Microanalysis of Amino Acids in Small Regions of the Rat Brain Using Dansyl Chloride

B. E. Leonard *, V. Neuhoff, and S. R. Tonge **

Max-Planck-Institut für Experimentelle Medizin, Arbeitsgruppe Neurochemie, Göttingen

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Dansyl-amino Acids, Microanalysis, Brain Regions

Ion exchange chromatographic methods have been used by several investigators to determine the concentration of free amino acids in the rat brain [1–3]. While this method is advantageous in that it allows the concentration of the individual substance to be accurately estimated, it has limited application when it is necessary to determine the concentrations of amino acids in very small regions of the rodent brain. For this reason, the microanalytical procedure for the estimation of amino acids in biological material using dansyl chloride [4, 5] is of particular interest. The present investigation was undertaken to determine the relative concentrations of the major amino acids in seven regions of the adult rat brain.

Male albino rats (180 – 200 g) were killed by decapitation. The brains were rapidly removed, placed on ice and dissected into the cortex, hippocampus, striatum, thalamus, hypothalamus, pons and "mid-brain" (tegmentum and colliculus). Immediately following dissection, the brain areas were frozen on solid carbon dioxide. Using a metal punch of the type described by Neuhoff [5], a small piece of each brain area weighing approximately 1 mg was removed and homogenized in 10 – 20 µl of 0.05 M sodium bicarbonate solution (pH 10). Following the dansylation of the amino acids in the supernatant with [^{14}C]dansyl chloride, the amino acids were separated by 2 dimensional chromatography on 3 cm × 3 cm polyamide sheets. After visualization of the dansylated products by ultraviolet light, the spots were marked, scrapped off and transferred to scintillation vials containing 10 ml liquid scintillator. The details of this method have been published elsewhere [4, 5]. In these experiments only those amino acids which were clearly separated were estimated; some 15 dansyl positive substances were determined in this way.

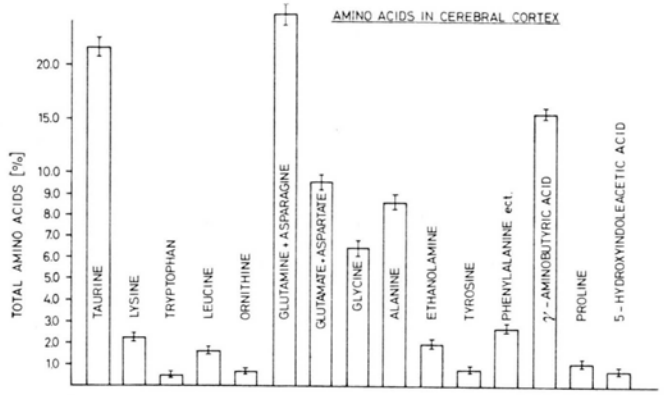
The results are shown in Fig. 1. In the two dimensional systems, used in this study, glutamine and asparagine, glutamate and aspartate could not be reproducibly separated and therefore have been estimated together. Phenylalanine, histidine and isoleucine was also estimated together.

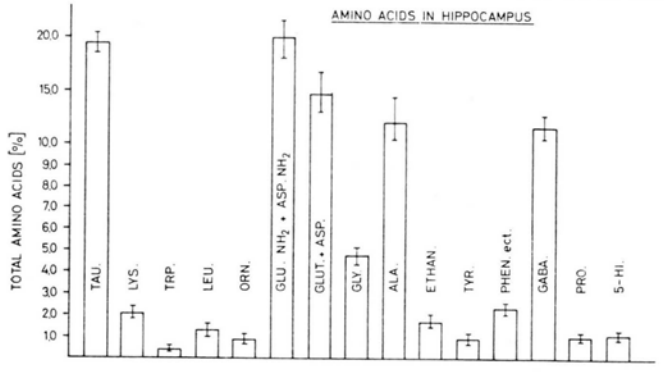
The concentration of taurine was found to be greatest in the striatum, cortex, thalamus and hippocampus; in these regions over 20% of the total radioactivity could be accounted for by this amino acid. In contrast, the pons, "mid-brain" had a taurine concentration which accounted for only about 10% of the total radioactivity. The relative concentrations of γ-amino butyric acid in these regions were inversely proportional to those of taurine; the pons, "mid-brain" and hypothalamus had a slightly higher concentration of γ-amino butyric acid than the other regions. It now seems possible that like γ-amino butyric acid, taurine may act as an inhibitory neurotransmitter in some areas of the mammalian brain [6–8]. There is also evidence that this amino acid has an effect on neuronal firing in some brain regions which is quite distinct from that of γ-amino butyric acid [8]. Thus the detailed distribution of taurine which the dansyl method makes possible may be advantageous in defining those brain regions where it may have a specific transmitter role.

