Roles of Tissue Transglutaminase in Ethanol-induced Inhibition of Hepatocyte Proliferation and α1-Adrenergic Signal Transduction*

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The mechanisms by which ethanol inhibits hepatocyte proliferation have been a source of some considerable investigation. Our studies have suggested a possible role for tissue transglutaminase (tTG) in this process. Others have shown that tTG has two distinctly different functions: it catalyzes protein cross-linking, which can lead to apoptosis and enhancement of extracellular matrix stability, and it can function as a G protein (Gα). Under that circumstance, we speculated that the cross-linking activity would be decreased and that it would function to enhance hepatocyte proliferation in response to adrenergic stimulation. Ethanol treatment inhibited hepatocyte proliferation and led to enhanced tTG cross-linking activity, whereas treatment of hepatocytes with an α1 adrenergic agonist, phenylephrine, enhanced hepatocyte proliferation while decreasing tTG cross-linking. However, phenylephrine treatment of several hepatoma cell lines had no effect on cellular proliferation or tTG cross-linking activity, and of note, Northern blot analysis demonstrated that whereas primary hepatocytes had high levels of the αβ adrenergic receptor (α1BAR) mRNA, the hepatoma cell lines did not have this mRNA. When the Hep G2 cell line was stably transduced with an expression vector containing the α1BAR cDNA, the cell line responded to phenylephrine treatment with enhanced proliferation and with decreased tTG cross-linking activity. Ethanol treatment of the α1BAR-transfected cells suppressed the phospholipase C-mediated signaling pathways, as detected in the phenylephrine-induced Ca2+ response. These results suggest that phenylephrine stimulation of hepatocyte proliferation appears to be occurring through the α1BAR, which is known to be coupled with the tTG G protein moiety, Gα, and that tTG appears to play a significant role in either enhancing or inhibiting hepatocyte proliferation, depending on its cellular location and on whether it functions as a cross-linking enzyme or a G protein.

It is well established that ethanol exposure inhibits hepatocyte regeneration in in vitro (1) and in vivo (2) systems. Although some associations have been found, the mechanism by which this inhibition occurs has not been delineated. One such association is that ethanol has been shown to decrease putrescine levels in the liver and that putrescine administration abrogates this inhibition of liver regeneration by ethanol administration (3). The mechanism by which putrescine has this effect is not known. One possibility is that putrescine may be exerting this effect on liver regeneration through its known action as a competitive substrate inhibitor of tissue transglutaminase (tTG)1 activity.

Tissue transglutaminase (transglutaminase II) is a member of a family of transglutaminases that are calcium-dependent enzymes that catalyze the posttranslational modification of proteins through cross-linking via εγ-glutamyl lysine bonds (4). Although its physiological significance has not been proven, tTG has been implicated to be involved in extracellular matrix organization (5) and in inhibition of cell growth and proliferation (6). We have demonstrated that its transglutaminase cross-linking activity is increased in humans with acute liver injury and in model systems of hepatic injury and fibrosis (7) and that tumor necrosis factor-α (TNF-α) up-regulated tTG gene expression in the Hep G2 cell line (8). In addition to its tissue transglutaminase cross-linking (tTGase) activity, tTG is unique in that it also is a GTP-binding protein, with GTPase activity referred to as Gα(9). In this role as a G protein, the Gα molecule is associated with a 50-kDa B subunit (Gβγ), from which it dissociates following stimulation through the α1B adrenergic receptor (α1BAR) and activates a 69-kDa phospholipase C (PLC-δ1) (10). It is thought that a switch mechanism may exist, with adrenergic stimulation activating its G-protein function and inhibiting its tTGase activity (9).

Adrenergic agonists such as epinephrine and phenylephrine have been shown by a series of authors to enhance hepatocyte proliferation (11–13). Adrenergic receptor activation by these agonists causes activation of phosphoinositide-specific phospholipase C, which produces the two intracellular messengers diacylglycerol and inositol 1,4,5-triphosphate, which mediate the activation of protein kinase C and intracellular Ca2+ release, respectively. Ethanol causes the desensitization of receptor-mediated PLC activation in agonist-treated hepatocytes (14–16), but the action of phenylephrine differs from the effects of the other agonists. Thus, the mechanisms by which phenylephrine and ethanol affect hepatocyte proliferation require further analysis.

