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THE INFLUX OF AMINO ACIDS INTO THE HEART OF THE RAT

By GUADALUPE BAÑOS, P. M. DANIEL,* S. R. MOORHOUSE O. E. PRATT AND PENELOPE A. WILSON

*From the Department of Neuropathology, Institute of Psychiatry, De Crespigny Park, London SE5 8AF, and * The Department of Applied Physiology and Surgical Science, Royal College of Surgeons, Lincoln's Inn Fields, London WC2A 3PN*

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SUMMARY

1. The influx of nineteen amino acids into the heart of the living rat was studied by a method specially devised for experiments under controlled conditions *in vivo*.

2. When, in separate experiments, the concentration of each amino acid in turn was artificially raised in the circulation, the influx of that amino acid into the heart increased.

3. Our data indicate that at least ten of these amino acids enter the heart *in vivo* by means of saturable carrier-mediated transport systems. The transport rates conform, at least approximately, to Michaelis kinetics and the transport systems are clearly, in the case of many amino acids, active, i.e. energy-dependent.

4. The amino acids which were studied had rates of influx into the heart which differed from each other over a range of more than 10 to 1, even when allowances were made for the difference in their concentration in the circulating blood. These differences in influx were not related to such factors as the molecular size of the individual amino acids.

5. The amino acids which have a high influx into the heart are mainly those which are needed either to re-synthesize contractile protein or as oxidizable substrates.

INTRODUCTION

We are not aware of any work in which the influx into the heart of a large range of amino acids has been studied in the living animal. Most of the work has been done *in vitro*, or on the isolated heart, with the disadvantage that the fluids used are devoid of many accessory factors, such as vitamins and hormones.

We have investigated the influx of nineteen amino acids into the heart of the living rat, using a technique by which a steady level of an amino acid and/or a steady level of specific activity of radioactive labelling can be maintained in the circulation. This method allows accurate, quantitative measurements of influx into cardiac muscle to be made. We have previously used this technique extensively to investigate the influx of amino acids and glucose into brain and skeletal muscle, as well as to study the influx into the brain of those amino acids which are precursors of neurotransmitters (Bachelard, Daniel, Love & Pratt, 1973; Baños, Daniel, Moorhouse & Pratt, 1973*a*, *b*, 1974, 1975; Daniel, Love & Pratt, 1975*b*, *c*, 1977; Daniel, Moorhouse &

Pratt, 1976; Pratt, 1976) and have made a preliminary note on the entry of amino acids into the heart of the growing rat (Baños, Daniel, Love, Moorhouse & Pratt, 1971).

METHODS

Young adult male Wistar rats, 7–10 weeks old and weighing between 150 and 250 g, were used. The animals were maintained on a commercial pelleted diet containing 20·4% of protein (Oxoid, breeding diet) given *ad libitum*. They were not fasted before the experiments. Amino acids (purchased from Koch–Light Laboratories Ltd, Bucks., England, or Cambrian Chemicals Ltd, Croydon, England) and all reagents used were of analytical grade. The amino acids (shown in Table 1) labelled with ^{14}C or ^{35}S and human serum albumin labelled with ^{125}I, were obtained from the Radiochemical Centre, Amersham, England.

TABLE 1. Influx of amino acids into the heart under normal conditions

Amino acid with nutritional category*		Influx (n-mole min^{-1} g^{-1} of heart)	$\frac{\text{Influx}}{\text{Concentration in plasma}}$ ($\frac{\mu\text{mole } min^{-1} g^{-1} \text{ of heart}}{\text{mM in plasma}}$)	Molecular weight	Radioactive label (with position in the molecule in parenthesis)
L-phenylalanine	E	12·1	0·243 ± 0·010 (12)	165	^{14}C(U)
L-tyrosine	N	10·8	0·188 ± 0·010 (7)	181	^{14}C(U)
L-dopa	NP	—	0·144 ± 0·011 (3)	197	^{14}C(3–)
L-leucine	E	15·9	0·136 ± 0·017 (4)	131	^{14}C(U)
L-tryptophan†	E	8·0	0·123 ± 0·009 (8)	205	^{14}C(methylene)
L-histidine	E	9·8	0·121 ± 0·012 (9)	157	^{14}C(ring 2-)
L-methionine	E	5·0	0·120 ± 0·008 (5)	149	^{14}C(methyl)
L-serine	N	16·6	0·106 ± 0·006 (5)	105	^{14}C(U)
L-cysteine	N	10·0	0·103 ± 0·018 (5)	121	^{14}C(U) or ^{35}S
L-lysine	E	29·8	0·085 ± 0·017 (5)	183	^{14}C(U)
L-valine	E	10·6	0·068 ± 0·004 (6)	117	^{14}C(U)
L-arginine	E	6·8	0·059 ± 0·005 (5)	211	^{14}C(U)
L-threonine	E	11·9	0·056 ± 0·008 (4)	119	^{14}C(U)
L-isoleucine	E	3·9	0·054 ± 0·007 (4)	131	^{14}C(U)
glycine	N	8·8	0·044 ± 0·009 (5)	75	^{14}C(U)
L-aspartate	N	0·48	0·034 (2)	133	^{14}C(U)
L-proline	N	4·4	0·032 ± 0·004 (5)	115	^{14}C(U)
taurine	NP	1·06	0·023 ± 0·0014 (5)	125	^{35}S
2-aminoisobutyrate	NP	—	0·020 ± 0·0026 (11)	105	^{14}C(1–)

* E = essential or essential for growth, N = non-essential, NP = not found in mammalian protein. Number of experiments in parentheses.

† The binding of tryptophan to albumin in the plasma has been ignored for the purpose of this comparison.

