ROLE OF ASCORBIC ACID ON TYROSINE HYDROXYLASE ACTIVITY IN THE ADRENAL GLAND OF GUINEA PIG

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Summary The decrease of tyrosine hydroxylase activity in adrenal homogenate in scurvy was recovered after the administration of ascorbic acid. The causes of the increase in the enzyme activity after the administration of ascorbic acid have been studied.

1. No significant elevation in the enzyme activity was observed after the administration of reserpine to the scorbutic guinea pig.

2. A dose of metal chelating agent, α, α'-dipyridyl, prevented the ascorbic acid-induced or reserpine-induced increase in enzyme activity in the scorbutic and the non-scorbutic guinea pigs, respectively.

3. Tyrosine hydroxylase activity was partially recovered by the administration of FeSO₄ to the scorbutic guinea pig.

From these results, it became clear that the induction of tyrosine hydroxylase which was not observed in scurvy was due to the deficiency of Fe²⁺. These results suggested that ascorbic acid affected the induction of this enzyme via Fe²⁺.

Previous investigations in this laboratory have shown that the activity of tyrosine hydroxylase in adrenal gland of guinea pig decreased in scurvy, and was recovered by the administration of ascorbic acid. This increase of the enzyme activity on the administration of ascorbic acid was found to be due to the increased amount of enzyme protein (1, 2).

MUELLOR et al. demonstrated that the administration of reserpine, which is known to cause an increase in preganglionic sympathetic neurone activity, produced an increase in tyrosine hydroxylase activity (3). This increase in the enzyme activity was also due to the increased amount of the enzyme protein (3, 4). It was suggested that this increased activity was the result of an increased amount of the enzyme protein caused by a reflex increase in sympathoadrenal activity (5, 6).
SHIMAN et al. and TAYLOR et al. showed that partially purified tyrosine hydroxylase from bovine adrenal medulla has been inhibited by α, α'-dipyridyl and o-phenanthroline, and stimulated by ferrous ion (7, 8). This suggested that Fe²⁺ participated in the hydroxylation reaction. Since ascorbic acid facilitates absorption of iron and its removal from ferritin, iron content in blood, liver, kidney, spleen and bone marrow was decreased in scorbutic guinea pig (9).

As shown previously, the amount of the protein of tyrosine hydroxylase was decreased in scorbutic guinea pig (2). In order to clarify the mechanism, we carried out an experiment on the following supposition. 1) The synthesis of tyrosine hydroxylase was inhibited by the decrease of Fe²⁺ which was necessary for the enzyme synthesis. 2) The enzyme synthesis was inhibited because sympathetic neurone activity was reduced by ascorbic acid deficiency.

In this paper, to confirm the supposition, we experimented with an increase in tyrosine hydroxylase activity by ascorbic acid, reserpine and iron, and the decrease by α, α'-dipyridyl. It was found that ascorbic acid activated tyrosine hydroxylase via Fe²⁺.

MATERIALS AND METHODS

Materials. L-Tyrosine-3,5-³H was obtained from Radiochemical Centre, Amersham, England. It was purified as described previously (1). DMPH₄ was purchased from Calbiochem. Reserpine was obtained Daiichi Seiyaku Co., Ltd.

Animals. An ascorbic acid deficient diet was prepared as described by NAKASHIMA et al. (1).

Male guinea pigs, weighing about 400 g were used in all the experiments. The scorbutic and the non-scorbutic groups were maintained on an ascorbic acid deficient diet for 20 days. The non-scorbutic groups were given 200 mg of ascorbic acid per day by intraperitoneal injection during the last 3 days. Normal diet groups received commercial guinea pig diet. Reserpine (0.05 mg/ml), α, α'-dipyridyl (7.5 mg/ml) and ferrous sulfate (10 mg/ml) were dissolved in saline solution. The animals were given reserpine (0.25 mg/kg), α, α'-dipyridyl (40 mg/kg) or ferrous sulfate (50 mg/kg) by intraperitoneal injection once a day during the last 3 days. They were killed by a blow on the head. The adrenal glands were removed, and homogenized with 3 volumes of ice-cold 0.32 M sucrose using a Potter-Elvehjem homogenizer at 0°C.

