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Effects of Some Prostaglandins on Liver Tryptophan Oxygenase Ativity in Mice

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Introduction

It has been suggested that the production of 5-hydroxytryptamine in the brain is decreased because of the fall of tryptophan contents there, when a main pathway of tryptophan metabolism is made vigorous by the induction or activation of tryptophan oxygenase (EC 1. 13. 1. 12, TO)[3,9,10]. It has been well known that 5-hydroxytryptamine is a mediator of inflammatory response, and glucocorticoids, a strong inducer of TO, have potent anti-inflammatory effects. Further, it has been suggested that the decrease of 5-hydroxytryptamine, when TO was induced by glucocorticoids, is related to anti-inflammatory actions of glucocorticoids[25]. Nakoneczna et al.[23] also have suggested a relation between tryptophan metabolism and inflammation. Thus, it is expected that there may be a close relation between tryptophan metabolism and inflammation.

Moreover, the increase of blood glucocorticoid level has been observed in animals after a peripheral administration of some prostaglandins (PGs), which have been regarded as one of the mediators of inflammatory effects[5,12,19,24]. Glenn and Rohloff[7] have shown that anti-inflammatory effects of PGsE_1 and E_2 are due to adrenal stimulation from high doses of them. From these points of view, the effects on mouse liver TO of some PGs were investigated in this study.

Materials and Methods

1. Animals

Male mice (ICR-JCU), 9 weeks of age, weighing between 32 and 36 g, were used. Five animals were kept in individual cages, fed a pelleted standard diet (Klea Japan Inc. CE-2) and tap water ad lib. Room temperature was 20 to 25°C.

The adrenalectomy was performed under ether anesthesia, usually 4 days before the mice were used. Drinking water was replaced by physiological saline for the adrenalectomized mice.

All animals were killed by decapitation at half past one to three o'clock in the afternoon.

2. Assay of TO

Since Miyao et al.[21] have suggested that normal mouse liver has less apoenzyme and more inactive forms of holoenzyme than rat liver, the enzyme activity in the homogenate was measured by the method modified for application to mice basing on the method of Knox et al.[18] in which inactive forms of TO are also activated. The activity by this method was decided to call whole TO activity toward total activity. Holo- and total enzymes activities also were measured in a part

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of this experiment. These were assayed by the method modified basing on the procedure of Knox[15].

3. Plasma corticosterone

Determined according to Riley and Spackman[27].

4. Adrenal ascorbic acid

Determined according to Maickel[20], provided that the adrenals were homogenized with metaphosphoric acid in place of trichloroacetic acid.

5. Drugs used and the administration method of them

(1) $PGsE_1$, E_2 and $F_{1\alpha}$(SIGMA)

Each 1 mg was dissolved in 0.1 ml of 95% ethanol and diluted to 1 ml with 0.2% Na_2CO_3 solution. These were kept at −20°C, diluted with saline when used, and injected intraperitoneally. The administered dose will be mentioned later.

(2) Dexamethasone (Orgadolone injection [Sankyo Co. LTD])

One ampul contains 5 mg of dexamethasone disodiumphosphate in 1 ml. 25 mg/kg as dexamethanosone was injected intraperitoneally.

(3) Phenobarbital (Phenobal [Sankyo Co. LTD.])

One ampul contains 100 mg of phenobarbital in 1 ml. In the continuously administered experiment, phenobarbital diluted with 2% Na_2CO_3 solution was freely administered in drinking water in increasing dose of 1 mg/ml on the 1st day, 1.5 on the 2nd and the 3rd days, 2 on the 4th day and 3 on the 6th day or later. In case of the intraperitoneal injection the drug was diluted with saline and 50 to 100 mg/kg of which were administered.

Results

1. Liver TO activity in the normal mice assayed by the method of Knox et al.[18]

TO has three types of enzyme, that is, a holoenzyme measured without its cofactor, hematin (a reduced form), an apoenzyme evaluated from a total activity measured with hematin and an inactive form of holoenzyme (an oxidized form)[17,18]. Knox et al.[18] have observed that maximal activation of rat liver TO is obtained by a 30-minutes preincubation with its substrate, L-tryptophan and ascorbic acid, in case of livers from tryptophan-treated rats, and by these additions plus methemoglobin as a source of the hematin prothetic group, in case of livers from the untreated and hydrocortisone-treated rats. As mentioned above, Miyao et al.[21] have suggested that normal mouse liver has less apoenzyme and more inactive form of holoenzyme than rat liver. From this viewpoint, most of the activities were measured by the method of Knox et al.[18]. Average TO activities by this method (whole TO activity) was 8.74 ± 1.67 in 5 male mice (Table 1). As average total and holoactivities were 2.59 and 2.41 in 7 animals, respectively (Table 1), the part regarded as an oxidized form is resulted in about 2.4 times as much as the total activity. As holo- and total activities were about 1.8 and 3.4 in the rat liver, respectively[22], it is assumed that the part regarded as an oxidized form is less than the total TO in the rat.

