Norepinephrine modulates the zonally different hepatocyte proliferation through the regulation of transglutaminase activity

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Hepatocytes are classified into periportal hepatocytes (PPH) and perivenous hepatocytes (PVH) on the basis of previous studies that demonstrated zonal differences in metabolism (13, 22) and cellular proliferation (12, 29, 36). In an experimental model induced by 70% partial hepatectomy, PPH and PVH show different growth capacities, and this regenerative growth process is regulated by endogenous growth factors and cyto- kines (11, 33). We and others have reported that cultured PPH and PVH show different responses to various mitogens such as epidermal growth factor (EGF) and hepatocyte growth factor (HGF) (14, 21, 29). However, the molecular mechanisms underlying the zonal differences in proliferative response are not well understood.

In addition, the liver regeneration is thought to be innervated by both the sympathetic and parasympathetic nerves. A sympathetic and/or parasympathetic denervation has previously been reported to cause a delay in the DNA synthesis and cellular proliferation after partial hepatectomy (15, 35, 42). A neurotransmitter, norepinephrine (NE), rises rapidly in the plasma within 1 h after hepatectomy (5) and induces secretion of EGF from Brunner’s glands of the duodenum (38). In hepatocyte culture, NE amplifies the mitogenic effects of EGF by acting on the α1-adrenergic receptor (4). The specificity to this receptor type was determined by the use of a selective antagonist. However, the molecular mechanism by which NE has this effect remains unclear.

Adrenergic agonists such as epinephrine and phenylephrine have been shown to enhance hepatocyte proliferation through binding to the α1-adrenergic receptor that is coupled with Gαh (32, 50). Gαh has the role of guanosine triphosphatase (GTPase) like other G proteins, which bind and hydrolyze GTP (20, 27). Gαh is also known as a transglutaminase 2 (TG2), which catalyzes a calcium-dependent amidation reaction, resulting in protein-protein cross-linking through the formation of ε-(γ-glutamyl)lysine isopeptide bonds (34). Thus Gαh (TG2) is a bifunctional enzyme with GTPase and cross-linking activities. The GTPase function of Gαh has been shown to be involved in regulation of cell cycle progression and receptor-mediated signaling (31). On the other hand, the cross-linking function of TG2 has been shown to be involved in cross-linking of the extracellular matrix (48), apoptosis (30), and downregulation of cellular proliferation (50). We reported that inhibition of de novo synthesis of TG2 resulted in promoting the growth of cultured rat hepatocytes in the presence of EGF (23) or HGF (24). Moreover, we have recently reported that TG2 is involved in the difference in growth capacities between PPH and PVH through downregulation of EGF receptor (EGFR) activation (29).

These findings raise the possibility that NE may modulate the zonal difference in proliferative capacity between PPH and PVH through regulation of switching between GTPase function of Gαh and cross-linking function of TG2.

In the present study, to test this possibility, we investigated the influence of NE on EGF-induced EGFR activation and DNA synthesis and TG2 activity in cultured PPH and PVH.

Materials and Methods

Animals and materials. Male Wistar rats (SLC, Hamamatsu, Japan) were kept at a controlled temperature (23 ± 1°C) under a 12-h...
light-dark cycle and were maintained on a standard diet and water. All animal experiments were approved by the Animal Care and Use Committee of the Tohoku Pharmaceutical University. [Methyl-\(^{3}H\)]thymidine, [\(^{3}H\)]NHE, [\(^{125}I\)]EGF, [\(^{14}C\)]putrescine, and inositol 1,4,5-triphosphate [\(^{3}H\)]radioceptor assay kit were obtained from Perkin-Elmer Life Sciences (Boston, MA). Collagenase was obtained from Nitta Gelatin (Osaka, Japan). Digitonin, NE, prazosin, yohimbine, metoprolol, and butoxamine were obtained from Sigma-Aldrich (St. Louis, MO). Mouse EGF was obtained from Biomedical Technologies (Stoughton, MA). Anti-ErbB-1 (EGFR) polyclonal antibody, anti-phospho-EGFR at Y1173 antibody and goat anti-rabbit IgG antibody conjugated with peroxidase were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). Anti-TG 2 antibody was obtained from Neomarker (Fremont, CA). NE (g-t-glutamyl)-l-lysine (Gln-Lys) isopeptide mouse monoclonal antibody (81D4) was obtained from Covalab (Villeurbanne, France).

