J. Physiol. (1981), **312**, *pp.* 551–562
With 1 text figure

Printed in Great Britain

THE EFFECT OF INSULIN UPON THE INFLUX OF TRYPTOPHAN INTO THE BRAIN OF THE RABBIT

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(*Received 2 June 1980*)

SUMMARY

1. The effect of hyperinsulinaemia upon the influx of tryptophan into the brain was determined. A raised level of insulin was maintained in the circulation of rabbits for periods of up to 120 min by means of a continuous, programmed intravenous injection of the hormone, given by an electronically controlled variable-drive syringe. A similar, appropriately programmed, intravenous injection of glucose, given simultaneously with the insulin, maintained the concentration of the blood glucose within normal limits throughout each experiment, so that the results were not vitiated by the development of hypoglycaemia.

2. Raised levels of insulin in the blood affect the supply of tryptophan to the brain in two opposing ways: (*a*) by increasing the binding of tryptophan to the albumin in the blood, thereby reducing the level of the free tryptophan in the circulation by about a half, which would *decrease* the influx of tryptophan into the brain; (*b*) by simultaneously reducing the levels in the blood of six or more of the amino acids which compete with tryptophan for transport carriers into the brain, which would *increase* the influx of tryptophan. The net result of these two opposing effects is that insulin causes only a slight increase in the influx of tryptophan into the brain.

3. To account in quantitative terms for the effect of insulin upon the influx of tryptophan into the brain it proved necessary to make one assumption. This assumption was that a predictable proportion of the tryptophan which is loosely bound to blood albumin is being stripped off this protein by the transport carrier located on the luminal surface membranes of the endothelial cells during the passage of the blood through the cerebral capillaries. If this assumption is accepted the work reported here explains adequately the effect of insulin on the influx of tryptophan into the brain.

INTRODUCTION

Tryptophan is one of the most important of the amino acids which have to be supplied to the brain from the blood (Curzon & Knott, 1977), but at present the factors which control its transport across the blood–brain barrier are incompletely understood, despite much work on the subject (Fernando, Knott & Curzon, 1976; Yuwiler, Oldendorf, Geller & Braun, 1977; Curzon & Knott, 1977; Green, 1978; Fernstrom, 1979; Pardridge, 1979; Pratt, 1979*a*). We have shown previously that

0022-3751/81/3950-0245 $07.50

the influx of tryptophan into the brain is related not only to the level of tryptophan in the circulation but also to the levels of other amino acids in the blood, since these compete with tryptophan for sites on the carrier molecule (Daniel, Moorhouse & Pratt, 1976, 1978*a*, *b*; Pratt, 1976, 1979*a*, *b*). In addition there is evidence that insulin stimulates amino acid transport into the brain, as there is a reduced uptake of tryptophan by the brain in diabetes (MacKenzie & Trulson, 1978), whilst de Montis, Olianas, Haber & Tagliamonte (1978) found that the hormone stimulates the transport of large neutral amino acids, including free tryptophan, into the brain. Accordingly we have now studied the way in which insulin affects the transport of tryptophan into the brain. In such a study a major problem is that the blood glucose level is lowered as a result of raised levels of insulin in the circulation. However we have been able to simplify the situation by means of technique (Daniel, Love & Pratt, 1975, 1977) by the use of which the level of the blood glucose is maintained within normal limits, despite the presence of steady raised levels of insulin in the circulation. A further complication in assessing the effects of abnormal levels of insulin is that the hormone alters the levels of many amino acids in the blood (Luck, Morrison & Wilbur, 1928).

A preliminary account of this work has been given (Daniel, Love, Moorhouse & Pratt, 1979).

METHODS

Animals

Young adult New Zealand White rabbits, fasted overnight and weighing 1·8–3·3 kg, were anaesthetized with sodium pentobarbitone, B.P.C. (35 mg kg^{-1}) I.P. Three catheters were inserted into a femoral vein (with gel foam between each catheter to prevent leakage) so that solutions of insulin, glucose and tryptophan could be injected simultaneously at various rates. A catheter was put into a femoral artery so that blood samples could be taken.

All chemicals were of analytical grade. The Radiochemical Centre, Amersham, Bucks., supplied the L-[^{14}C]tryptophan and A.B. Insulin Ltd, London, the bovine insulin, B.P. (40 u. $ml.^{-1}$).

