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EFFECT OF ETHANOL ON CHOLECYSTOKININ-INDUCED ENZYME SECRETION FROM ISOLATED RAT PANCREATIC ACINI*

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Abstract—Cholecystokinin octapeptide (CCK_8)-stimulated amylase release in isolated rat pancreatic acini was inhibited over 30% by 600 mM ethanol. The configuration of the dose–response curve for CCK_8, however, in the presence of ethanol was similar to that of the control. Amylase release elicited by maximal concentrations of CCK_8 (300 pM) was inhibited by increasing concentrations of ethanol (0.3 to 1.3 M), and this inhibition was concentration dependent. In addition, the binding of [^{125}I]CCK_{33} to specific membrane receptors on acini was inhibited by ethanol in a dose-dependent manner. A positive correlation between the inhibitory effects of ethanol on CCK binding and CCK-induced amylase release was observed. Furthermore, these inhibitory effects of ethanol were reversible. Basal amylase release, however, was increased 20–50% by ethanol between the concentrations of 0.3 and 1.3 M; higher concentrations caused a leakage of amylase from the acini both in the absence and presence of 300 pM CCK_8. This is confirmed by ^{51}Cr release from prelabeled acini which revealed no significant damage to acinar cell membrane between 0.3 and 1.6 M ethanol, but significant damage to acini at higher concentrations. These data suggest that the 600 mM ethanol-induced inhibition of CCK action in acini is due to reversible perturbation of the acinar cell membrane.

Ethanol is metabolized to acetaldehyde and other metabolites by the liver and other tissues [1–3]. Certain toxic effects of ethanol have been attributed to its major metabolite, acetaldehyde. In the pancreas it is difficult to ascertain whether the effect of ethanol is direct or via its metabolites, due mainly to the lack of conclusive evidence for the metabolism of ethanol by the pancreas [4–6]. In studies employing isolated pancreatic preparations, Steer *et al.* [7] and Solomon *et al.* [8] could not demonstrate the direct effects of ethanol on pancreatic exocrine cells responsible for enzyme secretion, whereas Iwatsuki and Chiba [9] reported the direct inhibitory effect of ethanol on perfused canine pancreas. An investigation of the effects of ethanol on an *in vitro* pancreatic preparation, in the presence and absence of the pancreatic secretagogue cholecystokinin (CCK), will facilitate our understanding of the direct effects of ethanol in this organ.

In this regard it will be of interest to note the report of Uhlemann *et al.* [10] who concluded that the ethanol-induced inhibition of CCK-stimulated enzyme secretion was on the exocytotic process or at some level not involving cyclic nucleotides. As these authors had not studied the effects of ethanol on radioiodinated CCK binding to its specific receptors on acinar cell membrane, the possibility of ethanol action on the binding process could not be ruled out. The aim of the present study was to investigate the primary mechanism by which ethanol inhibited CCK-induced enzyme secretion from acini.

We employed isolated rat pancreatic acini to determine the direct effects of ethanol on basal and CCK-induced enzyme secretion from the acini. To further elucidate the action of ethanol on CCK-induced enzyme secretion, we also studied the effect of ethanol on CCK binding to its specific membrane receptors on isolated acini.

EXPERIMENTAL PROCEDURES

All materials employed to prepare isolated acini and to radioiodinate CCK_{33} were obtained from sources previously described for similar studies [11, 12]. ^{51}Cr-sodium chromate (200–500 Ci/g chromium) was obtained from the New England Nuclear Corp., Boston, MA. CCK_8 was a gift from Dr. M. Ondetti, Squibb Institute for Medical Research, Princeton, NJ. CCK_{33} was obtained from Dr. V. Mutt, Karolinska Institute, Stockholm, Sweden. Since the two CCKs possess the same biological potency in binding and in releasing amylase from acini, CCK_{33} was used only for radioiodination and CCK_8 was used for amylase release and for the determination of nonspecific binding of tracer CCK_{33}. Krebs–Henseleit bicarbonate buffer (KHB) and *N*-2-hydroxyethyl-piperazine-*N'*-2-ethanesulfonic acid (HEPES)-buffered Ringer (HR) containing essential amino acid supplement and

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0.01% soybean trypsin inhibitor were prepared as previously described [11].