Assay of tyrosine hydroxylase activity. The enzyme activity was assayed according to NAGATSU et al. (10). Protein was assayed by the method of LOWRY et al. (11). Specific activity was defined as the activity of enzyme which catalyzed the release of ³H from ³H-tyrosine equivalent to the formation of 1 nmole of DOPA in 30 min per mg protein.

Assay of catecholamines. Adrenal homogenate (0.05 ml) was added to 2 ml of 0.05 N perchloric acid, and centrifuged at 2,500 rpm for 10 min. Catecholamines in the supernatant were separated by alumina according to the method
of ANTON and SAYRE (12). Catecholamines were determined fluorometrically according to THI method (13).

RESULTS

Effect of ascorbic acid and reserpine on tyrosine hydroxylase activity

It has been previously reported that tyrosine hydroxylase activity in the adrenal gland of the non-scorbutic guinea pig was 2-fold higher than that of the scorbutic guinea pig. The difference of the enzyme activity between the 2 groups was due to the difference in the amount of the enzyme protein (1, 2). Therefore, in this paper, we investigated the causes of the increase in the enzyme activity by ascorbic acid.

It is well known that reserpine, an adrenergic β-blocking agent causing an increase in preganglionic sympathetic neurone activity, stimulates tyrosine hydroxylase activities in adrenal gland, sympathetic ganglion and brain. Therefore, it was considered in scurvy, if sympathetic neurone activity was injured by ascorbic acid deficiency, the enzyme activity was not increased by the administration of reserpine. The increase in tyrosine hydroxylase activity was examined after the administration of ascorbic acid and reserpine to both normal and scorbutic guinea pigs.

![Fig. 1. Effect of ascorbic acid and reserpine on tyrosine hydroxylase activity in adrenal glands of normal guinea pig. Normal guinea pigs received ascorbic acid (200 mg/day) or reserpine (0.1 mg/day) for the last 3 days by intraperitoneal injection. Tyrosine hydroxylase activity was determined as described in MATERIALS AND METHODS.](image1)

![Fig. 2. Effect of ascorbic acid and reserpine on tyrosine hydroxylase activity in adrenal glands of the scorbutic guinea pig. The scorbutic guinea pigs received ascorbic acid (200 mg/day) or reserpine (0.1 mg/day) for the last 3 days by intraperitoneal injection. Tyrosine hydroxylase activity was determined as described in MATERIALS AND METHODS.](image2)
Normal guinea pigs received 0.1 mg of reserpine or 200 mg of ascorbic acid per day for last 3 days by intraperitoneal injection (Fig. 1). A dose of reserpine produced an increase in the enzyme activity in normal adrenal gland, although no significant changes in the enzyme activity was observed by the administration of ascorbic acid. Therefore, no increase in the enzyme activity was observed by the administration of an excess amount of ascorbic acid to normal guinea pig. Although the amount of reserpine administered to guinea pig (0.1 mg/kg) was smaller than that described for rat in many other references (1.25 mg/kg), about 1.5 fold increase in the enzyme activity occurred 3 days after the reserpine administration.

Scorbutic guinea pigs were administered 0.1 mg of reserpine, or 200 mg of ascorbic acid or both reserpine and ascorbic acid per day for the last 3 days (Fig. 2). No significant elevation in the enzyme activity was found by the administration of reserpine to the scorbutic guinea pig. However, a dose of ascorbic acid produced about a 2-fold increase in the enzyme activity. When ascorbic acid and reserpine were given at the same time, a 3-fold increase in the enzyme activity was observed. These data indicated that no increase in the enzyme activity was observed by the administration of reserpine in scurvy. Therefore, ascorbic acid was considered to be a necessary factor for the induction of tyrosine hydroxylase.

Effect of ascorbic acid and reserpine on catecholamine levels in adrenal glands

Administration of reserpine, a drug which diminishes the activity of the central nervous system, augments the release of catecholamine in adrenal glands (18). If the sympathetic neurone activity affected by reserpine was injured in scurvy, the release of catecholamine would not be augmented by the administration of reserpine. Therefore, to make clear why reserpine did not increase tyrosine hydroxylase activity in scurvy, catecholamine levels in adrenal glands were determined after the administration of ascorbic acid (200 mg/day) to the scorbutic guinea pig (Fig. 3). Catecholamine levels in adrenal glands were decreased by the administration of reserpine in both the scorbutic and the non-scorbutic guinea pigs. As ascorbic acid did not affect the release of catecholamines in adrenal gland, it became clear that the sympathetic neurone system was not injured in scurvy.

