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COMPARISON OF THE EFFECTS OF SOME THIAMINE ANALOGUES UPON THIAMINE TRANSPORT ACROSS THE BLOOD–BRAIN BARRIER OF THE RAT

BY J. GREENWOOD AND O. E. PRATT

From the Department of Neuropathology, Institute of Psychiatry, De Crespigny Park, London SE5 8AF

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SUMMARY

1. The flux of thiamine from the blood into the brain has been measured by a specially devised procedure in which a steady raised level of the vitamin, with or without radioactive labelling, was achieved rapidly and maintained steadily in the circulating blood plasma. This was done by a single rapid I.V. injection followed by a continuous injection given at a rate adjusted according to a pre-determined programme, so as to replace the injected material at the rate at which it had been found to leave the circulation in preliminary experiments.

2. A series of four chemical analogues of thiamine were given to see how each affected the flux of thiamine into the brain and the findings are compared with those for a fifth analogue studied in previous work.

3. Pyrithiamine, thiamine disulphide and acetylthiamine, like amprolium, inhibited thiamine transport across the blood–brain barrier. Kinetic analysis shows that they compete mainly for the saturable component of thiamine flux across the blood–brain barrier, with only a slight inhibition of the non-saturable component, most clearly seen with pyrithiamine.

4. Oxythiamine, despite its close chemical similarity to thiamine did not have any significant effect upon the flux of the vitamin into the brain.

5. These findings help to explain the efficacy of pyrithiamine administration, especially in conjunction with a thiamine-deficient diet, in rapidly producing central neurological signs of deficiency.

INTRODUCTION

The transport of free thiamine across the blood–brain barrier in the rat has been shown to be a two-component process (Greenwood, Love & Pratt, 1982; Reggiani, Patrini & Rindi, 1984), similar in this respect to the transport of the vitamin across the rat intestinal mucosa (Hoyumpa, 1982). One of the components is a carrier-mediated process saturable by an excess of thiamine (Spector, 1976) whilst the other is a process which does not appear to be saturable even by high concentrations of the vitamin. A chemical analogue of thiamine, 1-[(4′-amino-2′-propyl-5′-pyrimidinyl) methyl]-2-picolinium chloride hydrochloride (amprolium), acts selectively on these

two components, inhibiting the carrier-mediated, saturable component of the transport at the blood–brain barrier but not apparently the non-saturable process (Greenwood & Pratt, 1983). In so far as the apparently non-saturable component of thiamine transport across the blood–brain barrier represents passive diffusion, no inhibition by analogues would be expected. However, since thiamine is a polar, positively charged ion, it is at least questionable whether there is any appreciable passive diffusion at all. What is conveniently referred to as the 'non-saturable component' may well represent in the main transport by a low-affinity carrier, which is not easily saturated or inhibited. It seemed worthwhile, therefore, to see whether any of the other chemical analogues of thiamine appreciably inhibited this apparently non-saturable component of thiamine transport across the blood–brain barrier.

Two chemical analogues of thiamine, pyrithiamine and oxythiamine (Fig. 1), which have been widely used in work on thiamine metabolism, have rather differing effects. Pyrithiamine inhibits thiamine transport at various sites, including the rat intestine (Rindi & Ventura, 1967; Hoyumpa, Middleton, Wilson & Schenker, 1975), the cell membrane of Ehrlich ascites carcinoma cells (Menon & Quastel, 1966) and the uptake of thiamine into rat brain slices (Sharma & Quastel, 1965; Nose, Iwashima & Nishino, 1976). Oxythiamine, on the other hand, does not appear to enter the central nervous system (Rindi, De Giuseppe & Ventura, 1963; Ostrovsky, 1965) although peripheral signs of thiamine deficiency are seen when it is given to various species (Gubler, 1976). The present study compares the effects of these and some further analogues upon the two components of thiamine transport across the blood–brain barrier.

A preliminary account of this work has been given (Greenwood & Pratt, 1981).

METHODS

Animals

Young adult Wistar albino rats of either sex were used weighing 200–400 g. Water and food were given without restriction. The animals were fed on a commercial breeding diet containing 21·5% protein and 45% carbohydrate, with a thiamine content of 18·5 mg kg^{-1}. Under sodium pentobarbitone anaesthesia (BPC, 50 mg kg^{-1} body wt., I.P.), Portex nylon catheters were inserted, one (1·02 mm o.d.) into a femoral artery for withdrawing blood and one or two (0·63 mm o.d.) into femoral veins for giving the programmed I.V. injections. Radioactively labelled thiamine was given in isotonic NaCl solution in the form of [^{14}C]thiamine with a specific activity ranging up to 0·9 kBq μmol^{-1}, using an electronically controlled syringe drive (Pratt, 1974). A raised level of the tracer in the circulation was achieved rapidly and maintained steadily over a defined period by a well established procedure (Daniel, Donaldson & Pratt, 1975; Pratt, 1985) by giving a rapid (4 s) I.V. injection of the tracer, followed by a continuous I.V. infusion at a varying rate, according to a programme which had been determined from the results of preliminary experiments in which the rate of clearance of the tracer from the circulation had been measured.

In some experiments the specific activity was deliberately reduced by adding unlabelled thiamine and the rate of injection was scaled up so as to maintain the blood concentration of the vitamin at various levels considerably greater than normal.

