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SATURATION OF A SHARED MECHANISM WHICH TRANSPORTS L-ARGININE AND L-LYSINE INTO THE BRAIN OF THE LIVING RAT

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SUMMARY

1. The rate of entry of L-arginine and L-lysine into the brain of the rat was measured *in vivo* by a direct method in which the amino acid concentration was at a constant level in the blood plasma over the period of the experiment.

2. Both L-arginine and L-lysine enter the brain by a transport mechanism which can be saturated by a high concentration of the same amino acid in the bloodstream. The rate of entry can be explained by Michaelis–Menten kinetics.

3. The entry into the brain of L-arginine can be inhibited by raised plasma concentrations of L-lysine or L-ornithine and the entry of L-lysine by raised concentrations of L-arginine.

4. The inhibition of entry of an amino acid is most severe when its own concentration in the blood plasma is low and that of the inhibitor is high. The inhibition appears to be basically competitive in type, suggesting that common transport systems are shared by three dibasic amino acids.

5. It is suggested that the raised levels of amino acids found in various disorders of amino acid metabolism are likely to reduce the rate of entry into the brain of other amino acids and a way is suggested in which the dietary treatment of hyperlysinaemia may be made more effective.

INTRODUCTION

Blasberg & Lajtha (1965) obtained indications, *in vitro*, that high concentrations of one amino acid could inhibit the entry of another into brain slices. In an *in vivo* study we showed that the relative rates of entry of various amino acids were quite different from the rates reported *in vitro*. We found a wide spread in the rates of entry which could not be explained on the basis of differences in the physico-chemical properties of the amino

acids and we thus concluded that entry took place by means of selective transport processes (Baños, Daniel, Moorhouse & Pratt, 1973*a*). We had earlier obtained evidence that saturation of the transport system occurred at high concentrations of amino acids in the plasma (Baños, Daniel, Moorhouse & Pratt, 1970).

In this paper we have tried to find out in the living animal to what extent dibasic amino acids enter the brain by saturable transport processes and whether such transport processes are shared by more than one amino acid. We have chosen the dibasic amino acids L-arginine and L-lysine for special study since they are needed by the brain for the synthesis of basic proteins.

A preliminary note of the findings has already appeared (Baños, Daniel & Pratt, 1971).

METHODS

Albino rats aged 6–8 weeks of age were used, fed *ad libitum* on a commercial pelleted diet containing 17·6% of digestible protein (including 1·0% L-arginine and 1·2% w/w L-lysine). Amino acids (obtained from Koch-Light Laboratories Ltd., Bucks., England) and all other chemicals were of analytical grade. The ^{14}C-labelled L-arginine, L-lysine and L-ornithine were obtained from the Radiochemical Centre, Amersham, England. Under light ether anaesthesia (sodium pentobarbitone 35 mg/kg was used in a few experiments) a cannula was inserted into a femoral artery for withdrawal of blood and one or two femoral veins were cannulated for injections.

The maintenance of a steady level of radioactively labelled substances in the bloodstream

Experiments were carried out to determine the rate at which a radioactively labelled amino acid had to be injected in order to maintain a steady level in the blood over a short period. The rate at which the radioactively labelled substance disappeared from the bloodstream was determined as follows. A rapid I.V. injection of a known quantity of the radioactively labelled substance was given. Arterial blood samples were taken at intervals after this injection to record the fall in the level of radioactivity in the blood (Fig. 1). This experiment was repeated on three animals for each amino acid and at different plasma concentrations for each amino acid. From a mathematical analysis of these data (Daniel, Donaldson & Pratt, 1973) programmes were devised for each amino acid to specify the way in which the rate of injection had to be changed in order to maintain a constant concentration of the radioactively labelled material in the blood plasma. By means of an electronically driven syringe a loading dose was injected at high speed during the first 10 sec so as to raise the blood level rapidly. The rate of injection was then reduced progressively until the end of the experiment so that the follow-up dose maintained the blood level reached by the loading dose (Fig. 1). The reduction in the rate of injection needed to maintain a steady level is due to the increasing concentration of the radioactive material in the extravascular compartment or compartments (skeletal muscle forms one such compartment into which amino acids pass; Baños, Daniel, Moorhouse & Pratt, 1973*b*). Fluctuations in blood level were usually less than $\pm 3\%$ and the rate of drift was less than 1% per minute. The length of the injection was usually 3 min. This period was chosen because in so short a time it seemed unlikely that metabolic changes would have taken place which might lead to some of the radioactive label becoming detached. At the end of the period of injection the vascular