Techniques of injection

Injections of L-[^{14}C]tryptophan (10 μc $ml.^{-1}$ in 0·9% Na Cl), of insulin (B.P., 40 u. $ml.^{-1}$) and of D-glucose (50% w/v) were made by means of three syringes, each driven by an electronically controlled variable rate drive mechanism (Pratt, 1974). Each solution was injected at a rate controlled by a pre-set schedule, which caused the syringe to deliver the substance at a rate similar to that at which it left the bloodstream. In this manner a steady level of each substance could be maintained independently in the bloodstream. The technique for the maintenance of steady levels of substances in the circulation of living animals is given in Daniel, Donaldson & Pratt, 1974, 1975, 1976; Donaldson & Pratt, 1975, whilst examples of steady levels may be seen in Baños, Daniel, Moorhouse & Pratt, 1973, 1975; Baños, Daniel & Pratt, 1974.

The measurement of the influx of tryptophan into the brain

The influx of L-tryptophan into the brain was measured by the initial rate at which tracer amino acid entered the brain from the blood. A steady level of L-[^{14}C]tryptophan was maintained in the circulation for 1 min, after which time the blood was rapidly washed out of the vessels (Daniel, Love, Moorhouse, Pratt & Wilson, 1974), the head was cut off by means of a guillotine and the brain removed and rapidly frozen. Influx was calculated by the use of the following equation:

$$v = \frac{R_b s}{R_p t}$$

where R_b is the level of radioactivity in the cerebral tissue, R_p is the mean level of radioactivity in the blood plasma, and s is the total concentration of L-tryptophan in the plasma; t is the duration

of the injection of L-[^{14}C]tryptophan: we assume that the injected [^{14}C]tryptophan exchanges rapidly with the tryptophan that is bound to albumin.

The influx of L-tryptophan was also measured in separate experiments in which steady raised levels of insulin, between 0·2 and 0·4 u. ml.$^{-1}$ of plasma were maintained in the circulation for periods ranging from 20 min to 2 hr. During these experiments the blood glucose was maintained within normal limits by a continuous programmed injection of glucose solution. At intervals throughout the period of injection arterial blood samples were taken for estimation of amino acid levels. The samples were also analysed for glucose, to confirm that the levels were steadily maintained. Then, simultaneously, an injection of L-[^{14}C]tryptophan was given during the final minute of the injection of insulin and of glucose and finally, as in the earlier experiments, the blood was rapidly washed out of the vessels, the head was cut off and the brain frozen.

Analytical methods

Glucose in whole blood was estimated by a spectrophotometric method, using hexokinase and glucose-6-phosphate dehydrogenase (Slein, 1963). The concentration of insulin in the plasma was measured by means of a radioimmunoassay kit, using a human insulin standard (Radiochemical Centre, Amersham).

Estimation of total tryptophan in plasma

Tryptophan was estimated in plasma essentially as described by Denckla & Dewey (1967) except that the addition of the ferric chloride solution was delayed until just before heating, as recommended by Bloxam & Warren (1974). In this method tryptophan is converted by condensation with formaldehyde in acid solution to norharman, which fluoresces strongly in ultraviolet light. Samples of plasma standards and blanks, each in duplicate, were subjected to the following procedure. 2 ml. 0·67 M-trichloracetic acid was added to 0·2 ml. plasma. After mixing and centrifugation (1500 *g*, 10 min) to precipitate the plasma proteins, 0·5 ml. of the supernatant was pipetted into a boiling tube. 0·003 M-ferric chloride solution (0·05 ml.) was added and the contents were mixed using a vortex vibrator. 0·06 M-formaldehyde solution (0·05 ml.) was added and the contents again mixed. The boiling tube was stoppered with a glass marble to prevent excessive loss by evaporation and the tube was heated in a vigorously boiling water bath for 1 hr. At the end of this period the tube was cooled rapidly to room temperature and a portion of the solution was transferred to a microcuvette. The fluorescence activation and emission spectra were measured using an Aminco–Bowman spectrophotofluorimeter. Similar activation and emission spectra were obtained from extracts of blood plasma and from standard solutions of tryptophan, with a peak of activation at 370 nm and a peak emission at 450 nm, as reported by Denckla & Dewey (1967). Fluorescence was found to be linear with tryptophan concentration up to 500 μmole per sample.

