Temporal Activation of p42/44 Mitogen-Activated Protein Kinase and c-Jun N-Terminal Kinase by Acetaldehyde in Rat Hepatocytes and Its Loss after Chronic Ethanol Exposure

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ABSTRACT

Several cell-damaging effects of ethanol are due to its major metabolite acetaldehyde but its mechanisms are not known. We have studied the effect of acetaldehyde on p42/44 mitogen-activated protein kinase (MAPK) and p46/p54 c-Jun N-terminal kinase (JNK 1/2) in rat hepatocytes. Acetaldehyde caused peak activation of p42/44 MAPK at 10 min followed by JNK activation at 1 h. These responses were acetaldehyde dose-dependent (0.2–5 mM). There was a consistently higher activation of p46 JNK than p54 JNK. Ethanol also activated both p42/44 MAPK and p46/p54 JNK. The activation of JNK by ethanol, however, was not significantly affected by treatment of hepatocytes with 4-methylpyrazole, an alcohol dehydrogenase inhibitor. Cells treated with 200 mM ethanol for 1 h accumulated 0.35 ± 0.02 mM acetaldehyde, but the magnitude of JNK activation was greater than that expected with 0.35 mM acetaldehyde. Thus, ethanol-activated JNK may be both acetaldehyde-dependent and -independent. The activation of JNK by ethanol or acetaldehyde was insensitive to the treatment of hepatocytes with genistein (tyrosine kinase inhibitor) and 2-[1-(3-dimethylaminopropyl)-1H-indol-3-yl]-3-(1H-indol-3-yl)maleimide (GF109203X) (protein kinase C inhibitor). Remarkably, in contrast to the above-mentioned effects on normal hepatocytes, acetaldehyde was unable to increase JNK activity in hepatocytes isolated from rats chronically fed ethanol for 6 weeks and indicated a loss of this acetaldehyde response. Thus, temporal activation of the p42/44 MAPK and p46/p54 JNK, the greater activation of p46 JNK than p54 JNK, and loss of JNK activation after chronic ethanol exposure indicate that these kinases are differentially affected by ethanol metabolite acetaldehyde.

Alcohol-induced liver damage is one of the major causes of morbidity and mortality in alcoholics. Mechanisms and mediators responsible for this liver injury, however, are not clearly understood. Recent studies have suggested that ethanol alters cell functions via changes in multiple signaling pathways, especially mitogen-activated protein kinases (MAPKs) involved in cell proliferation, differentiation, and apoptosis (Su and Karin, 1996; Cross et al., 2000). We have reported ethanol potentiation of serum-stimulated p42/44 MAPK activation in BNLCL2 embryonic liver cells (Reddy and Shukla, 1996), and angiogenin II-stimulated p42/44 MAPK in primary culture of rat hepatocytes (Weng and Shukla, 2000). Ethanol also causes prolonged activation of p42/44 MAPK and p38 MAPK after stimulation of hepatocytes with various agonists (Chen et al., 1998). Ethanol and/or its metabolites are directly injurious to the liver (Lieber, 1990). Acetaldehyde is a highly reactive product of the oxidative metabolism of ethanol and causes induction of collagen gene expression in rat hepatic stellate cells (Chen and Davis, 2000) and inhibition of hormone-stimulated hepatocyte DNA synthesis (Carter and Wands, 1988). Acetaldehyde is also a critical mediator of ethanol-induced apoptosis (Zimmerman et al., 1995; Holownia et al., 1999). However, effects of acetaldehyde on hepatocyte p42/44 MAPK and JNK pathways are not known. We present results of such an investigation using primary cultures of hepatocytes from normal and 6-week ethanol-fed rat liver.

