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Regulation of Tissue-Type Plasminogen Activator (tPA) and Type-1 Plasminogen Activator Inhibitor (PAI-1) Gene Expression in Rat Hepatocytes in Primary Culture[1]

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We have performed a comparative study on tPA and PAI-1 mRNA expression in primary cultures of rat hepatocytes and elucidated the possible regulation of these factors by certain hormonal stimulation. The tPA mRNA increased 2- to 4-fold in the presence of cholera toxin (CT), dibutyryl cyclic AMP (dbcAMP), or 3-isobutyl-1-methyl xanthine (IBMX), but slightly decreased in the presence of dexamethasone. The tPA activity was also changed by these agents in a similar fashion. On the contrary, PAI-1 mRNA decreased with CT, dbcAMP, or IBMX, but increased transiently with dexamethasone. From results obtained with cycloheximide, ongoing protein synthesis was judged to be required for both PAI-1 induction with dexamethasone and PAI-1 suppression with IBMX, but not for the tPA induction with IBMX. Dexamethasone exerted opposite regulatory effects on the tPA mRNA expression depending on its concentration: at 10^{-8} to 10^{-6} M, it suppressed the expression; whereas at 10^{-10} M, it elevated the expression.

Key words: cAMP, dexamethasone, hepatocytes, tissue-type plasminogen activator, type-1 plasminogen activator inhibitor.

Plasminogen activators (PAs) are serine proteases that convert the inactive proenzyme plasminogen to its active form, plasmin (*1*). PA is an important enzyme as a trigger for fibrinolysis and is involved in other physiological and pathological processes: inflammation, tissue remodeling, ovulation, and implantation as well as tumor cell invasion and metastasis (*2, 3*). There are two types of PAs, tissue-type PA (tPA) and urokinase-type PA (uPA), and these PA activities are strictly regulated at the stage of its synthesis, or indirectly *via* specific inhibitors of PA: type-1 and -2 PA inhibitors (PAI-1 and PAI-2).

PAI-1, an approximately 50 kDa glycoprotein, is a major physiological inhibitor of PAs. PAI-1 is synthesized by a wide variety of cell types *in vitro*, and its gene expression is regulated by growth factors, tumor-promoting phorbol esters, cytokines, and hormones (*4-7*).

Although both tPA and PAI-1 can be found in several organs *in vivo*, the expression of these fibrinolytic components in normal rat liver is low or undetectable (*8, 9*). We have described the secretion of tPA into bile (*10*). Our previous studies revealed that the tPA in bile, bilokinase (BK), was of liver origin (*11*), and rat hepatocytes in primary culture produced tPA and secreted it into the culture medium (*12, 13*). Thus the liver is known to have the potential to synthesize/secrete tPA.

PAI-1 is produced by rat hepatocytes in primary cultures (*12*) as well as established hepatoma cell lines *in vitro* (*14, 15*). It is expressed not only in the regenerating rat liver *in vivo* (*16*), but also in normal liver after dexamethasone treatment (*17*), but it is not expressed in quiescent rat hepatocytes *in vivo* (*8, 16-18*). In the present study we examined tPA and PAI-1 gene expressions in primary cultures of rat hepatocytes as a means to investigate the regulatory mechanism of the PA-plasmin system in the normal rat liver. We now report that the gene expressions of these two factors are regulated by cAMP/cAMP-generating agents and by glucocorticoid.

MATERIALS AND METHODS

Isolation of Rat Hepatocytes and Primary Culture—Hepatocytes were isolated from male Wistar strain rats by perfusing the liver with collagenase (type I, Sigma Chem., MO, USA) (*19*). The isolated cells were plated on collagen-coated culture dishes (100 mm, Iwaki Glass, Chiba) and cultured in Williams' medium E (Dainippon Pharmaceutical, Osaka) supplemented with 5% FBS (JRH Bioscience, KS, Australia), 10^{-8} M insulin (Sigma), 10^{-8} M glucagon (Sigma), and 1.6 nM human recombinant epidermal growth

