BIOCHEMICAL LETTERS

Where do the depleted plasma amino acids go in phenylketonuria?

Although the phenylalanine accumulations of phenylketonuria (PKU) have long been held to interfere with the transport of various amino acids into the developing brain (Christensen, 1953; see also Guroff & Udenfriend, 1962; McKean *et al.*, 1968; McKean, 1972), an accompanying lowering of certain plasma amino acids (Linneweh & Ehrlich, 1962; Efron *et al.*, 1969; see also review by Knox, 1966) has remained a paradox. The list of affected amino acids includes threonine, glutamine, asparagine, proline, alanine and leucine. It has been suspected that the excess phenylalanine interferes with the net movement of such amino acids from the intestinal lumen. However, in discussing a probably related action of orally administered leucine to lower the plasma levels of valine, serine and isoleucine, not attributable to the loss of these amino acids into the urine, Nyhan *et al.* (1961) commented plausibly as follows: "... it seems likely that transport of amino acids into cells is responsible for their decreased concentration in the serum". To account for major increases in a few large amino acids in several organs, an effect largely specific to suckling rats, phenylalanine excesses have been held to stimulate "the use and accumulation of amino acids in developing tissue" (Huether & Neuheoff, 1983; Huether *et al.*, 1984). Neither of these suggestions explains, however, why amino acids come to be sequestered in tissues other than brain. Zanic-Grubisic & Lipovac (1981) showed that phenylalanine excess decreases influx of three ^{14}C -labelled amino acids into both liver and brain. This response by the liver we would expect only to compensate for the response by the brain. Unfortunately net changes in amino acid distribution were not measured.

Gradually I've come to see that my colleagues and I have long had in hand the evidence needed to explain such declines in the plasma amino acids (Christensen *et al.*, 1948). We sought in that year to perturb the distribution of endogenous amino acids between cells and the extracellular fluid by tube feeding to the guinea pig 10–12.5 mmol/kg body wt. of a single amino acid during an interval of 2 h. We noted first the behaviour we had expected to arise from transport competition by the administered amino acid. Seven out of nine amino acids that accumulated in the body at this dosage were found to have depressed the hepatic level of glycine relative to the plasma level. The lower half of Fig. 1 shows a similar effect on the glycine gradient maintained by skeletal muscle in nine out of ten tests with proline, threonine, serine, and alanine, each administered alone. But for muscle we long overlooked this effect, because a similar excess (upper half of Fig. 1) of tryptophan, leucine, phenylalanine, methionine, histidine or tyrosine had

Fig. 1. Amino acids that perturb the distribution of glycine between thigh muscles and plasma of the guinea pig when administered in excess fall into two populations: those that decrease the distribution ratio (below the line) and those selected for their high reactivity with System L, which instead intensify the muscle accumulation of glycine

raised rather than lowered the glycine gradient maintained by muscle. Clearly we need to treat the dipolar amino acids as two separate populations, one of which we see migrating principally by System A or other Na^+ -dependent systems (lower half of Fig. 1), and the other migrating principally by System L [see Oxender & Christensen (1963) and the upper part of Fig. 1]. Closer examination has indicated a similar differentiation applies to liver, with L-phenylalanine and DL-valine acting to intensify the hepatic retention of glycine.

What was needed to understand these two opposed transport effects is a consideration of the interaction between a highly concentrative transport system and a more weakly uphill transport system [see, for example, Christensen (1975) for an illustration of this concept]. The strongly uphill Na^+ -dependent systems, at least System A, maintain tissue gradients for each of a number of amino acids so steep that the less concentrative System L is forced to operate for exodus of these amino acids (see also Christensen & Handlogten, 1979). Experience (Christensen, 1975) has confirmed these separate affiliations of the two groups of amino acids differentiated in

Fig. 1. The central principle is that an amino acid supplied in excess can inhibit either the tissue uptake or release of other amino acids, depending in each case on the net directional role exerted by the transport system(s) suffering the larger competitive influence. Only occasionally can we expect the two opposed influences to be closely balanced.

We added support to the above interpretation by injecting intraperitoneally into rats a bicyclic amino acid ('BCH'; 2-aminoendobicyclo[3.2.1]carboxylic acid) built on the norbornane nucleus. This analogue was selected as a rather specific substrate for System L (Christensen & Cullen, 1981). In contrast with the widespread *lowering* of hepatic amino acid levels produced by the characteristic System A substrate analogues 2-aminoisobutyric acid or 2-(methylamino)isobutyric acid in parallel tests [Figs. 6, 8 and 10 in Christensen & Cullen (1968)], the bicyclic analogue proved to generate in the rat *elevated* gradients of seven amino acids for liver cells relative to plasma. No gradients were lowered significantly. We have not yet excluded an equally interesting alternative explanation, that phenylalanine or leucine accumulations might instead lower the plasma amino acids through their insulin-secretagogue action (Fajans *et al.*, 1967). One of the homologues of the norbornane amino acid might well serve to show the transport-competitive action dissociated from the secretagogue effect.