Thus, the mouse liver had more total quantity of the enzyme than in rat liver, but most of the quantity existed as inactive form, and the active form of TO in the mouse liver was only about 3/4 that in the rat liver.

Table 1. Normal mouse liver tryptophan oxygenase activity

TO activity (μM kynurenine/g/h)		
Whole enzyme	Total enzyme	Holoenzyme
8.74±1.67 (5)	2.59±0.48 (7)	2.41±0.42 (7)

Each value is the mean±standard deviation for the number of animals shown in parentheses.

Table 2. Mouse liver tryptophan oxygenase activity 4 hours after the intraperitoneal injection of various doses of PGE_1

Dose (μg/mouse)	Whole TO activity (μM kynurenine/g/h)
0	9.43±1.27 (8)
1	10.51±1.28 (4)
2	11.39±1.94 (6)
4	12.75±2.13 (7)*2
6	13.34±1.20 (5)*1
8	14.26±2.09 (4)*2
16	14.29±1.89 (5)*1

Each value is the mean±standard deviation for the number of animals shown in parentheses.
*1: Significantly different from 0 μg injection ($P<0.001$)
*2 Significantly different from 0 μg injection ($P<0.005$)

2. Changes of TO activity after the administration of various doses of PGE_1 and its time-course after a dose of PGE_1 given

Table 2 shows liver whole TO activities in mice 4 hours after the intraperitoneal injection of 1, 2, 4, 6, 8 and 16 μg/mouse of PGE_1. The highest activities were observed in the doses of 8 and 16 μg, and they were not elevated even in the case of more doses added. However, approximately linear log-dose response relationship existed between the doses of 1 to 8 μg/mouse of PGE_1 and the liver TO activities (Fig. 1). From this result, the later dose of PGE_1 was decided to be 8 μg/mouse.

Time-course of liver whole TO activity in mice injected with 8 μg/mouse of PGE_1 is shown in Fig. 2. Whole TO activities were already increased 2 hours after injection, reaching maximum after 4 hours, then began to be decreasing, and returned to the basal value 8 hours after injection. Judging from this result or from a rapid metabolism of PGE_1 in the body, effects of PGE_1 on TO are considered to be temporary.

3. Effects of $PGsE_2$ and $F_{1\alpha}$ on whole TO activity

Of many kinds of PGs, their relationship to the inflammatory responses has been chiefly observed with $PGsE_1$, E_2, $F_{1\alpha}$ and $F_{2\alpha}$. In this study, however, effects of $PGsE_2$ and $F_{1\alpha}$ on whole TO activity were compared with those of E_1. Results are shown in Table 3. The increase of whole TO activity 4 hours after PGE_2 was about half the increase after PGE_1, and $PGF_{1\alpha}$ showed no effects.

4. Effects of PGE_1 on whole TO activity in the adrenalectomized mice

Results are shown in Table 4. Whole TO activities were 8.74 in the intact animals without PGE_1, and 7.64 and 8.32 in the adrenalectomized ones without and with PGE_1, respectively. These values were not significantly different. On the other hand, the high value of 14.99 was shown in the sham operated mice treated with PGE_1.