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Abbreviations: BK, bilokinase; CT, cholera toxin; dbcAMP, N^6,2′-*o*-dibutyryladenosine 3′:5′-cyclic monophosphate; dCTP, deoxycytidine 5′-triphosphate; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HGF, hepatocyte growth factor; IBMX, 3-isobutyl-1-methyl xanthine; PA, plasminogen activator; PAI-1, type-1 PA inhibitor; PAI-2, type-2 PA inhibitor; tPA, tissue-type PA; uPA, urokinase-type PA; TGFβ, transforming growth factor β; UV, ultraviolet.

factor (EGF; Higeta Shoyu, Chiba) at 37°C in a humidified atmosphere of 5% CO_2:95% air as described previously (*13*). The medium was replaced with fresh medium of the same composition at 4 h after plating, and culture was continued until 24 h after plating. After this pre-culture with FBS and hormones, the medium was replaced with FBS- and hormone-free Williams' E medium, and the cells were cultured for 12 h. This medium was then replaced with the experimental medium containing the desired agent or hormone. The other agents used in this study were 3-isobutyl-1-methyl xanthine (IBMX; Sigma), N^6,2′-*o*-dibutyryladenosine 3′:5′-cyclic monophosphate (dbcAMP; Sigma), cholera toxin (CT; List Biological Laboratories, CA, USA), dexamethasone (Sigma), and cycloheximide (Sigma).

Assay of tPA Activity in the Condition Medium—The tPA activity in the conditioned medium was measured by the fibrin/agarose plate method (*20*). In this culture system, only tPA was detected by zymography of hepatocyte lysate and conditioned medium (*12, 13*), and the lysis area formed by the conditioned medium was completely quenched by anti-rat tPA rabbit antibody, but not by anti-uPA (data not shown). The conditioned medium (5 μl) was applied to a well (3 mm in diameter) formed on a fibrin/agarose gel plate, and the plate was incubated at 37°C for 72 h. The lysis zone formed on the plate during the incubation was measured, and the tPA activity was determined by referring to a standard curve prepared by use of human tPA (Techno Clone, Vienna, Austria).

Northern Blot Analysis—Total RNA was extracted from homogenates of hepatocytes according to the method of Chomczynski and Sacchi (*21*) and analyzed by Northern blotting as described previously (*22*). The total RNA (20 or 40 μg/lane) was electrophoresed on 1.5% agarose gels containing 1.8% formaldehyde (v/v), then transferred to Hybond-N nylon membranes (Amersham, Buckinghamshire, England), fixed by UV irradiation for 5 min, and baked in a vacuum oven at 80°C for 1 h. The DNA probes shown below were labeled by the random primer method (*23*) using [^{32}P]dCTP (3,000 Ci/mmol, Du Pont de Numoures, Dreieich, Germany). Then each blot was hybridized and washed under the conditions described previously (*22*). The radioactivity bound specifically was measured quantitatively by use of a Bio-Imaging analyzer (BAS-2000, Fuji, Tokyo). The amount of tPA or PAI-1 mRNA thus measured was normalized against that of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA, unless otherwise stated.

Probes—The rat tPA and PAI-1 cDNA probes (*24, 25*) were provided by Dr. T.D. Gelehrter (University of Michigan), and rat GAPDH cDNA probe (*26*) was a gift from Dr. R. Bravo (Bristol-Myers Squibb, NJ, USA).

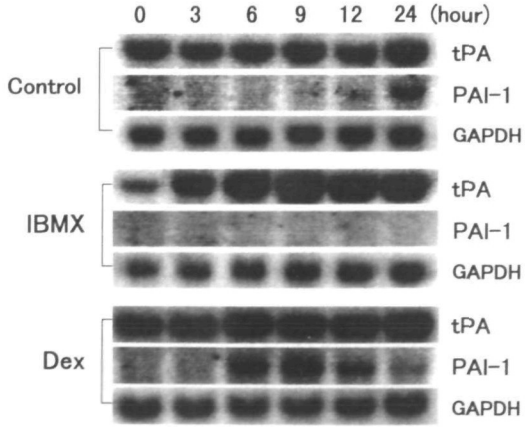

Fig. 1. **Northern blot analysis of the effects of IBMX and dexamethasone on tPA and PAI-1 mRNA accumulation in rat hepatocytes in primary culture.** The hepatocytes were incubated with 10^{-3} M IBMX or 10^{-6} M dexamethasone (Dex), harvested at the times indicated, and processed to isolate cellular RNA. Total RNA (40 μg) samples were subjected to the Northern blot analysis as described under "MATERIALS AND METHODS." The lower radiograms, Dex-treated cells; the middle radiograms, IBMX-treated cells; the top radiograms, agent-free control.