Measurement of influx

The influx, J, i.e. the unidirectional movement inwards of thiamine across the walls of the blood vessels into the extracellular space of the brain, was measured by the initial rate at which the tracer entered the tissue from the blood plasma. At the end of the short experimental period

(approximately 1 min) during which a steady level of the radioactive tracer was maintained in the blood plasma, the blood was rapidly washed out of the vessels by a forced intra-arterial injection of isotonic saline (Daniel, Love, Moorhouse, Pratt & Wilson, 1974), the head was cut off and the brain removed rapidly and frozen. The influx was calculated from the following equation:

$$J = \frac{R_t C_p}{R_c t}, \quad (1)$$

where R_t is the level of radioactivity in the cerebral tissue, R_c is the mean level of radioactivity in the capillary blood plasma, calculated from that in the arterial blood plasma as described previously (Greenwood *et al.* 1982), C_p is the mean concentration of thiamine in the blood plasma and, t, is the duration of the period during which the radioactive labelling was maintained in the circulation. Even though thiamine (like its analogues, except thiamine disulphide) is a positively charged ion, it does not seem to be bound appreciably by blood proteins since practically all of it is readily removed from plasma by dialysis (Rindi, De Giuseppe & Sciorelli, 1968). It has been assumed, therefore, that effectively the whole of the thiamine content of the blood plasma is available to high-affinity transport carriers at the blood–brain barrier.

Materials

Radioactively labelled thiamine ([thiazole-2-^{14}C]thiamine hydrochloride) was obtained from Amersham International, Amersham. Thiamine hydrochloride, 1-[(4′-amino-2′-methyl)-5′-pyrimidylmethyl]-2-methyl-3-[2-hydroxyethyl]-pyridinium bromide ('pyrithiamine') and *N*-(2′-methyl-4′-hydroxy-pyrimidyl-5′-methyl)-4-methyl-5-[2-hydroxyoxyethyl]-thiazolium chloride ('oxythiamine') from the Sigma Chemical Company, London. *N*,*N*′-dithio-bis[2-(2-hydroxyethyl)-1-methyl-vinylene]-bis-[(4′-amino-2′-methyl-5′-pyrimidinyl)methyl]-formamide dihydrate, ('thiamine disulphide') and *N*-(2′-methyl-4′-amino-pyrimidyl-5′-methyl)-4-methyl-5-acetoxyethyl-thiazolium chloride ('acetylthiamine') were kindly supplied by Roche Products, Welwyn Garden City, Herts. The structures are compared in Fig. 1. All other reagents were of analytical grade as far as available.

Radioactive counting

The blood samples were taken into heparinized syringes, the cells spun down, the plasma removed and stored at −20 °C until the radioactivity was assayed. The tissue samples (taken from the cerebral hemispheres, the cerebellum or the brain stem but excluding the choroid plexuses) were dissolved in a solution of an organic base (Soluene-350, Packard Instruments). Glacial acetic acid was then added to neutralize the solution, followed by 15 ml scintillation mixture containing 5 g 2,5-diphenyloxazole and 0·1 g 1,4-bis(2-(4-methyl-5-phenyloxazolyl)) benzene per litre toluene. Samples of plasma (10 μl) were prepared in a similar way. The radioactivity in the samples was measured in an automatic scintillation spectrometer (Tricarb, Model 2409, Packard Instruments). When necessary, quench corrections were done either by an external standard or by the channels ratio method.

Administration of thiamine analogues by a rapid intra-arterial injection

The effect of a rapid injection of either pyrithiamine, oxythiamine, thiamine disulphide or acetylthiamine upon the influx of thiamine into different regions of the brain was studied. This injection was conveniently given via the intra-arterial cannula already inserted in preparation for taking blood samples and for washing out the vascular system at the end of the experiment. All analogues were readily soluble in an aqueous medium. In each of a series of experiments, rapid 200 μl intra-arterial injections of a 0·1 M-solution of the analogue being studied were given via a cannula previously inserted into a femoral artery, 20 s prior to the start of a programmed [^{14}C]thiamine infusion. The concentration of thiamine maintained in each separate experiment was increased so as to cover a range of plasma concentrations. The influx of thiamine into the cerebral tissue was then estimated.

Dual infusion of thiamine and oxythiamine

In some experiments oxythiamine was given by a programmed injection. It was assumed that since oxythiamine differed only very slightly in structure (Fig. 1) from thiamine, the values of the injection parameters which were effective in maintaining a raised blood concentration of thiamine