Estimation of free tryptophan in the plasma

Tryptophan is the only naturally occurring amino acid which binds to an appreciable extent to plasma protein. Under normal conditions most of the amino acid in the circulating blood is bound to the albumin (McMenamy, Lund, Van Marcke & Oncley, 1961) leaving approximately 10–20% in the free form in the blood plasma. This is available for uptake by the tissues. This unbound fraction can be separated from the bound fraction by ultrafiltration or by dialysis. In this study the former method was used and the concentration of free tryptophan was measured in ultrafiltrates of blood plasma.

A volume of freshly separated plasma (approx. 1·0 ml.) was placed in an ultrafiltration membrane cone (No. CF 50, Amicon Ltd, High Wycombe, Bucks.). The cone and its plastic support were inserted into the neck of a suitably shaped plastic collecting tube and the assembly was centrifuged (800 *g* for 30 min) at room temperature. The ultrafiltration was started as soon as possible after the blood had been taken in order to minimize any rise in the pH of the plasma, since any rise would increase the degree of binding of the tryptophan and thus lead to results which would give too low a value for the free tryptophan. The bulk of the plasma had passed through the membrane in less than 3 min. Aliquots of the filtrate, as well as of the plasma before ultrafiltration, were taken for determination of free and total tryptophan respectively, using the method described in the previous section.

Estimation of free tryptophan in brain tissue

Tryptophan was extracted from a weighed sample (1–2 *g*) of brain tissue by the method of Curzon, Joseph & Knott (1972) and the concentration of the amino acid in the extract was measured in the same way as that described above for measuring the tryptophan content of blood plasma.

Estimation of concentration of amino acids in blood

The concentrations of the other free amino acids in the blood plasma were determined as follows: the blood was centrifuged and the protein was removed from the plasma by centrifugal filtration through an ultrafiltration membrane cone. Separation of the amino acids was performed on an ion-exchange resin column, using gradient elution. An automatic amino acid analyser (Chromaspek, Rank Hilger Ltd, Margate) was used with norleucine as an internal standard, eluting the amino acids from the column with lithium citrate buffers.

Assay of radioactivity

Samples of brain were brought into solution for radioactive counting as follows: a weighed aliquot of the sample (approximately 50–100 mg) was placed in a glass scintillation counting vial to which was added a solution of 2 ml. of an organic base (Soluene X-100; 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 $l.^{-1}$ in toluene were added. Samples of plasma were prepared in a similar way except that only 1 ml. Soluene X-100 was used to dissolve 50 μl. plasma and only 0·15 ml. glacial acetic was needed to neutralize the solution before adding the scintillation mixture. The radioactivity in each sample was measured in a liquid scintillation spectrophotometer (Model 3375, Packard Instruments), correcting for quenching by the channels ratio method.

RESULTS

The effects of insulin on the levels of amino acids in the arterial blood

When raised levels of insulin (0·2–0·4 mu. $ml.^{-1}$ of plasma) were maintained in the circulation for periods of up to 2 hr (the blood glucose being kept within normal limits), during the first hour there was a steady and significant fall in the concentrations of several amino acids in the arterial blood, especially those which are essential for normal nutrition (Fig. 1, Table 1). The levels of the branched chain amino acids, L-leucine, L-isoleucine and L-valine were reduced most severely, low values being reached within 1 hr after the beginning of the injection of insulin (Fig. 1).

The effect of raised levels of insulin on the concentration of L-tryptophan was more complex, since the level of the total tryptophan (i.e. the free tryptophan plus that bound to plasma albumin) was not reduced but the concentration of the free tryptophan was reduced to less than half of that which was present before insulin was given (Table 1). As the total tryptophan was not reduced, it is clear that more of the free tryptophan had become bound to albumin as a result of the insulin injection.

Rate of equilibration of free and bound L-tryptophan in the blood

In order to determine the rate at which L-[^{14}C]tryptophan, free in the plasma, became bound to plasma albumin, experiments were done both *in vivo* and *in vitro*.