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1 The abbreviations used are: tTG, tissue transglutaminase; tTGase, tissue transglutaminase cross-linking; α1BAR, α1B adrenergic receptor; GTPγS, guanosine 5′-O-(3-thiotriphosphate); PLC, phospholipase C; PNLP, phenylephrine; MHBS, modified Hanks' balanced salts.

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4 A single copy of this article is available online at http://www.jbc.org.
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In the present study, we have investigated the significance of both the tTGase and GTPase activity of tissue transglutaminase on hepatocyte proliferation. Our results suggest that whereas its tTGase activity is associated with inhibition of proliferation, its G protein activity appears to be a mechanism whereby hepatocyte proliferation is enhanced. The findings further indicate that phenylephrine stimulation of cellular proliferation may well be occurring through binding to the α1BAR that is coupled with the TGF molecule, Goi.

EXPERIMENTAL PROCEDURES

Animals—Rats (200–250 g) were purchased from Harlan Sprague-Dawley, Inc. (Indianapolis, IN). The animals were fed a commercial diet (Purina Chow 5001, Purina Mills, St. Louis, MO) and water ad libitum. All animal experiments were performed in accordance with a protocol approved by the Institutional Animal Care and Use Committee at the Thomas Jefferson University.

Isolation, Purification, and Culture of Rat Hepatocytes—Rat hepatocytes were isolated by two-step collagenase digestion (15), and the cells were seeded on 6- or 24-well Costar® plates precoated with collagen type I from rat tail (5 μg/cm2) and incubated in Williams’ E medium with 10% fetal calf serum, insulin (0.02 unit/ml), 10 mM HEPES, 10 mM NaHCO3, and antibiotics, under an atmosphere of 5% CO2/air. The cells were washed with 0.1 M phosphate-buffered saline 2 h after seeding, and the medium was replaced with fresh medium with the same supplements.

Determination of Hepatocyte Proliferation and tTG Activity—The cells seeded on 24-well Costar® plates were treated with phenylephrine at concentration indicated under “Results” for 24 h. After the cells were incubated with phenylephrine overnight, methyl-[^3]H]thymidine (Amersham Pharmacia Biotech) was added to reach a concentration of 1 μCi/ml in the culture medium, and the cells were incubated for an additional 4 h.[^3]H]Thymidine incorporation was determined according to the method described previously (17). The hepatocytes seeded on 6-well Costar® plates were used for determination of tTG activity. The assays were carried out on Hep G2 cell homogenate. The isotope was added to reach a concentration of 100 or 150 mM. GTPγS was added to cell extracts. Cells were incubated with the isotope for 30 min. After a series of washings, the ψ[^3]C]pipera-8ine incorporated into proteins was determined in 20 μl of the cell extracts. Cells were incubated with ethanol for 24 h at a final concentration of 100 or 150 mM. GTPγS and the cell homogenate were added separately to the culture medium to determine their effects on ethanol-induced inhibition of hepatocellular proliferation. A selective protein kinase C inhibitor, chelerythrine chloride, was first dissolved in dimethyl sulfoxide (Me2SO) as a stock solution and then added to culture medium to reach final concentrations indicated under “Results.” ET-18-OCH3, a selective phosphatidylinositol-specific phospholipase C inhibitor, was diluted in phosphate-buffered saline prior to being added to the culture medium.

RNA Extraction—Total RNA was extracted from isolated rat hepatocytes or Hep G2 cells using a Tri-reagent RNA extraction kit (Molecular Research Center, Cincinnati, OH), a modification of the acidic phenol/guanidine method of Chomczynski and Sacchi (18). The cells were homogenized in Tri-reagent, and the cell homogenate was filtered and combined with molecular grade chloroform. The RNA was then pelleted by centrifugation after precipitation in isopropanol and was purified further by resuspension/precipitation in ethanol. In all cases, total RNA was quantitated by A260 spectrophotometry and stored at −150 °C until being used for hybridization studies.

