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Stimulation of DNA synthesis and proliferation by prostaglandins in primary cultures of adult rat hepatocytes

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Abstract

We studied the effects of several prostaglandins on DNA synthesis and proliferation in serum-free primary cultures of adult rat hepatocytes. Maintained in short-term cultures (i.e., 3.5 h), the hepatocyte parenchymal cells synthesized DNA and proliferated in the presence of various prostaglandins in a dose-dependent manner. The half-maximal effective concentrations (ED_{50}) of prostaglandin $F_{2\alpha}$, prostaglandin E_1, prostaglandin E_2 and prostaglandin I_2 for proliferation were estimated to be 1.7×10^{-9}, 2.3×10^{-8}, 2.7×10^{-8} and 3.3×10^{-9} M, respectively. Prostaglandin E_2 and prostaglandin I_2 produced greater maximal responses than did either prostaglandin E_1 or prostaglandin $F_{2\alpha}$. The cells responded only weakly to prostaglandin D_2. The stimulatory effects of 10^{-6} M prostaglandin E_1 and 10^{-6} M prostaglandin E_2 on hepatocyte DNA synthesis and proliferation were inhibited by a specific antagonist of the EP_1 receptor, 8-chlorodibenz[b, f][1, 4]oxazepine-10(11H)carboxylic acid, 2-[3-[(2-furanylmethyl)-thio]-1-oxopropyl]hydrazide (SC-51322; 10^{-6} M). Specific inhibitors of signal transducing elements (e.g., 1-[6-[17β-3-methoxyestra-1, 3, 5(10)-trien-17-yl]amino] hexyl]-1H-pyrrol-2,5-dione (U-73122); 10^{-6} M), 10^{-6} M verapamil, 5×10^{-6} M genistein) almost completely blocked the growth-promoting effects of the prostaglandins. These results suggest that prostaglandins stimulate hepatocyte DNA synthesis and proliferation by their own receptors and exert their effects through both phospholipase C/Ca^{2+} and receptor tyrosine kinase pathways.

Keywords: DNA synthesis; Proliferation; Prostaglandin; Primary culture; Hepatocyte; (Adult rat)

1. Introduction

We reported previously that DNA synthesis and proliferation of adult rat hepatocytes are stimulated rapidly by multiple growth factors, such as epidermal growth factor (EGF), insulin, hepatocyte growth factor (HGF) and transforming growth factor-α (TGF-α), in serum-free primary cultures (Kimura and Ogihara, 1997a,b; Kimura and Ogihara, 1999). In addition, the effects of the major growth factors are modulated differently by α- or β-adrenoceptor agonists and by initial plating densities. According to the results of our and other studies, two classes of mitogens have been defined as complete (direct or primary) mitogens and incomplete (secondary) mitogens or co-mitogens (Refsnes et al., 1992; Michalopoulos, 1994; Michalopoulos and DeFrances, 1997). One group of the latter agents found to enhance growth responses in hepatocytes is the prostaglandins.

Prostaglandins are rapidly synthesized in several types of cells as oxygenated metabolites of arachidonic acid in response to various stimuli and tissue injury (Smith, 1989; Matsumoto et al., 1995). The prostaglandins are locally acting hormones that have a remarkable variety of physiological actions in nearly all mammalian tissues. The diverse biological functions of prostaglandins as chemical mediators for the maintenance of local homeostasis include regulatory functions linked to inflammation, platelet aggregation and contraction of smooth muscles. The diverse biological effects of prostaglandins are considered to be mediated through interaction with specific membrane-bound G-protein-coupled prostanoid receptors (Coleman et al., 1994). The effects of prostaglandin on inflammation have been studied extensively, but few studies have focused on the effects of prostaglandin on hepatocyte proliferation.

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Several lines of evidence suggest that prostaglandins play a role as stimulatory regulators of liver regeneration after the onset of tissue injury in vivo. For instance, partial hepatectomy in rats is followed by a rise in the level of prostaglandins, including prostaglandin E_2 and prostaglandin $F_{2\alpha}$, in the remaining liver tissue and in serum, and is associated with an increased release of prostaglandin E_2 from Kupffer cells (Callery et al., 1991; Tsujii et al., 1993). Indomethacin, a cyclooxygenase inhibitor, prevents this increase and inhibits DNA synthesis in the regenerating liver (Skouteris et al., 1988). Studies of the growth-promoting effects of prostaglandins in primary cultures of adult rat hepatocytes have been conducted primarily in the presence of complete mitogens, such as EGF or insulin (Refsnes et al., 1994; Refsnes et al., 1995; Dajani et al., 1996). However, these peptide growth factors, which strongly influence hepatocyte proliferation, may interfere with the intrinsic action of prostaglandins, even though they were used at relatively low concentrations (Kimura and Ogihara, 1997a,c). Therefore, the ability of prostaglandins on their own to promote the growth of cultured hepatocytes and the intracellular signaling mechanisms that mediate this effect have not been elucidated precisely.

The purpose of the present study was to investigate the effects of five naturally occurring prostaglandins on DNA synthesis and proliferation in serum-free primary cultures of adult rat hepatocytes, and to investigate pharmacologically the intracellular mechanisms that mediate these effects. The results demonstrate that prostaglandins on their own are capable of enhancing DNA synthesis and proliferation in the absence of complete mitogens. On the basis of experiments using specific inhibitors of major signal transducing elements (i.e., a phospholipase C inhibitor, U-73122; a Ca^{2+} channel blocker, verapamil and diltiazem; a receptor tyrosine kinase inhibitor, genistein; a phosphatydylinositol 3-kinase (PI3K) inhibitor, wortmannin; mitogen-activated protein (MAP) kinase kinase inhibitors, PD98059; and a ribosomal protein p70 S6 kinase (p70 S6K) inhibitor, rapamycin), the induction of hepatocyte DNA synthesis and proliferation by prostaglandins may be mediated via specific prostaglandin receptors coupled with G protein, possibly Gq, which stimulate the phospholipase C/Ca^{2+} system, and/or the entry of extracellular Ca^{2+} through Ca^{2+} channels. Furthermore, elements that seem to participate in the cascade induced by prostaglandins include those of the receptor tyrosine kinase pathway, such as PI3K, MAP kinase kinase and P70 S6K.

2. Materials and methods

2.1. Animals

Male Wistar rats (weight 200–220 g) were obtained from Saitama Experimental Animal (Saitama, Japan). They were allowed to adapt to a humidity- and temperature-controlled room for at least 3 days before the experiment was started. They were fed a standard diet and given tap water ad libitum. Rats used in the study were handled in accordance with the National Institute of Health guidelines for the care and use of laboratory animals.

2.2. Hepatocyte isolation and culture

The rats were anesthetized by intraperitoneal injection of sodium pentobarbital (45 mg/kg). Hepatocytes were isolated from normal liver by the two-step in situ collagenase perfusion technique created by Seglen to facilitate disaggregation of the adult rat liver as described previously (Seglen, 1975). In brief, hepatocytes were washed three times by slow centrifugation ($50 \times g$, 1 min) of the cell suspension in order to remove cell debris, damaged cells and non-parenchymal cells. Viability as tested by Trypan blue exclusion was more than 97%. Unless otherwise indicated, isolated hepatocytes were plated onto collagen-coated plastic culture dishes (Sumitomo Bakelite, Tokyo, Japan) at a density of 3.3×10^4 cells/cm^2 (3.1×10^5 cells/35-mm dish), and allowed to attach for 3 h on collagen-coated dishes in Williams' medium E containing 5% newborn calf serum, 0.1 nM dexamethasone, 100 U/ml penicillin, 100 μg/ml streptomycin and 0.10 μg/ml aprotinin in 5% CO_2 in air at 37°C. The medium was then changed, and the cells were cultured in serum- and dexamethasone-free Williams' medium E supplemented with various prostaglandins. When appropriate, the following agents were added: prostaglandins with or without inhibitors of signal transducing elements (e.g., genistein, wortmannin, PD98059, rapamycin, U-73122 and verapamil).

2.3. Measurement of DNA synthesis

Hepatocyte DNA synthesis was assessed by measuring the incorporation of [^{3}H]thymidine into acid-precipitable materials (Morley and Kingdon, 1972). Briefly, after an initial attachment period of 3 h, the hepatocytes were washed twice with serum-free Williams' medium E and cultured in a medium containing several prostaglandins for an additional 4 or 21 h. The cells were pulsed at 0, 1, 2 or 19 h post-prostaglandin stimulation for 2 h with [^{3}H]thymidine (1.0 μCi/well), followed by 10% trichloroacetic acid precipitation, as described previously. [^{3}H]Thymidine incorporation into DNA was measured in a liquid scintillation counter and normalized for cellular protein. Aphidicolin (10 μg/ml) was added to some wells to establish the level of non-replicative DNA synthesis. Hepatocyte protein content was measured by a modified Lowry procedure with bovine serum albumin as a standard (Lee and Paxman, 1972). The data are expressed as dis/min/h/mg cellular protein.

