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Activation of Mitogen-Activated Protein Kinase by Hepatocyte Growth Factor Is Stimulated by Both α_1- and β_2-Adrenergic Agonists in Primary Cultures of Adult Rat Hepatocytes

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Abstract. We investigated the effects of α_1- and β_2-adrenergic agonists on hepatocyte growth factor (HGF)-stimulated mitogen-activated protein kinase (MAPK) isoforms in primary cultures of adult rat hepatocytes. Hepatocytes were isolated and cultured with HGF (5 ng/ml) and/or α- and β-adrenergic agonists. Phosphorylated MAPK isoforms (p42 and p44 MAPK) were detected by Western blotting analysis using anti-phospho-MAPK antibody. The results show that HGF increased phosphorylation of p42 MAPK by 2.2-fold within 3 min. The HGF-induced MAPK activation was abolished by AG1478 treatment (10^{-7} M). The MEK (MAPK kinase) inhibitor PD98059 (10^{-6} M) completely inhibited the HGF-dependent increase in MAPK activity. Phenylephrine (10^{-6} M) and metaproterenol (10^{-6} M) alone had no effect in the absence of HGF, but significantly increased p42 MAPK induction by HGF. Moreover, the cell-permeable cAMP analog, 8-bromo cAMP (10^{-7} M), and phorbol 12-myristate 13 acetate (10^{-7} M) potentiated HGF-induced MAPK phosphorylation. The effects of these analogs were antagonized by the protein kinase A (PKA) inhibitor H-89 (10^{-7} M) and the protein kinase C (PKC) inhibitor sphingosine (10^{-6} M), respectively. These results suggest that direct or indirect activation of both PKA and PKC represent a positive regulatory mechanism for stimulating MAPK induction by HGF.

Keywords: mitogen-activated protein kinase (MAPK), hepatocyte growth factor (HGF), α_1- and β_2-adrenergic agonists, hepatocyte, cross-talk

Introduction

Hepatocyte growth factor (HGF) has multiple biological activities on a wide variety of cell types (1 – 4). HGF appears to trigger liver regeneration after partial hepatectomy and acute liver cell necrosis caused by chemicals in vivo (5 – 8). The response of adult rat hepatocytes to HGF has also been investigated extensively with respect to DNA synthesis and proliferation in vitro, and HGF is now known to be a potent hepatocyte mitogen (9). More recently, we reported that HGF is able to rapidly stimulate hepatocyte DNA synthesis and proliferation during short-term (e.g., 4 h) culture. In addition, the hepatocyte DNA synthesis and proliferation produced by HGF was inhibited depending on the initial plating density. Furthermore, we found that hepatocyte DNA synthesis and proliferation were potentiated by both α_1- and β_2-adrenergic agonists (10).

The signal transduction pathways activated in response to HGF in hepatocytes and other cell types have become more clearly understood (3, 11 – 15). Using specific inhibitors of signal transducers, we pharmacologically demonstrated that receptor tyrosine kinase, phosphoinositide 3-kinase, phospholipase C, and p70 ribosomal protein S6 kinase (p70S6K) activities are essential for HGF-induced DNA synthesis and proliferation in primary cultures of adult rat hepatocytes (10).

In addition, mitogen-activated protein kinase (MAPK) is now known to be activated in response to a large number of mitogenic stimuli, and the enzyme is a key participant in the response to various growth factors and cytokines (12, 16). In order to better understand

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the HGF-mediated signaling pathway, we investigated whether activation of MAPK isoforms, ERK1 and ERK2, is involved in HGF-induced DNA synthesis and proliferation in primary cultures of adult rat hepatocytes.

Catecholamines (e.g., norepinephrine and its analogs) have been shown to be involved in regulation of liver function (e.g., lipid metabolism, carbohydrate metabolism, and cell growth). There are several types of catecholamine receptors, β_1- and β_2-receptors, that stimulate adenylate cyclase, while α_2-receptors inhibit its activity (17). α_1-Receptors are related to phospholipase C activation and subsequent increases in inositolphosphate turnover and diacylglycerol production (10, 18). Although, some investigators have reported that the α_1- and β-adrenergic responses are related to adrenergic regulation of carbohydrate metabolism in liver from normal adult rats and cultured hepatocytes (17, 19, 20). There are few studies regarding the adrenergic regulation of MAPK activity induced by growth factors in liver cells. In the present study, therefore, we examined whether α_1- and β_2-adrenergic agonists can modulate HGF-induced MAPK isoform activities. The results obtained here indicate that HGF phosphorylates one of the MAPK isoforms, p42 MAPK, but not p44 MAPK, within 3 min. Moreover, both phenylephrine and metaproterenol potentiated p42 MAPK phosphorylation induction by HGF. The physiological significance of cross-talk between the HGF pathway and α_1- and β_2-adrenergic receptor-mediated pathway in regulating hepatocyte proliferation is also discussed.

Materials and Methods

Animals

Male Wistar rats (weight 200 – 220 g) were obtained from Saitama Experimental Animal Co. (Saitama). Rats were acclimated in the humidity- and temperature-controlled room for at least 3 days before experimentation, and were fed a standard diet and given tap water ad libitum. Rats were handled in accordance with the National Institutes of Health Guidelines for Care and Use of Laboratory Animals.

