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SPECIFIC INDUCTION BY GLUCOCORTICOIDS OF STEROID ESTERASE IN RAT HEPATIC MICROSOMES AND ITS RELEASE INTO SERUM

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Abstract—Steroid esterase hydrolysing methylprednisolone 21-hemisuccinate was induced specifically and markedly in hepatic microsomes and serum of rats by various glucocorticoids. Among the glucocorticoids examined, dexamethasone and betamethasone showed the highest potency to induce the hepatic steroid esterase, the induction ratio being 32 and 33 times higher than the basal level (about 160 mU/g liver), respectively. Steroid esterase in the serum was induced greatly by fluocinolone acetonide and betamethasone to 92 and 79 times of the basal level of about 16 mU/mL, respectively, followed by dexamethasone and methylprednisolone. When dexamethasone was given to rats, the enzyme in other tissues except for duodenum and small intestine (of which activity was lowered to 50% of the basal level) was also elevated, but the induction ratio was much lower than that in the liver and serum. The induction of the steroid esterase is probably due to stimulation of *de novo* synthesis of the enzyme by glucocorticoids, because the elevation of esterase activity was inhibited by treatment with cycloheximide (a translation inhibitor) and actinomycin D (a transcription inhibitor), and about 4- and 10-hr lag time was observed before the elevation of esterase activity in liver and serum, respectively. Coupled with these observations the following results indicate that the steroid esterase in serum is probably synthesized in the liver and subsequently released into the blood via the Golgi apparatus: (1) when the liver of rats treated with dexamethasone was subjected to perfusion with a recycling system, significant amounts of steroid esterase were released into the perfusate; (2) anti-hepatic esterase antibody inhibited the steroid esterase activity not only in the liver but also in serum; and (3) monensin, which prevents the secretion of various kinds of secretory proteins by disrupting the function of the Golgi apparatus, inhibited the elevation of the steroid esterase activity in serum by dexamethasone but did not affect the induction in liver.

During the course of investigations into the metabolism of methylprednisolone 21-hemisuccinate (MP-hemisuccinate†), which is a prodrug of methylprednisolone, we found that (1) this steroid ester was specifically hydrolysed by isozyme(s) of carboxylesterase (EC 3.1.1.1) [1, 2]; (2) the esterase (tentatively designated as steroid esterase) present in the rat liver was induced markedly not only by the substrate but also by the product, i.e. methylprednisolone; and (3) the activity of this esterase in the rat liver was regulated by pituitary hormones such as growth hormone and prolactin [3] as well as adrenocorticotropic hormone. Among these findings we have especially been interested in the induction of esterase, because the product *per se* induced the esterase. At the time of this induction study, Kauer *et al.* [4] reported hydrocortisone or its ester-type prodrugs elevated microsomal steroid esterase in rat liver. Based on the findings that methylprednisolone as well as hydrocortisone elevated the activity of the esterase, we have considered that the induction of the steroid esterase is a common feature of glucocorticoids and some of the pharmacological activities exerted by glucocorticoids are likely to be associated with the induced steroid esterase.

In this report we describe, firstly, the induction of steroid esterase by various glucocorticoids by the stimulation of *de novo* synthesis in the liver and secondly, that the induced hepatic steroid esterase was released into the systemic blood flow via the Golgi apparatus.

MATERIALS AND METHODS

Materials. Methylprednisolone 21-hemisuccinate (MP-hemisuccinate), methylprednisolone, hydrocortisone and fluoxymesterone were supplied from the Upjohn Co. (Kalamazoo, MI, U.S.A). Betamethasone, fluocinolone acetonide, dexamethasone, monensin, cycloheximide, actinomycin D, bovine serum albumin and Lubrol WX were purchased from the Sigma Chemical Co. (St Louis, MO, U.S.A.). Sephadex G-25 was obtained from Pharmacia Fine Chemicals (Uppsala, Sweden) and DEAE-cellulose (DE52) from Whatman BioSystems Ltd (Maidstone, U.K.). Freund's Complete Adjuvant was supplied from Difco Laboratories (Detroit, MI, U.S.A.). All other reagents used in the present study were of the highest analytical grade and were commercially obtained.