system was rapidly washed out with Krebs–Ringer solution (Krebs & Henseleit, 1932) at 37° C, the animal decapitated and the cerebral hemispheres removed for assay of the radioactive material present. Three arterial blood samples were taken during the injection, as in Fig. 1, to confirm that the mean concentration and specific activity in the blood plasma of the radioactively labelled amino acid were maintained steadily.

Two groups of experiments were done. In one group a single amino acid was injected, different concentrations, in separate experiments, being maintained in the bloodstream in order to study the effect of raised concentrations of the amino acid on its own entry into the brain. In the other group two amino acids were injected simultaneously in order to study the inhibitory effect of one on the entry into the brain of the other.

In some experiments the ^{14}C-labelled amino acid given was of high specific activity so that its concentration in the blood was not raised appreciably above the normal

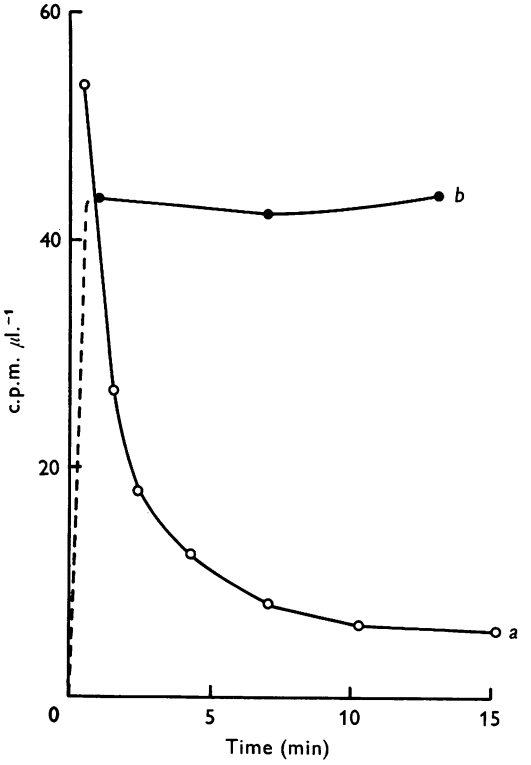

Fig. 1. Curve *a* shows rate at which [^{14}C]L-arginine (○), which is given as a rapid I.V. injection (at zero time), leaves the blood. Curve *b* shows the way in which a steady concentration of [^{14}C]L-arginine (●) may be maintained in the blood plasma by means of an injection at a variable rate by an electronically controlled syringe on the basis of calculations derived from the data of curve *a*. The I.V. injection starts at zero time. Although most experiments lasted only 3 min this longer experiment is shown in order to demonstrate that the steady concentration may be maintained for considerable periods.

level. In other experiments for each animal the injection consisted of a radioactively labelled amino acid of low specific activity prepared by diluting a high specific activity U-^{14}C labelled amino acid with the same amino acid but unlabelled. This injection did raise the blood level of the amino acid above normal and also provided tracer for determining the rate of entry into the brain. In different experiments a wide range of raised concentrations were maintained in the blood.

Analytical and assay methods. The cerebral hemispheres were rinsed briefly in saline, blotted, and weighed. Plasma was separated rapidly from heparinized blood. Samples of brain or of blood plasma were prepared in two different ways for radioactive counting. Brain: (*a*) to a weighed sample of brain a solution of 2 ml. of an organic base Soluene X-100 Packard Instruments) was added. When the brain was dissolved (usually 1–2 days) glacial acetic acid (0·1–0·2 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 per litre toluene were added; (*b*) the cerebral hemispheres were ground up in a Pyrex tube, using a Teflon pestle, in a known volume of 0·01 M sodium ethylene diamine tetraacetate and aliquots of this suspension were oxidized according to the method of Mahin & Lofberg (1966). Plasma samples were prepared in a similar way except that in method *a* 0·1 or 0·05 ml. plasma was dissolved in only 1 ml. Soluene X-100. The radioactivity in the samples of brain or plasma was measured in a scintillation spectrophotometer (Model 3375, Packard Intruments). No difference was found between the results obtained when separate aliquots of plasma were prepared in turn by methods *a* or *b*.

