Hypothyroidism of hypothalamic origin in pyridoxine-deficient rats

K. Dakshinamurti, C. S. Paulose and J. Vriend*

Department of Biochemistry and *Department of Anatomy, University of Manitoba, Winnipeg, Canada R3E 0W3

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ABSTRACT

Pyridoxine-deficient young rats (3 weeks old) had significantly reduced levels of pituitary TSH, serum thyroxine (T_4) and tri-iodothyronine (T_3) compared with pyridoxine-supplemented rats. The status of the pituitary-thyroid axis of normal, pyridoxine-supplemented and pyridoxine-deficient rats was evaluated by studying the binding parameters of [^{3}H](3-methyl-histidine^2)TRH in the pituitary of these rats. The effects of TRH and T_4 injections on pituitary TSH and serum TSH, T_4 and T_3 of these two groups were also compared. The maximal binding of TRH receptors in the pituitary of pyridoxine-deficient rats was significantly higher than that of pyridoxine-supplemented control and normal rats, but there was no change in the binding affinity. Treatment with TRH stimulated TSH synthesis and release. It also increased serum T_4 and T_3 in both pyridoxine-supplemented and pyridoxine-deficient rats. Treatment with T_4 decreased serum and pituitary TSH in both pyridoxine-supplemented and pyridoxine-deficient rats, compared with saline-treated rats. The increased pituitary TRH receptor content, response to TRH administration and the fact that regulation at the level of the pituitary is not affected in the pyridoxine-deficient rat indicates a hypothalamic origin for the hypothyroidism of the pyridoxine-deficient rat.

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INTRODUCTION

Reports of the monoamine modulation of thyroid hormone secretion through the hypothalamic pituitary pathway provided the rationale for studying thyroid function in the pyridoxine-deficient rat (DiRenzo, Quattrone, Schettini & Preziosi, 1978, 1979; Krulich, 1979; Chen & Ramirez, 1981; Dupont, Dussault, Rouleau *et al.* 1981; Morley, Brammer, Sharp *et al.* 1981; Smythe, Bradshaw, Cai & Symons, 1982). In the pyridoxine-deficient rat we have an animal model with a physiologically significant decrease in brain serotonin with no changes in the concentrations of dopamine and noradrenaline (Dakshinamurti, LeBlancq, Herchl & Havlicek, 1976; Dakshinamurti, 1982; Paulose & Dakshinamurti, 1985).

We have reported (Dakshinamurti, Paulose, Thliveris & Vriend, 1985) that serum thyroxine (T_4) and tri-iodothyronine (T_3) concentrations were significantly lower in the deficient rats than in pyridoxine-supplemented controls. No significant difference was detected between the two groups in the concentration of serum thyrotrophin (TSH). Highly significant decreases in the pituitary content of TSH and in the number of pituitary thyrotroph secretory granules were also found. We suggested that these observations would be consistent with a reduced hypothalamo-pituitary secretion in pyridoxine-deficient rats. In the present study the status of the pituitary-thyroid axis of pyridoxine-deficient rats was evaluated by examining the binding parameters of the ligand, [^{3}H](3-methyl-histidine^2)thyrotophin-releasing hormone ([^{3}H]MeTRH), to high-affinity TRH receptors in the pituitary, as well as the effects of TRH and T_4 injections on pituitary and serum TSH and serum T_4 and T_3 of pyridoxine-deficient rats.

MATERIALS AND METHODS

Materials

[^{3}H](3-methyl-histidine^2)thyrotrophin-releasing hormone was purchased from New England Nuclear,

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Boston, MA, U.S.A. Thyrotrophin-releasing hormone and pyridoxine hydrochloride were purchased from Sigma Chemical Co., St Louis, MO, U.S.A. Vitamin-free casein, anhydrous D-(+)-dextrose, Spector No. 446 salt mixture and choline chloride were purchased from ICN Nutritional Biochemicals, Cleveland, OH, U.S.A.