RESULTS

Northern Blot Analysis of tPA and PAI-1 mRNA Expression by Rat Hepatocytes in Primary Culture—The effects of a cAMP-generating agent and dexamethasone on tPA and PAI-1 mRNA expression were studied by Northern blot analysis. Figure 1 shows typical autoradiograms obtained by this study. Constitutive tPA expression was observed in the zero-time cells. These tPA signals were analyzed quantitatively by use of a Bio-Imaging analyzer™.

Effects of IBMX, dbcAMP, CT, and Dexamethasone on tPA mRNA Expression—The time courses of tPA mRNA expression in the presence of 10^{-3} M IBMX, 10^{-5} M dbcAMP, 10^{-7} M CT, or 10^{-6} M dexamethasone are shown in Fig. 2. The amount of tPA mRNA in the experimental culture was expressed as the ratio to that in the control

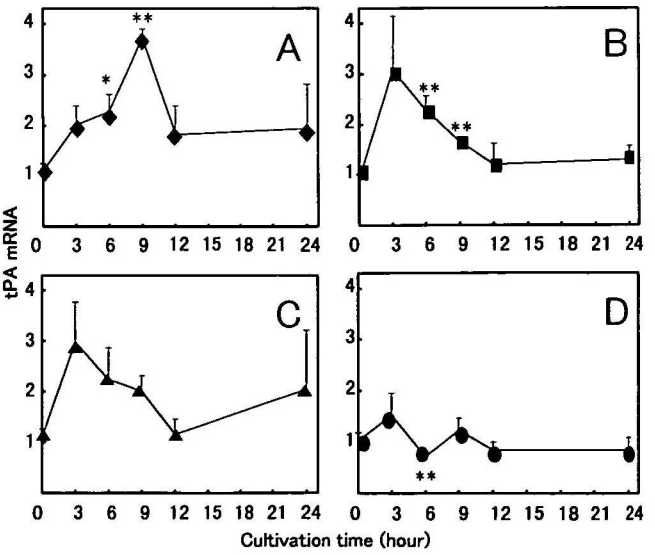

Fig. 2. **Time course of the effects of IBMX, dbcAMP, CT, and dexamethasone on tPA mRNA accumulation in rat hepatocytes in primary culture.** The hepatocytes were incubated with 10^{-3} M IBMX (panel A: ◆), 10^{-5} M dbcAMP (panel B: ■), 10^{-7} M CT (panel C: ▲), or 10^{-6} M dexamethasone (Dex; panel D: ●). The cells were harvested at the times indicated, then the tPA mRNA expression was measured by Northern blotting as described under "MATERIALS AND METHODS." The amount of tPA mRNA in these cells was normalized against that of GAPDH and expressed as the ratio to that expressed by control cells. Each point represents the average ± SE of 3-5 different experiments. $^*p<0.01$, $^{**}p<0.001$ *vs.* agent-free control cultere by Student's paired *t*-test.

culture (without agent), which was incubated for the same time as the experimental culture (base line). Incubation of the hepatocytes with dbcAMP or CT caused a maximal increase in the tPA mRNA level of the cells, approximately 3-fold at 3 h. IBMX also caused a 3.7-fold increase in the amount of tPA mRNA, although the time course, peaking at 9 h, differed from that with dbcAMP or CT. Although IBMX is an inhibitor of cAMP phosphodiesterase, some other mechanism(s) to induce tPA mRNA may also be involved in the induction of tPA by IBMX. In contrast to dbcAMP, CT, and IBMX, dexamethasone caused a slight reduction in the amount of tPA mRNA, which fell to 80% of the baseline at 12 and 24 h.

