ELSEVIER

European Journal of Pharmacology 317 (1996) 263–267

ejp

Short communication

Vesicular dysfunction during experimental thiamine deficiency is indicated by alterations in dopamine metabolism

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Received 18 July 1996; revised 17 October 1996; accepted 22 October 1996

Abstract

Experimental and clinical studies indicate that catecholamines play an important role in the neurobehavioural symptomatology of thiamine deficiency. Given the cerebral region-selective vulnerability and the behavioural impairment commonly encountered in thiamine deficiency, we undertook to investigate regional catecholamine metabolism in the brains of pyrithiamine-induced thiamine-deficient rats. Dopamine metabolism was unaffected in the striatum. In contrast, other regions also known to be involved in sensory processing and intellectual function (e.g., frontal cortex, hypothalamus, thalamus), but having a greater noradrenergic input, had increased levels of 3,4-dihydroxyphenylacetic acid (DOPAC) and decreased levels of other dopaminergic metabolites including noradrenaline. In these regions levels of the vesicular amine transporter, defined by tetrabenazine-sensitive [^{3}H]ketanserin binding, were also decreased. Our data suggest a region-selective vesicular dysfunction resulting in intraneuronal release, and subsequent degradation, of dopamine. These disruptions of dopamine and consequently noradrenaline metabolism may account for certain neurobehavioural deficits commonly encountered in thiamine deficiency.

Keywords: 3-Methoxytyramine; Monoamine oxidase; Wernicke-Korsakoff syndrome

1. Introduction

The roles of thiamine esters in enzymatic reactions related to carbohydrate metabolism and in membrane function have long been recognized. However, the exact pathogenesis of thiamine deficiency-induced brain dysfunction remains to be established. A variety of neurotransmitters which are believed to be implicated in the symptomatology of thiamine deficiency include the biogenic amines serotonin (5-hydroxytryptamine; 5-HT), dopamine and noradrenaline. Alterations in 5-HT receptor densities and 5-HT concentrations which either coincide or precede certain of the neurological symptoms such as anorexia and hypothermia commonly encountered in thiamine deficiency have recently been demonstrated (Mousseau et al., 1996a). Catecholamine dysfunction has been put forward as an important factor in the bradycardia, ataxia and sedation seen in experimental thiamine deficiency (Iwata et al., 1970), in addition to which altered catecholamine levels and/or turnover in specific brain nuclei of thiamine-deficient rats may account for the observed problems in task performance (Langlais et al., 1987, 1988).

Pyrithiamine-induced thiamine deficiency is routinely used as a model for the Wernicke-Korsakoff syndrome, a neuropsychiatric disorder associated with chronic alcoholism and thiamine deficiency. Korsakoff patients present reduced cerebrospinal fluid levels of the noradrenergic metabolite 3-methoxy-4-hydroxyphenyl glycol (McEntee and Mair, 1978) which are correlated with cognitive and memory impairments (Mair et al., 1985). Although debate surrounds the exact role of the noradrenergic system in the intellectual dysfunction associated with Korsakoff's syndrome (see Kopelman, 1995), improved test performances by Korsakoff patients following administration of the α-adrenoceptor agonist clonidine (McEntee and Mair, 1980; Mair and McEntee, 1986) and the adrenergic agonist methylphenidate (O'Donnell et al., 1986) have been observed.

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As part of a series of studies on the role of amine metabolism in experimental thiamine deficiency, we investigated the regional brain concentrations of noradrenaline as well as dopamine and its acid metabolites following treatment with the thiamine antimetabolite pyrithiamine. The effect of thiamine deficiency on the vesicular amine transporter was also investigated.

2. Materials and methods

2.1. Materials

All biogenic amine standards and buffer constituents were purchased from Sigma (St. Louis, MO, USA). [^{3}H]Ketanserin hydrochloride [*ethylene*-^{3}H] (spec. act.: 61.9 Ci/mmol) was purchased from New England Nuclear Research Products (Mississauga, Ontario, Canada). Tetrabenazine was generously donated by Dr. P.D. Hrdina (University of Ottawa, Ottawa, Ontario, Canada). The organic solvents (HPLC grade) were obtained from commercial sources.