Operative procedures

The rats were either anaesthetized with ether or I.P. sodium pentobarbitone, B.P.C. (35 mg/kg body wt.). A cannula for withdrawing blood, and for washing out the vascular system at the end of the experiment, was inserted into a femoral artery and one or both femoral veins were cannulated so that I.V. injections could be given.

The maintenance of a steady level of various substances in the bloodstream

Electronically controlled motor driven syringes (Pratt, 1974) were programmed to maintain constant levels of the various radioactively labelled or unlabelled amino acids, or of [^{125}I]human serum albumin, in the circulation over the experimental period. The programmes were developed

from a mathematical analysis of the rate at which each radioactively labelled amino acid, or at which human serum albumin, was removed from the bloodstream after a single rapid intravenous injection (Daniel, Donaldson & Pratt, 1974*a*, 1975*a*).

In order to determine the way in which raised concentrations of an amino acid in the blood affected the rate at which the same amino acid, radioactively labelled, left the circulation, a further series of experiments was done. In these experiments various raised concentrations of unlabelled amino acids were maintained in the circulation for 2 min, immediately before radioactively labelled amino acid was given as a rapid I.V. injection (for examples see Daniel *et al.* 1975*a*; Donaldson & Pratt, 1975). The rate at which an amino acid leaves the circulation and the way in which a steady level can be maintained in the circulation is shown in Fig. 1. Whenever it is stated that an injection is given, it means that a steady level of the substance was maintained in the circulation throughout the experiment.

Determination of the influx of the various amino acids into the heart

Influx was determined by maintaining a steady level of radioactively labelled amino acid, as a marker, in the circulation. A continuous I.V. injection of radioactively labelled amino acid was given and small arterial blood samples were taken at intervals throughout the injection to check that a constant level of radioactivity was maintained. Fluctuations in blood level were usually

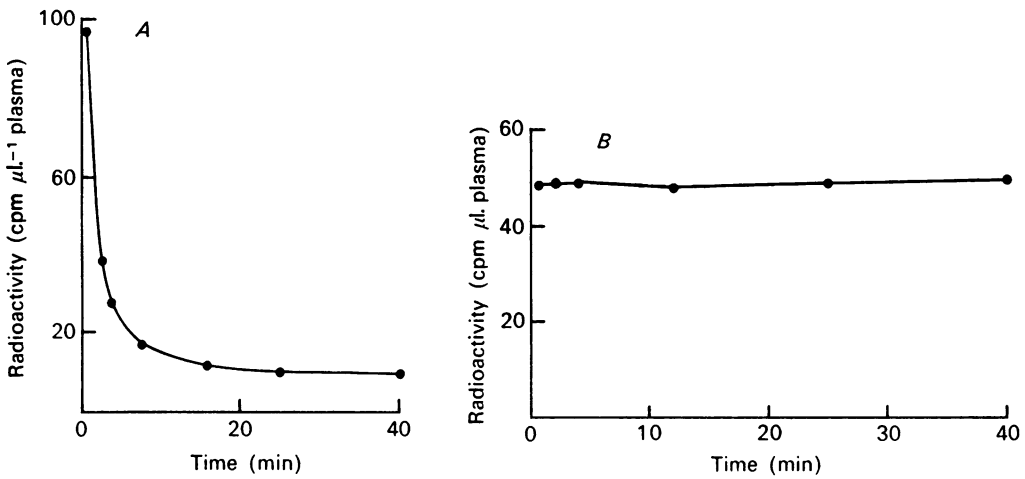

Fig. 1. *A*, the rate at which radioactivity leaves the circulation. A rapid I.V. injection of L-[^{14}C]valine was given at zero time and a series of arterial blood samples, ●, were taken. The rate at which the radioactivity left the blood plasma is shown by the curve. *B*, the accuracy with which a steady level of L-[^{14}C]valine could be maintained in the circulating blood. The continuous I.V. injection, given at a programmed rate, started at zero time. The radioactivity in each of a series of arterial blood samples, ●, which were taken at intervals during the injection, is shown.

less than ±3% and the rate of drift was less than 1% per min (Fig. 1*B*). At the end of an injection, lasting from 1 to 40 min, the jugular veins were opened and the blood was washed out of the vascular system (for 20 sec) with Krebs–Ringer solution (Krebs & Henseleit, 1932) or with normal saline, by means of a pressure apparatus (Daniel *et al.* 1974*b*). The animal was killed by decapitation. The heart was rapidly removed, rinsed briefly in saline, blotted and frozen in hexane at −70 °C (in less than 4 sec from the end of the washout). Samples were stored at −15 °C until required for assay.

In some experiments two syringes were used to give simultaneous injections. The programmes that controlled the rates of injection given by these syringes had been calculated by the methods described in Daniel *et al.* (1975*a*). Thus a particular unlabelled amino acid could be injected at such a rate that its concentration in the circulation was rapidly (within 5 sec) raised to a pre-

determined, steady level. After this injection had been given for 1–2 min, a second injection was begun which maintained a steady level of the same amino acid, radioactively labelled as a marker. By this means it was possible to measure the influx of an amino acid into the heart when the concentration of that amino acid in the blood varied over a wide range.

Measurement of the residual blood left in the heart after washing out

In a series of experiments a radioactive marker of blood protein was used. A steady level of [^{125}I]human serum albumin was maintained by I.V. injection in the circulating blood plasma during a short (usually 2 min) period. At the end of the injection a blood sample was taken and the heart was removed. The radioactivity in a given weight of heart muscle was measured and compared with that in a known volume of the blood plasma. The procedure was repeated in similar experiments after the blood had been washed out of the vascular system for various periods.

