaldehyde action, then, it would be expected that the curve for inhibition of binding of $[^{125}I]CCK_{33}$ should be to the left of the inhibition curve for CCK_8-induced amylase secretion, or at least the curves should be identical. On the contrary, the curves obtained are the exact opposite, suggesting, thereby, that the chemical interaction between the peptide and acetaldehyde was not the cause for the inhibitory effects. The mechanism for the irreversible inhibition by acetaldehyde of CCK_8-stimulated enzyme secretion is not certain. It may be due, however, to the high reactivity of acetaldehyde with intracellular components that mediate enzyme secretion from acini. In this context it might be of interest to note that A23187, a Ca^{2+} ionophore, reverses the inhibition by ethanol (and not by acetaldehyde) of CCK_8-stimulated enzyme secretion from acini [25].

Acetaldehyde and other aldehydes, low molecular weight alcohols, phenols and other organic substances are present as congeners in a variety of alcoholic beverages [26, 27]. Ingestion of these substances produces biological effects similar to ethanol [28]. Social or habitual drinking is often accompanied by smoking [29, 30], and since tobacco smoke contains considerable amounts of acetaldehyde [31], this would even further increase the levels of circulating acetaldehyde. It is questionable whether the concentration of acetaldehyde in blood after ethanol

ingestion or smoking or both will even attain the lowest concentration of acetaldehyde employed in our *in vitro* study. It is clear, however, that an accurate determination of acetaldehyde levels in blood has been difficult [32, 33] and, consequently, the levels of acetaldehyde that pass through liver, kidneys, pancreas and the brain are not known. Furthermore, the demonstration by Gaines *et al.* [6] that acetaldehyde reacts irreversibly with proteins of human erythrocytes may suggest that the free blood acetaldehyde levels estimated *in vivo* are an underestimation of the amount of acetaldehyde released by liver after ethanol ingestion.

In vitro research on the deleterious effects of acetaldehyde has focussed principally on liver slices, liver mitochondria, and erythrocyte membrane proteins [6]. In this study we employed isolated acini, structural and functional units of exocrine pancreas, associated with enzyme secretory activity. Although acetaldehyde exhibits inhibitory effects similar to ethanol, it is sixteen times more potent than ethanol and its mechanism of action appears to be different. Although we could observe an inhibitory effect on CCK_8-induced amylase secretion at acetaldehyde concentrations of 3–6 mM, the half-maximal inhibitory concentration of 45 mM is high considering the reported 50 μM blood level of acetaldehyde in chronic alcoholics [34]. Whether a 45 mM concentration of acetaldehyde is lethal or not is debatable since ^{51}Cr-release studies indicate that acetaldehyde at concentrations employed in this study do not cause apparent cell damage (LDH measurements provided similar data). Our recent studies reveal that the acini are viable after 45 mM acetaldehyde treatment and that 45 mM acetaldehyde inhibition of 6 μM A23187 (a Ca^{2+} ionophore)-induced amylase secretion is reversible. Furthermore, acetaldehyde-treated acini after wash and resuspension in fresh buffer secreted amylase with the same efficacy as that of control acini on restimulation with 6 μM A23187 (unpublished data). The possible experimental approach to overcome the use of high concentrations of acetaldehyde will be to increase the exposure (incubation) time of acini to lower concentrations of acetaldehyde. Unfortunately, experiments employing isolated acini have to be carried out within a 2- to 3-hr incubation time span as otherwise problems in regard to viability of acini and functional and secretory changes in acini arise. Hence, experiments dealing with longer exposure time do not provide consistent data from which meaningful conclusions could be drawn. The data presented in this study lend support to the conclusion that acetaldehyde effects may be intracellularly mediated. Thus, acetaldehyde, as a congener in alcoholic beverages, as a principal ethanol metabolite or as a toxicant absorbed from the tobacco smoke, even in low concentrations, may be harmful to *in vivo* exocrine pancreas. Our data, however, show that isolated pancreatic acini are resistant to the potentially toxic pharmacological concentrations of acetaldehyde employed in the *in vitro* investigation.

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