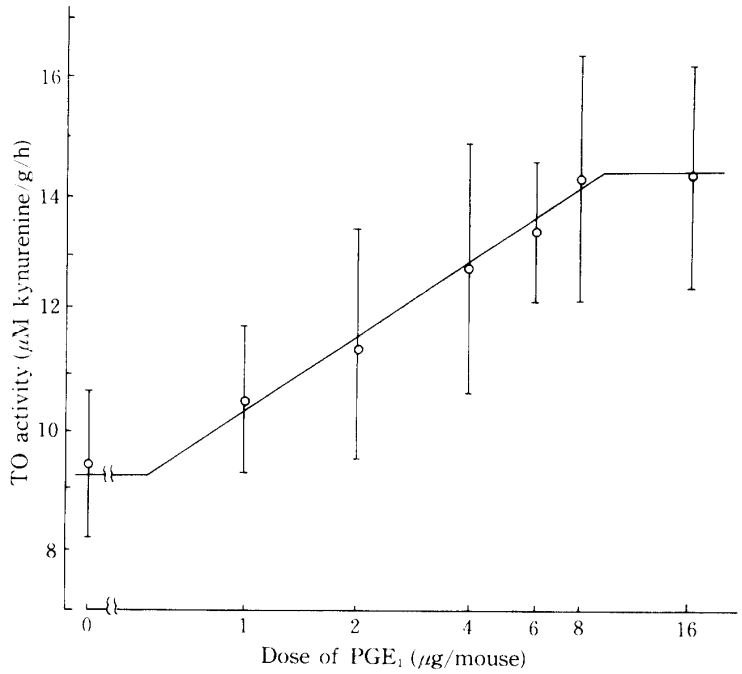

Fig. 1. Log-dose response relationship between PGE_1 intraperitoneally injected and liver whole tryptophan oxygenase activity in mice. Activities were assayed 4 hours after the injection. Vertical bars represent the standard deviation.

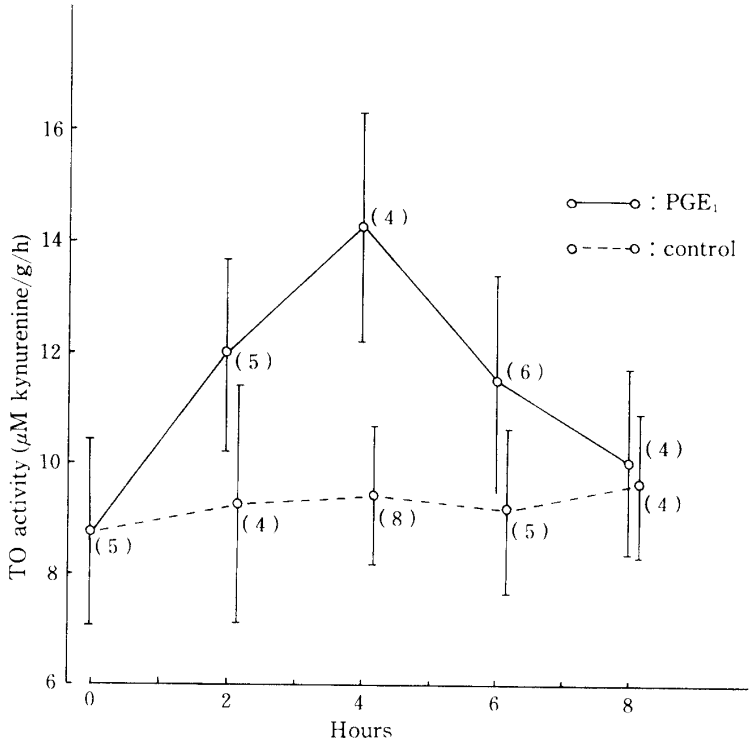

Fig. 2. Time-course of liver whole tryptophan oxygenase activity in mice injected with PGE_1 intraperitoneally (8 μg/mouse).
Vertical bars represent the standard deviation of the mean for the number of animals shown in parentheses.

Table 3. Effects of PGE_1, E_2 and $F_{1\alpha}$ on liver tryptophan oxygenase activity in mice 4 hours after the intraperitoneal injection (8 μg/mouse)

Injected PGs	Whole TO activity (μM kynurenine/g/h)
Control	9.43±1.27 (8)
PGE_1	14.26±2.09 (4)*
PGE_2	12.36±1.14 (5)*
$PGE_{1\alpha}$	9.55±1.56 (6)

Each value is the mean±standard deviation for the number of animals shown in parentheses.
*: Significantly different from the control ($P<0.005$)

Table 4. Effect of PGE_1 on liver tryptophan oxygenase activity in adrenalectomized mice 4 hours after the intraperitoneal injection (8 μg/mouse)

Treatment	PGE_1	Whole TO activity (μM kynurenine/g/h)
Sham operated	With	14.99±0.94 (4)
Adrenalectomized	Without	7.64±1.06 (6)
	With	8.32±0.56 (4)
Intact	Without	8.74±1.67 (5)

Each value is the mean±standard deviation for the number of animals shown in parentheses.

