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Changes in Neurotransmitter Amino Acids and Protein in CNS Areas of Mice Subjected to Differential Housing Conditions

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CORDOBA, F., B. YUSTA AND J. MUÑOZ-BLANCO. *Changes in neurotransmitter amino acids and protein in CNS areas of mice subjected to differential housing conditions.* PHARMACOL BIOCHEM BEHAV 21(3) 349–352, 1984.—The amino acid and protein content of mice exposed to enriched, restricted and impoverished environments have been studied in six discrete CNS areas. Differences between enriched and either restricted or impoverished groups were found whereas no difference was observed between restricted and impoverished ones. In the first case, a significant increase for aspartate was found in spinal cord, whereas glutamate significantly decreased in colliculi and cerebral cortex. Similarly, glycine increased in cerebral cortex and decreased in colliculi and pons-medulla, and γ-aminobutyrate (GABA) increased in spinal cord, pons-medulla and cerebellum and decreased in thalamus-hypothalamus. No changes in concentrations of five non-transmitter amino acids (serine, threonine, alanine, isoleucine, leucine) were observed. Significant increases of the protein concentration in cerebellum and spinal cord were found. The changes were due to enrichment, not to aggregation conditions. The results corroborate the proposed plasticity of the aminoacidergic system.

Brain plasticity Differentially-housed mice Neurotransmitter amino acids

ENVIRONMENTAL factors are decisive for the postnatal development of the brain. Many studies on the behavioral, morphological and neurochemical effects caused by differential housing of rodents have been reported [2, 6, 21]. However, the specific relationship between environment and CNS amino acids has been poorly investigated, in spite of the relevant role attributed to certain amino acids in the neurotransmission processes [7, 10, 24], and the partial modifications found in CNS aminoacidergic system induced by environmental changes [5, 11, 23].

In the present paper, the levels of non-transmitter amino acids (threonine, serine, alanine, isoleucine and leucine) as well as those of putative neurotransmitters (aspartate, glutamate, glycine and GABA) in several CNS areas of mice in different housing conditions were determined. The results indicate that only neurotransmitter amino acids were sensitive to environmental changes.

METHOD

Environmental Conditions

Male post-weanling Swiss mice, 28 days old, bred at our laboratory, were assigned randomly to each condition: (1) in impoverished environment, mice were housed separately in small cages (15×20×15 cm); (2) in restricted environment, three grouped mice were kept in medium-size cages (30×20×15 cm); and (3) in enriched environment, twelve-grouped mice were put in a large cage (60×30×25 cm) furnished with a variety of toys to provide the complexity conditions which define the environmental enrichment [1]. The toys, classified previously as forming several functional groups, were periodically renewed. Water and food vessel position was used as learning stimulus. All cages were placed in one laboratory-room because extracage stimulation is of little importance to define the environmental conditions [3]. All mice received food and water ad lib.

Anatomical Procedures

After 70 days in the above mentioned housing conditions, mice were decapitated under code number that did not reveal their environmental condition, their brains and cervical spinal cords rapidly removed and the cerebral cortex, thalamus-hypothalamus, cerebellum, colliculi and pons-medulla were dissected. The samples were weighed and homogenized in ice-cooled 0.32 M (10% w/v) sucrose solution. The homogenates were stored at −40°C until needed. Dissection and homogenization were performed at 0–4°C.

Analytical Procedures

After thawing, the homogenates were treated with ice-cold 5% (w/v) trichloroacetic acid (TCA), centrifuged at 5000 *g*, 10 min; the precipitate was washed and recentrifuged under the same conditions. The combined supernatant was dried under vacuum at 60°C and the resulting powder was dissolved in citrate buffer (0.1 M/pH 2.0). Amino acid

TABLE 1

EFFECTS OF DIFFERENT HOUSING CONDITIONS ON CONCENTRATION OF THREONINE (Thr), SERINE (Ser), ALANINE (Ala), ISOLEUCINE (Ile), LEUCINE (Leu), ASPARTATE (Asp), GLUTAMATE (Glu), GLYCINE (Gly) AND γ-AMINOBUTYRATE (GABA) IN VARIOUS CNS REGIONS OF MICE

