No Evidence for Individual Blood–Brain Barrier Phenylalanine Transport to Influence Clinical Outcome in Typical Phenylketonuria Patients

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Brain tissue concentrations of phenylalanine (Phe) can be measured by proton magnetic resonance spectroscopy[1–4] in patients with phenylketonuria (PKU). In combination with oral Phe challenges, the kinetics of Phe transport at the blood–brain barrier can be characterized. Weglage and colleagues[1] reported interindividual differences and an association of such kinetic parameters with IQ and magnetic resonance imaging (MRI) visible white matter changes in a group of 15 PKU patients. It was interpreted to indicate that "interindividually different blood–brain barrier transport characteristics [. . .] are major *causative* factors for clinical outcome in PKU."[1]

The results of Weglage and colleagues[1] contradict a recent study by Rupp and colleagues,[2] which yielded a close linear regression for blood and brain Phe concentrations and no correlation between blood and brain ratios (mean, 4.0 ± 0.4) and IQ ($r = 0.05$, not significant). Reanalysis of the data published by Weglage and colleagues[1] and comparison with earlier results of this group[3,4] were performed to clarify this discrepancy.

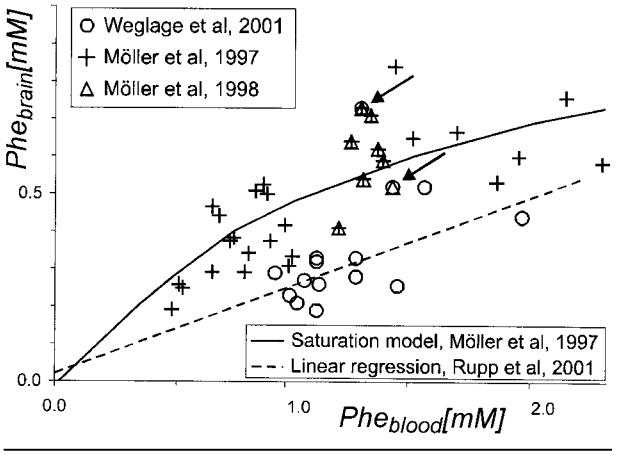
There is ample evidence that strictness of early dietary treatment, documented, for example, as blood Phe levels during the first 10 years of life ($[\text{Phe}]_{\text{blood} < 10y}$), determines IQ. As expected, Weglage and colleagues[1] found a strong negative correlation between $[\text{Phe}]_{\text{blood} < 10y}$ and IQ ($r = -0.68$; $p = 0.005$). The major conclusion of this study is based on associations between kinetic parameters and IQ ($K_{t,app}$: $r = 0.45$; $p = 0.09$; T_{max}/V_{met}: $r = -0.50$, $p = 0.06$). However, completing the correlational matrix in Table 3 in the study by Weglage and colleagues[1], it can be found that the kinetic parameters also are correlated with $[\text{Phe}]_{\text{blood} < 10y}$ ($K_{t,app}$: $r = 0.54$, $p = 0.04$; T_{max}/V_{met}: $r = 0.41$, $p = 0.13$; Spearman rank correlations based on the data presented in Tables 1 and 2, but not mentioned in Weglage and colleagues[1]). If the influence of $[\text{Phe}]_{\text{blood} < 10y}$ on IQ is statistically controlled for, the correlation coefficients of IQ with $K_{t,app}$ ($r = 0.13$, $p = 0.65$) and with T_{max}/V_{met} ($r = 0.32$, $p = 0.26$) substantially decrease and become statistically nonsignificant.

Numerous studies have shown that the severity of MRI changes is related to blood Phe levels during several weeks to months before investigation. The correlation between MRI changes and preload brain Phe ($r = 0.77$, $p = 0.001$)[1] therefore is not unexpected. In contrast with the literature, the association between MRI changes in adult life is substantially higher with $[\text{Phe}]_{\text{blood} < 10y}$ ($r = 0.75$, $p = 0.005$) than with $[\text{Phe}]_{\text{blood} > 10y}$ ($r = 0.31$, $p = 0.25$) or with preload $[\text{Phe}]_{\text{blood}}$ ($r = 0.02$, $p = 0.94$).