Figure 3 shows the effect of the same agents on tPA activity in the conditioned medium. Primary cultures of rat hepatocytes are known to produce only tPA and to secrete it into the culture medium (*12, 13*). The tPA activity detected in the agent-free control medium of the hepatocyte cultures at 24 h (which was calculated to be 24 U/ml) was augmented 1.3- to 3.2-fold by CT, dbcAMP, or IBMX, but repressed by dexamethasone to 60% of the baseline value.

These results suggest that tPA activity generation in rat hepatocytes in primary culture is up-regulated by cAMP-generating agents and down-regulated by dexamethasone.

Induction Mechanism of the tPA mRNA by IBMX and the Synergistic Effect with Dexamethasone—We observed that tPA mRNA was decreased slightly by incubation of cells with dexamethasone for 12 or 24 h, whereas it increased markedly when the cells were incubated for 3-9 h in the presence of both dexamethasone and IBMX, and reached a level 3- to 5-fold higher than the baseline level (data not shown). Therefore, we studied further the mechanism by which the expression of tPA mRNA is induced by IBMX and potentiated by IBMX plus dexamethasone, by introducing cycloheximide as an inhibitor of *de novo* protein synthesis. Under the experimental conditions used, the protein synthesis measured by use of [^{3}H]amino

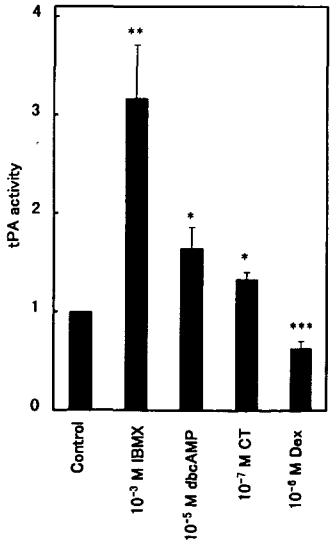

Fig. 3. **Effects of IBMX, dbcAMP, CT, and dexamethasone on the ability of the rat hepatocytes to secrete active tPA into the culture medium.** The hepatocytes were incubated with 10^{-3} M IBMX, 10^{-5} M dbcAMP, 10^{-7} M CT, or 10^{-6} M dexamethasone for 24 h. The tPA activity in the harvested medium was then measured by the fibrin/agarose method as described under "MATERIALS AND METHODS." The activity of the agent-free control is taken as 1. Each bar represents the average ± SE of 5-6 different experiments. $^*p<0.05$, $^{**}p<0.005$, $^{***}p<0.001$ *vs.* the control by Student's paired *t*-test.

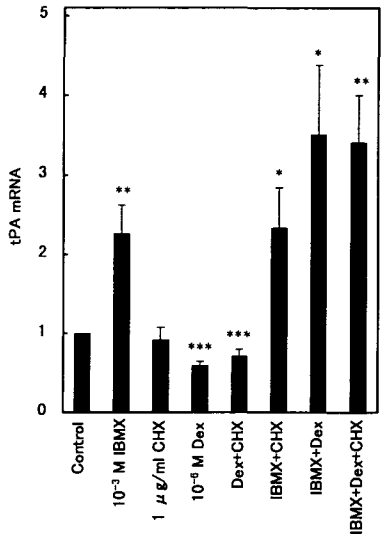

Fig. 4. **Effects of IBMX, cycloheximide and dexamethasone on tPA mRNA accumulation.** Primary cultures of rat hepatocytes were incubated with 10^{-3} M IBMX, 1 μg/ml cycloheximide (CHX), 10^{-6} M dexamethasone (Dex), Dex+CHX, IBMX+CHX, IBMX+Dex, or IBMX+Dex+CHX for 6 h, then the tPA mRNA expressed by the cells was measured by Northern blotting. The amount of tPA mRNA is presented as the ratio of experimental:control expression. Each bar represents the average ± SE of 4-5 different experiments. $^*p<0.05$, $^{**}p<0.01$, $^{***}p<0.005$ *vs.* the control by Student's paired *t*-test.