2.2. Animals and treatment groups

Male Sprague-Dawley rats (approximately 225 g initial weight) were randomly assigned to treatment groups: group 1 (pyrithiamine-treated) received a thiamine-deficient diet and pyrithiamine (i.p., 500 μg/kg per day) for 7 days (the presymptomatic rats; $n = 6$) at which time neurologic abnormalities were still absent. The symptomatic rats ($n = 6$) were allowed to progress until neurologic symptoms (i.e., loss of righting reflex) appeared; group 2 (pair-fed control) rats ($n = 12$) received the same diet paired to equal the consumption of pyrithiamine-treated rats but were supplemented with daily injections of thiamine (i.p., 100 μg/kg). Pair-feeding schedules were adjusted daily and these rats were killed at time points corresponding to rats in the pyrithiamine-treated groups.

2.3. Determination of regional catecholamine levels

The rats were decapitated and the brains were quickly removed and separated mid-sagitally. One half was immersed in ice-cold saline and dissected on ice into frontal cortex, thalamus, hypothalamus and striatum, whereas the other half, to be used for the quantitative autoradiography study, was immersed in isopentane cooled on dry ice. All tissue was frozen at −80°C until time of assay. Regional brain levels of putative catecholamine neurotransmitters (dopamine, noradrenaline) and selected acid metabolites (DOPAC, homovanillic acid, 3-methoxytyramine) were determined using high-pressure liquid chromatography as described previously (Mousseau et al., 1996a).

2.4. Quantitative autoradiography of tetrabenazine-sensitive [^{3}H]ketanserin binding

A series of 20 μm sections, thaw-mounted onto gelatin-coated glass slides, were collected using a cryomicrotome (IEC, Needham Heights, MA, USA; −20°C). the method of Leysen et al. (1987) based on tetrabenazine-sensitive [^{3}H]ketanserin binding was used to study the effect of thiamine deficiency on the vesicular amine transporter. Following a 15 min wash, tissue sections were incubated in 50 mM Tris buffer (pH 7.7, 40 min, 25°C) containing 3.0 nM [^{3}H]ketanserin and 1 μM methysergide. Nonspecific binding was determined by the addition of 2 μM tetrabenazine. The slides were washed twice in buffer for 10 min at 4°C, then rapidly air-dried. Autoradiograms were

Table 1
Concentrations of catecholamines and selected acid metabolites in brain regions of thiamine-deficient rats

Region		DA	DOPAC	3-MT	HVA	NA
Frontal cortex	Control	100.1 ± 15.9	70.6 ± 12.6	—	129.4 ± 11.9	517.3 ± 49.8
	Presymp	67.2 ± 9.6	143.7 ± 15.2 [b]	—	110.1 ± 11.4	470.6 ± 67.0
	Symp	76.7 ± 13.2	71.1 ± 6.9 [d]	—	136.5 ± 9.4	520.9 ± 43.8
Hypothalamus	Control	191.6 ± 15.5	59.0 ± 4.7	21.8 ± 2.6	121.8 ± 8.6	3228.1 ± 151.9
	Presymp	190.1 ± 39.0	92.4 ± 11.3 [a]	9.1 ± 1.6 [a]	151.6 ± 28.8	2613.1 ± 392.8
	Symp	213.9 ± 12.4	109.2 ± 8.4 [c]	15.4 ± 2.3	169.3 ± 8.4	2398.3 ± 158.3 [a]
Thalamus	Control	16.3 ± 7.2	52.9 ± 10.5	—	772.5 ± 89.9	1549.6 ± 159.3
	Presymp	44.7 ± 14.3	84.6 ± 6.5	—	807.8 ± 100.6	1936.3 ± 121.1
	Symp	121.6 ± 21.8 [c]	247.3 ± 46.9 [c]	—	142.0 ± 20.1 [c]	952.0 ± 87.27 [a,d]
Striatum	Control	14 322.8 ± 469.3	4987.4 ± 471.3	545.6 ± 40.4	2130.7 ± 152.8	334.8 ± 23.7
	Presymp	14 916.3 ± 585.1	4024.6 ± 396.5	481.4 ± 59.5	1709.5 ± 308.1	376.5 ± 70.1
	Symp	15 846.3 ± 492.4	4901.3 ± 534.1	283.8 ± 63.4 [a]	2315.0 ± 381.5	384.5 ± 57.8

DA, dopamine; DOPAC, 3,4-dihydroxyphenylacetic acid; 3-MT, 3-methoxytyramine; HVA, homovanillic acid; NA, noradrenaline; Control, pair-fed controls; Presymp, presymptomatic rats; Symp, symptomatic rats; —, below the level of detection; [a] $P < 0.05$; [b] $P < 0.01$; [c] $P < 0.001$, data (mean ± S.E.M.; ng/g wet weight tissue) significantly different from Control and [d] $P < 0.01$, significantly different from Presymp.

