Effects of L-Phenylalanine on Acetylcholinesterase and Na^+,K^+-ATPase Activities in Suckling Rat Frontal Cortex, Hippocampus and Hypothalamus

Artemis Doulgeraki[a], Zetta Papadopoulou-Daifoti[b] and Stylianos Tsakiris[a,*]

[a] Department of Experimental Physiology, Medical School, University of Athens, P. O. Box 65257, GR-154 01, Athens, Greece. Fax: 0030-1-7725295. E-mail: stsakir@cc.uoa.gr
[b] Department of Experimental Pharmacology, Medical School, University of Athens, Greece

* Author for correspondence and reprint requests

Z. Naturforsch. **57c,** 182–188 (2002); received September 4/October 16, 2001

Acetylcholinesterase, Na^+,K^+-ATPase, Frontal Cortex

The effect of different L-phenylalanine (Phe) concentrations (0.12–12.1 mM) on acetylcholinesterase (AChE), (Na^+,K^+)-ATPase and Mg^{2+}-ATPase activities was evaluated in homogenates of suckling rat frontal cortex, hippocampus and hypothalamus. Phe, at high concentrations, reduced AChE activity in frontal cortex and hippocampus by 18%-20%. On the contrary, the enzyme activity was unaltered in the hypothalamus. Na^+,K^+-ATPase was stimulated by high levels of the amino acid, both in the frontal cortex and the hypothalamus by 60%, whereas it was inhibited in the hippocampus by 40%. Mg^{2+}-ATPase was not influenced by Phe. It is suggested that: a) In the frontal cortex, the improper acetylcholine (ACh) release, due to AChE inhibition by Phe, combined with the stimulation of Na^+,K^+-ATPase, possibly explain tremor and the hyperkinetic behaviour in patients with classical phenylketonuria (PKU). b) In the hippocampus, inhibition of AChE by Phe could lead to problems in memory, while Na^+,K^+-ATPase inhibition by Phe may induce metabolic disorders and electrical instability of the synaptosomal membrane. c) In the hypothalamus, the behavioral problems in PKU "off diet" may be related to noradrenaline (NA) levels, which are probably correlated with the modulated Na^+,K^+-ATPase by Phe.

Introduction

PKU is an inherited metabolic disorder, characterized by the impaired conversion of Phe to tyrosine. Phe-hydroxylase shows reduced activity, resulting in abnormally high levels of Phe in body fluids (Missiou-Tsagaraki *et al.*, 1988). Phe influences neural excitability (Iarosh *et al.*, 1987), while experimental hyperphenylalaninemia in newborn rats leads to reduced myelinogenesis (Burri *et al.*, 1990), which could result in a decreased axonal conduction velocity.

Experimental data refer to the alterations of synaptic transmission because of the excessive Phe in plasma and consequently in the brain. Blau (1979) reported an impairment in the neurotransmitter amine synthesis, whereas high Phe levels decrease the availability of tryptophan and tyrosine (Aragon *et al.*, 1982) and cause serotonin and catecholamine depletion in PKU (Herrero *et al.*, 1983), influencing brain functions. Moreover, high Phe concentrations change the electrical brain function, which may be mediated, in part, through inhibition of catecholamine synthesis. Regarding cholinergic brain systems, hyperphenylalaninemia leads to a decrease in the density of muscarinic receptors of the hippocampus and other brain areas (Hommes, 1993).

The effect of different Phe concentrations on the activity of three brain enzymes was investigated: a) Acetylcholinesterase (AChE, EC 3.1.1.7), which plays a very important role in the ACh-cycle, including the release of ACh (Kouniniotou-Krontiri and Tsakiris, 1989). b) Na^+,K^+-ATPase (EC 3.6.1.3), an enzyme implicated in neural excitability (Sastry and Phillis, 1977), and metabolic energy production (Mata *et al.*, 1980) and c) Mg^{2+}-ATPase, which contributes to the maintenance of high brain intracellular Mg^{2+}, which is instrumental in controlling protein synthesis and cell growth (Sanui and Rubin, 1982). The above enzymes were examined in homogenates of frontal cortex, hippocampus and hypothalamus of suckling rats. Considering the mental retardation, seizures and hyperkinetic behaviour in children suffering from PKU, especially in neonatal period, a

0939–5075/2002/0100–0182 $ 06.00

correlation between the enzymatic changes in the above neural structures and neural dysfunctions, due to the excessive Phe, seems very challenging.