\textbf{PPH and PVH isolation and culture.} PPH and PVH were isolated from separate animals by the digitonin-collagenase perfusion technique; a detailed procedure has been described previously (36). Separation of PPH and PVH was confirmed by measuring enrichment in two specific marker enzymes, alanine aminotransferase for PPH (41) and glutamine synthetase for PVH (49). Viability of hepatocytes was determined by Trypan blue staining and was at a level of more than 90%. PPH and PVH were placed in 12-well collagen-coated plates (Iwaki, Tokyo, Japan) at a density of 0.8 × 10^5 cells/cm^2 in Williams’ E medium containing 10% fetal bovine serum, 10\(^{-4}\) M NE, 10\(^{-5}\) M prazosin, 10\(^{-5}\) M yohimbine, 10\(^{-5}\) M metoprolol, and 10\(^{-5}\) M butoxamine. The hepatocytes were treated 1 h before EGF treatment. The radioactivity was measured with a Beckman LS6500 liquid scintillation counter (Beckman Coulter, Fullerton, CA).

\textbf{Assay for binding of radiolabeled ligand to cultured hepatocytes.} Hepatocytes were plated in 12-well collagen-coated plates at a density of 0.8 × 10^5 cells/cm^2. After attachment, hepatocytes were incubated for 24 h at 37°C with serum-free medium. Culture medium was then replaced with binding buffer containing 50 mM HEPES (pH 7.4), 128 mM NaCl, 5 mM KCl, 1.2 mM CaCl_2, 5 mM MgSO_4, and 0.5% BSA. The reaction was started by the addition of radiolabeled ligand. Incubation was terminated at 4°C by adding ice-cold 20% trichloroacetic acid after removing the media as recommended by the manufacturer. The radioactivity of each filter paper was measured with a Beckman LS6500 liquid scintillation counter.

\textbf{Assay for PLC activity.} Phospholipase C (PLC) activity was assessed by the production of inositol 1,4,5-triphosphate (IP_3) in hepatocytes using IP_3-[\(^{3}H\)]radioceptor assay kit. The production of IP_3 was determined in the presence and absence of 10^{-4} M NE. IP_3 production in hepatocytes was stopped at 1 h by adding ice-cold 20% trichloroacetic acid after removing the media as recommended by the manufacturer. The radioactivity was measured with a Beckman LS6500 liquid scintillation counter.

\textbf{Statistical analysis.} For the binding assay of [\(^{125}I\)]EGF or [\(^{3}H\)]NHE, K_d values of PPH and PVH were compared as correlation coefficients. Student’s t-test was used for the statistical analysis of [\(^{14}C\)]putrescine incorporation into PPH and PVH and IP_3 production as PLC activity.

\textbf{RESULTS}

\textbf{Effect of NE and adrenoceptor antagonists on EGF-induced DNA synthesis in cultured PPH and PVH.} To examine the effect of NE on zonally different hepatocyte proliferation, we investigated DNA synthesis induced by EGF treatment with or without NE in cultured PPH and PVH (Fig. 1). NE markedly potentiated the EGF-induced DNA synthesis in PVH but not in PPH. Next, we investigated the effect of selective adrenoceptor antagonists on the ability of NE to stimulate DNA synthesis in hepatocytes in the presence of EGF (Fig. 1). Only the specific \(\alpha_1\)-adrenoceptor antagonist, prazosin, prevented the effect of NE on DNA synthesis in PVH. Yohimbine, an \(\alpha_2\)-adrenoceptor antagonist; metoprolol, a \(\beta_1\)-adrenoceptor antagonist; and bu-

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toxamine, a β2-adrenoceptor antagonist, were almost completely ineffective. The addition of 10 ng/ml of aphidicolin (a specific inhibitor of the replicative enzyme, DNA polymerase) to cultured hepatocytes completely abolished EGF stimulated [methyl-3H]thymidine incorporation without any effect on cell viability (data not shown).