Northern Blot Hybridization Analysis—Steady-state mRNA levels were determined by Northern blot hybridization analysis as described elsewhere (19). The filter membranes were prehybridized and then hybridized under stringent conditions with cDNA clones that were labeled to a specific activity of 0.2 to 1.0 × 106 cpm/μg DNA with [32P]dCTP by means of a primer extension kit (Amersham Pharmacia Biotech). The following cDNA clones were used. The tTG cDNA was provided by Dr. Peter Davies (Department of Medicine and Pharmacology, University of Texas-Houston Medical School). The α1BAR cDNA plasmid was kindly provided by Dr. R.M. Graham (Victor Chang Cardiac Research Institute, St. Vincent’s Hospital, Darlingherst, Sydney, Australia). The filter membranes were subsequently exposed to both a PhosphorImager screen (Molecular Dynamics) and to x-ray films. Densitometry tracings were recorded using a PhosphorImager and normalized against ribosomal RNA.

Protein Extraction and Western Blot Analysis of tTG Proteins—After treatments with ethanol, phenylephrine, or other combinations described under “Results,” total proteins from the hepatocyte homogenate were lysed by SDS, extracted by centrifugation, quantitated, and separated by electrophoreses in a 10% SDS-polyacrylamide gel and subsequently transferred to a nitrocellulose membrane. The membrane was described previously (18). After the membrane was washed in 5% nonfat dry milk, it was incubated with the goat-anti-guinea pig tTG antibody from Dr. Davies (7). Subsequently, the membrane was incubated with alkaline phosphatase-conjugated rabbit-anti-goat antibodies, and signals were detected with an alkaline phosphatase system (alkaline phosphatase conjugate substrate kit, Biogenex, San Ramon, CA).

Transfection of α1BAR cDNA in Hep G2 Cells—The Hep G2 cell line was co-transfected with Neo plasmids and α1BAR cDNA using Lipo- fectAMINE (Promega Corporation, Madison, WI) according to the manufacturer’s manual. Clones were examined by Northern blot analysis for the presence of α1BAR mRNA. Clones containing the transgene were then selected and treated with phenylephrine or ethanol to determine their response.

Evaluating Apoptosis in Ethanol-treated Hepatocytes—Isolated rat hepatocytes were seeded on collagen type I precoated Lab-Tek® chamber slides (Nalge Nunc International, Naperville, IL) and incubated under the same conditions as described for the cells seeded on the 6-well plates. After overnight incubation, the cells were treated with ethanol or ethanol with α1BAR-positive and -negative Hank’s salt solution (MHBS) twice. Acidine orange (Molecular Probes, Inc., Eugene, OR) dissolved in 0.1 M HCl (10 mg/ml) and further diluted in MHBS was added to the cells to a final concentration of 0.5 mg/ml. The cells were incubated for additional 20 min with the acidine orange solution and washed twice with MHBS. Green fluorescent images of nuclei were examined with a fluorescein isothiocyanate filter (emission, 525 nm) without any fixation under a fluorescence microscope (20).

Cytosolic Calcium Concentration and Oscillations in α1BAR-positive Hep G2 Cells—Changes in cytosolic calcium concentration [Ca2+]i, and oscillations in response to phenylephrine stimulation in these cells were examined using fura-2/AM as a fluorogenic indicator (21). The Hep G2 cells were seeded on polylysine-precocated coverslips overnight, washed with phosphate-buffered saline, and loaded with fura-2/AM (Molecular Probes, Inc.) at a concentration of 5 μM in Hanks’ balanced salt solution for 30 min. [Ca2+]i measurements were carried out by ratiometric fluorescence microscopic imaging on fura-2-loaded cells essentially as described previously (15, 22). Coverslips with attached fura-2-loaded Hep G2 cells were mounted in a chamber with 1 ml of Hanks’ medium and placed on the stage of an inverted microscope maintained at 37 °C. A fluorescence microscope equipped with an excitonic filter (emission, 525 nm) was used to observe the occurrence of calcium oscillations, basal cytosolic calcium concentration (nmol/liter), and peak concentration of cytosolic calcium of α1BAR-transfected cells with or without phenylephrine or ethanol treatment were calculated. The cells were treated with ethanol at 100 μM for 2 h before calcium measurement or 5 min after starting phenylephrine stimulation (20 μM).