Isolated pancreatic acini were prepared by the enzymatic digestion of the pancreas from female Wistar rats (150 g) fasted for 18 hr, as described by Williams *et al.* [11]. After 30 min of preincubation, the acini were stimulated with various concentrations of CCK_8 both in the absence and presence of ethanol, and the amylase released into the medium after 30 min at 37° was measured by the method of Jung [13] 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.* [14] 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.* [12]. 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 total binding. The effect of ethanol 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 ethanol 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.* [15]. Radioactive sodium chromate (5 μCi ^{51}Cr) was incubated with 5 ml of acini suspension for 1.5 hr at 37°. The suspension was then centrifuged, washed, and resuspended in the required volume of HR, and 1-ml aliquots were incubated with various concentrations of ethanol. Total membrane disruption was determined by resuspending acini in 1 ml of distilled water. After 30 min of incubation at 37°, an 800-μl aliquot was microcentrifuged, and 500 μl of supernatant fraction was saved. The pellet was processed as described in binding studies, and the radioactivity in the pellet and supernatant fraction was determined in a gamma counter. The radioactivity in the pellet is expressed as a fraction of the total radioactivity in the pellet and supernatant fraction. Apparent cell viability is indicated by the ability of the cell to retain ^{51}Cr. Non-viable cells release significantly more ^{51}Cr into the incubation medium.

Reversibility of ethanol effect. Two 8-ml acini suspensions were incubated with 300 pM CCK_8 in the absence and presence of 600 mM ethanol. At 30 min (first incubation), two 1-ml aliquots from each set were removed for amylase assay. Each of the remaining 6 ml of acini suspension 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 ethanol and without CCK_8 were the controls.

To study the reversibility of ethanol inhibition of CCK binding, two sets of acini (in the absence and presence of 600 mM ethanol) were incubated for 30 min, centrifuged, washed and resuspended in an identical volume 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, two 0.5 ml aliquots were removed, and the pellet was 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. Ethanol at 1.6 M and lower 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 1.6 M ethanol was significant. In comparison, the label release from acini in the presence of 3 M ethanol approached that of acini resuspended in distilled water (Table 1).

Ethanol on amylase release. In rat pancreatic acini, 30 pM CCK_8 caused a significant increase in amylase release; the stimulated response reached a peak at 300 pM (Fig. 1). Further increases in CCK_8 concentration resulted in a progressive fall in amylase

Table 1. Measurement of cell membrane damage in acini*

Ethanol (M)	^{51}Cr retained in cell	Significance
0	0.95 ± 0.005	
0.1	0.95 ± 0.004	NS†
0.3	0.94 ± 0.005	NS
0.6	0.92 ± 0.008	NS
1.0	0.91 ± 0.003	NS
1.3	0.91 ± 0.003	NS
1.6	0.86 ± 0.010	NS
2.0	0.47 ± 0.013	$P < 0.02$
3.0	0.42 ± 0.012	$P < 0.02$
Water	0.13 ± 0.020	$P < 0.01$

* Isolated rat pancreatic acini were incubated with ^{51}Cr for 90 min at 37°. Labeled acini were centrifuged, washed twice, and resuspended in HR. Aliquots (1 ml) of prelabeled acini were incubated in the presence of increasing concentrations of ethanol at 37° and ^{51}Cr release was followed over 30 min. ^{51}Cr retained by acini in the presence of various concentrations of ethanol is calculated as a fraction of ^{51}Cr retained by acini at the start of a 30-min incubation. At 0 time, greater than 99% of ^{51}Cr is retained by the cell and this value is designated 1. Acini spontaneously release 5% of ^{51}Cr over 30 min. Values are means ± S.E. of eight experiments.

† Not significant.

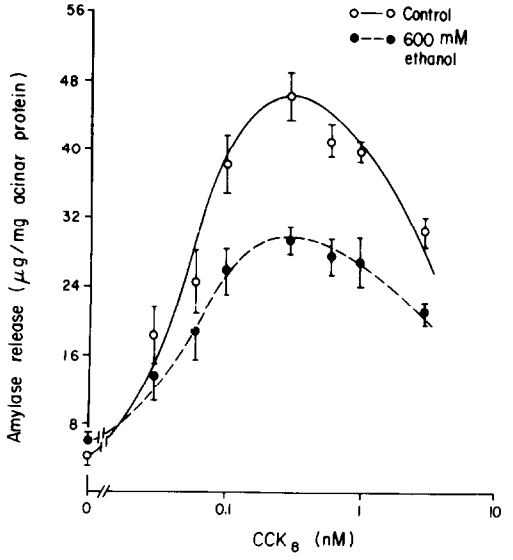

Fig. 1. Concentration-dependence of CCK_8-stimulated amylase release from isolated rat pancreatic acini in the presence and absence of ethanol. Amylase release from acini over 30 min at 37° is plotted as a function of CCK_8 (octapeptide of CCK) concentration in the medium. Each point is the mean ± S.E.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