Experimental Procedures

Materials. The protease inhibitors (aprotinin, leupeptin, and pepstatin A), cyanamide, 4-methylpyrazole, and p-hydroxybiphenyl (p-phenylphenol) were obtained from the Sigma-Aldrich (St. Louis, MO). The phospho-p42/44 MAPK antibody, p42/44 MAPK antibody, and stress-activated protein kinase/JNK antibody were purchased from Cell Signaling Technology, Inc. (Beverly, MA). U0126 and anti-active JNK antibody (phospho-JNK antibody), which recognizes p46/...
p54 JNK (or JNK1/2), were from Promega (Madison, WI), [γ-32P]ATP (3000 Ci/mmol) was from PerkinElmer Life Sciences (Boston, MA), myelin basic protein (MBP) was from Invitrogen (Carlsbad, CA), genistein was from Sigma/RBI (Natick, MA), and GF109203X was from BIOMOL Research Laboratories (Plymouth Meeting, PA).

Isolation and Treatment of Hepatocytes. Hepatocytes were isolated from male Sprague-Dawley rats (Weng and Shukla, 2000). The isolated hepatocytes were washed twice with 50 ml of Krebs-Ringer-bicarbonate buffer containing 0.5% bovine serum albumin. The hepatocytes (3 × 10^6 cells/60-mm dish) were plated onto collagen-coated culture dishes in Dulbecco’s modified Eagle’s medium containing 10% fetal bovine serum (FBS). After 2 h, the medium was changed to Dulbecco’s modified Eagle’s medium containing 1% FBS. After 24 h, either ice-cold acetaldehyde or ethanol was carefully added to the cells and the dishes were immediately sealed tightly with Parafilm (Pechiney Plastic Packaging, Inc., Neenah, WI). Addition of ice-cold acetaldehyde was carried out at 4°C to prevent its evaporation. Then cells were incubated for various times at 37°C. Cells were rinsed twice with ice-cold phosphate-buffered saline followed by addition of lysis buffer (20 mM HEPES, pH 8.0, 136 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% Triton X-100, 10 mM KCl, 2 mM MgCl₂, 1 mM phenylmethylsulfonil fluoride, 1 mM sodium orthovanadate, 1 mM dithiothreitol, 1 mM benzamidine, 10 mM β-glycerophosphate, 10 μg/ml aprotinin, 10 μg/ml leupeptin, and 10 μg/ml pepstatin). Cell lysates were collected and sonicated for 5 s using a microtip probe at a power setting of 5 in a Vibracell ultrasonic processor (model VC 600; Sonics and Materials, Inc., Danbury, CT). After centrifugation of the sonicated samples at 12,000g for 10 min at 4°C, the supernatant was collected, and protein concentrations were estimated using the Bio-Rad protein assay reagent (Bio-Rad, Hercules, CA).

Protein Kinases Assay. The p42/44 MAPK and JNK activities were determined by an in-gel kinase assay (Kyriakis and Avruch, 1990; Reddy and Shukla, 1996). Briefly, equal amounts (20 μg) of each sample were loaded on 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis gels containing 0.5 mg/ml MBP. SDS was removed by washing the gel with 50 mM Tris, pH 8.0, containing 20% isopropanol for 1 h at room temperature. Subsequently, proteins on the gel were denatured in 6 M guanidine HCl and renatured overnight at 4°C in 50 mM Tris, pH 8.0, containing 50 mM β-mercaptoethanol and 0.04% Triton X-100. The gel was incubated in kinase buffer containing 40 mM HEPES, pH 8.0, 0.5 mM EGTA, 40 μM ATP, 10 mM MgCl₂, 2 mM dithiothreitol, and [γ-32P]ATP (5 μCi/ml, 3000 Ci/mmol). The gel was washed with 5% trichloroacetic acid containing 1% sodium pyrophosphate, dried, and exposed to an X-ray film.

