Evidence for Inhibition of Exodus of Small Neutral Amino Acids from Non-brain Tissues in Hyperphenylalaninaemic Rats

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Summary: The mechanism of the depletion of several plasma amino acids in PKU has remained unexplained. In the present study, a statistically significant decrease in the plasma concentration of several amino acids was observed 2 h after the intraperitoneal injection of Phe to weanling rats. The pattern was very similar to the one observed in PKU patients. Statistically significant increases in the distribution ratios liver/plasma and, mainly, muscle/plasma ratios accompanied in most of the cases the corresponding decreases in plasma concentrations. Equimolar injection under the same conditions of the non-insulinogenic transport system L analogue, the a($\pm$) isomer of the 2-amino-norbornane-2-carboxylic acid, produced, in a parallel effect to Phe, statistically significant increases in the distribution ratios of Ala and Gly, and probably of Pro in muscle, as well as of Ala in liver. These results seem to indicate that the high intracellular Phe attained inhibits the exodus of small neutral amino acids through system L, causing their depletion in plasma and ultimately in the brain. This effect may be additive to the inhibition by Phe of the entry of bulky neutral amino acids at the level of the blood–brain barrier. Further study is needed to assess the relevance of these effects to PKU.

The underlying pathogenetic mechanism that leads to mental retardation in phenylketonuria (PKU) is not known (Scriver and Clow, 1980). An inadequate supply of certain amino acids to the brain with the ensuing impairment of protein and/or neurotransmitter synthesis is likely to be a major factor contributing to the brain damage (Tourian and Sidbury, 1983).

In 1953, one of us (H.N.C.) predicted that the systemic accumulation of phenylalanine in PKU might interfere with the transport of several amino acids into the brain. This hypothesis has received strong support. Depletion of amino acids in the brain has been observed in animals with experimental hyperphenylalaninaemia (McKean *et al.*, 1968) and at autopsy of PKU patients (McKean, 1972). Inhibition

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of influx of amino acids into the brain has been also observed in similar animal models (Partridge and Oldendorf, 1977), along with transport-competitive effects of phenylalanine in isolated human brain capillaries (Choi and Partridge, 1986). In general, the bulky neutral amino acids are mainly affected by Phe, a result that indicates that the inhibitory effect may occur at the level of transport system L in the blood–brain barrier (Wade and Katzman, 1975; Betz and Goldstein, 1978).

An accompanying paradox has received less attention. The plasma concentrations of several dipolar amino acids, the large ones less generally affected, decrease in plasma in PKU patients (Linneweh and Ehrlich, 1962; Efron *et al.*, 1969). The mechanism of this change is not known; malnutrition and loss in the urine have been excluded as possible explanations; decreased intestinal absorption or loss into the gut lumen is unlikely (Efron *et al.*, 1969).

Huether and colleagues (1984) have reported accumulation of several amino acids in various non-brain tissues of newborn rats chronically treated with phenylalanine and α-MePhe. The mechanism of this accumulative effect has likewise not been explained.

It was observed some years ago in this laboratory that the muscle/plasma ratio (Christensen *et al.*, 1948) or liver/plasma ratio (Christensen and Cullen, 1981) of some amino acids increase, rather than decrease, in the presence of certain system L substrates or analogues. The most likely explanation for this effect was taken to be an inhibitory action of the analogues on the exodus of the affected amino acids from tissues through system L. These observations have led Christensen to propose that the depletion of some plasma amino acids in PKU may be explained as a sequestering effect of Phe by a similar inhibition of the exodus of certain amino acids from non-brain tissues, causing their sequestration and possible accelerated catabolism (Christensen, 1986; 1987a, b). The aim of the present study was to test this hypothesis.

EXPERIMENTAL PROCEDURES

Materials

L-Phenylalanine was obtained from SIGMA. The racemic aminoexo isomer of the 2-aminonorbornane-2-carboxylic acid, often designated as a(±)BCH, but abbreviated here as aBCH, was our own preparation (Tager and Christensen, 1972). Aspartame was provided to us by the NutraSweet Company. All other reagents were of the best grade commercially available.

Animals

Twenty-two-day-old Sprague-Dawley male weanling rats, weighing 38–66 g were used for all the experiments. Rats were fasted for 20–24 h before each experiment.

Procedure

Phe or aBCH was injected intraperitoneally as an iso-osmotic solution at a dose of 10 mmol/kg body weight. Control rats received an intraperitoneal injection of

0.85% NaCl in the corresponding volume. Aspartame was administered by stomach tube only partially dissolved as a slurry, 1.5 g per kg, in 15 ml water. Two hours after an administration, the rats were anaesthetized with ether. Blood was taken from the exposed heart and collected in a heparinized tube. Plasma was separated and deproteinized with an equal volume of 12% sulphosalicylic acid. Approximately 1 g of liver and 0.5 g of muscle from the left thigh were weighed and ground in a mortar with sand and a 10% solution of sulphosalicylic acid in a volume 4 times the fresh weight of the tissue in grams. The homogenate was filtered through Whatman #1 paper. The protein-free filtrate was stored at −20°C until analysis.