Removal of mannitol from the non-cellular tissue of the heart by washing out the vascular system

In order to determine the extent to which substances could be washed out of the extracellular space, this space was first filled with a substance which does not enter the cells of the heart, [^{14}C]mannitol. This was done as described by Daniel *et al.* (1975*b*, *c*). In a series of experiments [^{14}C]mannitol was injected at a rate which raised its concentration in the circulation to a required level quickly (within 5 sec) and maintained it at that level. In successive experiments this injection was continued for progressively longer periods. At the end of each experiment the radioactivity in the heart, i.e. that in the blood within the cardiac tissue and in the extracellular space, was measured.

In a further series of experiments the [^{14}C]mannitol was injected during a period of 20 min and then the blood was washed out of the circulation for 20 sec. After this the radioactivity in the extracellular space was calculated in order to determine what proportion of it had been removed by washing out the vessels. The radioactivity in the residual blood in the cardiac vessels (as determined from the previous experiments in which [^{125}I]human serum albumin had been used) was subtracted from the total radioactivity in the heart, and, since mannitol does not enter the cells of the heart, this gave the radioactivity in the extracellular space.

The effect of washing out the vascular system on the concentration of amino acids in the cardiac tissue

In these experiments injections were not given, but the vascular system was washed out for periods up to 120 sec in order to determine the extent to which the washing out procedure removed amino acids from the cardiac tissue. At the end of each experiment the heart was removed rapidly and its content of free amino acids was measured chemically.

Preparation and analysis of tissues

Samples of the heart muscle, of about 100–200 mg, were weighed in duplicate in glass scintillation counting vials. Two methods were used to prepare the tissue and the plasma for radioactive counting. In one method, 2 ml. of a solution of an organic base (Soluene X 100; Packard Instruments) were added to the weighed samples of tissue. When the sample had 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 of toluene were added. Blood was taken into heparinized tubes and the plasma was separated rapidly; 0·1 ml. or 0·05 ml. aliquots were put into glass counting vials and 1 ml. Soluene X 100 was added. In the alternative method, samples of heart muscle or of plasma were oxidized with $HClO_4$ and H_2O_2 and the liquid scintillation reagents were made up to a total volume of 16 ml. with ethylene glycol monoethyl ether as described by Mahin & Lofberg (1966). The [^{14}C] or [^{35}S] radioactivity in the samples was measured in an automatic scintillation spectrophotometer (Tricarb model 3375, Packard Instruments) and [^{125}I] radioactivity was measured in a well-type scintillation counter (Model 5000, Packard Instruments). When necessary, quench correction was done by the channels ratio method.

The two different methods of preparation gave strictly comparable results but in the later stages of the work only the Soluene method was used, since it proved to be the more convenient.

To measure the proportion of the radioactivity incorporated into the cardiac protein, weighed

samples of heart muscle (about 0·2 g) were fragmented in a device described by Daniel, Love, Pratt & Reeves (1975*d*). They were then ground up in 2 ml. 0·3 M-$HClO_4$ and the protein was separated from the samples by centrifugation. It was dissolved in 2 ml. Soluene X 100 and the radioactivity was assayed as described for fresh tissue.

To determine the free amino acids in the blood plasma the protein was removed from freshly separated plasma by passing it through an ultrafiltration membrane cone (Diaflo membrane filters, C.F. 50, Amicon Ltd., High Wycombe, Bucks). The amino acids in the ultrafiltrate were separated and the concentration of each was measured after separation on an ion-exchange column, by means of an automatic amino acid analyser (Chromaspek, Rank Hilger, Margate), eluting with lithium citrate buffers (Atkin & Ferdinand, 1970).

RESULTS

The efficiency with which the blood could be washed out of the heart

In order to eliminate the uncertainty that would be introduced into the results by trying to take account of the relatively large amount of radioactivity present in the blood contained within the cardiac blood vessels the vascular system was washed out, at the end of each experiment, with Krebs–Ringer solution or with saline.

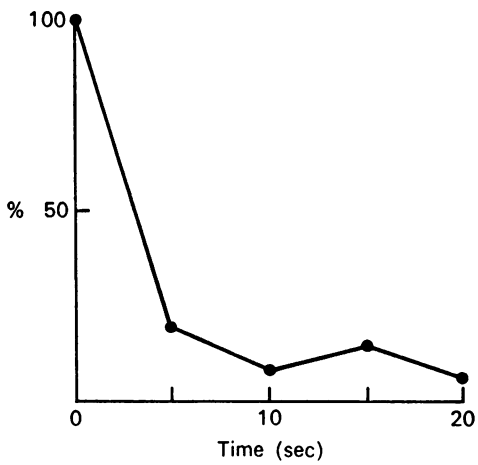

Fig. 2. The extent to which the quantity of blood contained within the vessels of the heart is reduced as the vascular tree is washed out. The percentage of blood remaining within the blood vessels after different periods of time have elapsed is shown. Each point represents the mean of three experiments (cf. with Fig. 1 in Daniel *et al.* 1975*b*).

The proportion of blood plasma in samples of heart muscle, taken under different conditions (i.e. normal hearts or hearts which had been washed out), was determined by injecting [^{125}I]human serum albumin as a plasma marker by a predetermined programme so as to maintain a steady level in the blood for a period long enough to ensure that the radioactivity in the heart tissue did not increase further. A period of 2 min proved to be adequate and at the end of this time the rat was killed. We removed the hearts from a series of nine rats, without first washing out the vascular system, and found that there was a mean blood plasma content of $32 \pm 0{\cdot}8$ (S.E.) μl. g^{-1}. In further groups of rats (twenty-one animals in all) the vascular system was washed out for 5, 10, 15, or 20 sec with Krebs–Ringer solution before the heart was removed. The mean content of plasma in the heart was reduced progressively as the

time of washing out was increased up to 10 sec (Fig. 2). After washing out the vascular system for 20 sec the mean content of plasma in the heart muscle was only $3{\cdot}7 \pm 0{\cdot}8$ (S.E.) μl. g^{-1}.

