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KINETICS OF THIAMINE TRANSPORT ACROSS THE BLOOD–BRAIN BARRIER IN THE RAT

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SUMMARY

1. By measurement of the rate of disappearance of injected tracer thiamine from the bloodstream, a programme for the continuous injection of thiamine at a variable rate has been devized by which a steady raised level can be achieved rapidly and maintained in the circulation. By this means the flux of radioactive thiamine across the blood–brain barrier has been measured.

2. In separate experiments progressively higher levels of thiamine were maintained in the bloodstream. Evidence was obtained that the transport of thiamine across the blood–brain barrier is a carrier-mediated process which can be saturated by raised levels of thiamine.

3. The saturation of the transport process was incomplete: kinetic analysis showed that there was a non-saturable component of the transport which was probably due to passive diffusion.

4. The contribution of the non-saturable component was normally small and is probably insufficient to meet the needs of the brain for the vitamin unless the concentration of the vitamin in the blood is raised considerably above normal.

5. This two-component transport process had substantially similar kinetic parameters in different regions of the brain.

INTRODUCTION

It has long been known that thiamine does not freely cross the blood–brain barrier (Asayama, Sakane & Yamamoto, 1954). Uptake of thiamine by carrier-mediated systems has been shown to occur in various tissues, including the intestinal wall (Rindi & Ventura, 1972) hepatocytes (Lumeng, Edmondson, Schenker & Li, 1979) and brain slices (Sharma & Quastel, 1965). More recently saturation effects have been observed during the re-establishment of equilibrium across the blood–brain barrier after I.V. injections of thiamine (Spector, 1976). Thiamine is of considerable importance for the functioning of the nervous tissue (Peters, 1936; Dakshinamurti, 1977) and there is a need to understand better how this water-soluble vitamin crosses the blood–brain barrier. In the present work a kinetic study has been made *in vivo* of the influx, that is of the unidirectional movement of thiamine into the brain. A preliminary account of this work has been given (Greenwood, Love & Pratt, 1980).

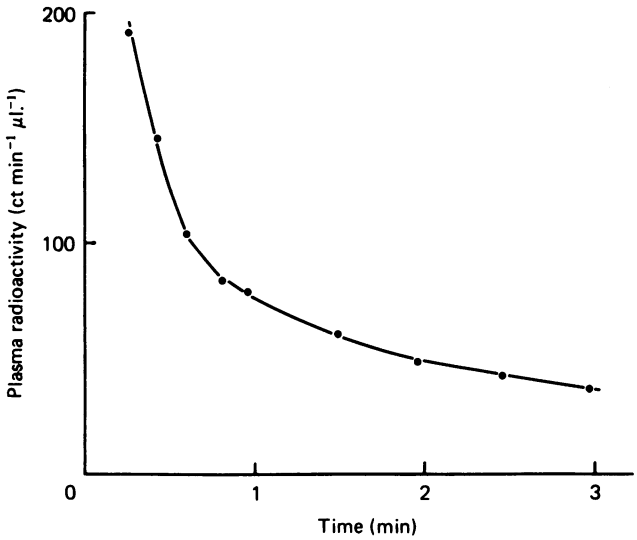

Fig. 1. The rate at which [^{14}C]thiamine disappeared from the circulation of a rat after a single rapid I.V. injection. A curve has been fitted to the points according to the method of Daniel *et al.* (1975).

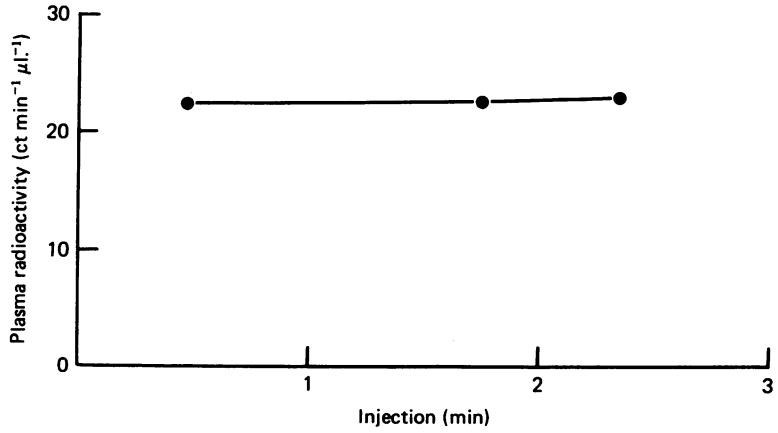

Fig. 2. Example of a steady level of [^{14}C)thiamine maintained in the blood plasma of a rat by a specially programmed I.V. injection.