2.4. Counting nuclei

The number of nuclei were counted utilizing a slightly modified version of the procedure previously described by Nakamura et al. (1983). Briefly, the primary cultured hepatocytes were washed twice with 2 ml of Dulbecco's phosphate-buffered saline (pH 7.4). Then, isolated liver cell nuclei were prepared for quantitation by exposure of hepatocyte cultures to 0.25 ml of 0.1 M citric acid containing 0.1% Triton X-100 for 30 min at 37°C. An equal volume of the nucleus suspension was mixed with 0.3% Trypan blue in Dulbecco's phosphate-buffered saline (pH 7.4) and the number of nuclei were counted in a hemocytometer. This procedure was performed because the hepatocytes firmly attached to the collagen-coated plastic culture dishes and were not dispersed sufficiently by 0.02% EDTA–0.05% trypsin treatment.

2.5. Materials

The following reagents were obtained from Sigma (St. Louis, MO, USA): dexamethasone, aprotinin, aphidicolin, diltiazem, verapamil, A23187, ionomycin, 2, 4-dideoxyadenosine, genistein, wortmannin, and D-sphingosine. Prostaglandin D_2, prostaglandin E_2, prostaglandin $F_{2\alpha}$, prostaglandin I_2, and prostaglandin $F_{2\alpha}$ dimethyl amine were from CAYMAN Chemical (Ann Arbor, MI, USA). Prostaglandin E_1, H-89 (N-[2-(p-bromocinnamylamino) ethyl]-5-isoquinolinesulfonamide dihydrochloride), 8-chlorodibenz[b, f][1, 4]oxazepine-10(11H)carboxylic acid, 2-

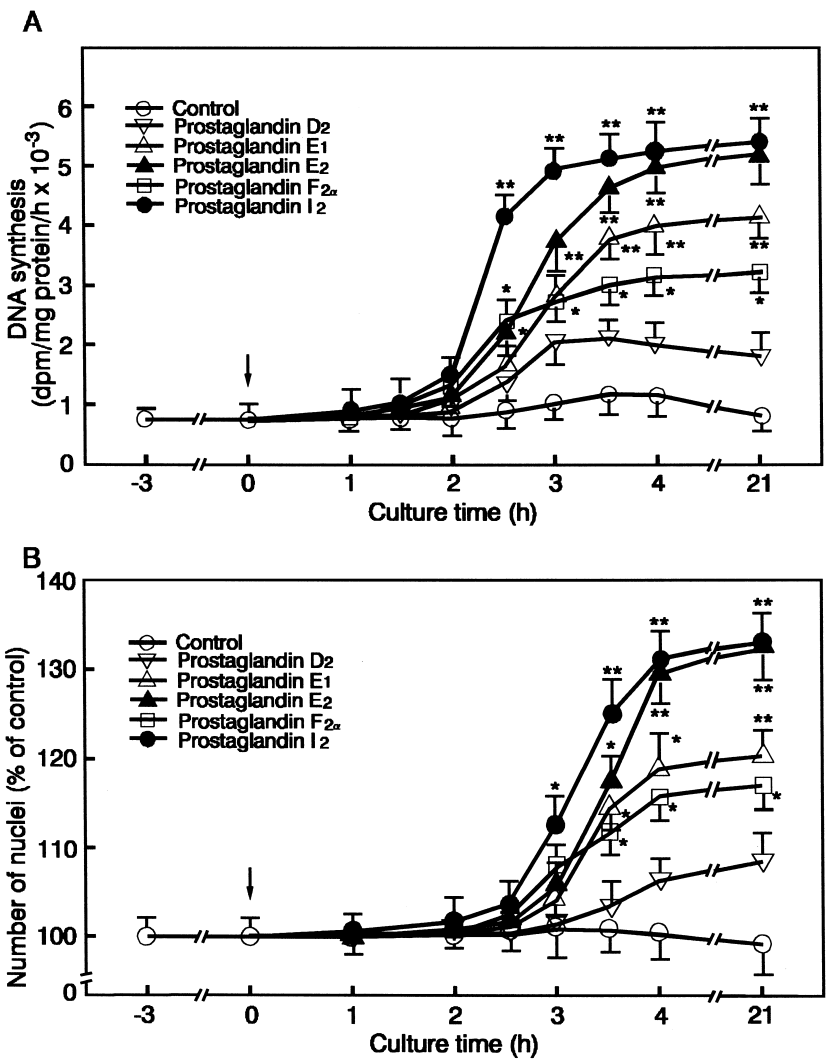

Fig. 1. Time course associated with the stimulation of hepatocyte DNA synthesis and proliferation induced by various prostaglandins. Hepatocytes at a density of 3.3×10^4 cells/cm^2 were plated and cultured in Williams' medium E supplemented with 5% newborn calf serum and 0.1 nM dexamethasone for 3 h. After an attachment period of 3 h (zero time), the medium was rapidly replaced with serum- and dexamethasone-free Williams' medium E with or without various prostaglandins (10^{-6} M prostaglandin D_2, 10^{-6} M prostaglandin E_1, 10^{-6} M prostaglandin E_2, 3×10^{-8} M prostaglandin $F_{2\alpha}$ and 10^{-6} M prostaglandin I_2) and cells were cultured for various lengths of time. Hepatocyte DNA synthesis and proliferation were determined as described in Materials and Methods. The data are expressed as means ± S.E.M. of three experiments. $^{*}P < 0.05$, $^{**}P < 0.01$ compared with the respective control (medium alone).

[3-[(2-furanylmethyl)-thio]-1-oxopropyl]hydrazide (SC-51322), 1-[6-[17β-3-ethoxyestra-1, 3, 5(10)-trien-17-yl]-amino] hexyl]-1H-pyrrol-2, 5-dione (U-73122), 1-[6-[17β-3-Methoxyestra-1, 3, 5(10)-trien-17-yl]amino] hexyl]-2, 5-pyrrolidine-dione (U-73343) were obtained from BIOMOL Research Laboratories, (Plymouth Meeting, PA, USA). 2′-Amino-3′-methoxyflavone (PD98059) was obtained from Calbiochem–Behring (La Jolla, CA, USA). Rapamycin was obtained from Research Biochemicals (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 (Freehold, NJ, USA). [Methyl-^{3}H]thymidine (20 Ci/mmol) was obtained from DuPont-New England Nuclear (Boston, MA, USA). All other reagents were of analytical grade.

2.6. Statistical analysis

The data are expressed as means ± S.E.M. and were analyzed by the unpaired Student's *t*-test. *P* values less than 0.05 were regarded as statistically significant.

3. Results

3.1. Time course associated with stimulation of hepatocyte DNA synthesis and proliferation induced by prostaglandins

We first examined the time course associated with stimulation of hepatocyte DNA synthesis and proliferation induced by various prostaglandins, including those of the