To determine the concentrations of the free amino acids in the blood plasma 1% picric acid solution (5 vol.) was added to freshly separated heparinized blood plasma. The picric acid was removed by adsorption on Dowex 50 resin and concentrations of the amino acids were determined in the extract after separation on an ion-exchange column (Stein & Moore, 1954) by means of a BC 200 automatic amino acid analyser (Biocal Instruments Ltd.), eluting with lithium citrate buffers (Atkin & Ferdinand, 1970). Arginine was also determined separately in some experiments using the Sakaguchi reaction (Sakaguchi, 1951; Jepson & Smith, 1953).

RESULTS

Calculation of rates of entry of amino acids into the brain

The rates of entry were calculated from the rate of accumulation of radioactive material in the brain.

The mean concentration of radioactive material in the brain (R_c) at the end of an injection lasting t min was determined, as was that in the arterial blood plasma (R_a) during the injection. The rate of entry (v) was calculated from

$$v = \frac{R_c s}{R_a t},$$

where s is the concentration of the amino acid in the arterial blood plasma during the injection.

Effect of different anaesthetics on the rate of entry of amino acids into the brain

In a short series of experiments in which sodium pentobarbitone was used as the anaesthetic instead of ether, no appreciable difference in the rate of entry was observed.

The rate of accumulation of radioactively labelled L-*arginine and* L-*lysine in the brain*

In a series of rats constant levels of [^{14}C]L-arginine were maintained in the blood plasma for times ranging from 2 to 20 min. It will be seen that accumulation occurred rapidly at first and then more slowly after 5–10 min (Fig. 2). In another series of rats sufficient unlabelled L-arginine was injected together with the radioactively labelled amino acid to raise its concentration in the blood plasma well above normal. The rate of accumulation of the [^{14}C]L-arginine in the brain was proportional to the length of the

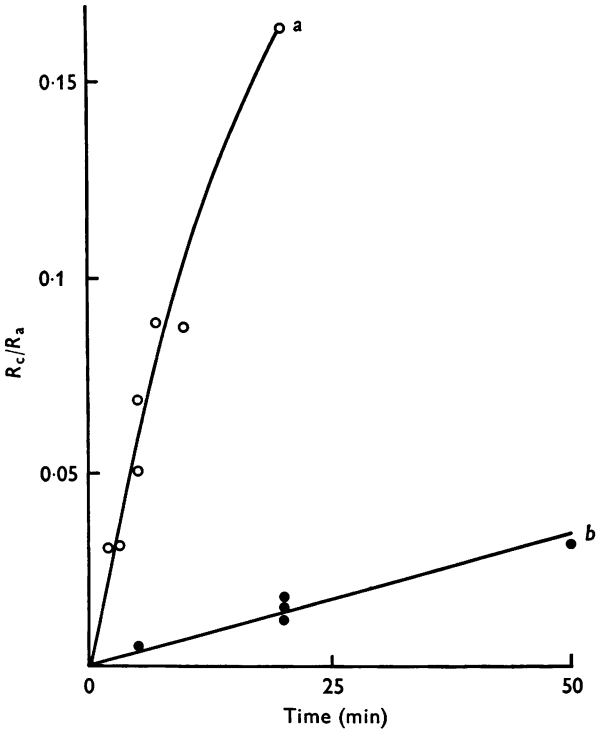

Fig. 2. Shows the rate of accumulation of [^{14}C]L-arginine in the brain during experiments in which steady concentrations were maintained in the blood plasma. The rate of accumulation is indicated by the ratio of radioactivity in the brain, R_c, to that in the arterial blood plasma, R_a.

