EFFECT OF PITUITARY HORMONES ON α -AMINO- β -CARBOXYMUCONATE- ϵ -SEMIALDEHYDE DECARBOXYLASE IN RAT¹

Hiroo SANADA and Motoyoshi MIYAZAKI²

*Division of Basic Nutrition, National Institute of Nutrition,
1 Toyamacho, Shinjuku-ku, Tokyo 162, Japan*

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Summary The hormonal regulation of α -amino- β -carboxymuconate- ϵ -semialdehyde decarboxylase (picolinic carboxylase) was studied to clarify the change of tryptophan-niacin metabolism in the different physiological states. Hypophysectomized rats were administered with predonine hemisuccinate and various kinds of pituitary hormones for 2 or 4 days every 12 hr. The activity of their liver picolinic carboxylase was then assayed. The injection of predonine alone elevated the enzyme activity in hypophysectomized rats. On the other hand, rat or bovine pituitary extract was found to suppress such enzyme induction when it was administered prior to predonine injection. The enzyme-suppressing activity of the pituitary extract was lost by heat treatment or addition of proteolytic enzyme such as trypsin and chymotrypsin. These results indicate that the effective substances in the extract may be polypeptides. By gel filtration of bovine pituitary extract, two effective fractions were obtained, which were able to suppress the predonine-dependent induction of liver picolinic carboxylase. The molecular weights of these substances were estimated to be about 23,000 and 2,000–4,000, respectively. Both of these effective substances were shown to be localized in the anterior lobe of hypophysis. In the many kinds of pituitary hormones tested, bovine somatotropin and mammalian prolactin were found to be effective in suppressing the predonine-dependent induction of liver picolinic carboxylase in hypophysectomized rats.

To obtain some information on the mechanism by which liver picolinic carboxylase activity is elevated in diabetic rats, bovine somatotropin or rat pituitary extract was administered to diabetic rats for 4 or 7 days every 12 hr. This hormone treatment was not observed to depress the enzyme activity in the diabetic rats. These results are considered to suggest that changes in pituitary hormone are not responsible for the elevation of the enzyme activity in the diabetic rat.

¹ Regulation of Tryptophan-Niacin Metabolism *in Vivo* (Part 2).

² 真田宏夫, 宮崎基嘉

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The metabolic pathway of tryptophan to nicotinamide derivatives has been well established by some investigators and the importance of α -amino- β -carboxymuconate- ϵ -semialdehyde decarboxylase [picolinic carboxylase; EC 4.1.1.45] has been described in their reports (1-4). In our previous experiments, it was found that the ratio of oral tryptophan to urinary *N*-methylnicotinamide (MNA) was roughly proportional to the activity of liver picolinic carboxylase in diabetic rats (5). Therefore, the activity of this enzyme was considered to be very important regarding aspects of niacin requirement. The elevated activity of liver picolinic carboxylase and reduced conversion rate of oral tryptophan to niacin in the diabetic rat were shown to be nearly normalized by long-term (3 weeks) insulin treatment (6). On other hand, 1 week of insulin treatment failed to normalize the enzyme activity and the conversion rate in chronically diabetic rats (5). Thus, insulin was considered to act indirectly on the enzyme activity. Whereas, Mehler *et al.* found that the liver picolinic carboxylase was induced by adrenocortical hormone only in hypophysectomized rats and suggested that pituitary hormone would play a role in the regulation of the enzyme (7). However, the effect of pituitary hormone on liver picolinic carboxylase has not yet been investigated. In the present report, we have described the role of pituitary hormones in the regulation of liver picolinic carboxylase.

MATERIALS AND METHODS

Predonine hemisuccinate (predonisolone sodium hemisuccinate), bovine somatotropin, bovine thyrotropin, ACTH and Pitressin (vasopressin) were purchased from Shionogi Co. (Osaka, Japan), Miles Laboratories, Inc. (Illinois, USA), Sigma Co. (Missouri, USA), Daiichi Seiyaku Co. (Tokyo, Japan) and Sankyo Co. (Tokyo, Japan), respectively. Prolactin, Atonin-O (oxytocin), Serotropin (FSH activity) and Gonatropin (LH activity) were obtained from Teikoku Zoki Co. (Tokyo, Japan).

Hypophysectomized male rats (Sprague Dawley strain, 4 weeks of age) were obtained from Clea Japan, Inc. (Tokyo, Japan). They were fed on a commercial ration *ad libitum* in an air-conditioned room. When they were 5 weeks old, the rats with poor growth were used for the following experiments. Predonine hemisuccinate was injected intraperitoneally 5 mg per rat 4 times every 12 hr. The pituitary hormones or extract were administered subcutaneously 30 min before predonine injection. The rats were killed by decapitation 12 hr after the last predonine injection. The liver was removed and homogenized with 3 volumes of 0.14 M potassium chloride solution at 0°C. After centrifugation at $105,000 \times g$ for 45 min, the activity of picolinic carboxylase in the supernatant was assayed as described in the previous paper (5).