Western Blotting. Cell lysates containing equal amounts of protein (20–30 μg) were fractionated on 10% SDS-polyacrylamide gel electrophoresis gel. After electrophoresis, proteins were transferred to nitrocellulose membrane (Bio-Rad). The membrane was washed with 25 mM Tris, pH 7.4, containing 137 mM NaCl and 0.1% Tween-20 and then blocked with 25 mM Tris, pH 7.4, containing 137 mM NaCl and 0.1% Tween-20 containing 5% nonfat dry milk for 2 h at room temperature. Blots were incubated with antibodies against phospho-p42/44 MAPK, phospho-JNK, p42/44 MAPK, or JNK overnight at 4°C. The blots were incubated with goat anti-rabbit horse-radish peroxidase. After washing, the blots were developed with enhanced chemiluminescence (Pierce Chemical, Rockford, IL) and exposed to X-ray film to detect the protein band.

Acetaldehyde Assay. Acetaldehyde level was measured by p-phenylphenol procedure as described previously (Dagani and Archer, 1978). Briefly, 50-μl aliquots of culture media obtained after 1 h of incubation with ethanol or acetaldehyde were mixed with 0.45 ml of 1.1 mg/ml semicarbazide hydrochloride. Subsequently, 25 μl of 5% copper sulfate was added, and 5 ml of concentrated sulfuric acid was added slowly with shaking in an ice bath followed by 0.1 ml of p-phenylphenol reagent. The resulting precipitate was dispersed using a vortex mixer. After incubation at 30°C for 30 min, the mixture was immersed in boiling water for 90 s and then cooled. The absorbance of violet color was measured at the 560 nm. A standard curve was generated using 0 to 300 μM acetaldehyde.

Chronic Ethanol Feeding. Male Sprague-Dawley rats (150 g) were fed a nutritionally adequate ethanol-containing liquid diet for 6 weeks, essentially as described previously (DeCarli and Lieber, 1967). Ethanol introduction was gradually increased starting with 1.25% (w/v) for the 1st day, 1.67% for the 2nd day, 2.5% for the 3rd and 4th days, and then increased to 5% for 6 weeks. The rats were given their total nutrient intake through graduated feeding tubes. The percentage of calories derived from ethanol was 36% of total calories. The control rats were fed on the same liquid diet, except that ethanol was replaced by dextrin-maltose and strictly pair-fed by administration of the same amount of liquid diet as taken by ethanol-fed rats on the previous day. To confirm continuing growth of the rats, the body weight of the animals was measured every week.

Statistical Analysis. Data are expressed as mean ± S.E.M. Differences between control and experimental groups were checked for statistical significance (p < 0.05) by the Student’s t test (two-tailed, unpaired).

Results

Acetaldehyde Stimulation of p42/44 MAPK and JNK. Initially, hepatocytes were treated with high concentration (10 mM) of acetaldehyde for different times. Whole cell extracts were prepared and in-gel kinase assay was performed (see Experimental Procedures). As shown in Fig. 1A, p42/44 MAPK activation occurred at 10 min. Interestingly, after 10 min, p42/44 MAPK activity decreased and then increased again at 2 h, showing a biphasic activation with two peaks at 10 min and 4 h (Fig. 1, B and C). In addition, significant phosphorylation of MBP was observed by an ~54-kDa protein at 30 min and 1 h (Fig. 1A). This band was consistent with the molecular mass of p54 JNK (JNK2) and was confirmed by immunoblotting with anti-phospho-JNK antibodies, which can detect activated JNK1 and JNK2 at approximately p46 and p54 kDa, respectively (Promega) (Fig. 1B). The immunoblot for phospho-JNK also showed increase in phosphorylation of p46 JNK (JNK1). Because the activity of p44 MAPK shown in in-gel kinase assay overlapped with the increase in p46 JNK activation, the p44 MAPK activation is not presented in Fig. 1C. Additional immunoblot studies with p42/44 MAPK and JNK antibody showed that acetaldehyde treatment for different times did not affect the amount of p42/44 MAPK and JNK protein (Fig. 1B). These data demonstrated a unique time-dependent activation of p42/44 MAPK followed by JNK and biphasic pattern of p42/44 MAPK activation by acetaldehyde. We measured the level of acetaldehyde in these experiments to account for any evaporation and the degree of its utilization by cells (Table 1). Based on observations in Fig. 1, in subsequent experiments, hepatocytes were treated with acetaldehyde for 10 min and 1 h for p42/44 MAPK and JNK activations, respectively. The activation of both p42/44 MAPK and JNK (monitored by anti-phospho-p42/44 MAPK antibody and anti-phospho-JNK antibody) by acetaldehyde occurred in a concentration-dependent manner (Fig. 3A). Appreciable changes in JNK activation were noted at 200 μM and higher concentrations of acetaldehyde. The activation of
JNK increased 11.0/3.06-fold at 1 mM and 50.4/11.98-fold at 5 mM acetaldehyde; n = 3 (Fig. 3B). The magnitude of p42/44 MAPK activation was much less than that of JNK activation. The p42/44 MAPK activation was 1.7 ± 0.04/3.1 ± 0.75-fold at 1 mM and 2.0 ± 0.04/5.4 ± 1.33-fold at 5 mM acetaldehyde; n = 3 (Fig. 3B).

