28. Nilsson T, Sjoling-Ericksson A, Deinum J. The mechanism of binding of fenton JW, Villanueva GB, Ofosu FA, Maraganore JM. Thrombin inhibition by Winn MJ, Jain K, Ku DD. Argatroban and inhibition of the vasomotor actions Nowak G, Bucha E. Quantitative determination of hirudin in blood and body Eichler P, Friesen H-J, Lubenow N, Jaeger B, Greinacher A. Antihirudin Song XH, Huhle G, Wang LC, Hoffmann U, Harenberg J. Generation of Harenberg J, Reichel T, Malsch R, Hirsh J, Rustagi P. Multicentric evaluation Elg M, Gustafsson D, Deinum J. The importance of enzyme inhibition Nar H, Bauer M, Schmid A, Stassen J, Wienen W, Priepke HW, et al. Phenylketonuria (PKU) is a disorder in which the aromatic amino acid Phe cannot be converted to Tyr (1, 2). Unfortunately, many PKU patients do not adhere to their low-Phe diet (off diet), which leads to high concentrations of the amino acid in their blood (1, 2). High Phe concentrations interfere with the production of adrenaline (A), noradrenaline (NA), and dopamine (DA) (1, 3). Furthermore, Krause et al. (4) reported an inverse relationship between NA and DA plasma concentrations and Phe because high Phe concentrations decrease the availability of the amino acids Tyr and Trp, the precursors of catecholamines and serotonin [5-hydroxytryptamine (5HT)], respectively (5–7).

Acetylcholinesterase (AChE) is a membrane-bound enzyme with its active side exposed at the external leaflet of the bilayer (ectoenzyme). When the enzyme is inhibited, it can no longer participate in the hydrolysis of acetylcholine (Ach) (8), involving parasym pathetic, sympathetic, peripheral, and central nervous system function (8–10). Alterations of the above substances in the cerebrospinal fluid are correlated with AChE activity in the cerebrospinal fluid of patients with mental impairment (11).

In our previous study (12), incubation of high Phe concentrations with human AChE type XIII led to inhibition of the enzyme (40–60%). The effect of Phe on AChE of rat diaphragm and rat brain showed a concentration-dependent enzyme inhibition (13, 14). We therefore aimed to evaluate AChE activities in the erythrocyte membranes from patients with PKU and to correlate the enzyme activities with blood concentrations of the biogenic amines A, NA, DA, and 5HT as well as with the precursors Tyr and Trp.

The study was approved by the Greek ethics committee and was conducted according to the principles expressed in the Helsinki Declaration.

The study population consisted of 23 PKU patients who were divided into two groups: group A (n = 12; mean age, 6.8 ± 1.2 years), who adhered strictly to their special therapeutic diet as evidenced by their almost normal plasma Phe concentrations (Phe, 180.4 ± 30.7 μmol/L); and group B (n = 11; mean age, 7.2 ± 2.0 years), who were off diet and had increased Phe concentrations (Phe, 1722 ± 286 μmol/L). Twenty-three healthy children of comparable age were the controls. All PKU patients were admitted to the day clinic of the Inborn Errors of Metabolism Department of the Institute of Child Health in Athens.

All blood samples were collected from an antecubital vein at the same time of day while both patients and controls were at rest. Blood samples (7.0 mL) were collected 3 h after participants arrived at our hospital, during which time the children fasted and were acclimatized to the hospital environment and staff.

Venous blood samples were collected into heparin-containing blood collection tubes from PKU patients and controls. The washed erythrocytes were lysed, as described by Galbraith and Watts (15) and Kamber et al. (16), after being frozen (−80 °C) and thawed (50 °C) five times. Membranes were suspended in 0.1 mol/L Tris-HCl, pH 7.4, to a final protein concentration of 2 g/L (17). The minor hemoglobin that remained attached to the membrane surface was measured by reagent set 527-A

Acetylcholinesterase Activity and Biogenic Amines in Phenylketonuria, Kleopatra H. Schulpis, 1* George A. Karikas, 2 Joanna Tjadamouris, 2 Helen Michelakakis, 1 and Stylianos Tsakiris 1 (1) Institute of Child Health and 2 Pharmacokinetics and Parenteral Nutrition Unit, Aghia Sophia Children’s Hospital, 11527 Athens, Greece; 3 Department of Experimental Physiology, Medical School, University of Athens, 15401 Athens, Greece; * address correspondence to this author at: Institute of Child Health, Aghia Sophia Children’s Hospital, PO Box 65257, 11527 Athens, Greece; fax 3010-7700111, e-mail inchild@otenet.gr)