Hepatocyte isolation and culture

Male Wistar rats were anesthetized by intraperitoneal injection of sodium pentobarbital (45 mg/kg). Two-step *in situ* collagenase perfusion was performed to facilitate disaggregation of adult rat livers, as described previously (21). After perfusion, cells were dispersed in Ca^{2+}-free Hanks' solution. Cells were then washed three times by slow centrifugation ($120 \times g$ for 1 min). Hepatocyte viability was monitored by Trypan blue dye exclusion. On average, more than 96% of the cells remained intact.

Isolated hepatocytes were plated onto collagen-coated plastic culture dishes (35-mm diameter) at a density of 3.3×10^4 cells/cm^2 in Williams' medium E containing 5% bovine calf serum and 0.1 nM dexamethasone for 3 h at 37°C in 5% CO_2 in air. The medium was then changed, and the cells were cultured in serum- and dexamethasone-free Williams' medium E containing HGF (5 ng/ml) with or without α_1- and β_2-adrenergic agonists and/or specific effectors or inhibitors of signal transducers for the indicated times at 37°C.

Measurement of MAPK activity

Phosphorylated MAPK isoforms (P-p42 MAPK [ERK 2] and P-p44 MAPK [ERK 1]) were identified by Western blotting analysis using anti-phospho-MAPK (ERK 1, 2) monoclonal antibody as described by Towbin et al. (22). Phosphorylated MAPK activity is normalized to the total MAPK. Briefly, cultured hepatocytes were washed once with ice-cold phosphate-buffered saline (pH 7.4) and 0.2 ml of lysis buffer added, after which the hepatocytes were harvested. After centrifugation at $16{,}300 \times g$ for 30 min at 4°C, cell lysates were denatured in boiling water for 5 min. Samples of the supernatant (30 μg of protein) were subjected to SDS-PAGE using a 10% acrylamide resolving gel according to the method of Laemmli (23). After electrophoresis, proteins were transferred to Immobilon-P membranes.

For detection of phosphorylated extracellular-regulated protein kinase p44 MAPK (ERK 1) and p42 MAPK (ERK 2), the membranes were immersed in Tris-buffered saline (pH 7.4) containing 1% bovine serum albumin. The membranes were then incubated with an antibody (1 μg/ml) against phospho-MAPK and/or MAPK and were washed as described previously (22). Antibody binding was visualized by incubation with a horseradish peroxidase-conjugated donkey anti-rabbit IgG secondary antibody [3000 dilution (24)] followed by enhanced chemiluminescence detection (ECL Kit; Amersham, Little Chalfont, UK). Densitometry analysis was performed using NIH Image version 1.68 for the Macintosh. Data were calculated in arbitrary units and are expressed as means ± S.E.M.

Cytosolic protein in hepatocytes was quantified by modification of the Lowry procedure with bovine serum albumin as a standard (25).

Materials

The following reagents were obtained from Sigma Chemical Co. (St. Louis, MO, USA): dexamethasone, aprotinin, aphidicolin, HGF (human recombinant), AG1478 (*N*-[3-chlorophenyl]-6,7-dimethoxy-4-quinazo-

linamine), 8-bromo-cAMP, wortmannin, H-89 (*N*-[2-(*p*-bromocinnamylamino)ethyl]-5-isoquinolinesulfonamide dihydrochloride), metaproterenol hemisulfate, sphingosine hydrochloride, phenylephrine hydrochloride. PD98059 (2'-amino-3'-methoxyflavone) was obtained from Calbiochem-Behring Corp. (La Jolla, CA, USA). Rapamycin and 12-*O*-tetradecanoylphorbol-13-acetate (phorbol ester, TPA) were obtained from Research Biochemicals, Inc. (Natick, MA, USA). Williams' medium E and newborn calf serum were purchased from Flow Laboratories (Irvine, Scotland). Collagenase (type II) was obtained from Worthington Biochemical Co. (Freehold, NJ, USA). Anti-phospho-p42/p44 MAPK monoclonal antibody and anti-p42/p44 MAPK monoclonal antibody were obtained from Cell Signaling Technology (Danvers, MA, USA). All other reagents were of analytical grade.

Statistical analysis

Group comparisons were made by ANOVA for unpaired data followed by post hoc analysis using Dunnett's multiple comparison test. Differences at the level of $P<0.05$ were considered to be statistically significant.

Results

Time course and patterns of MAPK isoform activity stimulated by HGF

It has been reported that MAPK plays an important role in the proliferation of hepatocytes and other cells induced by growth factors and cytokines (26 – 29). Figure 1A shows the typical detectable pattern of phospho-MAPK on Western blotting analysis. The phosphorylated p42 MAPK band was induced after only 1 min and peaked (about 2-fold increase) between 3 and 5 min after addition of 5 ng/ml HGF, a concentration that stimulated hepatocyte proliferation (Fig. 1B) (10). p44 MAPK was not significantly affected by either medium alone (control) or HGF treatment (Fig. 1C).