Sprague–Dawley male rats were purchased from

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† Abbreviations: MP-hemisuccinate, methylprednisolone 21-hemisuccinate; GOT, glutamate-oxaloacetate transaminase; GPT, glutamate pyruvate transaminase.

Charles River Japan (Kanagawa, Japan) and Japanese white male rabbits (about 3 kg) from Ichikawa Laboratory Co. Ltd (Tokyo, Japan). Animals were used after 1-week acclimation. The age and body weight of rats at each experimental day were 6–8 weeks old and 210–310 g, respectively, for all the studies except for the liver perfusion study in which 11-week old rats (360–390 g) were used.

Dosing. In the study examining the effect of various glucocorticoids (hydrocortisone, methylprednisolone, fluocinolone acetonide, betamethasone and dexamethasone) on the activity of steroid esterase, each glucocorticoid suspended in sesame oil (each 2 mL/kg) was given i.p. to groups of rats at 60 μmol/kg once a day for 2 days. In other studies the suspension of dexamethasone in sesame oil was administered i.p. to rats at a single dose of 60 μmol/kg. As controls, a group of rats received the same volume of sesame oil (2 mL/kg).

Sample preparation. Serum was obtained by centrifugation (1500 *g* for 15 min) of blood specimens, which were drawn from the aorta with a syringe under diethyl ether anaesthesia.

Hepatic microsomes were prepared as follows. The liver was perfused with 250 mM sucrose at room temperature to remove remaining blood, excised, rinsed in the ice-chilled 250 mM sucrose, and minced with scissors. The mince was homogenized in 4 vol. of the sucrose solution using a glass homogenizer with a teflon pestle in an ice-chilled bath. A portion of the homogenate was centrifuged at 9000 *g* for 20 min and the resultant supernatant was subsequently centrifuged at 105,000 *g* for 4° for 1 hr. The microsomal pellet was resuspended in the sucrose solution to a protein concentration of 20 mg/mL.

The homogenates of various tissues (heart, lung, pancreas, liver, spleen, kidney, adrenal gland, testis, stomach, duodenum, small intestine and large intestine) were prepared by the same method except that the perfusion was not conducted. A mechanical homogenizer (Physcotron, NS-50, Niti-on Co. Ltd, Chiba, Japan) was used instead of a glass homogenizer. The steroid esterase activity in each sample was determined within 6 hr of preparation.

The effect of anti-hepatic esterase antibody on the esterase was examined in serum, lung, liver, kidney, duodenum and small intestine. Each tissue homogenate was diluted with 250 mM sucrose to an appropriate esterase activity (less than 120 mU/mL) and thoroughly mixed with Lubrol WX (0.5%, w/v) and citrate–phosphate buffer (final concentration; 50 mM), pH 5.5. The mixture was centrifuged at 8000 *g* for 5 min after incubation at 25° for 45 min. The supernatant of each tissue and serum was incubated with anti-hepatic esterase antibody or normal rabbit IgG (140 μg, each) at 25° for 15 min and subsequently the mixture was centrifuged at 1500 *g* for 5 min. Resultant supernatant was used to determine the esterase activity for MP-hemisuccinate.

Assay method. For the assay of the esterase activity, the amount of methylprednisolone formed from MP-hemisuccinate was determined by HPLC. The reaction was initiated by adding MP-hemisuccinate (1 μmol) dissolved in 20 μL of *N,N*-dimethylformamide to the mixture composed of an appropriate volume of serum, tissue homogenates or liver microsomes and citrate–phosphate buffer (final concentration: 50 mM), pH 5.5, in a final volume of 1.0 mL. After a 15-min incubation at 37°, the reaction was terminated by adding 1.0 mL of acetonitrile containing 10% (w/v) trichloroacetic acid and 250 nmol of fluoxymesterone (an internal standard). The mixture was centrifuged at 1500 *g* for 10 min and an appropriate portion of the supernatant was applied to HPLC (Hitachi-655A, Tokyo, Japan) with a column (4.6 × 250 mm) packed with Nucleosil $7C_8$ (Macherey-Nagel, Düren, Germany). Methylprednisolone and the internal standard were eluted with acetonitrile/50 mM potassium phosphate buffer, pH 7.0 (1:1, v/v), at a flow rate of 1 mL/min and detected at 254 nm at the respective retention times of 4.5 and 5.6 min. Amounts of methylprednisolone formed were determined from a peak height ratio of methylprednisolone to the internal standard. The reaction was linear with respect to the time up to 15 min. The calibration curve was linear at the product concentration from 2 to 200 nmol/mL.