[3H]NE binding to cultured PPH and PVH. To characterize the zonal differences of NE-induced response between PPH and PVH in EGF-induced DNA synthesis, characteristics of [3H]NE binding to PPH and PVH were assessed. Scatchard plots comparing [3H]NE binding in both subpopulations are shown in Fig. 2. In both subpopulations, the Scatchard plots were curvilinear, indicating the presence of two classes of NE binding sites; one of high affinity and one of low affinity. Figure 2 (inset) shows saturation curves of [3H] NE-specific binding to its receptor. In PPH, dissociation constants (Kd) were 147 (high-affinity) and 1,500 (low-affinity) pM, and the numbers of binding sites (Bmax) were 2.52 (high-affinity) and 8.91 (low-affinity) pM, respectively. On the other hand, in PVH, Kd values were 112 (high-affinity) and 938 (low-affinity) pM, and Bmax values were 8.74 (high-affinity) and 31.9 (low-affinity) pM. PVH was found to have a greater affinity and number of receptors compared with PPH for NE.

Effect of NE on [125I]EGF binding to cultured PPH and PVH. To identify the locus of the NE-dependent alterations in EGF-induced DNA synthesis, the characteristics of [125I]EGF binding to PPH and PVH were assessed. As shown in Fig. 3A, a Scatchard plot of the binding data for control PPH was curvilinear and yielded two apparent Kd of 9.12 pM (high-affinity) and 107 pM (low-affinity). Bmax was 0.95 (high-affinity) and 6.15 (low-affinity) pM, respectively. In NE-treated PPH, Kd values were 8.06 (high-affinity) and 101 (low-affinity) pM, and Bmax values were 0.82 (high-affinity) and 6.71 (low-affinity) pM, respectively. In PPH, NE treatment showed no significant change in affinity of EGF for either of these sites or in receptor number. In control PVH, Kd values were 30.1 (high-affinity) and 212 (low-affinity) pM, and Bmax values were 1.36 (high-affinity) and 7.02 (low-affinity) pM, respectively (Fig. 3B). On the other hand, Kd values in NE-treated PVH were 9.04 (high-affinity) and 195 (low-affinity) pM, and Bmax values were 0.87 (high-affinity) and 6.27 (low-affinity) pM, respectively (Fig. 3B). In PVH, NE treatment led to a significant increase in the affinity of EGF to its receptor, whereas there was no significant difference in Bmax values.

Effect of NE on EGFR activation in cultured PPH and PVH. Next, we investigated the effect of NE on EGFR dimerization. Addition of EGF induced redistribution of the receptors from the 170- to 175-kDa band to the 340- to 350-kDa band, corresponding to the monomeric and dimeric forms of EGFR, respectively. As the control for EGFR activation, hepatic membranes from cultured hepatocytes without EGF treatment were used (Fig. 4A, lane 1). As shown in Fig. 4, the dimeriza-
Affinities were determined at a fixed concentration of [125I]EGF by competition. TG2 is a bifunctional enzyme possessing transglutaminase cross-linking and GTPase activities. The cross-linking activity of TG2 showed good correlations with its dimerization (Fig. 4). As shown in Fig. 5, the phosphorylation of EGFR on various time points following EGF treatment. As the control, in NE-untreated cells, the formation of isopeptide bonds in EGFR was identical to those in PPH. In NE-treated cells of both subpopulations, TG2 expression was the same as control level (Fig. 6B), whereas TG2-catalyzed cross-linking activity was significantly decreased in PVH but not in PPH (Fig. 6A). In all cases, EGF treatment showed no changes in TG2 expression and activity.

Effect of NE on TG2-catalyzed isopeptide cross-linking formation. Next, to investigate the effect of NE on TG2-catalyzed cross-linking formation of ε-(γ-glutamyl)lysine isopeptide bonds, we performed immunoprecipitation with anti-EGFR antibody and subsequent Western blotting with anti-isopeptide antibody. As shown in Fig. 7, the distribution of 170 kDa cross-linked isopeptide bonds in EGFR was observed. In the NE-untreated group, the formation of isopeptide bonds in EGFR in PVH was higher than that in PPH. By comparison, in the NE-treated group, the level of isopeptide bond in EGFR in PVH significantly decreased, but not in PPH. In both cases, EGF treatment showed no changes in formation of isopeptide bonds in EGFR (data not shown). Data showed a good correlation between TG2 activity and TG2-catalyzed cross-linked products, isopeptide bonds.

Effect of NE on PLC activity. PLC-61 is identified as an effector molecule in G_{G_{0/1}}-mediated signaling. Activated PLC catalyzes the hydrolysis of phosphatidylinositol 4,5-biphosphate to produce two second messengers: IP3 and diacylglycerol. Thus, to investigate whether Go_{G_{0/1}}-mediated PLC-61 is activated by NE treatment, we determined the production of IP3 by PLC. As shown in Fig. 8, in PPH, NE treatment showed almost no changes in PLC activity. By comparison, in PVH, its activity was significantly increased to seven times the level of NE-untreated cells. In both subpopulations, EGF treatment showed a slight increase in its activity (data not shown).