Effects of specific inhibitors of signal transducers on HGF-stimulated MAPK isoform activity

In order to characterize the involvement of MAPK in the mitogenic pathway induced by HGF, we investigated the effects of the receptor tyrosine kinase inhibitor AG1478 [10^{-7} M (30)], the phosphatidylinositol 3-kinase (PI3K) inhibitor wortmannin [10^{-7} M (31, 32)], the MEK inhibitor PD98059 [10^{-6} M (27)], and the p70S6K inhibitor rapamycin [10 ng/ml (33)] on HGF-induced MAPK activity. As shown in Fig. 2, the phosphorylation of ERK2 induced by HGF (5 ng/ml) at 5 min was almost completely blocked by AG1478, wortmannin, and PD98059. However, rapamycin did not affect the HGF-induced MAPK activity. Phosphorylation of p44 MAPK in the presence of 5 ng/ml HGF was not affected by these inhibitors.

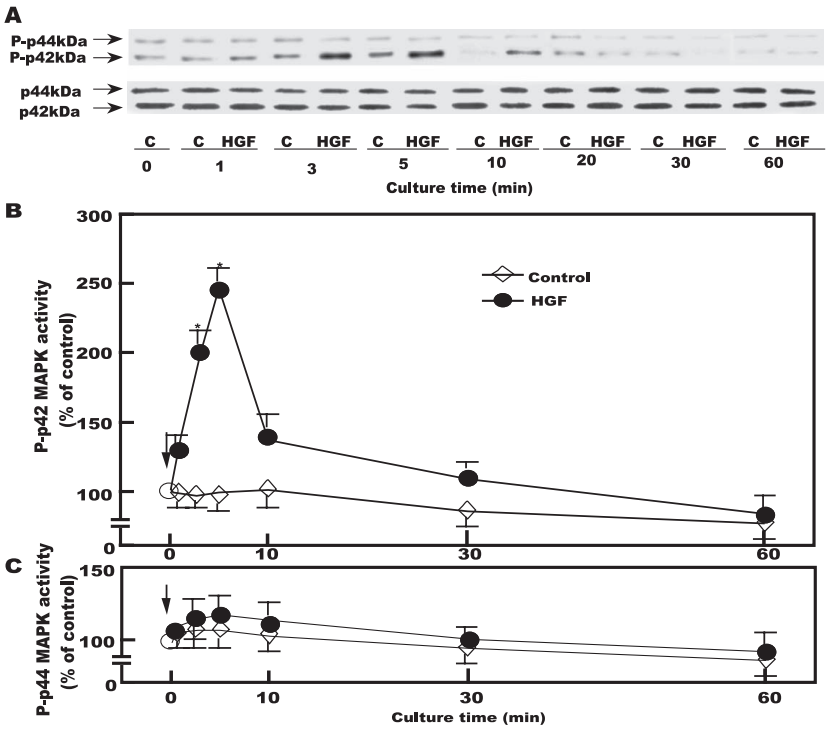

Fig. 1. Time course and patterns of MAPK isoform activity induced by HGF. Isolated hepatocytes cultured for 3 h were washed and incubated in either the absence (control, C: medium alone) or presence of 5 ng/ml HGF for the indicated times at 37°C. Phosphoyrlated MAPK isoforms (P-p42/P-p44 MAPK) were identified using anti-phospho MAPK antibody, as described in Materials and Methods. Intensity of the M_r 44- and 42-kDa bands corresponding to phosphorylated p44 MAPK and p42 MAPK was normalized to total MAPK [p42/p44 MAPK (p42/p44 MAPK)]. A: typical Western blotting band, phosphorylated p42/p44 MAPK (P-p42/P-p44 MAPK), total p42/p44 MAPK (p42/p44 MAPK); B: time-course of phosho-p42 MAPK (ERK2) activity; C: time-course of phosho-p44 MAPK (ERK1) activity. Results are expressed as a percentage of the respective control value (mean ± S.E.M. of three experiments). **P*<0.05, compared with the respective control.

Activation of p42 MAPK produced by HGF is potentiated by the β_2-adrerenergic agonist metaproterenol

The time course of effects of β_2-adrerenergic agonists on MAPK activity induction by 5 ng/ml HGF was investigated using the β_2-adrerenergic agonist metaproterenol. When hepatocytes were stimulated with HGF in the presence of the β_2-adrerenergic agonist metaproterenol (10^{-6} M), the agent caused a more rapid and significant increase in p42 MAPK activity than HGF alone, reaching a peak at 5 min after addition (about 3-fold increase from baseline) and rapidly declining to basal levels within 30 min (Fig. 3: A and B). In contrast, metaproterenol alone did not significantly stimulate p42 MAPK activity (data not shown). Metaproterenol in the presence of HGF did not significantly stimulate p44 MAPK phosphorylation (Fig. 3C). Therefore, it was found that metaproterenol (10^{-6} M) potentiated the HGF-induced p42 MAPK activity.