Protein concentrations were determined by the method of Lowry *et al.* [5], in which bovine serum albumin was used as standard.

A unit of the esterase activity was defined as the quantity of enzyme catalysing the formation of 1 μmol of methylprednisolone per min under the conditions of the assay.

Liver perfusion. At 40 hr after the administration of dexamethasone, rats (N = 3) were anaesthetized with diethyl ether and the liver was perfused at 37° with 60 mL of perfusion medium, which consisted of Krebs–Henseleit bicarbonate buffer, pH 7.4, and 3% (w/v) bovine serum albumin. After being isolated, the liver was connected to perfusion apparatus and perfused with 60 mL of the perfusion medium at 37° in a recycling system according to the procedure described by Tanaka and Hattori [6]. An aliquot of the perfusate (each 100 μL) was used to determine the steroid esterase activity for MP-hemisuccinate. Glutamate-oxaloacetate transaminase (GOT) and glutamate-pyruvate transaminase (GPT) activities were measured with assay kits (GOT-UV TEST Wako and GPT-UV TEST Wako, Wako Pure Chemicals, Tokyo, Japan) according to the procedures described by the supplier. GOT and GPT activities were indicated as the international unit of U/L.

Preparation of antibody. The steroid esterase purified from the rat liver [2] was dissolved in 50 mM potassium phosphate buffer, pH 6.8, at the concentration of 0.62 mg/mL and mixed with the same volume of Freund's Complete Adjuvant. The mixture was injected s.c. into the dorsum of rabbits at 0.3 mg/kg four times at 2-week intervals. Blood was drawn from the carotid artery at 2 weeks after the last injection and the serum obtained by centrifugation. Proteins in the serum were precipitated by the portionwise addition of powdered ammonium sulfate to result in 60% saturation. The precipitates were redissolved in a small volume of 15 mM potassium phosphate buffer, pH 8.0, and applied to a Sephadex G-25 column to remove ammonium sulfate. The antibody, which had been

Table 1. Effects of various glucocorticoids on esterase activity for MP-hemisuccinate in hepatic microsomes and serum

Treatment	Microsomes (mU/g liver)	Serum (mU/mL)
Control	157 ± 11	16 ± 1.9
Hydrocortisone	1270 ± 74*	20 ± 1.5
Methylprednisolone	3715 ± 322*	86 ± 13*
Fluocinolone acetonide	3891 ± 297*	1477 ± 150*
Betamethasone	5163 ± 222*	1261 ± 91*
Dexamethasone	5057 ± 389*	743 ± 58*

Values are the means ± SEM of six animals.
* Significantly different from the respective control values ($P < 0.05$).

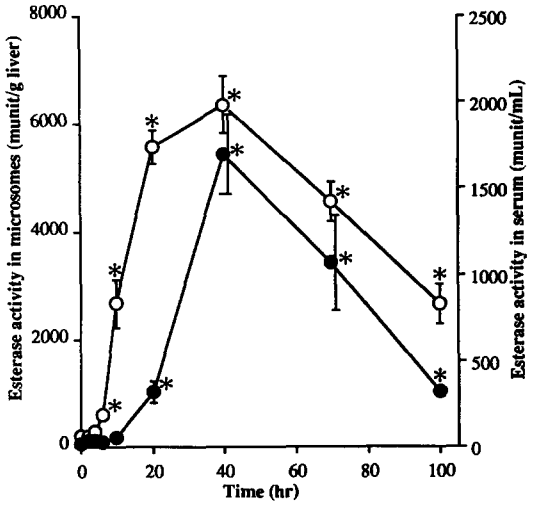

Fig. 1. Time course of induction of steroid esterase in hepatic microsomes and serum by dexamethasone. Values are the means ± SEM of four animals. Keys: (○) hepatic microsomes; and (●) serum. *Significantly different from the respective control values.

eluted with 15 mM potassium phosphate buffer, was applied to a DEAE-cellulose column and eluted with the same buffer. The steroid esterase antibody thus obtained was stored at −20° until use.

