Ethanol differentially regulates NF-κB activation in pancreatic acinar cells through calcium and protein kinase C pathways

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Although alcohol abuse is the most common cause of acute and chronic pancreatitis, the mechanism of alcohol’s effect on the pancreas is unknown (39). Research into the mechanism has been hampered by the fact that feeding ethanol to animals results in little or no pancreatic damage (23, 24, 31, 41). This lack of effect of ethanol feeding alone suggests that the effect of ethanol may be due to its ability to sensitize the pancreas to injurious actions of other agents. Considering this possibility, we recently reported (31) that ethanol feeding sensitized rats to pancreatitis caused by a low-dose CCK-8 infusion. That is, this low dose of CCK-8 caused acute pancreatitis in rats fed an ethanol diet by continuous intragastric infusion for 6 wk, whereas the same dose of CCK-8 did not cause pancreatitis in animals receiving control diet.
Ca\(^{2+}\) and PKC pathways in ethanol’s effects on NF-\(\kappa\)B activation in pancreas have not been addressed.

The mechanisms through which Ca\(^{2+}\) and PKC regulate NF-\(\kappa\)B in other systems are tissue and stimulus specific. For example, Ca\(^{2+}\) can regulate NF-\(\kappa\)B through Ca\(^{2+}\)-dependent protein phosphatase 2B (calcineurin) (9, 46, 49) or through Ca\(^{2+}\)-dependent (“conventional”) isoforms of PKC (12). Ca\(^{2+}\)-dependent (“novel” and “atypical”) PKC isoforms were also shown to mediate NF-\(\kappa\)B activation in several cell types (7, 25, 34). The PKC isoforms that mediate NF-\(\kappa\)B activation in the pancreatic acinar cell have not been determined. Of note, several PKC isoforms, including conventional \(\alpha\) and \(\gamma\), novel \(\delta\) and \(\epsilon\), and atypical \(\zeta\), were detected in pancreatic acinar cells (4, 33).

In the present study, we applied a model of isolated pancreatic acini to investigate in vitro the effect of ethanol on CCK-induced NF-\(\kappa\)B activation. We found that the in vitro ethanol treatment had similar effects to those that we observed in ethanol-fed rats, namely, augmentation of the CCK-8-induced activation of NF-\(\kappa\)B and attenuation of the basal NF-\(\kappa\)B activity in acinar cells. Ethanol prevented NF-\(\kappa\)B activation induced by thapsigargin, an agent that directly mobilizes the intracellular Ca\(^{2+}\). Our results indicate that the inhibitory effect of ethanol on NF-\(\kappa\)B is likely mediated through the Ca\(^{2+}\)/calcineurin pathway and that the augmentation effect is mediated through PKC signaling pathways. Furthermore, the results indicate that the nonconventional Ca\(^{2+}\)-independent isoforms of PKC mediate NF-\(\kappa\)B activation in pancreatic acinar cells. These divergent effects of ethanol on NF-\(\kappa\)B activation may play a role in the ability of ethanol to sensitize pancreas to the inflammatory response and pancreatitis.

**MATERIALS AND METHODS**

Preparation of dispersed pancreatic acini. Pancreatic acini were isolated using a collagenase digestion method as we described previously (6, 15, 16, 18, 19, 53) from the pancreas of Sprague-Dawley rats (75–100 g). Isolated acini were incubated for 3 h at 37°C in medium 199 containing 0.01% soybean trypsin inhibitor, with or without ethanol, and then treated for 30 min with the indicated concentrations of CCK-8, thapsigargin, or PMA.