Materials and Methods

Animals

Albino suckling (21 days) Wistar rats of both sexes (Saint Savvas Hospital, Athens, Greece) were used in all experiments. Under our *in vitro* experimental conditions, there were no differences of Phe action on AChE as well as on Na^+,K^+-ATPase activities in the brain areas between male and female rats. Body weight was 50 ± 3 g (mean ± SD). They were housed in a cage with their mother at a constant room temperature (22 ± 1 °C) under a 12h L:12h D (light 08:00–20.00 h) cycle. For the mothers, food and water were provided *ad lib*. Animals were cared for in accordance with the principles of the *"Guide to the Care and Use of Experimental Animals"*.

Tissue preparation

Animals were sacrificed by decapitation. The frontal cortex (33.9 ± 5.4 mg), hippocampus (54.2 ± 9.2 mg) and hypothalamus (32.9 ± 4.9 mg) of each of the thirty sucking rats were rapidly removed, weighed and thoroughly washed with isotonic saline. Tissues from thirty neonates were homogenized in 10 vol. ice-cold (0–4 °C) medium containing 50 mM tris(hydroxymethyl)-aminomethane-HCl (Tris-HCl), pH = 7.4 and 300 mM sucrose using an ice-chilled glass homogenizing vessel at 900 rpm (4–5 strokes). Then, the homogenate was centrifuged at 1,000 x*g* for 10 min to remove nuclei and debris. In the resulting supernatant, the protein content was determined according to the method of Lowry *et al.* (1951) and then, the enzymatic activities were measured. The enzyme incubation mixture was kept at 37 °C.

Phe preincubation

The enzymatic activity measurements were carried out on homogenized suckling rat frontal cortex, hippocampus and hypothalamus. The activity was determined after 1h preincubation of the homogenate with 0.12, 0.24, 0.48, 0.9, 1.8 and 12.1 mM of Phe at 37 °C. AChE activity was also investigated as a function of time of Phe action on the enzyme. These mentioned concentrations of Phe (0.12–1.8 mM) correspond to 6 times the plasma concentration (0.72–10.8 mM) (Scriver and Rosenberg, 1973). Phe concentrations of 0.3–10 mM are usually found in the plasma of untreated patients with PKU (Scriver and Rosenberg, 1973; Missiou-Tsagaraki *et al.*, 1988).

Determination of AChE activity

AChE activity was determined according to the method of Ellman *et al.* (1961). The reaction mixture (1 ml) contained 50 mM Tris-HCl, pH = 8 and 240 mM sucrose in the presence of 120 mM NaCl. Protein concentration was 80–100 μg/ml incubation mixture. Then, 0.030 ml 5,5′-dithionitrobenzoic acid (DTNB) and 0.050 ml acetylthiocholine iodide, used as a substrate, were added and the reaction started. The final concentrations of DTNB and substrate were 0.125 and 0.5 mM, respectively. The reaction was followed spectrophotometrically by the increase in absorbance ($\Delta\overline{OD}$) at 412 nm.

Determination of Na^+,K^+-ATPase and Mg^{2+}-ATPase activities

Na^+,K^+-ATPase activity was calculated as the difference between total ATPase activity (Na^+,K^+,Mg^{2+}-dependent) and Mg^{2+}-dependent ATPase activity. Total ATPase activity was assayed in an incubation medium which contained 50 mM Tris-HCl, pH 7.4, 120 mM NaCl, 20 mM KCl, 4 mM $MgCl_2$, 240 mM sucrose, 1 mM ethylene-diaminetetraacetic acid K_2-salt (K^+-EDTA), 3 mM disodium ATP and 80–100 μg protein of the homogenate, in a final volume of 1 ml.

