Inhibitory Effects of Dexamethasone on Epidermal Growth Factor-Induced DNA Synthesis and Proliferation in Primary Cultures of Adult Rat Hepatocytes

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We investigated the effects of dexamethasone on epidermal growth factor (EGF)-induced DNA synthesis and proliferation in serum-free primary cultures of adult rat hepatocytes. Isolated hepatocytes were cultured at a density of 3.3×10^4 cells/cm^2 in Williams' medium E containing 5% bovine calf serum and various concentrations of dexamethasone for 1, 2 and 3 h. After the 3-h attachment period, the medium was changed, and cells were cultured in serum-free and dexamethasone-free Williams' medium E with or without glucocorticoid receptor antagonists. The growth-stimulating effects of EGF (20 ng/ml) on the primary cultured hepatocytes were time- and concentration-dependently inhibited by dexamethasone added to the culture medium. The mineral corticoid aldosterone (10^{-7} M) did not produce the same growth-inhibitory effects as dexamethasone (10^{-8} M). The inhibitory effects of dexamethasone were reversed by treatment with the glucocorticoid receptor antagonist mifepristone (RU486, 10^{-6} M) or a monoclonal antibody against glucocorticoid receptor (100 ng/ml). In addition, the growth-inhibitory effects of dexamethasone did not affect EGF-induced p42 mitogen-activated protein (MAP) kinase phosphorylation. These results indicate that dexamethasone concentration-dependently delays and inhibits the EGF-induced DNA synthesis and proliferation through its own intracellular receptor in primary cultures of adult rat hepatocytes.

Key words dexamethasone; DNA synthesis; proliferation; cultured hepatocyte; epidermal growth factor

Much of the research on the proliferation of hepatocytes *in vitro* has been motivated by studies of liver regeneration *in vivo*,[1] and factors that initiate and regulate the replication of adult mammalian liver cells following 70% partial hepatectomy have been investigated. The signal transduction pathways of these growth factors have also been reviewed.[2—4] Maintained in short-term culture, in a defined medium, quiescent hepatocytes undergo one or two rounds of replication in response to mitogens (*e.g.*, epidermal growth factor (EGF), hepatocyte growth factor (HGF), platelet-derived growth factor (PDGF) and transforming growth factor (TGF)-α).[5—8] In addition to the growth factor effects on hepatocyte proliferation, various other factors and conditions modulate the response of hepatocytes to growth stimuli *in vitro* (*e.g.*, co-mitogens, component of basal culture medium, cell density, extracellular matrix materials, and cell-derived autocrine factors).[5,9—12] As *in vitro* systems have been useful for clarifying the action mechanisms of these factors, it is important for investigators to define the conditions under which primary hepatocytes are proliferation-competent *in vitro*.

Based on routine procedures, hepatocytes were seeded into serum-containing culture medium with a fixed concentration of dexamethasone. This was exchanged for serum-free, dexamethasone-free medium, and cells were treated with several agents before measurement of hepatocyte DNA synthesis and proliferation induced by growth factors such as EGF and HGF after further culture. With regard to glucocorticoids such as dexamethasone and hydrocortisone within the components added to culture medium, improved cell attachment and viability were observed, but no effects on proliferation were seen.[13,14] However, the concentrations and timing of addition of the glucocorticoid to culture have varied between investigators,[5—12] and optimal culture conditions remain to be determined.

The purpose of the present study was to investigate the effects of glucocorticoid dexamethasone on EGF-induced DNA synthesis and proliferation in primary cultures of adult rat hepatocytes. We also investigated the glucocorticoid receptor mediation of dexamethasone in relation to the mechanisms of intracellular signal transduction during primary culture. Our results show that higher concentrations of dexamethasone delay and inhibit EGF-induced DNA synthesis and proliferation in primary cultures of adult rat hepatocytes. Lower concentrations of dexamethasone are optimal for assessing DNA synthesis and proliferation in a defined medium, in short-term culture without affecting cell viability and attachment. Furthermore, glucocorticoid receptor is responsible for growth inhibition by dexamethasone in primary cultured hepatocytes.