In the first series of experiments (curve a) in which each experiment is represented by ○, the concentration of L-arginine in the blood plasma was within the normal range. In the second series (curve *b*) in which each experiment is represented by ●, the concentration of L-arginine in the blood plasma was raised to approximately 250 times the normal. The flatter slope of the second curve indicates the extent to which the transport system has been saturated by the high concentration of the amino acid in the blood plasma.

injection up to at least fifty minutes (Fig. 2). Similar results were obtained with [^{14}C]L-lysine.

The effect of raising the concentration of L-*arginine or of* L-*lysine in the blood plasma upon the rate of its entry into the brain*

The rate of entry of L-arginine (Fig. 3) was measured in seventeen rats and that of L-lysine (Fig. 4) in eleven rats. In each successive experiment a higher level of the amino acid was maintained in the bloodstream. The relation between the rate of entry into the brain and the plasma concentration of each amino acid was approximately linear at physiological concentrations, but when the blood concentration was raised above the normal range the relation soon became non-linear giving clear evidence that a transport mechanism that determined the rate of entry was becoming saturated (Figs. 3 and 4).

When the data shown in Figs. 3 and 4 were re-plotted as s/v against s (Dixon & Webb, 1964) the points could be fitted reasonably well by straight lines (Figs. 5 and 6).

The interference with the entry of one dibasic amino acid into the brain by a raised plasma concentration of another

Table 1 shows the effect of raised concentrations of L-lysine or of L-ornithine in the blood upon the rate of entry of radioactively labelled L-arginine into the brain and the effect of raised concentrations of L-arginine upon the rate of entry of radioactively labelled L-lysine. It will be seen that the rate of entry of L-arginine was reduced when the concentrations in the blood of either L-lysine or L-ornithine was raised. The mean reduction in the rate of entry of L-arginine into the brain in experiments 1 to 16 was $48 \pm 6\%$. This reduction was statistically significant ($P < 0{\cdot}001$, t test). The inhibition of entry of L-arginine tended to be greater at higher concentrations of L-lysine. Thus in experiments 1 to 6 (Table 1) in which the concentration of L-lysine in the blood plasma was raised to only moderately high levels, the mean reduction ($18{\cdot}8\%$) in the rate of entry of L-arginine was less than the mean reduction ($59{\cdot}4\%$) in experiments 7 to 14 in which the L-lysine concentration was raised to still higher levels. The difference between the mean reduction in entry rate in experiments 1 to 6 and that in experiments 7 to 14 was significant ($P < 0{\cdot}001$, t test). In experiments 17 to 19 (Table 1) in which L-ornithine was injected in place of L-lysine the mean reduction in the entry of L-arginine of 44% was also statistically significant ($P < 0{\cdot}05$, t test). In experiments 20 to 23 a raised concentration of L-arginine caused a considerable reduction in the entry rate of L-lysine, which was statistically significant ($P < 0{\cdot}001$, t test).

The results of experiments 8 to 14 (Table 1) in which the rate of entry of

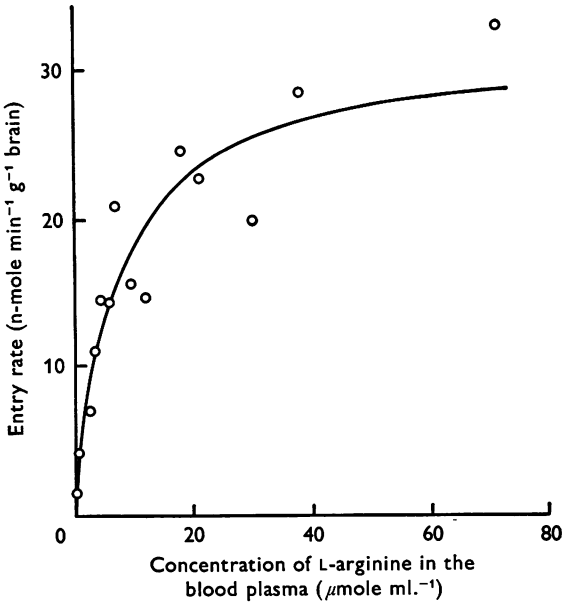

Fig. 3. The effect of raising the concentration of L-arginine in the blood plasma upon its rate of entry into the brain. The point nearest to the origin represents four separate experiments.