METHODS

Animals

Young adult Wistar albino rats of either sex and weighing 150–400 g were · 1. The animals were given water and food *ad libitum*, being fed on a commercial breeding diet containing 21·5% protein and 45% carbohydrates and with a thiamine content of 18·5 mg kg^{-1}. The animals were anaesthetized with sodium pentobarbitone B.P.C. (35 mg kg^{-1} body wt.) given into the peritoneum. A catheter was inserted into a femoral artery for withdrawing blood samples and, when necessary, another catheter into a femoral vein so that various I.V. injections of [^{14}C]thiamine could be given.

Preliminary experiments

A rapid injection was given I.V. of 2 kBq of [^{14}C]thiamine in 50 μl. saline. The rate of disappearance of the tracer from the bloodstream was determined by repeated small blood sampling. From these results (Fig. 1) a programme was worked out to replace the thiamine as fast as it left the circulation by a continuous injection at a rate controlled by the programme (Daniel, Donaldson & Pratt, 1975).

Maintenance of steady blood plasma levels of [^{14}C]thiamine

The programmed injection was given by means of an electronically controlled stepper-motor-driven syringe (Pratt, 1974). During the injection period three small blood samples were taken to check that a steady level was maintained over the experimental period, ranging from 0·5 to 15 min (Fig. 2).

Brain uptake of [^{14}C]thiamine

In a series of experiments the time, during which a steady level of [^{14}C]thiamine was maintained in the circulation, was increased progressively from under 1 min up to 15 min. At the end of the injection period the jugular veins were cut and the blood was rapidly washed out of the vascular system with physiological saline at 37 °C by means of a high pressure reservoir (Daniel, Love, Moorhouse, Pratt & Wilson, 1974).

At the end of this procedure (20 sec) the animals were killed by decapitation and the brain was rapidly excised, washed in isotonic saline, blotted and frozen in hexane cooled to −70 °C. The cerebrum, cerebellum and brain stem (medulla, pons and mid-brain) were taken separately and the heparinized blood samples were centrifuged and the plasma separated.

Measurement of influx

The influx of thiamine across the blood–brain barrier was measured by the initial rate at which the radioactive tracer entered the brain from the blood. A steady level of [^{14}C]thiamine was maintained in the circulation for a time (usually about 1 min). At the end of the experiment the level of radioactivity in the cerebral tissue was measured, as well as the mean level of radioactivity in the blood plasma sampled during the experiment. The mean thiamine concentration in the blood plasma during the experiment was calculated by adding to the mean normal level of the vitamin in the blood plasma (0·268 μmole l.$^{-1}$, Rindi, De Giuseppe & Sciorelli, 1968; Baker & Frank, 1969), the increase in its concentration due to the programmed injection. The latter value was obtained by dividing the mean level of radioactivity in the blood plasma by the specific activity of the injected solution. In a few blood plasma samples this calculation was confirmed by fluorimetric determination of the free thiamine (Patrini & Rindi, 1980). This value, together with the initial rate at which the radioactively labelled thiamine was entering the brain from the blood, was used to calculate the influx.

Effect of thiamine concentration

The [^{14}C]thiamine was available with a high specific activity (0·899 MBq μmole^{-1}) so that adequate labelling of thiamine could be obtained without raising the blood concentration much above the normal value which in the rat is about 0·3 μmole l^{-1} (Baker & Frank, 1969; Rindi *et al.* 1968). In some experiments the specific activity of the thiamine was reduced by adding a measured proportion of unlabelled thiamine. The quantity injected was thus increased so as to raise the blood level of the vitamin to progressively higher values above normal.

Materials

Radioactively labelled [2-^{14}C]thiazole thiamine hydrochloride was obtained from the Radiochemical Centre, Amersham and thiamine hydrochloride from Sigma Chemical Company, London. All other chemicals were analytical grade whenever available.