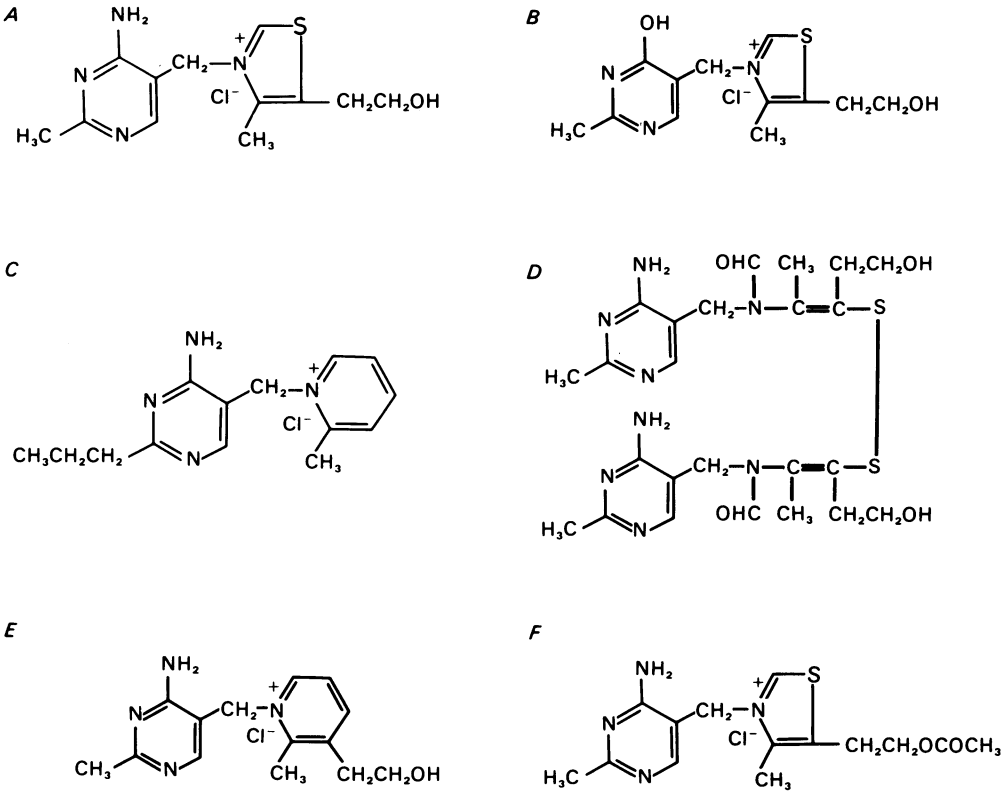

Fig. 1. The structure of thiamine and some of its closely related analogues. *A*, thiamine; *B*, oxythiamine; *C*, amprolium; *D*, thiamine disulphide; *E*, pyrithiamine; *F*, acetylthiamine.

would provide a reasonably good approximation to those which ought to be used in a programmed injection to maintain a steady raised concentration of the analogue. A second cannula was inserted into the other femoral vein of the rat and unlabelled oxythiamine solution injected from a syringe driven by a second independently controlled electronic injection apparatus. The programmed injection of 0·188 M-oxythiamine was begun 4 min prior to, and continued during the period of approximately 1 min during which the test tracer, [^{14}C]thiamine, was also injected. Although it was not possible to confirm by direct measurements how steadily the concentration of the analogue was maintained in the blood plasma, it seems reasonable to assume that the concentration would change less during the experiment than it did after a single rapid injection given shortly before the start of the [^{14}C]thiamine injection. At the end of the experiment both injections were stopped simultaneously, the vascular system washed out with isotonic NaCl and brain samples taken. The influx of thiamine into the brain was then calculated.

RESULTS

The effect of a chemical analogue upon thiamine influx

Pyrithiamine. In a series of experiments the influx of thiamine into the brain was measured after a single rapid intra-arterial injection of pyrithiamine (exceeding 50 μmol kg^{-1}) had been given 20 s prior to starting the thiamine infusion. The

measurement of thiamine influx into the brain was repeated with progressively higher concentrations of thiamine being maintained in the blood in successive experiments. In the presence of this dose of pyrithiamine it was found that influx was greatly reduced and that saturation of the transport system by high thiamine concentrations was no longer evident. Further increase in the quantity of inhibitor given did not reduce the thiamine flux any further. The data were fitted to eqn. (2) (see later) by the method of maximum likelihood (Bard, 1974), yielding the estimates of J_{max}, K_a and K_n shown in Table 1. The value of the function was largely determined by the latter parameter, showing little dependence upon J_{max} or K_a, the fitted curve approximating to a straight line (Fig. 2) for all brain regions studied.

TABLE 1. The effect of various analogues of thiamine upon its flux across the blood–brain barrier. Estimates of the maximum flux, J_{max}, the apparent Michaelis constant for transport, K_a, and the first-order rate constant for the apparently non-saturable component of the thiamine transport, K_n, were obtained by fitting eqn. (2) to the data (see Figs. 2–4) by the method of maximum likelihood (Bard, 1974). Mean values ± S.E. of mean, are shown with number of determinations in parentheses. Values in which the differences from the control group are statistically significant (*t* test) are indicated thus: ** $P < 0.001$, * $P < 0.01$, † $P < 0.05$.

Group	Cerebrum	Cerebellum	Brain stem
		J_{max} (pmol min^{-1} g^{-1})	
Pyrithiamine (6)	20·2 ± 10	21·1 ± 11	22·4 ± 12
Amprolium (24)	21·1 ± 4·2	21·9 ± 4·3	22·7 ± 4·5
Oxythiamine (17)	17·7 ± 1·3*	24·8 ± 1·8	23·7 ± 1·9
Control (78)	21·2 ± 0·6	24·9 ± 0·7	25·5 ± 0·7
		K_a (μmol l^{-1})	
Pyrithiamine (6)	217 ± 113**	824 ± 432**	1360 ± 713**
Amprolium (24)	149426 ± 29598**	169382 ± 3355**	2008 ± 398**
Oxythiamine (17)	0·57 ± 0·10	0·77 ± 0·13	0·66 ± 0·13
Control (78)	0·78 ± 0·05	0·68 ± 0·05	0·88 ± 0·06
		K_n (μl min^{-1} g^{-1})	
Pyrithiamine (6)	1·26 ± 0·078**	2·01 ± 0·20**	1·90 ± 0·21*
Amprolium (24)	1·93 ± 0·15†	2·91 ± 0·17	2·86 ± 0·16
Oxythiamine (17)	2·93 ± 0·28	3·09 ± 0·36	2·39 ± 0·37
Control (78)	2·33 ± 0·15	3·30 ± 0·21	2·65 ± 0·18