We consider that this residual radioactivity is not due to impurities, such as inorganic [^{125}I] in the human serum albumin, because, in comparable experiments on the entry of amino acids into the brain (Baños *et al.* 1973*a*, *b*) we found that the residual radioactivity, after washing out, was less than that in muscle, i.e. was under 1 μl. g^{-1} of brain.

The figures for the uptake of radioactively labelled amino acids into heart muscle, R_h, were corrected for the amount of radioactivity contained in the residual blood ($3{\cdot}7$ μl. g^{-1}), which had not been washed out of the tissue.

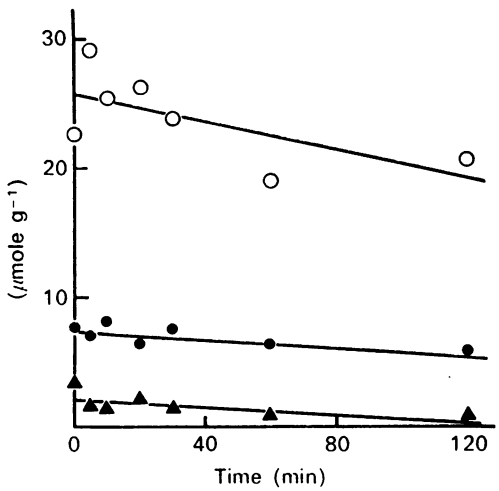

Fig. 3. The extent to which the acidic amino acids were removed from the cardiac cells by washing out the vascular system with saline. The neutral and basic amino acids were not significantly removed by this procedure. ○, taurine; ●, glutamate; ▲, aspartate. Straight lines were fitted to each set of points by linear regresion.

The effect of washing out the vascular system of the heart upon the amino acid content of the cardiac cells

In a series of experiments the vascular system was washed out for progressively longer periods, up to 120 sec. At the end of this wash out the heart was excised and its content of amino acids was determined chemically. In order to arrive at the amino acid content of the cellular tissue of the heart, two corrections had to be subtracted from each measurement, one to allow for the amino acid content of the residual blood and the other for any amino acid left in the extracellular space. The sizes of these corrections were calculated from the values for the residual blood after washing out for various periods (Fig. 2) and from the results of experiments on the removal of [^{14}C]mannitol from the extracellular space by the washout procedure. It was assumed that the concentration of the amino acid in the extracellular space before washing out the vascular system was equal to that in the blood plasma, since the heart is constantly pulsating.

For each amino acid the estimated concentration in the cardiac cells (i.e. after subtracting the corrections for amino acid in the residual blood and in the extracellular space) was plotted against the duration of the washing out period and a line was fitted to the points by linear regression. The slope of the line was significantly different from zero only for three amino acids (Fig. 3). These were the acidic ones, aspartate, glutamate and taurine. However, the proportion of these three which was washed out in 20 sec was less than 10 %. We therefore conclude that washing out for 20 sec is adequate to remove most of the blood from the cardiac vessels without appreciably lowering the levels of the amino acids in the heart tissue, except for a small proportion of the three acidic amino acids, and a 20 sec washout period was used in all subsequent experiments.

The experiments in which [^{14}C]mannitol was used as a marker of the extracellular space, showed that washing out the blood from the circulation for 20 sec removed from the extracellular space of cardiac muscle tissue 85 % of the radioactively labelled marker which had entered this space by the end of the period of injection, i.e. before the washout starts. This proportion is larger than the 68 % of this marker which is removed from the extracellular space of skeletal muscle by the washout procedure (Daniel *et al.* 1975*c*). A similar proportion (about 85 %) of the amino acids is removed from the extracellular space of this tissue by washing out the isolated heart (Scharff & Wool, 1965).

Thus, in our measurements of influx, we are able to calculate from our results the amount of the radioactivity which has entered the cells of the heart by substracting relatively small corrections for the radioactivity in the residual blood in this tissue and for that in the extracellular space. Thus from our results we obtain the true influx of amino acids into the cells of the heart.

The rate at which amino acids enter the cellular tissues of the heart from the circulation

A constant blood level of [^{14}C]2-aminoisobutyrate was maintained in the circulation for periods ranging up to 30 min. At the end of the period the residual radioactivity in the cellular tissue of the heart was determined after washing out the vascular system. The rate at which the radioactivity accumulated in the heart muscle is shown in Fig. 4*A*. Similar experiments were done for each amino acid studied and further examples are shown in Fig. 4*B*, *C*. In each experiment the level of radioactivity in the heart at the end of the period of infusion R_h (cpm mg^{-1} of fresh heart, after the corrections described above) was compared with that in the blood plasma R_p (cpm μl.$^{-1}$ of plasma). This ratio, R_h/R_p, increased in proportion to the length of the injection for a period which varied from 1 to 10 min for different amino acids (Fig. 4). A similar curve was obtained when the concentration of the amino acid was raised in the circulating blood by the injection of unlabelled amino acid (e.g. Fig. 4*B*).