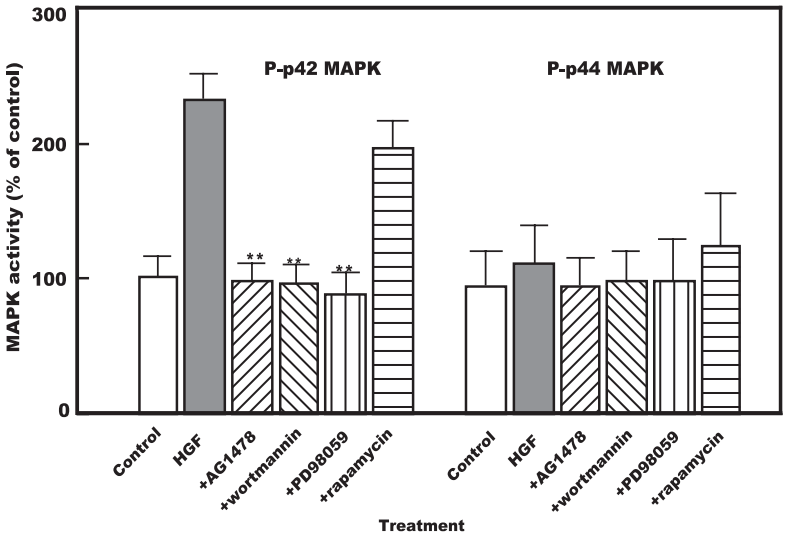

Fig. 2. Effects of specific inhibitors of signal transducers on MAPK isoform activity induction by HGF. Hepatocytes were stimulated with HGF (5 ng/ml) in the presence of specific inhibitors of signal transducers: AG1478 (10^{-7} M), PD98059 (10^{-6} M), wortmannin (10^{-7} M), and rapamycin (10 ng/ml). Phosphorylated MAPK isoforms (p44 kDa as ERK 1 and p42 kDa as ERK 2) were determined after incubation for 5 min with the test agents. Phosphorylated MAPK isoforms were identified using anti-phospho-MAPK antibody as described in Materials and Methods. Results are expressed as a percentage of the respective control value (mean ± S.E.M. of three experiments). $^{**}P<0.01$, compared with the HGF alone goup.

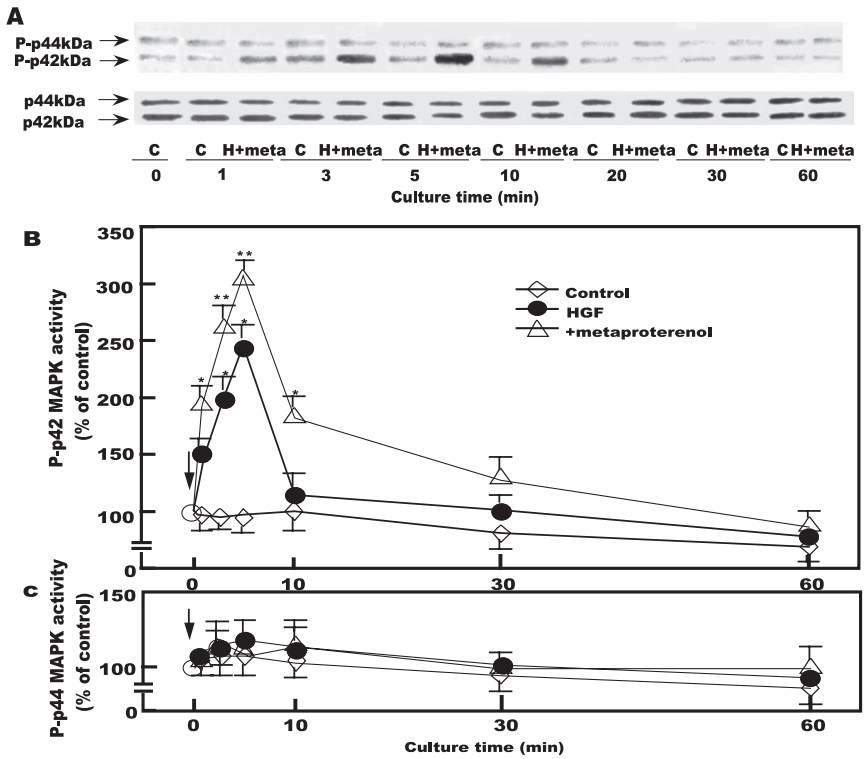

Fig. 3. Effects of metaproterenol on time course and patterns of MAPK isoform activity induced by HGF. Hepatocytes were stimulated with HGF (5 ng/ml) in the absence or presence of metaproterenol (10^{-6} M). Phosphorylated MAPK isoforms and/or total MAPK (A: typical Western blotting band, B: p42 MAPK, and C: p44 MAPK) were identified using anti-phospho-MAPK antibody and/or anti-MAPK, as described in Materials and Methods. Results are expressed as a percentage of the respective control value (mean ± S.E.M. of three experiments). $^{*}P<0.05$, $^{**}P<0.01$, compared with the respective control. H: HGF, meta: metaproterenol.