Ouabain (1 mM) was added to determine the activity of the Mg^{2+}-ATPase. The values of Mg^{2+}-dependent ATPase were similar in the presence of ouabain in the reaction mixture, as also in the absence of ouabain and without NaCl and KCl (in the presence of $MgCl_2$ only). The reaction started by adding ATP and stopped after a 20 min incubation period by the addition of 2 ml of a mixture which consisted of 1% lubrol and 1% ammonium molybdate in 0.9 M H_2SO_4 (Bowler and Tirri, 1974). The yellow colour was read at 390 nm.

Statistical analysis

The data were analyzed by two-tailed Student's *t*-test. P values <0.05 were considered statistically significant.

Results

The effect of different Phe concentrations on the enzymatic activities

Frontal cortex

The effects of different Phe concentrations on AChE and Na^+,K^+-ATPase activities were evaluated in frontal cortex homogenates of the suckling rats. The results of this study, as illustrated in Fig. 1A, showed that 1 h incubation of 0.1 mM Phe on AChE resulted in a slight decline of AChE activity about 8% ($p < 0.05$). Additionally, 10% inhibition ($p < 0.05$) was observed with 0.24 mM Phe and 18% ($p < 0.01$) for Phe concentrations ranging from 0.48–12.1 mM. Moreover, the same experiments were carried out on the eel *Electroforus electricus* pure AChE (not bound to the membrane) (Tsakiris *et al.*, 1998a). These results are also presented in Fig. 1A. It is obvious that the effect of Phe on AChE activity in the neonatal frontal cortex simulates the effect of Phe on the pure enzyme indicating a direct action of Phe on AChE.

Mg^{2+}-ATPase activity was found to be 9.83 ± 1.09 μmol Pi/h × mg protein, while Phe, in the concentrations used in this study, appeared unable to affect the enzymatic activity ($p > 0.05$).