Oxythiamine. In a further series of experiments the influx of thiamine into the brain was measured, the programmed injection of tracer thiamine starting 20 s after a single rapid intra-arterial injection of oxythiamine had been given. In all these experiments the dose of oxythiamine given exceeded 50 μmol kg^{-1}, and in some cases the amount injected was increased to more than 300 μmol kg^{-1}. In this series of experiments progressively higher concentrations of thiamine were maintained in the blood in successive experiments. It was found that, unlike pyrithiamine, oxythiamine appeared to have little or no effect upon thiamine influx into the brain (Fig. 3).

In view of this somewhat unexpected result, further measurements of thiamine flux were made in experiments in which raised blood plasma levels of oxythiamine were maintained by giving a programmed continuous I.V. injection. Although the exact levels of oxythiamine were not assayed it was assumed that the predicted levels would be similar to those of a thiamine infusion using the same injection parameters.

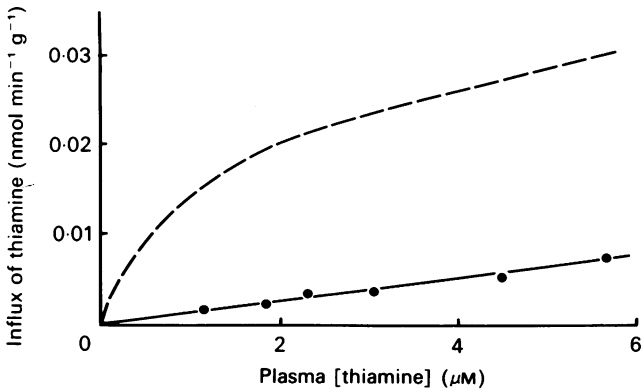

Fig. 2. The relation between the flux of thiamine across the blood–brain barrier into the brain and its concentration in the blood plasma. The dashed curve represents the flux with no inhibitor present and was obtained by fitting eqn. (2) to the data of Greenwood *et al.* (1982) for the cerebral hemispheres. The data points show the flux in the presence of pyrithiamine in the blood plasma and the continuous curve (which approximates closely to a straight line) is that obtained by fitting eqn. (2) to these points.

On this assumption, the concentration of oxythiamine in the blood plasma was greater than 0·4 M throughout an experiment in which the oxythiamine was given starting 4 min prior to, and continued during the 1 min thiamine infusion. The thiamine flux into the brain was similar in each group of experiments, i.e. after a single rapid intra-arterial injection of oxythiamine or during a programmed infusion of the inhibitor, and was not appreciably different from that in animals not given the inhibitor (Fig. 3).

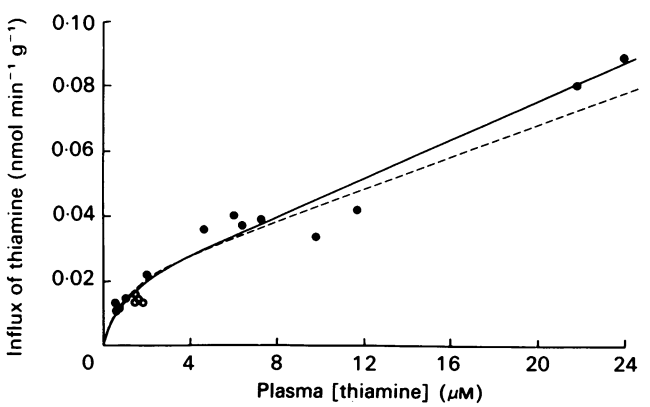

Fig. 3. The effect of increasing (in successive experiments) the concentration of thiamine in the blood plasma upon its influx into the cerebral hemispheres in the presence of a high concentration of oxythiamine (●, experiments in which a bolus dose was given; ○, experiments in which a programmed injection of oxythiamine was given). The continuous curve is that obtained by fitting eqn. (2) to the data by the method of maximum likelihood (Bard, 1974). The dashed curve, shown for comparison, is that obtained by fitting eqn. (2) similarly to the data obtained in the absence of oxythiamine.

Other analogues. The data obtained in previous work for inhibition of thiamine flux into the brain by amprolium (Greenwood & Pratt, 1983) were re-analysed by fitting them to eqn. (2) (see later), yielding the estimates of J_{max}, K_a and K_n shown in Table 1. The line fitted to the data for amprolium inhibition corresponded fairly closely to that for pyrithiamine inhibition.

Each of the other two thiamine analogues studied, thiamine disulphide and acetylthiamine, inhibited the transport of thiamine across the blood–brain barrier, affecting mainly the easily saturable component. The inhibition caused by each of these analogues closely resembled that produced by pyrithiamine at corresponding concentrations of that inhibitor, in that the value of the flux appeared to be only slightly dependent upon J_{max} or K_a. It was not possible to obtain meaningful estimates of these two parameters from the limited data available, but the data could be fitted quite well by the line used to fit the data for inhibition of the thiamine transport by pyrithiamine or by amprolium (Fig. 4).