As catechol compounds inhibits the activity of tyrosine hydroxylase, it was considered that catecholamines inhibits the enzyme activity to catalyze the rate-limiting step in the biosynthesis of catecholamines by end-product inhibition in vivo (14–17). As catecholamine levels in adrenal glands decreased in both the scorbutic and the non-scorbutic guinea pigs, no increase in the enzyme activity in scurvy was not caused by the difference of the end-product inhibition by catecholamines.

In the scurvy, the adrenal catecholamine levels decrease to about 60% of that of the ascorbic acid-supplemented group. Therefore, the increase in the enzyme
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Fig. 3. Effect of ascorbic acid and reserpine on catecholamine levels in adrenal glands of the scorbutic guinea pig. Catecholamine levels in adrenal glands were determined after administration of ascorbic acid (200 mg/day) or reserpine (0.1 mg/day) for the last 3 days by intraperitoneal injection to the scorbutic guinea pig. Catecholamine contents were determined as described in MATERIALS AND METHODS.

activity caused by ascorbic acid must be due to some other mechanism than reserpine.

Effect of $\alpha$, $\alpha'$-dipyridyl on tyrosine hydroxylase activity

Ascorbic acid plays an important role in the absorption and the transport of iron through the intestinal mucosa, the utilization of iron from storage organs such as liver and spleen, and the utilization of iron for hemoglobin synthesis. In the scorbutic guinea pigs, the iron levels in plasma were reduced to about 50% of the normal guinea pig (9).

Tyrosine hydroxylase is an Fe$^{2+}$ enzyme, and Fe$^{2+}$ participates in the hydroxylation reaction (19). Then, it was possible that ascorbic acid affected the enzyme synthesis through the Fe$^{2+}$.

Reserpine, administered to the scorbutic guinea pigs, decreased the catecholamine levels but did not increase tyrosine hydroxylase activity in adrenal gland. In order to clarify the reasons, the enzyme activity was assayed after the administration of $\alpha$, $\alpha'$-dipyridyl, a chelating agent of Fe$^{2+}$ (Fig. 4). After the administration of $\alpha$, $\alpha'$-dipyridyl (15 mg/day) and ascorbic acid (200 mg/day) to the scorbutic guinea pig by intraperitoneal injection for the last 3 days, tyrosine hydroxylase activity in adrenal gland was assayed. The enzyme activity was increased by the administration of ascorbic acid, however, no increase was observed by the simultaneous administration of ascorbic acid and $\alpha$, $\alpha'$-dipyridyl to the reaction mixture. The inhibition was not caused by the deficiency of Fe$^{2+}$ as a cofactor.

To determine whether chelating agents inhibit tyrosine hydroxylase activity in adrenal gland of normal guinea pig, $\alpha$, $\alpha'$-dipyridyl (15 mg/day) and reserpine (0.1 mg/day) were administered intraperitoneally for the last 3 days to the normal
Fig. 5. Effect of reserpine and α,α′-dipyridyl on tyrosine hydroxylase activity in adrenal glands of scorbutic guinea pig. Normal guinea pigs received reserpine (0.1 mg/day) or α,α′-dipyridyl (0.1 mg/day) for the last 3 days by intraperitoneal injection. Tyrosine hydroxylase activity was assayed as described in MATERIALS AND METHODS.

As shown in Fig. 5, the enzyme activity was increased by the administration of reserpine, but was decreased by α,α′-dipyridyl. In the reserpine and α,α′-dipyridyl-administered guinea pig, α,α′-dipyridyl blocked the increase of the enzyme activity by reserpine. As α,α′-dipyridyl, a chelating agent of Fe²⁺, prevented the induction of tyrosine hydroxylase by reserpine or ascorbic acid, it was concluded that Fe²⁺ was required for the induction of the enzyme. When the enzyme activity was assayed, an excess amount of Fe²⁺ was added to the reaction mixture far above the concentration of α, α′-dipyridyl in the tissue. Therefore, the decrease of the enzyme activity by α, α′-dipyridyl was not caused by the deficiency of Fe²⁺ in the reaction mixture.