Effect of specific inhibitors of signal transducers on metaproterenol and/or 8-bromo cAMP-induced MAPK isoform activity in the presence of HGF

In order to investigate the potentiating mechanisms for the β_2-adrenergic receptor/protein kinase A (PKA) pathway in induction of p42 MAPK activity by HGF, we examined the effects of metaproterenol [indirect PKA stimulator (34)] and the cell-permeable cAMP analog 8-bromo cAMP (direct PKA stimulator) on phosphorylation of p42 MAPK induced by HGF. Both metaproterenol- and 8-bromo cAMP-induced potentiation of p42 MAPK phosphorylation in the presence of HGF were almost completely blocked by AG1478, wortmannin, and PD98059. However, they were not blocked by rapamycin. In contrast, pretreatment of hepatocytes with the PKA inhibitor H-89 [10^{-7} M (35)] blocked the potentiating effects of metaproterenol or 8-bromo cAMP on the phosphorylation of p42 MAPK in the presence of HGF. Metaproterenol (10^{-6} M) or 8-bromo cAMP (10^{-7} M) alone had no significant effect on the phosphorylation of p44 MAPK and p42 MAPK (Fig. 4)

Activation of p42 MAPK produced by HGF is potentiated by α_1-adrenergic agonist phenylephrine

The potentiating effects of α_1-adrerenergic agonists on MAPK pathway induction by 5 ng/ml HGF were investigated with the α_1-adrerenergic agonist phenylephrine. HGF in the presence of phenylephrine (10^{-6} M) caused a more rapid and significant increase in p42 MAPK activity than HGF alone. p42 MAPK activity peaked at 5 min (about 3.5-fold increase from baseline) after addition and rapidly decreased to basal levels within 30 min (Fig. 5: A and B). In contrast, phenylephrine alone did not significantly stimulate p42 MAPK activity (data not shown). Phenylephrine (10^{-6} M) in the presence of HGF did not significantly stimulate p44 MAPK phosphorylation (Fig. 5C). The present findings indicate that phenylephrine potentiates HGF-induced p42 MAPK activity.

Effects of specific inhibitors of signal transducers on α_1-adrenergic agonist- and/or TPA-induced MAPK isoform activity in presence of HGF

In order to investigate the potentiating mechanism for the α_1-adrenergic receptor/protein kinase C (PKC) pathway in induction of p42 MAPK activity by HGF, we examined the effects of phenylephrine [indirect PKC

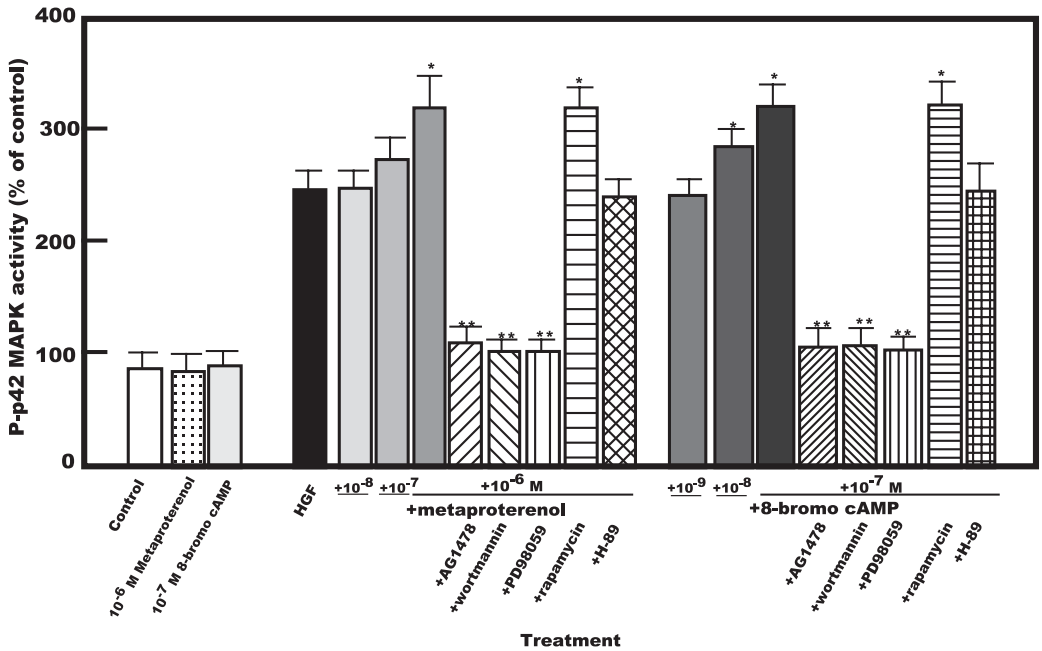

Fig. 4. Effects of specific inhibitors of signal transducers on metaproterenol and/or 8-bromo cAMP-induced MAPK isoform activity in the presence of HGF. Hepatocytes were stimulated with HGF (5 ng/ml) plus metaproterenol (10^{-8} – 10^{-6} M) and/or 8-bromo cAMP (10^{-9} – 10^{-7} M) in the presence of specific signal transducer inhibitors: AG1478 (10^{-7} M), PD98059 (10^{-6} M), wortmannin (10^{-7} M), rapamycin (10 ng/ml), and H-89 (10^{-7} M). Phosphorylated MAPK isoforms (ERK 1 and ERK 2) were determined after incubation for 5 min with the test agents. Phosphorylated MAPK isoforms (ERK 1 and ERK 2) were identified using anti-phospho-MAPK antibody, as described in Materials and Methods. Results are expressed as a percentage of the respective control value (mean ± S.E.M. of three experiments). *P<0.05, **P<0.01, compared with the HGF alone group.