Statistical analysis. All data expressed as mean ± SEM were evaluated statistically by Student's *t*-test.

RESULTS

Effects of glucocorticoids on the activity of steroid esterase

Groups of rats (N = 6 each) were treated with hydrocortisone, methylprednisolone, betamethasone, fluocinolone acetonide or dexamethasone. At 16 hr after the second injection, the steroid esterase activity in the hepatic microsomes and serum was determined (Table 1).

The steroid esterase activity for MP-hemisuccinate in the hepatic microsomes was elevated to the range of about 1300–5200 mU/g liver by either one of the glucocorticoids examined, the values being about 8–32 times higher than the basal level (about 160 mU/g liver). The increment in the serum esterase activity was only 1.3-fold (which was not statistically significant) by hydrocortisone and 5.4-fold by methylpredinosolone over the basal level (16 mU/mL serum), whereas dexamethasone, betamethasone and fluocinolone acetonide elevated the serum esterase activity up to 46, 79 and 92 times.

Time course of induction of steroid esterase by dexamethasone

The time course of induction of the esterase activity for MP-hemisuccinate was examined in the hepatic microsomes and serum of rats (N = 4) after injection with dexamethasone (Fig. 1).

In the microsomes, the activity of the steroid esterase rapidly increased and plateaued at a level ranging from 5600 to 6400 mU/g liver between 20 and 40 hr after a 4-hr lag period. The plateau level was 29–34 times higher than the basal level of 190 mU/g liver. At 100 hr after the dosing, the activity was still high and about 2700 mU/g liver. The activity in the serum ranged in the basal level (about 20 mU/mL) up to 10 hr after dosing and then increased to reach a maximum activity of 1700 mU/mL (85 times higher than the basal level) at 40 hr. Thereafter, the serum activity rapidly decreased, but 16 times higher activity was observed even at 100 hr after the treatment with dexamethasone.

Inhibition of dexamethasone-dependent induction of steroid esterase by actinomycin D or cycloheximide

To clarify whether the elevation of steroid esterase activity by glucocorticoids was attributed to the stimulation of *de novo* synthesis of the esterase protein, we gave actinomycin D (0.3 mg/kg, a transcription inhibitor) or cycloheximide (0.6 mg/kg, a translation inhibitor) intraperitoneally to rats (N = 4) at 0, 3, 6 and 9 hr after the administration of dexamethasone. The steroid esterase activity was determined at 24 hr after the dexamethasone treatment (Fig. 2).

When the animals were treated with dexamethasone alone, the steroid esterase activity for MP-hemisuccinate increased up to 6300 mU/g liver and 320 mU/mL in the serum, each value being about 33 and 16 times greater than the respective basal levels. The steroid esterase activity in the hepatic microsomes was significantly decreased to 2800 mU/g liver by the treatment with actinomycin D and 2100 mU/g liver by cycloheximide ($P < 0.05$) compared to the dexamethasone-treated rats. These activities were approximately 44% and 33%, respectively, of those in the rats injected with dexamethasone alone. In the serum, the activity was reduced to 77 mU/mL (24% of the activity of rats treated with dexamethasone alone) by actinomycin D and 80 mU/mL (25%) by cycloheximide. The decreases were statistically significant ($P < 0.05$).

Release of hepatic steroid esterase into perfusate

Forty hours after treatment with dexamethasone, liver was isolated from rats (N = 3) and

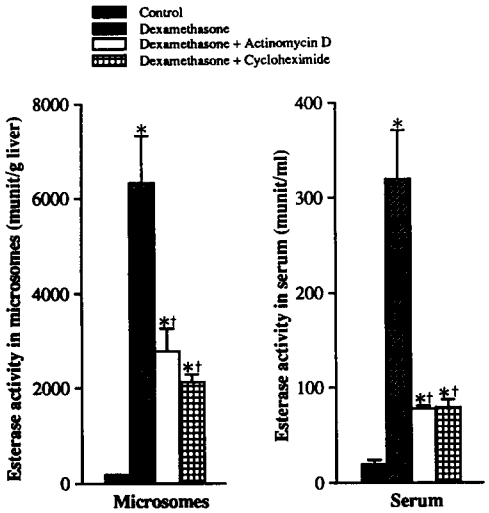

Fig. 2. Effect of actinomycin D and cycloheximide on induction of steroid esterase in hepatic microsomes and serum by dexamethasone. Values are the means ± SEM of four animals. *Significantly different from the values of respective control ($P < 0.05$). †Significantly different from the values of dexamethasone-treated group ($P < 0.05$).