5. Effects of PGE_1 on total and holo- TO activities

Total and holo- TO activities were 3.47 and 3.41, respectively, 4 hours after the injection of PGE_1 (8 μg/mouse), and were elevated by about 40% higher than the control values (Table 5). That the ratios of holoenzyme/apoenzyme were 13.4 and 56.8 in animals without and with PGE_1, respectively, shows a significant conversion of apoenzyme to holoenzyme by PGE_1. The holoenzyme/whole enzyme ratio was approximately similar to be 0.24 and 0.26 in animals without and with PGE_1.

Table 5. Effect of PGE_1(8 μg/mouse) on holo-, total and whole tryptophan oxygenase activities in mice 4 hours after the intraperitoneal injection

Treatment	TO activity (μM kynurenine/g/h)			Holo/apo	Holo/whole
	Holoenzyme	Total enzyme	Whole enzyme		
Control	2.41±0.42 (7)	2.59±0.48 (7)	9.43±1.27 (8)	13.4	0.26
PGE_1	3.41±0.56 (5)*2	3.47±0.53 (5)*3	14.26±2.09 (4)*1	56.8	0.24

Each value is the mean±standard deviation for the number of animals shown in parentheses.
*1: Significantly different from the control ($P<0.005$)
*2: Significantly different from the control ($P<0.01$)
*3: Significantly different from the control ($P<0.025$)

6. Effects of dexamethasone on effects of PGE_1 on mouse liver TO

Instead of hypophysectomy were investigated the effects of dexamethasone (DM), which depresses ACTH secretion from the hypophysis, on the effects of PGE_1 on mouse liver TO activity. First, plasma corticosterone level after the injection of DM (25 mg/kg) was determined in order to examine a depressing effect of ACTH secretion of DM on the pituitary (Fig. 3). The level decreased to 2/3 of the first level 5 hours after DM, then retained itself unaltered until 15 hours, and was considerably restored 20 hours after injection. Fig. 4 shows the time-course of liver whole TO activity in mice after DM. The activity increased linearly until 6 hours after DM. It was considerably decreased to 12.4 after 15 hours. This reduction is considered to be an apparent one derived from the augmentation of liver weight due to the accumulation of glycogen by much amount

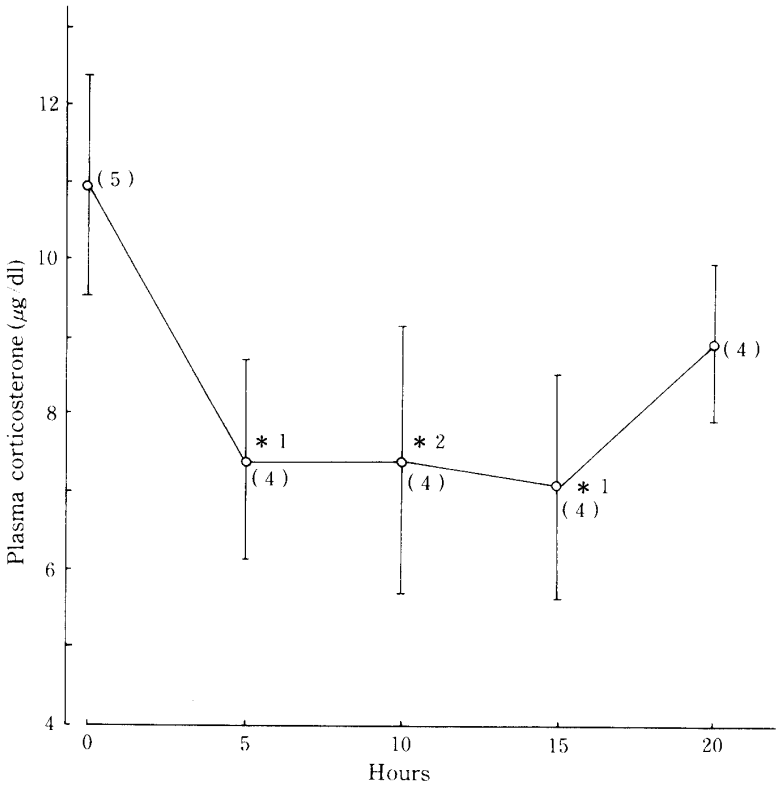

Fig. 3. Time course of plasma corticosterone level in mice injected with dexamethasone intraperitoneally (25 mg/kg).
Vertical bars represent the standard deviation of the mean for the number of animals shown in parentheses.
*1: Significantly different from the 0 hour ($P<0.02$)
*2: Significantly different from the 0 hour ($P<0.025$)