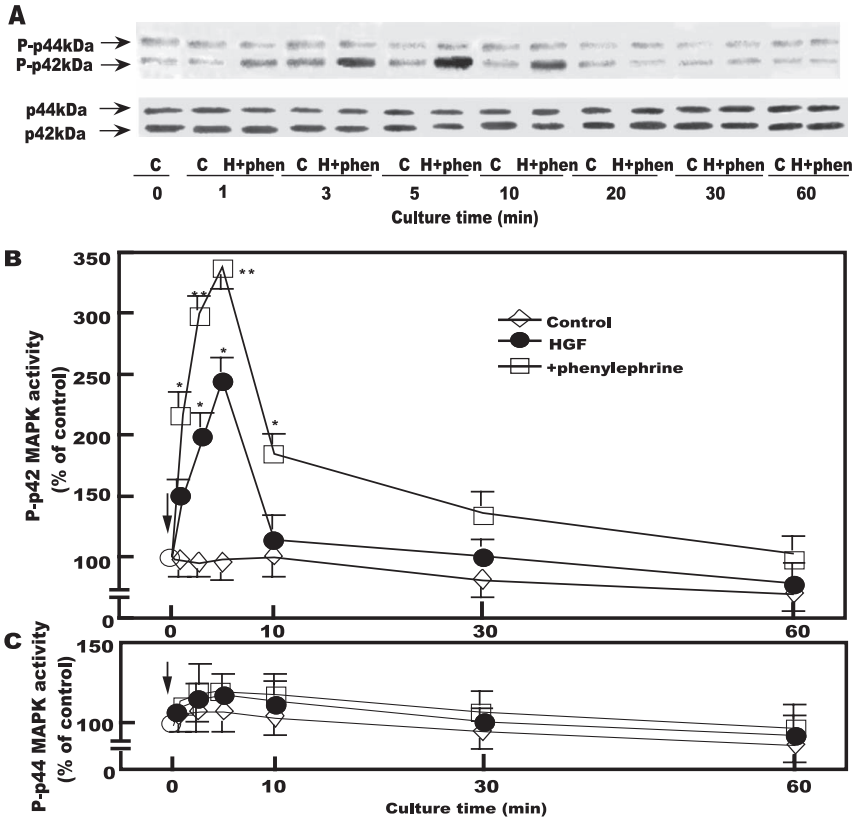

Fig. 5. Effects of phenylephrine on time course and patterns of MAPK isoform activity induced by HGF. Hepatocytes were stimulated with HGF (5 ng/ml) in the absence or presence of phenylephrine (10^{-6} M). Phosphorylated MAPK (A: typical Western blotting band, B: p42 MAPK, and C: p44 MAPK) were identified using anti-phospho-MAPK antibody and/or anti-MAPK, as described in Materials and Methods. Results are expressed as a percentage of the respective control value (mean ± S.E.M. of three experiments). **P*<0.05, ***P*<0.01, compared with the respective control. H: HGF, phen: phenylephrine.