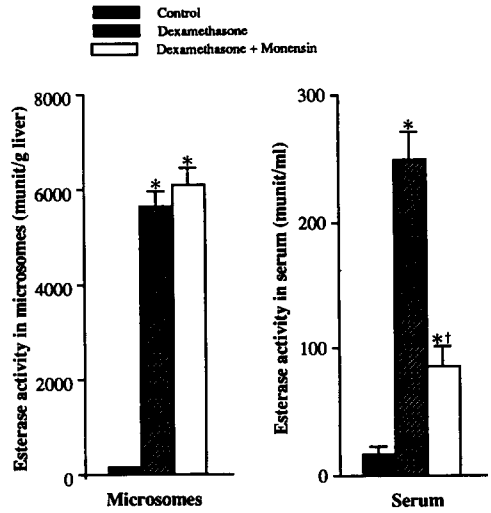

Fig. 3. Inhibition of esterase release into the blood from liver. Values are the means ± SEM of three to four animals. *Significantly different from the values of respective control ($P < 0.05$). †Significantly different from the values of dexamethasone-treated group ($P < 0.05$).

perfused. The activities of steroid esterase for MP-hemisuccinate, GPT, and GOT and concentration of protein were measured in the perfusate 30 min after the initiation of perfusion.

The steroid esterase was found to be present in the perfusate and the cumulative amounts during the first 30-min were 2800 ± 390 mU. In the perfusate, proteins derived from the liver were virtually negligible and the activities of GOT and GPT were 34 ± 0.9 and 13 ± 1.5 U/L perfusate, respectively, which were in the normal range observed in perfusion experiments [7]. These results indicate that the steroid esterase in the perfusate was actively released from the hepatic cells and did not leak due to damage or necrosis during the perfusion.

Inhibition of release of hepatic steroid esterase into blood by monensin

We examined whether the serum steroid esterase was secreted from the liver by using monensin, a univalent ionophore, which prevents the secretion of various kinds of secretory proteins by disrupting the function of the Golgi apparatus [8–10]. Rats (N = 3) were injected s.c. with monensin suspended in sesame oil (5 mg/mL) at 5 mg/kg at 0, 3, 6 and 9 hr after the injection with dexamethasone. At 24 hr after the administration of dexamethasone, the steroid esterase activity in the hepatic microsomes and serum was determined (Fig. 3).

In the rats (N = 4) which were given dexamethasone alone, the microsomal esterase activity increased to 5600 mU/g liver and the serum activity to 250 mU/mL. These values are 37 and 15 times higher than the respective basal levels ($P < 0.05$). When the animals were co-treated with monensin and dexamethasone, the microsomal esterase activity, at 6100 mU/g liver, was virtually the same as that found in the dexamethasone-treated rats, while the serum activity was significantly lowered to 86 mU/mL ($P < 0.05$) in comparison with the activity in the animals injected with dexamethasone alone. This indicates that the serum esterase is probably secreted from the liver via the Golgi apparatus.

Effects of dexamethasone on steroid esterase activity in various tissues

The effects of dexamethasone on the activity of steroid esterase were examined in various tissues at 40 hr (the peak time for the serum steroid esterase activity) after the administration of dexamethasone to rats (N = 4) (Table 2).

In the control rats which were administered sesame oil, the duodenum and small intestine showed conspicuously high basal activity of about 15,000 and 12,000 mU/g tissue, respectively, followed by kidney, lung and liver, of which basal activity was around 300–400 mU/g tissue. When dexamethasone was given to rats, the steroid esterase in the liver and serum was induced markedly as was observed in the time course study (Fig. 1). Induction was found in the heart—six times greater than and significantly different ($P < 0.05$) from the basal level of 18 mU/g tissue. Increases in the enzyme activity in the stomach, pancreas, kidney and testis were significant but much lower in comparison with the liver, serum or heart. Unexpectedly, the activities in the duodenum and small intestine were lowered by dexamethasone to approximately 7300 and 6000 mU/g tissue. These activities were 50% lower than the respective basal levels.