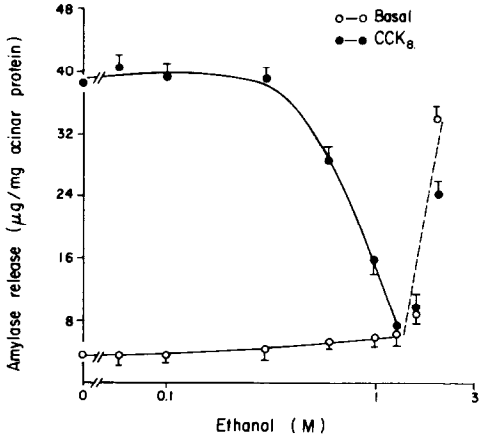

Fig. 2. Effect of ethanol on CCK_8 (300 pM)-induced amylase release from isolated rat pancreatic acini. Amylase release from acini over 30 min at 37° is plotted as a function of ethanol concentration in the medium. Each point is the mean ± S.E. of six experiments. The broken line indicates the enhanced release of amylase, both in the presence and absence of 300 pM CCK_8, due mainly to cell membrane damage.

release. In the presence of 600 mM ethanol, basal amylase release was increased by approximately 50%, whereas CCK_8-stimulated response was diminished significantly. The dose–response curve, in the presence or absence of ethanol, exhibited the characteristic biphasic shape with a peak at 300 pM CCK_8. The reduction in the magnitude of amylase release without a parallel rightward shift in the dose–response curve is suggestive of non-competitive type of inhibition.

Ethanol between 300 mM and 1.3 M concentrations produced a progressive increase in the basal release of amylase [amylase expressed in μg/mg acinar protein (mean ± S.E.) in the absence and presence of 300 mM, 600 mM, 1, 1.3, and 1.6 M, respectively, was 3.3 ± 0.6, 4.4 ± 0.3, 5.2 ± 0.7, 5.9 ± 0.8 and 6.3 ± 1.1. Amylase values for 600 mM to 1.6 M ethanol were significantly different (P values ranged from 0.05 to 0.01) from amylase value obtained in the absence of ethanol]. Identical concentrations of ethanol produced an inhibition of 300 pM CCK_8 (maximal dose)-induced amylase release from acini (Fig. 2). This inhibition by ethanol was half-maximal at 760 mM and maximal at 1.3 M. Further increases in ethanol concentration caused a pronounced increase in basal amylase release. This ethanol-augmented amylase release observed both in the absence and presence of 300 pM CCK_8 (Fig. 2) presumably is due to significant acinar cell membrane damage which is characterized by the leakage of the enzymes (compare Table 1 and Fig. 2).

Ethanol on binding of CCK to acini. CCK binding to the acinar cell membrane correlates with CCK-induced amylase release from the acini [16]. In the present study, increasing concentrations of ethanol (between 300 mM and 1.6 M) caused a progressive fall in the binding of $[^{125}I]CCK_{33}$ to its specific receptors on acini. There was no significant change in the binding of $[^{125}I]CCK_{33}$ in the presence of 30–100 mM ethanol. The apparent ethanol concentration for half-maximal inhibition of $[^{125}I]CCK_{33}$ binding was 600 mM. This concentration required for half-maximal inhibition of binding of CCK_{33} correlates with the concentration of ethanol (760 mM) required for half-maximal inhibition of amylase release (compare Figs. 2 and 3).

Reversibility of ethanol inhibition. Ethanol, 600 mM, inhibited 300 pM CCK_8-induced amylase release from acini by 30% after a 30-min incubation (Table 2). At the end of the second incubation, the ethanol control and basal amylase values were com-

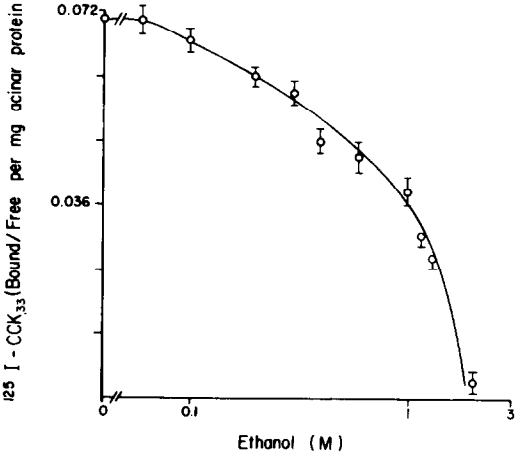

Fig. 3. Effect of ethanol on binding of $[^{125}I]CCK_{33}$ to receptors on isolated rat pancreatic acini. Effect of increasing concentrations of ethanol on $[^{125}I]CCK_{33}$ binding to membrane receptors on acini is represented. Each point is the mean ± S.E. of seven experiments.