Measurement of free cytosolic Ca\(^{2+}\) concentration. Cytosolic Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]\(_{i}\)) was monitored by the change in the fluorescence intensity of fura-2-loaded cells, as we described previously (32). Acini were incubated for 3 h at 37°C with or without 100 mM ethanol and for the last 30 min with 2 \(\mu\)M fura-2 AM. Cells were then washed twice by centrifugation and resuspended in a buffer containing (in mM) 20 HEPES (pH 7.4), 120 NaCl, 5 KCl, 1 MgCl\(_2\), 1 CaCl\(_2\), 10 glucose, 10 sodium pyruvate, and 10 ascorbic acid with 0.1% BSA and 0.01% soybean trypsin inhibitor, with or without ethanol. Ca\(^{2+}\) responses to CCK-8 or thapsigargin were measured at 37°C by monitoring fura-2 fluorescence in a Shimadzu RF-1501 spectrofluorimeter with excitation at 340 and 380 nm and emission at 510 nm.

Preparation of protein extracts. Nuclear and cytosolic protein extracts were prepared as we described previously (6, 16, 18, 19, 31, 48, 53). Briefly, pancreatic acini were lysed on ice in a hypotonic buffer A (16) supplemented with 1 mM PMSF, 1 mM DTT, and protease inhibitor cocktail containing 5 \(\mu\)g/ml each of pepstatin, leupeptin, chymostatin, antipain, and aprotinin. Cells were left to swell on ice for 20–25 min, then 0.3% Igepal CA-630 was added, and the nuclei were collected by microcentrifugation. The supernatant (cytosolic protein) was saved for Western blot analysis of I\(\kappa\)B, and the nuclear pellet was resuspended and incubated at 4°C for up to 1 h in a high-salt buffer C (16) supplemented with 1 mM PMSF, 1 mM DTT, and the protease inhibitor cocktail described above. Membrane debris were pelleted by microcentrifugation for 10 min, and the clear supernatant (nuclear extract) was aliquoted and stored at −80°C.

**RESULTS**

Effects of ethanol on CCK-8-induced NF-\(\kappa\)B activation. Our first aim in the present study was to establish an in vitro model using isolated rat pancreatic acini to determine the effects of ethanol on CCK-induced NF-\(\kappa\)B activation. We incubated isolated rat pancreatic acini at 37°C for 3 or 5 h with and without 50 or 100 mM ethanol and then stimulated them with either 0.1 nM CCK-8, which causes maximal digestive enzyme secretion, or 100 nM CCK-8, which is supramaximal for enzyme secretion and causes inhibition of enzyme secretion (15, 50). By measuring LDH release, we tested that neither ethanol nor the combination of ethanol and CCK-8 decreased cell viability (data not shown). Also, we previously reported (40) that such treatment with ethanol did not affect CCK-induced amylase secretion in pancreatic acinar cells.
In acini treated with ethanol, 0.1 nM CCK-8 induced NF-κB binding activity, whereas it did not activate NF-κB in acini not treated with ethanol (Fig. 1, A and B). Furthermore, in ethanol-treated acini, 100 nM CCK-8 produced greater NF-κB activation than without ethanol (Fig. 1, B and C). Thus ethanol potentiates NF-κB activation caused by both maximal and supramaximal doses of CCK-8. The effect of ethanol on CCK-induced NF-κB activation was dose and time dependent. At 3 h incubation, the sensitization was only observed with 100 mM but not with 50 mM ethanol. However, the sensitizing effect of 50 mM ethanol was detected at 5 h incubation with ethanol (Fig. 1C).

Figure 1, A and B, also shows that ethanol treatment decreased basal NF-κB binding activity by ~30%. Thus ethanol had two effects on NF-κB in pancreatic acinar cells: it decreased the basal NF-κB activity and potentiated the CCK-8-induced NF-κB activation. As a result, in ethanol-treated cells, the NF-κB response to 100 nM CCK-8 was two times greater than in cells not treated with ethanol (Fig. 1C).

The effects of ethanol on NF-κB in isolated pancreatic acini are similar to those we found in vivo in an experimental model of ethanol + CCK pancreatitis (31). Although the conditions of the in vivo and in vitro applications of ethanol are different, in both models ethanol potentiates CCK-8-induced NF-κB activation while having an inhibitory effect on NF-κB basal activity. Of note, in our in vivo model, alcohol concentration in the blood of rats continuously fed ethanol is in the range of 20–120 mM (1, 3, 31, 47).