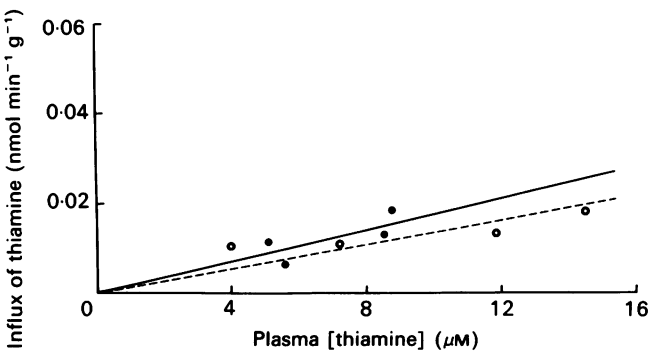

Fig. 4. The effect of increasing (in successive experiments) the concentration of thiamine in the blood plasma upon its influx into the cerebral hemispheres in the presence of high concentrations of acetylthiamine (○) or thiamine disulphide (●). The two curves shown are those obtained by fitting eqn. (2) to the data for thiamine flux in the presence of high concentrations of pyrithiamine (dashed line) or of amprolium (continuous line) by the method of maximum likelihood (Bard, 1974). It will be seen that either of these curves (which approximate to straight lines) provide at least an approximate fit to the data for the flux in the presence of acetylthiamine or thiamine disulphide.

Kinetic analysis

The flux, J, for a combination of a saturable, carrier-mediated system and an apparently non-saturable component of transport can be represented (Greenwood *et al.* 1982) by

$$J = \frac{J_{max} C_p}{C_p + K_a} + K_n C_p, \qquad (2)$$

where K_n is an apparent transfer constant for the second seemingly non-saturable component, J_{max} is the maximum carrier-mediated flux in the presence of excess substrate and K_a, the apparent affinity constant, is equal to the Michaelis constant for transport, K_t.

The effect of a competitive inhibitor of thiamine transport (e.g. amprolium) upon the process (Greenwood & Pratt, 1983) is to make K_a in the denominator of eqn. (2) a function of the inhibitor conditions, increasing its value above that of K_t, so that the flux is now given by

$$J = \frac{J_{max} C_p}{C_p + K_t[1 + (i/K_i)]} + K_n C_p, \quad (3)$$

where i is the concentration of the competitive inhibitor in the blood plasma and K_i is the Michaelis-type constant, inversely proportional to the affinity of the inhibitor for the carrier. From eqn. (3) it will be seen that the failure to find a significant difference (Table 1) between the estimate of the value of K_a, the apparent affinity constant in the presence of oxythiamine and that of K_t, equal to the value of K_a from control experiments without any inhibitor present, must mean that the term i/K_i in the denominator of the first term of eqn. (3) is not significantly different from zero.

The data shown for thiamine flux alone or in the presence of one of the chemical analogues were analysed kinetically by estimating the values of the parameters of the saturable transport component which gave the best fit of the data to eqn. (2) by the method of maximum likelihood (Bard, 1974) using an iterative microcomputor algorithm (Pratt, 1985). The maximum likelihood function was calculated from normalized deviations and the standard errors were calculated numerically by the radex procedure.

The data for thiamine flux inhibited by a large concentration of pyrithiamine (Fig. 2), or of amprolium, when fitted by eqn. (2) yielded estimates of K_a which were considerably larger than those obtained in the absence of any inhibitor or when oxythiamine was given (Table 1). From a comparison of eqns. (2) and (3) it seems clear that at the concentrations of the analogues pyrithiamine, amprolium, acetylthiamine or of thiamine disulphide used, K_i was much less than i, so that the value of i/K_i in the denominator of the first term of eqn. (3) was large enough to make the first term of eqn. (2) comparatively small. That this is so is confirmed by the way in which the inhibited flux was largely determined by the second term in this equation and only slightly dependent upon the values of J_{max} or K_a.

The data shown in Fig. 3 for thiamine flux in the presence of oxythiamine were analysed kinetically in a similar manner by fitting them to eqn. (2). They could be fitted also reasonably well by the curve used previously to fit the control data for thiamine flux in the absence of any analogue, as can be seen from Fig. 3. Oxythiamine, unlike pyrithiamine, did not appear to have any consistent effect upon the values of any of the three parameters for thiamine transport into the brain. The slight reductions in the values of J_{max} (statistically significant in only one of the three brain regions) may well be due to random errors. All changes were small and the differences from those obtained from measurements of thiamine influx in control animals not given any analogue were not consistent between the brain regions and generally not significant (Table 1).

It seems likely that the four thiamine analogues which severely inhibited the first, easily saturable, component of thiamine transport into the brain also inhibited to some extent the second, apparently non-saturable, component of the thiamine

transport. All the estimates of K_n in the presence of pyrithiamine and two out of three of those in the presence of amprolium fell below the corresponding control values, and of these reductions all those due to pyrithiamine and one of those due to amprolium were statistically significant.

DISCUSSION

The results from the administration of pyrithiamine, acetylthiamine and thiamine disulphide provide further support for the existence of at least two separate components in the transport of free thiamine across the blood–brain barrier. It seems clear that it is mainly the first, easily saturable, process which is susceptible to inhibition, and that in the presence of a sufficiently high concentration of these inhibitors free thiamine can only be transported across the blood–brain barrier to any appreciable extent by the second, relatively non-saturable process which is not easily suppressed (Fig. 2; Table 1).