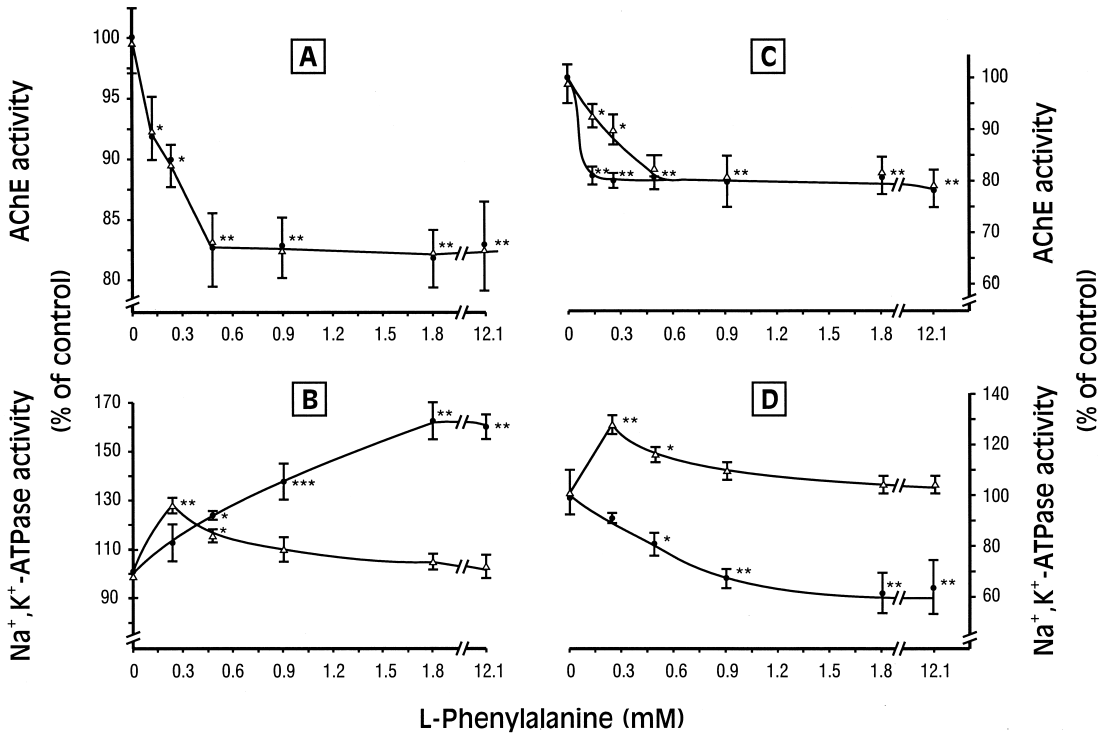

Fig. 1. Effect of different concentrations of Phe on AChE and Na^+,K^+-ATPase activities, which were determined in suckling rat frontal cortex (A,B) or hippocampus (C,D) homogenates (●) and in pure AChE from eel *E.electricus* (△) (A,C) or in pure Na^+,K^+-ATPase from porcine cerebral cortex (△) (B,D). Under the same experimental conditions, the effect of Phe on the activities of pure enzymes have also been studied (Tsakiris *et al.*, 1998a). The control value for AChE activity in the frontal cortex was 0.269 ± 0.010 $\Delta\overline{OD}$/min × mg protein, in hippocampus 0.397 ± 0.016 $\Delta\overline{OD}$/min × mg protein and in pure enzyme 1.23 ± 0.04 $\Delta\overline{OD}$/min × μg protein. The control value for Na^+,K^+-ATPase activity in the frontal cortex was 5.30 ± 0.49 μmol Pi/h × mg protein, in hippocampus 5.71 ± 0.57 μmol Pi/h × mg protein and in pure enzyme 14.8 ± 1.6 μmol Pi/h × mg protein. Points and vertical bars represent mean values ± SD of five experiments. The average value of each experiment derives from three determinations. *$p < 0.05$, **$p < 0.01$, compared to control.

As shown in Fig. 1B, one hour Phe (0.24 mM concentration) preincubation with frontal cortex homogenates, stimulated Na^+,K^+-ATPase by 18% ($p > 0.05$), 24% ($p < 0.05$) with 0.48 mM Phe, 37% ($p < 0.001$) with 0.9 mM Phe and 60% ($p < 0.01$) with Phe concentration ranging from 1.8 to 12.1 mM (plateau). Furthermore, in the same figure it is presented the effect of Phe on the pure Na^+,K^+-ATPase of the porcine cerebral cortex (Tsakiris *et al.*, 1998a). Under the same experimental conditions, the activity of the pure enzyme had been increased by 28% ($p < 0.01$) and by 16% ($p < 0.05$) when Phe concentration was 0.24 and 0.48 mM, respectively. When the enzyme was preincubated with higher levels of Phe, the enzymatic activity had been unchanged ($p > 0.05$). Conclusively, the action of low Phe concentrations (0.24–0.48 mM) on Na^+,K^+-ATPase is direct, whereas the high concentrations of the amino acid (0.9–12.1 mM) influence the tissue enzyme indirectly.

Hippocampus

As far as the hippocampus homogenates are concerned, Phe caused a statistically significant decrease in the AChE activity after 1h of amino acid preincubation. Interestingly, different concentrations of Phe (0.12–12.1 mM) resulted in an average decline of enzymatic activity about 20% ($p < 0.01$) (Fig. 1C). In other words, the statistically significant decline of AChE activity due to Phe seemed not to be concentration-dependent. Moreover, the measurements, regarding the pure AChE from the eel *E. electricus*, are also presented in Fig. 1C (Tsakiris *et al.*, 1998a). Comparison of the results from the neonatal hippocampus and the pure enzyme shows that Phe inhibits AChE indirectly at low concentrations (0.12–0.48 mM). On the contrary, high Phe concentrations (0.9–12.1 mM) resulted in a decline of the enzyme activity (18–20%) in both the hippocampus and the pure enzyme, suggesting a direct effect of Phe on AChE.