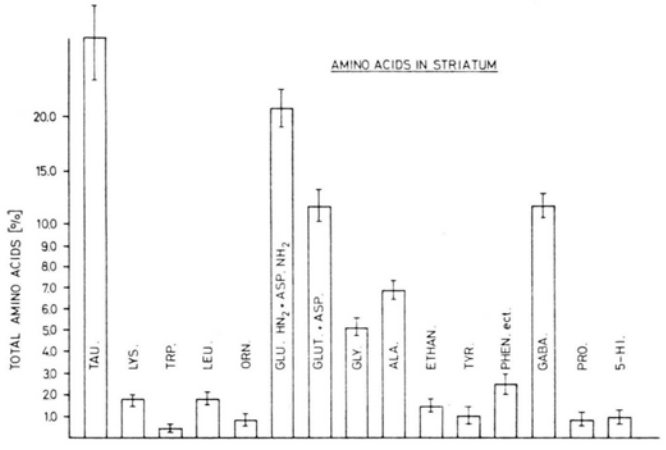
Glycine appears to be present in the same relative concentration in most of the regions (10%) apart from the pons where the concentration of this amino acid was approximately 15% of the total. Using the dansyl chloride method, other investigators [4] have demonstrated that glycine is present in a particularly high concentration in the ventral horn region of the mammalian spinal cord. These findings help to confirm the view that glycine is an inhibitory transmitter in the hind brain and spinal cord [9]. Although serotonin was not estimated in this study, its metabolite 5-hydroxyindoleacetic acid was determined. The concentration of this substance was found to only vary slightly in most of the regions studied although there was evidence that the concentration was higher in those brain stem regions which have been shown in contain serotonergic fibres [10]. The excitatory amino acids, glutamate and aspartate, accounted for approximately 10% of the radioactivity in most brain regions except the hippocampus where the concentration was 15% of the total. Whether the higher concentration of these substances in the hippocampus implies that they have a special physiological function in that brain region remains a matter of speculation.

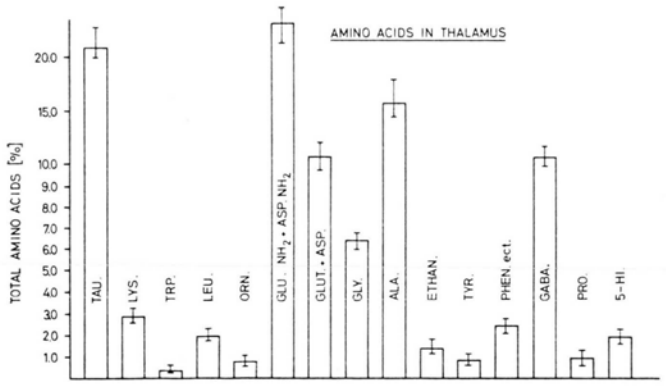
* Present adress: Pharmacology Department, Organon Scientific Development Group, Oss, The Netherlands.
** Pharmacology Department, School of Pharmacy, Liverpool Polytecnic, Byrom Street, Liverpool, U.K.