Analysis
Amino acids were measured with a 119 CL Beckman automatic amino acid analyser using a high-sensitivity amino acid standard solution from SIGMA.

Calculations
For calculation of apparent concentrations of amino acids in cell water, the liver and muscle samples were taken to contain, respectively, 49% and 62% intracellular and 22% and 15% extracellular water (Christensen and Cullen, 1981; Lowry and Hastings, 1942). The distribution ratio, tissue/plasma, was the ratio $\mu mol\, kg^{-1}$ cell water/$\mu mol\, kg^{-1}$ extracellular water (Lowry and Hastings, 1942).

## Statistical analysis
Differences in plasma levels, intracellular concentrations and distribution ratios between Phe- or aBCH-treated and control rats were calculated by the *t* test using separate variances when the number of controls and treated animals differed (Wilkinson, 1986).

RESULTS
Plasma levels of Phe and tyrosine at various intervals after Phe injection are shown in Figure 1. Maximum levels of Phe, about 40 times the levels of the controls, were observed at 2 h, although about 75% of the increase was seen by 30 min. By 4 h, a 75% decline from the peak was observed, and, by 8 h, the levels had returned to control values. Presumably through the action of the uninhibited hepatic hydroxylase, Tyr increased, although at a slower rate than Phe, during the first 2 h, increasing further as Phe decreased. On the basis of these results, all subsequent experiments were performed taking the samples at 2 h after injection.

   Plasma amino acid levels and net intracellular concentrations in liver and muscle after Phe injection are shown in Table 1. There was a statistically significant increase in the plasma concentrations of both Phe and Tyr; the ratio [Phe]/[Tyr] at 2 h was about 8/1. All other amino acids except Glu⁻ showed decreased plasma levels 2 h after Phe administration, the difference being statistically significant for Leu, Ile, Ala, Pro, Gly, His, Orn and Arg. The valine decrease showed a *p* value of 0.055
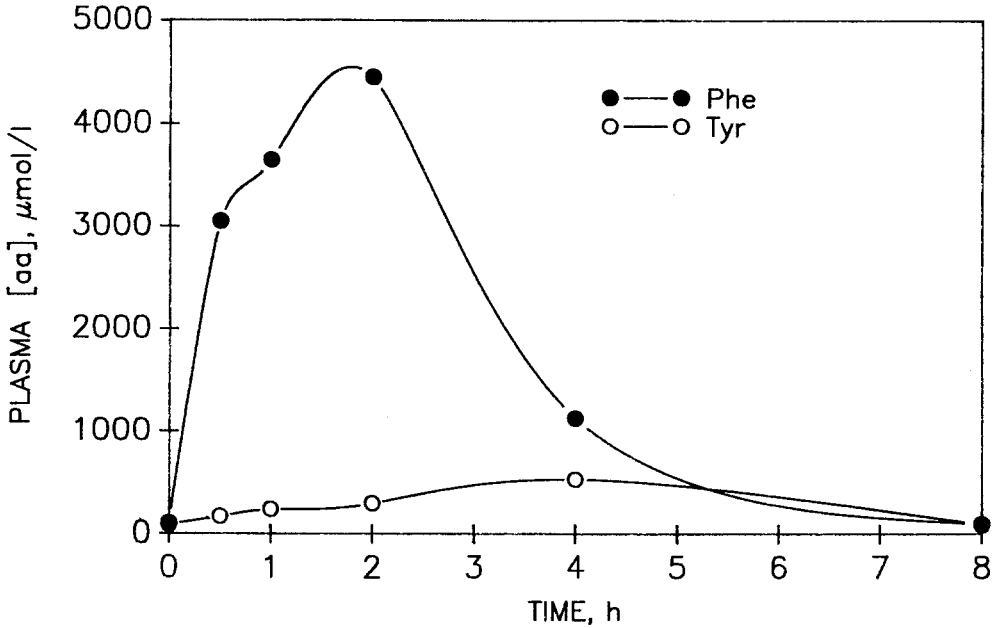
Figure 1 Time course of Phe and Tyr plasma levels after Phe administration. Each pair of points represents one animal. Values at 0 and 2 h in the same range of the means found in further experiments

Table 1 Plasma and net intracellular amino acid concentrations in controls and after Phe administration (for details see *Experimental procedures*). Values are means±S.D., $n = 8$ controls, 8 experimental animals. Plasma and tissue levels are expressed in µmol/L and µmol/kg tissue, respectively