This conclusion is consistent with kinetic analysis of the data. The curve fitted to the data in the presence of a high concentration of the inhibitor, pyrithiamine, relating the residual non-inhibited flux to the concentration of thiamine in the blood plasma, approximates to a straight line. The finding that the flux is dependent mainly upon the value of K_n the first-order constant for the relatively non-saturable component of thiamine flux, and largely independent of the other two parameters (provided that the ratio of K_a/J_{max} is large enough to make the first term in eqn. (2) relatively small) suggests that the inhibition is conforming to the competitive model represented by eqn. (3) with the ratio i/K_i considerably greater than unity. Increases of the order of 300–2000 times in the values of K_a suggest that the concentrations of pyrithiamine achieved in the blood plasma (of the order of 0·4 mM) largely suppressed the first component of the flux, as represented by the first term in eqn. (3) whereas the inhibition of the second component of the flux, represented by the second term in eqn. (3), measured by the reductions in K_n, was significant but less severe (Table 1).

The results of reanalysis of previous data (Greenwood & Pratt, 1983) for thiamine influx in the presence of another thiamine analogue, amprolium (Fig. 1) correspond closely with those for the effects of pyrithiamine (Table 1), showing that the effects of these two analogues upon thiamine transport across the blood–brain barrier are similar. In addition, the ease with which the data for the effects upon the flux of two other analogues of thiamine, acetylthiamine and thiamine disulphide (Table 1) can be fitted by either the curve used to fit the data for pyrithiamine or that for amprolium (Fig. 4) suggests that all four of these inhibitors conform to a broadly similar pattern in their effects upon thiamine transport across the blood–brain barrier. The fact that the second component of the thiamine flux across the blood–brain barrier is susceptible to inhibition by at least two of the thiamine analogues suggests that this component must be due, in part at least, to a carrier-mediated process which is not easily saturable, probably because of a rather low affinity for thiamine of the second carrier. It might be, for example, the thiamine monophosphate carrier reported by Reggiani *et al.* (1984). The impracticality of saturating the second carrier makes it difficult to estimate how much of the second component may be

due to passive diffusion, but it is worth noting that the values of K_n of 1–2 μl min^{-1} g^{-1} are approaching the values of 0·5–1 μl min^{-1} g^{-1} reported for the diffusion of radioactively labelled mannitol across the blood–brain barrier (Deane, Greenwood, Lantos & Pratt, 1984). It seems likely therefore that there will prove to be three components of the transport of free thiamine across the blood–brain barrier, as represented by

$$J = \frac{J_{max} C_p}{C_p + K[1 + (i/K_i)]} + \frac{J'_{max} C_p}{C_p + K'[1 + (i/K'_i)]} + K_n C_p, \quad (4)$$

in which J'_{max} and K_t are the corresponding parameters for the second transport carrier and K'_i is the inhibitor constant expressing the affinity between the second carrier and the inhibitor (which almost certainly will differ from K_i, the inhibitor constant for the first carrier). The third term would now represent only passive diffusion, but on currently available data it is difficult to resolve the second and third terms.

The failure of oxythiamine to have any consistent, appreciable effects upon thiamine influx into the central nervous system as shown by the mostly small, probably random, changes in the kinetic parameters of transport of thiamine in the presence of high plasma oxythiamine (Table 1) are explicable if oxythiamine is not transported across the blood–brain barrier by the thiamine carrier. Although this conclusion is somewhat unexpected, as this analogue resembles thiamine structurally more closely than the other analogues which did inhibit the thiamine transport carrier (Fig. 1), it is consistent with a number of previous findings. Thus oxythiamine fails to enter the brain (Rindi *et al.* 1963; Ostrovsky, 1965), does not inhibit thiamine uptake by brain slices (Nose *et al.* 1976) and does not inhibit brain transketolase even though it does inhibit this enzyme in other tissues (Brin, 1962). Moreover, the failure of oxythiamine to inhibit thiamine transport across the blood–brain barrier is consistent with its failure to alter brain thiamine levels when it is administered to various species, and the finding that its administration leads to peripheral rather than central nervous system signs of thiamine deficiency (Gubler, 1976).

The failure of oxythiamine to interfere with thiamine transport across the blood–brain barrier suggests that the amino group on the pyrimidine moiety is essential for combination between the substrate and the transport carrier (Fig. 1). In contrast, the thiazole moiety does not seem to be essential for high affinity for the carrier since both amprolium and pyrithiamine, whose thiazole rings are substituted by pyridine moieties, are highly effective inhibitors of the thiamine transport system. It seems clear that the requirements for combination with the carrier differ from that for the catalytic site in the thiamine molecule which appears to be situated within the thiazolium ring (Rogers, 1982). It can be concluded that the combination of thiamine with the transport carrier at the blood–brain barrier is a process which is distinct from its function as a coenzyme.