The rate of accumulation of radioactivity in the cardiac tissue became slower as the injection was prolonged, presumably due to efflux of labelled material, possibly as metabolites leaving the heart. The slope of the initial linear part of the curves of the type shown in Fig. 4, was assumed to provide a measure of the influx into the cellular tissue of the heart and, therefore, subsequently only the results of experiments of no longer than 1–3 min were used when determining influx. The calculation of the influx, v, was based upon the mean specific activity of the radioactive amino acid

in the blood plasma during the experiment, R_p/s. Thus, for an experiment lasting a time, t, the influx of the amino acid was given by

$$v = \frac{sR_h}{tR_p}, \quad (1)$$

where s is the mean concentration of the amino acid in the plasma (this formula is similar to that which we have used in previous studies of this kind Baños *et al.* 1973*a*, 1975; Daniel *et al.* 1975*c*).

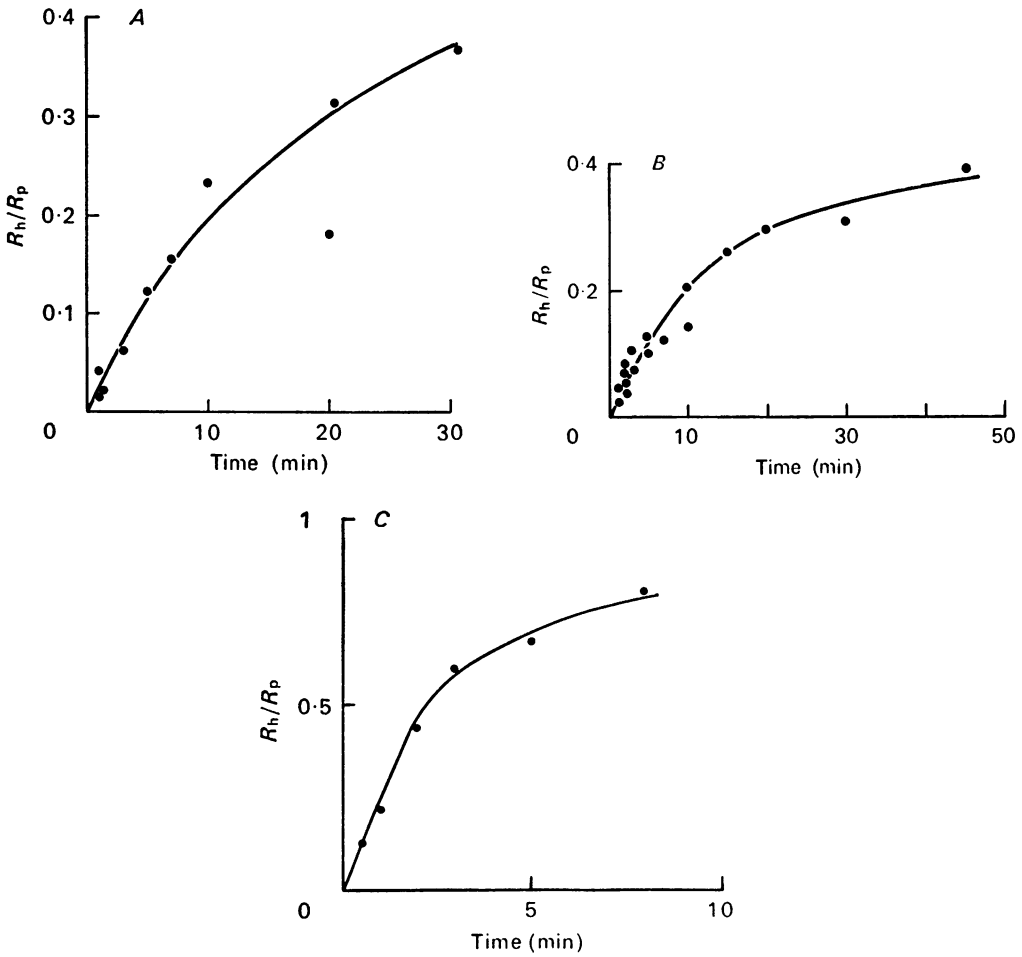

Fig. 4. The rate at which radioactivity accumulated within the cardiac tissue in the time during which a steady specific activity of labelling of an amino acid was maintained in the circulating blood. Radioactivity in the heart muscle, R_h, compared with radioactivity in the blood plasma, R_p. Each point represents a separate experiment. *A*, [^{14}C]aminoisobutyrate. *B*, L-[^{14}C]serine with the blood serine also raised by an injection to a level between 2 and 3 mM. *C*, L-[^{14}C]arginine.

When prolonged injections were given (Fig. 4) in order to study how rapidly a steady state could be reached in which efflux partly counterbalanced influx, it is likely that at the end of the experiment not all of the radioactivity in the blood was

still in the form of the amino acid. For this study the fact that metabolic changes had altered the attachment of the radioactivity was not important. The short injections used to study influx ensured that errors did not arise as a result of metabolism or of protein binding of the labelled amino acid.

The influx given by eqn. (1) represents the total amount of amino acid entering the cardiac cells, regardless of whether or not it becomes incorporated into protein. In a few experiments additional measurements were made to determine the proportion of the total radioactivity which had become incorporated into protein.

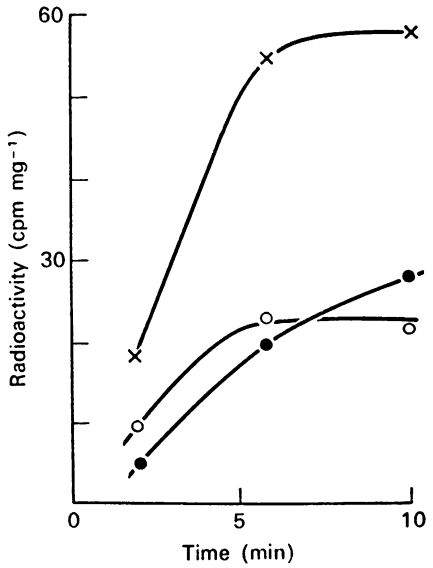

Fig. 5. Rate of incorporation of radioactivity into the protein of cardiac muscle when a steady level of ^{14}C-labelled phenylalanine is maintained in the circulation for progressively longer periods. The results of three different series of experiments are shown in each curve (the points were fitted by eye). ×, total radioactivity in the heart, at the end of each experiment; ●, radioactivity which had become incorporated into the cardiac protein, at the end of each experiment; ○, the rest of the radioactivity, i.e. that which is not incorporated into cardiac protein, at the end of each experiment. Ordinate: radioactivity per unit weight of fresh tissue.