In vitro, tritium-labelled L-leucine was used as a reference substance, since it is a non-volatile material which does not bind appreciably to blood proteins. An aliquot of a reference solution, containing a mixture of [^{14}C]tryptophan and [^{3}H]leucine, in a previously determined ratio, was added to 1 ml. of freshly separated rabbit blood plasma contained in an ultrafiltration membrane cone. The solution was mixed rapidly with a vibrator and the ultrafiltrate was obtained as quickly as possible by centrifugation at 800 *g*. Further ultrafiltrates were prepared at intervals of up to an hour. In less than 0·5 min after mixing the solutions sufficient ultrafiltrate was obtained for assay of the two radioisotopes. Even in this first sample the proportion

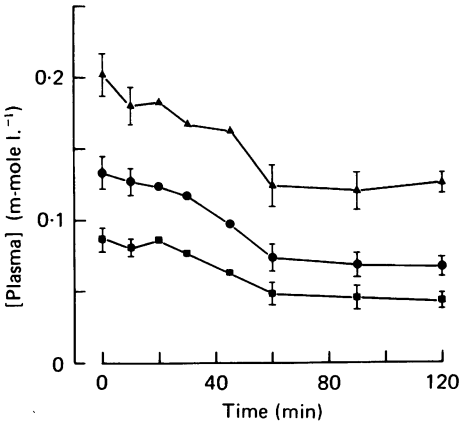

Fig. 1. The way in which a raised level of insulin which was maintained in the circulation over a two hour period by means of a specially programmed intravenous injection, caused a steady fall in the levels of branched-chain amino acids in the blood. The points represent either the results of single measurements or where bars are shown the mean and S.E. of mean of five experiments. ■, isoleucine; ●, leucine; ▲, valine.

TABLE 1. The effect of maintaining a steady raised level of insulin in the circulation (0·2–0·4 mu. ml.$^{-1}$) on various amino acids in the blood plasma. The development of hypoglycaemia was prevented by a simultaneous, continuous I.V. injection of glucose. Mean values ± S.E. with number of determinations in parentheses

	Concentration of amino acids in blood plasma (m-mole l.$^{-1}$)		
	Before insulin injection	After insulin had been injected for 60–120 min	Statistical significance of difference if any (*t* test)
*L-valine	0·198 ± 0·012 (12)	0·125 ± 0·007 (14)	$P < 0·001$
*L-isoleucine	0·086 ± 0·006 (12)	0·046 ± 0·004 (14)	$P < 0·001$
*L-leucine	0·132 ± 0·009 (12)	0·070 ± 0·004 (14)	$P < 0·001$
*L-lysine	0·116 ± 0·006 (12)	0·083 ± 0·004 (15)	$P < 0·001$
*L-methionine	0·022 ± 0·002 (10)	0·015 ± 0·002 (15)	$P < 0·02$
L-tyrosine	0·034 ± 0·003 (9)	0·023 ± 0·002 (11)	$P < 0·02$
*L-tryptophan†			
total	0·038 ± 0·008 (5)	0·041 ± 0·003 (4)	—
free	0·0066 ± 0·0004 (5)	0·0032 ± 0·0005 (4)	$P < 0·005$
*L-threonine	0·093 ± 0·005 (12)	0·077 ± 0·004 (15)	$P < 0·025$
*L-phenylalanine	0·046 ± 0·003 (12)	0·037 ± 0·003 (12)	$P < 0·05$
L-alanine	0·158 ± 0·018 (11)	0·179 ± 0·018 (15)	—
L-arginine	0·056 ± 0·006 (11)	0·052 ± 0·005 (12)	—
L-glutamate	0·071 ± 0·005 (12)	0·064 ± 0·005 (15)	-
L-glutamine	0·391 ± 0·114 (5)	0·311 ± 0·043 (12)	—
glycine	0·510 ± 0·060 (11)	0·509 ± 0·057 (14)	—
L-histidine	0·096 ± 0·006 (12)	0·089 ± 0·006 (14)	—
L-proline	0·081 ± 0·013 (4)	0·075 ± 0·004 (11)	—
L-serine	0·088 ± 0·008 (11)	0·086 ± 0·004 (15)	—
L-aspartate	0·018 ± 0·003 (11)	0·016 ± 0·002 (15)	—
L-cysteine	0·076 ± 0·006 (11)	0·069 ± 0·005 (12)	—

* Total = free plus bound tryptophan. Free tryptophan is that which is not bound to albumin.
† Nutritionally essential amino acids.

of [^{14}C]tryptophan which was not bound to plasma albumin had already been reduced to less than 17 % and this proportion did not change significantly over the next hour. Further experiments were done *in vivo* in which [^{14}C]tryptophan was given to a rabbit by a programmed I.V. injection and blood samples were withdrawn at timed intervals. The proportion of tryptophan which was not bound to plasma albumin did not alter appreciably from about 2 to 10 min. These results indicated that equilibration of the [^{14}C]tryptophan with the tryptophan already present in the blood plasma was substantially complete in well under 2 min, and probably in less than 0·5 min.