Table 2. Effect of dexamethasone on esterase activity for MP-hemisuccinate in various tissues and inhibition by anti-liver esterase-rabbit antibody

Tissues	Esterase activity (mU/g tissue or mL serum)		Antibody inhibition Residual activity (%)†	
	Control	Dexamethasone	Control	Dexamethasone
Serum	17 ± 6.1	1064 ± 104*	50.5 ± 15.5	10.3 ± 1.3
Heart	18 ± 2.8	115 ± 18.0*	ND	ND
Lung	313 ± 33.1	436 ± 42.9	119.7 ± 2.3	67.0 ± 3.5
Pancreas	79 ± 3.1	105 ± 6.7*	ND	ND
Liver	304 ± 56.4	13,300 ± 504*	53.5 ± 3.2	5.2 ± 0.5
Spleen	46 ± 9.3	73 ± 6.0	ND	ND
Kidney	386 ± 53.6	619 ± 57.1*	35.9 ± 2.5	24.3 ± 0.9
Adrenal gland‡	51	124	ND	ND
Stomach	142 ± 5.2	292 ± 9.0*	ND	ND
Duodenum	14,844 ± 264	7342 ± 1625	80.8 ± 12.9	77.4 ± 10.6
Small intestine	12,241 ± 2163	6018 ± 758*	82.8 ± 13.2	85.5 ± 13.9
Large intestine	213 ± 38.4	281 ± 74.5	ND	ND
Testis	42 ± 1.5	119 ± 19.5*	ND	ND

Values are the means ± SEM of four animals.

* Significantly different from the respective control values ($P < 0.05$).

† Values represent as percentage relative to the control activity of solubilized enzyme treated with normal rabbit IgG as described in Materials and Methods.

‡ Adrenal glands from four rats were combined and homogenized.

ND, not determined.

Inhibition of steroid esterase by hepatic steroid esterase antibody

Tissue homogenates with basal activity of steroid esterase higher than 300 mU/g tissue were treated with Lubrol WX to solubilize the esterase and the inhibitory effect of the anti-hepatic esterase antibody on the solubilized enzyme was examined (Table 2). About 90% and 95% of the esterase in the serum and liver, which were obtained from the rats treated with dexamethasone, were inhibited by the antibody, respectively, whereas about 50% of the esterase activity in both tissues of the control rats was retained after treatment with the antibody. In the small intestine and duodenum the antibody inhibited only 23% or less of the activity before and after treatment with dexamethasone. These results indicate that the induced esterase present in the serum and liver was immunologically identical, but different from the esterase(s) in the intestine or duodenum. In the case of kidney the basal esterase activity was about 390 mU/g tissue and was higher than all other tissues except for duodenum and small intestine. About 65% of basal and induced esterase activity in the kidney were inhibited by the antibody.

DISCUSSION

Kauer *et al.* [4] reported that the steroid esterase activity in rat liver microsomes was induced up to 2–4 times by the oral administration of hydrocortisone or its ester-type prodrugs (hydrocortisone 21-acetate and hydrocortisone 21-hemisuccinate) at a single dose of 25 μmol/kg. In the present study, we clarified that not only hydrocortisone but also other kinds of glucocorticoids elevated the activity of the steroid esterase both in the liver and serum (Table 1). These results indicate that the capability to elevate the activity hydrolysing steroid esters (at least in these two tissues) is probably common to all glucocorticoids.

Interestingly, the elevation of the activity of the serum esterase was not proportional to the induction of the hepatic esterase (Table 1). The induction potency of methylprednisolone was comparable to that of fluocinolone acetonide for the hepatic esterase, but the activity in the serum of rats treated with methylprednisolone was much lower than the serum activity elevated by fluocinolone acetonide. Additionally, dexamethasone possessed a comparable potency to induce hepatic esterase with betamethasone, although the serum esterase activity elevated by dexamethasone was about 40% lower than that by betamethasone. While the reasons for this different induction ratio between the liver and serum are obscure at present, the most probable explanation is the respective glucocorticoids possess different potencies to stabilize the serum esterase and/or each glucocorticoid has different potency to release the hepatic esterase into the serum (see below with respect to the origin of serum esterase).