	Plasma		Liver		Muscle	
	Control	+Phe	Control	+Phe	Control	+Phe
Phe	104±28	4421±868*	60±39	1348±786**	138±73	3560±670*
Tyr	93±23	537±108*	39±21	327±106*	124±47	509±117*
Trp	116±27	103±34	42±39	56±32	67±44	94±83
Leu	254±27	139±37*	151±89	134±38	228±91	198±77
Ileu	175±37	105±32†	53±50	61±28	180±63	141±61
Val	332±49	268±71††	209±128	184±65	285±106	257±84
Ala	419±120	219±128†	1056±494	1756±674**	1897±1369	2261±1481
Pro	188±38	99±42†	67±60	77±37	367±103	644±318**
Gly	625±121	391±134†	2392±1127	2381±680	5287±3030	6807±3921
His	83±20	59±11†	376±224	377±137	369±102	428±94
Orn	82±23	53±27**	233±118	193±66	285±91	316±134
Arg	167±26	100±21*	N.D.	N.D.	1029±319	1018±108
Lys	436±176	317±146	403±361	439±181	3166±1380	2877±1587
Gln	608±195	471±186	1728±848	1013±532	3853±2792	3642±2051
Glu	184±61	226±195	1592±1030	1548±688	2172±637	2933±614**

$*p<0.001$; $†p<0.01$; $**p = <0.05$; $††p = 0.055$; N.D. = undetectable by the analysis used

(Table 1). In general, the amino acid pattern in plasma was very similar to the one reported by Linneweh and Ehrlich (1962) and Efron and colleagues (1969) in PKU children (Figure 2).
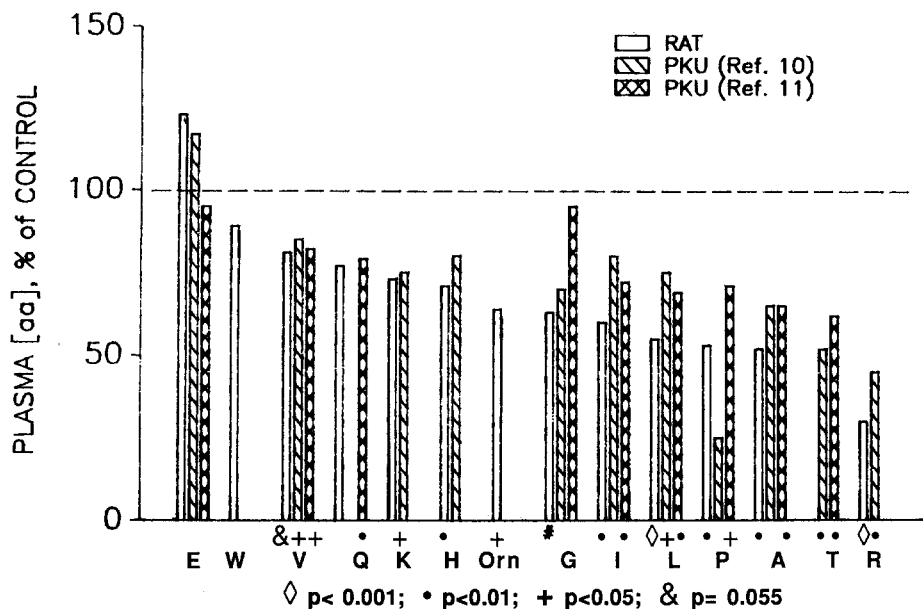
Figure 2 Plasma amino acid levels after Phe administration in rats and in PKU children off treatment. Plasma concentrations from Table 1 and from Linneweh and Ehrlich (1962) and Efron and colleagues (1969) are shown here as per cent of control. Non-essential amino acids were not included in the statistical calculations in Linneweh and Ehrlich. The one-letter abbreviations are Glu, E; Trp, W; Val, V; Gln, Q; Lys, K; His; H; Gly, G; Ileu, I; Leu, L; Pro; P; Ala, A; Thr, T; Arg, R

Statistically significant increases in net intracellular concentrations were found for Phe and Tyr in both organs studied (Table 1); the ratio [Phe]/[Tyr] reached at 2 h was 4/1 and 7/1 in liver and muscle, respectively. In liver, aside from Phe and Tyr, Ala was the only amino acid that showed a statistically significant increase in net intracellular concentration. In muscle, only Pro and Glu⁻ showed statistically significant increases in their net intracellular concentrations (Table 1).