Tryptophan influx into brain

The influx of tryptophan into the brain was measured in normal animals and in a separate group of animals in which a raised level of insulin was maintained in the blood for 1–2 hr (the level of glucose being kept within normal limits). Despite the sharp fall in the free tryptophan level in the blood after the blood insulin had been raised, the influx of tryptophan into the brain increased slightly but not significantly (Table 2). However the increase of some three to four times in the ratio: influx/concentration of free tryptophan in the blood after insulin had been given was highly significant (Table 2). Like the influx, the concentration of tryptophan in the brain rose only slightly after giving insulin although the rise in the ratio of brain tryptophan: free tryptophan in the blood, was highly significant (Table 2).

DISCUSSION

We have shown previously that the transport mechanism that carries tryptophan across the blood–brain barrier has two components: a major saturable process, which is carrier-mediated, and a minor process which is not readily saturated when the concentration of tryptophan in the circulation is raised (Daniel *et al.* 1978*a*).

These two processes can be described by the following equation:

$$v = \frac{Vs}{s+K_a} + sD \tag{1}$$

where v is the observed influx when the concentration of tryptophan in the blood is s; D is the first order rate constant of the non-saturable process (possibly diffusion), V is the maximum rate of the saturable transport process and K_a is the apparent Michaelis constant of the latter process, i.e. the effective affinity constant under *in vivo* conditions. Pratt (1979*a*) has shown that K_a is related to the real Michaelis constant of the carrier K_t, as in the following equation:

$$K_a = K_t\left(1 + \sum_{n=1}^{n=n} I_n\right), \tag{2}$$

where $I_n = i_n/K_{i_n}$. The second term within the bracket represents the sum of the effects of a series of a number, n, of other inhibitory amino acids which are normally present in the bloodstream, each in a concentration of i_1, i_2, i_3, etc. and each having an inhibitor constant of K_{i_1}, K_{i_2}, K_{i_3}, etc.

We have found that fifteen amino acids compete, to a greater or lesser degree, with tryptophan for the transport carrier and have determined the value of K_i for each

TABLE 2. The effect of maintaining a steady, raised level of insulin in the circulation (0·2–0·4 mu. ml.$^{-1}$ of blood plasma) by continuous I.V. injection (for 1–2 hr) upon the influx of tryptophan into the brain and also upon the concentration of free tryptophan in the cerebral tissue. A normal level of blood glucose was maintained throughout each experiment. Mean values ± S.E. with number of determinations in parentheses

	Influx into brain (n-mole min^{-1} g^{-1} of brain)	Free tryptophan in plasma (n-mole ml.$^{-1}$ of plasma)	Ratio: $\frac{\text{influx into brain}}{\text{concentration of free tryptophan in plasma}}$ (n-mole min^{-1} g^{-1} of brain per μmole l.$^{-1}$ of free tryptophan in plasma)	Free tryptophan in brain (n-mole g^{-1} of brain)	Ratio: $\frac{\text{free tryptophan in brain}}{\text{free tryptophan in plasma}}$ (n-mole g^{-1} of brain per μmole l.$^{-1}$ of free tryprophan in plasma)
Before insulin	1·53 ± 0·20 (5)	6·6 ± 0·4	0·23 ± 0·03 (5)	19·8 ± 2·0 (4)	3·0 ± 0·3 (4)
After insulin	1·85 ± 0·74 (4)	3·2 ± 0·5	*0·58 ± 0·22 (4)	22·2 ± 0·7 (5)	*6·9 ± 1·1 (5)

* The difference between the ratios before and after giving insulin was statistically significant (t test, $P < 0·01$).

of these (Pratt, 1979*a*; Daniel *et al.* 1978*a*). From these data and the concentrations of the amino acids shown in Table 1 it can be calculated, using eqn. (2), that when insulin is given for 2 hr, the value of K_a for tryptophan is reduced from 1·07 to 0·83 n-mole $l.^{-1}$.