**DISCUSSION**

Numerous studies using in vivo and in vitro models have provided evidence that a neurotransmitter, NE, amplifies the mitogenic effect of growth factor, including EGF in the liver. However, the molecular mechanism of NE-induced mitogenic effect at the proliferative state of the liver is not well understood. To understand this phenomenon in detail, we examined hepatocytes isolated in periportal and perivenous regions of the liver, which differ in proliferative capacity. Several previous reports have indicated that NE has been shown to act via the α_{1}-adrenoceptor on parenchymal hepatocytes to enhance their responsiveness to EGF in a serum-free primary cultured system (3, 19). These findings are consistent with our present data on DNA synthesis using selective adre-
noceptor antagonists (Fig. 1). In the present results, we found here that NE significantly increased EGF-induced DNA synthesis only in PVH. To confirm this zonal specificity, we provide data on the binding of \[^{3}H\]NE to both subpopulations. Our data indicate that PVH has a greater affinity and number of receptors than PPH for NE (Fig. 2). This higher affinity for NE in PVH may be involved in enhancement of EGF-induced DNA synthesis. We and others reported that PPH responded to EGF with higher sensitivity than PVH (10, 12, 29). However, the present data indicated that the zonal differences in EGF-induced DNA synthesis in both subpopulations disappeared with NE treatment. A possible reason for a drastic change in EGF-induced DNA synthesis in PVH is that the characteristics of EGFR may be changed by NE treatment. Thus, to investigate this, we studied the characteristics of \[^{125}I\]EGF binding to PPH and PVH. Our results indicated that only in PVH did NE significantly increase in the affinity of EGF to its receptor, without a significant change in the number of receptors (Fig. 3). These results suggest that a subclass of high- but not low-affinity EGFR is upregulated by NE treatment. However, Cruise et al. (3) reported that NE decreased the number of EGFR, without a change in receptor affinity to its ligand, regardless of the enhancement of EGF-induced DNA synthesis. A possible reason for the discrepancy is the different conditions used for culture: Cruise et al. cultured hepatocytes under low-density conditions, whereas we cultured them under high-density but not confluent conditions. It has been reported that culturing hepatocytes at low density gives rise to a rapid loss of liver-specific functions (9). In fact, the previous study indicated that specific markers in PPH and PVH were abolished in cells cultured under low-density conditions (29). Therefore, we think that hepatocytes cultured under high-density conditions reflect the in vivo conditions to some extent.

In general, it is assumed that EGF preferentially binds to high-affinity EGFR, which is in equilibrium with low-affinity EGFR, and thereafter the EGF-EGFR complexes undergo dimerization, resulting in activation of the downstream signaling pathway (7, 17). Indeed, the present results show that the expression of EGF-induced dimerization was highly correlated with the appearance of high-affinity EGFR in both cases: the control group and the NE-treated group (Fig. 4). Moreover, the pattern of phosphorylation of EGFR at tyrosine 1173, which is essential for EGF-induced hepatocyte growth, was nearly parallel to that of dimerization of its receptor (Fig. 5). Therefore, we think that NE-induced EGFR upregulation as well as affinity for its ligand, dimerization, and phosphorylation must

Fig. 4. Effect of NE on EGF receptor (EGFR) dimerization induced by EGF treatment in cultured PPH and PVH. After EGF (10^{-8} M) treatment at intervals (1, 5, 10, 30, and 60 min), the proteins were cross-linked with bis-(sulfosuccinimidyl) suberate (BS'). The cell membranes obtained from cultured hepatocytes were then lysed and the protein samples were immunoblotted with anti-EGFR antibodies. The band quantitation was performed with National Institutes of Health image software. A: immunodetections of EGFRs (monomer and dimer) are shown. Results represent 1 typical experiment. WB, Western blot. B: histogram represents means ± SE of 3 independent experiments, expressed as a relative percentage to the peak of EGFR monomer in untreated PPH, taken as 100%.
be related to hepatocyte proliferation. In the present study, NE stimulates EGFR dimerization and tyrosine phosphorylation in the absence of EGF stimulation and that this effect is greater in PVH than in PPH. Previous reports have suggested that the EGFR may be utilized by G protein-coupled receptor (GPCR) as intermediate signaling protein (8). GPCR-induced activation of EGFR signaling is the so-called EGFR transactivation that has been originally designated as ligand-independent tyrosine phosphorylation of EGFR (6). Moreover, GPCR has been demonstrated to stimulate the activity of a membrane-spanning matrix metalloprotease, resulting in the release of heparin-binding EGF and transforming growth factor-β, and subsequently, in a ligand-mediated paracrine activation of EGFR (40, 44). Therefore, the present results suggest that NE treatment alone may stimulate EGFR dimerization and phosphorylation by GPCR/EGFR cross talk as a mechanism for NE potentiation of EGFR signaling, but this effect is only modest in the present primary cultured system.

