On the Glutamine and γ-Aminobutyric Acid Contents of Various Regions of the Cat Brain

It has been well established that amino acids are not only the building blocks for the synthesis of brain proteins, but also the basic substances for the production of many active amines and enzymes, taking an important part in the metabolism of this organ[1].

The exceptional ability of the glutamic acid to undergo oxidation, transamination and decarboxilation, the relatively high concentrations of the glutamic acid, glutamine and γ-aminobutyric acid in the brain as compared with the other organs, and the possibilities of their mutual transformations, determine, according to some authors[2], the central position of these compounds in the dynamics of brain metabolism and reveal their very important role in the functioning of the central nervous system.

Previous information concerning the glutamic acid, glutamine and γ-aminobutyric acid contents of the brain are derived from the analyses of the total organ[3-5], or of the gray and white matter of the brain[6]. However, many investigations, including some recent protein studies[7], showed that because of the high morphological and functional organisation of the brain, the values of a given metabolite, obtained from the total brain under given conditions, could not be considered representative of all the different parts of this complex organ.

This series of experiments have been undertaken to investigate amino acid metabolism of the brain under various physiopathological conditions. In this preliminary communication, however, only the values concerning the normal distribution of glutamine and γ-aminobutyric acid in various regions of the cat's brain will be presented.

Adult cats were used in the experiments. Bearing in mind the findings of HAKKINEN et al.[8] that for the estimation of glutamic acid glutamine and γ-aminobutyric acid, the fixation in the liquid air was not necessary, the tissue samples excised from various regions of the brain after decapitation at room temperature, were immediately homogenized in ice cold 75% ethanol. After being kept for at least 1 h at 4°C, the homogenates were centrifuged at 24000 g for 1 h at 0°C. The precipitates were suspended in 5 ml of cold 75% ethanol and centrifuged again for 30 min. After evaporation of the combined supernatants, the dry residues were dissolved in water (1 ml/g of original fresh brain tissue). Glutamine and γ-aminobutyric acid were separated by means of two-dimensional paper chromatography, according to the slightly modified method of ROBERTS et al.[9], and their contents quantitatively estimated using a Beckman DU spectrophotometer. Unfortunately, the glutamic acid was not separated clearly enough to permit the quantitative determination. Standards containing the mixture of equal amounts of 5 μg of glutamic acid, glutamine, γ-aminobutyric acid, taurine, serine, alanine and aspartic acid were run through the whole chromatographic procedure with each experimental series. Chromatograms of brain tissue extracts were found to be qualitatively identical with the standards.

Results of the experiments are presented in the Table. The highest concentrations of glutamine were found to be present in the caudate nucleus and the cerebellar cortex, somewhat lower in the frontal cortex, hippocampus, thalamus and hypothalamus, still lower in the pons, medulla and the spinal cord and the lowest in the cerebral white matter.

The distribution of the γ-aminobutyric acid followed different pattern: the highest concentrations were found in the hypothalamus and the caudate nucleus, somewhat lower in the thalamus and pons and the lowest in the frontal cortex, hippocampus, cerebellar cortex, medulla spinal cord and the cerebral white matter. It seems to be worth emphasizing that while the concentrations of glutamine of the cortical and the diencephalic structures were remarkably higher than those of the lower parts of the brain stem, and particularly of the white matter, the concentrations of the γ-aminobutyric acid were rather uniformly distributed among these structures.

It would be, of course, extremely difficult and premature, on the basis of experiments of such static type, to draw any definite conclusion as to the functional significance of the results obtained, especially when one has in mind that in the brain, there is a constant and highly dynamic interplay of a variety of factors determining the metabolism and the turnover rate of proteins. Nevertheless it is tempting to make some very general remarks in this respect.

It is perfectly clear today that glutamine and γ-aminobutyric acid must be considered together with glutamic acid to which they are both chemically and metabolically very closely related. Glutamine is known to be the depot of glutamic acid[3] and to participate in the detoxication of ammonia accumulated during the functional activity of the brain[2]. γ-aminobutyric acid, on the other hand, is found to be a specific inhibitor of neuronal transmission. There is a good deal of evidence now that glutamate-GABA system plays an important role in the maintainance of the excitability of the brain and consequently in its convulsive activity[2].

There can hardly be any doubt that the significant differences in the content of glutamine and γ-aminobutyric acid in different regions of the brain examined in our experiments reflect the variations in the metabolism of these compounds related to the functional activities of the respective regions of the central nervous system. *A priori* the uniquely high concentrations in some parts of the brain might possibly be due to the greater rate of

Brain region	Number of animals	Glutamine mg %	GABA mg %
Frontal cortex	7	47.1 ± 11.0	37.0 ± 16.2
Hippocampus	7	45.9 ± 9.3	39.1 ± 9.0
Cerebral white matter	7	27.7 ± 4.4	32.7 ± 12.1
Cerebellar cortex	7	56.7 ± 9.0	35.7 ± 10.4
Caudate nucleus	7	66.2 ± 13.3	51.6 ± 19.0
Thalamus	7	47.0 ± 9.1	46.2 ± 11.9
Hypothalamus	7	47.7 ± 4.9	61.9 ± 17.1
Pons	7	37.9 ± 7.0	41.3 ± 13.8
Medulla	7	32.7 ± 7.0	36.3 ± 10.6
Spinal cord	6	28.4 ± 5.6	35.9 ± 8.0

