Zonal differences in effects of HGF/SF and EGF on DNA synthesis in hepatocytes under fed or starved conditions

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Imai, Kimiko, Tetsuya Mine, Megumi Tagami, Kazuo Hanaoka, and Toshiro Fujita. Zonal differences in effects of HGF/SF and EGF on DNA synthesis in hepatocytes under fed or starved conditions. Am. J. Physiol. 275 (Gastrointest. Liver Physiol. 38): G1394–G1401, 1998.—Zonal differences of DNA synthesis in hepatocytes induced by hepatocyte growth factor and/or scatter factor (HGF/SF) and epidermal growth factor (EGF) were investigated using male Wistar rats under fed or starved conditions. Overall, DNA synthesis was greater in fed rats than in starved rats. The predominance of EGF in perivenular hepatocytes (PPH) on zonal DNA synthesis was reversed by starved conditions, but the predominance of HGF/SF on zonal DNA synthesis in perivenous hepatocytes (PVH) was not influenced by nutritional conditions. 125I-labeled EGF and 125I-labeled HGF/SF-receptor binding studies revealed no significant difference between PPH and PVH in starved or fed rats. To investigate the mechanism of the signal transduction pathway, we used genistein, an inhibitor of tyrosine kinase. Genistein had different effects on zonal difference in EGF and HGF/SF. In EGF, 1 µg/ml genistein abolished zonal differences, but in HGF/SF 1 µg/ml genistein did not abolish zonal differences. These data suggest that, in contrast to HGF/SF, zonal difference of DNA synthesis by EGF was dependent on nutritional conditions and DNA synthesis induced by HGF/SF and EGF might be related to tyrosine kinase, but the influence of tyrosine kinase on DNA synthesis was different between HGF/SF and EGF.

hapatocyte growth factor and/or scatter factor; epidermal growth factor

Numerous studies indicate that there is a morphological, biochemical, and functional (transport and metabolic) heterogeneity between peripoortal hepatocytes (PPH) and perivenous hepatocytes (PVH). As for carbohydrate metabolism, processes of both gluconeogenesis and glycogenolysis seem to occur predominantly in PPH, whereas glycolysis and glycogenesis appear to occur mainly in PVH (10, 11, 30). With regard to the metabolism of amino acid, urea synthesis seems to occur predominantly in PPH and glutamine synthetase (GS) is mainly distributed in PVH (7, 11, 30).

On the other hand, hepatocyte growth factor and/or scatter factor (HGF/SF), one of the major agents for proliferation of hepatocytes, was originally purified from rat platelets (16, 22) and plasma from patients with fulminant hepatic failure (5, 23). It is a disulfide-linked heterodimer protein consisting of a 55- to 60-kDa heavy (α-) chain and a 32- to 34-kDa light (β-) chain (5, 23). HGF/SF induces DNA synthesis in primary cultured hepatocytes. Epidermal growth factor (EGF), which is also one of the major agents for proliferation of hepatocytes, is a single chain polypeptide that consists of 53 amino acids and stimulates DNA synthesis in primary cultured hepatocytes (8). Both polypeptides induce proliferation of hepatocytes, but there have been few studies regarding zonal differences with respect to proliferative effect.

Recently, it has been reported (27, 28) that liver-specific immediate-early genes encode proteins important for glucose homeostasis and cellular metabolism and that genes encoding enzymes that oppose gluconeogenesis, such as pyruvate kinase, decrease rapidly at the time of partial hepatectomy. The expression of gluconeogenic genes is also controlled by hepatic transcription factors, such as hepatic nuclear factor-1 (HNF-1) and CAAT enhancer binding protein (C/EBP). Furthermore, EGF is well known to induce gluconeogenesis (9). Considering these facts, it seems to be possible that nutritional conditions might affect the efficacy of these growth factors in PPH and PVH.