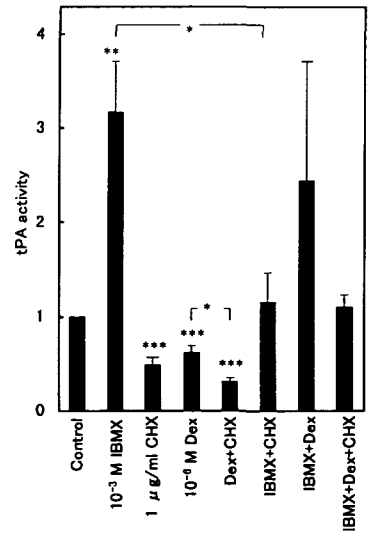

Fig. 5. **Effects of IBMX, cycloheximide, and dexamethasone on the ability of the hepatocytes to release active tPA into the culture medium.** Primary cultures of rat hepatocytes were incubated with 10^{-3} M IBMX, 1 μg/ml cycloheximide (CHX), 10^{-6} M dexamethasone (Dex), Dex+CHX, IBMX+CHX, IBMX+Dex, or IBMX+Dex+CHX for 24 h. The activity of the agent-free control is taken as 1. Each bar represents the average ± SE of 3-4 different experiments. $^*p<0.05$, $^{**}p<0.005$, $^{***}p<0.001$ *vs.* the control (or as indicated) by Student's paired *t*-test.

acid was inhibited more than 95% (*27*). As Fig. 4 shows, neither cycloheximide, dexamethasone nor their mixture caused any induction of tPA mRNA over its baseline level. However, the induction by IBMX was observed whether the agent was used alone or combined with dexamethasone or cycloheximide, and the potentiation by IBMX plus dexamethasone was not suppressed by the addition of cycloheximide. These results suggest that the tPA induction mechanism of IBMX or IBMX plus synergistic dexamethasone does not require concomitant protein synthesis.

Effects of IBMX and Dexamethasone on tPA Activity—The tPA activity in the conditioned media of the hepatocyte cultures in the presence of IBMX (10^{-3} M) and/or dexamethasone (10^{-6} M), with or without cycloheximide is shown in Fig. 5. IBMX caused a 3-fold increase in tPA activity, whereas dexamethasone decreased it to 60% of the baseline. The synergistic effect of IBMX and dexamethasone that was observed in the induction of tPA mRNA (Fig. 4) was not observed in the appearance of tPA activity.

Regulation of PAI-1 mRNA Accumulation by IBMX, dbcAMP, CT, and Dexamethasone—The time courses of the PAI-1 mRNA accumulation in the cultures containing IBMX, dbcAMP, CT, or dexamethasone were studied next (Fig. 6). The amount of PAI-1 mRNA was expressed as an arbitrary unit by assuming the baseline value of the cultures without reagent at 24 h to be 1.0. The zero-time cells hardly expressed PAI-1 mRNA, as was the case for hepatocytes *in vivo* (*8, 16-18, 22*) although they gradually began to express it over the course of 24 h.

Dexamethasone induced PAI-1 mRNA rapidly and markedly, and the level peaked about 4-fold higher than the