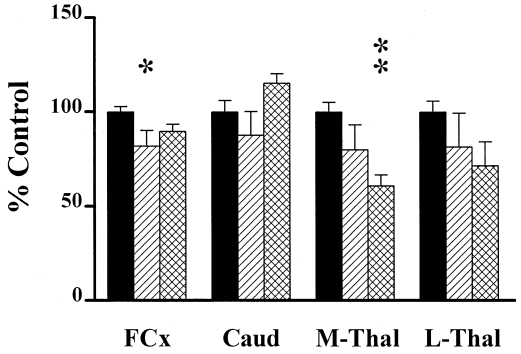

Fig. 1. Effect of pyrithiamine-induced thiamine deficiency on regional levels of the vesicular amine transporter defined by tetrabenazine-sensitive [^{3}H]ketanserin binding. Black columns: pair-fed controls ($n = 7–8$); hatched columns: presymptomatic rats ($n = 4–5$); cross-hatched columns: symptomatic rats ($n = 4–5$); FCx: frontal cortex; Caud: caudate; M-Thal: medial thalamic nuclei; L-Thal: lateral thalamic nuclei. Values represent mean ($\pm$S.E.M.) percent of control relative optical densities. Quantitative autoradiography was performed using 20 μm-thick brain slices. Binding was determined in the presence of 3 nM [^{3}H]ketanserin and 1 μM methysergide. Non-specific binding was defined by 2 μM tetrabenazine.

prepared and analyzed as described previously (Mousseau et al., 1996b).

2.5. Statistics

Effect of treatment was determined by analysis of variance followed by multiple comparisons of the means using the post-hoc Tukey test. $P < 0.05$ was defined as the criterion for statistical significance.

3. Results

The effects of thiamine deficiency on catecholamine metabolism varied between regions (Table 1). Concentrations of dopamine were increased in the thalamus, whereas those of noradrenaline were decreased in the thalamus and hypothalamus. Depending on the region studied, levels of DOPAC either remained unchanged or increased, whereas levels of homovanillic acid and 3-methoxytyramine either remained unchanged or decreased in comparison to pair-fed control levels. Tetrabenazine-sensitive [^{3}H]ketanserin binding was significantly decreased in the frontal cortex of presymptomatic rats and in medial (e.g., anteromedial, centromedial, mediodorsal, gelatinosum) thalamic nuclei of symptomatic rats (Fig. 1). Lateral (e.g., anteroventral, centrolateral, ventrolateral) thalamic nuclei displayed marginal decreases in binding site densities in symptomatic animals whereas binding was unaffected in the caudate putamen.

4. Discussion

Dopamine metabolism is now well understood. Intracellular (mitochondrial) monoamine oxidase (MAO) and/or extracellular (synaptic) catechol-*O*-methyltransferase (COMT) are the two primary catabolic enzymes in dopamine metabolism. Dopamine is oxidized by MAO to DOPAC which, in turn, is metabolized by COMT to homovanillic acid. Alternatively, dopamine can be methylated by COMT to 3-methoxytyramine which is then metabolized to homovanillic acid by MAO following uptake into the nerve terminal. In adrenergic neurons a third metabolic route involves β-hydroxylation of dopamine to noradrenaline within the storage vesicle. The seemingly redundant nature (i.e., multiple degradative pathways) of dopamine metabolism enables a compensatory degradation in the event that one pathway is affected and also allows for a more accurate interpretation of events that transpire at the nerve terminal, within the terminal itself as well as within the synapse. We now report regionally selective alterations in dopamine metabolism in pyrithiamine-treated rat brains.