Phenylketonuria (PKU) is a disorder in which the aromatic amino acid Phe cannot be converted to Tyr (1, 2). Unfortunately, many PKU patients do not adhere to their low-Phe diet (off diet), which leads to high concentrations of the amino acid in their blood (1, 2). High Phe concentrations interfere with the production of adrenaline (A), noradrenaline (NA), and dopamine (DA) (1, 3). Furthermore, Krause et al. (4) reported an inverse relationship between NA and DA plasma concentrations and Phe because high Phe concentrations decrease the availability of the amino acids Tyr and Trp, the precursors of catecholamines and serotonin [5-hydroxytryptamine (5HT)], respectively (5–7).

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impairment in biogenic amine synthesis suggest that the principal cause for this dysfunction is the brain dysfunction in PKU, and several experimental data strongly inverse correlations with Phe concentrations (18).

Additionally, Tyr, Trp, DA, NA, 5HT, and AChE showed amines, and AChE activities were significantly decreased. The CVs for DA, A, and NA were 3.4%, 2.9%, and 3.2%, respectively. 5HT concentrations were measured in a platelet-rich plasma with a new HPLC method (22). The CV for 5HT was 2.8%.

Data were analyzed by t-test and multiple regression analysis for the correlation coefficients. All analyses were performed with the SPSS 10.0 statistical package on an IBM personal computer.

As shown in Table 1, blood Tyr, Trp, DA, NA, A, 5HT, and AChE concentrations in group A of the PKU patients were not statistically different from controls, whereas in group B, plasma amino acids (except Phe), their biogenic amines, and AChE activities were significantly decreased. Additionally, Tyr, Trp, DA, NA, 5HT, and AChE showed strong inverse correlations with Phe concentrations (P <0.001).

Alterations in synaptic transmission are implicated in brain dysfunction in PKU, and several experimental data suggest that the principal cause for this dysfunction is the impairment in biogenic amine synthesis (5). Increased plasma Phe concentrations, such as we found in PKU patients (group B), by decreasing the availability of the precursors Tyr and Trp, which are also decreased in the same group of patients, might be the primary cause of their catecholamine and 5HT depletion (6, 7, 23). It is highly likely that the decreased Tyr and Trp seen in the plasma of PKU patients (group B) might cause decreased uptake by the adrenal medulla and platelets, leading to low production of catecholamine and 5HT. Additionally, a large excess of large neutral amino acids, such as Phe, in the same group of patients will saturate the carrier system across the blood–brain barrier, excluding Tyr and Trp from entry into the brain (4, 23). Thus, conversions of the above amino acids to the biogenic amines are possibly lowered in the central nervous system (4, 5, 23).

Because high Phe concentrations in the plasma of PKU patients (group B) could lead to brain dysfunction (2) and AChE inhibition can influence cholinergic transmission, a more detailed study of Phe action on AChE seemed worthwhile. In our in vitro previous studies (12–14), various concentrations of Phe on human AChE, rat homogenized diaphragm, pure eel (Electrophorus electricus) AChE, and rat homogenized brain AChE showed that Phe induced a similar concentration–dependent inhibition of AChE activities. We therefore assumed that Phe directly inhibited AChE, possibly interacting with its positively charged sites, and/or indirectly by changing the membrane lipid-bilayer microenvironment, causing functional modulation of the enzyme (8, 13). It could be also that the high degree of AChE inhibition in erythrocyte membranes from PKU patients off diet may be caused by the long-term indirect influence of high Phe concentrations on the enzyme membrane bilayer through lipid–protein interactions (24). Experiments on the effects of incubation of red cells with various Phe concentrations and evaluation of AChE protein concentration, such as by Western blot measurements or direct antigen assays, would be useful for understanding the mechanism of this effect.