Statistical Analysis—Data of [3H]thymidine incorporation and tTG activity were calculated as percentage of controls from each independent experiment and expressed as means ± S.E. These data were normally distributed and evaluated by means of the one-way variance test and the Newman-Keuls test for multiple comparisons between groups. A p value of less than 0.05 was considered statistically significant.

Chemicals and Reagents—Phenylephrine, GTPγS, collagenase, phenol/chloroform/isoamyl alcohol mixture (25:24:1), William’s E medium, and MHBS were purchased from Sigma. Minimum Eagle’s culture medium and fetal calf serum was from Life Technologies, Inc. Chelerythrine chloride and ET-18-OCH3 were purchased from Alexis Biochemicals (San Diego, CA). All other chemicals used were commercially available reagents of analytical grade.

RESULTS

Because tTG cross-linking activity (tTGase) has been associated with apoptosis and inhibition of cellular proliferation in other systems, we first investigated its activity when primary rat hepatocytes were treated with ethanol. Our hypothesis was...
that ethanol would enhance tTG expression, leading in turn to an inhibition of proliferation, and that putrescine may enhance hepatocyte proliferation by its action as a substrate inhibitor of tTG activity. Rat hepatocytes were isolated by standard techniques and then treated with 100 or 150 mM ethanol for 24 h. This treatment did not cause liver cell necrosis as monitored by lactate dehydrogenase leakage (data not shown), but it led to selective inhibitor of tTGase activity, at a concentration of 20 mM. A fluorescein isothiocyanate filter was used for generation of green images. A, hepatocytes without any treatment; no cells with fragmented nuclei were observed. In B, some cells with fragmented nuclei (arrows) after treatment with ethanol at 100 mM for 4 h were recorded (>630).

To explore the relationship between enhanced tTGase activity and inhibition of hepatocyte proliferation in ethanol-treated rat hepatocytes, two inhibitors of the cross-linking activity that act by completely different mechanisms were employed in this assay system. Treatment of the hepatocytes with GTPγS, a selective inhibitor of tTGase activity, at a concentration of 20 μM abrogated the ethanol-induced inhibition of hepatocyte proliferation, and at the same time it reduced ethanol-induced elevation of tTG activity (Fig. 3, top panel). Putrescine treatment at a concentration of 50 μM also significantly decreased tTGase activity at the same time that it strikingly reduced the inhibition of hepatocyte proliferation due to ethanol treatment (Fig. 3, bottom panel).

To further investigate the role of tTGase in hepatocyte proliferation, the cells were treated with an α1 adrenergic receptor agonist, phenylephrine (PNLP). As expected, PNLP at concentrations of 1–20 μM caused an enhancement of cell proliferation. At the same time, PNLP decreased tTGase activity in the cells (Fig. 4, top panel). These effects were abrogated by ethanol treatment at concentrations of 100–150 mM (Fig. 4, bottom panel). Next, a series of selective inhibitors were employed to demonstrate that this effect of PNLP was indeed occurring through the adrenergic receptor system. ET-18-OCH3, a selective phosphatidylinositol-specific phospholipase C inhibitor, reduced the phenylephrine-induced increase in [3H]thymidine incorporation at a concentration of 1.0–5.0 μM. In Hep G2, cells using a Neo-selectable system. Lines were produced that were transduced with the selectable marker vector alone or with the addition of an expression plasmid that contained the α1BAR cDNA. Fig. 6, bottom panel, demonstrates the presence or absence of α1BAR mRNA in control cells or in cells transduced with the plasmids. Representative clones were grown and treatment of the new cell lines was undertaken with phenylephrine. It is shown in Fig. 7 that treatment of the Hep

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**Fig. 1. Ethanol-induced apoptosis in rat hepatocytes.** Rat hepatocytes were seeded on collagen type I-precoated chamber slides and treated with ethanol at 100 mM for 4 h. Then, the cells were evaluated for the presence of apoptosis as described under “Experimental Procedures.” A fluorescein isothiocyanate filter was used for generation of green images. A, hepatocytes without any treatment; no cells with fragmented nuclei were observed. In B, some cells with fragmented nuclei (arrows) after treatment with ethanol at 100 mM for 4 h were recorded (>630).
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G2 cells that were transduced with the αBAR cDNA with phenylephrine led to hepatocyte proliferation and decreased tTGase activity. Treatment of the cell line containing only the Neo marker plasmid with phenylephrine had no effect on hepatocyte proliferation or tTGase activity.