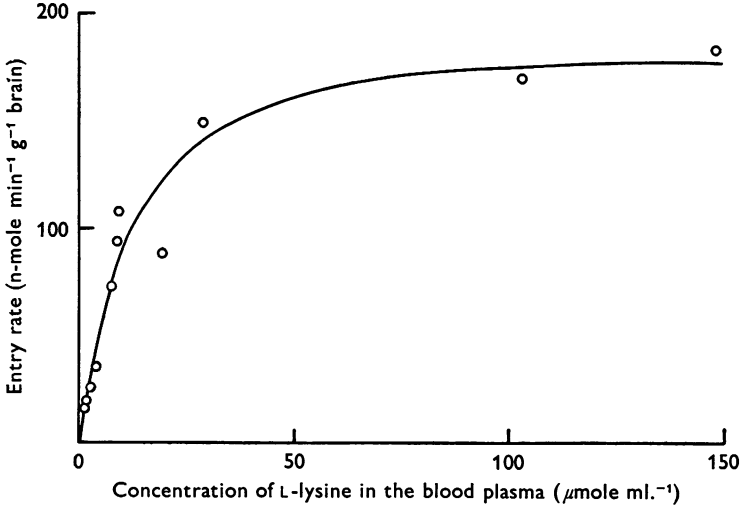

Fig. 4. The effect of raising the concentration of L-lysine in the blood plasma upon its rate of entry into the brain.

L-arginine had been measured in the presence of a high concentration of L-lysine as an inhibitor (of the order of 16 μmole ml.$^{-1}$) were plotted as the reciprocal of the entry rate against the reciprocal of the concentration of L-arginine in the plasma (Dixon & Webb, 1964). In the different experiments the concentration of L-arginine in the plasma was raised and it was found that the rate of entry of L-arginine increased in spite of the high

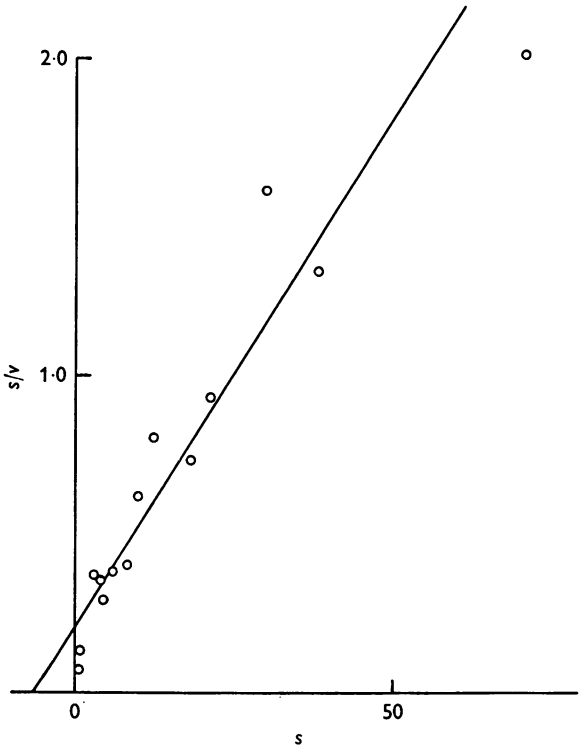

Fig. 5. The ratio of the concentration of L-arginine in the blood plasma to its entry rate, s/v, plotted against its concentration in the blood plasma, s (Dixon & Webb, 1964). The best straight line has been fitted to the points by the method of least squares.

concentration of L-lysine in the plasma (Fig. 7). For comparison a similar plot is shown of the results of experiments in which the level of L-lysine in the plasma was not raised. The best straight line was fitted to each of these sets of points by linear regression. The line fitted to the points when the plasma concentration of L-lysine was raised had a steeper slope than when the L-lysine was at a normal level (Fig. 7).