Pyrithiamine-induced inhibition of thiamine transport has previously been demonstrated at a number of other sites, the rat intestine (Rindi & Ventura, 1967; Hoyumpa *et al.* 1975), the cell membrane of Ehrlich ascites carcinoma cells (Menon & Quastel, 1966) and for uptake into rat brain slices (Nose *et al.* 1976). It seems likely that the efficacy of pyrithiamine as a transport inhibitor at least partly

explains the effectiveness and frequency of its administration either on its own or in conjunction with a thiamine-deficient diet as a method of rapidly producing a deficiency with neurological symptoms in a variety of species: rats (Troncoso, Johnston, Hess, Griffin & Price, 1981; Hakim & Pappius, 1983; Aikawa, Watanabe, Furuse, Iwasaki, Satoyoshi, Sumi & Moroji, 1984), mice (Woolley & White, 1943; Collins, Kirkpatrick & McDougal, 1970; Watanabe, 1978; Watanabe, Tomita, Hung & Iwasaki, 1981), cats (Irle & Markowitsch, 1982) and cattle (Markson, Lewis, Terlecki, Edwin & Ford, 1972). Because pyrithiamine readily enters the brain (Rindi, Perri & De Caro, 1961; Rindi & Perri, 1961) the effects of the resulting thiamine deficiency within the central nervous system may be aggravated by intracellular inhibition of thiamine pyrophosphokinase, the enzyme that converts thiamine to thiamine pyrophosphate (Sharma & Quastel, 1965). However, it is unlikely that such inhibition of phosphorylation played any significant part in the reduction of thiamine flux across the blood–brain barrier seen in the presence of thiamine analogues in our results because of the short experimental time of approximately 1 min.

It is important to assess the effects of competitive inhibition in relation to variations in blood thiamine levels. Competitive inhibition of the first, easily saturable, carrier-mediated component of blood–brain barrier thiamine transport will be especially effective in excluding the vitamin from the brain when plasma thiamine levels are low so that virtually no thiamine enters the brain by the less readily saturable process or processes. This explains why experimental pyrithiamine administration in conjunction with a deficient diet produces central neurological signs of deficiency so rapidly. Thus, in a direct comparison of pyrithiamine-induced deficiency and dietary-induced deficiency in rats (Murdock & Gubler, 1973) dietary lack alone gradually reduced thiamine phosphates and total thiamine in brain whereas pyrithiamine administration much more rapidly reduced the brain thiamine content. During dietary deficiency thiamine is lost more slowly from the brain than from other tissues, but this is not so when the deficiency is induced by pyrithiamine administration (DeCaro, Rindi, Perri & Ferrari, 1958). Our finding that pyrithiamine inhibits transport of thiamine across the blood–brain barrier means that, although the turnover and consequent natural wastage of the vitamin in the brain is likely to remain the same, replenishment of thiamine from the circulation will be greatly reduced due to the transport inhibition. Thus the greater rate of loss of the vitamin from the brain when thiamine deficiency is induced by giving pyrithiamine is likely to be an increased net loss caused by the vitamin failing to reach the tissue from the blood. Relevant to this problem is the evidence of Rindi *et al.* (1961) that not only the influx of thiamine or its analogues into the brain, but also equally their efflux from the brain, is likely to be limited by the endothelial barrier, since after a single injection of pyrithiamine the analogue is first distributed evenly throughout the tissues of the animal, but after a few days its concentration in the brain continues to rise whilst that in other tissues falls. These findings, like our own, affirm the importance of the crossing of the capillary endothelial barrier as the rate-limiting step in the flux of thiamine between the blood plasma and the brain.

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REFERENCES

AIKAWA, H., WATANABE, I. S., FURUSE, T., IWASAKI, Y., SATOYOSHI, E., SUMI, T. & MOROJI, T. (1984). Low energy levels in thiamine-deficient encephalopathy. *Journal of Neuropathology and Experimental Neurology* **43**, 276–287.

BARD, Y. (1974). *Nonlinear Parameter Estimation*. New York: Academic Press.

BRIN, M. (1962). Effects of thiamine deficiency and of oxythiamine on rat tissue transketolase. *Journal of Nutrition* **78**, 179–183.

COLLINS, R. C., KIRKPATRICK, J. B. & MCDOUGAL, D. B. (1970). Some regional pathologic and metabolic consequences in mouse brain of pyrithiamine-induced thiamine deficiency. *Journal of Neuropathology and Experimental Neurology* **29**, 57–69.

DANIEL, P. M., DONALDSON, J. & PRATT, O. E. (1975). A method for injecting substances into the circulation to reach rapidly and to maintain a steady level: with examples of its application in the study of carbohydrate and amino acid metabolism. *Medical and Biological Engineering* **13**, 214–227.

DANIEL, P. M., LOVE, E. R., MOORHOUSE, S. R., PRATT, O. E. & WILSON, P. A. (1974). A method for rapidly washing the blood out of an organ or tissue of the anaesthetized living animal. *Journal of Physiology* **237**, 11–12*P*.

DEANE, B. R., GREENWOOD, J., LANTOS, P. L. & PRATT, O. E. (1984). The vasculature of experimental brain tumours. Part 4. The quantification of vascular permeability. *Journal of the Neurological Sciences* **65**, 59–68.

DE CARO, L., RINDI, G., PERRI, V. & FERRARI, G. (1958). Avitaminosi B_1 alimentare e da antivitamine (neopiritiamina ed ossitiamina) nei roditari. *Internationale Zeitschrift für Vitaminforschung* **28**, 252–273.

GREENWOOD, J., LOVE, E. R. & PRATT, O. E. (1982). Kinetics of thiamine transport across the blood–brain barrier in the rat. *Journal of Physiology* **327**, 95–103.

GREENWOOD, J. & PRATT, O. E. (1981). Inhibition of thiamine transport across the blood–brain barrier by thiamine analogues. *Journal of Physiology* **317**, 65*P*.