MATERIALS AND METHODS

Materials The following reagents were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.): aldosterone, aphidicolin, aprotinin, dexamethasone, human-recombinant epidermal growth factor (EGF), hydrocortisone and mifepristone (RU486). Monoclonal antibody against glucocorticoid receptor (raised against amino acids 395—411) was obtained from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, U.S.A.). 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, U.S.A.). [Methyl-^{3}H] thymidine (20 Ci/mmol) was purchased from DuPont-New England Nuclear (Boston, MA, U.S.A.). All other reagents were of analytical grade.

Animals Male Wistar rats weighing 200—220 g were

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obtained from Tokyo Experimental Animal Co. (Tokyo, Japan). Adaptation to a light-, humidity- and temperature-controlled room occurred over a minimum 3-d period prior to the start of the experiments. Rats were fed a standard diet and given tap water *ad libitum*. The animals used in this study were handled in accordance with the Guidelines for the Care and Use of Laboratory Animals of Josai University and/or the Guiding Principle for the Ministry of Education, Culture, Sports, Science and Technology of Japan.

Hepatocyte Isolation and Culture Rats were anesthetized by intraperitoneal injection of sodium pentobarbital (45 mg/kg). Hepatocytes were isolated from normal livers by the two-step *in situ* collagenase perfusion technique devised by Seglen to facilitate disaggregation of the adult rat liver.[5,15] Briefly, dispersed hepatocytes were washed three times by slow centrifugation ($50\times\boldsymbol{g}$, 1 min) of the cell suspension to remove cell debris, damaged cells and non-parenchymal cells. Viability, as tested by Trypan blue exclusion, was more than 97%. Unless otherwise indicated, freshly isolated hepatocytes were plated onto collagen-coated plastic culture dishes (Asahi Techno Glass Co., Tokyo, Japan) at a density of 3.3×10^4 cells/cm^2 (3.0×10^5 cells/35-mm dish), and were allowed to attach for 3 h on collagen-coated dishes in Williams' medium E containing 5% newborn calf serum, 10^{-10} M dexamethasone, 0.2 mM penicillin, 70 mM streptomycin and 0.1 mM aprotinin in 5% CO_2 in air at 37 °C. Medium was then replaced by aspiration, and cells were cultured further in serum- and dexamethasone-free Williams' medium E supplemented with epidermal growth factor (EGF, 20 ng/ml). When appropriate, the following agents were added: EGF with or without growth-related signal transducer inhibitors (*i.e.*, mifepristone,[16] PD98059,[17] and a monoclonal antibody against glucocorticoid receptor[18]).

Measurement of DNA Synthesis Hepatocyte DNA synthesis was assessed by measuring the incorporation of [^{3}H]thymidine into acid-precipitable materials. Briefly, after an initial attachment period of 3 h, hepatocytes were washed twice with serum-free, dexamethasone-free Williams' medium E and were cultured in medium containing EGF (20 ng/ml) with or without testing agents for an additional 4 or 21 h. The cells were pulsed at 1, 2, 3 or 19 h after a 2-h EGF stimulation with [^{3}H]thymidine (1.0 μCi/well) followed by 10% trichloroacetic acid precipitation, as described previously.[19] [^{3}H]Thymidine incorporation into DNA was counted using a scintillation counter in terms of counts per min, and was normalized against cellular protein. Aphidicolin (3.0×10^{-5} M) was added to some wells in order to establish a level for non-replicative DNA synthesis. Hepatocyte protein content was measured by a modified Lowry procedure using bovine serum albumin as a standard.[20] Data are expressed as dpm/h/mg cellular protein.

Nuclei Counting Number of nuclei, rather than number of cells, was counted using a modified version of the procedure described previously.[5,9] Briefly, primary cultured hepatocytes were washed twice with 2 ml of Dulbecco's phosphate-buffered saline (PBS, pH 7.4), and isolated liver cell nuclei were prepared for quantitation by exposing cultured hepatocytes to 0.25 ml of citric acid (0.1 M) containing Triton X-100 (0.1%) for 30 min at 37 °C. An equal volume of the nuclear suspension was mixed with Trypan blue (0.3%) in PBS (pH 7.4) and the number of nuclei was counted in a hemocytometer.