Table 2. Reversibility of ethanol inhibition of amylase release and of [^{125}I]CCK$_{33}$ binding in isolated rat pancreatic acini*

	(1) No ethanol	(2) 600 mM Ethanol	(1 and 2) Significance	(3) 600 mM Ethanol and wash	(2 and 3) Significance
Basal amylase release (as %)	100	144 ± 11	$P < 0.02$	87 ± 7	$P < 0.01$
300 pM CCK$_8$-induced amylase release (as %)	529 ± 20	368 ± 29	$P < 0.005$	509 ± 53	$P < 0.05$
[^{125}I]CCK$_{33}$ specifically bound (% of maximum)	100	51 ± 4	$P < 0.02$	95 ± 10	$P < 0.02$

* Basal and CCK$_8$-induced amylase release over 30 min at 37° was measured. To determine the reversibility of ethanol effect (600 mM ethanol and wash), both basal and CCK$_8$-induced acini in the presence of 600 mM ethanol were centrifuged, washed, and reincubated for a further 30 min. At this time acini were centrifuged, washed, resuspended in fresh HR, and incubated in the absence and presence of 300 pM CCK$_8$ for 30 min, and the amylase release was measured. Values are means ± S.E. of six experiments. [^{125}I]CCK binding in the absence and presence of ethanol over 30 min was determined. Another set of binding experiments without the addition of tracer, but in the presence of ethanol, was allowed to proceed for 1 hr with centrifugation and wash procedures at 30 and 60 min. At 60 min acini were pulsed with [^{125}I]CCK, and B/F was determined after a further 30-min period of incubation. Appropriate nonspecific binding was determined in the presence of 10^{-6} M CCK$_8$. Values are means ± S.E. of five experiments. For details refer to Materials and Methods.

parable to the first 30 min amylase levels. CCK$_8$ and CCK$_8$ plus ethanol-treated acini showed identical residual stimulation, suggesting the reversal of ethanol inhibition. This is further documented by the third incubation where the two groups of acini (CCK$_8$ and CCK$_8$ plus ethanol-treated) showed similar increases in amylase release to restimulation with 300 pM CCK$_8$. Similar data were obtained in reversibility studies on ethanol inhibition of [^{125}I]CCK$_{33}$. Acini treated with 600 mM ethanol and incubated for 30 min, compared to untreated acini, showed 30–40% inhibition of binding. However, acini treated with and without 600 mM ethanol and incubated for 30 min, washed and reincubated with [^{125}I]CCK$_{33}$ and with no ethanol added, showed identical binding (Table 2).

DISCUSSION

The specific binding of radioiodinated CCK to receptors on isolated pancreatic acini [12, 16–20] and on brain cortical particulate preparations [21–24] has been well documented. Further work has demonstrated the correlation between CCK binding to receptors and CCK-stimulated biological actions in isolated acini [18]. The presence of CCK silver grains, determined by autoradiography, on the basolateral plasma membrane of acini has been demonstrated at 30 min [25], the time point employed for CCK binding–biological function correlation studies.