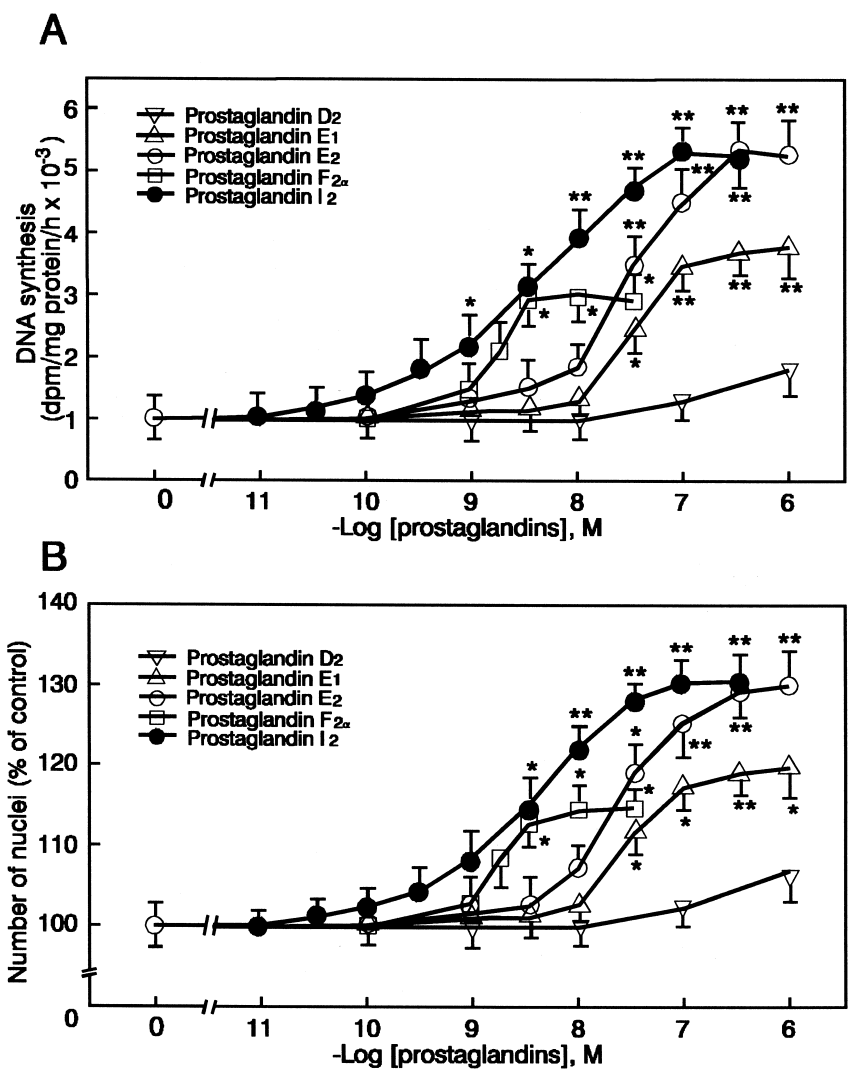

Fig. 2. Dose-dependent effects of various prostaglandins on hepatocyte DNA synthesis and proliferation. Hepatocytes at a density of 3.3×10^4 cells/cm^2 were plated and cultured as described in the legend for Fig. 1. After an attachment period of 3 h (zero time), the medium was rapidly replaced with serum- and dexamethasone-free Williams' medium E with or without various prostaglandins (10^{-11}–10^{-5} M) and cultured for 4 h. Hepatocyte DNA synthesis and proliferation were determined as described in Materials and Methods. The data are expressed as means ± S.E.M. of three experiments. $^{*}P < 0.05$, $^{**}P < 0.01$ compared with the respective control (medium alone).

D, E, F and I series, in the absence of any additional primary growth factors. Maintained in short-term cultures in a serum-free defined medium, the hepatocyte parenchymal cells synthesized DNA and proliferated in the presence of various prostaglandins. Significant stimulation of hepatocyte DNA synthesis was observed about 2.5 h after addition of prostaglandin I_2 (10^{-6} M), prostaglandin E_2 (10^{-6} M), and prostaglandin $F_{2\alpha}$ (3×10^{-8} M) (Fig. 1A). Significant DNA synthetic responses of cultured hepatocytes to addition of 10^{-6} M prostaglandin E_1 were observed during the first 3.0 h. The DNA synthetic activity of each prostaglandin reached a plateau at about 4 h, and was sustained for an additional 17 h. In particular, no significant increase was obtained after addition of 10^{-6} M prostaglandin D_2. The number of nuclei (proliferation) induced by prostaglandins significantly increased at about 3 (prostaglandin I_2) and 3.5 h (prostaglandin E_1, prostaglandin E_2 and prostaglandin $F_{2\alpha}$) after prostaglandin addition, reached a plateau around 4 h, and was sustained for an additional 17 h (Fig. 1B). No significant increase in proliferation was obtained after addition of 10^{-6} M prostaglandin D_2.

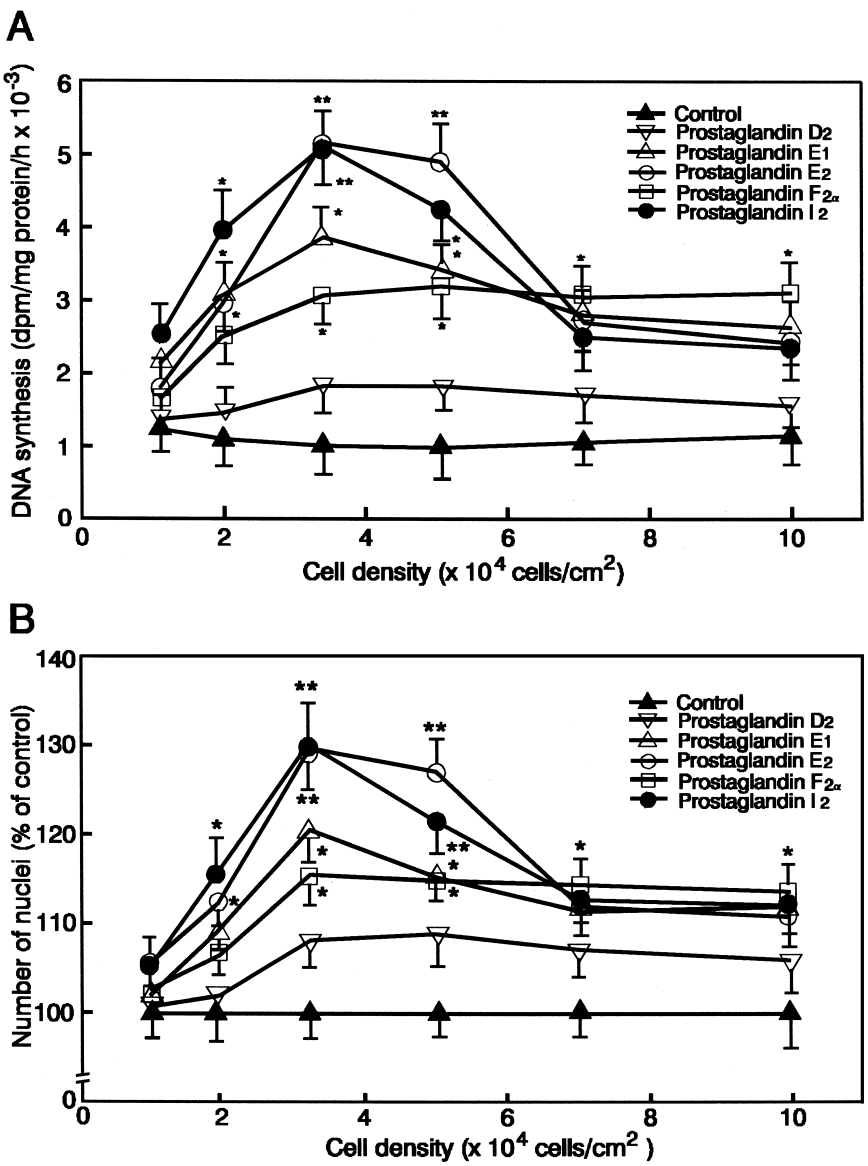

Fig. 3. Influence of cell density on prostaglandin-stimulated hepatocyte DNA synthesis and proliferation. Hepatocytes at various plating densities (1.0×10^4–1.0×10^5 cells/cm^2) were cultured for 3 h as described in the legend for Fig. 1. After an attachment period of 3 h (zero time), the medium was rapidly replaced with serum- and dexamethasone-free Williams' medium E with or without various prostaglandins (10^{-6} M prostaglandin D_2, 10^{-6} M prostaglandin E_1, 10^{-6} M prostaglandin E_2, 3×10^{-8} M prostaglandin $F_{2\alpha}$ and 10^{-6} M prostaglandin I_2) and cultured for 4 h. Hepatocyte DNA synthesis and proliferation were determined as described in Materials and Methods. The data are expressed as means ± S.E.M. of three experiments. $^{*}P < 0.05$, $^{**}P < 0.01$ compared with the respective control (medium alone).