Determination of Mitogen-Activated Protein (MAP) Kinase Activity Phosphorylated MAP kinase isoforms (p42 and p44) were identified by Western blot analysis using a 1 : 1000 dilution of rabbit polyclonal dual phospho-specific antibodies (1 mg/ml) with horseradish peroxidase (HRP)-conjugated goat anti-rabbit immunoglobulin G (IgG) as a secondary antibody, as previously described.[21] Briefly, cultured hepatocytes were washed with ice-cold PBS (pH 7.4) and 0.2 ml of lysis buffer (10 mM Tris–HCl buffer (pH 7.4), 150 mM NaCl, 2 mM ethylene glycol-bis(2-aminoethylether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA), 2 mM dithiothreitol, 1 mM sodium orthovanadate, 1 mM phenylmethylsulphonyl fluoride, 22 μM leupeptin, 11 μM aprotinin) were added, and hepatocytes were harvested. After centrifugation, cell lysates were denatured in boiling water for 5 min. Samples of supernatant (20 μg/lane) were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) on a 10% polyacrylamide resolving gel using the method of Laemmli.[22] After electrophoresis, proteins were transferred to a polyvinylidene fluoride (PVDF) membrane at 4 °C for 10 h. Proteins were quantified by densitometry after the membrane was developed with an enhanced chemiluminescence reagent and exposure to Hyperfilm (Kodak Japan, Tokyo, Japan). Densitometric analysis was performed using the NIH image program (ver. 1.6 for Macintosh). Data were calculated in arbitrary units and are expressed as means±S.E.M. ($*p<0.05$ *vs.* EGF-treated control). The autodiagram is a representation of three experiments using different cell preparations.

Statistical Analysis Data are expressed as means± S.E.M. Group comparisons were made by analysis of variance (ANOVA) for unpaired data followed by *post-hoc* analysis using Dunnett's multiple comparison test. Differences of $p<0.05$ were considered to be statistically significant.

RESULTS

Inhibitory Effects of Dexamethasone on EGF-Induced Hepatocyte DNA Synthesis and Proliferation: Time-Course Study We first examined the effects of glucocorticoid dexamethasone on DNA synthesis and proliferation in primary cultures of adult rat hepatocytes in the presence of epidermal growth factor (EGF, 20 ng/ml). Freshly isolated hepatocytes were plated at a low cell density (3.3×10^4 cells/cm^2). Lower concentrations of dexamethasone (10^{-10} M) were added 3 h before EGF addition (time zero), and changes to serum-free, dexamethasone-free or dexamethasone-containing culture medium were then made. At various culture times, hepatic parenchymal cells underwent time-dependent DNA synthesis and proliferation (*i.e.*, an increase in the number of nuclei) in the presence of 20 ng/ml EGF alone. The onset of DNA synthesis was first observed about 2.5 h after the addition of EGF (Fig. 1A), while the mitotic activity of the hepatocytes was first observed at about 3.0 h, and peaked at 4.0 h (Fig. 1B). Maximal stimulation for hepatocyte DNA synthesis and proliferation seen with EGF were approximately 6.0- and 1.3-fold, respectively. In contrast, treatment with higher concentrations of dexamethasone (10^{-8} M) almost completely suppressed the EGF-induced DNA synthesis and proliferation. Mineral corticoid aldosterone (10^{-7} M) and hydrocortisone (10^{-7} M, a low efficacy