Assay of radioactivity

Samples of brain were brought into solution for radioactive counting as follows: a weighed aliquot of the sample (approximately 0·1–0·2 g) was placed in a glass scintillation counting vial to which was added a solution of 1 ml. of an organic base (Soluene 350, Packard Instruments). When the tissue was dissolved (usually after 1–2 days) glacial acetic acid (0·3 ml.) was added to neutralize the

solution and 15 ml. of a scintillation mixture containing 5 g 2,5-diphenyloxazole and 0·3 g 1,4-bis (2-(4-methyl-5-phenyloxazolyl) benzene l.$^{-1}$ of toluene were added. Samples of plasma were prepared in a similar way except that only 0·5 ml. of Soluene 350 was used to dissolve 50 μl. plasma and only 0·15 ml. glacial acetic acid was needed to neutralize the solution before adding the scintillation mixture. The radioactivity in each sample was measured in a liquid scintillation spectrophotometer (Model 2409, Packard Instruments), being corrected for quenching when necessary by the channels-ratio method.

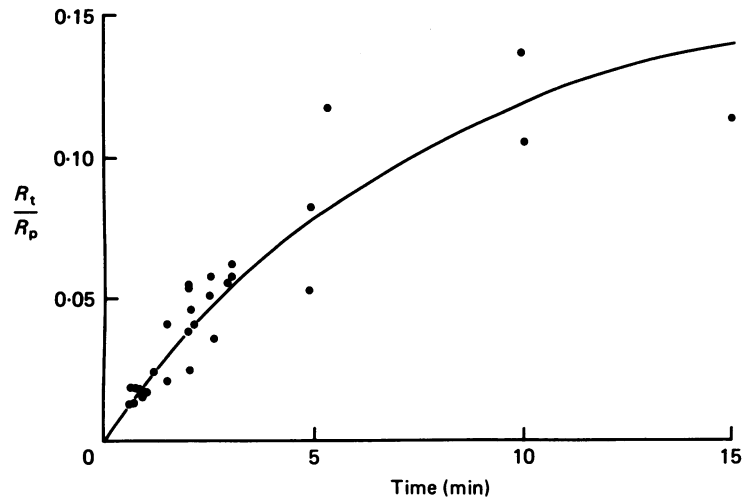

Fig. 3. The accumulation of radioactivity in the rat cerebrum during successive experiments in which a steady level of [^{14}C]thiamine was maintained in the blood plasma by a programmed I.V. injection. The ratio R_t/R_p is the radioactivity in the brain tissue at the end of the experiment, divided by the mean radioactivity in the blood plasma during the experiment. A curve has been fitted to the results by the method of maximum likelihood (Bard, 1974).

RESULTS

The time course of the movements of [^{14}C]thiamine between the blood and the brain

Steady levels of [^{14}C]thiamine were maintained in the circulation of a series of rats for periods ranging from 30 sec to 15 min. The ratio of radioactivity g^{-1} brain/radioactivity ml.$^{-1}$ plasma (R_t/R_p) was calculated for each experiment. For the first 2 min the ratio increased almost in direct proportion to the length of the period of injection (Fig. 3). As the period of the experiment was prolonged further the ratio increased less rapidly. It was concluded that the efflux of the tracer from the brain back into the blood was small for periods up to 2 min. In subsequent experiments, periods of 2 min or less (usually 1 min) were used to provide a measure of the initial rate of movement into the brain, that is of unidirectional flux of the thiamine from the blood into the brain. Such a short period has the additional advantage of minimizing the effects of any metabolic conversion of the injected tracer.

Corrections were made in calculating the influx from the observed uptake of labelled thiamine into the brain tissue. The concentration of the tracer in the capillary blood (C_c) was taken to be slightly less than the mean value found in the arterial blood plasma (C_a)

$$C_c = C_a - 0{\cdot}63\frac{J_0}{F} \quad (1)$$

using the approximate empirical equation of Pappenheimer & Setchell (1973) and the

Fick principle, where J_0 is the observed flux and F is the blood plasma flow, taken to be 0·72 ml. min^{-1} g^{-1} of tissue (Gjedde, Hansen & Siemkowicz, 1980). No appreciable correction was needed for the efflux of tracer from the brain over the short period of the experiments (less than 2 min.).