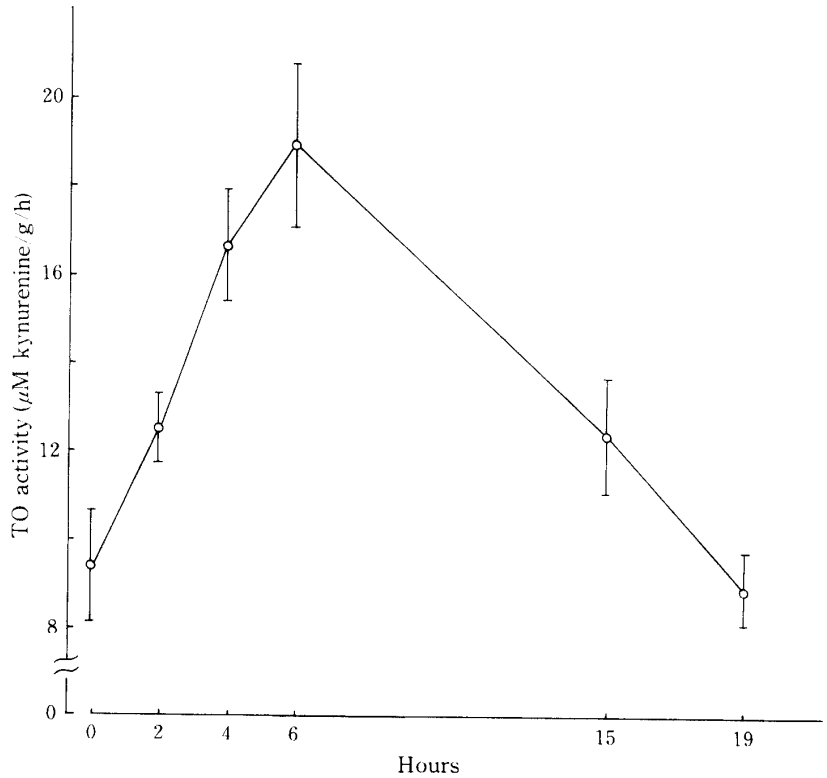

Fig. 4. Time course of liver whole tryptophan oxygenase activity in mice injected with dexamethasone intraperitoneally (25 mg/kg).
Each point represents the mean of 4 animals and vertical bars do the standard deviation.

of DM (The data are not tabulated). This is understood because TO activities per liver are unaltered, showing 36.79 and 32.13 6 and 15 hours after DM, respectively.

Basing on both the fact that the function of the hypophyseal-adrenocortical axis was considered to be depressed 15 to 20 hours after DM (Fig. 3) and the change of whole TO activity after DM (Fig. 4), PGE_1 was administered 15 hours after DM and the activities were investigated further after 4 hours. The value of 11.33 after PGE_1 was significantly different from the value of 8.91 in the control. No difference was recognized in the adrenalectomized animals (Table 6).

Table 6. Effect of PGE_1 on liver tryptophan oxygenase activity in intact or adrenalectomized mice injected with dexamethasone

Treatment	Whole TO activity (μM kynurenine/h/g) Control	PGE_1 (8 μg/mouse)
Intact	8.91 ±0.73	11.33±0.94*
Adrenalectmized	11.71 ±1.60	12.14±1.54

PGE_1 injections were given 15 hours after dexamethasone.
Animals were sacrificed 4 hours after the injection of PGE_1.
Each value is the mean±standard deviation of 5 animals.
*: Significantly different from the control ($P<0.005$)

The results of adrenal ascorbid acid determination applicable as an index of the hypophyseal-adrenocortical axis function are shown in Table 7. Though the administration of PGE_1 gave no effect on adrenal ascorbic acid amounts in animals treated with DM, the amount per bilateral adrenals decreased by about 30% 1 hour after PGE_1 in the animals without DM.

Table 7. Effect of PGE_1 on adrenal ascorbic acid amounts in mice injected with dexamethasone

Hours after the PGE_1 (8 μg/mouse)	No dexamethasone		Dexamethasone injection (25 mg/kg)		
	0	1	0	1	2
Ascorbic acid μg/mg adrenal	1.64±0.13 (9)	1.14±0.09 (5)	2.02±0.17 (5)	1.68±0.20 (5)	1.46±0.23 (4)
Ascorbic acid μg/bilateral adrenals	5.75±0.97 (9)	4.06±0.42 (5)*	5.52±0.82 (5)	5.13±0.44 (5)	5.20±0.84 (4)
Adrenal weight (mg, bilateral)	3.5 ±0.2 (9)	3.5 ±0.1 (5)	2.8 ±0.5 (5)	3.1 ±0.5 (5)	3.6 ±0.2 (4)