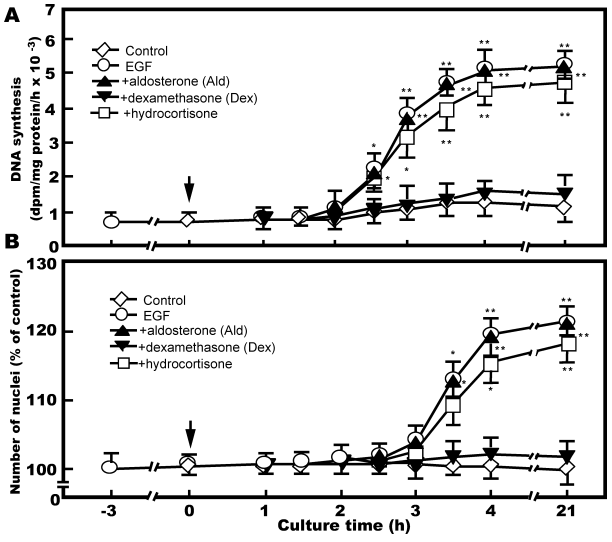

Fig. 1. Inhibitory Effects of Dexamethasone on EGF-Induced Hepatocyte DNA Synthesis and Proliferation: Time-Course Study

Freshly isolated hepatocytes were cultured in Williams' medium E containing 5% newborn bovine serum, 10^{-10} M dexamethasone, 0.1 μM aprotinin, and antibiotics (0.2 mM penicillin and 70 mM streptomycin) at a cell density of 3.3×10^4 cell/cm^2. After a 3-h attachment period (time zero), the medium was rapidly replaced with serum-free Williams' medium E supplemented with 20 ng/ml EGF and/or aldosterone (10^{-7} M) or hydrocortisone (10^{-7} M), and cultured for various lengths of time. Hepatocyte DNA synthesis and proliferation were determined as described in the Materials and Methods. Hepatocyte DNA synthesis is expressed in terms of dpm/mg protein/h (A). Hepatocyte proliferation is expressed as the percent increase in total number of nuclei, as compared to the control culture (B). The results are expressed as means±S.E.M. of three experiments. $*p<0.05$, $**p<0.01$, *vs.* respective controls.

glucocorticoid) did not affect EGF-induced DNA synthesis and proliferation in primary cultures of adult rat hepatocytes. Cell viability and attachment were not affected by the various concentrations of dexamethasone treatment.

Inhibitory Effects of Dexamethasone on EGF-Induced Hepatocyte DNA Synthesis and Proliferation: Timing of Dexamethasone Addition The effects of the timing of dexamethasone addition to the culture on EGF-induced hepatocyte DNA synthesis and proliferation were investigated. Higher concentrations of dexamethasone (10^{-8} M) were added at 1, 2 or 3 h before EGF addition, followed by a change to serum-free, dexamethasone-free Williams' medium E supplemented with 20 ng/ml EGF (time zero). When isolated hepatocytes were treated with lower concentrations of dexamethasone (10^{-10} M, control) for 3 h in serum-containing medium, followed by changing to serum-free, dexamethasone-free culture medium, maximum stimulation of EGF-induced hepatocyte DNA synthesis and proliferation were obtained, as shown in Figs. 1A and B. As compared with controls, when higher concentrations of dexamethasone (10^{-8} M) were added at 1 and 2 h before 20 ng/ml EGF addition, the onset of DNA synthesis was significantly delayed, depending on pretreatment time. In contrast, when dexamethasone (10^{-8} M) was added at 3 h before 20 ng/ml EGF addition, the onset of hepatocyte DNA synthesis was markedly delayed (Fig. 2A). In addition, hepatic parenchymal cells did not undergo significant proliferation until 21 h in the presence of 20 ng/ml EGF (Fig. 2B). Treatment with dexamethasone (10^{-8} M) during 24 h culture almost completely suppressed EGF-induced hepatocyte DNA synthesis and proliferation. Cell viability and attachment were not affected by pretreatment with dexamethasone (10^{-8} M).