In the present study, we first investigated zonal differences of hepatocyte proliferation caused by HGF/SF and EGF under various nutritional conditions. Second, we investigated the 125I-labeled HGF/SF and 125I-labeled EGF specific binding to PPH and PVH under each condition to examine mechanisms by which these differences occurred. Third, we investigated the role of postreceptor signaling in zonal difference. Multiple step signaling events are involved in signal transduction by activation of the cell surface receptor leading to nuclear events. Inhibitors in signaling pathways at each step could be useful in elucidating mechanisms of signaling of growth factor. An isoflavonoid compound, genistein, was an inhibitor of tyrosine-specific protein kinase. HGF/SF receptor (c-Met protooncogene) possesses tyrosine kinase, and HGF/SF induced phosphorylation of tyrosine kinase after binding to the receptor. The EGF receptor also possesses tyrosine kinase, and EGF induces phosphorylation of tyrosine kinase after binding to the receptor. Therefore, it seems possible to investigate the mechanism of zonational dependency of EGF- or HGF/SF-induced DNA synthesis by using genistein. In the present study, zonal differences of hepatocyte proliferation caused by HGF/SF and EGF under various nutritional conditions, where multiple-step signaling events of transduction of growth factor activation existed, were investigated.

Materials and Methods

Preparation and culture of PPH and PVH. Fed or starved (48 h) male Wistar rats, weighing 170 g, were used for experiments. The study was approved by the Institutional Animal Care and Use Committee of Tokyo University.
For PPH preparation, the abdomen was incised, and the portal vein was cannulated. Immediately, the liver perfusion was started with perfusate at a flow rate of 25 ml/min (Krebs-Ringer HEPES buffer containing 0.5 mM EDTA, 37°C), and the inferior vena cava was incised. The chest was opened, and the thoracic portion of the inferior vena cava was cannulated. Then the abdominal portion of the inferior vena cava was ligated. After 10 min of anterograde (portal-to-caval) perfusion, the direction of flow was changed to retrograde (caval-to-portal), and perfusate was then changed to the solution containing digitonin (7 nM) at a rate of 5 ml/min for 40 s to selectively destroy perivenous cells. The direction was changed to anterograde again, and perfusate was changed to solution containing collagenase (0.05 wt/vol). After the digitonin solution was washed out in the opposite direction, the perfusate was recirculated.

PPH preparation was performed by perfusion in the opposite direction to PPH. The abdomen and chest were incised, and the thoracic portion of the inferior vena cava was cannulated. After the beginning of perfusion, the portal vein was cannulated. After 10 min of retrograde perfusion, the direction of flow was changed to anterograde, and the liver was perfused with the solution containing digitonin at a rate of 5 ml/min for 40 s to selectively destroy portal cells. Then the direction was changed to retrograde again, and perfusate was changed to solution containing collagenase. After the digitonin solution was washed out in the opposite direction, the perfusate was recirculated.

After 10 min of perfusion with solution containing collagenase, the liver lobes were carefully transferred in DMEM (Nissui, Tokyo, Japan) and minced gently. The minced liver tissue was filtered through a 100-nylon mesh. After centrifugation at 500 rpm for 1 min, the supernatant was removed, and the tissue was filtered through a 100-nylon mesh. After centrifugation at 2,000 rpm for 10 min. The supernatant was discarded, and the pellet was resuspended in 100 µl of 1% gelatin and 5 mM KI. Then 10% TCA was added to, and the precipitate was collected by centrifugation at 2,000 rpm for 10 min. The supernatant was discarded, and the pellet was resuspended in 100 µl of 1% gelatin and 5 mM KI. The mixture was incubated for 10 s at room temperature. The reaction was stopped by adding 50 µl of 2.5 mg/ml sodium metabisulfite in 50 mM sodium phosphate buffer (pH 7.4) containing 0.5 M NaCl. The labeled protein was separated by free iodine by gel filtration on Sephadex G-25 fine column (0.8 × 25 cm) equilibrium with PBS containing 0.25% gelatin and 5 mM KI.