Möller and colleagues[3] described saturation kinetics for Phe transport based on blood–brain Phe ratios (mean 2.1 ± 0.5 for blood Phe values <1.5mM, in which a significant linear correlation was found by the researchers[3]). In their recent work,[1] the corresponding preload ratio was 3.8 ± 1.1. This suggests a systematic difference between the present[1] and earlier studies[3,4] (Fig). The data of the two patients with lowest $K_{t,app}$ and highest T_{max}/V_{met} (no.1 and 2), and hence largest influence on the apparent "individuality" of the kinetic parameters, already were included in earlier studies (nos. 8 and 9 in Möller and colleagues[4]). These two patients, who previously have been characterized as "typical" PKU patients,[4] now seem to represent "particular" patients compared with the remaining 13 patients (eg, T_{max}/V_{met} is >6 standard deviations above the mean of the 13 new patients), whereas the kinetic parameters of three previously "atypical" patients[4] fit well with the new data set. Thus, the statistics in the recent article appear to be based on an inhomogeneous sample (Fig) composed of 13 recently investigated patients and 2 patients added from an earlier study. If the latter are excluded from analysis, the correlations of IQ with $K_{t,app}$ ($r = 0.23$, $p = 0.46$) and with T_{max}/V_{met} ($r = -0.31$, $p = 0.31$) decrease, corroborating the results of Rupp and colleagues.[2]

In summary, clinically significant interindividual differences in blood–brain barrier Phe transport and an influence of kinetic characteristics determined in adult life on outcome parameters is not confirmed for most "typical" PKU patients. The conclusion that the reported observations ultimately could lead to individual dietary recommendations[1] is currently not tenable.

Fig. Juxtaposition of recent[1] and earlier[2–4] data on brain versus blood Phe content in patients with phenylketonuria (PKU) determined by proton magnetic resonance spectroscopy Möller and colleagues[3] reported saturation kinetics (plus sign, solid nonlinear regression line) *on the basis of steady state measurements and oral Phe challenges in some patients. Blood and brain concentrations of Phe in eight "typical" PKU patients* (triangles), *reported in 1998,[4] were in agreement with the earlier data. However, the latest data* (circles) *presented in this journal[1] (accuracy of brain Phe was reported as ±0.1mM) fit neither the earlier steady state nor the earlier kinetic data from this group but rather corroborate the linear model suggested by Rupp and colleagues[2]* (dashed linear regression line) *(individual data omitted for clarity). Two data points in the latest report[1]* (arrows) *originate from the earlier studies and largely govern the suggested[1] influence of individual blood–brain barrier transport kinetics on clinical outcome.*



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Reply

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Different clinical outcome despite comparable dietary control is well known in patients with phenylketonuria (PKU).[1] Single case reports have described untreated patients with classic PKU and blood phenylalanine (Phe) levels consistently above 1.2mmol/L and with normal intelligence.[2] Exact mechanisms for this most interesting phenomenon are still not well understood.

Recently, researchers at the University of Bern/Heidelberg, Yale, and University of Münster (cited according to Möller and colleagues[2]) demonstrated independently that in vivo proton magnetic resonance spectroscopy offers a novel strategy for quantifying intracerebral Phe concentrations. Slightly different examination techniques (eg, point resolved spectroscopy versus stimulated echo acquisition mode) and, more importantly, different strategies used for data evaluation probably contribute to some systematic inconsistencies in the concentrations estimates from different groups as examined recently by Kreis.[3] Regarding potential interstudy inconsistencies, we have repeatedly pointed out that current limitations of the experimental technique used for quantifying brain Phe at concentrations well below 1mmol/L cause considerable variability, even when comparing studies from the same laboratory.[2,4,5] Because of the notoriously poor signal-to-noise ratio of the Phe signal under such conditions, estimates of $[Phe]_{brain}$ derived from nuclear magnetic resonance spectroscopy are associated with relatively large errors. These uncertainties in the $[Phe]_{brain}$ data unavoidably lead to substantial standard deviations of the estimated kinetic parameters by error propagation.[2,4]

The figure presented by Pietz and colleagues[6] might be misleading, suggesting that our kinetic analysis[5] was based on single spectroscopic measurements under steady state conditions. Thus, note that all kinetic parameters were, in fact, extracted from oral loading experiments and linear fits of the observed time-dependent variations of blood and brain Phe concentrations. Consequently, potential interstudy variability of $[Phe]_{brain}$ is not an issue for the individual time courses, which were used in calculations of the parameters $K_{t,app}$ and T_{max}/v_{met} and our subsequent analysis.