Mg^{2+}-ATPase activity was 6.30 ± 0.63 μmol Pi/h× mg protein, while, the above mentioned concentrations of Phe, failed in altering the enzymatic activity ($p > 0.05$).

Finally, as it is clearly shown in Fig. 1D, one hour Phe preincubation of the hippocampus homogenates, resulted in an inhibition of Na^+,K^+-ATPase by about 9% ($p > 0.05$) with 0.24 mM Phe concentration, 20% ($p < 0.05$) with 0.48 mM Phe, 35% ($p < 0.01$) with 0.9 mM Phe and 40% ($p < 0.01$) with 1.8–12.1 mM Phe (plateau). Furthermore, it has also been found (Tsakiris *et al.*, 1998a) an increased enzymatic activity by 28% ($p < 0.01$) and 16% ($p < 0.05$) at Phe concentration 0.24 and 0.48 mM, respectively, as showed the results of the experiments with Phe on pure Na^+,K^+-ATPase. No influence on the pure Na^+,K^+-ATPase activity was observed with higher Phe concentrations (0.9–12.1 mM). Therefore, the high concentrations of the amino acid may influence the tissue enzyme indirectly.

Hypothalamus

The last part of experiments concerned the hypothalamus homogenates of suckling rats. After 1–3 h preincubation with Phe, there was found no effect on the AChE activity ($p > 0.05$), regardless of Phe concentrations (0.12–12.1 mM). Moreover, under the same experimental conditions, the effect of Phe on the pure AChE activity had been studied (Tsakiris *et al.*, 1998a). Phe concentration (0.12–0.48 mM) slightly inhibited by 10%-15% ($p < 0.05$) the pure AChE activity. In comparison to the effect of Phe on the hypothalamus homogenates, it seems that low concentration of the amino acid acts directly on the AChE. However, higher Phe concentration (0.9–12.1 mM) caused a remarkable decline (20%, $p < 0.01$) in the pure AChE activity. Since the same concentrations of Phe had no effect on the AChE of the hypothalamus, Phe possibly acts indirectly at high concentrations.

The control value of Mg^{2+}-ATPase activity was estimated at 4.98 ± 0.58 μmol Pi/h × mg protein and the effect of different Phe concentrations was not statistically significant ($p > 0.05$). Therefore, Mg^{2+}-ATPase activity did not change after the preincubation with Phe.

As shown in Fig. 2, one hour Phe 0.24 mM preincubation with hypothalamus homogenates resulted in a stimulation of Na^+,K^+-ATPase about 10% ($p > 0.05$), 27% ($p < 0.01$) with 0.48 mM Phe and 60% ($p < 0.01$) with 0.9–12.1 mM Phe (plateau). Fig. 2 also presents the results of the experiments of Phe action on pure Na^+,K^+-ATPase (Tsakiris *et al.*, 1998a). Phe concentration 0.24 and

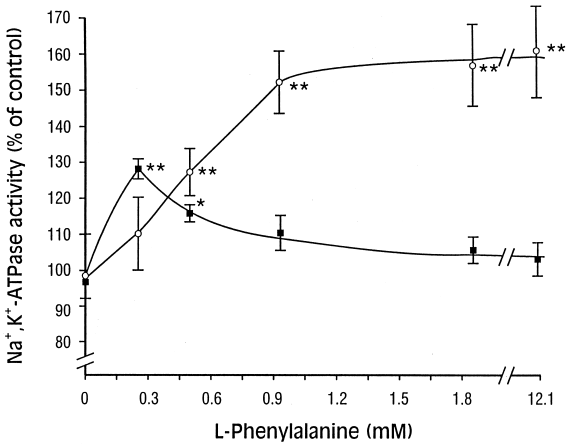

Fig. 2. Effect of different Phe concentrations on the Na^+,K^+-ATPase activity, which was determined in suckling rat hypothalamus homogenate (○) and in pure enzyme from porcine cerebral cortex (■) (Tsakiris *et al.*, 1998a). The control value of Na^+,K^+-ATPase activity in the hypothalamus was 2.44 ± 0.17 μmol Pi/h × mg protein and of the pure enzyme was 14.8 ± 1.6 μmol Pi/h × mg protein. Points and vertical bars represent the mean values ± SD of five experiments. The average value of each experiment results from three determinations. *$p < 0.05$, **$p < 0.01$, compared to control.