GREENWOOD, J. & PRATT, O. E. (1983). Inhibition of thiamine transport across the blood–brain barrier in the rat by a chemical analogue of the vitamin. *Journal of Physiology* **336**, 479–486.

GUBLER, C. J. (1976). Biochemical changes in thiamine deficiency. *Thiamine*, ed. GUBLER, C. J., FUJIWARA, M. & DREYFUS, P. M., pp. 121–139. New York: John Wiley & Sons.

HAKIM, A. M. & PAPPIUS, H. M. (1983). Sequence of metabolic, clinical, and histological events in experimental thiamine deficiency. *Annals of Neurology* **13**, 365–375.

HOYUMPA, A. M. (1982). Dual system of intestinal transport in humans. *Journal of Laboratory and Clinical Medicine* **99**, 701–708.

HOYUMPA, A. M., MIDDLETON, H. M., WILSON, F. A. & SCHENKER, S. (1975). Thiamine transport across the rat intestine. I. Normal characteristics. *Gastroenterology* **68**, 1218–1227.

IRLE, E. & MARKOWITSCH, H. J. (1982). Thiamine deficiency in the cat leads to severe learning deficits and to widespread neuroanatomical damage. *Experimental Brain Research* **48**, 199–208.

MARKSON, L. M., LEWIS, G., TERLECKI, S., EDWIN, E. E. & FORD, J. E. (1972). The aetiology of cerebrocortical necrosis: the effects of administering antimetabolites of thiamine to preruminant calves. *British Veterinary Journal* **128**, 488–499.

MENON, I. A. & QUASTEL, J. H. (1966). Transport and metabolism of thiamine in Ehrlich ascites-carcinoma cells. *Biochemical Journal* **99**, 766–775.

MURDOCK, D. S. & GUBLER, C. J. (1973). Effects of thiamine deficiency and treatment with the antagonists, oxythiamine and pyrithiamine on the levels and distribution of thiamine derivatives in rat brain. *Journal of Nutrition Science and Vitaminology* **19**, 237–249.

NOSE, Y., IWASHIMA, A. & NISHINO, H. (1976). Thiamine uptake by rat brain slices. *Thiamine*, ed. GUBLER, C. J., FUJIWARA, M. & DREYFUS, P. M., pp. 157–168. New York: John Wiley & Sons.

OSTROVSKY, Y. M. (1965). Impermeability of the blood–brain barrier to oxythiamine (in Russian). *Voprosi Meditsinskoi Khimii* **11**, 95–97.

PRATT, O. E. (1974). An electronically controlled syringe drive for giving an injection at a variable rate according to a preset programme. *Journal of Physiology* **237**, 5–6*P*.

PRATT, O. E. (1985). Continuous injection methods for the measurement of flux across the blood–brain barrier: the steady-state, initial-rate method. *Research Methods in Neurochemistry* **6**, 117–150.

REGGIANI, C., PATRINI, C. & RINDI, G. (1984). Nervous tissue thiamine metabolism *in vivo*. I. Transport of thiamine and thiamine monophosphate from plasma to different brain regions of the rat. *Brain Research* **293**, 319–327.

RINDI, G., DE GIUSEPPE, L. & SCIORELLI, G. (1968). Thiamine monophosphate a normal constituent of rat plasma. *Journal of Nutrition* **94**, 447–454.

RINDI, G., DE GIUSEPPE, L. & VENTURA, U. (1963). Distribution and phosphorylation of oxythiamine in rat tissues. *Journal of Nutrition* **81**, 147–154.

RINDI, G. & PERRI, V. (1961). Uptake of pyrithiamine by tissue of rat. *Biochemical Journal* **80**, 214–216.

RINDI, G., PERRI, V. & DE CARO, L. (1961). The uptake of pyrithiamine by cerebral tissue. *Experientia* **17**, 546–547.

RINDI, G. & VENTURA, U. (1967). Phosphorylation and uphill intestinal transport of thiamine, *in vitro*. *Experientia* **23**, 175–176.

ROGERS, E. F. (1982). General discussion of antithiamine compounds and thiamine antagonists. *Annals of the New York Academy of Sciences* **378**, 157–160.

SHARMA, S. K. & QUASTEL, J. H. (1965). Transport and metabolism of thiamine in rat brain cortex *in vitro*. *Biochemical Journal* **94**, 790–800.

SPECTOR, R. (1976). Thiamine transport in the central nervous system. *American Journal of Physiology* **230**, 1101–1107.

TRONCOSO, J. C., JOHNSTON, M. V., HESS, K. M., GRIFFIN, J. W. & PRICE, D. L. (1981). Model of Wernicke's Encephalopathy. *Archives of Neurology* **38**, 350–354.

WATANABE, I. (1978). Pyrithiamine-induced acute thiamine deficient encephalopathy in the mouse. *Experimental Molecular Pathology* **28**, 381–394.

WATANABE, I., TOMITA, T., HUNG, K-S. & IWASAKI, Y. (1981). Edematous necrosis in thiamine-deficient encephalopathy of the mouse. *Journal of Neuropathology and Experimental Neurology* **40**, 454–471.

WOOLLEY, D. W. & WHITE, A. G. C. (1943). Production of thiamine deficiency disease by the feeding of a pyridine analogue of thiamine. *Journal of Biological Chemistry* **149**, 285–289.