In order to determine whether this reduction in the value of K_a for tryptophan accounts for the increase in influx from 1·53 to 1·85 n-mole $min^{-1} g^{-1}$ of brain after giving insulin (Table 2), the influx rates must be calculated from eqn. (1). In this equation the mean value of V was taken to be 49 n-mole $min^{-1} g^{-1}$ of brain and of D to be 5 n-mole $min^{-1} g^{-1}$ of brain per mmole $l.^{-1}$ of tryptophan in the blood plasma (Pratt, 1979*a*). It is not clear, however, whether the value of s to be used in eqn. (1) should be the total concentration of tryptophan (i.e. both free and albumin bound) in the blood plasma or only that small part of the tryptophan which is not bound to albumin. Fernstrom, Hirsch, Madras & Sudarsky (1975) consider that the total concentration of tryptophan in the blood plasma is the important factor which determines the rate at which tryptophan enters the brain. They believe that much of the tryptophan can be stripped off the albumin to which it is bound, even during the very short time that the blood takes to pass through the cerebral capillaries.

Attempts to use in turn in eqn. (1) either the value for free or that for total tryptophan from Table 1 showed that our results were not consistent with either of the two extreme assumptions, i.e. that the influx of tryptophan into the brain depends only upon the level of free tryptophan in the blood or that it depends upon the total tryptophan level regardless of whether or not the tryptophan is albumin bound.

If we take s to be the concentration of free tryptophan in the blood, then when eqn. (1) is used with our data we should expect to find a fall in influx after insulin had been given, since the effect of the reduction in s would outweigh the increase in influx due to the reduction in value of K_a. On the other hand, if s were taken to be the total tryptophan in the blood, i.e. the concentration of bound plus free tryptophan then eqn. (1) would predict an increase in the influx of tryptophan of 37 % after insulin had been given, whilst we found only an influx of 21 % after insulin (Table 3). Thus, when we assess the supply of tryptophan to the brain we have to take into account three separate factors: (*a*) the degree to which the levels of other amino acids in the blood, which compete for the transport carrier, alter the influx of tryptophan, (*b*) the levels of free and of bound tryptophan in the blood plasma and (*c*) the ease with which the bound tryptophan can be stripped from the albumin in the plasma as the blood passes through the cerebral capillaries.

The solution to this problem was indicated by W. Pardridge (personal communication) who suggested that the carrier molecules in the walls of the cerebral capillaries are able to strip the protein-bound tryptophan away from the albumin to an extent which is determined by the relative affinities of the carrier molecule and of the albumin for the tryptophan. This, he suggests, works according to the following equation:

$$\nu_t = \frac{Vs_f}{K_a + s_f} + \frac{VFs_b}{K_a + Fs_b}, \tag{3}$$

where ν_t is the carrier-mediated influx, i.e. omitting the second term in eqn. (1), s_f is the concentration of tryptophan which is not bound to albumin and s_b is the

TABLE 3. Values of the variables for the calculation of F, the proportion of the tryptophan which is stripped from albumin during the passage of the blood through the cerebral capillaries, using eqn. (4)

	Before insulin	After insulin
[1]Influx of tryptophan into brain (ν)	1·53	1·85
[2]Concentration of free tryptophan in the blood (s_f)	0·0066	0·0032
[2]Concentration of albumin-bound tryptophan in the blood (s_b)	0·0314	0·0378
[3,4]Maximum influx of tryptophan into the brain (V)	49	49
[3]Michaelis constant (K_t)	0·2	0·2
[3,4]Apparent coefficient of diffusion (D)	5	5
[5]Sum of terms representing competition (ΣI_n)	4·33	3·13
[6]Apparent Michaelis constant (K_a)	1·07	0·83
[7]Fraction of tryptophan stripped off from albumin (F)	0·85	0·74

[1] From Table 2; [2] from Table 1; [3] from Pratt (1979*a*); [4] Daniel, Moorhouse & Pratt (1978*a*); [5] from values of i in Table 1, using values of K_i from Pratt (1979*a*, 1980); [6] from eqn. (2); [7] see below.

concentration of albumin-bound tryptophan. F is a factor which can vary between 0 and 1, depending upon the experimental conditions, and which represents the fraction of the tryptophan which is stripped off the albumin by the carrier-molecule. This explanation rests upon the assumption that tryptophan can be dissociated from the albumin molecule to an appreciable extent during the time that the blood takes to make one passage through the brain. That dissociation can occur very rapidly is confirmed by our results which show that free and bound tryptophan reach equilibrium very rapidly in the blood.