3.2. Dose-dependent effects of prostaglandins on hepatocyte DNA synthesis and proliferation

We next examined the dose-dependent effect of various prostaglandins on hepatocyte DNA synthesis and proliferation. Prostaglandin E_2 and prostaglandin I_2 dose-dependently stimulated hepatocyte DNA synthesis. DNA synthesis reached a plateau at concentrations of 10^{-7} M prostaglandin E_2, and 1.5×10^{-8} M prostaglandin I_2; about five-fold stimulation was seen with these agents. Prostaglandin E_1 and prostaglandin $F_{2\alpha}$ also produced a dose-dependent stimulation of hepatocyte DNA synthesis; however, the maximal responses were significantly lower than those of prostaglandin E_2 and prostaglandin I_2 (Fig. 2A). The half-maximal effective concentrations (ED_{50}) of prostaglandins to induce hepatocyte DNA synthesis were estimated to be prostaglandin $F_{2\alpha}$ (1.7×10^{-9} M) < prostaglandin I_2 (3.7×10^{-9} M) < prostaglandin E_2 (2.7×10^{-8} M) < prostaglandin E_1 (3.2×10^{-8} M), while only slight induction was found with prostaglandin D_2. In addition, prostaglandin E_2 and prostaglandin I_2 dose-dependently stimulated hepatocyte proliferation (Fig. 2B). The maximal proliferating activities of the cultured hepatocytes induced by 10^{-7} M prostaglandin E_2 and 1.5×10^{-8} M prostaglandin I_2 were 1.3 times higher than the values in control cultures, and those of 1.5×10^{-7} M prostaglandin E_1 and 3×10^{-9} M prostaglandin $F_{2\alpha}$ were 1.2 times higher than the control. The cells responded only weakly to prostaglandin D_2, and plateau effects were not attained for prostaglandin D_2 even at this high concentration (10^{-6} M). The maximal effective concentrations of prostaglandin E_2 and prostaglandin $F_{2\alpha}$ were estimated to be 6.5×10^{-8} and 2.0×10^{-7} M, respectively. The ED_{50} of prostaglandins to induce hepatocyte proliferation was estimated to be prostaglandin $F_{2\alpha}$ (1.7×10^{-9} M) < prostaglandin I_2 (3.4×10^{-9} M) < prostaglandin E_2 (2.7×10^{-8} M) < prostaglandin E_1 (2.5×10^{-8} M). Therefore, the relative magnitude of the proliferating effects of the different prostaglandins roughly corresponded to their activities as stimulators of DNA synthesis.

3.3. Influence of cell density on hepatocyte DNA synthesis and proliferation induced by prostaglandins

To determine whether or not the proliferative effects of various prostaglandins are affected by initial plating densi-

Table 1
Effects of specific prostaglandin receptor antagonists, 2, 4-dideoxyadenosine and H-89 on hepatocyte DNA synthesis and proliferation in the presence of prostaglandins

Treatment	DNA synthesis (dis/min/mg protein/h $\times 10^{-3}$)		Number of nuclei (% of control)	
	Culture time (h)		Culture time (h)	
	4	21	4	21
Control	1.165 ± 0.210	1.090 ± 0.338	100.1 ± 2.4	99.9 ± 2.7
Prostaglandin E_1	3.893 ± 0.301^{b}	4.213 ± 0.203^{b}	120.3 ± 2.0^{b}	120.7 ± 2.1^{b}
+ SC-51322	$2.253 \pm 0.206^{a,d}$	$2.486 \pm 0.216^{a,e}$	$110.8 \pm 2.7^{a,d}$	$112.0 \pm 1.9^{a,d}$
+ Prostaglandin $F_{2\alpha}$ dimethyl amine	3.778 ± 0.322^{b}	4.236 ± 0.310^{b}	120.5 ± 2.8^{b}	120.2 ± 2.7^{b}
+ 2, 4-Dideoxyadenosine	3.802 ± 0.225^{b}	4.307 ± 0.156^{c}	120.7 ± 1.3^{b}	122.0 ± 2.6^{b}
+ H-89	3.787 ± 0.221^{b}	4.102 ± 0.211^{b}	120.3 ± 2.7^{b}	119.8 ± 2.4^{b}
Prostaglandin E_2	5.203 ± 0.313^{c}	5.410 ± 0.603^{b}	131.1 ± 2.6^{c}	134.7 ± 2.4^{c}
+ SC-51322	1.053 ± 0.126^{f}	1.158 ± 0.221^{e}	100.7 ± 1.9^{f}	101.0 ± 2.9^{f}
+ Prostaglandin $F_{2\alpha}$ dimethyl amine	5.707 ± 0.415^{c}	5.426 ± 0.333^{c}	130.7 ± 2.4^{c}	130.9 ± 4.7^{b}
+ 2, 4-Dideoxyadenosine	5.335 ± 0.323^{c}	5.306 ± 0.512^{b}	133.9 ± 1.8^{c}	133.3 ± 2.3^{c}
+ H-89	5.255 ± 0.309^{c}	5.402 ± 0.451^{b}	130.9 ± 2.4^{c}	133.6 ± 2.9^{b}
Prostaglandin $F_{2\alpha}$	3.106 ± 0.121^{b}	3.210 ± 0.189^{b}	116.1 ± 2.6^{a}	116.7 ± 3.4^{a}
+ SC-51322	3.035 ± 0.168^{b}	3.208 ± 0.138^{b}	116.7 ± 1.4^{b}	117.0 ± 3.7^{a}
+ Prostaglandin $F_{2\alpha}$ dimethyl amine	1.070 ± 0.102^{f}	1.063 ± 0.110^{f}	100.5 ± 1.8^{e}	100.2 ± 3.0^{d}
+ 2, 4-Dideoxyadenosine	3.008 ± 0.133^{b}	3.307 ± 0.262^{b}	117.5 ± 3.8^{a}	117.3 ± 3.2^{a}
+ H-89	3.150 ± 0.243^{b}	3.211 ± 0.311^{b}	117.7 ± 2.6^{b}	117.6 ± 3.9^{a}
Prostaglandin I_2	5.313 ± 0.422^{c}	5.437 ± 0.503^{b}	132.6 ± 3.5^{b}	133.3 ± 4.2^{b}
+ SC-51322	5.183 ± 0.421^{c}	5.232 ± 0.332^{c}	130.1 ± 3.3^{b}	131.0 ± 3.6^{b}
+ Prostaglandin $F_{2\alpha}$ dimethyl amine	5.007 ± 0.327^{c}	5.213 ± 0.391^{b}	130.8 ± 2.7^{b}	130.2 ± 2.8^{b}
+ 2, 4-Dideoxyadenosine	5.045 ± 0.237^{c}	5.220 ± 0.502^{b}	130.0 ± 2.8^{b}	131.7 ± 3.7^{b}
+ H-89	5.166 ± 0.317^{c}	5.263 ± 0.389^{b}	130.5 ± 2.7^{b}	132.4 ± 2.6^{c}

Hepatocytes were plated at a density of 3.3×10^4 cells/cm^2. After an attachment period of 3 h, the medium was changed and the cells were cultured further for 4 and 21 h with 10^{-6} M prostaglandin E_1, 10^{-6} M prostaglandin E_2, 3×10^{-8} M prostaglandin $F_{2\alpha}$ or 10^{-6} M prostaglandin I_2 on their own or with various agents: SC-51322, 10^{-7} M; prostaglandin $F_{2\alpha}$ dimethyl amine, 10^{-6} M; 2, 4-dideoxyadenosine, 10^{-6} M; H-89, 10^{-7} M. Each value is expressed as the mean ± S.E.M. from three independent preparations. Values significantly different from control are indicated by $^{a}P < 0.05$, $^{b}P < 0.01$, or $^{c}P < 0.001$. Values significantly different from prostaglandin E_1, prostaglandin E_2, prostaglandin $F_{2\alpha}$ or prostaglandin I_2 on their own are indicated by $^{d}P < 0.05$, $^{e}P < 0.01$, or $^{f}P < 0.001$.

ties, we investigated the density dependence of hepatocyte DNA synthesis and proliferation induced by various prostaglandins at 4 h of culture. Fig. 3A shows that hepatocyte DNA synthesis induced by prostaglandins significantly increased with increasing initial plating density ($1.0–3.3 \times 10^4$ cells/cm^2). It reached a maximum value at a cell density of 3.3×10^4 cells/cm^2 and decreased only slightly at a higher cell density. In general, there was a good correlation between the ability of prostaglandins to stimulate DNA synthesis and the increase in the number of nuclei (proliferation) at various cell densities (Fig. 3B). The apparent cell density-independent effect of prostaglandins at high plating densities is similar to that of insulin, platelet-derived growth factor (PDGF) (Kimura and Ogihara, 1998a), insulin-like growth factor-II (IGF-II) (Kimura and Ogihara, 1998b) and TGF-α (Kimura and Ogihara, 1999).