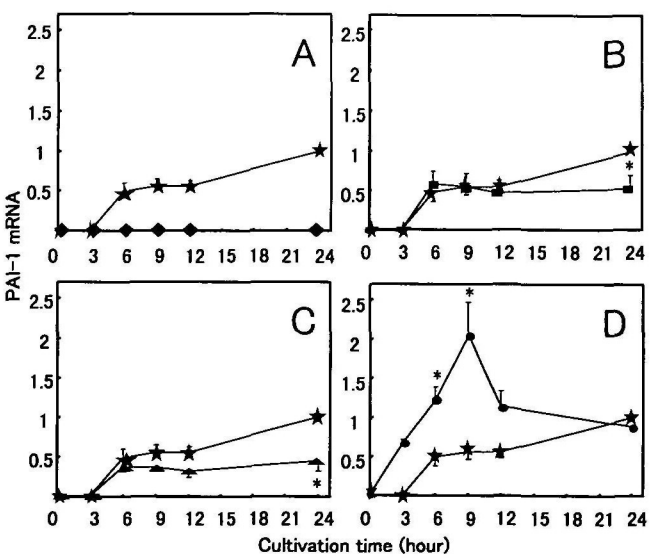

Fig. 6. **Time course of the effect of IBMX, dbcAMP, CT, or dexamethasone on PAI-1 mRNA accumulation in primary cultures of rat hepatocytes.** The hepatocytes were incubated with each agent for up to 24 h, and the amount of PAI-1 mRNA was measured at the times indicated. The amount of PAI-1 mRNA was normalized against that of GAPDH mRNA and expressed as the ratio to the expression of agent-free control culture (★) at 24 h. Panel A: 10^{-3} M IBMX (◆), panel B: 10^{-5} M dbcAMP (■), panel C: 10^{-7} M CT (▲), or panel D: 10^{-6} M dexamethasone (●). Each datum point represents the average±SE of 3-5 experiments. $^{*}p<0.05$ *vs.* agent-free control by Student's paired *t*-test.

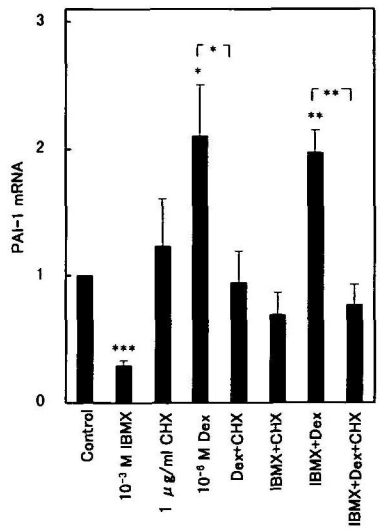

Fig. 7. **Effects of IBMX, cycloheximide, and dexamethasone on PAI-1 mRNA accumulation.** Primary cultures of rat hepatocytes were incubated with 10^{-3} M IBMX, 1 μg/ml cycloheximide (CHX), 10^{-6} M dexamethasone (Dex), Dex+CHX, IBMX+CHX, IBMX+Dex, or IBMX+Dex+CHX for 6 h; then PAI-1 mRNA expressed in these cells was measured as described in the legend of Fig. 1. Amount of mRNA in the cells is presented as the ratio to that of control cells. Each bar represents the average±SE of 4-5 experiments. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.005$ *vs.* the control (or as indicated) by Student's paired *t*-test.

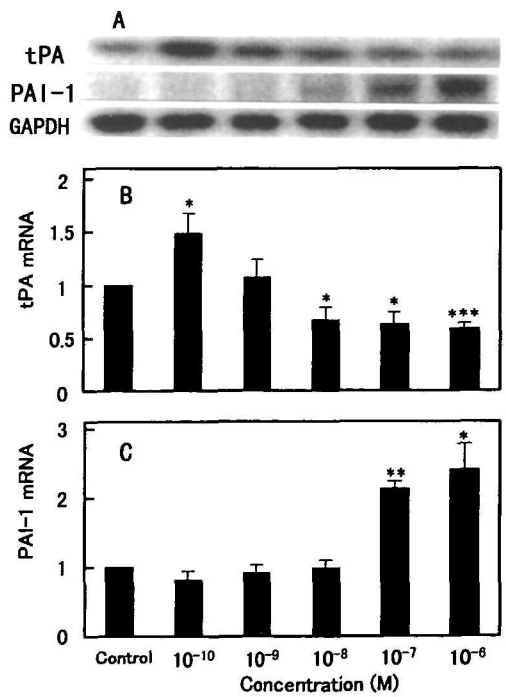

Fig. 8. **Effect of dexamethasone concentration on the induction of tPA and PAI-1 mRNAs.** Primary cultures of rat hepatocytes were incubated with various concentrations of dexamethasone for 6 h. Panel A shows a typical autoradiogram of the Northern blotting using 20 μg of total RNA. Panel B shows the amount of tPA mRNA presented as the ratio of experimental: control expression, and panel C, the amount of PAI-1 mRNA presented in the same way. Each bar represents the average±SE of 4 different experiments. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ *vs.* the control by Student's paired *t*-test.