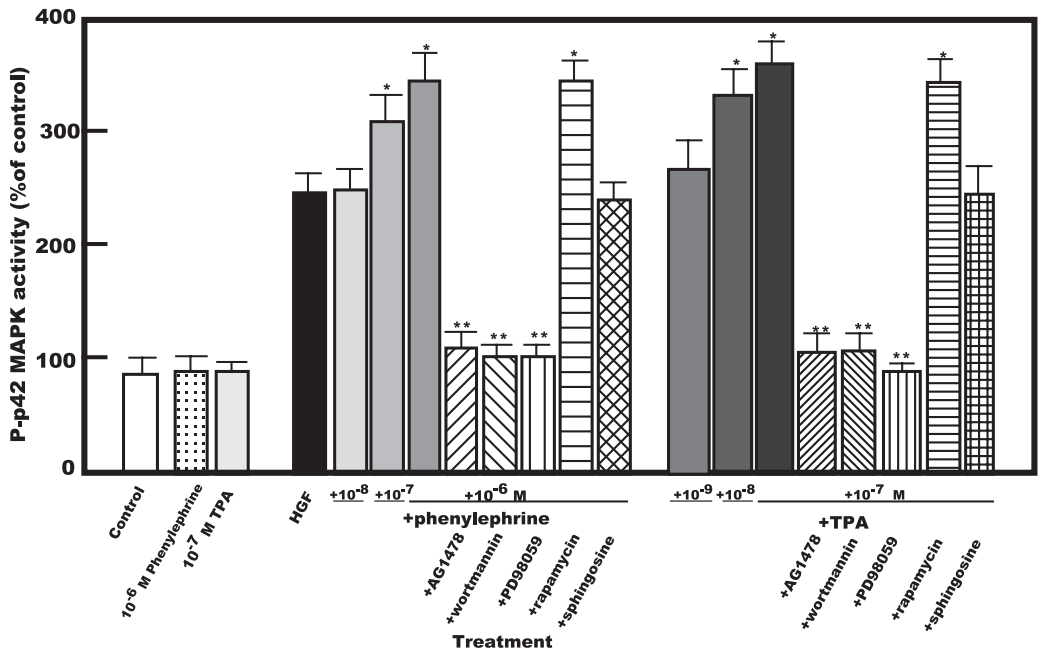

Fig. 6. Effects of specific inhibitors of signal transducers on phenylephrine and/or TPA-induced MAPK isoform activity in the presence of HGF. Hepatocytes were stimulated with HGF (5 ng/ml) plus phenylephrine (10^{-8} – 10^{-6} M) and/or TPA (10^{-9} – 10^{-7} M) in the presence of specific inhibitors of signal transducers: such as AG1478 (10^{-7} M), PD98059 (10^{-6} M), wortmannin (10^{-7} M), rapamycin (10 ng/ml), and sphingosine (10^{-7} M). Phosphorylated MAPK isoforms (ERK 1 and ERK 2) were determined after incubation for 5 min with test agents. Phosphorylated MAPK isoforms (ERK 1 and ERK 2) were identified using anti-phospho-MAPK antibody, as described in Materials and Methods. Results are expressed as a percentage of the respective control value (mean ± S.E.M. of three experiments). **P*<0.05, ***P*<0.01, compared with the HGF alone group.

activator (7)] and TPA, (phorbol ester, direct PKC activator) on the phosphorylation of MAPK isoforms induced by HGF. Both phenylephrine (10^{-6} M)- and TPA (10^{-7} M)-induced potentiation of p42 MAPK phosphorylation in the presence of HGF were almost completely blocked by AG1478, wortmannin, and PD98059. However, they were not blocked by rapamycin. In contrast, pretreatment of hepatocytes with the PKC inhibitor sphingosine [10^{-7} M (36)] blocked the potentiative effects of phenylephrine and TPA on the phosphorylation of p42 MAPK induced by HGF. Phenylephrine (10^{-6} M) or TPA (10^{-7} M) alone had no significant effect on the phosphorylation of p44 MAPK and p42 MAPK (Fig. 6).

Discussion

The proliferative pathway by which HGF activates the receptor tyrosine kinase c-Met/MAPK cascade (ERK pathway) has been described in many normal cells and transformed cells (4, 14, 15, 37). The MAPK pathway is one of the most important and intensively studied signaling pathways. However, there are few studies regarding the HGF-induced activation of MAPK isoforms in primary cultured adult rat hepatocytes. Therefore, we investigated the possible roles of the MAPK isoforms induced by HGF in primary cultures of adult rat hepatocytes. As shown in Figs. 1 and 2, we demonstrated that HGF (5 ng/ml) rapidly stimulates p42 MAPK activity, but not p44 MAPK activity. p42 MAPK activity was blocked by the MEK inhibitor PD98059. Moreover, hepatocyte p42 MAPK activity induction by HGF was almost completely blocked by specific inhibitors of signal transducers such as AG1478 and wortmannin, but not rapamycin (Fig. 2). These results suggest that signal transducers such as receptor tyrosine kinase, p42 MAPK, and PI3K play an essential role in the mitogenic activity induced by HGF under our experimental conditions. The present results are consistent with reports that p42 MAPK acts upstream of p70S6K and downstream of receptor tyrosine kinase and PI3K in primary cultures of adult rat hepatocytes (15, 38, 39).

There have been very few studies on the adrenergic agonist-induced regulation of MAPK activity. Thus, we examined whether α_1- and β_2-adrenergic agonists modulate the HGF-induced changes in the activity of each MAPK isoform. We found that the HGF-induced hepatocyte p42 MAPK activity was enhanced by metaproterenol (10^{-6} M), a β_2-adrenergic receptor agonist and an indirect adenylate cyclase activator (Fig. 3) (34). In contrast, metaproterenol alone had no significant effects on hepatocyte MAPK activity in the absence of HGF (Fig. 4). The enhancing effects of metaproterenol in the presence of HGF were inhibited by the PKA inhibitor, H-89 (35), thus suggesting the involvement of PKA in the effects of metaproterenol (Fig. 4). The involvement of PKA in the effects of metaproterenol is supported by our previous results indicating that metaproterenol-induced potentiation of hepatocyte DNA synthesis and proliferation in the presence of HGF (5 ng/mL) was completed inhibited by a specific PKA inhibitor, H-89 (10). However, the role of the second messenger, cAMP, in the control of hepatocyte DNA synthesis and proliferation remains uncertain (39, 40). Cyclic AMP can either stimulate or inhibit DNA synthesis depending on culture conditions (41 – 44). For example, elevated hepatocyte cAMP levels have been reported to inhibit HGF-stimulated DNA synthesis and proliferation (3). We demonstrated previously that the cell-permeable cAMP analog, 8-bromo cAMP also enhanced the p42 MAPK activity and hepatocyte proliferation induced by HGF (Fig. 4). These results indicate that the β_2-adrenergic receptor/cAMP pathway cross-talks with the HGF receptor/MAPK pathway to potentiate hepatocyte growth. Because both metaproterenol- and 8-bromo cAMP-induced potentiation of p42 MAPK activity in the presence of HGF were completely inhibited by AG1478, wortmannin and PD98059, but not rapamycin, β_2-adrenergic pathway may cross-talk with upstream of ERK2. The point(s) of convergence between the two signaling cascades may be raf or ras, an upstream element of ERK2 (45). A possible scheme for cross-talk between the HGF receptor/MAPK pathway and the β_2-adrenergic receptor/PKA pathway is shown in Fig. 7. The discrepancy between other data and our own is unclear at present. Thus, more detailed mechanisms of the cross-talk between the HGF-signaling pathway and the β_2-adrenergic receptor/cAMP/PKA pathway should be explored.

