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CHANGES INDUCED BY AGE AND STRESS IN THE BEHAVIOUR AND BRAIN METABOLISM OF FREE AMINO ACIDS IN WISTAR RATS. INFLUENCE OF ASLAVITAL TREATMENT

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Summary. The experiments were carried out on 90 Wistar rats, 45 males and 45 females, 1 year old. Each sex-group was made up of three subgroups: control rats, rats under stress treated with Aslavital and untreated rats under stress.

The treatment consisted in intramuscular injections of 0.2 ml/kg body-weight, three times a week, for 30 days.

The stress was applied as electric shocks (50 Hz, 1 mA, 0.5 s), three minutes daily, for 30 days.

All the rats were examined with a complex maze in order to estimate the learning and memorizing capacity.

At the end of the experiments, the rats were sacrificed, and their brains were cut off for biochemical determinations.

The amino acids extracted from the cerebral hemispheres were identified by circular paper chromatography.

As regards the effect of stress on the learning and memorizing processes, the results showed significant differences between the studied groups: the maze was solved by: 80% control females; 7% stressed untreated females; 27% stressed treated females; in male rats, the percentages were 67%, 0 and 13%, respectively.

Under stress the content of free amino acids in the cerebral hemispheres increased.

In stressed animals treated with Aslavital, the amino acid content was lower than in untreated animals under stress, the difference being statistically significant.

With regard to the stress-age relation, some authors [1] consider aging itself the result of stress-induced accumulations in the course of life, whereas others [2, 3, 4] claim that the process of aging is accelerated by repeated exposure to stressing stimuli. The clinical observations and the laboratory investigations have pointed out the age-induced decrease in the organism's adaptability to stress.

The researches into the effect of stress on animals have pointed out behavioural changes, such as apathy, drowsiness, anorexia, phobism, etc. [5–10].

Other studies have investigated the biochemical changes in the brain of stressed animals [7, 8, 11]. Mention should be made of the studies on brain protein synthesis inhibition [12], with the subsequent increase in the amount of free amino acids [13].

Taking into consideration the age-stress interaction we decided to investigate the influence of age and stress on learning, memory and content of glutamic acid, glutamine, gamma-aminobutyric acid in the brain. As known, the first two

amino acids mentioned above play an important part in the metabolic process involved in maintaining the morphological and functional integrity of the nervous cell [14]; the gamma-aminobutyric acid (GABA) is a membrane modulator involved in inhibitory processes.

We studied also the effect of the biotrophic substance Aslavital on the above-mentioned functional and biochemical nervous processes in stressed rats at different ages. Aslavital is a solution containing procaine 2%, glutamic acid and an increased amount of potassium ions (as against Gerovital H_3).

As known, Aslavital was elaborated by Ana Aslan in order to potentiate the psychotropic and lipotropic action of procaine. Beside the clinical studies on 1,400 patients [15], which pointed out these characteristics of Aslavital, a number of experimental researches were conducted, having similar goals. Mention should be made of the studies on the effect of Aslavital on learning ability, memory and passive avoiding behaviour in rats [16].

The electroencephalographic investigations have pointed out that the changes resulting from advancing age, such as the slowing down of the basic rhythm and the decrease in the amplitude of the tracings, are less marked in the Aslavital treated rats as against controls, in which slow waves were detected [17].

MATERIAL AND METHOD

The experiment was conducted on 270 Wistar rats, males and females, equal in number; three age-groups were investigated, each including controls, stressed — untreated and stressed — Aslavital treated animals.

The stress consisted in electric shocks (2—6 mA; 50 Hz) to which the animals were exposed 0.5 sec. every 10 sec. intervals for 3 min. daily, during 30 days.

Verzar Mc Douglas' maze method [18] was used in evaluating the changes induced by stress, age and treatment on rats' learning ability and memory.

At the end of the experiment, the animals were sacrificed in order to make the necessary biochemical determinations on the brain. The glutamic acid, glutamine and gammaaminobutyric acid (GABA) were extracted. The circular paper chromatography for the identification of amino acids and the spectrophotometric method for quantitative determinations [19, 20] were used.

The Aslavital treatment consisted in i.m. injections with 0.2 ml/kg body weight, 3 times per week for 30 days.

RESULTS

a) *In relation to age.* A significantly decreased ability of solving the maze problem was noticed in 12- and 24-month-old rats, both males (from 67% to 20%) and females (from 80% to 40%; $p < 0.05$), (Table 1).

With regard to the concentration of free amino acids in the brain, a constant and significant increase with age of glutamic acid, glutamine and GABA was pointed out (Tables 2 and 3). For instance, the glutamic acid level which was 64.3 mg/100 g wet tissue in 4-month-old male rats reached 123.4 mg and 297.2 mg in 12- and 24-month-old rats, respectively ($p < 0.01$).

Glutamine levels increased from 14.4 mg in 4-month-old rats to 190 mg and 167.8 mg in 12- and 24-month-old rats, respectively. The corresponding values for GABA were 28.2 mg and 78.3 mg ($p < 0.01$).

Male rats

Female rats

Fig. 1. — Free amino acids in the cerebral hemispheres of control and stressed (treated and untreated) rats.