Normal Sprague Dawley male rats (7 weeks of age) were made diabetic by intraperitoneal injection of alloxan (180 mg/kg body weight). After 80 days, they were administered with protamine zinc insulin (Shimizu Seiyaku Co., Shimizu, Japan) (5 units/rat) and pituitary extract (0.5 ml/rat) intraperitoneally 14 times every 12 hr.

For the preparation of pituitary extract, 208 hypophyses from normal adult rats were homogenized with 26 ml of saline using a Potter-teflon homogenizer. After centrifugation at $105,000 \times g$ for 45 min in cold conditions, the supernatant was passed through membrane-filter (millipore filter, $0.45 \mu\text{m}$) to sterilize the extract. This solution was stored at -20°C until use. The bovine pituitary extract was prepared in the same way as that for rat except the bovine tissue was homogenized with 9 volumes of saline.

The molecular sieve column chromatography of bovine pituitary extract was performed using Sephadex G25 ($2.5 \times 70 \text{ cm}$) or Sephadex G100 ($2.0 \times 90 \text{ cm}$). The pituitary extract (4 ml) was passed through the column with 0.01 M phosphate buffer (pH 6.8) and 10-ml fractions were collected.

The activity of tryptophan pyrrolase was measured by the method of Knox *et al.* (8). Protein was assayed by the method of Lowry *et al.* (9).

RESULTS

The activity of liver picolinic carboxylase was shown to be increased slightly more in the hypophysectomized rats ($1.1 \pm 0.2 \mu\text{mol/hr/g}$ liver, $n=6$) than in normal rats ($0.6 \pm 0.2 \mu\text{mol/hr/g}$ liver, $n=5$). The administration of predonine hemisuccinate for 2 days every 12 hr resulted in the significant elevation of the enzyme activity (Table 1). However, prior injection of rat pituitary extract 30 min before each predonine administration was found to reduce the enzyme activity to lower than that of the saline-treated control. When the amount of pituitary extract was reduced, the degree of suppression of the enzyme activity was also decreased correspondingly (Fig. 1). After the high and low molecular weight fractions were prepared from rat pituitary extract by Sephadex G25 column chromatography, each fraction was injected into the hypophysectomized rat prior to the administration of predonine. Both high and low molecular weight fractions were shown to be able to suppress the predonine-dependent induction of liver picolinic carboxylase (Fig. 2). These results indicated that some kinds of effective factors were present in the rat pituitary extract. Similar results were obtained when bovine pituitary extract was used (Fig. 3). The nature of the picolinic carboxylase-suppressing factors in the bovine pituitary extract was next examined.

As shown in Fig. 4, the enzyme-suppressing activity of bovine pituitary extract was lost by heat treatment (100°C , 5 min) or by the addition of proteolytic enzyme. From these results, it is considered that the effective factors are heat-unstable polypeptides. Molecular sieve fractionation was then carried out to determine the molecular weights of the picolinic carboxylase-suppressing factors using bovine

Table 1. Effect of predonine and rat pituitary extract on the activity of liver picolinic carboxylase in hypophysectomized rats.

Group	Rat No.	Body weight gain (g/4 days)	Liver weight (g)	Picolinic carboxylase ($\mu\text{mol/hr/g liver}$)
Control	1	5	4.2	0.88
	2	8	2.9	1.60
+ Predonine	1	2	4.1	2.64
	2	3	4.4	2.10
+ Pituitary extract + predonine	1	16	4.4	0.08
	2	15	4.5	0.40

The hypophysectomized rats were injected with predonine hemisuccinate and rat pituitary extract for 2 and 4 days, respectively. The group receiving both hormones was injected with pituitary extract only for the first 2 days. The control hypophysectomized rats were administered with 0.2 ml of saline intraperitoneally for 2 days every 12 hr. Other details in the experiments are described in METHODS.

Fig. 1. Effect of the amount of rat pituitary extract on the predonine-dependent induction of liver picolinic carboxylase. The rat pituitary extract equivalent to one or half a hypophysis was injected into the hypophysectomized rats prior to predonine administration for 2 days. Each point represents the mean $\pm$ S.E.M. of 3 rats.

serum albumin, insulin, cytochrome *c* and cyanocobalamin as the standard substances. Two active peaks to suppress the predonine-induced picolinic carboxylase were observed (Fig. 5). The molecular weights of the high molecular and comparatively low molecular weight factors were estimated to be about 23,000 and 2,000–4,000, respectively. In the next experiments, the localization of the

Fig. 2.