These results indicated that JNK activation (-fold increase) by acetaldehyde is greater than p42/44 MAPK activation, and that p46 JNK activation by acetaldehyde was more sensitive than p54 JNK activation.

**Table 1**

<table>
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<th>Added Conc. (mM)</th>
<th>Measured Conc. (mM)</th>
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<tr>
<td>Without cells</td>
<td>5.26 ± 0.13</td>
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<tr>
<td>With cells</td>
<td>0.68 ± 0.04</td>
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<td>5</td>
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**Fig. 2.** Absorbance of various concentrations of acetaldehyde. Acetaldehyde (0–300 μM, 50 μl) was mixed with 0.45 ml of 1.1 mg/ml semicarbazide hydrochloride. The level of acetaldehyde was measured as described under Experimental Procedures. Values shown are mean ± S.E.M. from three independent experiments. Absorbance toward these concentration was significantly correlated \( r = 0.99 \).

p46/p54 JNK increased 11.0 ± 3.06/3.6 ± 0.49-fold at 1 mM and 50.4 ± 11.98/9.1 ± 0.81-fold at 5 mM acetaldehyde; n = 3 (Fig. 3B). The magnitude of p42/44 MAPK activation was much less than that of JNK activation. The p42/44 MAPK activation was 1.7 ± 0.04/3.1 ± 0.75-fold at 1 mM and 2.0 ± 0.04/5.4 ± 1.33-fold at 5 mM acetaldehyde; n = 3 (Fig. 3B).

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**Ethanol Stimulation of p42/44 MAPK and JNK.** To determine whether the effects of acetaldehyde on MAPKs can also be observed with ethanol, hepatocytes were treated with 200 mM ethanol, and then alterations in the activities of p42/44 MAPK and JNK were monitored. Immunoblot studies showed that the exposure of hepatocytes to 200 mM ethanol increased phosphorylation of both p42/44 MAPK and JNK after ethanol treatment for 1 h (Fig. 4A). The increases in phosphorylation of both p42/44 MAPK and JNK remained higher than the control at 4 h. However, the activity at 24 h was not significantly different from control values or, in some instances, was slightly lower than the control. The basal
levels of phosphorylation do vary among experiments but their protein levels do not change. The activities of these kinases were confirmed by in-gel kinase assay (data not shown). The activation of JNK by ethanol was concentration-dependent (Fig. 4B). When exposed to increasing concentrations of ethanol for 1 h, the activity of p46/p54 JNK increased 4.2/2.2-fold at 100 mM, and 10.1/3.2-fold at 200 mM ethanol (Fig. 4C). The p42/44 MAPK activation by 200 mM ethanol treatment for 1 h increased 1.57 ± 0.05; **, P < 0.01 (compared with corresponding unstimulated samples).