Although the changes in acid metabolite levels can be interpreted initially to suggest altered catabolic enzyme activity, brain MAO activity determined as either total MAO activity (Iwata et al., 1969) or as specific regional MAO-A and -B activities (Mousseau et al., 1996a) is unaltered during thiamine deficiency. Furthermore COMT activity, at least in the periphery, also remains unchanged (Iwata et al., 1970). Local cerebral blood flow is increased during experimental thiamine deficiency (Vogel and Hakim, 1988) and while this could explain the decreased levels of homovanillic acid and 3-methoxytyramine it does not account for the accumulation of DOPAC.

Regions representing important noradrenergic terminal fields exhibited decreased concentrations of noradrenaline and concurrent increases in levels of DOPAC (cf., the striatum where generalized changes were not observed). These changes coincided with a decrease in tetrabenazine-sensitive [^{3}H]ketanserin binding site densities which suggests a local effect on the vesicular amine transport and, by extension, on vesicular function. These data strongly suggest that dopamine is being released from the vesicle, before it can be hydroxylated to noradrenaline, directly into the cytoplasm where it is readily degraded to DOPAC by MAO. Furthermore, the levels of the COMT-mediated metabolite 3-methoxytyramine, the predominant metabolite of released dopamine (Karoum et al., 1994), were not increased to detectable levels in these regions and were actually decreased in the hypothalamus thus suggesting that dopamine release from the nerve terminal is also apparently impeded in our model of thiamine deficiency. The present data indicate a potential disruption of vesicular and cellular membrane function in specific brain regions during the course of thiamine deficiency and further underscore the importance of thiamine esters in membrane function (for further discussion, see Mousseau et al., 1996a,b).

The observations of Iwata et al. (1969) also appear to indicate vesicular disruption in experimental thiamine defi-

ciency: (i) Treatment of thiamine-deficient rats with reserpine, which disrupts vesicles containing monoamines, did not induce any differences in the animals' behavioural responses when compared to untreated thiamine-deficient rats, although the expected reserpine-mediated sedation was observed in the control rats. (ii) Treatment with amphetamine, which promotes cytosolic rather than vesicular (i.e., exocytotic) monoamine release, induced a more marked locomotor activity as well as more stereotypic behaviours in thiamine-deficient rats than in amphetamine-treated control rats. These observations could be explained respectively by (i) fewer intact vesicles targeted for disruption and (ii) an increase in cytosolic dopamine in the thiamine-deficient rats. Unfortunately, only total tissue catecholamine content was measured (Iwata et al., 1969) thereby limiting the interpretation of the role of specific catecholamines in the observed behaviours. Unrelated studies have demonstrated that beyond its generalized effect on biogenic amine storage reserpine also induces an increase in the activity and expression of tyrosine hydroxylase, the rate-limiting enzyme in catecholamine synthesis, with this increase being regionally specific and limited to areas innervated by noradrenergic neurons (see Pasinetti et al., 1990). If one assumes this to be a compensatory response to vesicular dysfunction, then the present increase in thalamic dopamine levels coinciding with a reduction in the density of the vesicular amine transporter further implies thiamine deficiency-induced vesicular disruption.

It is interesting that all of the measurable amines and metabolites as well as the density of the vesicular amine transporter were affected in the thalamus, a structure extremely vulnerable during the normal course of thiamine deficiency. Although the pattern of changes observed during the course of these studies does imply altered dopamine and consequently noradrenaline homeostasis as a direct consequence of vesicular disruption, the possibility that these changes are strictly secondary to ongoing degenerative processes cannot be discounted. Our findings, however, may have significant bearing on the clinical reports of decreased cerebrospinal fluid levels of noradrenergic metabolites as well as the reports of beneficial properties of adrenergic agonists in Korsakoff patients. Based on the positive effects of amphetamine and other catecholaminergic enhancing compounds in the behavioural recovery following cortical injury (for further discussion, see Krobert et al., 1994) and in models of neuroprotection (see Yu et al., 1994 and references therein), we suggest that the region-selective alterations in catecholamine availability during the course of thiamine deficiency may be an integral determinant for the accompanying behavioural impairment (Langlais et al., 1988; Mair et al., 1988) and the region-selective development of histological lesions (Zhang et al., 1995).

Acknowledgements

The studies described were funded by a grant from The Medical Research Council of Canada (MA 9156). D.D.M. and V.L.R.R. are MRC Fellows.

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