In the previous study, we have found that TG2, which catalyzes the cross-linking reaction between proteins by the formation of isopeptide bonds, regulates the binding affinity of high-affinity EGFR but not that of low-affinity receptor (29). Previous reports have suggested that the EGFR may be utilized by G protein-coupled receptor (GPCR) as intermediate signaling protein (8). GPCR-induced activation of EGFR signaling is the so-called EGFR transactivation that has been originally designated as ligand-independent tyrosine phosphorylation of EGFR (6). Moreover, GPCR has been demonstrated to stimulate the activity of a membrane-spanning matrix metalloprotease, resulting in the release of heparin-binding EGF and transforming growth factor-α and, subsequently, in a ligand-mediated autocrine and/or paracrine activation of EGFR (40, 44). Therefore, the present results suggest that NE treatment alone may stimulate EGFR dimerization and phosphorylation by GPCR/EGFR cross talk as a mechanism for NE potentiation of EGFR signaling, but this effect is only modest in the present primary cultured system.

In the previous study, we have found that TG2, which catalyzes the cross-linking reaction between proteins by the formation of isopeptide bonds, regulates the binding affinity of high-affinity EGFR but not that of low-affinity receptor (29). Moreover, we have shown that TG2 downregulates EGFR activation through intramolecular cross-linking of its receptor, resulting in inhibition of hepatocyte proliferation (28). Thus, to investigate the effect of NE on TG2 activity, we measured its protein expression and cross-linking activity. As a result, TG2-catalyzed cross-linking activity represented by [1,4-14C]putrescine incorporation and by detection of isopeptide bonds was decreased in PVH but not in PPH, whereas TG2 protein expression showed no change in both subpopulations (Figs. 6 and 7). The present data suggest that this decrease in TG2 cross-linking activity in PVH may lead to amplification of the DNA synthesis. In addition to its cross-linking activity, TG2 has a role performing the GTPase function of a G protein (Gβγ). It has been reported that Gβγ-adrenergic stimulation of hepatocyte proliferation appears to act, at least in part, through activated GTPase performing as Gβγ (50). Adrenergic signaling occurs through activation of PLC, which in turn produces the two intracellular messengers, diacylglycerol and IP3. These intermediate messengers mediate the activation of protein kinase C and intracellular calcium elevation. The downstream events include activation of cell cycling genes and cell proliferation in malignant hamster fibrosarcoma (31). Wu et al. reported that Gβγ-dependent activation of PLC-61 by phenylephrine was preferentially involved in the enhancement of rat hepatocytes proliferation (50). In the present study, NE treatment alone significantly increased the PLC activity in PVH but not in PPH (Fig. 8) and did not influence the DNA synthesis in either subpopulation (Fig. 1). Kimura and Ogihara (25, 26) indicated that α- and β-adrenoceptor agonists alone did not significantly influence hepatocyte DNA synthesis and proliferation. Thus, considering the results of previous reports in addition to our present findings, it is possible that NE-induced switching from TG2 to Gβγ, i.e., decrease of TG2 cross-linking activity, is far more important for the early stage of cell proliferation than Gβγ-dependent downstream signaling events.