3.4. Effect of prostaglandin receptor antagonists, specific inhibitors of adenylate cyclase and protein kinase A on hepatocyte DNA synthesis and proliferation induced by prostaglandins

To investigate an association between prostanoid receptors and second messenger-generating systems, such as adenylate cyclase and protein kinase A, we examined the effects of specific prostaglandin receptor antagonists, specific inhibitors of adenylate cyclase and protein kinase A on prostaglandin-stimulated hepatocyte DNA synthesis and proliferation (Table 1). Prostaglandin E_2-induced hepatocyte DNA synthesis and proliferation were completely blocked by the specific EP_1 receptor antagonist SC-51322 (10^{-7} M) (Coleman et al., 1994; Hallinan et al., 1994), but the prostaglandin E_1-induced response was only partially blocked by the antagonist during 4 and 21 h of culture. Prostaglandin $F_{2\alpha}$- and prostaglandin I_2-induced hepatocyte DNA synthesis and proliferation were not affected by SC-51322 (10^{-6} M). The specific FP receptor antagonist prostaglandin $F_{2\alpha}$ dimethyl amine (10^{-6} M) (Maddox et al., 1978) completely blocked the hepatocyte DNA synthesis and proliferation induced by prostaglandin $F_{2\alpha}$ (3×10^{-8} M) during 4 and 21 h of culture. However, this agent did not affect the mitogenic responses induced by prostaglandin E_1, prostaglandin E_2 and prostaglandin I_2. There are currently no selective antagonists available for prostaglandin I_2 (IP) receptors.

To characterize the possible involvement of the adenylate cyclase/ protein kinase A system in prostaglandin-induced hepatocyte DNA synthesis and proliferation, we investigated the effects of the direct inhibitor of adenylate cyclase 2, 4-dideoxyadenosine (Holgate et al., 1980), and the specific protein kinase A inhibitor H-89 (Zusick et al., 1994) on these responses. As shown in Table 1, treatment of hepatocytes with either 10^{-6} M 2, 4-dideoxyadenosine

Table 2
Effects of specific inhibitors of phospholipase C, protein kinase C, Ca^{2+} channel blockers and Ca^{2+} ionophores on hepatocyte DNA synthesis and proliferation in the presence of prostaglandin E_1 or E_2

Treatment	DNA synthesis (dis/min/mg protein/h $\times 10^{-3}$)		Number of nuclei (% of control)	
	Culture time (h)		Culture time (h)	
	4	21	4	21
Control	1.105 ± 0.216	1.138 ± 0.264	100.3 ± 3.4	100.2 ± 3.1
Prostaglandin E_1	3.783 ± 0.321^{b}	4.110 ± 0.603^{a}	120.2 ± 2.7^{a}	120.8 ± 2.8^{b}
+ U-73122	1.234 ± 0.327^{e}	1.282 ± 0.238^{d}	100.2 ± 2.8^{e}	101.5 ± 2.9^{e}
+ U-73343	3.722 ± 0.356^{b}	4.113 ± 0.355^{b}	120.3 ± 3.6^{a}	121.3 ± 3.7^{a}
+ Sphingosine	3.737 ± 0.320^{b}	4.306 ± 0.402^{b}	121.4 ± 3.2^{a}	121.2 ± 2.9^{b}
+ A23187	$6.065 \pm 0.382^{c,d}$	$6.168 \pm 0.430^{c,d}$	$138.2 \pm 3.6^{b,d}$	$140.0 \pm 3.8^{b,d}$
+ Ionomycin	$6.151 \pm 0.325^{c,e}$	$6.211 \pm 0.421^{c,d}$	$140.9 \pm 2.9^{c,e}$	$142.1 \pm 3.2^{c,e}$
+ Verapamil	1.708 ± 0.115^{e}	1.608 ± 0.125^{d}	106.2 ± 3.5^{d}	105.6 ± 3.2^{d}
+ Diltiazem	1.561 ± 0.637^{d}	1.632 ± 0.626^{d}	102.4 ± 2.3^{e}	103.0 ± 2.7^{d}
Prostaglandin E_2	5.222 ± 0.356^{c}	5.313 ± 0.375^{c}	130.3 ± 3.6^{b}	131.3 ± 3.7^{b}
+ U-73122	1.452 ± 0.303^{e}	1.524 ± 0.229^{f}	100.9 ± 2.7^{e}	100.2 ± 3.3^{e}
+ U-73343	5.255 ± 0.289^{c}	5.618 ± 0.456^{b}	130.4 ± 3.0^{b}	131.2 ± 3.2^{b}
+ Sphingosine	5.167 ± 0.517^{b}	5.506 ± 0.423^{c}	129.7 ± 4.2^{b}	130.6 ± 2.8^{b}
+ A23187	$6.518 \pm 0.302^{c,d}$	$6.564 \pm 0.241^{c,d}$	$142.2 \pm 2.3^{c,d}$	$144.0 \pm 2.6^{c,d}$
+ Ionomycin	$6.333 \pm 0.385^{c,d}$	$6.819 \pm 0.362^{c,d}$	$144.1 \pm 3.4^{b,d}$	$145.1 \pm 3.3^{c,d}$
+ Verapamil	1.399 ± 0.135^{c}	1.501 ± 0.105^{c}	103.6 ± 3.0^{c}	104.2 ± 3.1^{c}
+ Diltiazem	1.500 ± 0.187^{b}	1.507 ± 0.190^{b}	103.4 ± 1.8^{b}	103.9 ± 2.5^{b}

Hepatocytes were plated at a density of 3.3×10^4 cells/cm^2. After an attachment period of 3 h, the medium was changed and the cells were cultured further for 4 and 21 h with 10^{-6} M prostaglandin E_1 or 10^{-6} M prostaglandin E_2 on their own or with various agents: U-73122, 10^{-6} M; U-73343, 10^{-6} M; sphingosine, 10^{-6} M; A23187, 10^{-6} M; ionomycin, 10^{-7} M; verapamil, 10^{-6} M; diltiazem, 10^{-6} M. Each value is expressed as the mean ± S.E.M. from three independent preparations. Values significantly different from control are indicated by $^{a}P < 0.05$, $^{b}P < 0.01$, or $^{c}P < 0.001$. Values significantly different from prostaglandin E_1 or prostaglandin E_2 on their own are indicated by $^{d}P < 0.05$, $^{e}P < 0.01$, or $^{f}P < 0.001$.

or 10^{-7} M H-89 did not produce any significant inhibition of the hepatocyte DNA synthesis and proliferation induced by various prostaglandins during 4 and 21 h of culture. These inhibitors on their own did not significantly affect hepatocyte mitogenesis (data not shown).

3.5. Effects of specific inhibitors of phospholipase C, protein kinase C, Ca^{2+} channel blockers and calcium ionophores on hepatocyte DNA synthesis and proliferation induced by prostaglandins

To characterize the involvement of the phospholipase C/protein kinase C system in hepatocyte DNA synthesis and proliferation induced by various prostaglandins, we investigated the effects of the specific phospholipase C inhibitor U-73122 (Thompson et al., 1991) and the protein kinase C inhibitor sphingosine (Merrill et al., 1989) on these responses (Tables 2 and 3). U-73122 (10^{-6} M) was found to markedly attenuate the action of 10^{-6} M prostaglandin E_1 or 10^{-6} M prostaglandin E_2 on hepatocyte DNA synthesis and proliferation during 4 and 21 h of culture. U-73343 (10^{-6} M), a close structural analog of U-73122, which has no such inhibitory action on phospholipase C, did not significantly affect 10^{-6} M prostaglandin E_1- or 10^{-6} M prostaglandin E_2-induced hepatocyte DNA synthesis and proliferation. However, sphingosine (10^{-6} M), a protein kinase C inhibitor, alone had no significant effect on hepatocyte DNA synthesis and proliferation induced by 10^{-6} M prostaglandin E_1 or 10^{-6} M prostaglandin E_2 during 4 and 21 h of culture. The pharmacological profiles of the above-mentioned inhibitors, U-73122 (10^{-6} M) and sphingosine (10^{-6} M), on prostaglandin $F_{2\alpha}$- and prostaglandin I_2-induced hepatocyte DNA synthesis and proliferation during 4 and 21 h of culture were very similar to those of prostaglandin E_1 and prostaglandin E_2 (Table 3).