Combining eqns. (1), (2) and (3) we obtain an equation for influx:

$$\nu = \frac{Vs_f}{K_t\left(1+\sum_{n-1}^{n-n} I_n\right)+s_f} + \frac{VFs_b}{K_t\left(1+\sum_{n-1}^{n-n} I_n\right)+Fs_b} + Ds_f \qquad (4)$$

We have applied this equation to our data both before and after giving insulin in order to determine the value of F. All the other variables are known or could be calculated from the data in Table 3. Thus, the values of ν, s_f and s_b were those given in Tables 1 and 2. The values of V, K_t, D and K_i for each inhibitor were known from previous work (Pratt, 1979*a*, 1980). $\sum_1^n I_n$ was calculated from the known values of K_i and the value of i in Table 1 for each of the fifteen competing amino acids, before and after giving insulin. A value of F of about 0·8 was consistent with the interpretation of our data by eqn. (4). The slight fall in the value of F caused by

insulin (Table 3) is not unexpected since the fall in the ratio of K_{diss} (the dissociation constant of the tryptophan–albumin complex) to K_a, would lead to a relative increase in the affinity of the albumin in the blood for tryptophan compared with that of the carrier molecule in the wall of the endothelial cell of the brain capillary, which transports it across the blood–brain barrier (Pardridge, personal communication & 1979).

The values of K_{diss} will be given by:

$$K_{diss} = \frac{nAs_f}{s_b} \tag{5}$$

where n is the number of binding sites per molecule of albumin and A is the concentration of albumin, taken to be 1 and 0·5 mole $l.^{-1}$ respectively (c.f. McMenamy *et al.* 1961). This fall in the value of K_{diss} is due to the removal by insulin of a proportion of the free fatty acids which compete with tryptophan for the binding site on the albumin molecule (Curzon & Knott, 1974, 1975, 1977).

In assessing the effect of changes in the levels of tryptophan in the blood, allowance has to be made for a predictable proportion of the tryptophan which is bound to the albumin being stripped off from this protein during the passage of the blood through the cerebral capillaries, since the carrier molecule in the cell surface membrane of the endothelial cells has a greater affinity for the tryptophan molecule than has the albumin in the blood.

The figures given in Table 3 indicate that about 80 % of the tryptophan which bound to albumin is stripped off from the albumin during the passage of the blood through the capillaries of the brain. Under such conditions a fairly good correlation would be expected between influx of tryptophan and the *total* concentration of tryptophan in the plasma and this we have found to be the case. When Bloxam & Curzon (1978) compared the levels of tryptophan in the blood with the levels of tryptophan in the brain of rats which had had a portocaval anastomosis, they found, in contrast to our findings, a high correlation between the tryptophan *free* in the plasma and that in the brain, but a low correlation between the *total* tryptophan in the plasma and that in the brain. These correlations were also found in the control groups. The difference between our findings and those of Bloxam & Curzon may well be explained by a species difference (between rats and rabbits) in the value of F (the fraction of the bound tryptophan which is stripped off the albumin, eqn. 3). The value of F is dependent upon the relative affinity for tryptophan of the transport carrier in the endothelium of the cerebral capillaries and that of the albumin in the blood plasma (Pardridge, 1978). One or other of these affinities may be changed by a variety of experimental conditions.

In conclusion, insulin removes from the bloodstream a large proportion of those amino acids that would otherwise compete with tryptophan for the shared transport system which operates across the blood–brain barrier, thus increasing the influx of tryptophan into the brain. On the other hand insulin not only increases the proportion of the blood tryptophan that is bound to albumin, but, what is more important, makes it more difficult for the transport carriers in the walls of the cerebral capillaries to strip off the tryptophan from the albumin to which it is bound. Thus the increase caused by insulin in the influx of tryptophan into the brain across the blood–brain barrier is less than might be expected.

The net result of these two opposing effects is that insulin causes only a slight increase in the supply of tryptophan to the brain.

This work was supported by grants from the Wellcome and Kennedy Trusts and the National Fund for Research into Crippling Diseases. P. M. Daniel held an Emeritus Fellowship from the Leverhulme Tust.

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