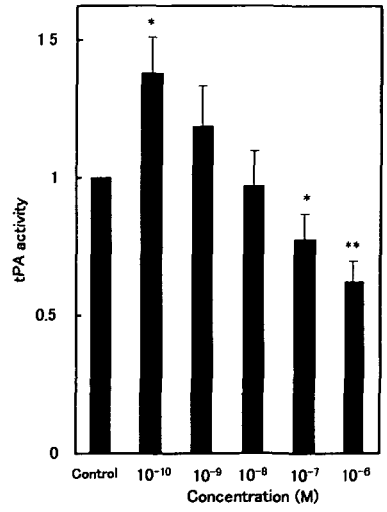

Fig. 9. **Effect of dexamethasone concentration on the generation of tPA activity.** Primary cultures of rat hepatocytes were incubated for 24 h with various concentrations of dexamethasone as indicated. The activity of the agent-free control is taken as 1. Each bar represents the average ± SE of 4 different experiments. $^{*}p<0.05$, $^{**}p<0.001$ *vs.* the control by Student's paired *t*-test.

baseline at 9 h, then gradually decreased to the baseline (Fig. 6D). IBMX completely repressed the expression (Fig. 6A), and both dbcAMP and CT weakly repressed it (Fig. 6, B and C).

The effect of cycloheximide on PAI-1 mRNA expression, which was measured in 6 h cultures, was also studied (Fig. 7). Although cycloheximide hardly affected the baseline level of PAI-1 mRNA expression, it repressed dexamethasone-induced and dexamethasone/IBMX-induced PAI-1 mRNA levels to the baseline, showing that in the induction mechanisms with these agents required that some protein be synthesized *de novo*.

Opposite Effects of Dexamethasone on the Expression of mRNAs of tPA and PAI-1—The effects of dexamethasone concentration on tPA and PAI-1 mRNAs were studied by Northern blot analysis using the cells cultured for 6 h (Fig. 8). At lower concentrations of 10^{-8}–10^{-10} M, dexamethasone had no effect on PAI-1 mRNA expression, but at higher concentration (10^{-7} and 10^{-6} M), it caused a 2- to 2.4-fold increase in the expression (Fig. 8, panels A and C). At the lowest concentration studied (10^{-10} M), dexamethasone caused a 1.5-fold increase in tPA mRNA, but at concentrations higher than 10^{-8} M, it caused about 50% repression of the mRNA level as compared with the baseline value (Fig. 8, panels A and B). Dexamethasone concentration had a similar effect on the level of tPA activity in the conditioned medium from 24 h cultures (Fig. 9). The tPA activity is more likely to change reflecting both the tPA and PAI-1 mRNA expressions; at the lower concentration of dexamethasone, tPA activity was slightly augmented, but at the higher concentration, dexamethasone repressed tPA activity in the same manner as observed in tPA mRNA.

DISCUSSION

The liver is well characterized as an organ responsible for the clearance of PAs and PA/PAI-1 complexes from the circulation *via* specific receptors on hepatocytes (*28-31*). On the other hand, the normal mammalian liver has long been known as an exceptional organ in that it fails to produce any PA. Our previous study performed to determine the origin of BK revealed that hepatocytes in primary culture produce tPA and secrete it into the culture medium (*12, 13*). We also reported that tPA production in primary cultures of rat hepatocytes was increased by cAMP-generating hormones, and decreased by glucocorticoid (*12*).

In this study, we employed primary cultures of rat hepatocytes to study the regulation of tPA and PAI-1 gene expression in the normal liver. Rat hepatocytes in primary culture have been shown to maintain the normal features and functions observed for the cells *in vivo* (*32*).

The results of this study illustrate that tPA and PAI-1 mRNA levels are regulated by cAMP/cAMP-generating agents and glucocorticoid: *i.e.*, dbcAMP, CT, and IBMX caused an increase in tPA mRNA accumulation. In contrast, these agents caused a 50, 60, and 100% decrease, respectively, in the PAI-1 mRNA level. Induction of tPA mRNA by IBMX was not affected by a protein synthesis inhibitor, cycloheximide, but the suppression of PAI-1 mRNA by IBMX was inhibited by cycloheximide. These results suggest that the tPA induction by IBMX does not require *de novo* protein synthesis, whereas the mechanism of PAI-1 mRNA suppression by IBMX does require it. On the contrary, PAI-1 mRNA induction by dexamethasone with or without IBMX was inhibited by cycloheximide; thus ongoing *de novo* protein synthesis is required for the PAI-1 mRNA induction by dexamethasone.