Ethanol was reported to affect osmotic balance of the incubation medium (2, 27). To test whether the observed effects of ethanol were caused by changes in osmolarity, we measured the effects of hyperosmolarity on the CCK-8-induced NF-κB activation. Pancreatic acini were incubated for 3 h in the presence or absence of 100 mM mannitol and then stimulated for 30 min with 100 nM CCK-8. In the presence of mannitol, CCK-8 increased NF-κB binding activity 1.5 ± 0.2-fold, and in the absence of mannitol increased it 1.7 ± 0.2-fold (n = 3), the responses being statistically not different. These results suggest that changes in osmolarity are not a major factor in the effect of ethanol on NF-κB activation in pancreatic acinar cells.

We and others (18, 20, 45) reported that CCK-8-induced NF-κB activation in the pancreatic acinar cell is associated with degradation of the inhibitory IκB proteins. Figure 1D shows that incubation with ethanol potentiated the CCK-8-induced degradation of IκBα. In particular, 0.1 nM CCK-8 stimulated IκBα degradation in ethanol-treated but not in control acini, and 100 nM CCK-8 produced a greater degra-

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**Fig. 1.** Ethanol augments the CCK-8-induced but attenuates the basal NF-κB binding activity and IκBα degradation in pancreatic acinar cells. Dispersed rat pancreatic acini were incubated for 3 h (A, B, D) or indicated time (C) with and without 50 (C) or 100 mM (A–D) ethanol. Cells were then stimulated for 30 min with 0.1 or 100 nM CCK-8 (or vehicle). A: NF-κB binding activity was measured in nuclear extracts by EMSA. Representative of at least 4 independent experiments on different acinar preparations. B, C: NF-κB band intensities were quantified in the PhosphorImager. In B, the intensity of the NF-κB band was normalized on that in untreated acini. In C, the NF-κB response to 100 nM CCK-8 in the absence of ethanol was considered as 100%. Values are means ± SE (n = 4–23). *P < 0.05 compared with untreated acini; #P < 0.05 compared with acini treated with the same dose of CCK-8 in the absence of ethanol (paired t-test); ∼P < 0.05 compared with acini treated with ethanol alone. D: IκBα degradation was measured in cytosolic extracts by Western blot analysis. Numbers on the bottom show the intensity of the IκBα band quantified by densitometry and normalized on that in untreated cells. Representative of 3 independent experiments. EtOH, ethanol.
dation of IκBα in acini treated with ethanol than in control acini. The greater degradation of IκBα in cells treated with CCK-8 in the presence of ethanol (Fig. 1D) correlates with the increased NF-κB binding activity in these preparations (Fig. 1, A–C). We also observed increased basal levels of IκBα (Fig. 1D) in acini treated with ethanol, correlating with the diminished basal NF-κB binding activity (Fig. 1A).

Effects of ethanol on NF-κB activation induced by Ca2+ mobilization. As stated in the introduction, previous studies (21, 45) have demonstrated that Ca2+ mobilization mediates, in part, the effect of CCK-8 on NF-κB activation. To determine whether ethanol regulates NF-κB binding activity through effects on the Ca2+-mobilization pathway, we measured the effects of ethanol on NF-κB activation induced by thapsigargin, an agent that directly causes intracellular Ca2+ mobilization bypassing the receptor. Figure 2 shows that thapsigargin stimulated NF-κB activation in acini incubated for 3 h in the absence of ethanol. The stimulation of NF-κB binding activity by thapsigargin was ~1.5-fold compared with a twofold increase with 100 nM CCK-8. Previously, thapsigargin has been reported to induce NF-κB activation in freshly isolated rat pancreatic acini (21) or lobules (45).