Similarly, to determine the possible involvement of Ca^{2+} mobilization in prostaglandin E_1- or prostaglandin E_2-stimulated hepatocyte DNA synthesis and proliferation, the cells were treated with the Ca^{2+} ionophore ionomycin or A23187 (Xiaomei et al., 1995) for 4 and 21 h of culture. Significant potentiation of both hepatocyte DNA synthesis and proliferation induced by 10^{-6} M prostaglandin E_1 or 10^{-6} M prostaglandin E_2 was observed with 10^{-7} M ionomycin or 10^{-6} M A23187 during 4 and 21 h of culture. Conversely, the ability of these prostaglandins to stimulate hepatocyte DNA synthesis and proliferation was almost completely blocked by Ca^{2+} channel blockers, such as 10^{-6} M verapamil or 10^{-6} M diltiazem (Table 2). The pharmacological profiles of the Ca^{2+} ionophores and Ca^{2+} channel blockers on prostaglandin $F_{2\alpha}$- and prostaglandin I_2-induced hepatocyte DNA synthesis and proliferation during 4 and 21 h of culture were very similar to those of prostaglandin E_1 and prostaglandin E_2 (Table 3).

Table 3
Effects of specific inhibitors of phospholipase C, protein kinase C, Ca^{2+} channel blockers and Ca^{2+} ionophores on hepatocyte DNA synthesis and proliferation in the presence of prostaglandin $F_{2\alpha}$ or I_2

Treatment	DNA synthesis (dis/min/mg protein/h $\times 10^{-3}$)		Number of nuclei (% of control)	
	Culture time (h)		Culture time (h)	
	4	21	4	21
Control	1.108 ± 0.201	1.144 ± 0.259	100.2 ± 3.6	100.3 ± 3.0
Prostaglandin $F_{2\alpha}$	3.134 ± 0.257^{b}	3.314 ± 0.306^{b}	117.3 ± 1.8^{a}	118.0 ± 1.9^{b}
+ U-73122	1.366 ± 0.114^{e}	1.236 ± 0.175^{e}	102.8 ± 2.0^{e}	103.0 ± 3.1^{d}
+ U-73343	3.233 ± 0.199^{b}	3.377 ± 0.329^{b}	117.0 ± 4.3^{a}	118.2 ± 3.9^{a}
+ Sphingosine	3.361 ± 0.233^{b}	3.227 ± 0.298^{b}	118.7 ± 2.6^{a}	119.2 ± 3.2^{a}
+ A23187	$6.007 \pm 0.229^{c,e}$	$6.013 \pm 0.411^{c,e}$	$138.1 \pm 3.4^{b,e}$	$139.4 \pm 3.5^{b,e}$
+ Ionomycin	$6.030 \pm 0.328^{c,e}$	$6.156 \pm 0.331^{c,e}$	$146.1 \pm 2.8^{c,e}$	$146.3 \pm 3.0^{c,e}$
+ Verapamil	1.516 ± 0.101^{e}	1.506 ± 0.113^{e}	105.7 ± 3.5^{d}	106.1 ± 2.0^{d}
+ Diltiazem	1.442 ± 0.123^{e}	1.318 ± 0.703^{e}	103.4 ± 1.9^{e}	105.2 ± 2.7^{d}
Prostaglandin I_2	5.313 ± 0.364^{c}	5.277 ± 0.302^{c}	131.0 ± 3.0^{d}	133.2 ± 3.4^{d}
+ U-73122	1.225 ± 0.250^{f}	1.533 ± 0.296^{f}	100.2 ± 2.6^{e}	100.6 ± 3.1^{e}
+ U-73343	5.239 ± 0.418^{c}	5.197 ± 0.436^{b}	131.2 ± 3.4^{b}	131.4 ± 3.1^{b}
+ Sphingosine	5.192 ± 0.358^{c}	5.201 ± 0.427^{b}	130.1 ± 4.0^{b}	130.3 ± 2.5^{b}
+ A23187	$6.515 \pm 0.233^{c,d}$	$6.489 \pm 0.312^{c,d}$	$144.4 \pm 3.6^{c,d}$	$145.9 \pm 3.2^{c,d}$
+ Ionomycin	$6.436 \pm 0.302^{c,d}$	$6.371 \pm 0.176^{c,d}$	$145.3 \pm 2.5^{c,d}$	$145.6 \pm 2.8^{c,d}$
+ Verapamil	1.881 ± 0.346^{e}	1.525 ± 0.276^{f}	104.2 ± 3.6^{e}	103.8 ± 2.6^{e}
+ Diltiazem	2.019 ± 0.330^{e}	2.008 ± 0.243^{e}	105.3 ± 3.2^{e}	105.5 ± 2.9^{e}

Hepatocytes were plated at a density of 3.3×10^4 cells/cm^2. After an attachment period of 3 h, the medium was changed and the cells were cultured further for 4 and 21 h with 3×10^{-8} M prostaglandin $F_{2\alpha}$ or 10^{-6} M prostaglandin I_2 on their own or with various agents: U-73122, 10^{-6} M; U-73343, 10^{-6} M; sphingosine, 10^{-6} M; A23187, 10^{-6} M; ionomycin, 10^{-7} M; verapamil, 10^{-6} M; diltiazem, 10^{-6} M. Each value is expressed as the mean ± S.E.M. from three independent preparations. Values significantly different from control are indicated by $^{a}P < 0.05$, $^{b}P < 0.01$, or $^{c}P < 0.001$. Values significantly different from prostaglandin $F_{2\alpha}$ or prostaglandin I_2 on their own are indicated by $^{d}P < 0.05$, $^{e}P < 0.01$, or $^{f}P < 0.001$.

3.6. Effects of specific inhibitors of receptor tyrosine kinase and related signal transducers on hepatocyte DNA synthesis and proliferation induced by prostaglandins

We investigated whether or not the mitogenic responses of hepatocytes to various prostaglandins, including those of the E, F and I series, were mediated by signal transducers, such as receptor tyrosine kinase, PI3K, MAP kinase kinase and p70 S6K by using corresponding specific inhibitors of the signal transducers. As shown in Fig. 4, the hepatocyte DNA synthesis and proliferation induced by 10^{-6} M prostaglandin E_1 and 10^{-6} M prostaglandin E_2 during 4 and 21 h was almost completely blocked by 5×10^{-6} M genistein (Akiyama et al., 1987), 10^{-7} M wortmannin (Baggiolini et al., 1987), 10^{-5} M PD98059 (Alessi et al., 1995) and 10 ng/ml rapamycin (Price et al., 1992), suggesting that the hepatocyte mitogenesis induced by these prostaglandins was also stimulated through recep-