Animals

Sperm-positive female Sprague–Dawley rats were housed individually and fed a pyridoxine-supplemented diet containing 50 mg pyridoxine/kg deficient diet, for the period of gestation. At the time of delivery the mothers were divided into two groups. The number of pups left with the mother was manipulated soon after birth so that each mother in the deficient group (fed a pyridoxine-deficient diet) had eight pups. Each mother was left with 16 pups in the group fed the pyridoxine-supplemented diet. The percentage composition of the pyridoxine-deficient diet (Dakshinamurti & Stephens, 1969) was as follows: vitamin-free casein, 30·0; dextrose, 59·85; corn oil, 5·0; salt mix No. 446, 4·0; vitamin mix without pyridoxine, 1·0 and choline chloride, 0·15. All rats were allowed free access to their respective diets. However, because of the large number of pups in the pyridoxine-supplemented group, they were getting less milk and thus were subjected continually to a generalized malnutrition so that their body weights were close to those of the deficient pups. At the end of the second week, pyridoxine-supplemented and pyridoxine-deficient pups were each divided into three groups. One group received physiological saline, the second TRH (15 µg/100 g body wt per day i.p.) and the third T_4 (8 µg/100 g body wt per day i.p.) for a period of 1 week. The last injection was given 1 h before the rats were killed. Pups from all the groups were killed when 21 days old, between 16.00 and 18.00 h. Blood was collected after decapitation. Serum was used for assay of TSH, T_4 and T_3. Pituitary glands were sonicated in phosphate-buffered saline (pH 7·6), the homogenate was centrifuged at 2000 *g* for 20 min and the supernatant fraction used for TSH assay. Protein content in the pituitary homogenate was measured according to Lowry, Rosebrough, Farr & Randall (1951).

Assay of hormones

Pituitary and serum TSH were assayed using reagents and protocol provided by NIADDK, Bethesda, MD, U.S.A. The TSH values were expressed in terms of the RP-2 standard which is 176 times more potent than the NIADDK-rTSH-RP-1 previously supplied. Concentrations of T_4 and T_3 were determined using T_4 and T_3 solid-phase radioimmunoassay kits purchased from Becton-Dickinson & Co., Orangeburg, NY, U.S.A. Serum T_4 and T_3 concentrations were expressed in nmol/l. The data from different groups of animals were analysed statistically by analysis of variance followed by Duncan's multiple range test.

Assay of pituitary TRH receptor

Three groups of rats were used for the pituitary TRH receptor study. In addition to the pyridoxine-deficient and malnourished, but pyridoxine-supplemented, groups a normal group of rats (eight pups/mother; fed the pyridoxine-supplemented diet throughout the study) was included. All animals were killed at 21 days of age between 16.00 and 18.00 h. Ten pituitaries were pooled from the pyridoxine-deficient group and eight from the pyridoxine-supplemented and normal groups. Thyrotrophin-releasing hormone receptor binding was assayed using [^{3}H]MeTRH as ligand, according to Burt & Taylor (1983). The ligand used ([^{3}H]MeTRH, an analogue of TRH) is known to have a higher binding affinity than TRH and appears to bind to the same class(es) of TRH receptors in the pituitary as [^{3}H]TRH (Wei, Loh & Way, 1976; Taylor & Burt, 1981). Specific binding data were analysed according to Scatchard (1949), from which maximal binding (B_{max}) and the dissociation constant (K_d) were derived by linear regression analysis. The data were analysed statistically by analysis of variance and Student's unpaired *t*-test. Protein was measured according to Lowry *et al.* (1951).

RESULTS

Mean body weights (g) of rat pups in the experimental groups were as follows: normal, 53·8 ± 4·8 (S.D.); pyridoxine-supplemented, 30·9 ± 3·2 and pyridoxine-deficient, 23·3 ± 3·8. The body weights of TRH- and T_4-treated pyridoxine-supplemented and pyridoxine-deficient rats were not significantly different from those of non-treated groups. We have previously shown (Dakshinamurti *et al.* 1985) that there was no significant decrease in the pituitary and serum TSH as well as in serum T_4 and T_3 levels between malnourished pyridoxine-supplemented (controls) and normal rats. In the present study, therefore, we compared the pyridoxine-deficient rats with malnourished pyridoxine-supplemented rats. There was a significant ($P < 0·01$) decrease of hypothalamic serotonin content in pyridoxine-deficient rats: pyridoxine-supplemented, 1·82 ± 0·19 (S.E.M.) nmol/g; pyridoxine-deficient 1·03 ± 0·26 nmol/g (Dakshinamurti *et al.* 1985). Pituitary TSH content, serum T_4 and serum T_3 were significantly decreased in pyridoxine-deficient rats (Table 1).

TABLE 1. Effects of TRH and thyroxine (T_4) on pituitary TSH and serum TSH, T_4 and tri-iodothyronine (T_3) in pyridoxine-supplemented and pyridoxine-deficient 3-week-old rats. Values are means ± S.E.M.; numbers of experiments are shown in parentheses