Radioiodination of HGF/SF. HGF/SF was iodinated by the chloramine-T method (21). HGF/SF (10 µg) was mixed with 0.5 mCi of 125I-labeled sodium in 50 µl of 0.5 M sodium phosphate buffer (pH 7.4) containing 0.5 M NaCl, and then 50 µl of 1 mg/ml chloramine-T in 50 mM sodium phosphate buffer (pH 7.4) containing 0.5 M NaCl were added, and the mixture was incubated for 10 s at room temperature. The reaction was stopped by adding 50 µl of 0.1, 1, 5, and 10 µM. At the end of incubation, cells were washed four times with ice-cold binding buffer and dissolved in 1 ml of a solution containing 2% SDS, 2 mM EDTA, and 10 mM NaHCO3. Radioactivity of 125I-HGF/SF in the presence or absence of unlabeled agents (5 × 10⁻¹⁰ M). At the end of incubation, cells were washed four times with ice-cold binding buffer and dissolved in 1 ml of a solution containing 2% SDS, 2 mM EDTA, and 10 mM NaHCO3. Radioactivity of 125I-HGF/SF bound to cells was measured with a gamma counter (Aloca ARC-380, Tokyo, Japan). The specific binding was calculated after subtracting the radioactivity of the samples containing an ~40- to 1,600-fold excess of unlabeled HGF/SF.

Assay for binding of 125I-EGF to cultured hepatocytes. Hepatocytes were plated in a 24-well collagen-coated dish at a density of 10⁴ cells/well. After attachment, monolayer cells were incubated for 4 h with binding buffer containing 50 mM HEPES (pH 7.4), 128 mM NaCl, 5 mM KCl, 1.2 mM CaCl₂, 5 mM MgSO₄, and 0.5% BSA. After 4 h, the monolayer cells were incubated for 24 h at 4°C with a binding buffer containing various concentrations of 125I-HGF/SF in the presence or absence of unlabeled agents (5 × 10⁻¹⁰ M). At the end of incubation, cells were washed four times with ice-cold binding buffer and dissolved in 1 ml of a solution containing 2% SDS, 2 mM EDTA, and 10 mM NaHCO3. Radioactivity of 125I-EGF in the presence or absence of unlabeled agents (10⁻³ M) in the presence or absence of unlabeled agents (10⁻³ M) in 100 µl of solution containing 2% SDS, 2 mM EDTA, and 10 mM NaHCO3. Radioactivity of 125I-EGF bound to cells was measured with an Aloca ARC-380 gamma counter.
Materials. Male Wistar rats were purchased from Tokyo Jikken Dobutsu (Tokyo, Japan). Collagenase was purchased from Wako Junyaku (Tokyo, Japan). Digitonin, EGF, HGF/SF, and genistein were purchased from Sigma Chemical. [3H]thymidine and [125I]-EGF were purchased from DuPont NEN (Boston, MA).

Statistical analysis. The Mann-Whitney test was used for statistical analysis of [3H]thymidine incorporation of PPH and PVH. For the binding assay of [125I]-HGF/SF and [125I]-EGF, dissociation constant (Kd) values of PPH and PVH were compared as correlation coefficients. When appropriate, P < 0.05 was considered to be significant.

RESULTS

Separation of PPH and PVH. The ALT activity of PPH was approximately twice that of PVH under both fed and starved conditions, as previously reported by Morrison et al. (20). BUN synthesis of PPH was about two times greater than that of PVH in both fed and starved rats, as described by Quistorff et al. (26). The activity of GS in PVH was 13 times greater than that in PPH under fed conditions and 9 times greater than that in PPH under starved conditions, as described by Burger et al. (2). These results indicated successful separation of PPH and PVH in this study.