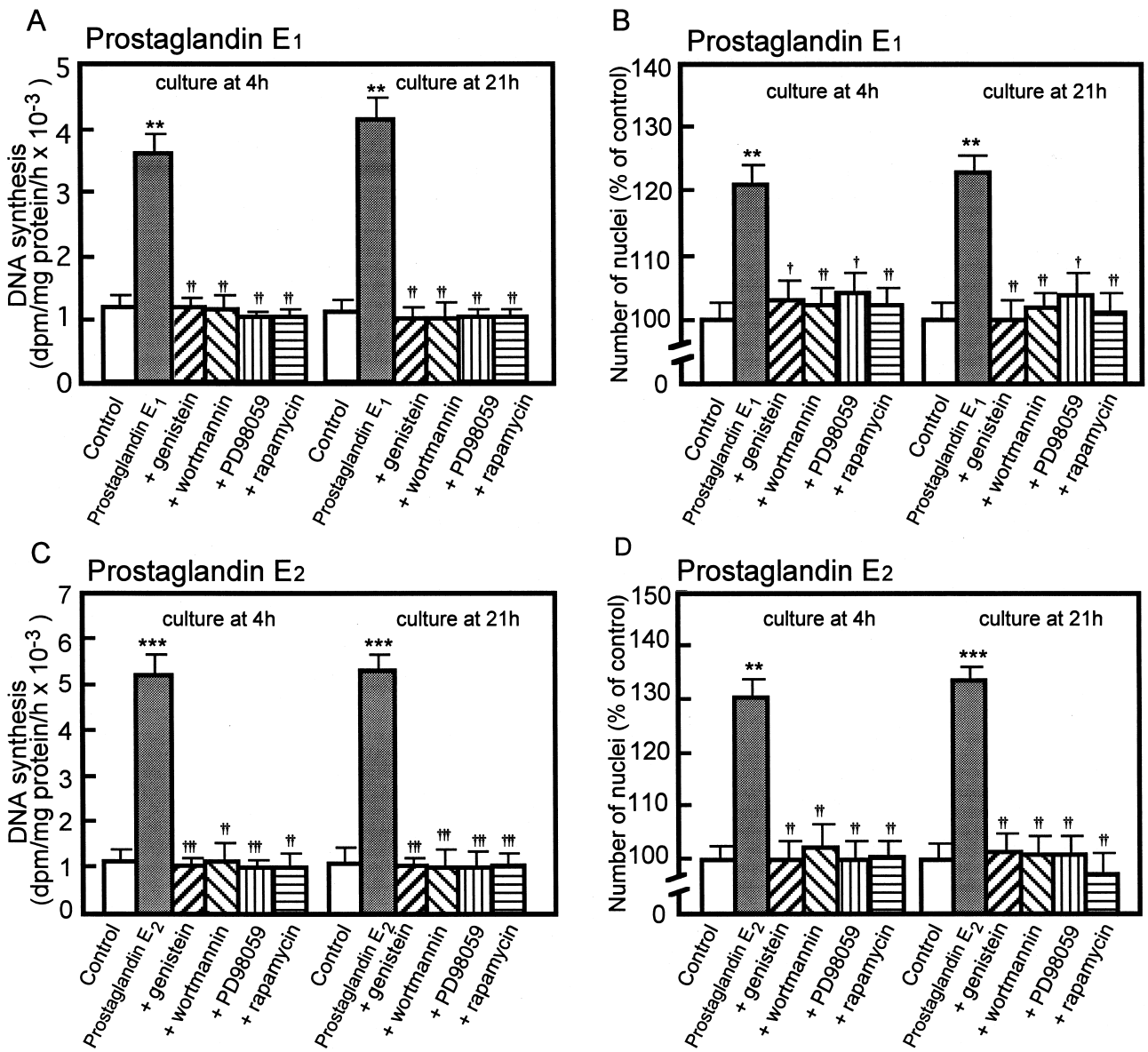

Fig. 4. Effects of specific inhibitors of signal-transducing elements on hepatocyte DNA synthesis and proliferation induced by prostaglandin E_1 or prostaglandin E_2. Hepatocytes at a density of 3.3×10^4 cells/cm^2 were plated and cultured as described in the legend for Fig. 1. After an attachment period of 3 h (zero time), the medium was rapidly replaced with serum- and dexamethasone-free Williams' medium E with or without various prostaglandins and/or specific inhibitors of signal transducers. The cells were cultured further for 4 and 21 h with 10^{-6} M prostaglandin E_1 and 10^{-6} M prostaglandin E_2 on their own or with various specific inhibitors of signal-transducing elements: genistein, 5×10^{-6} M; wortmannin, 10^{-7} M; PD98059, 10^{-5} M; rapamycin, 10 ng/ml. The data are expressed as means ± S.E.M. of three experiments. Values significantly different from control (medium alone) are indicated by $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$. Values significantly different from 10^{-6} M prostaglandin E_1 and 10^{-6} M prostaglandin E_2 on their own are indicated by $^{\dagger}P < 0.05$, $^{\dagger\dagger}P < 0.01$ or $^{\dagger\dagger\dagger}P < 0.001$.

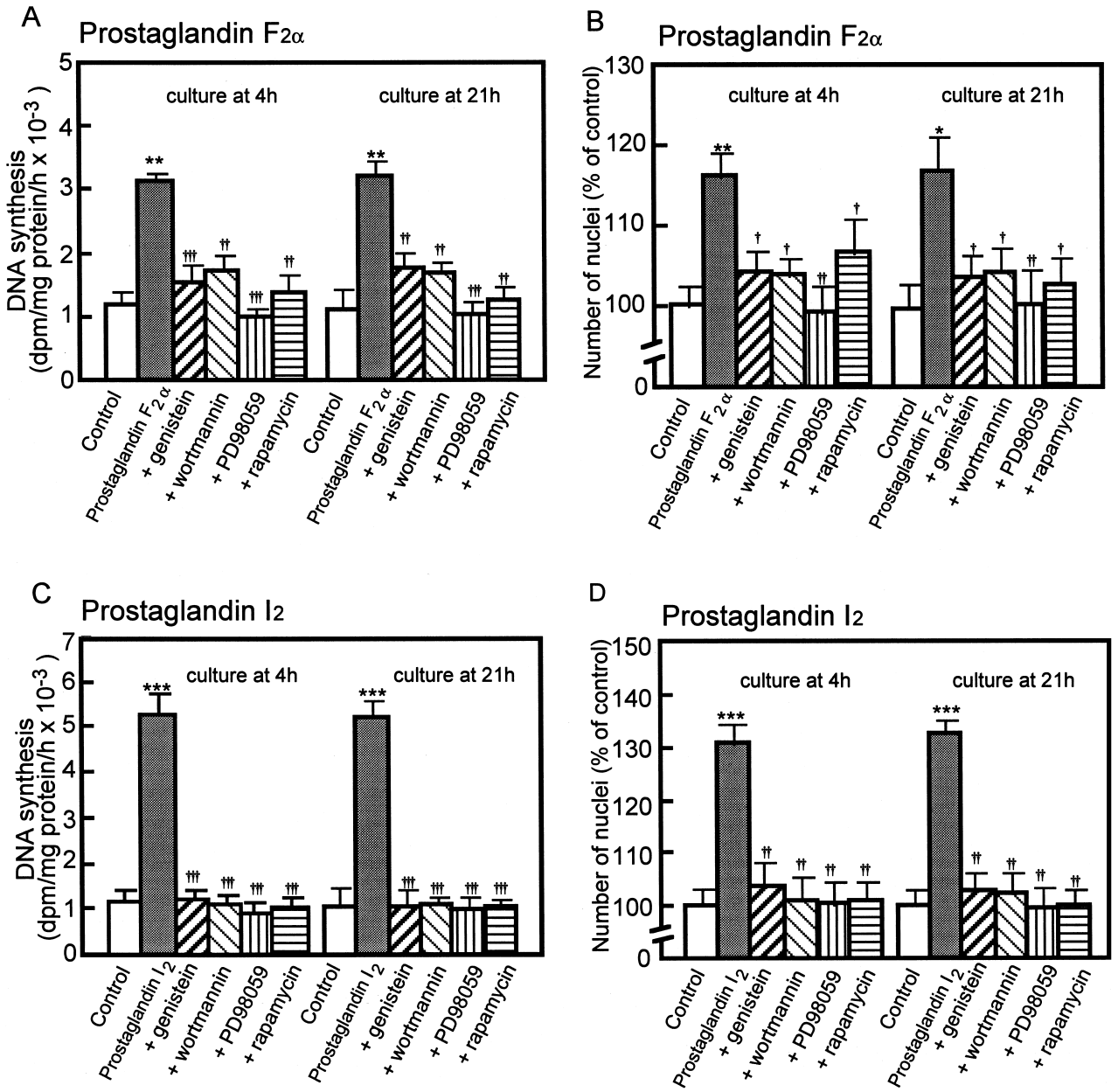

Fig. 5. Effects of specific inhibitors of signal-transducing elements on hepatocyte DNA synthesis and proliferation induced by prostaglandin $F_{2\alpha}$ or prostaglandin I_2. Hepatocytes at a density of 3.3×10^4 cells/cm^2 were plated and cultured as described in the legend for Fig. 1. After an attachment period of 3 h (zero time), the medium was rapidly replaced with serum- and dexamethasone-free Williams' medium E with or without various prostaglandins and/or specific inhibitors of signal transducers. The cells were cultured further for 4 and 21 h with 3×10^{-8} M prostaglandin $F_{2\alpha}$ and 10^{-6} M prostaglandin I_2 on their own or with various specific inhibitors of signal-transducing elements: genistein, 5×10^{-6} M; wortmannin, 10^{-7} M; PD98059, 10^{-5} M; rapamycin, 10 ng/ml. The data are expressed as means ± S.E.M. of three experiments. Values significantly different from control (medium alone) are indicated by $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$. Values significantly different from 3×10^{-8} M prostaglandin $F_{2\alpha}$ and 10^{-6} M prostaglandin I_2 on their own are indicated by $^{\dagger}P < 0.05$, $^{\dagger\dagger}P < 0.01$ or $^{\dagger\dagger\dagger}P < 0.001$.

tors that are associated with tyrosine kinase, and mediated via PI3K, MAP kinase kinase and p70 S6K. The pharmacological profiles of the above-mentioned inhibitors of signal transducers on 3×10^{-8} M prostaglandin $F_{2\alpha}$- and 10^{-6} M prostaglandin I_2-induced hepatocyte DNA synthesis and proliferation during 4 and 21 h of culture were very similar to those of prostaglandin E_1 and prostaglandin E_2 (Fig. 5). These findings suggest that these prostaglandins use a common pathway to stimulate hepatocyte DNA synthesis and proliferation.