The data presented here provide evidence for the inhibition by ethanol of both CCK binding and CCK-stimulated enzyme release from isolated rat pancreatic acini. These inhibitory effects were concentration dependent and were reversible. The inhibitory effect of ethanol on CCK binding to acinar membrane receptors correlated with the inhibition of CCK-stimulated amylase release from acini. The inhibition by ethanol of CCK binding was observed at lower concentrations (60–100 mM) compared to ethanol inhibition of CCK-induced amylase release (100–300 mM). Since CCK binding is associated with CCK-stimulated biological actions in acini [18], the inhibition by ethanol of CCK binding to its specific membrane receptors may be the cause of the inhibition of CCK-induced amylase release from acini. Uhlemann *et al.* [10] have reported the action of ethanol, higher alcohols, and branched chain alcohols on adenylate cyclase activity and cellular cyclic AMP levels in isolated pancreatic acinar cells. Straight chain alcohols with fewer than seven carbon atoms potentiated secretin and vasoactive intestinal peptide (VIP)-induced adenylate cyclase activity and cellular cyclic AMP levels. At sufficiently high concentrations, straight chain alcohols with more than two carbon atoms inhibited secretin and VIP-induced cyclase activity and cellular cyclic AMP. In the case of VIP, ethanol inhibited VIP-induced amylase secretion (although it potentiated VIP-induced cyclase activity and cellular cyclic AMP) without altering the binding of [^{125}I]VIP to acinar cells [10]. These authors showed that ethanol inhibited secretin, CCK$_8$ and VIP-induced amylase release from pancreatic acinar cells. They also observed potentiation by ethanol of cyclic nucleotide levels induced by the above peptides. Since an increase in cyclic AMP level is associated with an increase in enzyme release, the authors suggested that this paradoxical dual effect of ethanol (increase in cAMP levels and inhibition of enzyme release) may be due to ethanol action on either the exocytotic process or at some level not involving cyclic nucleotides [10]. In our study we have shown the inhibition by ethanol of both [^{125}I]CCK$_{33}$ binding to, and CCK$_8$-induced amylase release from, acini. Furthermore, since the inhibition of CCK binding was observed at lower concentrations of ethanol than those required to

inhibit CCK_8-induced amylase release, it is most apparent that inhibition of binding is the primary event in ethanol inhibition of CCK-induced actions in acini.

Inhibition of CCK action by dibutyryl cyclic GMP [26] and by 27–32 amide of CCK [27] in isolated pancreatic acinar cells has been described as competitive antagonism since these antagonists cause a parallel rightward shift in the dose–response curve for the agonist (CCK) on enzyme secretion. In our study we observed that ethanol did not shift the dose–response curve for CCK on acinar amylase release but reduced the magnitude of CCK-induced amylase release. This suggests the ethanol-induced inhibition of CCK action to be non-competitive. Our findings reveal that CCK_8-stimulated amylase release is inhibited by ethanol concentrations between 100 mM and 1 M. This inhibition was not due to an apparent cell membrane damage, as is illustrated by ^{51}Cr studies in which no significant increase in ^{51}Cr release was observed. Ethanol, however, at concentrations >1.3 M caused a profound increase in amylase release both in the absence (basal) and presence of 300 pM CCK_8 (stimulated). This enhanced amylase release presumably is due to leakage of the enzyme from the acini, consequent to acinar cell membrane damage by ethanol. This inference is supported by ^{51}Cr studies in which ethanol at >1.3 M concentrations caused over 50% loss of ^{51}Cr from prelabeled acini. Although other membrane and intracellular effects of ethanol, such as increases in cAMP and adenylate cyclase levels [10], at concentrations lower than that causing cell membrane disruption (<1.3 M) in acini cannot be discounted, our studies show a direct inhibitory effect of ethanol on CCK binding to pancreatic acini. The data also suggest that the inhibitory effects of ethanol on CCK action in acini are direct because: (1) there is a lack of evidence for ethanol metabolism by the pancreas, (2) there is a positive correlation between the inhibitory effects of ethanol on CCK binding to, and CCK-induced amylase release from, the acini, the former preceding the latter, and (3) the inhibitory effects of ethanol on CCK binding and CCK-stimulated enzyme secretion are reversible.

Ethanol has been reported to affect protein and lipid components of biological membranes [6]. The inhibition by ethanol of both basal and insulin-stimulated alpha amino isobutyric acid uptake in cultured hepatocytes was suggested to be a physical effect, probably reflecting alterations in membrane fluidity caused by ethanol [6]. The authors did not exclude the possibility of ethanol-induced changes in the conformation of protein receptors. These ethanol-induced membrane alterations in hepatocytes might provide an explanation for the ethanol inhibition of CCK binding, observed in our study, to its specific receptors on acini.

In conclusion, our data indicate that ethanol inhibited the action of CCK on acini, at least in part, by inhibiting CCK binding. This decrease in hormone binding may be the result of alterations in the acinar cell membrane caused by ethanol. It is not practicable to determine the concentration of ethanol that bathes pancreatic acinar cells. At best, a comparison could be made between the levels of ethanol attainable in chronic alcoholics and the concentrations of ethanol employed in the present study. Ethanol levels of 80–100 mM are attainable in chronic alcoholics [6]. Although concentrations of 100, 300 and 600 mM ethanol may be toxic in an *in vivo* system, ethanol at the concentration employed (600 mM) in the present study to correlate inhibition of CCK binding to the inhibition of CCK-induced enzyme release in isolated acini does not appear to be toxic since both the inhibitory effects were totally reversible.

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