Effect of Fe³⁺ on tyrosine hydroxylase activity

To determine whether the induction of tyrosine hydroxylase was responsible or not to the deficiency of Fe³⁺, the enzyme activity was determined after the administration of Fe³⁺ in the scorbutic guinea pig. The increase in the enzyme activity was observed after the administration of ferrous sulfate (20 mg/day) by intraperitoneal injection for 3 days to the scorbutic guinea pigs (Fig. 6). Therefore,
Fig. 6. Effect of Fe$^{2+}$ on tyrosine hydroxylase activity in adrenal glands of the scorbutic guinea pig. Tyrosine hydroxylase activity was determined after administration of ferrous sulfate (20 mg/day) for the last 3 days by intraperitoneal injection to the scorbutic guinea pigs. The enzyme activity was assayed as described in MATERIALS AND METHODS.

The results of our experiment demonstrated an increase in tyrosine hydroxylase activity in adrenal gland after the administration of ascorbic acid to the scorbutic guinea pigs. However, no rise in the enzyme activity was observed by administration of ascorbic acid to the non-scorbutic guinea pigs. In a previous paper, we have shown that the rise in the enzyme activity elicited by ascorbic acid can be blocked by treatment with protein synthesis inhibitors such as puromycin and actinomycin D. Immunochemical titration of the enzyme in both adrenal gland of the scorbutic and the non-scorbutic guinea pigs demonstrated that enhanced enzyme activity is entirely attributed to the accumulation of the specific enzyme protein (2).

On the other hand, recent studies have demonstrated that after the stimulation of the nerve to peripheral organs or the destruction of postganglionic sympathetic nerve endings by reserpine, not only the increase in the activity of tyrosine hydroxylase, but also the increase in the amount of the enzyme protein occurred (3–5). The reserpine induced increase of tyrosine hydroxylase activity can be blocked by cycloheximide and actinomycin D (21, 22). By immunochemical titration, Toh et al. demonstrated that the increase of the enzyme activity in adrenal gland elicited by reserpine resulted from accumulation of specific enzyme protein (4). However, as shown in this paper, chronic administration of reserpine to the scorbutic guinea pig did not increase the activity of tyrosine hydroxylase.
Two reasons were considered as the causes. 1) In the scurvy, the enzyme synthesis was inhibited by the deficiency of Fe$^{2+}$. 2) The enzyme synthesis was inhibited because sympathetic neuron activity was reduced by ascorbic acid deficiency.

It is well known that retention of adrenomedullary catecholamines was decreased by activation of the sympathetic nervous system after reserpine administration (18). As shown in this paper, catecholamine levels in adrenal gland was decreased after administration of reserpine in both the scorbutic and the non-scorbutic guinea pigs. Therefore, transmission of sympathetic nervous system appears not to be injured in the scurvy.

However, in spite of the decrease in adrenal catecholamine levels, the reserpine-induced increase in tyrosine hydroxylase activity was not observed in the scurvy. Therefore, it became clear that the ascorbic acid-induced increase in this enzyme activity was due to some other causes than the reserpine-induced increase in this enzyme.

NAGATSU et al. and TAYLOR et al. suggested that tyrosine hydroxylase required Fe$^{2+}$ and the enzyme activity was inhibited by the variety of chelating agents in vivo and in vitro (14, 20). In addition, adrenal catecholamine levels were markedly decreased after the administration of $\alpha$, $\alpha'$-dipyridyl (8). These observations further suggest metal ion participation in tyrosine hydroxylase.

This paper reports, the increase of tyrosine hydroxylase by the administration of ascorbic acid to scurvy or reserpine to normal guinea pigs inhibited by the administration of $\alpha$, $\alpha'$-dipyridyl. The fact revealed that Fe$^{2+}$ was necessary for the induction of tyrosine hydroxylase. From these results we conclude that the reserpine-induced increase in the enzyme was not observed in the scurvy on account of the deficiency of Fe$^{2+}$.

REFERENCES