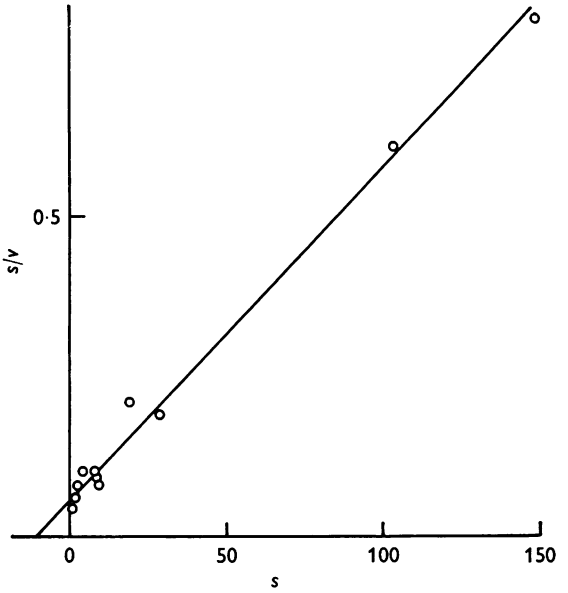

Fig. 6. The ratio of the concentration of L-lysine in the blood plasma to its entry rate, s/v, plotted against its concentration in the blood plasma, s (Dixon & Webb, 1964). The best straight line has been fitted to the points by the method of least squares.

DISCUSSION

We have shown in earlier work that *in vivo* there is a great variation in the rates of entry into the brain of various amino acids and that a moderate increase in the concentration of any one of them leads to a more or less proportionate increase in the rate of its entry. Since the differences in the rates of entry of these amino acids could not be explained by any obvious differences in their molecular weights or by any other of their physicochemical properties we concluded that diffusion plays little part in the process of entry and that specific carrier-mediated transport mechanisms must be operating in the living animal (Baños, Daniel, Moorhouse & Pratt, 1973*a*).

In the experiments described here we confirmed our earlier findings that the rate of entry of either one of the dibasic amino acids L-arginine or L-lysine into the brain was proportional to its own concentration in the blood throughout a range of normal levels. However, when the blood concentration of either of these amino acids was raised above physiological levels the rate of entry into the brain ceased to be proportional to the blood level and increased less rapidly than at lower concentrations (Figs. 3 and 4).

TABLE 1. The effect of raising the concentration of one amino acid in the blood stream upon the rate of entry of another amino acid into the brain (concentrations of amino acids in blood plasma in μmole ml.$^{-1}$)

Effect of a raised concentration of L-lysine upon the entry of L-arginine into the brain

Expt. no	Concentration of L-arginine (radioactively labelled) in the blood plasma	Concentration of L-lysine in the blood plasma	Rate with raised L-lysine / Rate with normal L-lysine (%)	
1	0·22	1·4	110	
2	0·48	1·8	66	
3	4·2	1·96	84	
4	8·8	2·6	96	Mean = 81·2 ± 7·9 %
5	2·9	2·5	72	
6	1·8	3·6	59	
7	0·1	11·4	45	
8	0·1	14·3	43	
9	0·40	14·7	15	
10	1·99	14·8	40	
11	8·4	16·3	69	Mean = 40·6 ± 6·1 %
12	4·5	17·0	51	
13	0·1	17·1	43	
14	0·88	19·3	19	
15	0·36	24·8	8·9	
16	0·1	52	8·8	

Effect of a raised concentration of L-ornithine upon the entry of L-arginine into the brain

	Concentration of L-arginine (radioactively labelled) in the blood plasma	Concentration of L-ornithine in the blood plasma	Rate with raised L-ornithine / Rate with normal L-ornithine (%)	
17	1·07	5·75	79	
18	1·16	6·8	42	Mean = 56 %
19	1·95	7·85	46	

Effect of a raised concentration of L-arginine upon the entry of L-lysine into the brain

	Concentration of L-lysine (radioactively labelled) in the blood plasma	Concentration of L-arginine in the blood plasma	Rate with raised L-arginine / Rate with normal L-arginine (%)	
20	0·3	3·8	4·5	
21	0·3	4·2	5·3	
22	0·3	4·0	6·7	Mean = 6·8 ± 1·4 %
23	0·3	4·8	10·7	

The saturation of the mechanism of transport into the brain of these two amino acids which occurs when the plasma concentration of either is raised above the physiological range confirms that carrier mediated transport mechanisms are operating. Michaelis–Menten kinetics (Figs. 5 to 7) represent the behaviour of these transport mechanisms reasonably well.