It has been shown that the rat and mouse tPA genes have potential cAMP-responsive element (CRE) in their 5′-flanking region (*33, 34*). We have also revealed that the augmentation of tPA production by dbcAMP is completely inhibited by a specific inhibitor of cAMP-dependent protein kinase (A-kinase) (*12*); thus the phosphorylation of transcription factor, CRE-binding protein, by A-kinase (*35*) may be required for the augmentation of tPA production/mRNA expression by cAMP/cAMP-generating agents. Interestingly, augmentation of tPA mRNA by IBMX was potentiated by dexamethasone (Fig. 4). Dexamethasone sometimes potentiates gene expression induced *via* a signal transduction pathway by cAMP/A-kinase (*36*). It has also been reported that dexamethasone stabilizes the mRNA (*37*). Such functions of dexamethasone might contribute to the potentiation of tPA mRNA induced by IBMX.

PAI-1 mRNA was hardly detectable in zero-time primary cultures of rat hepatocytes by Northern blot analysis. However, this mRNA was gradually expressed as the culture period increased up to 24 h (Fig. 6). Because PAI-1 mRNA expression is augmented by increasing cell density (*38*), the spontaneous expression of PAI-1 mRNA during the culture may be caused by augmented cell-cell interactions. IBMX, CT, and dbcAMP suppressed PAI-1 induction in culture. The 3′-untranslated region of the PAI-1 gene contains AU-rich sequences similar to functionally characterized 3′ AU-rich sequences implicated in mRNA turnover (*39, 40*). Because the cyclic nucleotide decreases the rate of PAI-1 transcription and stimulates the rate of PAI-1 mRNA decay in HTC cells (*5*), the mechanism(s) for the suppression of PAI-1 mRNA induction might involve both transcriptional and posttranscriptional stages.

Here we also showed the dual effect of dexamethasone on tPA activity (Fig. 9). The changes in tPA activity were well correlated with the changes in tPA and PAI-1 mRNAs by dexamethasone (Fig. 8).

We reported earlier that both tPA and PAI-1 mRNAs are induced in the liver injured by carbon tetrachloride *in vivo* (*22*), and that tPA and PAI-1 are classified respectively as a delayed early growth response gene and an immediate early growth response gene in primary cultures of rat hepatocytes (*38*). It has also been reported that PAI-1 mRNA is induced in the regenerating rat liver as an immediate early growth response gene after partial hepatectomy (*16*). These studies on the regulation of tPA and PAI-1 expression *in vivo* suggest that tPA and PAI-1 may play important roles in the process of hepatocyte growth and proliferation *in vivo*. Indeed, a component of the PA-plasmin system is known to be involved in the processing of factors governing hepatocyte proliferation and liver regeneration *in vitro* (*41*): both tPA and uPA activate an inactive hepatocyte tropic growth factor, pro-HGF (*42, 43*) to become active HGF (*44-47*), and such activation is prevented by PAI-1 (*44, 45*). Plasmin activates latent TGFβ to active TGFβ (*48*), which is a potent suppressor of hepatocyte proliferation and liver regeneration (*49, 50*) and a potent inducer of PAI-1 production (*51*). HGF is also reported as an inducer of PAI-1 in hepatoma cells, HepG2 (*52*).

In this study, we demonstrated opposite effects in regulation of tPA and PAI-1 by both cAMP-generating agents and glucocorticoid in primary cultures of rat hepatocytes. Cyclic AMP is known to stimulate DNA synthesis in cultured hepatocytes and the cell cycle in the regenerating liver (*53, 54*). As the cAMP concentration in the liver is known to be increased before liver regeneration (*54, 55*), the hepatic tPA production in response to cAMP may stimulate the liver regeneration, and the PAI-1 produced by hepatocytes may represent a negative feedback regulation in the regeneration process (*16*). Studies to elucidate the pathophysiologic role(s) of tPA and PAI-1 produced by hepatocytes are now under way in both *in vitro* and *in vivo* systems in our laboratory.

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