Ethanol Metabolism and Activation of JNK in Hepatocytes. To investigate the requirement for ethanol metabolism in ethanol-induced JNK activation, hepatocytes were incubated with 4-methylpyrazole (4-MP), an inhibitor of alcohol dehydrogenase (Kurose et al., 1997; Bailey and Cunningham, 1998), and cyanamide, an inhibitor of acetaldehyde dehydrogenase (Bailey and Cunningham, 1998). Cyanamide treatment (0.2 mM) itself increased basal JNK activity by 3-fold (data not shown) and therefore could not be used as a tool. At 0.1 mM 4-MP, it is known that alcohol metabolism is reduced by 65% (Carter and Wands, 1988). Pretreatment with up to 8 mM 4-MP had no significant effect on ethanol-induced JNK activation (Fig. 5). Acetaldehyde accumulation can occur by other pathways, including catalase-mediated acetaldehyde production (Eysseric et al., 2000). We therefore measured the accumulation of acetaldehyde after treatment of hepatocytes with ethanol. Treatment of hepatocytes with 200 mM ethanol for 1 h caused 0.35 ± 0.02 mM of acetaldehyde accumulation and there is no noticeable decrease in the acetaldehyde concentration in the presence of 4 and 8 mM 4-MP. The magnitude of JNK activation by 200 mM ethanol (Fig. 4B) was higher than that would be expected by 0.35 mM acetaldehyde (Fig. 3A). These results suggest that, besides acetaldehyde-mediated, ethanol may also activate JNK by other pathways. Thus, ethanol-activated JNK in hepatocytes may be both acetaldehyde-dependent and independent.

Effects of Genistein and GF109203X on JNK Activation in Hepatocytes. Tyrosine kinases are involved in the activation of JNK induced by growth factors and various stress stimuli (Rosette and Karin, 1996; Fanger et al., 1997). It is also negatively regulated by tyrosine kinases (Croisy-Deley et al., 1997), or is tyrosine kinase-independent (Uchida et al., 1999). To address the involvement of tyrosine kinase in the activation of JNK in hepatocytes, we used genistein, an inhibitor for both receptor and nonreceptor tyrosine kinases. Hepatocytes were preincubated with 30 μM genistein for 2 h followed by either 200 mM ethanol or 5 mM acetaldehyde for 1 h. Whole cell lysates were prepared for Western blotting assay using anti-phospho-JNK antibodies. Genistein at this concentration has been shown to inhibit hepatocyte growth-factor-stimulated MAPK activation in hepatocytes (Adachi et al., 1996). JNK activation was not inhibited by genistein (Fig. 6A). It has been reported that PKC regulates JNK pathway both positively and negatively in rat hepatocytes (Jarvis et al., 1997). Pretreatment of hepatocytes with maximal inhibitory dose of the PKC inhibitor
GF109203X (10 μM) had no significant effect on ethanol- and acetaldehyde-induced JNK activation (Fig. 6B). Together, these results indicate that the activation of JNK by ethanol or acetaldehyde was not affected by tyrosine kinase or PKC inhibitors.

Effects of Chronic Ethanol Consumption on JNK Activation in Hepatocytes. We next investigated the effects of chronic ethanol consumption on the JNK activation by acetaldehyde. Hepatocytes were isolated from pair-fed and ethanol-treated (for 6 weeks) rats and exposed to acetaldehyde. The basal p46 JNK and p54 JNK activities in hepatocytes from chronically ethanol-fed rats decreased 67 and 37%, respectively, compared with pair-fed control rats (Fig. 7, A and B). Interestingly, acetaldehyde-induced JNK activation was abolished in chronic ethanol-treated hepatocytes (Fig. 7, A and C).