Treatment	Pituitary TSH (µg/mg protein)	Pituitary TSH (µg/pituitary)	Serum TSH (µg/l)	Serum T_4 (nmol/l)	Serum T_3 (nmol/l)
Saline					
Pyridoxine-supplemented	6·83 ± 0·14 (11)	1·09 ± 0·04 (11)	1·74 ± 0·13 (9)	81·21 ± 1·92 (14)	1·51 ± 0·08 (14)
Pyridoxine-deficient	4·12 ± 0·15** (8)	0·81 ± 0·05** (8)	1·92 ± 0·23 (13)	52·00 ± 2·95* (16)	0·96 ± 0·05* (16)
TRH					
Pyridoxine-supplemented	4·55 ± 0·14†† (12)	0·74 ± 0·03†† (12)	5·54 ± 0·76†† (11)	104·29 ± 7·70† (7)	1·76 ± 0·08 (7)
Pyridoxine-deficient	5·88 ± 0·15**†† (10)	1·04 ± 0·05** (10)	5·82 ± 0·57†† (7)	86·29 ± 4·66*† (7)	2·37 ± 0·20*†† (7)
T_4					
Pyridoxine-supplemented	1·28 ± 0·08†† (7)	0·20 ± 0·02†† (7)	1·13 ± 0·05† (7)	901 ± 29†† (7)	24·86 ± 1·22†† (7)
Pyridoxine-deficient	3·76 ± 0·34** (5)	0·62 ± 0·05**† (5)	1·23 ± 0·13† (6)	1330 ± 104**†† (5)	21·60 ± 1·03**†† (5)

*$P<0·05$, **$P<0·01$ compared with pyridoxine-supplemented group.
†$P<0·05$, ††$P<0·01$ compared with saline-treated group (Duncan's multiple range test).

TABLE 2. [^{3}H](3-methyl-histidine^2)TRH ([^{3}H]MeTRH) binding in the pituitary of 3-week-old rats. Values are means ± S.E.M. of eight separate determinations in each group

Animal status	[^{3}H]MeTRH binding B_{max} (fmol/mg protein)	K_d (nmol/l)
Normal	141·40 ± 3·34	1·78 ± 0·25
Pyridoxine-supplemented (control)	144·38 ± 6·15	2·22 ± 0·49
Pyridoxine-deficient	181·20 ± 7·39*	2·38 ± 0·31

*$P<0·005$ compared with control. (Student's unpaired *t*-test).
B_{max}, maximal binding; K_d, dissociation constant.

Effects of TRH injections

Thyrotrophin-releasing hormone treatment significantly increased serum TSH ($P<0·01$) and T_4 ($P<0·05$) in both pyridoxine-supplemented and pyridoxine-deficient rats, compared with saline-treated animals (Table 1). A significant increase in serum T_3 after TRH treatment was observed in pyridoxine-deficient rats but not in pyridoxine-supplemented rats. Thyrotrophin-releasing hormone treatment significantly ($P<0·01$) decreased the pituitary TSH content in pyridoxine-supplemented rats whereas in pyridoxine-deficient rats a significant ($P<0·01$) increase in pituitary TSH content was observed compared with saline-treated animals.

Effects of T_4 injections

This experiment was designed to examine the integrity of the feedback regulation of TSH secretion by high levels of circulating T_4. Hence a non-physiological dose of T_4 was injected which resulted in over a ten-fold increase of serum T_4 and T_3 in both the pyridoxine-deficient and pyridoxine-supplemented rats. Thyroxine treatment significantly ($P<0·05$) decreased serum TSH in both pyridoxine-supplemented and pyridoxine-deficient rats compared with the saline-injected groups. Pituitary TSH was also reduced by T_4 injections. The decrease in pituitary TSH content of T_4-treated rats was significantly ($P<0·01$) less in pyridoxine-deficient rats than in pyridoxine-supplemented rats (Table 1). The reduction in pituitary TSH content after T_4 injections was significantly greater in pyridoxine-supplemented rats (82% reduction) than in pyridoxine-deficient rats (24% reduction). Similar results were also obtained when the data were expressed as TSH/mg protein (Table 1).

Effect of pyridoxine-deficiency on TRH receptors

Scatchard analysis of [^{3}H]MeTRH binding to membrane preparations from pituitary glands of pyridoxine-deficient and pyridoxine-supplemented control rats is given in Fig. 1. There was no significant difference between the normal and malnourished pyridoxine-supplemented control group of rats for either of the binding parameters studied (Table 2). There was, however, an increase in TRH receptor content ($P<0.005$), with no significant change in the binding affinity, in the pyridoxine-deficient group.