	Spinal Cord			Pons-Medulla		
	(I)	(II)	(III)	(I)	(II)	(III)
Thr	0.33 ± 0.02	0.30 ± 0.02	0.34 ± 0.03	0.32 ± 0.02	0.35 ± 0.01	0.33 ± 0.03
Ser	0.83 ± 0.06	0.79 ± 0.03	0.85 ± 0.06	1.12 ± 0.05	1.13 ± 0.03	1.08 ± 0.07
Ala	0.46 ± 0.02	0.46 ± 0.02	0.47 ± 0.02	0.48 ± 0.02	0.49 ± 0.03	0.50 ± 0.02
Ile	0.05 ± 0.002	0.06 ± 0.004	0.06 ± 0.003	0.04 ± 0.002	0.04 ± 0.002	0.05 ± 0.004
Leu	0.09 ± 0.003	0.10 ± 0.008	0.09 ± 0.002	0.09 ± 0.004	0.09 ± 0.006	0.09 ± 0.005
Asp	2.20 ± 0.07*	2.19 ± 0.08*	2.43 ± 0.04 ↑	2.26 ± 0.04	2.27 ± 0.10	2.12 ± 0.11
Glu	4.98 ± 0.20	4.93 ± 0.18	5.08 ± 0.10	6.08 ± 0.36	6.02 ± 0.24	5.88 ± 0.27
Gly	4.45 ± 0.17	4.31 ± 0.07	4.58 ± 0.09	3.74 ± 0.06‡	3.96 ± 0.16‡	3.00 ± 0.11 ↓
GABA	0.80 ± 0.04†	0.79 ± 0.04†	0.94 ± 0.02 ↑	1.39 ± 0.02†	1.39 ± 0.02†	1.51 ± 0.03 ↑
	Colliculi			Thalamus-Hypothalamus		
	(I)	(II)	(III)	(I)	(II)	(III)
Thr	0.31 ± 0.02	0.36 ± 0.03	0.27 ± 0.02	0.40 ± 0.03	0.44 ± 0.03	0.42 ± 0.02
Ser	1.15 ± 0.08	0.97 ± 0.06	1.00 ± 0.05	1.00 ± 0.05	1.10 ± 0.03	1.01 ± 0.03
Ala	0.69 ± 0.04	0.58 ± 0.02	0.62 ± 0.02	0.67 ± 0.03	0.70 ± 0.03	0.64 ± 0.01
Ile	0.06 ± 0.004	0.04 ± 0.002	0.05 ± 0.004	0.04 ± 0.003	0.05 ± 0.004	0.04 ± 0.003
Leu	0.11 ± 0.01	0.09 ± 0.007	0.10 ± 0.006	0.09 ± 0.007	0.09 ± 0.004	0.09 ± 0.007
Asp	2.33 ± 0.12	2.34 ± 0.18	2.12 ± 0.08	2.77 ± 0.06	2.79 ± 0.10	2.58 ± 0.07
Glu	8.63 ± 0.45†	8.39 ± 0.21†	6.61 ± 0.33 ↓	8.12 ± 0.16	8.18 ± 0.31	7.64 ± 0.15
Gly	1.81 ± 0.10*	1.86 ± 0.11†	1.46 ± 0.05 ↓	1.14 ± 0.04	1.14 ± 0.04	1.07 ± 0.04
GABA	3.64 ± 0.20	3.57 ± 0.18	3.41 ± 0.14	2.56 ± 0.03‡	2.59 ± 0.02‡	2.32 ± 0.02 ↓
	Cerebellum			Cerebral Cortex		
	(I)	(II)	(III)	(I)	(II)	(III)
Thr	0.32 ± 0.02	0.30 ± 0.02	0.37 ± 0.02	0.41 ± 0.01	0.38 ± 0.03	0.42 ± 0.01
Ser	0.83 ± 0.05	0.80 ± 0.03	0.90 ± 0.04	1.31 ± 0.08	1.26 ± 0.04	1.27 ± 0.02
Ala	0.61 ± 0.02	0.60 ± 0.01	0.63 ± 0.009	0.65 ± 0.02	0.64 ± 0.03	0.66 ± 0.02
Ile	0.05 ± 0.002	0.05 ± 0.002	0.05 ± 0.003	0.06 ± 0.005	0.05 ± 0.004	0.06 ± 0.005
Leu	0.07 ± 0.005	0.08 ± 0.002	0.08 ± 0.005	0.08 ± 0.006	0.08 ± 0.006	0.09 ± 0.009
Asp	2.02 ± 0.09	1.93 ± 0.06	2.16 ± 0.05	2.98 ± 0.13	2.92 ± 0.08	2.96 ± 0.10
Glu	10.38 ± 0.44	9.61 ± 0.60	10.52 ± 0.13	9.11 ± 0.23†	9.17 ± 0.20‡	8.05 ± 0.14 ↓
Gly	0.73 ± 0.03	0.68 ± 0.01	0.73 ± 0.02	0.31 ± 0.007‡	0.32 ± 0.007‡	0.37 ± 0.008 ↑
GABA	1.45 ± 0.06*	1.47 ± 0.06*	1.66 ± 0.04 ↑	2.17 ± 0.13	2.13 ± 0.09	2.18 ± 0.18

Results are expressed as μmol/g wet weight ± S.E.M. (N=6 animals for each condition). Statistical significance between enriched (III) and either impoverished (I) or restricted (II) groups was calculated by using the Student's *t* test.

*$p<0.05$.

†$p<0.01$.

‡$p<0.001$.

The arrows on significant changes indicate whether change was an increase or an decrease of (III) vs. (I) or (II).

analysis was performed in a Technicon Amino Acid Analyzer (TSM-1). Norleucine (2.5 μmol/ml) was used as internal standard. Protein of total homogenate was determined according to Lowry *et al.* [18].