Pretreatment with ethanol greatly inhibited both the thapsigargin-induced NF-κB activation (Fig. 2, A and B) and IκBα degradation (Fig. 2C). Thus ethanol produced opposite effects on the CCK-8- and thapsigargin-induced NF-κB activation in pancreatic acini: ethanol potentiated the former but inhibited the latter response (Figs. 1 and 2). These results show that the augmenting effect of ethanol on CCK-8-induced NF-κB activation cannot be through ethanol’s effect on the Ca2+ mobilization pathway alone.

To further elucidate the contribution of Ca2+ mobilization to the NF-κB responses, we applied BAPTA-AM, an intracellular Ca2+ chelator. In separate experiments, we tested that 40 μM BAPTA-AM prevented both the CCK-8- and thapsigargin-induced [Ca2+]i responses in pancreatic acini incubated for 3 h (not illustrated). The results in Fig. 3 show that BAPTA partially inhibited NF-κB activation induced by CCK-8 and almost completely inhibited NF-κB activation induced by thapsigargin. The extent of the inhibitory effect of BAPTA on the CCK-8-induced NF-κB activation was the same in cells treated and not treated with ethanol (not illustrated). These results indicate that Ca2+ mobilization contributes to both the CCK- and thapsigargin-induced NF-κB activation. However, as distinct from thapsigargin, CCK-8-induced NF-κB activation is also mediated through Ca2+-independent mechanisms.

To test the possibility that ethanol could affect Ca2+-dependent pathways of NF-κB activation by directly affecting Ca2+ signals, we measured the effects of ethanol treatment on [Ca2+], changes induced by CCK-8 and thapsigargin in pancreatic acini. The [Ca2+]i responses to both 0.1 and 100 nM CCK-8 are composed of the initial peak, resulting from intracellular Ca2+ mobilization, followed by an elevated [Ca2+]i plateau, resulting from Ca2+ influx (32, 50). The [Ca2+]i response to thapsigargin also shows the peak-and-plateau pattern (26, 52), but the peak is less abrupt and smaller in magnitude than in the CCK-induced [Ca2+]i response. The data in Table 1 show that ethanol had no significant effects on either the peak or plateau of the CCK-8-induced [Ca2+]i, response. The [Ca2+]i response to thapsigargin was also not significantly affected by ethanol. Ethanol did not affect the basal [Ca2+]i level in rat pancreatic acini (Table 1).

These data indicate that ethanol affects Ca2+-dependent pathways of NF-κB activation by targeting signals downstream of Ca2+ mobilization. Indeed, under conditions used, ethanol had no effect on the thapsigargin- or CCK-8-induced [Ca2+]i signals, whereas it affected the NF-κB responses.

One mediator that can link NF-κB activation and intracellular Ca2+ mobilization is the Ca2+-dependent phosphatase calcineurin (9, 21, 45, 46, 49). Calcineurin inhibitors, cyclosporin A and FK506, have been shown to inhibit amylase secretion by both CCK-8 and thapsigargin (14). We found that cyclosporin A and FK506 markedly inhibited NF-κB response induced by thapsigargin in pancreatic acini (Fig. 4A), the extent

Fig. 2. Ethanol prevents NF-κB activation induced by thapsigargin (TG) in pancreatic acinar cells. Pancreatic acini were incubated for 3 h with and without 100 mM ethanol and then stimulated for 30 min with 10 μM TG or vehicle. A: NF-κB binding activity was measured in nuclear extracts by EMSA (representative of 9 independent experiments). B: NF-κB band intensities were quantified in the PhosphorImager and normalized on the intensity of the NF-κB band in untreated acini. NF-κB response to TG in the absence of ethanol was considered as 100%. TG (10 μM) in the absence of ethanol increased NF-κB binding activity 1.5 ± 0.2-fold (n = 9). Values are means ± SE (n = 9); *P < 0.05 compared with the NF-κB response to TG in the absence of ethanol. C: IκBα degradation was measured in cytosolic extracts by Western blot analysis (representative of 3 independent experiments). Numbers on the bottom show the intensity of the IκBα band quantified by densitometry and normalized on that in untreated cells. Representative of 3 independent experiments.