Time course of DNA synthesis in PPH and PVH. As we described previously (33), DNA synthesis by primary cultured hepatocytes increased gradually, and peak DNA synthesis was at 72 h. In this study, we further investigated DNA synthesis of primary cultured PPH and PVH. We measured [3H]thymidine incorporation from 0 to 24 h, 24 to 48 h, 48 to 72 h, and 72 to 96 h under various conditions. Figure 1A shows the time course without growth factors. The maximum DNA synthesis of PPH and PVH with HGF/SF was observed between 48 and 72 h under both fed and starved conditions. Figure 1B shows the time course after the addition of 5 × 10^{-10} M HGF/SF. The maximum DNA synthesis of PPH and PVH with HGF/SF was observed between 48 and 72 h under both fed and starved conditions. Figure 1C shows the time course after the addition of 10^{-8} M EGF. The maximum DNA synthesis of PPH and PVH with EGF was observed between 48 and 72 h under both fed and starved conditions. In these experiments, the maximum DNA synthesis was observed from 48 to 72 h. Furthermore, both PPH and PVH showed the same time course of DNA synthesis as typical primary cultured hepatocytes under both fed and starved conditions.

Efficacy of HGF/SF and EGF on DNA synthesis in cultured PPH and PVH under fed conditions. Figure 2A shows the efficacy of HGF/SF on DNA synthesis in cultured PPH and PVH under fed conditions. Peak values of DNA synthesis in PPH and PVH were obtained by 5 × 10^{-10} M HGF/SF. HGF/SF at 5 × 10^{-10} M induced a 12-fold increase in DNA synthesis assumed by [3H]thymidine incorporation in PVH but a 7-fold increase in PPH. Similar results were obtained by 10^{-11} and 10^{-10} M HGF/SF; 10^{-9} M HGF/SF also induced the same results as 5 × 10^{-10} M HGF/SF.

Next, we examined the efficacy of EGF on DNA synthesis in cultured PVH and PPH under fed conditions (Fig. 2B). Peak values of DNA synthesis in PPH and PVH were obtained by 10^{-8} M EGF. Unlike HGF/SF, 10^{-8} M EGF induced a 16-fold increase in DNA synthesis in PPH but a 10.5-fold increase in PVH. Similar results were obtained by 10^{-10} and 10^{-9} M EGF; 10^{-7} M EGF also induced the same results as 10^{-8} M EGF.

Efficacy of HGF/SF and EGF on DNA synthesis in cultured PPH and PVH under starved conditions. Next, we examined the efficacy of HGF/SF and EGF on DNA synthesis in cultured PPH and PVH under starved conditions (Fig. 3). After a 48-h starvation period, PVH was more sensitive to HGF/SF than PPH (Fig. 3A). More than 5 × 10^{-10} M HGF/SF induced a 7.1-fold increase in DNA synthesis in PVH and a 4.6-fold increase in PPH. Similar results were obtained by 5 × 10^{-11} and 10^{-10} M HGF/SF.

In contrast to the fed conditions, the efficacy of EGF on the DNA synthesis was greater in PVH than PPH (Fig. 3B). More than 10^{-8} M EGF induced a 9.2-fold increase in DNA synthesis in PVH and a 4.9-fold increase in PPH. Similar results were obtained by 10^{-10} M and 10^{-9} M EGF.

Specificity of [125I]-HGF/SF binding to hepatocytes and Scatchard plot analysis. To study the specificity of [125I]-HGF/SF binding, we added various concentrations...
of unlabeled HGF/SF to assay the ability to compete with \(^{125}\text{I}\)-HGF/SF for binding to hepatocytes. Figure 4 shows a Scatchard plot of bound/free and \(^{125}\text{I}\)-EGF binding rate to the receptor on hepatocytes. Figure 5A shows the results under fed conditions, and Fig. 5B shows those under starved conditions. These data suggest that more than two types of EGF receptors exist on rat hepatocytes. Figure 5 (insets) shows saturation curves of \(^{125}\text{I}\)-EGF-specific binding to its receptor. \(K_d\) values of PPH under fed conditions were 1.41 and 26.7 pM. The receptor numbers were 251 and 2,209 cpm/well. Under starved conditions, \(K_d\) values of PPH were 1.37 and 15.76 pM. The receptor numbers were 193 and 2,574 cpm/well. \(K_d\) values of PVH under fed conditions were 1.46 and 6.58 pM. The receptor numbers were 268 and 2,229 cpm/well. Under starved conditions, \(K_d\) values of PVH were 1.38 and 5.09 pM. The receptor numbers were 320 and 3,616 cpm/well. There was no significant difference in \(K_d\) values between PPH and PVH under fed and starved conditions.