PGE_1 injections were given 15 hours after dexamethasone.
Each value is the mean±standard deviation for the number of animals shown in parentheses.
*: Significantly different from the value at 0 hour ($P<0.01$)

7. Effects of phenobarbital on mouse liver TO

From the above results, it is anticipated that the hypophyseal-adrenocortical axis might be concerned in the effects of PGE_1 on TO. Furthermore, effects of phenobarbital (PB), which depresses the ascending activating system of brain-stem-reticular-formation and is assumed to depress rat TO activity[1], were investigated in order to examine whether the hypothalamus also might be concerned or not.

In the experiment PB was administered continuously, whole TO activities significantly rose from the next day after the administration and remained increased until the 12th day (Table 8).

Table 8. The change of liver tryptophan oxygenase activity in mice and rats after the oral administration of phenobarbital

Animal	Treatment	Days after administration	TO activity (μM kynurenine/g/h) Whole enzyme	Total enzyme	Holoenzyme	Adrenal weight (mg, bilateral)
Mouce	Intact	0	8.74±1.67 (5)	2.59±0.48 (7)	2.41±0.42 (7)	3.5±0.2 (9)
		1	14.03±1.20 (5)	3.95±0.63 (5)	3.74±0.53 (5)	4.2±0.4 (5)
		3	12.03±0.92 (4)	6.35±1.21 (5)	6.00±1.26 (5)	3.8±0.3 (5)
		6	16.53±2.32 (5)	3.00±0.53 (4)	2.66±0.65 (4)	4.5±0.4 (9)
		9	15.59±2.81 (5)	3.77±1.04 (4)	3.57±1.05 (4)	4.3±0.4 (7)
		12	15.19±2.47 (4)	4.07±0.22 (4)	3.64±0.24 (4)	4.6±0.5 (4)
	Adrenal-ectomized	0	7.64±1.06 (6)	2.10±0.46 (6)	1.71±0.29 (6)	—
		1	11.41±3.00 (5)	3.02±0.55 (5)	2.51±0.45 (5)	—
Rat	Intact	0	—	3.77±0.35 (5)	2.36±0.12 (5)	—
		9	—	8.00±2.31 (4)	6.93±1.77 (4)	—

The method of administration of phenobarbital is described under "Materials and methods".
Each value is the mean±standard deviation for the number of animals shown in parentheses.

As Badawy and Evans[1] have shown that chronic phenobarbitone administration particularly inhibits apoenzyme activity in the rat, holo- and total activities also were assayed, but both of them rose similarly. For trial, PB was administered continuously for 9 days in the rat, and holo- and total activities were assayed on the 9th day, but both rose similarly as in mice (Table 8). Thus, these results were quite reverse to those of Badawy and Evans[1]. Because some increases were observed in adrenal weights (Table 8), whole, total and holo- activities were assayed 1 day after the administration of PB in the adrenalectomized mice. They showed the same increase as in the intact mice (Table 8). In some mice, PB was freely administered in drinking water (1 mg/ml) for 24 hours and then animals were sacrificed. Ethionine (40 mg/kg) was injected intraperitoneally just prior to PB treatment and 4 hours before the sacrifice. Liver whole TO activities decreased by about 16% in these mice in comparison with the control (Table 9).

Table 9. Effect of ethionine on liver whole tryptophan oxygenase activity in mice treated with phenobarbital

	Treatment		Inhibition ratio (%)
	Phenobarbital	Phenobarbital + Ethionine	
Whole TO activity (μM kynurenine/g/h)	13.56±1.40	11.44±1.54	15.6

Phenobarbital was freely administered in drinking water of 1 mg/ml for 24 hours and then animals were sacrificed.
Ethionine (40 mg/kg) was injected intraperitoneally just prior to phenobarbital treatment and 4 hours before the sacrifice.
Each value is the mean±standard deviation for 5 animals.

For the comparison with the case of continuous administration of PB whole TO activities were measured after a single injection of PB. No increases were recognized 4 hours after the injection

of 50 or 100 mg/kg of PB (Table 10). The activity increased to about 1.3 times that of the control, 24 hours after the injection of 50 mg/kg of PB. This increase was depressed after ethionine.

From these results it was considered that PB stimulated the TO synthesis. Although it was expected that TO activity might be depressed by PB, the opposite results were obtained, so the experiment of the administration of PGE_1 after PB was given up.