Because the patient group examined in our last study[5] is relatively small, nonparametric tests (rank correlations according to Spearman) were used for statistical evaluation. This is appropriate. Classification of PKU patients into "atypical," "particular," and "typical" categories as proposed by Pietz and colleagues[6] is unreasonable and contradicts basic physiological understanding. Hence, we do not see sufficient reason to exclude patients from analysis. Preselection of patients to achieve a homogeneous sample may be appropriate for a metabolic characterization of most PKU patients. However, it is not likely to lead to an understanding of the heterogeneity in the clinical outcome, which is known to vary significantly in a subgroup of patients. Nevertheless, the numbers of patients included in both our most recent study[5] and the one by Rupp and colleagues[7] are low, and additional research certainly is indicated to clarify potential inconsistencies among the results.

There is no doubt that all large neutral amino acids are transported across the blood–brain barrier by a single facilitated system, which is stereospecific and follows Michaelis-Menten kinetics. Numerous studies in animals and cell cultures have underlined that different affinities do exist for the large neutral amino acids to the same carrier system. Results of previous investigations by Möller and colleagues[2,4] suggested a saturation kinetics for Phe at this carrier system. This is consistent with kinetic data measured in hyperphenylalaninemic rabbits.[8] In addition, direct support for this hypothesis arises from permeability surface area products for amino acid transport into human brain measured by the double-indicator method.[9] Furthermore, Pietz and colleagues[10] demonstrated that Phe influx into the brain via the L-type amino acid carrier can be competitively blocked by supplementing high doses of all other large neutral amino acids. Although it is an open question at which Phe concentration significant saturation occurs in individual patients, there is no doubt from basic physiological understanding that a close linear regression for blood and brain Phe concentrations will not persist beyond some threshold of $[Phe]_{blood}$. Again, more investigations are clearly warranted to answer this question.

Intracerebral Phe levels measured by magnetic resonance spectroscopy under steady state conditions in PKU patients clearly remained below blood concentrations with ratios $[Phe]_{blood}$ to $[Phe]_{brain}$ varying between 3.45 and 4.47 in the study of Rupp and colleagues[7] and between 1.7 and 5.6 in our recent study.[5] Our measurements compare very well with a recent study in 29 PKU patients by Koch and colleagues,[11] indicating that interindividual differences in $[Phe]_{brain}$ in patients with similar blood concentrations of Phe are not limited to a few exceptional cases but may be more common. However, some inconsistencies between the results, reported by Rupp and colleagues,[7] Weglage and colleagues,[5] and Koch and colleagues[11] remain currently unexplained. Further research is absolutely needed.

There is no doubt that the quality of dietary control during the first years of life is the most important factor for the

patients' development. In addition, some studies suggest that the severity of magnetic resonance imaging changes is related to blood Phe levels during weeks to months before investigation. However, correlations between blood Phe levels and different parameters of clinical outcome generally were found to be low.[12] This might be explained by our observation that patients' brain Phe levels may be different despite comparable blood Phe levels.

Finally, in view of the discussion of potential error sources including such issues as the small patient population, we feel that the results were interpreted with sufficient caution: "… we hypothesize that, in addition to the quality of diet during the first decade of life, favorable transport parameters may additionally protect the brain from uptake of high amounts of the neurotoxin Phe. … BBB Phe transport and $[Phe]_{brain}$ seem to be important factors for individual clinical outcome in PKU. … Our observations may ultimately lead to individual dietary recommendations in the future; however, numerous questions remain to be answered …."[5]

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Mild Cognitive Impairment and the Cholinergic Hypothesis: A Very Different Take on Recent Data

Martin Sarter, PhD, and John P. Bruno, PhD

In his recent editorial, Morris[1] concluded that the very interesting data presented by DeKosky and colleagues[2] challenge the hypothesis that decline in the integrity of basal forebrain cholinergic projections is associated with the emergence of cognitive impairments associated with the initial stages of Alzheimer's disease (AD). We take issue with this interpretation and, in the context of other recent data, suggest that, in fact, quite the opposite is true: the case for an early involvement of the cholinergic system in the age-related cognitive decline has never been stronger.