The proposed action of excess phenylalanine or leucine in causing tissue sequestration of various amino acids, thus lowering their plasma levels, adds a second action unfavourable to the nutrition of the brain, beyond the direct competition with the net inward flow across the blood/brain barrier. The fact that System L catalyses *inward* flow across that barrier (Wade & Katzman, 1975, with the probable exception of the counterflow of glutamine; James *et al.*, 1979), but *outward* flow across the plasma membrane of many other tissues, causes, I propose, a summation of competitive effects on System L. This added effect of the lowered mass action of certain amino acids at the blood/brain barrier makes it harder to predict the full list of amino acids that may suffer depletion in the brain in PKU or in maple-syrup-urine disease. Note also that when the liver is stimulated to maintain an unusual accumulation of certain amino acids, their hepatic catabolism will probably be accelerated. A correspondingly accelerated catabolism of branched-chain amino acids might arise from their intensified retention by muscle. Indeed, stimulated amino acid catabolism has been reported to be a feature of hyperphenylalaninaemia (Lipovac *et al.*, 1979). The accelerated catabolism might readily mask a moderate cellular amino acid sequestration unless the tissue level in each case is referred appropriately to the corresponding plasma level. This frame of reference makes use of a relation shown, for example, for glycine in the guinea pig; however widely the free glycine varies, it tends to vary in

association in the plasma, in the liver, and in the muscle unless transport is perturbed (Christensen *et al.*, 1948). The complication thus introduced into our argument may, however, be avoidable, because preliminary tests made in collaboration with Dr. J. G. Thoene on weanling rats fed 5% phenylalanine in their diet showed increases in the hepatic levels of various amino acids concurrent with declines in the corresponding plasma levels. Hence amino acid sequestration was obvious whether the tissue level was or was not referred to the plasma level.

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- Christensen, H. N. (1953) *Annu. Rev. Biochem.* **22**, 235
 Christensen, H. N. (1975) *Biological Transport*, 2nd edn., p. 381, W. A. Benjamin, Reading, MA
 Christensen, H. N. & Cullen, A. M. (1968) *Biochim. Biophys. Acta* **150**, 237–252
 Christensen, H. N. & Cullen, A. M. (1981) *Life Sci.* **29**, 749–753
 Christensen, H. N. & Handlogten, M. E. (1979) *J. Neural Transm. Suppl.* **15**, 1–13
 Christensen, H. N., Streicher, J. A. & Elbinger, R. L. (1948) *J. Biol. Chem.* **172**, 515–524
 Efron, M. L., Song Kong, E., Visakorpi, J. & Feller, F. X. (1969) *J. Pediatr.* **74**, 399–405
 Fajans, S. S., Floyd, J. C., Jr., Knopf, R. F. & Conn, J. W. (1967) *Recent Prog. Horm. Res.* **23**, 617–660
 Guroff, G. & Udenfriend, S. (1962) *J. Biol. Chem.* **237**, 803–806
 Huether, G. & Neuhoff, V. (1983) *J. Neurochem.* **41**, S26
 Huether, G., Schott, K., Sprotte, U., Thoemke, F. & Neuhoff, V. (1984) *Int. J. Dev. Neurosci.* **2**, 43–54
 James, J. H., Jeppson, V., Ziparo, V. & Fischer, J. E. (1979) *Lancet* **ii**, 772–775
 Knox, W. E. (1966) in *The Metabolic Basis of Inherited Disease* (Stanbury, J. B., Wyngaarden, J. B. & Fredrickson, D. S., eds.), 2nd edn. pp. 258–278, McGraw-Hill, New York
 Linneweh, F. & Ehrlich, M. (1962) *Klin. Wochenschr.* **40**, 225–226
 Lipovac, K., Zanic-Grubisic, T., Juretic, D. & Mihaljeric, I. (1979) in *Models for the Study of Inborn Errors of Metabolism* (Hommes, F. A., ed.), pp. 133–140, Elsevier/North-Holland, Amsterdam
 McKean, C. M. (1972) *Brain Res.* **47**, 469–476
 McKean, C. M., Boggs, D. E. & Peterson, N. A. (1968) *J. Neurochem.* **15**, 235–241
 Nyhan, W. L., Borden, M. & Childs, B. (1961) *Pediatrics* **27**, 539–545
 Oxender, D. L. & Christensen, H. N. (1963) *J. Biol. Chem.* **238**, 3686–3699
 Wade, L. A. & Katzman, R. (1975) *J. Neurochem.* **25**, 837–842
 Zanic-Grubisic, T. & Lipovac, K. (1981) *J. Inherited Metab. Dis.* **4**, 105–106

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