Table 10. Liver whole tryptophan oxygenase activity 4 and 24 hours after a single intraperitoneal injection of phenobarbital in mice and the effect of etionine on those activities

Hours after injection of phenobarbital		4	24
		(μM kynurenine/g/h)	
Treatment	Control	8.48±1.05 (4)	8.96±1.01 (5)
	Phenobarbital 50 mg/kg	8.62±1.09 (6)	11.73±1.07 (6)*1
	Phenobarbital 100 mg/kg	8.87±1.04 (5)	—
	Phenobarbital (50 mg/kg) + Ethionine	—	9.72±1.46 (5)*2
Inhibition ratio (%)			17.2

Ethionine (40 mg/kg) was injected intraperitoneally 4 and 24 hours before the sacrifice.
Each value is the mean±standard deviation for the number of animals shown in parentheses.
*1: Significantly different from the value of control ($P<0.005$)
*2: Significantly different from the value of phenobarbital injection (50 mg/kg) ($P<0.05$)

Discussion

Knox et al.[17,18] and Chytil et al.[4,13,14] have suggested the fairly more existence of inactive forms of TO in the soluble liver supernatant in the rat. Therefore, quantitative changes of TO molecule and the variation of proportion of active parts may be considered for the induction or inhibition of this enzyme by various drugs.

In this study, TO activities were assayed on the basis of the method[18] which was said to be capable of activating the inactive form of this enzyme. Whole TO activity by this method was about 9 in the liver homogenate in normal mice (As mentioned above, called whole TO for total TO), which was nearly 4 times as much as holo- TO or total TO activity (Table 1). Judging from the results of Knox et al.[18], whole TO activity is about 4.5 in rat liver homogenates, which is about half of that in mouse cases, and the inactive part of this enzyme in rat homogenates is considered to be less than the active part or be about the same. The suggestion by Miyao et al.[21] that more inactive form of holoenzyme exists in mouse liver homogenates than in rat ones appears to be correct from the results in this experiment.

Further studies should be carried out on the participation of xanthine oxidase which is being thought to be one of the activation factors of inactive form of this enzyme on this problem.

By adrenalectomy, whole TO activity was not so much decreased in the mouse liver (Table 4), but it has been observed to be lowered by about 50% in the rat liver[18]. The decrease of total and holo- TO activities also is observed in the adrenalectomized rats[16,29], but not in the adrenalectomized mice[21]. Accordingly, it is conisdered that adrenocortical hormone is relatively less participated on the maintenance of normal TO level in mice.

It has been suggested that blood glucocorticoid level rises after the peripheral administration of PGs (particularly E_1)[5,12,19,24] and PGE_1 and PGE_2 produce anti-inflammatory effects when given at high dosages to rats[7]. Therefore, it is expected that PGs affect TO activities. Peng et al.[24] have suggested that PGE_1 has no ACTH-like effects on the adrenal cortex but acts by stimulating ACTH-release, from the observations that the log-dose relationship between PGE_1 given intravenously and the adrenal ascorbic acid concentration was linear between 0.5 and 2.0 μg as well as that PGE_1 did not affect adrenal ascorbic acid in the cortisol-pretreated or hypophysectomized rats. On the contrary, Hedge[12] has suggested that PGs stimulate ACTH secretion indirectly by acting at the hypothalamus, presumably via CRF. Accordingly, it is considered that effects of PGs on TO should be investigated from the viewpoint not only of the adrenal but of the hypopshyseal-adrenocortical and the hypothalamo-hypophyseal-adrenocortical axes.

Effects on whole TO activity were twice as large as PGE_2 in PGE_1 and negligible in $PGF_{1\alpha}$ (Fig. 2, Table 3). No increase of the activity was observed in the adrenalectomized mouse (Table 4). The linear log-dose response relationship between doses of 1 to 8 μg/mouse of PGE_1 and whole TO activities (Fig. 1) agrees with the observation of Peng et al.[24] on the log dose relationship between doses of PGE_1 and adrenal ascorbic acid concentration. Thus it seems clear that the increase of whole TO activities in mouse liver by PGE_1 and PGE_2 results via glucocorticoid seretion from the adrenal stimulation, and this action of PGE_1 is about twice PGE_2 because PGsE are said to be comparatively stable in blood[6]. It is also apparent from the results in Table 5 that the apoenzyme is appreciably altered to the holoenzyme by PGE_1, and because the ratio of holoenzyme to whole enzyme is almost the same as the one in the control 4 hours after PGE_1, the increase of TO activitity is considered to be due to the acceleration of enzyme protein synthesis. That this action of PGE_1 is quite temporary may be related to the fact that inactivation of PGs in the lung or excretion from the kidney is extremely active[6]. No action was observed in $PGF_{1\alpha}$. The cause of this may be due to that $PGF_{1\alpha}$ injected intraperitoneally was more rapidly degraded in blood, not that it has less intrinsic activity, like the suggestion of Hedge[12].

