Acetylcholinesterase Activity and Biogenic Amines in Phenylketonuria, Kleopatra H. Schulpis, 1* George A. Karikas, 2 Joanna Tjamouranis, 1 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; * Department of Experimental Physiology, Medical School, University of Athens, 15401 Athens, Greece; 16 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 inchil@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).

Acetylcholinesterase (AChE) is a membrane-bound enzyme with its active site 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 parasympathetic, 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
impairment in biogenic amine synthesis suggests that the principal cause for this dysfunction is the brain dysfunction in PKU, and several experimental data have shown their possible involvement during Phe action. 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.

In conclusion, high plasma Phe concentrations caused marked in vivo inhibition of erythrocyte-membrane AChE. This finding implies that high plasma Phe concentrations in 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.

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

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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 urine 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).

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<th>Table 1. Recovery of total β-hCG from urine by the Abbott AxSYM.</th>
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