RESULTS

The effects of environmental conditions on amino acid contents (expressed as μmole/g wet weight) in various CNS regions of mice are shown in Table 1.

The different housing conditions produced significant changes in the level of aspartate (spinal cord), glutamate (colliculi and cerebral cortex), glycine (pons-medulla, colliculi and cerebellum) and GABA (spinal cord, pons-medulla, colliculi and cerebellum). The levels of threonine, serine, alanine, isoleucine and leucine remained practically unaltered.

No significant differences in amino acid contents between the impoverished and restricted (aggregated) groups were ever observed.

Protein content in spinal cord and cerebellum of enriched mice, was significantly higher than that of impoverished and restricted groups (Table 2). In the other regions studied, the protein content remained constant after differential housing conditions. When amino acid concentrations of spinal cord and cerebellum were expressed on the basis of protein con-

TABLE 2

EFFECTS OF DIFFERENTIAL HOUSING CONDITIONS ON PROTEIN CONTENT IN VARIOUS CNS REGIONS OF MICE

	(I)	(II)	(III)
Spinal Cord	83.8 ± 1.0 (11)‡	82.4 ± 1.6 (7)†	90.2 ± 1.2 (8) ↑
Pons-Medulla	93.1 ± 1.4 (9)	94.0 ± 1.6 (6)	92.3 ± 1.8 (8)
Colliculi	98.5 ± 1.4 (11)	100.4 ± 2.4 (7)	100.4 ± 1.9 (8)
Thalamus-Hypothalamus	89.8 ± 0.8 (8)	90.6 ± 2.1 (6)	87.5 ± 0.5 (7)
Cerebellum	100.8 ± 1.2 (11)‡	102.4 ± 3.2 (6)*	111.7 ± 1.6 (8) ↑
Cerebral Cortex	105.1 ± 2.7 (8)	102.9 ± 2.2 (6)	104.1 ± 1.9 (8)

Results are expressed as mg protein/g wet weight ± S.E.M. Number of animals is expressed between brackets. Conditions and symbols as in Table 1.

tent, no statistically significant differences were observed (results not shown).

No changes in mice and brain weight were found between conditions.

DISCUSSION

The above results on the effects of different housing conditions on CNS amino acid levels in mice can be explained in terms of the complexity of environmental enrichment, since no significant changes between impoverished (isolated) and restricted (aggregated) mice were ever observed. This is consistent with the proposal that environmental enrichment, and not the social grouping, accounts for the brain responses to environment [13,22]. Bennett and co-workers [4] proposed that plasticity is a consequence of learning processes resulting from environmental enrichment. We have found changes in overall studied regions, including pons-medulla and spinal cord, the lower levels of morphofunctional organization of CNS. These results support the proposal of Oakley [19] that learning ability is a fundamental property of all levels of mammalian CNS.

Only the amino acids proposed as neurotransmitters (aspartate, glutamate, glycine and GABA) [10,24] changed significantly in their concentrations as a consequence of differential housing conditions, whereas the levels of other amino acids, not considered as neurotransmitters (serine, alanine, threonine, isoleucine and leucine), remained unaltered after differential housing conditions, which suggest that the environmental control is specifically exerted on the mechanism of aminoacidergic transmission.

It has been reported little or no difference in brain protein concentration between enriched and impoverished rats [1]. However, we have found significant increases of protein concentration in cerebellum and spinal cord of enriched mice. When amino acid concentrations of these areas were expressed on the basis of protein content, no significant differences were observed. Previously, it had been found in brain of isolated mice lower protein content and GABA and glycine binding capacity of synaptosomal fractions than in that of their aggregated counterparts, whereas no differences appeared in the same binding capacities on a protein basis [11]. Similar results were reported for the binding of several pharmacological agents to membrane fractions from cortex of differentially-reared rats [20]. According to DeFeudis and colleagues [11] this parallel modification in protein and total binding capacities indicates a morphological change in the brain. Our results can be interpreted similarly, in terms of a structural modification in cerebellum and spinal cord, caused by enrichment and resulting in the higher levels of protein and amino acids. Actually, the existence of morphological changes in dendritic branching and synaptic boutons [12, 15, 16] as well as the protein content of certain synaptic junctions [17] seem to support this interpretation.

DeFeudis [8,9] reported a higher requirement of mannose and glucose for the brain of aggregated than for isolated mice, and he proposed that such differences can be used to study the synthesis and turnover of the central neurotransmitters related with the energetics of the brain. In a parallel way, modifications in the brain amino acid contents caused by environmental changes can be explained in terms of a change of the energetic metabolism of the brain, following the enrichment conditions. Our results do not exclude the latter hypothesis because the observed changes of amino acid levels are exclusively restricted to the amino acids considered as neurotransmitters, linked to cerebral energy metabolism. However, our results show different and specific pattern of response to environment, for each analyzed region. According to Greenough and Juraska [14], if general effects on CNS metabolism were the cause of changes following complex rearing, they would be more evenly distributed throughout the brain. Thus, our results can be also attributed to specific mechanisms related to neurotransmission other than those dependent on cerebral energy metabolism.

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