Although PGE_1 increased significantly whole TO activities in intact mice, this increase was depressed to some extent after DM and not found in the adrenalectomized animals after DM (Table 6, Fig. 2). Furthermore, since PGE_1 had no effect on adrenal ascorbic acid concentration in mice treated with DM, showing a significant decrease of the acid in the DM-untreated mice (Table 7), it seems clear that the peripheral administration of PGE_1 accelerates ACTH secretion and the pituitary also takes part in the induction of TO by PGE_1. Hedge[12] has observed that $PGsE_1$, $F_{1\alpha}$ and $F_{2\alpha}$ increase ACTH secretion when injected into the hypothalamic median eminence in the rat, but don't show this effect when injected into the lateral hypothalamus, anterior pituitary and a tail vein. These findings differ from the present results. The release of so many pituitary hormones including ACTH, however, has been recognized by the peripheral administration of PGs[28] that it may be considered that the intraperitoneal injection of PGE_1 also stimulates ACTH secretion from the hypophysis.

Since it is found that the TO activity increases by the stimulation of hypothalamus too[31], it is expected that the hypothalamus also may play a role in the increase of the activity, from the suggestion of Hedge[12] that ACTH secretion by PGE_1 may act via CRF. Because it was impossible to inject with PGE_1 into the hypothalamus directly, effects of PGE_1 on TO were tried to be investigated in PB-pretreated mice from the reason as mentioned above. All of whole, total and holo- TO activities were increased to the same extent in the mice injected with PB continuously (Table 8). These results are quite different from the observation of Badawy and Evans[1] and

agree with the results of Seifert[30] in rats. As the similar tendency was found in the rat liver too (Table 8), it is considered that these findings are not due to the difference of species but to the fact that continuous administration of PB elevates TO activity. It is not considered that this increase by PB is induced by the stress via the adrenal because it was found after the adrenalectomy too.

Judging from the facts that (1) the increase of TO 24 hours after PB is inhibited by ethionine (Table 9, 10), (2) the continuous administration of PB has no effects on the degradation of the enzyme induced by cortisone or tryptophan[30], (3) PB induces microsomal enzymes and promotes the synthesis of heme, a cofactor of TO[8,11,26], (4) PB increases both the total and holo- activities as well as the degree of saturation of the enzyme by heme (Table 7 as well as reference 30), (5) the similar findings are obtained after the administration of δ-aminolaevulinate, a precursor of heme, too [21], (6) PB particularly increases whole To activity (Table 8), it is considered that the increase of TO activities by PB may be due to the increase of heme by microsomal enzymes induced with PB and the resulting stimulation TO synthesis by heme.

As no decrease of TO activity was found after the administration of PB, the experiment of PGE_1 administration was discontinued in the PB-pretreated mice. Therefore, although whether the hypothalamus takes part on the activation of TO after the peripheral administration of PGE_1 or not is obscure at the present stage of this study, its possiblity may exist because it has been suggested that effects of PGE_1 on blood corticosterone and adrenal ascorbic acid levels are prevented in the rats treated with PB or morphine[24].

Summary

Effects of PGs on mouse liver TO were investigated in connection with the hypothalamo-hypophyseal-adrenocortical axis.

Although the activity of mouse liver TO was much larger than that of rat one, it became clear that the larger part of the enzyme existed as an inactive form in the mouse liver.

The linear log-dose response relationship existed between doses of 1 to 8 μg/mouse of PGE_1 and TO activities in mice. This increase after PGE_1 was temporary.

On the induction of TO by PGs, PGE_2 had half the effect of PGE_1, and $PGF_{1\alpha}$ no effect.

The participation of the hypophyseal-adrenocortical axis was recognized on the induction of TO by PGE_1. This increase of TO activities appeared to be due to the increase of enzyme synthesis.

All of whole, total and holo- activities increased in the mice administered PB in drinking water for 9 days. Accordingly, since the experiment of the administration of PGE_1 was discontinued in the PB-pretreated mice, the participation of hypothalamus on the induction of TO by PGE_1 remained unclear.

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