Fig. 3.

Fig. 2. Effect of high and low molecular weight fractions of rat pituitary extract on the predonine-dependent induction of liver picolinic carboxylase in hypophysectomized rats. Rat pituitary extract was fractionated on a Sephadex G25 column. The first high molecular weight fraction at the void volume and the other low molecular weight fraction were collected. Each fraction was concentrated by lyophilization and sterilized by membrane filter. The amount equivalent to 2 hypophyses was injected for 4 days. Predonine was administered 30 min after the injection of pituitary extract for the last 2 days. Each bar represents mean $\pm$ S.E.M. of 2 rats.

Fig. 3. Suppressing effect of bovine pituitary extract on the activity of picolinic carboxylase. The low molecular weight fraction of bovine pituitary extract was prepared as described in Fig. 2. Hypophysectomized rats were injected with the extract equivalent to 0.86 mg of protein (this amount is almost the same as that contained in the extract of 2 rat hypophyses) prior to predonine administration. Other methods were the same as described in Fig. 2. Each bar represents the mean $\pm$ S.E.M. of 2 rats.

factors in bovine hypophysis was investigated. The saline extracts of bovine anterior or median-posterior lobes of hypophysis were fractionated by Sephadex G25 column chromatography and the picolinic carboxylase suppressing activity of each fraction was assayed using hypophysectomized rats (Fig. 6). Both of the high and low molecular weight factors were found to be contained in the anterior lobe of bovine hypophysis.

Further, the picolinic carboxylase-suppressing activity of many kinds of pituitary hormones were assayed. As shown in Fig. 7(A) and (B), bovine somatotropin and mammalian prolactin were found to have activity for suppressing the predonine-induced liver picolinic carboxylase. None of the posterior hormones tested showed such suppressing activity.

The activity of liver picolinic carboxylase is well known to be elevated in

Fig. 4. Stability of the picolinic carboxylase-suppressing factors in bovine pituitary extract. After the various treatments described below, the extract was injected into the hypophysectomized rats prior to predonine administration for 2 days. The activity of liver picolinic carboxylase was measured 12 hr after the last injection of hormone. Each bar represents the mean $\pm$ S.E.M. of 2 rats.

pH 3: The pituitary extract (2 ml) was acidified to pH 3 by adding acetic acid. After 1 hr at room temperature, the solution was neutralized by adding sodium hydroxide. It was then sterilized by membrane filter after centrifugation (total volume 4 ml).

pH 10: The pituitary extract (2 ml) was adjusted to pH 10 by adding 1 N sodium hydroxide solution and 1 hr later, it was neutralized by adding hydrochloric acid solution. It was then sterilized by membrane filter after centrifugation. (total volume 4 ml)

Heat: The pituitary extract (2 ml) was added to 2 ml of saline and the mixture was heated in boiling water for 5 min. Denaturated protein was then removed by centrifugation.

Trypsin: The pituitary extract (2 ml), 0.02 ml of 1 M Tris buffer solution (pH 8), 0.2 ml of 0.1 M CaCl_2 and 0.5 mg of trypsin were mixed and incubated at 25°C for 1 hr. Saline was added to make the final volume 4 ml and the solution was sterilized by membrane filter.

Chymotrypsin: The pituitary extract (2 ml), 0.02 ml of 1 M Tris buffer (pH 8) and 0.5 mg of chymotrypsin were mixed and incubated at 25°C for 1 hr. Total volume was adjusted to 4 ml with saline and sterilized by membrane filter.

Intact extract: Saline (2 ml) was added to the pituitary extract (2 ml) and the solution was incubated at 0°C or 25°C for 1 hr.

diabetic animals. However, the mechanism of such increase in enzyme activity has not yet been elucidated. In the present experiments, we studied the effect of pituitary hormone on the liver picolinic carboxylase of diabetic rats. When bovine somatotropin was injected for 4 days into the rats in the early stage of alloxan diabetes, the activity of liver picolinic carboxylase was rather increased (Fig. 8).

Fig. 5. Molecular sieve chromatography of the picolinic carboxylase-suppressing factors in bovine pituitary extract. Bovine pituitary extract was fractionated on a Sephadex column by elution with 0.01 M phosphate buffer (pH 6.8). The enzyme-suppressing activity of each fraction was assayed as described in METHODS. The volume of the eluate injected at one time was 0.75 ml per rat. Each point represents the mean of 2 or 3 rats. A, Sephadex G100; B, Sephadex G25.