**Discussion**

Acute treatment of primary cultures of rat hepatocytes with acetaldehyde caused a temporal activation of p42/44 MAPK followed by JNK. The phosphorylation of JNK was of higher magnitude than that of p42/44 MAPK. In addition, p42/44 MAPK showed biphasic activation and the time of decrease in p42/44 MAPK activity coincided with that of increase in JNK activity. This suggested that p42/44 MAPK might be regulated by JNK activity. Other studies have shown that activation of JNK can inactivate MAPK (Yan et al., 1994; Bokemeyer et al., 1996). The effects of acetaldehyde and ethanol on the pattern of activations of p42/44 MAPK and JNK were similar. This may suggest that acetaldehyde is a possible mediator of ethanol-induced increase in MAPK signaling in rat hepatocytes. It must be noted that ethanol potentiation of angiotensin II-activated MAPK is not affected by acetaldehyde (Weng and Shukla, 2000). Thus, there is a clear distinction between “direct” and “modulatory” effects of ethanol on p42/44 MAPK. In the present study, the effect of ethanol on p42/44 MAPK activation was observed at 1 h, whereas acetaldehyde-induced peak activation of p42/44 MAPK occurred at 10 min. This delayed activation of p42/44 MAPK by ethanol may suggest that a threshold acetaldehyde accumulation is necessary to mediate the effects of ethanol on MAPKs. This is also supported by a significant amount of 0.35 mM acetaldehyde accumulated in hepatocytes after 200 mM ethanol treatment for 1 h.

In the hepatocytes, there are three known enzymes capable of ethanol oxidation to acetaldehyde: cytosolic alcohol dehydrogenase (ADH), microsomal ethanol oxidizing system (e.g.,

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**Fig. 6.** Effects of genistein and GF109203X on the activation of p46/p54 JNK by ethanol or acetaldehyde. Hepatocytes were incubated with (+) or without (−) 30 μM genistein (A) and 10 μM GF109203X (B) for 2 h and 30 min, respectively, then stimulated with 200 mM ethanol (EtOH) or 5 mM acetaldehyde (Acet). Whole cell extracts were prepared and 20 μg of protein from each sample was subjected to Western blotting with anti-phospho-JNK antibody.

**Fig. 7.** Effects of chronic ethanol consumption on activation of p46/p54 JNK by acetaldehyde. Hepatocytes were isolated from pair-fed and ethanol (EtOH)-fed rats. After 3-h culture of hepatocytes in 0.1% FBS, cells were treated with 5 mM of acetaldehyde (Acet) for 1 h. Whole cell extracts were prepared and 20 μg of protein from each sample was subjected to Western blotting using anti-phospho-JNK antibody. A, data are from one of three similar experiments. Basal (B) and acetaldehyde-induced (C) p46/p54 JNK activity was quantitated by densitometric analysis, where values in histograms are mean ± S.E.M. (bars), n = 3. *, P < 0.05; **, P < 0.01 (compared with corresponding unstimulated samples).
CYP2E1), and peroxisomal catalase (Nomura et al., 1983; Tampler and Mardones, 1986; Crabb et al., 1987). To examine the role of ADH, hepatocytes were treated with an ADH inhibitor, 4-MP, but this had no effect on ethanol activation of JNK and acetaldehyde accumulation after ethanol treatment (Fig. 5). It has been reported that ADH activity in rat hepatocytes decreased approximately 90% during the first 24 h (Carter and Wands, 1988) and this may account for the observed lack of effect of 4-MP after 24-h culture in the present study. Acetaldehyde accumulation (0.35 mM) after 200 mM ethanol treatment in our experiment seems to occur through pathways other than ADH pathway. However, JNK activation at 1 h by 200 mM ethanol was higher than that expected with 0.35 mM acetaldehyde. Thus, ethanol may activate JNK by both acetaldehyde-dependent and -independent pathways. It would be interesting in the future to investigate its mechanisms in detail. It must be mentioned that the effects of 0.2 to 1 mM acetaldehyde can be relevant in vivo because hepatocytes are exposed to additional acetaldehyde present in the portal vein absorbed from colon. Alcohol ingested orally is transported to the colon, where ethanol is oxidized to acetaldehyde by a bacteriocolonic pathway (Salaspuro, 1996). During ethanol oxidation, the levels of acetaldehyde in the colon can reach up to 3.0 mM due to the low aldehyde dehydrogenase activity of colonic mucosa (Seits et al., 1990; Koivist and Salaspuro, 1997). Intracellular acetaldehyde can be thus absorbed to the portal vein (Matysik-Budnik et al., 1996) and give higher levels in vivo. In addition, acetaldehyde concentration in hepatic vein has been reported to be 10 to 30 times higher (0.1–67.9 μM) than that in the peripheral vein (<2 μM) (Nuuinen et al., 1984) and chronic alcohols exhibit high levels (30 μM) of acetaldehyde (Hatake et al., 1990) in peripheral vein. Furthermore, more prominent and remarkable elevation of blood acetaldehyde has been observed after a high than a low dose of ethanol (Nuuinen et al., 1983), suggesting a significant amount of acetaldehyde accumulation in hepatocytes after heavy alcohol consumption.