Amino acid distribution ratios, liver/plasma and muscle/plasma, after Phe injection are shown in Table 2. Owing to the extremely high plasma levels attained, hepatic Phe showed an almost statistically significant decrease in its distribution ratio in spite of the high intracellular concentration reached ($p = 0.051$). All other amino acids except Glu⁻ showed an increase in their distribution ratio in liver, although the difference was statistically significant only for Ala, Gly and Lys (Table 2, column 3).

In muscle, both Phe and Tyr showed decreases in their distribution ratios after Phe injection, but the difference was statistically significant only for Tyr. All other amino acids showed increases in their distribution ratios; the difference was

Table 2 **Changes in relative concentrations and distribution of each of 15 amino acids produced after injecting L-Phe or aBCH into weanling rats**

	Phe injection			aBCH injection		
	Plasma	*Liver*	*Muscle*	*Plasma*	*Liver*	*Muscle*
Phe	4300*	2250†	2580*	124	105	109
D.R.		0.50	0.62		1.09	0.84
Tyr	580*	840*	410*	99	82	93
D.R.		1.41	0.72†		1.18	1.03
Trp	89	133	140			
D.R.		1.52	1.34			
Leu	55*	89	87	121	84	93
D.R.		1.31	1.56†		1.06	0.88
Ileu	60**	115	78	108	68	89
D.R.		2.19	1.29		1.03	0.84
Val	81	88	90	113	71	92
D.R.		1.24	1.25		1.01	1.19
Ala	52**	167†	119	91	92	101
D.R.		3.74**	2·62†		1.53††	1.68†
Pro	53**	115	175†	90	80	102
D.R.		2.19	3.93*		0.56	1.32
Gly	63**	100	129	71†	70**	107
D.R.		1.88†	2.18†		1.05	1.80**
His	71**	100	116	126	85	87
D.R.		1.50	1.56†		0.84	0.76
Orn	65†	83	111	94	77	79
D.R.		1.50	1.88**		1.07	0.98
Arg	60*	Not	99	99	Not	120
D.R.		detected	1.98**		detected	1.20
Lys	73	109	90	101	114	99
D.R.		1.87†	1.30		1.58	0.92
Gln	77	58	95			
D.R.		1.10	1.34			
Glu	124	97	135†			
D.R.		1.03	1.62			

The 3 values in each column parallel with the abbreviation (Column 1) of the amino acids show, for plasma, liver and muscle, respectively, the percentage of the control value attained in 2 h for each of these tissues. The number just below the percentage value for each amino acid shows the calculated relative distribution ratio (D.R.) between the concentrations in the cellular and extracellular water, as described under *Methods*, for the indicated solid tissue. The number of animals represented by each value varies from 7 to 13, both for control and experimental groups. See Tables 1 and 3 for the analytically determined concentrations which served for calculating these amino acid distributions. The superscript symbols indicate the statistical significance of changes
*$p<0.001$, **$p<0.01$, †$p<0.05$, ††$p<0.07$

statistically significant for Leu, Ala, Pro, Gly, His, Orn and Arg (Table 2, column 4).

Parallel experiments to those with Phe were made with aBCH (Table 2). This isomer of BCH was selected because it is not an insulin secretagogue (Tager and

Christensen, 1971) and could serve to dissociate that action from the suspected direct effects on amino acid transport.

Gly showed a statistically significant decrease in both plasma and liver concen-

Table 3   Plasma and net intracellular amino acid concentrations in controls and after aBCH administration (for details see *Experimental procedures*). Values are means±S.D.; $n = 5$ controls, 7 experimental animals. Plasma and tissue levels are expressed in μmol/L and μmol/kg tissue, respectively

|	Plasma		Liver		Muscle	
	Control	*+aBCH*	*Control*	*+aBCH*	*Control*	*+aBCH*
Phe	72±14	89±22	61±12	64±12	96±22	102±22
Tyr	71±18	70±21	56±16	46±10	118±26	106±21
Leu	224±65	271±119	265±84	223±59	249±94	231±84
Ileu	309±274	335±306	142±86	97±58	220±145	196±105
Val	206±44	230±89	258±61	183±71	250±59	230±59
Ala	318±50	289±93	1605±555	1470±445	3107±961	3145±715
Pro	156±28	141±40	146±103	116±68	378±123	385±178
Gly	556±106	393±108†	2882±43	2019±535*	6755±1626	7243±1646
His	65±26	82±18	510±79	432±115	394±109	342±60
Orn	52±17	49±17	269±58	202±36	260±117	206±68
Arg	138±16	133±38	N.D.	N.D.	684±242	822±221
Lys	402±170	405±145	788±426	895±431	3162±1031	3138±949

*$p<0.01$; †$p<0.05$; N.D. = undetectable by the analysis used

trations after aBCH injection (Table 3). With the exception of Ala, Gly, Pro and Arg in muscle, there was a tendency of most amino acids to be decreased intracellularly after aBCH injection in both organs studied. In no case, however, were the changes statistically significant (Table 3).