Effect of genistein on DNA synthesis in PPH and PVH. We investigated the effect of genistein on DNA synthesis induced by HGF/SF and EGF in PPH and PVH. Genistein at 10 µg/ml inhibited the increasing
efficacy of HGF/SF or EGF completely under both fed and starved conditions. However, 1 and 5 µg/ml genistein inhibited the increasing efficacy of DNA synthesis by EGF to the degree that zonal difference disappeared (Figs. 6B and 7B). In contrast, zonal differences in DNA synthesis stimulated by HGF/SF did not disappear at 1 and 5 µg/ml genistein (Figs. 6A and 7A). We already demonstrated that, although the efficacy of EGF on DNA synthesis was greater in PPH than PVH under fed conditions, it was greater in PVH than PPH under starved conditions. Moreover, it became obvious that 1 and 5 µg/ml genistein abolished zonal difference by inhibition of the EGF-induced increase in PPH, compared with PVH under fed condition (Fig. 6B). Similar results with genistein were obtained in the EGF-induced increase in PVH, compared with PPH under starved condition (Fig. 7B). In contrast to EGF, the efficacy of HGF/SF on DNA synthesis in both PPH and PVH during fed conditions was inhibited by genistein with preservation of PVH dominance (1 and 5 µg/ml).

Then zonal difference by HGF/SF did not disappear even after addition of genistein (1 and 5 µg/ml).

DISCUSSION

We investigated DNA synthesis in hepatocytes induced by HGF/SF and EGF, with special reference to spatial zone and nutritional condition. BUN synthesis, ALT, and GS activities were examined to confirm separation of PVH and PPH, and the separation was considered successful. We then investigated differences of cell cycles between PPH and PVH under both fed and starved conditions. As DNA synthesis assay, [3H]thymidine incorporation from 0 to 24 h, 24 to 48 h, 48 to 72 h, and 72 to 96 h was measured under various conditions, i.e., with or without growth factor, and under fed or starved conditions. The maximum DNA synthesis induced by HGF/SF and EGF was observed between 48 and 72 h after the addition of the growth factors in both PPH and PVH. From these data, it was concluded that the G1-M phase entry of PVH and PPH occurred at the same time under both fed and starved conditions.

Fig. 4. Scatchard plot of bound/free and 125I-labeled HGF/SF-specific binding to receptors on hepatocytes. With or without unlabeled agents, 10⁵ cells/well of rat hepatocytes were incubated for 4 h at 0°C with various concentrations of 125I-HGF/SF. A: Scatchard plot of bound/free and 125I-HGF/SF-specific binding to receptors on hepatocytes in fed state. A, inset: saturation curves of 125I-HGF/SF-specific binding to its receptor on hepatocytes under fed conditions. B: Scatchard plot of bound/free and 125I-HGF/SF-specific binding to receptors on hepatocytes in starved state. B, inset: saturation curves of 125I-HGF/SF-specific binding to its receptor on hepatocytes under starved conditions.