0.48 mM increased the activity of Na^+,K^+-ATPase by 28% ($p < 0.01$) and 16% ($p < 0.05$), respectively. Higher Phe concentration had no effect on the enzymatic activity. In other words, low Phe concentrations (0.24–0.48 mM) act directly on the Na^+,K^+-ATPase, whereas higher concentrations of Phe (0.9–12.1 mM) influence the tissue enzyme indirectly.

Discussion

Frontal cortex

Comparison of the effects of frontal cortex lesions between rats, monkeys and humans has shown that the behavioral symptoms are remarkably similar (Kolb, 1984).

The observed decline by Phe of AChE activity could lead to an increase of ACh concentration in the synaptic cleft, which possibly explains the tremor in untreated PKU patients. Moreover, it has been mentioned that a long-term inhibition of AChE can result in a progressive neuronal deprivation of ACh (Kouniniotou-Krontiri, 1985; Kouniniotou-Krontiri and Tsakiris, 1989). In addition, high levels of Phe reduce the binding sites of ACh in the rat frontal cortex (Hommes, 1993). ACh is closely related to associative learning, by enhancing the neuronal response and the associations with the motor response (Pirch *et al.*, 1992). A possible reason for the reduced IQ of PKU patients, derives from the importance of AChE in the brain development of the neonate. This enzyme is instrumental in the neuronal maturation, cellular differentiation and intercellular connections (Hommes, 1993). Thus, the observed reduction in enzymatic activity might lead to a reduced rate of brain development and a low IQ, combined with behavioral disorders.

Moreover, it was found that Na^+,K^+-ATPase activity was increased by Phe. This stimulatory effect demonstrates a possible Phe transformation to NA (Doulgeraki *et al.*, 1999), which is bound to NA-receptors, affecting thereafter the function of Na^+,K^+-ATPase (Swann, 1983 and 1984). As frontal cortex is a structure, which contains many adrenergic receptors, the high NA levels derived from Phe might stimulate Na^+,K^+-ATPase.

Hippocampus

This structure is the centre of coding and establishment of new information in the context of the memory process. It is the location where the new stimuli are evaluated, and the decisions about their inclusion is one's memory are made (Guyton, 2000). Apart from this, hippocampus is often a focus of seizures. Untreated PKU patients, usually present memory disorders and epileptic seizures. Due to the fact that the memory process is controlled through cholinergic and adrenergic interactions (Ohno *et al.*,1997), AChE and Na^+,K^+-ATPase were studied.

Hippocampus is a structure with prominent cholinergic innervation. The observed inhibition of AChE by Phe in the hippocampus is similar to that reported in the rat diaphragm (Tsakiris *et al.*, 1998b), due to a possible interconnection between the hippocampus and the diaphragm (Wainer *et al.*, 1985). Moreover, in the developing cholinergic system of the hippocampus, high levels of Phe reduce the ACh-binding sites in the cellular layers CA_1 and CA_3 (Hommes, 1993). As a result, synaptic dysfunction is expected. Since the cholinergic synapses belong to the endogenous controlling systems of memory (storage and establishment of

new data), the impairment of the synaptic transmission caused by Phe, might lead to disturbances of the memory.

Moreover, the observed inhibition of Na^+,K^+-ATPase by Phe could lead to high levels of ACh in rat brain (Meyer and Cooper, 1981). Since ACh may be increased in the neonatal hippocampus (through the AChE inhibition by Phe), consequently Na^+,K^+-ATPase activity can be also decreased.