Fig. 5. Effect of NE on EGFR phosphorylation induced by EGF treatment in cultured PPH and PVH. After EGF (10−8 M) treatment for the indicated times, the cell membranes obtained from cultured hepatocytes were lysed and the protein samples were immunoblotted with antibodies to EGFR phosphorylated at Y1173 and EGFR. To determine specific tyrosine phosphorylation of the EGFR, PVDF membranes probed with anti-phosphotyrosine antibody were stripped with a solution of 0.1 M glycine (pH 2.1) and reprobed with anti-EGFR antibody. The band quantitation was performed with National Institutes of Health image software. A: immunodetections of EGFR phosphorylation and EGFR are shown. Results represent 1 typical experiment. B: histogram represents means ± SE of 3 independent experiments, expressed as a relative percentage to the peak of EGFR phosphorylation in NE-treated PVH at 5 min following EGF treatment, taken as 100%.
We have demonstrated that the level of TG2 in PVH is higher than that in PPH, and this higher level of TG2 in PVH leads to lower EGF-induced DNA synthesis than PPH through downregulation of EGFR by TG2 (28, 29). The present results suggest that NE-induced amplification of EGF-induced DNA synthesis in PVH is caused by upregulation of EGFR activation through decrease of TG2 cross-linking activity, i.e., NE-induced switching from TG2 to G

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It has been reported that EGF induces the PLC-γ activation by the autophosphorylation of EGFR at Y1173 (2). Indeed, EGF slightly increased IP3 production in both subpopulations (data not shown). IP3 mediates calcium release from intracellular stores, affecting a host of calcium-dependent enzymes such as Ral (18) and nuclear factor-kB (45). However, in the present study, the activity of TG2 that catalyzes a calcium-dependent cross-linking reaction was decreased by NE treatment (Fig. 6B). Therefore, IP3-mediated calcium oscillation may not be related to the activation of TG2.

Recent studies have shown that the alcohol-induced switching of function from G0 to TG2 may play an important role in ethanol-induced liver disease (50, 51). Tatsukawa et al. (46) reported that ethanol-induced enzymatic TG2 induced hepatocyte apoptosis via transcription factor.
Sp1 cross-linking and inactivation, with resultant downregulation of c-Met expression required for hepatocyte viability. Moreover, TG2, acting as a G protein, has recently been reported to have a protective role against Fas-mediated death pathway in hepatocytes (43). These reports together with ours suggest switching from enzymatic to nonenzymatic TG2 function may be involved in the recovery program of the liver after various kinds of hepatic injury. In the present study, the switching was mediated by NE, a neurotransmitter. Thus the nervous system may play an important role in homeostasis or disease onset of the liver.

The manipulation of TG2/Gαo activity will modulate the NE-EGFR pathway and may thus be exploited therapeutically. A variety of compounds such as cysteamine, 5-(bioninamido) pentylamine, and gluten peptides have been used to suppress TG2-catalyzed protein cross-linking. However, since several of these compounds contain primary amines or potential inhibitory motifs, it remains unclear whether the observed effects are due to an excess of competing amine donor or by blocking TG2 turnover (16). On the other hand, TG2 inducers such as ethanol and retinoic acid have not only inducible effect of TG2, but also multiple possible effects (39, 47). Thus resolution of this issue will require further detailed studies using the highly selective reagents to manipulate the TG2 activity or a more direct method.

Adrenergic regulation is now thought to be involved in the hepatic regenerative process in vivo. Until now, effect of NE on liver regeneration was not well understood. In the present hepatic regenerative process in vivo. Until now, effect of NE-EGFR pathway and may thus be exploited therapeutically. A variety of compounds such as cysteamine, 5-(bioninamido) pentylamine, and gluten peptides have been used to suppress TG2-catalyzed protein cross-linking. However, since several of these compounds contain primary amines or potential inhibitory motifs, it remains unclear whether the observed effects are due to an excess of competing amine donor or by blocking TG2 turnover (16). On the other hand, TG2 inducers such as ethanol and retinoic acid have not only inducible effect of TG2, but also multiple possible effects (39, 47). Thus resolution of this issue will require further detailed studies using the highly selective reagents to manipulate the TG2 activity or a more direct method.

Adrenergic regulation is now thought to be involved in the hepatic regenerative process in vivo. Until now, effect of NE on liver regeneration was not well understood. In the present study, we demonstrate the possibility that NE affects hepatocyte proliferation by the modification of EGF receptor activation through the switching of function between TG2 and Gαo. Moreover, these results raise the possibility that NE may regulate the characteristic pattern of cell growth for PPH and PVH. However, the increase in GTPase activity as Gαo may be associated with a concomitant decrease in the TG2 cross-linking activity. What is more clear is that NE-mediated change in relative amount of TG2 vs. GTPase acting as Gα may well play a significant role in the regulation of zonally different hepatocyte proliferation.

DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the author(s).

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