High Phe concentrations could also induce changes in brain electrical function, which may be mediated in part through inhibition of biogenic amine production (4). Regarding cholinergic brain systems, experimental results showed their possible involvement during Phe action. Additionally, an increase in Phe concentration can cause an increase in the GTP-cyclohydrolase-stimulating protein. The latter increases de novo the synthesis of tetrahydrobiopterin, leading to its high uptake into the red cells. 6R-1-Erythro-5,6,7,8-tetrahydrobiopterin, a natural cofactor for Phe hydroxylases, has direct Ach-releasing action in vivo in the rat hippocampus (25).

In conclusion, (a) high plasma Phe concentrations caused marked in vivo inhibition of erythrocyte-men-

<table>
<thead>
<tr>
<th>Table 1. Phe, Tyr, Trp, and biogenic amine concentrations and AChE activities in PKU patients vs controls.</th>
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<tbody>
<tr>
<td><strong>Group A (n=12)</strong></td>
</tr>
<tr>
<td>Phe, µmol/L</td>
</tr>
<tr>
<td>Tyr, µmol/L</td>
</tr>
<tr>
<td>Trp, µmol/L</td>
</tr>
<tr>
<td>DA, pmol/L</td>
</tr>
<tr>
<td>NA, nmol/L</td>
</tr>
<tr>
<td>A, pmol/L</td>
</tr>
<tr>
<td>5HT, d</td>
</tr>
<tr>
<td>AChE activity, e</td>
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</tbody>
</table>

*Values are expressed as mean ± SD.

1. P<0.0001 for group A vs group B, group A vs controls, and group B vs controls.

2. P<0.001 for group A vs group B and group B vs controls; differences between group A and controls were not significant.

3. Platelet 5HT content is expressed as nmol of 5HT/10^9 platelets.

4. AChE activity is expressed as Δabsorbance/min · mg protein.
brane AChE activity in PKU (the latter is reinforced by our studies on the in vitro effect of Phe on AChE), (b) AChE inhibition could affect ACh hydrolysis and its consequences in nervous system functions, (c) high Phe concentrations may explain the decreased concentrations of biogenic amines in PKU, and (d) our data showed for the first time that the evaluation of erythrocyte-membrane AChE activity in relation to biogenic amine blood concentrations could be a useful peripheral marker for evaluation of the effects of high Phe concentrations in the brains of PKU patients.

We thank Anna Stamatis for assistance with this manuscript.

References

Total β-Human Chorionic Gonadotropin Measured in Urine by an Automated Method, Mary O. Carayannopoulos, David G. Grenache, and Ann M. Gronowski (Department of Pathology and Immunology, Division of Laboratory Medicine, Washington University School of Medicine, 660 South Euclid Ave., Box 8118, St. Louis, MO 63110; * author for correspondence: fax 314-362-1461, e-mail gronowski@pathology.wustl.edu)

Methods to quantify human chorionic gonadotropin (hCG) in serum are well established (1), but automated quantitative urine assays are not readily available. When the validity of point-of-care qualitative urinary hCG results are called into question, a rapid quantitative method for urine hCG could be useful. In the present study, we have validated the Abbott AxSYM Total β-hCG (Abbott Laboratories) assay (approved for serum use only) for use in the quantitative determination of urinary concentrations of total β-hCG.

Recovery studies were performed by adding hCG (US Pharmacopeia) to urine from premenopausal, nonpregnant females. Recovery studies were carried out in quadruplicate in two separate experiments. Recovery was 99–112% at concentrations of 26–725 IU/L (Table 1). Urine protein at concentrations up to 7.4 g/L had no effect on recovery (not shown).

Table 1. Recovery of total β-hCG from urine by the Abbott AxSYM.

<table>
<thead>
<tr>
<th>β-hCG, IU/L</th>
<th>Expected</th>
<th>Observed, mean (SD)</th>
<th>Recovery, %</th>
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<tbody>
<tr>
<td></td>
<td>(n = 8)</td>
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<td></td>
</tr>
<tr>
<td>26</td>
<td>26 (4.6)</td>
<td>99</td>
<td></td>
</tr>
<tr>
<td>52</td>
<td>54 (5.6)</td>
<td>104</td>
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</tr>
<tr>
<td>78</td>
<td>82 (8.2)</td>
<td>105</td>
<td></td>
</tr>
<tr>
<td>155</td>
<td>167 (10.5)</td>
<td>107</td>
<td></td>
</tr>
<tr>
<td>725</td>
<td>815 (34.0)</td>
<td>112</td>
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