The adrenergic receptor-mediated signaling response in HepG2 cells was analyzed by determining the phenylephrine-induced Ca²⁺ elevation in αBAR transduced cells and in cells transfected with the Neo marker plasmid (Table I). Phenylephrine stimulation of αBAR cells resulted in a transient Ca²⁺ elevation in the majority of the cells (ranging from 50 to 80% of total cells in different experiments) but had no significant effect on Neo cells. The elevation of [Ca²⁺], in individual cells occurred after a lag time of several minutes, with a relatively broad peak elevation of approximately 300 nM over the basal [Ca²⁺], often with superimposed irregular oscillations (Fig. 8A). In a small fraction (<20%) of the cells, a more regular Ca²⁺ oscillation pattern was obtained, in which the Ca²⁺ concentration returned to basal levels between individual peaks. No significant differences in basal Ca²⁺ concentrations were observed between cells that responded to phenylephrine treatment and those that did not, or between the αBAR cells and the Neo cells (Table I). Treatment of the αBAR cells with ethanol at 100 mM for 5 min prior to phenylephrine stimulation markedly suppressed the Ca²⁺ response, with only 13% of the cells responding to phenylephrine (Table I). Thus, ethanol suppressed α1B adrenergic receptor-mediated signaling in these cells by almost 80%. The small fraction of cells that retained a response to phenylephrine was distinct in that the lag phase prior to the onset of the Ca²⁺ transient was significantly shorter than in the untreated cells and the responding cells had much shorter transients, occurring either singly or as multiple oscillations (>80% of the responding cells; see Fig. 8B), suggesting that this may represent a subfraction of cells with distinct characteristics of Ca²⁺ signaling. Surprisingly, when αBAR cells were pretreated with ethanol for 2 h, the suppression of the phenylephrine-induced Ca²⁺ signaling was overcome, and the cells recovered their Ca²⁺ response. Although the recovery resulted in many cells showing broad Ca²⁺ transients that were similar to those in untreated cells, a large fraction (close to 50%) of the recovered cells had more regular oscillation characteristics with shorter Ca²⁺ transients than the untreated αBAR cells (Fig. 8C). Because prolonged ethanol exposure did not reverse the ethanol enhancement of tTG activity, the recovery data may indicate that the cells can adapt to generate a Gq conformation that couples to αBAR while still retaining tTG activity. However, an alternative explanation is that the α1B adrenergic receptor under these conditions can be induced to couple to other G proteins, e.g. Gq, which activates a different PLC isofrom, namely PLC-β1 (23). The α1B receptor coupling to Gq (or closely related members of the G protein family) has been demonstrated in cotransfection studies (24), although the factors that control the choice by the receptor of its G protein partner have not been elucidated. If this suggestion is correct, it is likely that the signaling pathways that are linked to the enhancement of proliferation are selective for the Gq-coupled PLC-β1 activation mechanism. This may also explain previous reports that vasopressin, which is a potent activator of PLC-β1 in hepatocytes through V1a receptors that are coupled to Gq, is much less effective than phenylephrine in enhancing hepatocyte proliferation (25).
DISCUSSION

We determined previously that tTG cross-linking activity is increased in animal models of acute liver injury and during fibrogenesis (7), and that it appears that TNF-α enhances the expression of tTG (8). In the present report, our results suggest that tTG may manifest two distinct and somewhat conflicting activities in hepatocytes depending on the cellular milieu. During acute or chronic CCl₄ intoxication or during acute ethanol administration, tTG cross-linking activity was enhanced, probably contributing to the often-described inhibitory effects of toxins (or the fibrotic process) on hepatocyte proliferation. On the other hand, α-adrenergic stimulation of hepatocyte proliferation appears to act, at least in part, through tTG G protein activation (GTPase activity).