4. Discussion

The diversity of prostaglandin action is due to prostanoid receptor subtypes coupling to different signal transduction

pathways (Coleman and Kennedy, 1985; Coleman et al., 1994). Molecular cloning has identified a number of different prostanoid receptor subtypes and differently spliced isoforms. For example, there are at least three subtypes of prostaglandin E (EP) receptors, termed EP_1, EP_2 and EP_3, which have been defined on the basis of their pharmacological profiles and signal transduction pathways in many cell types (Honda et al., 1993; Watabe et al., 1993; Coleman et al., 1994). Generally, the effects of prostaglandin are mediated through G-protein-coupled receptors. However, relatively little is known about subtypes and effector mechanisms for prostaglandin receptors, which may regulate hepatocyte mitogenesis.

As shown in Figs. 1 and 2, we demonstrated for the first time that prostaglandin E_1, prostaglandin E_2, prostaglandin $F_{2\alpha}$ and prostaglandin I_2, on their own, stimulate hepatocyte DNA synthesis and proliferation in the absence of exogenous growth factors (e.g., EGF and insulin). The sensitivity and the magnitude of the stimulatory response elicited by each prostaglandin differed significantly. Among the prostaglandins we tested, prostaglandin $F_{2\alpha}$ showed the most potent proliferative effect, followed by prostaglandin I_2, prostaglandin E_2, prostaglandin E_1, and prostaglandin D_2 as judged by ED_{50} values. These differences are likely to depend on the affinity of each prostaglandin for its own receptor. In addition, the differences in efficacy may be due to a difference in the intrinsic activity of individual prostaglandins. In order to clarify the mechanism by which prostaglandins caused proliferation of hepatocytes, we tried to pharmacologically determine the role of membrane-bound effectors for prostanoid receptors. The EP_1 receptor antagonist, SC-51322 (10^{-7} M), almost completely blocked the proliferating action of prostaglandin E_2, and only partially inhibited the action of prostaglandin E_1, indicating that the effects of prostaglandin E_2 are mediated mainly via the EP_1 receptor, and that the effects of prostaglandin E_1 are only partly mediated by the EP_1 receptor (Table 1). The specific prostaglandin $F_{2\alpha}$ (FP) receptor antagonist prostaglandin $F_{2\alpha}$ dimethyl amine (10^{-6} M) completely blocked hepatocyte DNA synthesis and proliferation induced by prostaglandin $F_{2\alpha}$, but did not affect those responses induced by other prostaglandins, also suggesting that the action of prostaglandin $F_{2\alpha}$ is mediated through its own receptor (Table 1).

Butcher et al. (1967) were the first to demonstrate an association between prostaglandins and cAMP. Prostaglandins of the E and I series slightly stimulate adenylate cyclase in many cells, via Gs-protein, but those of the F series markedly inhibit, via Gi-protein, the response to glucagon or epinephrine in some cells (Melien et al., 1988). Therefore, the involvement of these pathways in hepatocyte growth is not known. Since submaximal concentrations of 2, 4-dideoxyadenosine (10^{-6} M) and H-89 (10^{-7} M) did not inhibit hepatocyte DNA synthesis and proliferation (Table 1), we think that the activation of adenylate cyclase/the second messenger cAMP and protein kinase A signaling system may not be involved in the stimulation of hepatocyte mitogenesis induced by various prostaglandins.

However, prostaglandins, acting through other G-protein (possibly Gq)-coupled receptors, stimulate phospholipase C-dependent signaling pathways, leading to phosphatidylinositol-1, 4, 5-trisphosphate (IP_3)-induced elevation of intracellular Ca^{2+} and diacylglycerol activation of protein kinase C. These mechanisms have been reported to be involved in mitogenesis in certain cell types and hepatocytes (Nishizuka, 1986; Berridge, 1993). As shown in Tables 2 and 3, activation of phospholipase C by prostaglandins is an essential step in hepatocyte mitogenesis, since prostaglandin-stimulated hepatocyte DNA synthesis and proliferation were blocked by a phospholipase inhibitor U-73122, but not by U-73133. However, the second messenger diacylglycerol/protein kinase C signaling pathway appears less important, because a submaximal concentration of a protein kinase C inhibitor, sphingosine (10^{-6} M), had no significant effect on prostaglandin-induced hepatocyte DNA synthesis and proliferation (Tables 2 and 3). In contrast, intracellular and/or extracellular mobilization of Ca^{2+} are obligatory for the prostaglandin-mediated induction of hepatocyte DNA synthesis and proliferation, because hepatocyte mitogenesis was abolished by the addition of Ca^{2+} channel blockers to the cultures and potentiated by Ca^{2+} ionophores (Tables 2 and 3). As for the role of Ca^{2+} in prostanoid receptor activation, it has been reported that prostaglandin E_2 contracts guinea pig trachea via EP_1 receptors depending on extracellular Ca^{2+} (Coleman and Kennedy, 1985). Watabe et al. (1993) demonstrated that prostaglandin E_2 caused an increase in intracellular Ca^{2+} concentration in cells expressing the recombinant murine EP_1 receptor. However, the phosphoinositide response evoked by this EP_1 receptor was weak and occurred slowly, making it difficult to assess the contribution of phosphoinositide turnover to the rapid transient increase in free Ca^{2+} concentration in the cells. It has also been reported that activation of Ca^{2+} mobilization occurs in Swiss mouse 3T3 cells where prostaglandin $F_{2\alpha}$ acts as a mitogen (Nakada et al., 1990; Nakao et al., 1993; Woodward and Lawrence, 1994). In addition, there have been several reports of prostaglandin I_2 increasing intracellular Ca^{2+} concentrations and evoking smooth muscle contraction (Lawrence et al., 1992). Overall, these reports and our results suggest that EP, FP, and IP receptors couple directly to G-protein to cause phospholipase C activation, IP_3-dependent Ca^{2+} mobilization and/or activation of Ca^{2+} channel, which mediated the entry of extracellular Ca^{2+} (Tables 2 and 3).

The involvement of receptor-tyrosine kinase pathways in prostaglandin-stimulated hepatocyte DNA synthesis and proliferation is of particular interest, since activation of this signaling pathway is essential for the initiation of DNA synthesis and mitosis in most cells (Ullrich and Schlessinger, 1990; Davis, 1993). The results of our stud-

ies with specific inhibitors of major signal transducing elements participating in this cascade suggest that, among the elements that link the cell surface receptor to the nucleus, tyrosine kinase, PI3K, MAP kinase kinase and p70 S6K mediate the action of the various prostaglandins tested here (Figs. 4 and 5). The patterns of inhibition of prostaglandin-stimulated hepatocyte mitogenesis by these specific inhibitors of signal transducers were very similar among the naturally occurring prostaglandins we tested. Although the activation of receptor tyrosine kinase results in the stimulation of activity of other cellular components, such as PI3K, MAP kinase kinase and p70 S6K, and the induction of hepatocyte DNA synthesis and proliferation, stimulation of Ca^{2+}-mobilizing receptors by α_1-adrenoceptor agonists or vasopressin did not stimulate hepatocyte DNA synthesis and proliferation (Kimura and Ogihara, 1997b). Therefore, whether or not an association exists between the prostaglandin receptors-Gq-phospholipase C/Ca^{2+} pathway and the receptor tyrosine kinase system remains unknown. To our knowledge, direct activation of the receptor tyrosine kinase system by the phospholipase C/Ca^{2+} pathway has not been reported previously. At present, therefore, it seems reasonable that the prostaglandin receptor-Gq-phospholipase C/Ca^{2+} signaling pathway is closely associated with the tyrosine kinase pathway through an unknown mechanism that stimulates DNA synthesis and proliferation in primary cultures of adult rat hepatocytes. As to the further mechanism of prostaglandin action, it is tempting to speculate that the action of prostaglandin is mediated by a hepatocyte-derived autocrine growth factor. Studies to support this notion are now in progress in our laboratory.

The following hypothesis on the control of hepatocyte DNA synthesis and proliferation by various prostaglandins is therefore proposed: elevated levels of prostaglandins in the early stages of liver regeneration may play a key role as intrinsic paracrine (or autocrine) trigger substances for neighboring hepatocytes. The action of prostaglandin may be mediated by an increase in intracellular Ca^{2+}, whether of intracellular or extracellular origin, after which, it activates the receptor tyrosine kinase pathway via an unknown mechanism. Insight into these mechanisms may shed light not only on basic mechanisms of hepatic growth regulation, but also on therapeutic use of various prostaglandins for hepatic injury.

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