Influx of thiamine into the brain and the effect of raising the concentration of the vitamin in the plasma upon this process

In eleven experiments in which the concentration of thiamine in the blood plasma was within the normal range (0·2–0·6 μmole l^{-1}) its influx into the cerebrum was

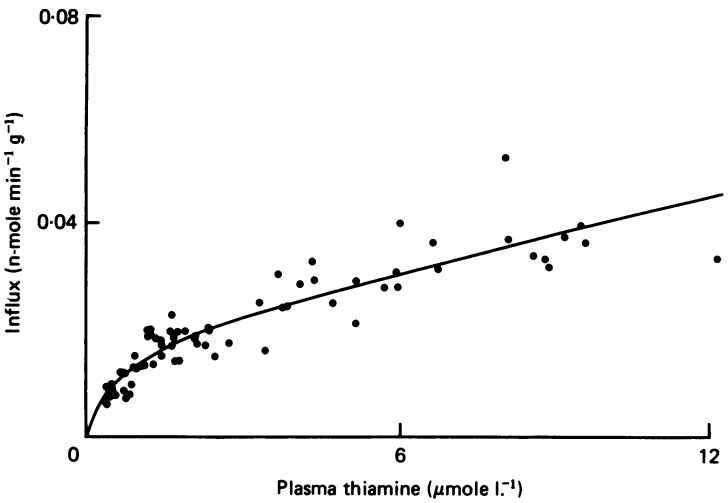

Fig. 4. The effect of increasing (in successive experiments) the concentration of thiamine in the blood plasma upon its influx into the cerebral hemispheres. A curve has been fitted to the results by the method of maximum likelihood (Bard, 1974) by the use of eqn. (3).

8·4 ± 0·3, into the cerebellum 10·5 ± 0·5, into the brain stem 9·3 ± 0·4 and into the brain as a whole 9·4 ± 0·4 p-mole min^{-1} g^{-1} of tissue.

When the concentration of thiamine in the plasma (C_p) was raised it was found that the influx (J) did not rise in direct proportion to this plasma concentration but showed evidence of the saturation (Fig. 4) which is associated with carrier-mediated transport. Such a process can be described by the simple Michaelis–Menten equation:

$$J = \frac{J_{max} C_p}{C + K_t}. \tag{2}$$

Where J_{max} and K_t represent the kinetic parameters of transport, maximum influx and Michaelis constant respectively.

The statistical method of Wilkinson (1961) based on eqn. (2) was used to analyse the experimental data but the predicted curve did not provide a very good fit to the observed values of influx. Equation (2) was therefore modified to allow for an additional non-saturable component:

$$J = \frac{J_{max} C_p}{C_p + K_t} + D' C_p, \tag{3}$$

where D' is a transfer constant for diffusion. The data were re-analysed fitting them to eqn. (3) by the method of maximum likelihood (Bard, 1974) to obtain the best estimates of J_{max}, K_t and D' (Table 1).

DISCUSSION

Our results show that thiamine crosses the blood–brain barrier mainly by a saturable transport process which must be carrier-mediated (Fig. 4). The order of magnitude of the specific influx of thiamine across the blood–brain barrier is much

TABLE 1. Estimated values of the kinetic parameters for the transport of thiamine across the blood–brain barrier, obtained by fitting eqn. (3) to the data from seventy-eight separate experiments using the method of maximum likelihood (Bard, 1974). Mean ± S.E. of mean

Brain region	Maximum influx J_{max} (p-mole min^{-1} g^{-1})	Michaelis constant of transport K_t (μmole l.$^{-1}$)	Transfer constant for diffusion D' (μl. min^{-1} g^{-1})
Cerebral hemispheres	18·6 ± 0·6	0·61 ± 0·05	2·4 ± 0·1
Cerebellum	16·5 ± 1·5	0·24 ± 0·15	4·1 ± 0·2
Brain-stem	18·5 ± 0·8	0·46 ± 0·07	3·2 ± 0·1
Whole brain	17·6 ± 1·3	0·40 ± 0·15	3·3 ± 0·2

TABLE 2. Comparison of the specific flux, that is the ratio of influx across the blood–brain barrier to the normal concentration in the blood plasma for thiamine and various other substances of small molecular weight

Substance	Molecular weight	Specific flux (μl. min^{-1} g^{-1})
Thiamine	265*	44 ± 11
D-glucose†	180	129 ± 7
L-lysine†	147*	26 ± 5
L-tryptophan†	204*	33 ± 1
D-3-hydroxy-butyrate	103*	22 ± 6
D-mannitol‡	182	1·4 ± 0·1