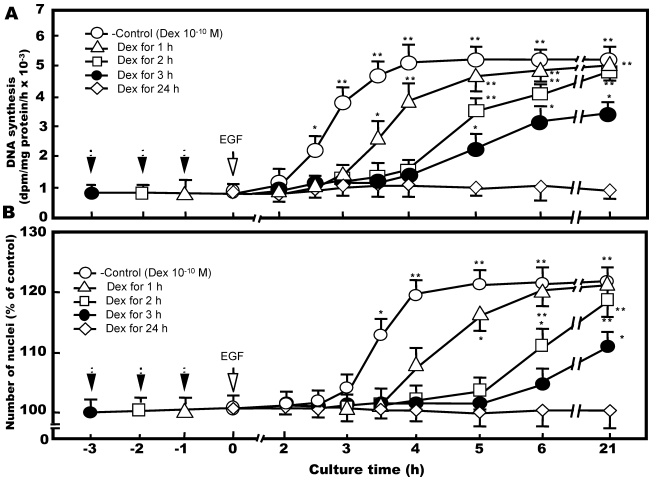

Fig. 2. Inhibitory Effects of Dexamethasone on EGF-Induced Hepatocyte DNA Synthesis and Proliferation: Timing of Dexamethasone Addition

Freshly isolated hepatocytes were plated at a density of 3.3×10^4 cells/cm^2 in Williams' medium E containing 5% newborn bovine serum, 0.1 μM aprotinin, and antibiotics. Dexamethasone (10^{-8} M) was added at 0, 1 and 2 h after plating, followed by culture for 3 h. Medium was then replaced by aspiration, and cells were further cultured in serum- and dexamethasone-free or dexamethasone (10^{-8} M)-containing Williams' medium E supplemented with 20 ng/ml EGF, as described in the legend of Fig. 1. Hepatocyte DNA synthesis is expressed in terms of dpm/mg protein/h (A). Hepatocyte proliferation is expressed as the percent increase in total number of nuclei, as compared to the control culture (B). Results are expressed as means±S.E.M. of three experiments. $*p<0.05$, $**p<0.01$ *vs.* respective controls.

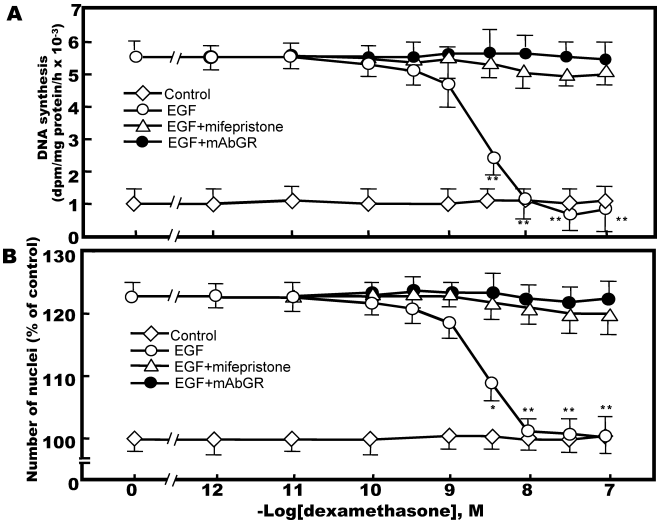

Fig. 3. Concentration-Dependent Inhibitory Effects of Dexamethasone on EGF-Induced Hepatocyte DNA Synthesis and Proliferation: Influence of Mifepristone (RU486) and Monoclonal Antibody against Glucocorticoid Receptor

Freshly isolated hepatocytes were plated at a density of 3.3×10^4 cells/cm^2, and were cultured as described in the legend of Fig. 1. After changing the medium, hepatocytes were cultured with various concentrations of dexamethasone with or without mifepristone, 10^{-6} M or monoclonal antibody against glucocorticoid receptor, 100 ng/ml in the presence of 20 ng/ml EGF for 4 h. Hepatocyte DNA synthesis is expressed in terms of dpm/mg protein/h (A). Hepatocyte proliferation is expressed as the percent increase in total number of nuclei, as compared with control culture (B). Results are expressed as means±S.E.M. of three independent experiments. $*p<0.05$, $**p<0.01$ *vs.* EGF-treated controls (EGF alone).