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of their effect being similar to that of BAPTA (Fig. 3). These results suggest that thapsigargin-induced NF-κB activation is predominantly mediated by calcineurin. To further demonstrate that thapsigargin activates calcineurin in pancreatic acinar cells, we measured the effect of thapsigargin on the transcription factor nuclear factor of activated T cells (NFAT), a downstream target of calcineurin (9). Thapsigargin stimulated NFAT binding activity, which was prevented by both cyclosporin A and FK506 (Fig. 4). These data indicate that thapsigargin was engaged in the CCK-8-induced NF-κB activation in rat pancreatic acinar cells. For this purpose, we applied both broad-spectrum (GF-109203X and Ro-32-0432) PKC inhibitors and an inhibitor of the conventional Ca2+-dependent PKC isoforms, Go-6976 (28, 29). We tested that under conditions used in our experiments, these inhibitors prevented activation of their target PKC isoforms in rat pancreatic acini (37).

Table 1. Effects of cell pretreatment with ethanol on [Ca2+]i responses to CCK-8 and thapsigargin in rat pancreatic acinar cells

<table>
<thead>
<tr>
<th>Basal [Ca2+]i, nM</th>
<th>0.1 nM CCK-8</th>
<th>100 nM CCK-8</th>
<th>10 μM Thapsigargin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Peak</td>
<td>Plateau</td>
<td>Peak</td>
</tr>
<tr>
<td>Control</td>
<td>105±7</td>
<td>5.8±0.2</td>
<td>2.9±0.1</td>
</tr>
<tr>
<td>EtOH</td>
<td>103±5</td>
<td>5.4±0.3</td>
<td>3.1±0.3</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 4. Pancreatic acini were incubated for 3 h without (control) or with 100 μM ethanol and for the last 30 min with 2 μM fura-2 AM. Then cells were washed, resuspended in a buffer with or without 100 μM ethanol (EtOH), transferred into the cuvette, and stimulated with CCK-8 or thapsigargin. Free cytosolic Ca2+ concentration ([Ca2+]i) was determined at the maximum of the peak and at the plateau (at 100 s after CCK-8 and 220 s after thapsigargin addition).
and without ethanol (Fig. 6). Also, Go-6976 did not inhibit the CCK-8-induced IκB degradation (Fig. 7). These results indicate that the conventional Ca\(^{2+}\)-dependent PKC isoforms do not mediate the CCK-8-induced NF-κB activation in rat pancreatic acinar cells.

To further explore the role of PKC in NF-κB activation in ethanol-treated pancreatic acinar cells, we used PMA, a general activator of PKC. PMA activates both Ca\(^{2+}\)-dependent and -independent PKC isoforms in rat pancreatic acini (33). Figure 8 shows that cell treatment with ethanol potentiated PMA-induced NF-κB activation in pancreatic acini, providing further evidence that PKC mediates ethanol’s effects on NF-κB. We next examined the effects of PKC inhibitors on PMA-induced NF-κB activation. PMA-induced NF-κB activation in ethanol-treated acini was prevented by GF-109203X and Ro-32-0432 but was unaffected by Go-6976 (Fig. 9). Similar results were obtained in cells not treated with ethanol (not illustrated). The results with Go-6976 further indicate that activation of Ca\(^{2+}\)-dependent PKC isoforms (which occurs with PMA treatment) does not contribute to NF-κB activation in pancreatic acinar cells.

The data presented in Figs. 6–9 indicate that it is Ca\(^{2+}\)-independent (novel and/or atypical) but not the Ca\(^{2+}\)-dependent (conventional) PKC isoforms that mediate NF-κB activation induced by both CCK-8 and PMA in pancreatic acinar cells and the augmentation of NF-κB responses by ethanol.