Hypothalamus

This brain area has prominent adrenergic innervation. Its multifunctional role includes behavioral changes: anger, rage, satisfaction, reward and punishment reactions (Guyton, 2000). PKU rats could not perform their tasks properly (during behavioral tests), and their answers to the stimuli were delayed (García-Verra *et al.*, 1998), resulting in failure to adapt to the environmental conditions.

It was mentioned that high Phe concentrations have a possible indirect effect on the hypothalamic AChE. It is suggested that AChE is "protected" from the harmful effect of Phe, possibly by a cellular lipophilic factor which could change the lipid(s)-protein interactions in the membrane.

According to these results, the cholinergic system seems not to be affected by Phe in this brain area. As a consequence, the behavioral disorders of PKU should be examined in relation to Na^+,K^+-ATPase changes which reflect the NA levels of this structure (NA is the main transmitter of the hypothalamus) (Spencer *et al.*, 1985; Mefford, 1988). It was observed that there was a remarkable stimulation of Na^+,K^+-ATPase by Phe (Fig. 2). This result was found similar to that obtained from the experiments on the neonatal frontal cortex (see Fig. 1B). NA levels were enhanced by Phe (Doulgeraki *et al.*, 1999), consequently, the activity of Na^+,K^+-ATPase was increased by this biogenic amine (Swann, 1983 and 1984).

It is worth noticing that these conclusions refer to the rats and should be cautiously related to human behavior, because the function of NA, as well as the location and density of NA-receptors are different in the human hypothalamus as compared to those of the rat hypothalamus (Little *et al.*, 1992).

Acknowledgements

This study was funded by the University of Athens.

Aragon M. C., Gimenez C. and Valdivieso F. (1982), Inhibition by phenylalanine of tyrosine transport by synaptosomal plasma membrane vesicle: implications in the pathogenesis of phenylketonuria. J. Neurochem. **39**, 1185–1187.

Blau K. (1979), Phenylalanine hydroxylase deficiency: Biochemical, physiological and clinical aspects of phenylketonuria and related phenylalaninemias. In: Aromatic Amino Acids Hydroxylases and Mental Disease (Youdim M. B. H., ed.). Wiley, New York, pp. 77–139.

Bowler K. and Tirri R. (1974), The temperature characteristics of synaptic membrane ATPases from immature and adult rat brain. J. Neurochem. **23**, 611–613.

Burri R., Steffen C., Stieger S., Brodbeck U., Colombo J. P. and Herschkowitz N. (1990), Reduced myelinogenesis and recovery in hyperphenylalaninemic rats. Correlation between brain phenylalanine levels, characteristic brain enzymes for myelination and brain development. Mol. Chem. Neuropathol. **13**, 57–69.

Doulgeraki A., Tsakiris S., Paraschou E. and Papadopoulou-Daifoti Z. (1999), In vitro effect of the noradrenaline neurotoxin DSP-4 and L-phenylalanine on the rat brain Na^+,K^+-ATPase activity. Res. Commun. Biol. Psychol. Psychiatry **24**, 59–62.

García-Vera C., Baldellou-Vaquez A., García Romero J., Ventura-Faci P. and Perez-Gonzalez J. (1998), An experimental study on the effects of maternal hyperphenylalaninemia on the central nervous system and behavior of offspring rats. Ann. Esp. Pediatr. **48**, 11–16.

Guyton A. C. (2000), Textbook of Medical Physiology. WB Saunders, Philadelphia, pp 850–856.

Herrero E., Aragon M. C., Gimenez C. and Valdivieso F. (1983), Inhibition by L-phenylalanine of tryptophan transport by synaptosomal plasma membrane vesicles: implications in the pathogenesis of phenylketonuria. J. Inher. Metab. Dis. **6**, 32–35.

Hommes F. A. (1993), The effect of hyperphenylalaninemia on the muscarinic acetylcholine receptor in the HPH-5 mouse brain. J. Inher. Metab. Dis. **16**, 962–974.

Iarosh A. K., Goruk P. S. and Luk'ianov E. A. (1987), Comparative characteristics of the functioning of brain structures exposed to morphine and D-Phe. Farmacol. Toksicol. **50**, 20–23.