Rate of incorporation of radioactivity from L-[^{14}C]*phenylalanine into the protein of cardiac muscle*

In a series of experiments a steady level of L-[^{14}C]phenylalanine was maintained in the circulation for periods ranging up to 10 min. The heart was removed at the end of each experiment and not only the total radioactivity which had entered the cardiac tissue, but also the proportion of it which had become incorporated into protein was determined. This proportion was small at first but increased steadily as the period of the experiment was lengthened, reaching about 50% after 10 min (Fig. 5).

TABLE 2. The extent to which the transport systems carrying amino acids into the heart became saturated as their concentration in the blood was raised, as indicated by deviation from a linear relationship between influx and concentration. Where possible the maximum influx, V, and the Michaelis constant, K_t, for half saturation, have been estimated by the method of Wilkinson (1961) with the number of experiments in parentheses. The concentrations of the amino acids in the blood plasma and in the water of the cardiac cells are also compared

Amino acid	Range of concentrations in the blood over which influx was measured (mM)	Approximate concentration above which deviation from linearity became apparent (mM)	Maximum influx V (μmole min^{-1} g^{-1} of heart)	Concentration in the blood needed to half saturate the transport mechanism, K_t (mM)	Concentration in normal plasma* (mM)	Concentration in the intracellular water of the heart † (mM)	Ratio of concentrations $\frac{\text{heart water}}{\text{blood plasma}}$
L-phenylalanine	0·05–10·2	0·6	0·76 ± 0·07	3·7 ± 0·9 (39)	0·050 ± 0·002	0·085 ± 0·021	1·7
L-tyrosine	0·05–0·2	—	—	—	0·057 ± 0·003	0·099 ± 0·025	1·7
L-leucine	0·12–20	2	1·9 ± 0·2	12 ± 2 (29)	0·117 ± 0·005	0·141 ± 0·027	1·2
L-tryptophan	0·06–8·3	0·7	0·50 ± 0·07	3·0 ± 0·9 (38)	0·065 ± 0·003	0·044 ± 0·015	0·68
L-histidine	0·08–21·5	0·3	0·43 ± 0·03	5·0 ± 0·8 (41)	0·081 ± 0·004	0·354 ± 0·021	4·4
L-methionine	0·04–0·06	—	—	—	0·041 ± 0·002	0·098 ± 0·021	2·4
L-serine	0·39–37	2	1·6 ± 0·3	30 ± 7 (12)	0·157 ± 0·009	1·13 ± 0·16	7·2
L-cysteine	0·09–0·2	—	—	—	0·097 ± 0·060	0·089 ± 0·014	0·92
L-lysine	0·35–29	3	1·2 ± 0·3	6 ± 3 (8)	0·351 ± 0·026	0·512 ± 0·077	1·5
L-valine	0·08–60	10	3·0 ± 0·3	50 ± 7 (24)	0·156 ± 0·006	0·145 ± 0·021	0·93
L-arginine	0·12–39	2	0·17 ± 0·02	4·8 ± 1·6 (16)	0·115 ± 0·014	0·091 ± 0·023	0·79
L-threonine	0·48–78	8	1·9 ± 0·3	51 ± 14 (22)	0·213 ± 0·008	0·575 ± 0·041	2·7
L-isoleucine	0·03–50	1·5	1·06 ± 0·19	28 ± 8 (17)	0·072 ± 0·003	0·091 ± 0·021	1·3
L-alanine	0·4–0·8	—	—	—	0·31 ± 0·04	1·9 ± 0·8	6·1
L-aspartate	0·01–0·03	—	—	—	0·014 ± 0·004	2·1 ± 0·4	150
L-glutamate	0·14–0·2	—	—	—	0·16 ± 0·02	7·6 ± 1·0	48
L-glutamine	—	—	—	—	0·94 ± 0·04	8·7 ± 0·4	9·3
glycine	0·17–0·4	—	—	—	0·199 ± 0·010	0·89 ± 0·07	4·5
ornithine	—	—	—	—	0·054 ± 0·005	0·17 ± 0·05	3·0
L-proline	0·11–0·2	—	—	—	0·139 ± 0·010	2·4 ± 0·9	17
taurine	0·04–0·1	—	—	—	0·046 ± 0·011	29 ± 1	630

The binding of tryptophan to albumin in the plasma has been ignored for the purpose of this comparison.

* 9–12 determinations for each amino acid.

† 6–7 determinations for each amino acid.