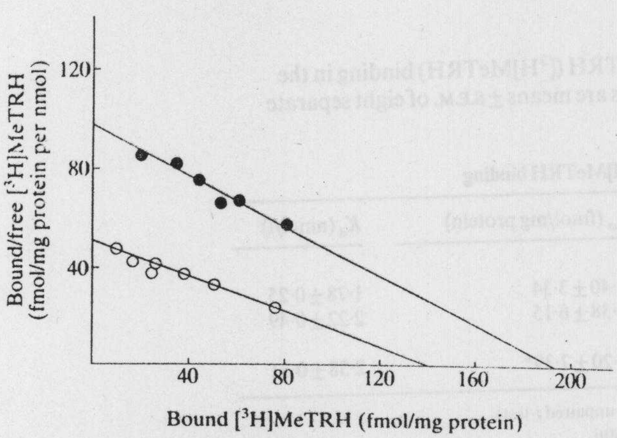

FIGURE 1. Scatchard analysis of [^{3}H](3-methyl-histidine^2)-TRH ([^{3}H]MeTRH) binding in crude membrane preparations from pituitary glands of pyridoxine-supplemented (○) and pyridoxine-deficient (●) 3-week-old rats. The pituitary was sonicated in 50 volumes of sodium phosphate buffer (20 mmol/l; pH 7·4) and centrifuged at 48 000 *g* for 15 min. The pellet was resuspended and the procedure repeated twice. The final pellet was resuspended in sodium phosphate buffer (pH 7·4), and 0·05–0·08 mg protein was used in each assay. The incubation mixture containing [^{3}H]MeTRH (0·2–5 nmol/l) with and without excess unlabelled TRH (10 μmol/l) was incubated for 1 h on ice. The contents of the incubation tubes were rapidly filtered under partial vacuum through GF/B filters and washed three times with 5 ml ice-cold sodium phosphate buffer (20 mmol/l; pH 7·4). The [^{3}H]MeTRH bound to the membranes in the filter was determined by liquid scintillation spectrometry. Specific binding was determined by subtracting non-specific from total binding.

DISCUSSION

Pyridoxine-deficiency in the rat has been shown to result in decreased levels of hypothalamic serotonin, with no concomitant decreases in levels of brain norepinephrine or dopamine (Dakshinamurti *et al.* 1985). The pyridoxine-deficient rat has been used as an animal model to examine the effects of decreased brain serotonin on central regulation of thyroid hormone secretion.

Although significantly reduced levels of serum T_4 and T_3 have been found in the pyridoxine-deficient rat (Dakshinamurti *et al.* 1985), the present results show that the pituitary thyrotroph response to TRH was not impaired (Table 1). These results indicate that the readily releasable pool of TSH in the pituitary of pyridoxine-supplemented and pyridoxine-deficient rats was not different. The pituitary TSH content of pyridoxine-deficient rats injected with saline was significantly reduced compared with similar pyridoxine-supplemented rats (Table 1). On stimulation with TRH, however, the pituitary content of TSH decreased significantly in pyridoxine-supplemented rats, but increased significantly in pyridoxine-deficient rats. These results should be interpreted in the context of an increased pituitary TRH receptor content in pyridoxine-deficient rats (Table 2). An increased sensitivity of hypothalamic hypothyroid rats to TRH has been shown by Aizawa, Kobayashi, Komiya *et al.* (1984).

In experiments with rats bearing hypothalamic lesions the TSH response to acute TRH may be either unchanged or significantly increased (Fukuda & Greer, 1977; Aizawa & Greer, 1981; Aizawa *et al.* 1984). In the present study, chronic TRH administration (7 days of injections) resulted in a serum TSH response in deficient rats that was not significantly different from the response of pyridoxine-supplemented rats. Pituitary TSH contents of pyridoxine-deficient rats treated with TRH were greater than those of TRH-treated pyridoxine-supplemented rats and saline-treated pyridoxine-deficient rats. Pituitary TSH content is determined by a balance between its synthesis and release, both of which are modulated by TRH and a number of other factors. Serum levels, as well as pituitary concentrations, of TSH are responsive to exogenously administered T_4 in both pyridoxine-deficient and pyridoxine-supplemented control rats. Thus the secretory response to administered TRH or T_4 by the pituitary of pyridoxine-deficient rats seems to be intact and comparable to that of control rats. It is possible that the increase in the number of TRH receptors in the deficient pituitary elicits a greater hormone-synthetic response to the administered TRH. The results presented here are interpreted as being consistent with a hypothalamic type of hypothyroidism in pyridoxine-deficient rats. Primary hypothyroidism was ruled out by the lack of increased serum TSH; secondary hypothyroidism was ruled out by the normal pituitary response to TRH. Since pyridoxine deficiency results in low serotonin levels in the hypothalamus, the present study provides evidence for the role of serotonin in the synthesis and release of TSH from the pituitary. Presumably this effect of serotonin occurs through TRH secretion.

In the normally growing rat the thyroid becomes fully developed during weaning (Dussault & Labrie, 1975). The highest serum concentration of thyroid hormones occurs during weeks 3 and 4 of life and subsequently decreases to adult levels. A higher TSH response to TRH has been found in young rats during the early developmental period than in adult rats, with a progressive decrease as the animals age (Strbak & Greer, 1981). The present results suggest that during this developmental period the neuroendocrine-thyroid axis is stimulated by serotonergic neurones.

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