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Monoamine oxidase activities in tissues of thiamine-deficient rats*

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The oxidation of monoamine by homogenates of tissues of thiamine-deficient rats was measured with noradrenaline as substrate. Monoamine oxidase activities were depressed in tissues in which the catecholamine content had previously been found raised during thiamine deficiency, with the exception in the cerebral cortex. The impaired enzyme activities were restored to the control level by repeated thiamine injections. An accumulation of pyruvic acid did not inhibit monoamine oxidase activity and thiamine pyrophosphate is not implicated as a co-factor in the oxidation of monoamine substrate.

We have previously reported (Iwata, Fujimoto & others, 1968) that the concentration of catecholamines in the cerebral cortex, in the atria and ventricles of the heart and in the spleen, are significantly increased in thiamine-deficient rats compared with the levels found in these organs of pair-fed control rats and of rats fed the thiamine-deficient diet supplemented with thiamine.

It is well known that catecholamine is mainly inactivated by monoamine oxidase and catechol-*O*-methyltransferase (COMT). It is possible that in the brain, as in the rest of the body, COMT is largely responsible for the degradation of extracellular catecholamine, while monoamine oxidase is responsible for the degradation of the amine existing intracellularly near the site of catecholamine synthesis and storage (Carlsson, 1960; Kopin & Gordon, 1962; Potter, Cooper & others, 1965).

That the raised levels of catecholamine in thiamine-deficient rats could be due to decrease in the activities of these enzymes was therefore considered, and so monoamine oxidase activities in the tissues of thiamine-deficient rats have been measured.

EXPERIMENTAL

Animals. Male Sprague-Dawley rats, 80 to 100 g were used. Thiamine deficiency was induced by feeding a synthetic thiamine-deficient diet *ad libitum*. Control animals were fed the same diet supplemented by 3 mg thiamine hydrochloride per kg of the basal diet. As previously (Iwata & others, 1968), only those animals possessing heart rates less than 70% of those of the control group were considered acutely deficient in thiamine and suitable for use.

Diet. The composition of the thiamine-deficient basal diet was (g): vitamin-free casein 20, soy bean oil 9, cod liver oil 1, McCullum's salt 4, sucrose 64·6, choline chloride 0·1, DL-methionine 0·3 and vitamin solution 1 ml.

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The vitamin solution contained (mg): riboflavin 40, pyridoxine HCl 30, cyanocobalamin 3, ascorbic acid 2000, menadione 50, nicotinic acid 400, calcium pantothenate 400, inositol 1000, *p*-aminobenzoic acid 1000, folic acid 20, biotin 4 and 50% ethanol to 100 ml.

Assay of monoamine oxidase. Tissue homogenates, buffered at pH 7·4 with 0·1M phosphate buffer, were incubated with substrate for 1 h at 37°. (±)-Noradrenaline (Nakarai Chemicals Ltd.) was used at 3×10^{-3}M. The production of ammonia from (±)-noradrenaline was measured by the method of Cotzias & Dole (1951) modified by Tokawa & Takahashi (1966).

Assay of catecholamine. Tissue catecholamine was extracted by the methods of Crout, Creveling & Udenfriend (1961), and measured fluorometrically as described by Euler & Floding (1955), using a Hitachi spectrofluorometer, model 203.

RESULTS

The effects of thiamine deficiency and administration of thiamine to the deficient animals on monoamine oxidase activity of rat tissue homogenates are summarized in Table 1. Noradrenaline oxidase activity was found to be higher in the liver than

Table 1. *Monoamine oxidase activities* (μmol/g h^{-1}) *by homogenates of the tissues of control and thiamine-deficient rats with noradrenaline as substrate*

Tissue	Control (5)	Thiamine deficient (5)	Thiamine deficient †Thiamine (4·0 mg/kg)	Thiamine deficient ‡Thiamine (1·0 mg/kg × 4)
Cerebral cortex	4·85 ± 0·61	4·80 ± 0·57	4·93 ± 0·81 (5)	4·80 ± 0·75 (5)
Brain stem	5·09 ± 0·59	4·98 ± 0·59	5·50 ± 0·73 (6)	5·37 ± 0·52 (6)
Heart atrium	5·32 ± 0·58	3·69 ± 0·52*	3·60 ± 0·55*(6)	4·99 ± 0·51**(7)
ventricle	4·09 ± 0·32	2·90 ± 0·33*	3·14 ± 0·44*(6)	4·30 ± 0·54**(6)
Spleen	4·68 ± 0·59	3·01 ± 0·51*	3·01 ± 0·59*(6)	4·66 ± 0·53**(5)
Liver	8·32 ± 1·01	8·25 ± 0·99	8·31 ± 0·92 (5)	8·72 ± 1·09 (5)

The values are mean ± s.e. Figure in brackets refer to number of experiments.
† Thiamine (4·0 mg/kg): In these experiments, 4·0 mg/kg thiamine was injected subcutaneously 5 h before the animals were killed.
‡ Thiamine (1·0 mg/kg × 4/2 days): 1·0 mg/kg thiamine was injected subcutaneously every 12 h for 2 days and 12 h after the last injection the animals were killed.
* Significant difference from control group ($P<0·05$).
** Significant difference from thiamine-deficient group ($P<0·05$).

in other tissues; the heart and brain appeared to contain one half to two thirds as much monoamine oxidase activity per g of tissue as the liver.

A significant reduction of noradrenaline metabolism was demonstrable in the atria and ventricles of the heart and in the spleen of thiamine-deficient animals.

The rates of oxidation of the amine by homogenates of the tissues of thiamine-deficient rats were unaffected 5 h after subcutaneous injection of 4·0 mg/kg thiamine hydrochloride. Correction of the deficiency required 4 injections each of thiamine, 1·0 mg/kg, at 12 h intervals. This increased the rate of oxidation of noradrenaline by these three organs to control levels.