The effect of raising the concentration of an amino acid in the circulation upon its influx into the heart

When the concentration of any of the amino acids in the circulating blood was raised above normal its influx into the heart was increased. We studied the effect of raising the concentration of some of the amino acids, one at a time, to progressively higher levels in the circulation, to determine the extent to which the transport system for each could be saturated (Table 2). Over a limited range of concentrations this increase in influx was approximately proportional to the increase in the concentration in the blood. This range (Table 2) was generally greater than the normal variation in the concentration of the amino acid in the blood of different animals

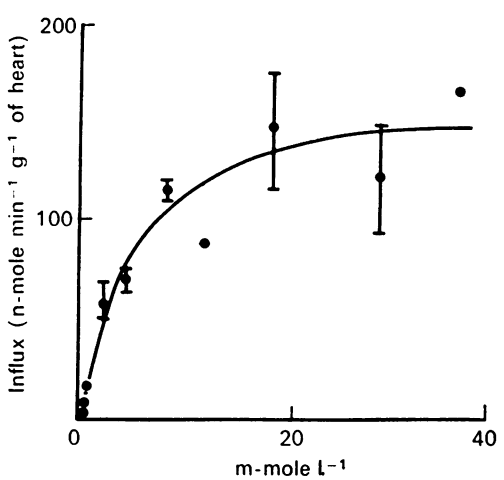

Fig. 6

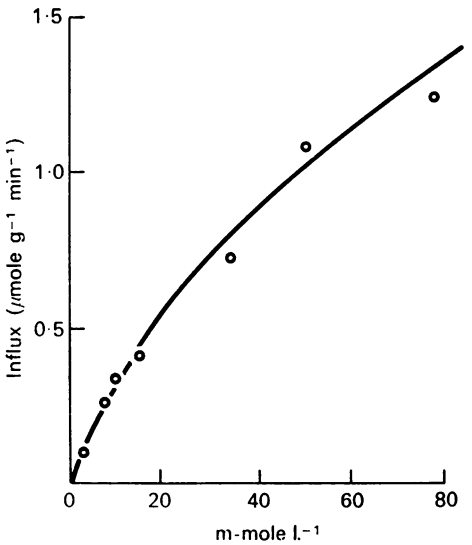

Fig. 7

Fig. 6. The effect of raising the concentration of L-arginine in the circulating blood upon the influx of this amino acid into the cardiac muscle. Some points represent the means of two or three experiments and these are marked with bars to represent the S.E. A curve has been fitted to the points by the method of Wilkinson (1961). Note that when the concentration of the amino acid is raised to a high level in the circulating blood the curve becomes nearly horizontal, indicating that the carrier which transports this amino acid into cardiac tissue has become more fully saturated than that which transports L-threonine (see Fig. 7).

Fig. 7. The effect of raising the concentration of L-threonine in the circulating blood upon the influx of this amino acid into the cardiac muscle. Each point represents the mean of two to four experiments. Note that when the level in the blood is relatively low the influx increases in proportion to this level. However, when the concentration in the circulation is raised above about 10 mM influx fails to increase *pari passu* with the concentration in the blood, a finding which indicates that saturation of a transport system is taking place.

(Table 2). The effect of raising the concentration of each of ten amino acids (phenylalanine, leucine, tryptophan, histidine, serine, lysine, valine, arginine, threonine and isoleucine) in the circulation was studied. For each of these amino acids the pro-

portional relationship between the influx into the heart and the concentration of the amino acid in the blood (Table 2) broke down when its concentration in the blood was raised to a high enough level. It proved impossible to determine whether the transport systems of three other amino acids (tyrosine, methionine and cysteine) could be saturated because their limited solubility in aqueous solutions made it difficult to raise the concentration in the blood to a high level rapidly by I.V. injection. As the concentration of the amino acid in the blood was increased in different experiments, progressively smaller increases in influx were found (Figs. 6, 7). This failure to maintain a proportional increase in influx with increasing concentrations in the circulation, indicated that saturation of a transport system was taking place. The results conformed, at least approximately, to Michaelis kinetics. The maximum rate of influx, V, as well as the concentration in the blood at which the transport system was half saturated, K_t, (Table 2) were estimated by the method of Wilkinson (1961) in which linear regression with statistical weighting of the data is employed. For some amino acids the standard errors, especially of K_t, were large and the figures must be regarded as indicating merely the order of magnitude.

DISCUSSION

The ability to maintain a radioactively labelled amino acid at a steady concentration in the circulating blood (Fig. 1*B*), over a defined period of time, makes possible quantitative measurement of the influx of individual amino acids into the heart *in vivo*. Influx is dependent upon the concentration of the amino acid in the blood (Table 1) being approximately proportional to the blood concentration over a range which is usually at least as wide as that of the normal variation between animals. However, when the concentration of an amino acid in the circulating blood is raised well above normal, its influx into the heart usually fails to increase in strict proportion to the increase in its concentration in the blood (Fig. 6), although complete saturation was not observed for all amino acids (Fig. 7). This failure of influx to keep pace with an increasing concentration of amino acid in the blood is an indication that saturation of a transport mechanism is occurring, and provides evidence that carrier-mediated transport processes are involved in the movement of amino acids across some barrier to permeability between the blood and the heart. That this barrier must be at the membrane of the muscle cell is shown by the ability of the cells of the heart to retain amino acids within themselves at concentrations which are higher than those at which the amino acids are present in the circulating blood, e.g. in the case of aspartate, glutamate, glutamine, proline and taurine the concentrations are some ten times or more greater in the heart than in the circulating blood; the concentrations of alanine, glycine, histidine, ornithine, serine and threonine are some three times or more and those of methionine, phenylalanine and tyrosine are about twice the concentrations found in the circulating blood (Table 2). Similar concentrations of many of these amino acids, free in cardiac tissue, have been reported by Posner, Mierzwinski & Fallen (1973). Some of these amino acids may conceivably be synthesized within cardiac cells, e.g. alanine, glutamate and proline, but others such as methionine, phenylalanine, threonine and, almost certainly, also histidine and tyrosine, cannot be made by the cells of the heart and our results show

that they must enter the cardiac cells against a concentration gradient by means of a transport process which is not only carrier-mediated but is also an active mechanism, i.e. it is energy-dependent (Christensen, 1975).