It is noticeable that the capacity to elevate the serum esterase activity correlated well with the anti-inflammatory activity of the glucocorticoids. The glucocorticoids can be classified into three groups, tentatively designated as Class I, II and III according to the potency of their anti-inflammatory activities [11, 12]. Thus, hydrocortisone, of which anti-inflammatory activity is markedly low, is in Class I, methylprednisolone belongs to Class II, while the glucocorticoids in Class III such as dexamethasone, betamethasone and fluocinolone acetonide exert highest anti-inflammatory activity. The gluco-

corticoids in Class III elevated the activity of the serum esterase to greater than 740 mU/mL, whereas induction by the glucocorticoids in Class II and Class I was merely 86 and 20 mU/mL, respectively (Table 1). Based on these results, we predict that the esterase studied is responsible for some physiological or pharmacological activities such as anti-inflammatory activity in addition to the esterase activity. It is well known that glucocorticoids exert their anti-inflammatory activity by inducing various protein factors such as lipocortin (a phospholipase A_2 inhibitor) [13–15], superoxide dismutase (a superoxide scavenger) [16–18], and vasoregulin (a vascular permeability inhibitor) [17, 18], which interact with various substances involved in inflammatory processes. In analogy with these protein factors, the steroid esterase released into the blood may also interact with some factor(s) involved in inflammatory processes and thereby exerts the anti-inflammatory activity. The physiological significance of the steroid esterase is under investigation in our laboratory.

The elevation of the enzyme activity by glucocorticoids is most probably attributable to the stimulation of *de novo* synthesis of the enzyme; the administration of potent inhibitors of peptidyltransferase (cycloheximide) or of transcription (actinomycin D) inhibited the elevation of the esterase activity by dexamethasone (Fig. 2) and a 4-hr lag time needed for *de novo* synthesis of the esterase was observed before the elevation of the esterase activity in the hepatic microsomes by dexamethasone (Fig. 1). Many investigators have reported the involvement of glucocorticoids in the induction of various enzymes such as tyrosine hydroxylase [19], alcohol dehydrogenase [20], tryptophan oxygenase [21] and glutamine synthetase [22] by increasing the corresponding mRNA and transcriptional activity. Together with these experimental observations the present results with actinomycin D and cycloheximide suggest that the esterase was induced by a similar type of mechanism.

Various proteins (including enzymes) in the serum such as albumin are synthesized in the liver and subsequently released into the systemic blood [23], therefore we assumed that the steroid esterase was such a secretory protein. Indeed, several lines of evidence indicate that the steroid esterase is probably synthesized in the liver and then secreted into the blood: firstly, when the liver of rats treated with dexamethasone was subjected to perfusion, the enzyme was released into the perfusate but markers of cellular damage were not, secondly, monensin, which inhibits the release of secretory proteins from cells by disrupting the function of the Golgi apparatus [8–10], markedly suppressed the increase in the serum steroid esterase, but did not effect the amount of hepatic esterase (Fig. 3), and thirdly, anti-hepatic esterase antibody inhibited the induced esterase in the hepatic microsomes and also the serum (Table 2).

More than 90% of the esterase activity in the liver and serum in the dexamethasone-treated rats was inhibited by the antibody prepared by using purified esterase [2]. On the other hand, 50% of the esterase activity in these tissues was not affected by the presence of antibody (Table 2). The result suggests that in the liver and serum two or more kinds of esterases exist and one of the esterases was specifically induced by dexamethasone. Indeed, when we attempted to purify the esterases responsible for hydrolysis of MP-hemisuccinate from normal rat liver according to the same procedure described in the accompanied report [2], we found that two esterases in the same amounts were separated by DEAE-cellulose column chromatography (data not shown). One of the enzymes was identical with the purified esterase induced by dexamethasone [2], while another was not induced by dexamethasone nor affected by the antibody to any extent. The latter enzyme is being purified for characterization in our laboratory.

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