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formation than in the others, slower rate of removal, or the combination of both. With this in view, the exceptionally high concentration of γ-aminobutyric acid in the hypothalamus, for example, might indicate the presence of greater amount of enzyme and the higher rate of α-decarboxilation of the glutamic acid in this highly functional part of the central nervous system. The lowest concentrations of investigated compounds in the cerebral white matter could be accounted for by the improbability, in view of the electron microscopic evidence, that the axoplasm contains the systems responsible for protein synthesis[1]. However, what the functional significance of the highest glutamine contents in the caudate nucleus and cerebellum may be, whether the second highest concentration of γ-aminobutyric acid in the caudate nucleus could be related to the fact that this nucleus represents an integral part of 'supressor system' in the brain[10], remain questions awaiting an answer from further investigations[11].

Résumé. Les auteurs ont déterminé par la méthode de la chromatographie à deux dimensions, la teneur en glutamine et en acide γ-aminobutyrique des différentes parties du cerveau du chat. La concentration la plus élevée de l'acide γ-aminobutyrique a été trouvé dans l'hypothalamus, tandis que celle de la glutamine a été constatée dans le noyau caudé. Les concentrations les plus basses de ces deux protéines apparaissent dans la substance blanche du cerveau.

Lj. Kržalić, V. Mandić, and Lj. Mihailović

Institute of Pathological Physiology, Medical School, University of Belgrade (Yugoslavia), March 11, 1962.

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Nucleoside Phosphorylase Activity in Guinea Pig Polymorphonuclear Leukocytes

Nucleoside phosphorylase activity has been the subject of a number of investigations in the red cells of man and other mammals[1]. On the other hand, no data are available concerning leukocytes: the present report deals with some studies performed on guinea pig neutrophilic granulocytes.

The cells were obtained from adult female guinea pigs by the peritoneal sterile exudate technique, using 7.2% sodium caseinate, as described by Hirsch[2]; grossly hemorrhagic samples were discarded. The peritoneal fluid was collected with an anticoagulant (1.2% ammonium sulphate in 0.8% potassium oxalate) and centrifuged for 10 min at $1000 \times g$. The sedimented cells were additioned with 1 vol of 0.2% NaCl; after 2 min 1 vol of 1.6% NaCl was added, in order to insure lysis of the rare red cells with minimum damage to the leukocytes. After centrifuging, the slightly pinkish supernatant was discarded, the cells were re-suspended in about 9 vol of water and homogenized in a Potter-Elvehjem apparatus at 0°C. The soluble fraction was obtained from this whole homogenate by centrifuging at 2°C for 1 h at $30000 \times g$. Differential centrifugation was performed in some experiments in 0.25 *M* sucrose according to Montreuil[3].

Nucleoside phosphorylase activity was estimated, with inosine as substrate, by measuring the rate of uric acid formation in the presence of xanthine oxidase[4]. The latter enzyme was prepared from buttermilk according to Horecker and Heppel[5]: a 20–30-fold purification, corresponding to the ammonium sulphate step, was generally satisfactory[6]. For a semi-quantitative estimation of nucleoside phosphorylase, and for purposes of comparison with activity on guanosine, the determination of the disappearance of NaOH-fast pentose[7] has been found suitable, and in good agreement with the spectrophotometric technique. Proteins were determined by Bücher's method[8].

The results obtained with rising concentrations of inosine are shown in Figure 1. When the enzyme is saturated with respect to both substrates (inosine and inorganic phosphate), the activity exhibited corresponds to the splitting of 2 µmoles nucleoside per mg protein in

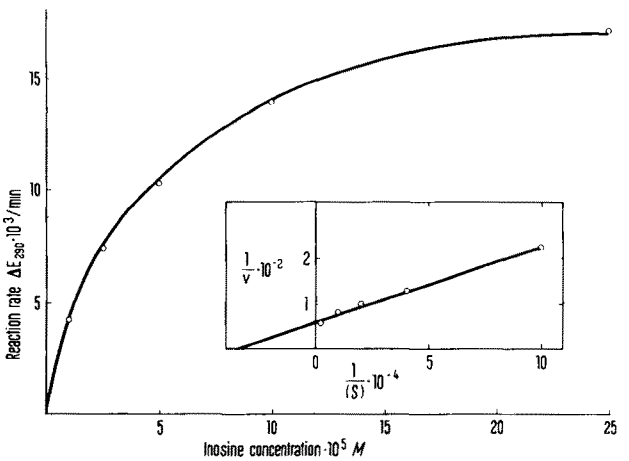

Fig. 1. Inosine phosphorylase activity of guinea pig leukocytes. The reaction mixture was prepared in 1.35 ml 1 cm path spectrophotometric cuvettes and contained 23 µmoles of sodium phosphate buffer, pH 7.6, 30 µl of xanthine oxidase, 10 µl of the leukocyte soluble fraction (corresponding to 0.17 µg of protein), and inosine at the concentrations indicated, in a final volume of 1 ml. Readings were taken every minute at 290 mµ using an Optica CF 4 spectrophotometer. Reaction rate was constant for at least 10 min. In the central part of the Figure, data are plotted according to Lineweaver and Burk[12].

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