DISCUSSION
In the present study, we have developed an in vitro model using isolated rat pancreatic acini to determine the effects of ethanol on CCK-8-induced NF-κB activation. We found that ethanol treatment augmented the CCK-8-induced activation of NF-κB while at the same time attenuating the basal NF-κB activity in pancreatic acinar cells. These effects are similar to those we observed in vivo in ethanol-fed rats treated with CCK-8 (31). Although the conditions of the in vivo and in vitro treatments (e.g., the duration of exposure to ethanol) are different, the results indicate that in both models, ethanol decreased the basal and potentiated the CCK-induced NF-κB activation.
Our results show that ethanol has diverse effects on the signaling pathways mediating NF-κB activation in pancreatic acinar cells. Ethanol can cause both stimulation and inhibition of NF-κB activation, and it affects both Ca²⁺- and PKC-mediated pathways of NF-κB activation.

As was recently shown (21, 45) and corroborated by the results of the present study, CCK-8 causes NF-κB activation through both Ca²⁺- and PKC-mediated pathways. Our data indicate that there are at least two different mechanisms through which Ca²⁺ mobilization causes activation of NF-κB in pancreatic acinar cells, one of which is employed by thapsigargin and the other by CCK-8. Our results with cyclosporin A and FK506 indicate that thapsigargin-induced NF-κB activation is mediated by the Ca²⁺-dependent protein phosphatase 2b (calcineurin). With the calcineurin inhibitors, we also found an inhibition of thapsigargin-induced activation of NFAT, a downstream target of calcineurin. Of note, calcineurin has recently been shown to mediate Ca²⁺-dependent NF-κB activation in other cell types (46, 49).

![Image](Fig. 6. Broad-spectrum PKC inhibitors but not inhibition of conventional PKC isoforms prevent the CCK-8-induced NF-κB activation in pancreatic acinar cells treated with and without ethanol. Pancreatic acini were incubated for 3 h with and without 100 mM ethanol, the broad-spectrum PKC inhibitors GF-109203X (GF) and Ro-32–0432 (Ro), or the inhibitor of Ca²⁺-dependent PKC isoforms, Go-6976 (Go; each 10 μM), and then stimulated for 30 min with 100 nM (A-C) or 0.1 nM (D) CCK-8. A: NF-κB binding activity was measured in nuclear extracts by EMSA. Representative of 3 independent experiments. B–D: NF-κB band intensities were quantified in the PhosphorImager and normalized on the intensity of the NF-κB band in nonstimulated acini. NF-κB response to CCK-8 in the absence of the inhibitors was considered as 100%. Values are means ± SE (n = 3–4). *P < 0.05 compared with the NF-κB response to CCK-8 in the absence of the inhibitors.)

![Image](Fig. 7. Broad-spectrum PKC inhibitors but not inhibition of conventional PKC isoforms prevent the CCK-8-induced IκBα degradation in pancreatic acinar cells treated with and without ethanol. Pancreatic acini were incubated for 3 h with and without 100 mM ethanol, 10 μM GF-109203X, or 10 μM Go-6976 and then stimulated for 30 min with 100 nM CCK-8. IκBα degradation was measured in cytosolic extracts by Western blot analysis. Numbers on the bottom show the intensity of the IκBα band quantified by densitometry and normalized on that in untreated cells. Representative of 3 independent experiments.)
By contrast, the inhibitory analysis indicated that calcineurin is not engaged in the CCK-8-induced pathway of NF-κB. The reason for these differences in the involvement of calcineurin is unknown. One possibility is that CCK-8 but not thapsigargin elicits a signal blocking the Ca^{2+}/calcineurin pathway of NF-κB activation. The mechanisms through which Ca^{2+} mediates the CCK-8-induced NF-κB activation in pancreatic acinar cells remain to be determined.