Concentration-Dependent Inhibitory Effects of Dexamethasone on EGF-Induced Hepatocyte DNA Synthesis and Proliferation: Influence of Mifepristone (RU486) and Monoclonal Antibody against Glucocorticoid Receptor The concentration-dependent effects of dexamethasone on EGF-induced hepatocyte DNA synthesis and proliferation

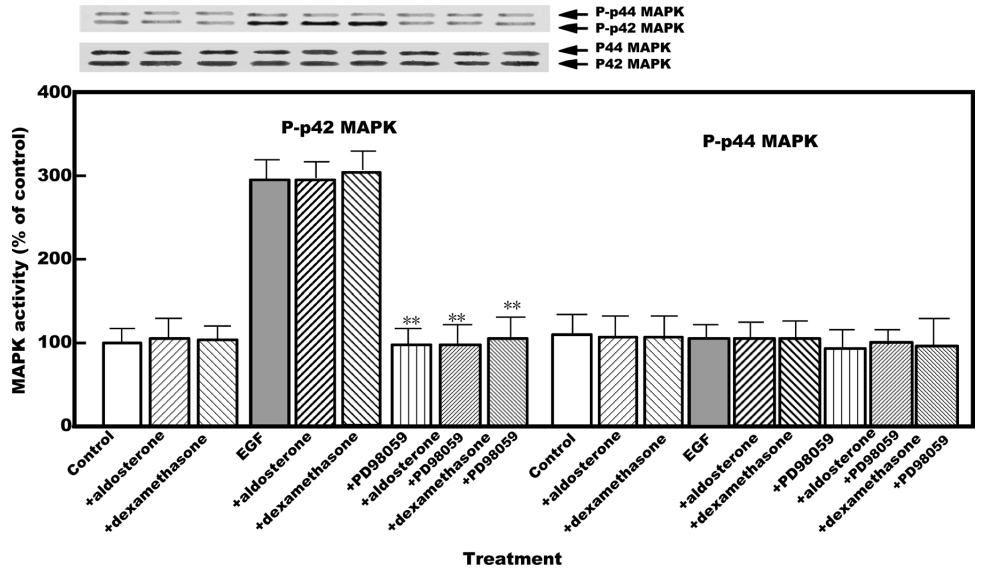

Fig. 4. Effects of Dexamethasone on EGF-Induced MAP Kinase Activity

Hepatocytes were plated at a cell density of 3.3×10^4 cells/cm^2, and were cultured as described in the legend of Fig. 1. Specific inhibitors were added with 20 ng/ml EGF immediately after the medium change, and hepatocytes were cultured for 5 min. Hepatocyte p42 MAP kinase (ERK2) and p44 MAP kinase (ERK1) activity was determined as described in the Materials and Methods. Concentrations of the test agents were as follows: dexamethasone, 10^{-8} M; PD98059, 10^{-6} M; and aldosterone, 10^{-7} M. Results are expressed as means±S.E.M. of three independent experiments. $**p<0.01$ *vs.* respective EGF-treated controls.

were investigated after 4 h of culture in the presence of various concentrations of dexamethasone. In addition, to confirm glucocorticoid receptor mediation of the inhibitory dexamethasone action, we investigated the effects of a glucocorticoid receptor antagonist, mifepristone (RU486),[16] and a monoclonal antibody against glucocorticoid receptor (raised against amino acids 395—411[18]) on EGF-induced-hepatocyte DNA synthesis and proliferation. As shown in Figs. 3A and B, the DNA synthesis and proliferating effects of 20 ng/ml EGF on primary cultured hepatocytes were significantly inhibited by dexamethasone (3×10^{-9}—10^{-7} M) in a concentration-dependent manner. The inhibitory effects of dexamethasone (3×10^{-9}—10^{-7} M) on the EGF-induced hepatocyte DNA synthesis and proliferation were almost completely reversed by treatment with mifepristone (10^{-6} M) or monoclonal antibody against glucocorticoid receptor (100 ng/ml).