The rate of carrier-mediated influx, v, into the cardiac cells will depend upon the concentration of the amino acid in the extracellular space, s, in a manner which can be defined by the Michaelis–Menten equation:

$$v = \frac{Vs}{K_t + s} \tag{2}$$

where V is the maximum influx when the carrier mechanism is saturated and K_t is the Michaelis constant (that concentration of a substance in the circulation which half saturates the carrier mechanism). Under normal conditions (Table 2) not only will the concentration of the amino acid in this extracellular space be close to the concentration of the amino acid in the blood but it will also be much less than K_t (Table 2), so that eqn. (2) can be simplified to

$$v = \frac{Vs}{K_t}. \tag{3}$$

The approximate value of the ratio V/K_t for each amino acid will be given by v/s. The amino acids in Table 1 are arranged in descending order of this ratio (shown in the third column). In the case of ten of the amino acids it is possible to compare ratios of V to K_t, calculated in two different ways. First, there is the approximate estimate of the ratio, using eqn. (3) only. This estimate is derived from the results of experiments in which the concentration of the amino acid in the blood was low (Table 1, column 3). Secondly, the ratio can be calculated directly from those experiments in which the individual values of V and K_t (Table 2) can be estimated separately by the method of Wilkinson (1961). The second estimate of the ratio is based upon the whole group of experiments, including those in which the concentration of the amino acid in the circulation was raised considerably above normal. Agreement between these two different estimates of the V/K_t ratio is good in the case of some amino acids (e.g. leucine and valine for which the ratios calculated from Table 2 are 0·158 and 0·060 compared with the corresponding ratios in Table 1, 0·136 and 0·068 respectively), while for all of the amino acids the ratios are at least of a similar order of magnitude, confirming that the relation between influx and blood concentration can be represented, at least approximately, by the Michaelis kinetics of eqn. (2).

The ratio of V to K_t provides an index of the activity of the transport system for that amino acid. These ratios, and also the values of the two parameters, when estimated separately, vary over a wide range for different amino acids. It can be seen from Tables 1 and 2 that this variation is not related to the molecular weights of the amino acids. Differences in influx may, however, be related to lipid solubility since the four aromatic amino acids as well as leucine have a high influx (Table 1) and also have high lipid solubilities.

Waterlow (1969) has shown that recycling of the amino acids which are derived from protein breakdown takes place in many tissues. The heart, which has to synthesize protein at a high rate (Petersen & Lesch, 1972), doubtless recycles some of

the amino acids which are released from the breakdown of its own muscle protein. However, the cardiac muscle cells also need an additional supply of amino acids from the circulating blood in order to replace not only the amino acids which have not been recycled but also to replace the amino acids which have been metabolized. There are ten amino acids (lysine, phenylalanine, leucine, threonine, isoleucine, valine, methionine, tryptophan, histidine and arginine) that are essential for the nutrition or normal growth of the rat (Rose & Cox, 1924; Borman, Wood, Black, Anderson, Oesterling, Womack & Rose, 1946; Rose, Oesterling & Womack, 1948) and it will be seen from Table 1 that the influx of each of these amino acids into the heart is high. Morgan, Earl, Broadus, Wolpert, Giger & Jefferson (1971) showed that the amount of lysine and leucine in cardiac myosin is twice as great as that of any other of the essential amino acids. Our finding that there is a high influx of these two amino acids (as well as of the other essential amino acids) into the heart suggests that all these amino acids are entering the heart rapidly to meet the need for resynthesis of cardiac protein (Fig. 5).

Table 1 shows that the amino acids of the aromatic group, i.e. phenylalanine, tyrosine, tryptophan and histidine enter the heart, in general, more rapidly than any other group of amino acids. Another aromatic amino acid, levadopa, (which is not normally present in the blood, but is given therapeutically) also has a high rate of entry into the heart (Table 1). It is perhaps significant that some of these aromatic amino acids (including levadopa, L-dihydroxyphenylalanine) are precursors of the monoamine transmitters (Daniel *et al.* 1976) which play a part in the conduction mechanism of the heart. The aromatic amino acids are also important donors of amino-groups in the metabolism of cardiac muscle (Krebs, 1975).

Four amino acids (aspartate, proline, taurine and 2-aminoisobutyrate) have a low influx into the heart, of the order of 10 % of that of phenylalanine, when influx is measured at comparable concentrations of the amino acids in the blood. Since 2-aminoisobutyrate is not normally found in the blood and taurine is not normally found in protein, it is probable that there is no special transport system for these substances and that the low influx of these two amino acids may be due to passive diffusion. The influx of aspartate or of proline is only a little greater than that of taurine or of 2-aminoisobutyrate so that aspartate and proline may also enter the heart mainly or entirely by passive diffusion.

However, the importance of carrier-mediated transport systems for the influx of many amino acids into the cardiac muscle, especially the aromatic and dibasic amino acids, is indicated by the fact that there is evidence of saturation of the transport mechanisms for these particular amino acids when their concentration in the blood is raised only moderately above normal (Table 2). In various diseases in which amino acid metabolism is disturbed (e.g. various aminoacidurias) the levels of one or more amino acids in the blood may be raised to remarkably high levels (Stanbury, Wyngaarden & Fredrickson, 1972; Daniel *et al.* 1976).

The heart needs amino acids mainly for protein synthesis but also, probably, as oxidizable substrates for energy metabolism. It is clear that carrier-mediated transport mechanisms ensure an adequate supply of amino acids to the cardiac muscle cells and thus enable the heart to function properly. The dependence of the heart upon carrier-mediated transport for the supply of amino acids means that the organ

is vulnerable to any disturbance which interferes with these transport processes, especially to abnormal levels of amino acids in the circulation.

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