Ala showed an increase in its liver/plasma distribution ratio after aBCH injection on the verge of statistical significance ($p = 0.07$). In no case were the changes in distribution ratios, if any, for other amino acids in liver statistically significant (Table 2, column 6).

Ala and Gly showed statistically significant increases in their muscle/plasma distribution ratios after the administration of aBCH. Pro, although the amino acid whose distribution ratio increased the most next to Ala and Gly (Figure 3, panel D), showed a p value of 0.2 for the increase in its muscle/plasma distribution ratio. The combined distribution ratios, muscle/plasma of the three small neutral amino acids, showed, however, a statistically significant difference when considered collectively (Figure 3). Despite the considerable variance, we find the general downward trends impressive.

Aspartame administered by stomach tube as a 10% slurry in a dose of 1.5 g per kg body weight, approximately equivalent to the phenylalanine injected, led, in 2 h (4 animals), to increases of the plasma phenylalanine by about four-fold, to 0.2 mmol/L, and of the tyrosine as a significant contrast by about ten-fold, to 0.5 mmol/L. Several of the same amino acids lowered in the plasma by phenylalanine (e.g Gly, Pro, Met, Arg, Val) tended downward. Observations on one animal
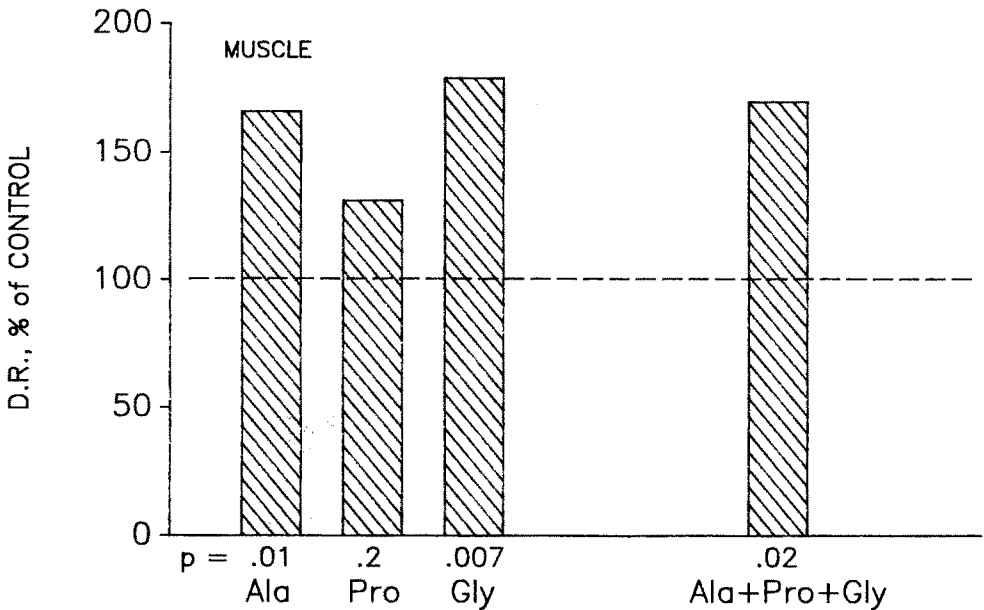
Figure 3 Distribution ratios muscle/plasma for the small neutral amino acids, Ala, Pro and Gly, after aBCH administration. Results are expressed as per cent of control; *n* varied from 7 to 13 animals for both control and experimental observations

each at 1 and 1.5 h already indicated the same tendencies. Because of the complications of interpretation arising from possible effects of aspartate released from aspartame (e.g. distinct rises seen for plasma Ala and Gln), we did not, in this case, extend our results to the point of general statistical significance for the declines in plasma animo acid levels.

DISCUSSION

We lack an animal model to represent PKU and the usual experimental approach by inducing hyperphenylalaninaemic states in rats has important drawbacks. Phe administered chronically results in a higher concentration of Tyr in plasma, an effect opposite to the change observed in the human disease. Inhibitors of Phe hydroxylase, in particular p-ClPhe, are not entirely specific and show considerable toxicity manifested as loss of weight and high mortality (Vorhees *et al.*, 1981). Therefore, we used no inhibitor of the hydroxylase in the present study. Under our experimental conditions, statistically significant increases in the concentration of Phe in plasma and tissues were nevertheless obtained. Although Tyr also increased significantly in plasma and tissues, the mean [Phe]/[Tyr] ratios were 8/1, 4/1, and 7/1 in plasma, liver and muscle, respectively (Table 1). Moreover, the statistically significant decrease of the muscle/plasma ratio for Tyr (Table 2, column 4), consistent with the slower rise in plasma Tyr concentration than Phe (Figure 1),

indicates that, at least in muscle, Tyr had not yet attained a steady-state distribution by the time our experiments were terminated. Therefore, we believe that the effects observed here were caused mainly by elevation of Phe and not of Tyr.