Effects of Dexamethasone on EGF-Induced Mitogen-Activated Protein Kinase Activity In order to confirm the notion that EGF induces hepatocyte DNA synthesis and proliferation through MAP kinase activation, we investigated whether 20 ng/ml EGF is able to stimulate MAP kinase isoform activity. The effects of dexamethasone (10^{-8} M) on MAP kinase phosphorylation in the presence of EGF were also examined. Figure 4 (Western blot data; upper panel) shows that EGF stimulation caused an increase in phosphorylation of p42 MAP kinase, but not p44 MAP kinase. Phosphorylation of p42 MAP kinase peaked at about 3-fold (*vs.* control) at 5 min after addition of 20 ng/ml EGF. Pretreatment with higher concentrations of dexamethasone (10^{-8} M) did not affect the EGF-induced increase in p42 MAP kinase phosphorylation. In addition, p42 MAP kinase phosphorylation induced by 20 ng/ml EGF was abolished by MAP kinase kinase inhibitor PD98059 (10^{-6} M), but was not affected by aldosterone (10^{-7} M) treatment.

Proposed Mechanism for Inhibitory Effects of Dexamethasone on EGF-Induced Hepatocyte DNA Synthesis and Proliferation Based on the above pharmacological and biochemical analysis, we speculated that a higher concentration of dexamethasone inhibits EGF-induced hepatocyte DNA synthesis and proliferation downstream of p42 MAP kinase by binding to its own intracellular glucocorticoid receptor. Dexamethasone may act by inhibiting the expression of cell cycle-related protein mRNAs. The proposed mechanism for the inhibitory effects of dexamethasone on

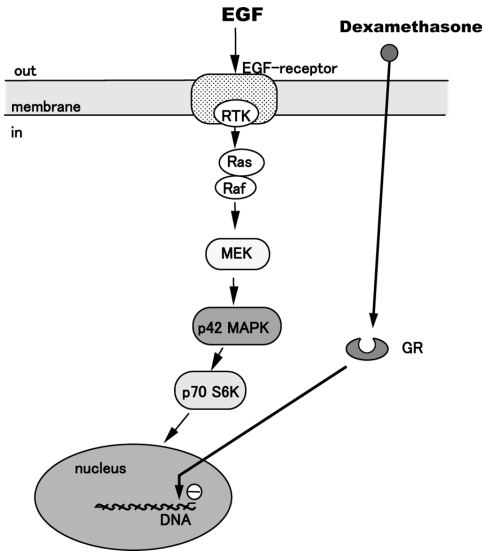

Fig. 5. Proposed Mechanism for Inhibitory Effects of Dexamethasone on EGF-Induced Hepatocyte DNA Synthesis and Proliferation

EGF interacts with its own receptor, and activates the receptor tyrosine kinase/MAP kinase pathway to induce hepatocyte DNA synthesis and proliferation. Pretreatment with higher concentrations of dexamethasone (10^{-8} M) inhibits EGF-induced hepatocyte DNA synthesis and proliferation downstream of MAP kinase through binding to its own intracellular glucocorticoid receptor. Abbreviations: RTK, receptor tyrosine kinase; MEK, MAP kinase kinase; p42 MAPK: 42 kDa MAP kinase; p70 S6K, p70 ribosomal S6 kinase; and GR, glucocorticoid receptor.

the EGF-induced hepatocyte DNA synthesis and proliferation is shown in Fig. 5.

DISCUSSION

Primary mitogens (*e.g.*, EGF and HGF) reportedly possess growth-stimulating effects on primary cultured hepatocytes through intracellular signal transduction, which in turn brings about changes in the plasma membrane receptor leading to nuclear DNA synthesis and proliferation.[2—4] The signal transduction pathway of the growth factors has been investigated *in vitro*, while additional substances that modulate the response of hepatocytes to growth stimuli have also been studied. Glucocorticoids such as dexamethasone and hydrocortisone exhibit effects on cell attachment and viability, but not proliferation.[8—10] However, the concentration (about 10^{-9}—10^{-5} M) and timing of glucocorticoid addition have varied between investigators.[5,9,23—26] Especially, glucocorticoid concentrations used by some investigators are very high compared with our experimental condition.[5—8] We therefore investigated the effects of dexamethasone on DNA synthesis and proliferation induced by EGF (20 ng/ml) in primary cultures of adult rat hepatocytes.