Kolb B. (1984), Functions of the frontal cortex of the rat: a comparative review. Brain Res. Rev. **8**, 65–98.

Kouniniotou-Krontiri P. (1985), Effects du lithium sur la libération quantique spontanée de transmetteur par

les terminaisons motrices du diaphragm de rat. J. Physiol. (Paris) **80**, 340–348.

Kouniniotou-Krontiri P. and Tsakiris S. (1989), Time dependence of Li^+ action on acetylcholinesterase activity in correlation with spontaneous quantal release of acetylcholine in rat diaphragm. Jpn. J. Physiol. **39**, 429–440.

Little K. Y., Duncan G. E., Breese G. R. and Stumpf W. E. (1992), β-adrenergic receptor binding in human and rat hypothalamus. Biol. Psychiatry **32**, 512–522.

Mata M., Fink D. J., Gainer H., Smith C. B., Davidson L., Savakis H., Schwartz W. J. and Sokoloff L. (1980), Activity-dependent energy metabolism in rat posterior pituitary primarily reflects sodium pump activity. J. Neurochem. **34**, 213–215.

Mefford I. N. (1988), Epinephrine in mammalian brain. Prog. Neuropsychopharmacol. Biol. Psychiatry **12**, 365–388.

Meyer E. M. and Cooper I. R. (1981), Correlations between Na^+,K^+-ATPase activity and acetylcholine release in rat cortical synaptosomes. J. Neurochem. **36**, 467–475.

Missiou-Tsagaraki S., Soulpi K. and Loumakou M. (1988), Phenylketonuria in Greece: 12 years' experience. J. Mental Deficiency Res. **32**, 271–287.

Ohno M., Kobayashi M., Kishi A. and Watanabe S. (1997), Working memory failure by combined blockade of muscarinic and β-adrenergic transmission in the rat hippocampus. Neuroreport **8**, 1571–1575.

Sanui H. and Rubin H. (1982), The role of magnesium in cell proliferation and transformation. In: Ions, Cell Proliferation and Cancer (Boynton A. L., McKeehan W. L., Whitfield J. P., eds). Academic Press, New York, 517–537.

Sastry B. S. R. and Phillis J. W. (1977), Antagonism of biogenic amine-induced depression of cerebral cortical neurons by Na^+,K^+-ATPase inhibitors. Can. J. Physiol. Pharmacol. **55**, 170–180.

Scriver C. and Rosenberg R. (1973), Amino Acid Metabolism and its Disorders. WB Saunders, Philadelphia.

Spencer S., Saper C. B., Joh T., Reis D. J., Goldstein M. and Raese J. D. (1985), Distribution of catecholamine-containing neurons in the normal human hypothalamus. Brain Res. **328**, 73–80.

Swann A. C. (1983), Stimulation of brain Na^+,K^+-ATPase by norepinephrine *in vivo*: prevention by receptor antagonist and enhanced by repeated stimulation. Brain Res. **260**, 336–341.

Swann A. C. (1984), (Na^+,K^+)-adenosine triphosphatase regulation by the sympathetic nervous system: effects of noradrenergic stimulation and lesion *in vivo*. J. Pharmacol. Exp. Ther. **228**, 304–311.

Tsakiris S., Kouniniotou-Krontiri P., Schulpis K. H. and Stavridis J. C. (1998a), L-phenylalanine effect on rat brain acetylcholinesterase and Na^+,K^+-ATPase. Z. Naturforsch. **53c**, 163–167.

Tsakiris S., Kouniniotou-Krontiri P. and Schulpis K. H. (1998b), L-phenylalanine effect on rat diaphragm acetylcholinesterase and Na^+,K^+-ATPase. Z. Naturforsch. **53c**, 1055–1060.

Wainer B. H., Levey A. I., Rye D. B., Mesulam M. M. and Mufson E. J. (1985), Cholinergic and non-cholinergic septohippocampal pathways. Neurosci. Lett. **54**, 45–52.