In the present study, the upstream regulation of JNK activation was also examined. JNK activation has been reported to be regulated by upstream kinases, i.e., tyrosine kinase (Rosette and Karin, 1996; Fanger et al., 1997) and PKC (Jarvis et al., 1997). However, genistein and GP109203X, which are selective tyrosine kinase and PKC inhibitors, respectively, and which have been shown by us to inhibit these kinases in hepatocytes (Y. Weng and S. D. Shukla, unpublished data), were ineffective. This implies that acetaldehyde may elicit this effect through pathways independent of the above-mentioned kinases. Other pathways regulating JNK activation have also been reported. H2O2 significantly activates JNK in rat liver epithelial cells (UCHIDA et al., 1999). In rat myogenic cell line (H9c2), ethanol activates JNK via inhibition of JNK dephosphorylation (MERRIN et al., 1999). Thus, the mechanism of JNK activation by acetaldehyde can be by multiple pathways.

The JNK and p42/44 MAPK are involved in various biological responses. For example, JNK activation has been linked to both hepatocyte proliferation and apoptosis (AUER et al., 1998; CRENESSE et al., 2000). In the present study, both acetaldehyde and ethanol caused robust activation of JNK compared with p42/44 MAPK. Although the basal activity of p46 JNK was lower than that of p54 JNK (Figs. 3 and 4), the increase in p46 JNK was ~5- and ~3-fold higher than that of p54 JNK by 5 mM acetaldehyde and 200 mM ethanol, respectively. It has been reported that JNKI (p46 JNK) is strongly activated by γ radiation and UV-C and that JNKI is involved in the initiation of the apoptosis process (CHEN et al., 1996). It suggests that the pronounced activation of p46 JNK by either ethanol or acetaldehyde may be involved in ethanol-induced hepatotoxicity.

The present study also compared acetaldehyde activation of JNK in hepatocytes from pair-fed rats with ethanol-fed rats. Chronic ethanol decreased basal JNK activity and surprisingly, acetaldehyde activation of JNK was almost abolished. Whether this loss is due to cellular adaptation or to desensitization to acetaldehyde remains to be known. Diehl and colleagues have shown that chronic ethanol consumption decreased responses, including JNK stimulation of hepatocytes after partial hepatectomy (ZELDIN et al., 1996). The pathophysiological significance of the differential effects of acetaldehyde on JNK in acute versus chronic ethanol treatment remains unknown. Is it protective or damaging? Future studies must be pursued to correlate this effect to liver growth, apoptosis, fibrosis, regeneration, and remodeling to fully understand the JNK involvement in alcoholic liver injury.

In conclusion, we have demonstrated, for the first time, that short-term exposure of hepatocytes to acetaldehyde directly elicited a unique temporal activation of p42/44 MAPK followed by JNK, and that JNK activation was greater than p42/44 MAPK. Furthermore, p46 JNK was more sensitive to acetaldehyde than p54 JNK. In contrast to these acute effects, chronic ethanol consumption for 6 weeks was accompanied by a decreased basal JNK activity and absence of acetaldehyde-induced JNK activation. Thus, acetaldehyde-mediated JNK activation must be considered as a crucial component in the mechanisms of ethanol-induced alterations in liver in vivo.

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References


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