From previous work in this laboratory, we have learned that, under normal conditions, changes in amino acid concentrations in plasma and tissues tend to go in the same direction (Christensen *et al.*, 1948). Only when selective inhibitors e.g. amino acid analogues, are present do changes in the distribution ratio tend to occur.
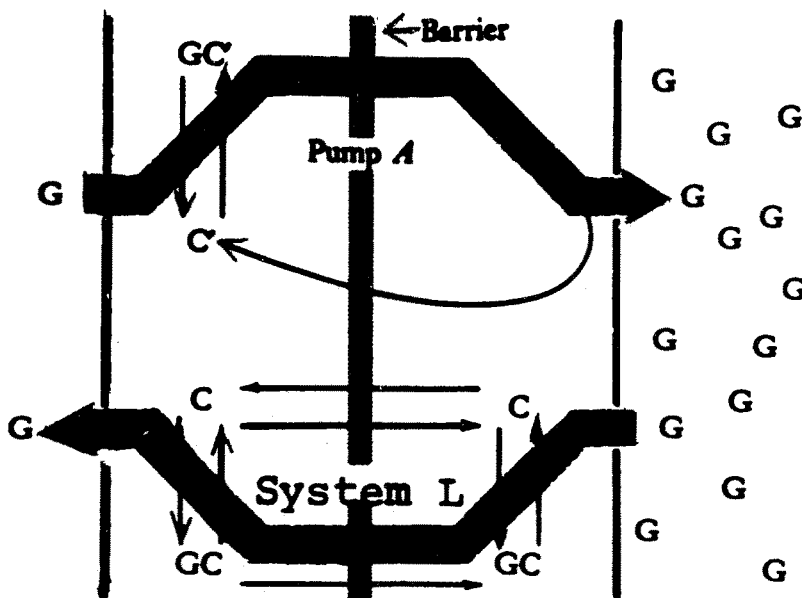
Figure 4   Diagram to illustrate the parallel operation of two transport systems in non-brain tissues. The Na^+-dependent systems, such as A, tend to be highly concentrative for G, so that Na^+-independent, system L is caused to serve mainly for its exodus. G may represent Gly, Ala or Pro in this study, but may represent any other system L substrate that is also concentrated by system A, as is Phe itself. BCH is also transported by system L. Either Phe or BCH inhibits the exodus of G through system L.

These effects are illustrated in the diagram of Figure 4. The parallel operation of two transport systems is shown for an amino acid that is pumped into the cell by a strongly concentrative transport system, such as system A. System L, generally being weakly concentrative, is forced to operate for net exodus by the high amino acid concentration attained inside the cell through the action of the Na^+-dependent systems (Christensen, 1986; 1987a; 1987b; Christensen and Handlogten, 1979). In fact, it has been observed in this laboratory that the usually specific system A inhibitor, 2-aminoisobutyric acid, or its more uniformly specific *N*-methyl derivative, causes *decreases* of the hepatic levels of amino acids relative to their plasma levels (Christensen and Cullen, 1968), whereas the relatively specific system L inhibitor, BCH, causes corresponding *increases* (Christensen and Cullen, 1981). Phe, as well as other amino acids preferring system L for passage, has been shown

to increase the distribution ratio, tissue/plasma, for Gly in the liver and in the thigh muscle of the guinea pig (Christensen *et al.*, 1948).

At the level of the blood–brain barrier, however, system L was long the only transport system clearly recognized to function for neutral amino acids, the inward operation of system A at this site being apparently negligible (Wade and Katzman, 1975; Betz and Goldstein, 1978). Therefore, Phe or similar system L substrates will, we hold, tend to decrease the brain/plasma distribution ratio of a number of amino acids by transport competition.

In the present study, in all cases except that of Lys in liver, a statistically significant increase in distribution ratio after Phe injection was always accompanied by a statistically significant decrease in the plasma concentration of the corresponding amino acid (Table 2). Ala and Pro in liver and muscle, respectively, were the only two cases where an increase in net intracellular concentration accompanied their respective opposite changes in both plasma levels and distribution ratios. Only Glu⁻ in muscle showed a statistically significant increase in the intracellular concentration without any other significant change (Table 2, column 4).