A series of studies has shown that both acute and chronic ethanol exposure inhibits hepatic regeneration (3, 26, 27). Ethanol has also been shown to inhibit putrescine levels in the liver (3, 28), and the administration of putrescine (27) or its precursors, alanine or glutamine (28), abrogates this inhibition of liver regeneration by ethanol administration. The mechanism by which putrescine abolishes this effect of ethanol on regeneration is unknown. The hypothesis that we have investigated is that putrescine may be exerting this effect on liver regeneration through its known action as a competitive substrate inhibitor of tTGase activity (29). This action of putrescine is important because tTG has been shown to inhibit cell proliferation by a number of investigators in other systems. This is thought to occur by delaying the progression of the cells from S-phase to G₂/M. (30, 31). Our speculation is that ethanol or other profibrogenic agents may enhance tTG expression and

![FIG. 5. A phospholipase C inhibitor and a protein kinase C inhibitor abrogated PNLP-stimulated rat hepatocyte proliferation. Top panel, PNLP-stimulated rat hepatocyte regeneration was reduced by the addition of ET-18-OCH₃ (ET) at a dose of 1.0–5.0 μM during overnight incubation. Bottom panel, PNLP-stimulated rat hepatocyte proliferation was also evaluated with or without the addition of a protein kinase inhibitor, chelerythrine chloride (CC) at a dose range of 1–5 μM. The data were summarized from three or four independent experiments, each performed in triplicate. **, p < 0.01 compared with the controls; #, p < 0.01 compared with PNLP alone (20 μM).]

![FIG. 6. Expression of α1BAR in rat hepatocytes and hepatoma cell lines with or without transduction with an α1BAR cDNA. Top panel, RNA was extracted from cultured rat hepatocytes, Hep G₂ cells, and Alexander cells and probed with an α1BAR cDNA. In the bottom panel, Hep G₂ cells were not transfected (Hep G₂) or transfected with the control plasmid (Neo) or with the α1BAR expression plasmid (clones 1–5). An α1BAR cDNA probe was employed to evaluate the transcripts by Northern blot analysis. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was employed as the control.]

![FIG. 7. Responses of a clone of Hep G₂ cells stably transduced with the α1BAR cDNA to PNLP treatment. The α1BAR-positive cells were treated with PNLP at 20 μM, and [³H]thymidine or [¹⁴C]putrescine incorporation was determined as described under "Experimental Procedures." The data were summarized from two or three independent experiments.]

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<td>Ethanol (2 h)</td>
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* p < 0.001.

² P < 0.01 compared to α1BAR control.
tTGase activity, leading in turn to an inhibition of the proliferation cascade, and that putrescine may enhance hepatocyte proliferation by inhibiting tTGase activity.

Our results in this report indicate that ethanol administration enhances tTGase activity at concentrations that inhibit hepatocyte proliferation in vitro. Although ethanol administration slightly but reproducibly enhanced tTG mRNA levels (see Fig. 2, bottom panel), its effects on tTGase activity were greater (Fig. 2, top panel), suggesting an additional posttranslational mechanism. Moreover, two inhibitors of tTGase, putrescine, and GTP\textsubscript{S} abrogated the ethanol inhibition of hepatocyte proliferation at the same time that they inhibited the cross-linking activity. These findings suggest that ethanol-induced inhibition of hepatocyte proliferation could be caused, at least in part, by enhanced tTGase activity. This effect is likely to be due not only to the inhibition by tTG of cell proliferation but also to the known association of tTG with apoptosis. Our results indicate that hepatocytes demonstrate both diminished proliferation and apoptosis with ethanol treatment (Figs. 1 and 2). This finding of apoptosis induced by ethanol administration may explain the conflicting results concerning the toxic effects of ethanol on hepatocytes. It is consistent with reports of others that ethanol-induced hepatocyte cytotoxicity may be due to apoptosis (32), whereas other have reported cell necrosis with ethanol treatment under special circumstances (33).