Ethanol produced opposite effects on NF-κB activation induced by thapsigargin and CCK-8. Ethanol inhibited the thapsigargin-induced while potentiating the CCK-8-induced NF-κB activation. Ethanol’s effects were not due to its direct action on [Ca^{2+}], signals elicited by thapsigargin or CCK-8. One mechanism of the inhibitory effect of ethanol on thapsigargin-induced NF-κB activation could be through inhibition of the Ca^{2+}/calcineurin pathway by ethanol. Indeed, both ethanol and the calcineurin inhibitors markedly inhibited thapsigargin-induced NF-κB activation (as did the intracellular Ca^{2+} chelator BAPTA).

As stated above, our results indicate that the Ca^{2+}/calcineurin pathway of NF-κB activation does not contribute...
significantly to the NF-κB response to CCK-8. This may be one reason why ethanol does not inhibit (but instead potentiates) the NF-κB response to CCK-8. Another factor underlying the differential effects of ethanol on thapsigargin- and CCK-8-induced NF-κB activation, as discussed below, is the involvement of the PKC pathway in the latter but not the former response.

The mechanisms through which ethanol inhibits the basal NF-κB activity remain to be determined. As we have shown (6), this activity is caused by stresses of the process of acinar cell isolation from the pancreas. We have also reported that ethanol and its oxidative metabolite, acetaldehyde, decreased basal NF-κB activity in isolated acini (17). One explanation for the decreased basal NF-κB activity in ethanol-treated cells, compared with cells not treated with ethanol, could be an inhibitory effect of ethanol on the Ca^{2+}/calcineurin pathway of NF-κB activation. However, this mechanism remains to be determined.

Our data suggest that the potentiation of the CCK-8-induced NF-κB activation by ethanol is mediated by ethanol’s effect on the PKC pathway. We found that the CCK-8-induced NF-κB activation in cells both treated and not treated with ethanol was blocked by general PKC inhibitors. In contrast, a specific inhibitor of conventional Ca^{2+}-dependent PKC isoforms did not inhibit the CCK-8-induced NF-κB activation. We also observed similar effects of the PKC inhibitors on NF-κB activation induced by PMA. Thus our results indicate that the conventional isoforms of PKC are not involved in either CCK-8-induced or PMA-induced NF-κB activation. It is the Ca^{2+}-independent (novel and/or atypical) PKC isoforms that regulate NF-κB activation in pancreatic acinar cells. Of note, both the conventional and the novel and atypical isoforms of PKC are expressed in pancreatic acinar cells (4, 33, 37). In other cell types, both Ca^{2+}-dependent and -independent isoforms of PKC have been shown to mediate NF-κB activation (7, 12, 25, 34). However, the mechanisms through which PKC isoforms regulate NF-κB are not well understood.

Our findings suggest that the effect of ethanol to augment CCK-8-induced NF-κB activation results from ethanol’s actions on the PKC pathway. First, we found that ethanol treatment augmented the effect of PMA on NF-κB activation. Second, PKC inhibitors prevented the augmented NF-κB activation by CCK-8 in ethanol-treated acini. The mechanisms through which ethanol affects PKC-mediated pathways of NF-κB activation in pancreatic acinar cells remain to be determined. Ethanol could directly affect PKC activity or regulate PKC-mediated signaling. The finding that ethanol augmented the CCK-8-induced degradation of IkBo suggests that ethanol’s effects on PKC are upstream of IkBo degradation, possibly at the level of IKK activation. Recent evidence indicates that IKKs are regulated through phosphorylation or protein-protein interaction by a variety of kinases, including PKC isoforms (12). Our results do not exclude the possibility that pathways other than those that are PKC mediated contribute to the potentiation of CCK-8-induced NF-κB activation by ethanol. In particular, ethanol may directly affect IKKs or upstream kinases unrelated to PKC.

In conclusion, the results of the present study demonstrate that ethanol augments the NF-κB response caused by CCK-8 in pancreatic acinar cells while inhibiting the basal NF-κB activity and NF-κB activation induced by thapsigargin. These

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**REFERENCES**