The effect of Phe in increasing the distribution ratios of Ala and Gly in liver and also those of Ala, Gly and Pro in muscle (Table 2, column 4) is produced also by the system L analogue, aBCH, for Ala and Gly and probably also for Pro in muscle (Table 2, column 7; Figure 3) as well as for Ala in liver (Table 2, column 6). We think that these results can be explained by the mechanism discussed above. Ala, Gly and Pro, as preferred substrates of system A, are accumulated into the cells by this route and then the elevated intracellular Phe (Table 1) inhibits their exodus through system L. In a parallel effect to the one observed for Phe, aBCH caused a statistically significant decrease in the plasma concentration of Gly accompanying the increase in the distribution ratio of this amino acid in muscle. Although the decreases in the plasma concentrations of Ala and Pro after the administration of aBCH failed to show statistical significance, on the basis of their increased tissue/plasma ratios attained, we think that this trapping effect is a likely contributory mechanism for the decrease of these two amino acids in plasma in hyperphenylalaninaemia. It appears to be a major mechanism for the decrease in the plasma concentration of Gly under that condition. Previous results in this laboratory when Phe was fed to weanling rats at 5% in the diet for 6 days support the same conclusion (Christensen, 1987b).

The statistically significant decrease in the net intracellular concentration of Gly in liver after aBCH injection can best be explained by its rapid catabolism through increased mass action arising from strong inhibition of exodus. A similar effect may explain the lack of increase in net intracellular concentrations of other affected amino acids.

We have no compelling explanation at this time for the statistically significant increases by Phe of the distribution ratios in muscle of other amino acids, namely Leu and His and the cationic amino acids, Orn and Arg, all of which also showed a statistically significant decrease in plasma. The apparent parallelism in the effects of Phe and aBCH on Lys in liver (Table 2, columns 3 and 6) and Arg in muscle (Table 2, Columns 4 and 7) deserves, however, further study (see Oldendorf *et al.*, 1988).

It is not clear why the unexplained accumulations of various amino acids in non-brain tissues of newborn rats treated with Phe and α-MePhe observed by Huether and colleagues (1984) are seen only in the first perinatal days. These authors propose that the consequent depletion in plasma is the main factor contributing to the unavailability of amino acids to the brain and speculate that no competitive transport effects of Phe occur either at the blood–brain barrier (Huether *et al.*, 1984) or elsewhere (Huether, 1987). We think that a direct effect of Phe on the blood–brain barrier need not be excluded in proposing a separate likely contributory effect of the sequestration in other tissues on the depletion of amino acids in the brain in hyperphenylalaninaemic states. Neither does an increased utilization of certain amino acids exclude transport effects of the kind we are describing here for muscle and liver. Moreover, these transport effects may prove to be the first step that leads to an increased utilization of the trapped amino acid by mass action, as is probably the case for Gly in liver as illustrated in this study (Table 2, columns 3 and 6).

We propose instead that Phe causes a depletion of neutral amino acids in the brain by means of a dual competitive effect, one affecting directly the entry especially of bulky neutral amino acids at the level of the blood–brain barrier, and another, according to the results in this study, inhibiting the exodus of small neutral amino acids from non-brain tissues.

Transport mechanisms in non-brain tissues have been proposed but not experimentally tested to explain the decrease in the plasma concentrations of isoleucine and valine after oral (Nyhan *et al.*, 1961) and intravenous (Erikson *et al.*, 1981) loads of leucine in humans. On the specific question faced here, Efron and colleagues (1969), on the basis of the already known inhibitory effect of Phe on the entry of some amino acids into the brain, excluded transport effects as an explanation for the decrease of plasma amino acids in PKU. It was not fully understood at that time, however, that a specific transport system tends to be given directionality in its operation by the flows of metabolism in various tissues.

Insulin concentrations in plasma have been found to be increased in PKU patients after a glucose load (Antonozzi *et al.*, 1987). In fact, Phe is an effective insulin secretagogue in man (Fajans *et al.*, 1967), although it appears be a very weak one in the rat, according to studies in the perfused pancreas (Landgraff *et al.*, 1974) and also according to preliminary observations in our laboratory. In the present study, we purposely used a non-insulinogenic species of BCH, the a(±) isomer (Tager and Christensen, 1971). Hence, the effect of Phe on the small neutral amino acids observed here seems to be related mainly to direct transport competitive effects. System A proves, however, a selective target of insulin in stimulation of amino acid transport in isolated preparations of rat liver and muscle (Shotwell *et al.*, 1983). Even if secreted to a slightly increased extent in the pretreated rat, insulin could play a role to enhance the trapping effect by some administered amino acids described above. In man, the plasma levels of the branched-chain amino acids fell most during regulated insulin administration, although Pro and Ile were the most sensitive to insulin dose (Fukagawa *et al.*, 1986). We are currently exploring possible contributory effects of insulin in the changes of plasma and tissue amino

acids observed here, as well as after administration of Leu, the latter a clear insulin secretagogue in the rat (Landgraff *et al.*, 1974).