Table 2 shows the effects of thiamine treatment on catecholamine content in tissues of thiamine-deficient rats. In these rats, catecholamine contents in the cerebral cortex, in the atria and ventricles of the heart and in the spleen were significantly

Table 2. *Catecholamine content* (μg/g) *in tissues of thiamine-deficient rats treated with thiamine*

Tissue	Control¶	Thiamine deficient	Thiamine deficient † thiamine (4·0 mg/kg)	Thiamine deficient ‡ thiamine (1·0 mg/kg)
Cerebral cortex	0·26 ± 0·04 (4)	0·52 ± 0·09*(5)	0·43 ± 0·05*(4)	0·38 ± 0·06**(5)
Brain stem	0·58 ± 0·09 (4)	0·63 ± 0·10 (5)	0·60 ± 0·07 (5)	0·60 ± 0·15 (5)
Heart: atrium	1·31 ± 0·14 (6)	2·60 ± 0·39*(5)	2·50 ± 0·31*(5)	2·02 ± 0·35**(4)
ventricle	0·34 ± 0·12 (3)	0·94 ± 0·12*(5)	0·81 ± 0·10*(5)	0·41 ± 0·06**(4)
Spleen	0·40 ± 0·03 (6)	1·95 ± 0·15*(4)	1·67 ± 0·12*(6)	0·86 ± 0·20**(5)
Adrenal gland	1063·0 ± 107·9 (6)	1107·7 ± 155·0 (5)	1110·3 ± 121·9 (6)	1079·7 ± 74·0 (5)

* Significant difference from control group ($P < 0.05$).
** Significant difference from thiamine-deficient group ($P < 0.05$).
Value: Mean ± s.e., Each group: 3–4 rats, Number of groups are in parentheses.
†‡ See footnotes to Table 1.
¶ The data are those previously reported (Iwata & others, 1968).

increased compared with those of control animals. Catecholamine concentration of tissues from thiamine-deficient rats 5 h after the subcutaneous injection of 4·0 mg/kg thiamine hydrochloride remained unaffected, but, as found with monoamine oxidase activity, thiamine, 1·0 mg/kg, injected 4 times at 12 h intervals, returned catecholamine levels to near control levels in all tissues in which amine concentration was raised by thiamine deficiency.

No significant effects of pyruvate were detected on the rates of oxidation of noradrenaline by homogenates of tissues taken from control rats 5 h after sodium pyruvate, 600 mg/kg, i.p., or 300 mg/kg injected twice daily for 30 days.

To find possible direct effects of pyruvic acid, thiamine and thiamine pyrophosphate on the rates of amine oxidation, experiments were made *in vitro* on control and thiamine-deficient cerebral cortex, brain stem, heart, spleen and liver using sodium pyruvate (1×10^{-4}M), thiamine (1×10^{-6}M) and thiamine pyrophosphate (1×10^{-6}M) added with substrate. But the oxidation rate was not influenced.

DISCUSSION

Gal & Drewes (1961) investigated monoamine oxidase activity in the tissues of thiamine-deficient rats. They found an increase in this activity in mitochondrial fractions isolated from the whole brain and the small intestine of thiamine-deficient animals, but no change in the monamine oxidase activity of liver, spleen and kidney, when it was assayed manometrically (Creasey, 1956) with 5-hydroxytryptamine as substrate. We find that, using tissue homogenate as the source of enzyme and noradrenaline as substrate, thiamine deficiency depresses monoamine oxidase activity in the cardiac atria and ventricles and in the spleen. The reason for this disagreement is not known but may be due to differences in substrate and method. It is likely that tissue homogenates contain enzymes responsible for alternative pathways for the metabolism of amines and therefore give a better picture of the overall effects of thiamine deficiency on amine metabolism. In addition, support for the hypothetical

existence of more than one system capable of metabolizing monoamines in some, at least, of our tissue homogenates has been given by the discrepancies found in the monoamine oxidase activity of the various tissues from normal animals using the different substrates (Iwata, Nishikawa & Fujimoto, to be published).

Overall, except the cerebral cortex, those tissues in which the catecholamine content had been previously found raised during thiamine deficiency (Iwata & others, 1968) are the tissues in which amine metabolism is now found depressed by lack of thiamine. In the accumulation of catecholamine in the tissues, especially in the cerebral cortex of thiamine-deficient animals, other factors also must be taken into consideration such as acceleration of synthesis, enhancement of uptake and inhibition of spontaneous release of the amine. Reduction of spontaneous release of catecholamines from their storage sites was seen and this could be another factor contributing to the accumulation of the amine (Iwata, Nishikawa & Watanabe, 1969).

Previous work has also shown elevation of pyruvic acid levels in the cerebral cortex, cardiac atria and ventricles and spleen during thiamine deficiency (Iwata & others, 1968). Five h after the subcutaneous injection of 4·0 mg/kg thiamine hydrochloride, the concentration of pyruvic acid in all these tissues, except the spleen, fell to normal (Iwata & others, 1968) but the catecholamine content and rates of metabolic destruction of noradrenaline (Table 1) in the tissues were not restored. Two days' treatment with a total of 4·0 mg/kg in 12 hourly doses were required to restore catecholamine levels (Table 2) and amine metabolism to normal. There is therefore no parallel between the changes in the levels of pyruvic acid in the tissues on the one hand and the change in catecholamine levels and amine metabolism, on the other, during recovery from thiamine deficiency. The present work demonstrates that an accumulation of pyruvic acid cannot itself inhibit the catecholamine metabolism and that thiamine pyrophosphate is not implicated as a co-factor in the oxidation of the monoamine.

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