While phenylephrine alone had no effect on hepatocyte MAPK activity in the absence of HGF, HGF-induced hepatocyte p42 MAPK was enhanced by phenylephrine, an α_1-adrenergic agonist (Fig. 6). The enhancing effects of phenylephrine in the presence of HGF were inhibited by the PKC inhibitor, sphingosine (10^{-6} M) (36), thus suggesting the involvement of PKC in the potentiating effects of the α_1-adrenoceptor (Fig. 6). The involvement of PKC in the effects of phenylephrine is supported by our previous results indicating that phenylephrine-induced potentiation of hepatocyte DNA synthesis and proliferation in the presence of HGF (5 ng/mL) was completed inhibited by a specific PKC inhibitor, sphingosine (10). However, the roles of the second messengers, inositol 1,4,5-triphosphate (IP_3) and diacylglycerol, in the control of

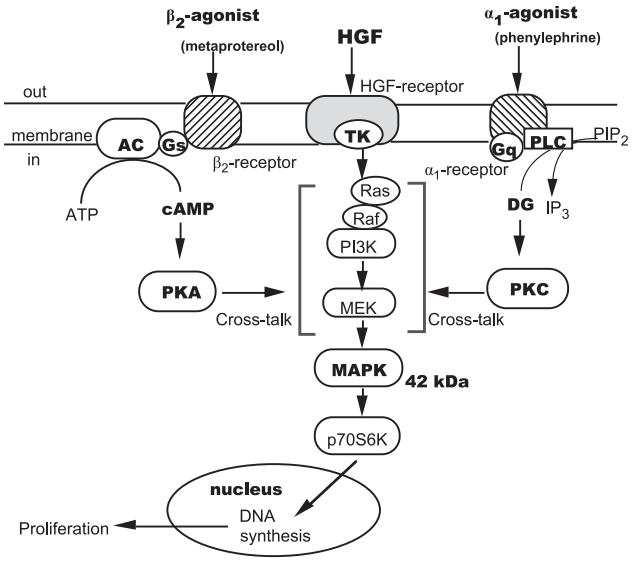

Fig. 7. Possible model for cross-talk between the HGF receptor /MAPK pathway and α_1- or β_2-adrenergic receptor-mediated pathways. HGF, hepatocyte growth factor; TK, receptor tyrosine kinase; AC, adenylate cyclase; PLC, phospholipase C; PI3K, phosphatidylinositol 3-kinase; MEK, MAP kinase kinase; MAPK, mitogen-activated protein kinase; p70S6K, p70 ribosomal protein S6 kinase; PKA, protein kinase A; PKC, protein kinase C; PIP_2, phosphatidylinositol 4,5-bisphosphate; DG, diacylglycerol; IP_3, inositol 1,4,5-triphosphate.

hepatocyte DNA synthesis and proliferation remain uncertain (46). Phorbol ester, TPA (10^{-7} M), is a direct activator of PKC, and can either directly or indirectly stimulate DNA synthesis depending on culture conditions (10, 11, 26, 28). In addition, α_1-adrenergic receptor-mediated signals may interact with upstream signal transducers, such as the MEK, raf or ras pathways (47), and have a positive influence normal liver growth and liver regeneration in vivo (7, 8, 48, 49). Therefore, the potentiating effects of phenylephrine are likely, at least in part, to cross-talk with the HGF receptor/MAPK pathway; both phenylephrine- and TPA-induced potentiation of p42 MAPK activity induced by HGF were completely inhibited by AG1478, wortmannin and PD98059, but not by rapamycin. Thus, the α_1-adrenergic pathway may interact with upstream elements such as raf or ras (45). A possible scheme for cross-talk between the HGF receptor/MAPK pathway and the α_1-adrenergic receptor/PKC pathway is shown in Fig. 7. More detailed mechanisms of cross-talk between the HGF-signaling pathway and the α_1-drenergic receptor /PKC pathway remain to be explored.

In conclusion, the present study demonstrates that the cross-talk signals by extracellular α_1- and β_2-adrenoceptor agonists, such as phenylephrine and metaproterenol, potentiate HGF-induced p42 MAPK activity in primary cultured adult rat hepatocytes. The present findings support the notion that endogenous catecholamine-induced potentiation of hepatocyte DNA synthesis and proliferation in the presence of HGF play an important role in the activation of the MAPK cascade during liver regeneration after partial hepatectomy or liver necrosis caused by toxic chemicals in vivo (6, 7). There are few clear examples of how cells handle or manage various signal pathways. Thus, we should also examine whether there is cross-talk between catecholamine pathways and those of other growth factors [e.g., epidermal growth factor, platelet-derived growth factor, and insulin] in primary cultured hepatocytes.

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