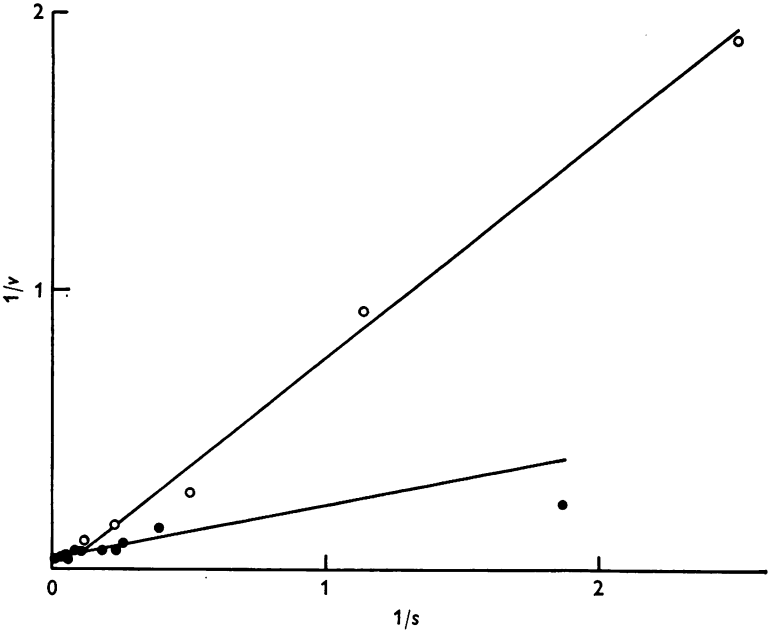

Fig. 7. To show the inhibition of the entry of L-arginine into the brain when the L-lysine concentration in the blood plasma is raised to a high level. The reciprocal of the rate of entry of L-arginine into the brain, $1/v$, is plotted against the reciprocal of its concentration in the blood plasma $1/s$ (Dixon & Webb, 1964).

The series of experiments in which the concentration of L-lysine in the plasma was raised to levels of the order of 16 μmole ml.$^{-1}$ are shown by ○. Control experiments in which the concentration of L-lysine was within normal limits are shown by ●. The best straight line has been fitted to each set of points by the method of least squares.

In addition we have found that there is inhibition of the entry of L-arginine into the brain if the concentration of L-lysine or of L-ornithine is raised to levels above the physiological while inhibition of the entry of L-lysine is caused by a raised plasma level of L-arginine (Table 1). This shows that these dibasic amino acids share one or more common transport systems (similar shared transport mechanisms for the same group of amino acids are found in the kidney; see Rosenberg, Downing & Segal, 1962) and that a high plasma concentration of one of them saturates the

mechanism and thus causes a slowing down of the rates of entry of the others. However, the rates of entry of the inhibited amino acids may be increased to some extent by raising their levels in the blood plasma, i.e. the effect of the inhibitor can be overcome.

The competitive nature of the inhibition when transport mechanisms are shared may be of practical importance in the treatment of the amino-acidurias. These are disorders in which raised plasma levels of one or more amino acids are associated with mental retardation (Crome & Stern, 1972). In hyperlysinaemia, for example (Woody, 1964) the mental retardation may be due to the excess of lysine entering the brain (there is often a ten-fold increase in the concentration of lysine in the blood) but may also be due in part to a diminished rate of entry of other amino acids, e.g. arginine. Both L-arginine and L-lysine are needed by the brain for the synthesis of protein throughout life especially during the first weeks after birth, in the rat, when the brain is growing rapidly (Dobbing, 1968).

The findings reported in this paper suggest possible means of treatment. If amino acid A is present at a high concentration in the plasma and is inhibiting the entry into the brain of amino acids B and C, then raising the concentrations of B and C (possibly by dietary means) should ensure that the supply of these two amino acids to the brain is increased. This step combined with a lowering of the level of A in the plasma might diminish the risk of the development of mental retardation in such a hypothetical disorder of amino acid metabolism.

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