Fig. 5. Scatchard plot of bound/free and 125I-labeled EGF-specific binding rate to receptor on hepatocytes. With or without unlabeled agents, 10⁵ cells/well of rat hepatocytes were incubated for 4 h at 0°C with various concentrations of 125I-EGF. A: Scatchard plot of bound/free and 125I-EGF-specific binding to receptors on hepatocytes in fed state. A, inset: saturation curves of 125I-EGF-specific binding to its receptor on hepatocytes under fed conditions. B: Scatchard plot of bound/free and 125I-EGF-specific binding to receptors on hepatocytes in starved state. B, inset: saturation curves of 125I-EGF-specific binding to its receptor on hepatocytes under starved conditions.
We next examined zonal differences in DNA synthesis induced by HGF/SF and EGF between PPH and PVH. It was suggested that PVH was more sensitive to HGF/SF than PPH under both fed and starved conditions and that PPH was more sensitive to EGF than PVH under fed conditions, although this was not the case under starved conditions.

In addition, we examined the mechanism by which these differences occurred. We investigated the $^{[125]}$-HGF/SF- and $^{[125]}$-EGF-specific binding to PPH and PVH under each condition. However, there was no significant difference in $K_d$ values between PPH and PVH under each condition.

The data suggested that different responses to growth factors of PPH and PVH might be caused by sensitivities that might change according to the nutritional condition rather than by receptor numbers. We then investigated the effects of genistein, an inhibitor of tyrosine kinase, to learn how zonal difference appeared in postreceptor networks. Our results show that zonal difference disappeared after the addition of genistein. Genistein at 1 µg/ml abolished zonal difference completely by inhibition of increase in PPH induced by EGF under fed condition or by inhibition of increase in PVH under starved condition. The efficacy of HGF/SF on DNA synthesis was inhibited by any doses of genistein in PPH as well as in PVH to the same ratio. Then zonal difference by HGF/SF did not disappear even after addition of genistein.

Gebhardt and Jonitza (4) reported that PPH responded to EGF with higher sensitivity than PVH, although they did not investigate under starved conditions. The results of Gebhardt and Jonitza (4) are consistent with ours under fed conditions, whereas PVH was more sensitive to EGF than PPH under starved conditions. Gebhardt and colleagues (3, 13) also reported that EGF receptors were unevenly distributed among hepatocytes, that is, PPH apparently had a higher number of these receptors than PVH, but they did not investigate EGF receptors under starved conditions. In the present study, there was no significant spatial difference of EGF receptors between PPH and PVH under each condition. A possible explanation for the discrepancy is the different strains of rat used in the experiments: Gebhardt et al. (3, 4) used Sprague-Dawley rats in their studies, while we used Wistar rats.

**Fig. 6.** Effect of genistein (0.1, 1, 5, or 10 µg/ml) on DNA synthesis in PPH and PVH under fed conditions. $^{[3]H}$thymidine was included from 48 to 72 h. Values are means ± SD for 8 determinations. A: effect of genistein on DNA synthesis by HGF/SF ($5 \times 10^{-10}$ M) under fed conditions. B: effect of genistein on DNA synthesis assumed by EGF ($10^{-8}$ M) under fed conditions. *P < 0.05, **P < 0.01, PPH vs. PVH at same concentration.

**Fig. 7.** Effect of genistein (0.1, 1, 5, or 10 µg/ml) on DNA synthesis in PPH and PVH under starved conditions. $^{[3]H}$thymidine was included from 48 to 72 h. Values are means ± SD for 8 determinations. A: effect of genistein on DNA synthesis by HGF/SF ($5 \times 10^{-10}$ M) under starved conditions. B: effect of genistein on DNA synthesis by EGF ($10^{-8}$ M) under starved conditions. *P < 0.05, **P < 0.01, PPH vs. PVH at same concentration.
Our additional study showed that, in Sprague-Dawley rats, PPH apparently had a higher number of EGF receptors than PVH, in contrast to male Wistar rats (data not shown). The distribution pattern of the EGF receptor on hepatocytes might be different among rat strains and might cause a different proliferative effect in PPH and PVH.