* Mol. wt. of ion present at physiological pH.
† Data from Pratt (1980).
‡ Data from Daniel, Lam & Pratt (1981).

greater than that of a molecule like mannitol which only crosses the blood–brain barrier by passive diffusion. It is comparable with that of other brain cell nutrients, for example the nutritionally essential amino acids (Table 2) which have been shown to cross the blood–brain barrier by carrier-mediated transport processes (Baños, Daniel & Pratt, 1971; Baños, Daniel, Moorhouse & Pratt 1973).

In addition there is a normally small but appreciable non-saturable component of the transport of thiamine across the blood–brain barrier (Fig 4). In all probability this is passive diffusion or it may be due to a second system with a low affinity for thiamine. In having a minor non-saturable component the flux of thiamine across the blood–brain barrier resembles that of many neutral amino acids (Daniel, Pratt & Wilson, 1977*a*,*b*,*c*; Daniel, Moorhouse & Pratt, 1978; Pratt, 1979).

The movement of thiamine across the blood–brain barrier may differ in one respect from the carrier-mediated processes for the uptake of thiamine which have been described in the membranes of various animal cells, including those of the cerebral cortex (Sharma & Quastel, 1965; Rindi & Ventura, 1967). The uptake of thiamine into these cells is an energy-dependent process of active transport which can work against an adverse concentration gradient and in which the vitamin may become phosphorylated. Such cells are able to continue to take up thiamine over a prolonged period, whereas in our experiments an equilibrium is approached after a relatively short time (Fig. 3). Our results are consistent with carrier-facilitated diffusion (Christensen, 1976) of thiamine across the blood–brain barrier.

That the brain cells need a continuous supply of thiamine has been clear since Peters (1936) demonstrated the part played by the vitamin in cerebral pyruvate metabolism. In the form of its diphosphate the vitamin serves as an essential co-enzyme for several important enzymes of cerebral metabolism, and the triphosphate has been implicated in nerve function (Itokawa, Schulz & Cooper, 1972). Experimental depletion of thiamine from the brain causes neuromuscular disturbances, especially ataxia and convulsions, and the rates at which the vitamin is lost from the brain can be as high as 1 n-mole g^{-1} brain tissue per day (Murdock & Gubler, 1973; McCandless & Schenker, 1968; Dreyfus, 1961). From our results (Fig. 4 and Table 1) it is clear that the normal rate of influx of thiamine across the blood–brain barrier is of the order of ten times this maximal rate of loss of the vitamin from the brain, i.e. of the order of 8·4–10·5 p-mole min^{-1} g^{-1} brain tissue. This rate is of a similar order of magnitude to that of thiamine turnover in different brain regions, found by Rindi, Patrini, Comincioli & Reggiani (1980) to range from 7·9 to 27·2 p-mole min^{-1} g^{-1} brain tissue. These comparisons show that, although transport across the blood–brain barrier is normally adequate to meet the needs of the brain cells for thiamine and to replace the obligatory losses, there is little, if any, margin of spare capacity. Thus, if anything interferes with the carrier-mediated component of thiamine transport across the blood–brain barrier, as represented by the first term in eqn. (3), the supply of the vitamin will become inadequate. It can be calculated from our results (Table 1) that the movement across the barrier by the non-saturable process, represented by the second term in eqn. (3), normally provided less than 10% of the total flux. This means that influx across the blood–brain barrier by the non-saturable process alone could not meet the needs of the cerebral cells for thiamine unless the concentration of the vitamin in the blood was very much higher than normal.

These conclusions about the critical nature of thiamine transport across the blood–brain barrier have a bearing not only upon thiamine deficiency but also in all probability upon a number of other metabolic abnormalities in which the need for thiamine may be increased, including subacute necrotizing encephalopathy (Leigh, 1951) in which an abnormal metabolism of thiamine triphosphate has been implicated (Cooper, Itokawa & Pincus, 1969), as well as a disease of the nervous system which causes intermittent ataxia and shows thiamine dependency due to a partial block of the thiamine-requiring enzyme, pyruvate decarboxylase (Lonsdale, Faulkner, Price & Smeby, 1969).

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