Fig. 6. Localization of the picolinic carboxylase-suppressing factors. The pituitary extract of anterior or median-posterior lobe (4 ml) was supplied on the Sephadex G100 column and eluted with 0.01 M phosphate buffer (pH 6.8). Other methods were the same as described in Fig. 5. Each point represents the mean of 2 rats. The enzyme activity induced by predonine in the absence of any enzyme suppressing factors is indicated by shaded area.

Further, the simultaneous administration of rat pituitary extract and insulin to the diabetic rats for 7 days every 12 hr also failed to depress the enzyme activity of their livers (Table 2). These findings suggest that the pituitary factors, which can suppress the predonine-dependent induction of picolinic carboxylase in hypophysectomized rat, are not able to depress the enzyme activity in diabetic rats.

Fig. 7. Effect of various hormones on the predonine-dependent induction of liver picolinic carboxylase. The amount of hormone given at a time per rat was as follows: ACTH, 400 mU; somatotropin, 1 mg; thyrotropin, 0.5 IU; Gonatropin, 100 IU (LH activity); Serotropin, 100 IU (FSH activity); prolactin, 20 IU; Pitressin, 20 mU (vasopressin activity); Atonin-O, 400 mU (oxytocin activity); the bovine pituitary extract, 0.2 ml (equivalent to 20 mg of hypophysis). Each bar represents the mean $\pm$ S.E.M. of 2 rats.

Fig. 8. Effect of somatotropin on the liver picolinic carboxylase in diabetic rats. Male Sprague Dawley rats (about 100 g of body weight) were rendered diabetic with alloxan. After 3 days, the activity of liver picolinic carboxylase was measured in 3 rats (before treatment). Other rats were injected with protamine zinc insulin (10 IU/rat/12 hr) or somatotropin (0.5 mg/rat/12 hr) for 4 days every 12 hr. Twelve hr after the last hormone injection, the enzyme activity was assayed. The enzyme activity of normal rat is indicated by shaded area. Number of animals in each group is indicated in parenthesis.

Table 2. Effect of rat pituitary extract and insulin on the activity of liver picolinic carboxylase in diabetic rats.

Group	Picolinic carboxylase	
	Activity per g liver ($\mu\text{mol/hr/g liver}$)	Total activity ($\mu\text{mol/hr/liver}$)
Diabetic	6.7 ± 2.4	54 ± 15
Diabetic + insulin + pituitary extract	5.5 ± 2.3	53 ± 17
Control	0.6 ± 0.2	8 ± 2

Male rats rendered diabetic by alloxan at 7 weeks of age were injected with both rat pituitary extract and insulin for 7 days every 12 hr at the age of about 5 months old. The activity of liver picolinic carboxylase was assayed 12 hr after the last injection of hormone. Values represent mean $\pm$ S.E.M. of three rats.

DISCUSSION

In the present studies, somatotropin and prolactin were shown to suppress the induction of liver picolinic carboxylase by predonine. In the earlier reports, the induction of some enzymes such as tyrosine aminotransferase and those of the urea cycle, was described as being partially suppressed by somatotropin (10, 11). Although the enzymes mentioned in those reports were induced by adrenocortical hormone even in non-hypophysectomized rats, liver picolinic carboxylase was reported never to be induced in normal rats (7). Thus, the present results may suggest that the level of the pituitary hormone in normal rat would be in the range sufficient to suppress the adrenocortical hormone-dependent induction of picolinic carboxylase.

The high molecular weight fraction of the picolinic carboxylase-suppressing factors is considered to contain somatotropin and prolactin, because the molecular weights of these factors are about 26,000 and 23,000, respectively. In addition, both hormones are known to be localized in the anterior lobe of hypophysis. On the other hand, the active substance in the low molecular weight fraction has not yet been identified. Further study is necessary for the identification of this active peptide in the anterior lobe of hypophysis.

The administration of pituitary extract or somatotropin to diabetic rats failed to suppress the activity of liver picolinic carboxylase. These results suggest that the suppressing effect of these hormones on the liver picolinic carboxylase is prevented in diabetic rats. Although the somatomedin activity in sera from streptozotocin-induced diabetic rats was reported to be significantly low, administration of insulin caused rapid recovery in the activity (12). Since simultaneous administration of pituitary extract and insulin for 7 days failed to normalize the activity of liver picolinic carboxylase in diabetic rats, disappearance of the effect of the enzyme-suppressing hormones in these rats was not considered to be caused by the low level of somatomedin. The mechanism by which the pituitary hormones suppress

the liver picolinic carboxylase activity must be studied further to elucidate the elevation of the enzyme activity in the diabetic animal.

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