As shown in Fig. 1 and Fig. 2, when lower concentrations of dexamethasone (10^{-10} M) were added 3 h before 20 ng/ml EGF addition, the hepatic parenchymal cells underwent time-dependent DNA synthesis and proliferation (*i.e.*, increase in the number of nuclei) in the presence of EGF. These results suggest that higher concentrations of dexamethasone (10^{-8} M), but not high concentrations of hydrocortisone (10^{-7} M, a low-efficacy glucocorticoid) or aldosterone (10^{-7} M, mineralcorticoid), significantly suppress cell cycle progression. In support of this notion, it has been reported that regeneration is crucial for the recovery of hepatic mass following liver regeneration, and the glucocorticoids and immunosuppressive agents commonly used in transplantation are known to inhibit the expression of specific cytokines and growth factors (*e.g.*, tumor necrosis factor a and interleukin 6).[27] Debonera *et al.* demonstrated that administration of dexamethasone at the time of transplantation delayed expression and nuclear translocation of cyclin D1 and impaired DNA synthesis and mitosis, and inhibited the initiation of the regenerative process *in vivo*.[28—30] These findings may be particularly relevant for the present results.

However, intracellular signal transduction mechanisms of the growth inhibition by dexamethasone in the presence of 20 ng/ml EGF remain to be clarified. Specific inhibitors of growth-related signal transducers, mifepristone (10^{-6} M) and monoclonal antibody against glucocorticoid receptor were found to reverse the inhibitory effects of dexamethasone on EGF-stimulated hepatocyte DNA synthesis and proliferation (Figs. 3A, B). At present time, we have no direct evidence that anti-glucocorticoid receptor antibody enter into cultured hepatocytes. However, it has been reported that this antibody was used as cell permeable/endocytotic character of glucocorticoid receptor in the previous study, and similar to the clearance of cholesterol *via* lipoproteins.[31] These pathways involve the recognition of carrier proteins by endocytic receptors on the surface of target cells, followed by internalization and cellular delivery of the bound sterols.[32] The anti-glucocorticoid receptor antibody may be transported through similar pathways. The present results suggest that higher concentrations of dexamethasone (10^{-8} M) inhibit EGF-induced hepatocyte DNA synthesis and proliferation by binding to its own receptor.

MAP kinases are serine/threonine kinases that mediate intracellular phosphorylation events triggered by a variety of extracellular stimuli including growth factors, cytokines, and hormones.[33] MAP kinases play a role in various intracellular signal transduction pathways.[34,35] Therefore, we examined the mechanisms of growth inhibition by dexamethasone on EGF-induced MAP kinase activity. As shown in Fig. 4A, EGF stimulation caused an increase in the phosphorylation of p42 MAP kinase, but not p44 MAP kinase. Pretreatment with higher concentrations of dexamethasone (10^{-8} M) did not affect the EGF-induced increase in p42 MAP kinase phosphorylation. These results suggest that higher concentrations of dexamethasone (10^{-8} M) inhibit the EGF-induced hepatocyte DNA synthesis and proliferation downstream of p42 MAP kinase. Scheving *et al.*, reported that high-concentration (10^{-6} M) dexamethasone in hepatocytes stabilized or up-regulated several inhibitory effectors of EGF receptor/ErbB/MAP kinase, including receptor-associated late transducer (RALT) and MAPK phosphatase-1 (MKP-1), respectively. Thus high-concentration dexamethasone exerts a time-dependent and redundant inhibitory effect on EGF receptor-mediated proliferative signaling in hepatocytes, targeting not only the ErbB proteins but also their various positive and negative effectors.[26] The present results support the notion that dexamethasone (10^{-8} M) suppresses EGF-induced DNA synthesis and proliferation (*i.e.*, cell cycle progression) at the nuclear level.[23,36]

In conclusion, we provide evidence that lower concentrations of dexamethasone (10^{-10}, 10^{-9} M) are useful to assess hepatocyte DNA synthesis and proliferation without affecting cell viability and attachment, thereby minimizing the length of time that hepatocyte cultures are maintained. In addition, the glucocorticoid receptor is responsible for the inhibitory effects of dexamethasone on the EGF-induced DNA synthesis and proliferation in primary cultures of adult rat hepatocytes.

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