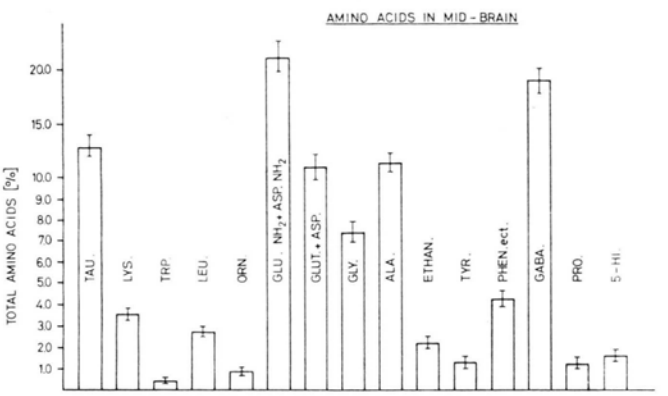
Requests for reprints should be sent to Prof. Dr. V. Neuhoff, Max-Planck-Institut für Experimentelle Medizin, Arbeitsgruppe Neurochemie, *D-3400 Göttingen*, Hermann-Rein-Str. 3.

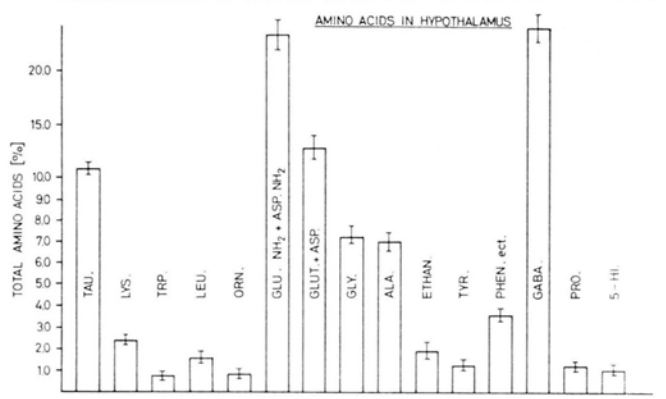












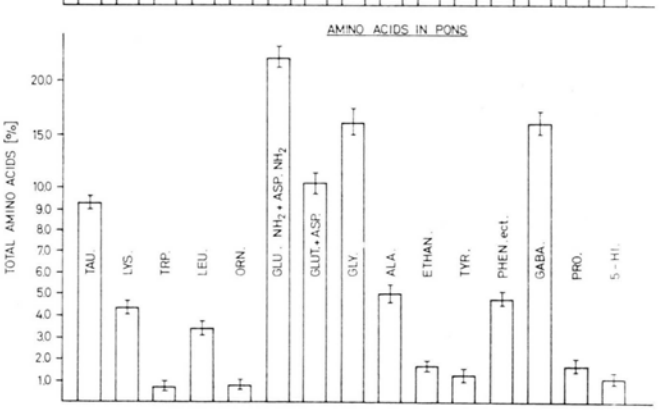


Fig. 1. Relative concentration of some free amino acids in 7 regions of the adult rat brain. The results for each amino acid are expressed as the mean ± s.e.m. radioactivity (as dpm) as a percentage of the total activity of all the dansylated products estimated. Triplicate estimations were made on each brain region. The amino acids estimated were: Tau. = taurine, Lys. = lysine, Trp. = tryptophan, Leu. = leucine, Orn. = ornithine, $GlutNH_2 + AspNH_2$ = glutamine + asparagine, Glut. + Asp. = glutamate + aspartate, Gly. = glycine, Ala. = alanine, Ethan. = ethanolamine, Tyr. = tyrosine, Phen.etc. = phenylalanine + histidine + isoleucine, Pro. = proline, HI = 5-hydroxyindoleacetic acid. GABA = γ-aminobutyric acid.

This investigation shows, that the dansyl chloride technique can be applied with some success to the study of the relative distribution of amino acids in small, discrete regions of the rodent brain.

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