It is a well established finding that a series of \(\alpha_1\) adrenergic agonists, such as epinephrine and phenylephrine, enhances hepatocyte proliferation (11, 12). Adrenergic signaling occurs through activation of PLC, which in turn produces the two intracellular messengers, diacylglycerol and inositol 1,4,5-triphosphate. These intermediate messengers mediate the activation of protein kinase C and intracellular [Ca\textsuperscript{2+}] elevation, resulting in calcium oscillations in the cells. The downstream events include activation of cell cycling genes and cell proliferation (see schematic illustration of the cascade, Fig. 9). A wide variety of hormones and other agonists act through specific receptors coupled to different G proteins to stimulate distinct classes of PLC isozymes. For instance, hepatocytes not only contain \(\alpha_{1B}\) adrenergic receptors that couple to \(G_h\) to activate PLC-\(\delta_1\) but are also rich in vasopressin V\textsubscript{1a} receptors that activate the PLC-\(b_1\) isoform through \(G_{\alpha_q}\). By contrast, the PLC-\(b_2\) isoform is preferentially activated by \(\beta\gamma\) subunits that can be generated by different G proteins (34). Growth factors, such as epidermal growth factor or hepatocyte growth factor,
activate the PLC-γ1 isofrom through receptor tyrosine kinases, an activity that involves Gα1 (35). All these PLC isoforms hydrolyze phosphatidylinositol 4,5-bisphosphate to generate the second messenger inositol 1,4,5-trisphosphate, which causes the release of intracellular Ca²⁺ stores by binding to the inositol 1,4,5-trisphosphate receptor in the endoplasmic reticulum. Yet, for reasons that have not been elucidated, different agonists give rise to substantially distinct Ca²⁺ oscillation patterns. This may reflect specific features of control or subcellular localization of the different PLC isoforms. The functional consequences of these different PLC signaling branches are not known. However, previous studies established that the enhancement of cell proliferation by G protein coupled receptor agonists is much more potent for phenylephrine than for vasopressin or other hormones acting through different receptors (25). Thus, it is likely that the different PLC isoforms activate distinct downstream signaling pathways, leading to different functional consequences for the cell. Our data suggest that the Gαq-dependent activation of PLC-δ1 by phenylephrine is preferentially involved in the enhancement of cell proliferation and accounts for the ethanol sensitivity of this process.

Our findings indicate that phenylephrine administration did enhance hepatocyte proliferation, while decreasing tTGase cross-linking activity, and that these effects were abolished by the use of selective PLC or protein kinase C inhibitors, indicating that G protein signaling was crucial. The adrenergic agonist had no effect on proliferation or tTGase cross-linking activity in hepatoma-derived cell lines, despite the fact that the cell lines contained tTG mRNA (8). In an attempt to explain this result, we demonstrated that whereas hepatocytes had substantial amounts of α1BAR mRNA, the hepatoma cell lines lacked the mRNA for the receptors as determined by Northern blot analysis. However, when a Hep G2 cell line with stably transduced α1BAR cDNA was established, it responded to phenylephrine administration with increased proliferation and with decreased tTGase activity. In addition, Ca²⁺ oscillations were induced in the transduced cells with phenylephrine treatment. Ethanol treatment inhibited the α1BAR-mediated Ca²⁺ signals.

These experiments suggested that phenylephrine stimulates hepatocyte proliferation, at least in part, through binding the α1BAR. Moreover, other authors have demonstrated that tTG acts as the novel GTP-binding protein (Gαq) that transmits the α1B adrenergic receptor signal to PLC-δ1 through its GTPase activity (9), and they have identified the interactive sites on Gαq for the α1BAR (36). Thus, the combination of our results and the findings of others suggest that tTG may well serve as a primary G protein intermediate in phenylephrine-induced stimulation of hepatocyte proliferation.

A question that remains to be resolved is the mechanism responsible for the "switch" that appears to occur between tTG cross-linking activity located in the cytosol and the GTPase activity residing in the membrane fraction. The explanation of how the increase in the GTPase activity may be associated with a concomitant decrease in the tTGase activity is not clear. It is necessary to determine whether there is an associated translocation of the protein or a posttranslational change in the receptor-G protein complex, or whether there is some other explanation. The mechanism responsible for this effect needs further elucidation. What is more clear is that tTG may well play a significant role in the regulation of hepatocyte proliferation in both physiological and pathophysiological conditions.

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