The main finding by DeKosky and colleagues[2] indicates that cortical choline acetyltransferase (ChAT) activity is unchanged in subjects with mild cognitive impairment (MCI) when compared with subjects with no cognitive impairment (NCI) and, in fact, is increased in hippocampal regions and the superior frontal cortex. As DeKosky and colleagues[2] point out, ChAT activity does not represent the rate-limiting step in the synthesis and release of acetylcholine (ACh). Changes in ChAT activity likely indicate substantial changes in the density of presynaptic cholinergic neurons. Although DeKosky and colleagues[2] and Morris appear to consider the absence of decreases in ChAT activity in MCI an unexpected result, this result is predicted by previous studies indicating no decline in the number of ChAT-immunoreactive neurons in the basal forebrain of subjects with MCI.[3]

There is now ample evidence for the hypothesis that cholinergic neurons in MCI are not normally regulated. Mufson and colleagues[4,5] reported that in MCI the number of neurons in the nucleus basalis showing immunoreactivity for trkA, the high-affinity receptor for nerve growth factor (NGF), as well as neurons immunoreactive for the low-affinity p75 NGF receptor, is significantly decreased when compared with NCI. Moreover, the numbers of immunoreactive neurons in MCI were statistically similar to the low number of trkA- or p75-immunoreactive neurons counted in AD.[4,5] In situ hybridization further confirmed that the number of neurons expressing trkA is decreased in MCI and is indistinguishable from Alzheimer's disease.[6] Furthermore, the number of basalis neurons bearing NGF receptors correlated with the cognitive status of the subjects in these reports.[4–6] Finally, in animal studies, ChAT activity changes are, as expected, a poor predictor of changes in the regulation of ACh release, specifically for the capacity of (residual) cholinergic neurons to respond to behavioral or pharmacological challenges.[7]

The exact status of cholinergic transmission in the forebrain of subjects with MCI is unclear. Although it is unlikely that any ex vivo histochemical or neurochemical method might be capable of showing such changes, emerging positron emission tomography[8] or single-photon emission

computed tomography[9] methods to monitor cholinergic activity may provide insights into the functional status of cholinergic neurons in MCI. The extensive experimental evidence provides overwhelming support for the hypothesis that disruption of trophic factor support necessarily, and strikingly, affects the function of cholinergic neurons.[10–13]

Thus, the available data suggest that although the number of cholinergic terminals is either unchanged or even increased in MCI, their trophic factor regulation is disrupted. Even subtle changes in telencephalic cholinergic transmission, if persistent, may trigger initially limited but eventually escalating cognitive impairments.[14] Thus, the available data, in fact, do not challenge the cholinergic hypothesis, as suggested by Morris,[1] but instead provide increasingly impressive support for the hypothesis that possibly early abnormalities in the regulation of the cholinergic system trigger cognitive limitations that per se, and even more so in conjunction with an accelerating decline in cholinergic functioning, contribute to the emergence of dementia.[15]

Finally, the therapeutic limitations of cholinesterase inhibitors may reflect the limited efficacy of these drugs to enhance or reinstate phasic cholinergic transmission[14] and/or the possibility that postsynaptic signaling mechanisms are also disrupted,[16] rather than rejecting hypotheses about the role of abnormal cholinergic transmission in the manifestation of age-related cognitive impairments, as suggested by Morris.[1] Clearly, "the cholinergic hypothesis" remains a simplistic and likely incomplete account of the neuronal bases of cognitive decline, particularly if the discussion remains focused on cell loss and ignores changes in cholinergic signal regulation. However, the available evidence from subjects with MCI and Alzheimer's disease, together with the extensive experimental literature,[17] indicates that it remains one of the most viable hypotheses in this field.

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