It is apparent in this study that the small neutral amino acids are the most generally affected. Experimental studies by others have also shown significant changes in several small neutral amino acids after Phe treatment. Ala has been found to be the most severely depleted of the non-essential amino acids in the brain of hyperphenylalaninaemic 10-day-old rats (Lowden and LaRamee, 1969), although the occasionally overlooked brain metabolism of branched chain amino acids also received appropriate emphasis in these results. Gly uptake into rat liver and brain has been found to be stimulated by Phe in short-term experiments (Zanic-Grubisic and Lipovac, 1981). The relationship of these effects to our findings is, however, not clear at this time.

Ala, Gly and Pro are non-essential according to the classical meaning of this term. The possibility that the brain may need to receive non-essential amino acids supplied by other tissues must, however, not be overlooked. This point brings us to the subject of interorgan nutrition (Christensen, 1987b; 1982). For example, in discussing the availability of neurotransmitters in PKU, it is natural to think first only of Trp and Tyr. It is not known, however, if a shortage in the supply of blood Gly may also affect the concentration and further function of this neurotransmitter in the brain (Sershen *et al.*, 1987). The exaggerated classical concept of essentiality of amino acids was so ingrained at the time of the publication of Linneweh and Ehrlich's paper that the non-essential amino acids were excluded from the calculations (1962 Figure 2).

The significance of the present results to an understanding of the pathogenesis of the brain damage in PKU has to be assessed by further work. We are currently looking for changes in amino acid distribution in rats treated chronically with Phe and α-MePhe. A better animal model of PKU would, however, still be highly desirable. Complete plasma amino acid analysis in PKU patients at some times during treatment and at different metabolic states may also be enlightening to explain the significance of changes in other amino acids aside from Phe and Tyr in PKU.

It may be relevant that under our relatively acute experimental as well as more prolonged (Christensen, 1987b) conditions, we have observed a pattern of plasma amino acids very similar to the one reported in PKU children (Figure 2; Linneweh and Ehrlich, 1962; Efron *et al.*, 1969). As far as we are aware, the plasma amino acid pattern characteristic of PKU had not previously been reproduced in animals (Dolan and Golin, 1967; Kaufman, 1977). It may also be relevant that Efron and colleagues (1969) found an enhanced decrease of the amino acids affected in plasma in two of their PKU patients after an intravenous infusion of Phe. The differences were, however, also statistically significant when the patients were in their chronic condition, as was also apparently the case in the patients of Linneweh and Ehrlich (1962). On the other hand, Snyderman and colleagues (1981), in measuring several amino acids in fasting newborn PKU patients, found that only Thr showed a statistically significant decrease in the plasma, although Ala and also Arg were decreased along with Thr in the cerebrospinal fluid. Age of the patients and length

of fasting before sampling may be likely causes for the discrepancy between the different groups of PKU patients. Unfortunately, Thr always co-eluted with Ser in our analyses.

Small neutral amino acids are also, in general, prominently depleted in plasma in PKU (Figure 2). Ala, Pro and Gly were found to be sharply decreased in the Linneweh and Ehrlich study, but, as noted above, the significance of the changes was not assessed statistically. On the other hand, Ala and Pro were found to be significantly decreased in plasma in the study of Efron and colleagues; Gly showed a *p* value of 0.05 as well as an additional decrease after Phe infusion (Efron *et al.*, 1969). Moreover, Ala together with Gln and Asn have been considered to be the only 'non-essential' amino acids whose depressed concentrations in plasma are corrected during the usual dietary treatment of PKU (Perry *et al.*, 1970). In the present study, Gln, once considered an importantly depleted amino acid in PKU (Perry *et al.*, 1970), was found to be decreased in plasma, although not to a statistically significant level ($p = 0.17$) (Table 1). Although significantly decreased in plasma in PKU patients, Gln was one of the less effected amino acids in the study of Efron and colleagues (1969). Under our experimental conditions, we did not find statistically significant changes either in the distribution ratio or in the intracellular concentration of Gln in both liver and muscle (Tables 1 and 2).

Perturbed transport processes are likely to be a prime cause of the deleterious imbalances in PKU and related inborn errors of amino acid metabolism. The identification of the transport mechanisms involved may be relevant to the possibility of specific interventions through the administration of selected amino acids or even relatively inert amino acid analogues. Such a step might help to correct the imbalances and may also have the additional benefit of allowing a less stringent and more palatable diet. Although this approach has been proposed by other investigators (Brunner *et al.*, 1979; Pratt, 1980), the focus previously has been directed exclusively to the brain and to the classical essential amino acids. We urge that, under our working hypothesis, other tissues and the amino acids usually considered as non-essential receive attention.

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