Volk et al. (31) reported that PPH also responded to HGF/SF with higher sensitivity than PVH, although they did not investigate receptor numbers of 

\[ \text{HGF/SF} \]

on PPH and PVH, including GS-positive hepatocytes. They emphasized that GS-positive hepatocytes, probably part of PVH, showed the lowest response to HGF/SF. However, in the study by Volk et al. (31), even the control value of DNA synthesis (bromodeoxyuridine index) in GS-positive hepatocytes was much lower than that in PPH and other PVH. In our study, PVH is more sensitive to HGF/SF than PPH under both fed and starved conditions. For \( K_d \) values obtained by binding assay of 

\[ \text{HGF/SF} \]

, there was no significant difference between PPH and PVH under each condition. Our data suggest that sensitivity to HGF/SF in PVH is greater than in PPH, although HGF/SF receptors distributed evenly among hepatocytes, and these did not change according to the nutritional state.

Many present studies indicated that zonal heterogeneity changed according to the nutritional conditions. As for the carbohydrate metabolism, gluconeogenesis has been thought to occur mainly in PPH, and glycogenesis has been thought to occur mainly in PVH (10, 11, 30). Under starved conditions, the glycogenic area located mainly in PPH extended to the PVH area (10, 11, 30). In isolated perfused livers of starved rats, glycogen was synthesized from pyruvate via the indirect pathway in the periportal zone and from glucose via the direct pathway in the perivenous zone (1). Mohn et al. reported that liver-specific immediate-early genes encoded proteins that were important for glucose homeostasis and cellular metabolism. These include phosphoenolpyruvate carboxykinase (PEPCK), glucose-6-phosphatase (G-6-Pase), and insulin-like growth factor binding protein-1 (IGFBP-1), which had been implicated as having both metabolic and growth effects. These proteins are important in increasing hepatic glucose production (6, 18, 19). On the other hand, mRNAs encoding enzymes that oppose gluconeogenesis, such as pyruvate kinase, decrease rapidly at the time of partial hepatectomy (27, 28). These changes in gene expression are paralleled by changes in insulin and glucagon levels that are consistent with the known regulation of these genes by those hormones. For example, insulin positively regulates the glucokinase and pyruvate kinase genes and downregulates the expression of PEPCK, IGFBP-1, and G-6-Pase (24). However, the expression of gluconeogenic genes is also controlled by hepatic transcription factors such as HNF-1 and C/EBP. EGF is also well known to induce gluconeogenesis (9). Considering these facts, it seems to be possible that nutritional conditions might affect the efficacy of EGF in PPH and PVH, even though details of its mechanism have yet to be investigated.

The present study shows that the sensitivity of PPH and PVH to growth factors may also change according to the nutritional conditions. However, the number of each receptor was not affected by the conditions of nutrition. Furthermore, zonal difference induced by EGF that varied according to nutritional conditions disappeared through inhibition of tyrosine kinase activity. It was then suspected that zonation of the sensitivity to growth factors was controlled by postreceptor signal transduction of growth factor, partly by tyrosine kinase activity. In contrast to HGF/SF, in the case of EGF, 1 μg/ml genistein abolished zonal difference completely by inhibition of the increase in PPH under fed conditions or by inhibition of the increase in PVH under starved conditions. Although receptors of both HGF/SF and EGF have tyrosine kinase activity, the effects of HGF/SF and EGF on cellular activity of rat hepatocytes do not seem to be consistent (17). These data are compatible with the present data.

In conclusion, there are zonal differences in DNA synthesis induced by HGF/SF and EGF. Furthermore, the efficacy of EGF changes according to conditions of nutrition. Under fed conditions, PPH are more sensitive to EGF, but, under starved conditions, PVH are more sensitive. In contrast, PVH show a greater response to HGF/SF under starved conditions as well as under fed conditions than PPH. These zonal differences seem to be regulated not by receptor numbers but by tyrosine kinase, which was a candidate for trigger of zonal difference.

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