Table 1

Ability to solve the maze problem

Group	Sex	Rats constantly solving the maze %	Rats inconstantly solving the maze %	Rats which do not solve the maze %
Control	females	80±3.63	20±3.63	–
	males	67±3.11	13±2.21	20±2.64
Stressed, untreated	females	7±2.21	20±2.64	73±2.92
	males	–	13±2.21	87±2.21
Stressed, treated with Aslavital	females	27±2.92	27±2.92	46±3.29
	males	13±2.21	20±2.64	67±3.11

Table 2

Free amino acid contents in male rats' cerebral hemispheres

Amino acids mg/100 g wet tissue / Group	Controls I	Stressed, untreated II	Stressed, treated with Aslavital III	Statistic significance I–II	Statistic significance II–III
Glutamic acid	62.0±0.44	117.0±9.87	74.0±1.19	$p < 0.01$	$p < 0.01$
Glutamine	9.5±0.25	16.9±2.01	10.9±0.30	$p < 0.01$	$p < 0.01$
GABA	39.1±1.30	70.3±6.58	56.5±0.81	$p < 0.01$	$p < 0.01$

The data were analysed statistically by means of Student's "t" test.

Table 3

Free amino acid contents in female rat cerebral hemispheres

Amino acids mg/100 g wet tissue / Group	Controls I	Stressed, untreated II	Stressed, treated with Aslavital III	Statistic significance I–II	Statistic significance II–III
Glutamic acid	43.5±0.52	77.5±0.96	71.0±0.89	$p < 0.01$	$p < 0.01$
Glutamine	1.9±0.03	7.3±0.14	6.1±0.89	$p < 0.01$	$p < 0.01$
GABA	14.7±0.16	44.4±0.75	28.0±1.19	$p < 0.01$	$p < 0.01$

The data were analysed statistically by means of Student's "t" test.

b) *Stress-induced changes at different ages.* The results in solving the maze problem were greatly inferior in all the stressed groups, e.g.: from 60% to 27% in 4-month-old rats, from 67% to 0% in 12-month-old rats and from 20% to 0% in 24-month-old rats (Table 1).

With regard to the 3 amino acids investigated, a more prominent value increase was noticed, sometimes by 80—100, even 200% as compared to the unstressed animals of the same age (Tables 2 and 3, Fig. 1).

c) *The influence of Aslavital treatment on age and stress-induced changes.* Better results were obtained in solving the maze problem (Table 1), such as:

— from 27% to 40% in 4-month-old male rats, from 0% to 13% in 12-month-old rats, from 0% to 30% in 24-month-old rats;

— from 20% to 47% in 4-month-old female rats, from 7% to 27% in 12-month-old rats, from 0% to 40% in 24-month-old rats.

With regard to the concentration of the 3 free amino acids investigated (Tables 2 and 3), a tendency was noticed to reaching the levels pointed out in unstressed rats of the same age; e.g.:

— in 4-month-old male rats = glutamic acid levels decreased from 91.7 mg/100 g wet tissue to 71.2 mg (64.3 mg in controls); glutamine levels decreased from 27.6 mg to 16.9 mg (14.4 mg in controls); GABA levels decreased from 59.4 mg to 30.6 mg (28.2 mg in controls);

— in 12-month-old male rats = glutamic acid levels decreased from 146.8 mg to 126.6 mg (123.4 mg in controls); glutamine levels decreased from 21.7 mg to 18.0 mg (19.0 in controls); GABA levels decreased from 112.6 mg to 81.4 mg (78.2 mg in controls);

— according to the above data, as a result of the treatment, the concentrations of the 3 amino acids reached values close or equal to those pointed out in younger controls.

Similar results were obtained in female rats. (Table 3).

DISCUSSION

The changes in the process of learning, memory and brain biochemistry, resulting from advancing age and stress were pointed out in the course of the experiment. The fact was expressed by the modifications in the concentration of 3 amino acids (glutamic acid, glutamine, GABA) which play an important part in the development of the brain metabolic processes.

The different aspects of the behavioural changes in old animals have been described by numerous investigators [16, 21, 22].

The stress-related changes in the concentration of certain brain amino acids may be correlated with the well-known sensitiveness to exogenous and endogenous factors, of the brain biochemical mechanisms which incorporate amino acids into the proteins.

According to some authors [23, 24], the adaptability of the brain biochemical mechanisms to stress as well as protein synthesis diminish with advancing age.

Under these circumstances, the use of the biotrophic treatment with Aslavital is particularly significant since it improves learning ability and memory in stressed aged rats. The decrease in the concentration of the investigated amino acids could be due to the stimulated protein synthesis resulting from the treatment.

These data confirm experimentally the results obtained at the National Institute of Gerontology and Geriatrics on aged subjects treated with Aslavital, some of whom had an accelerated aging syndrome due to environmental stress factors (family, profession, accidents, etc.).

Generally, 3—5 months of treatment resulted in a changed attitude of the elderly towards the surrounding environment. They became more optimistic, active, got along better with each other. Memory improved considerably and the behavioural disturbances of some elderly patients either diminished or disappeared. Meanwhile, psychopathic tendencies were reduced, the depressive states disappeared and a good psychic balance was reached.

CONCLUSIONS

The Aslavital treatment, administered to Wistar rats induced important improvements in some brain functions, such as learning ability and memory, which had previously been diminished by age and stress. It also improved the functioning of certain biochemical brain mechanisms, fact which was pointed out by the decrease in the concentration of free amino acids, probably due to an increased protein synthesis.

Résumé. Les expérimentations ont été effectuées sur 90 rats Wistar, 45 mâles et 4 femelles, âgés d'un an. Chaque groupe par sexe a été formé par trois sous-groupes: des rats témoins, des rats stressés et traités à l'Aslavital et rats stressés non traités.

Le traitement a été composé par des piqûres intramusculaires 0,2 ml/kg corps, trois fois par semaine, pendant 30 jours.

Le stress a été appliqué sous forme de chocs électriques (50 Hz, 1 mA, 0,5 s), trois minutes par jour, pendant 30 jours.

Tous les rats ont été testés dans un labyrinthe complexe, pour pouvoir estimer l'apprentissage et la mémoire.

Les rats ont été sacrifiés à la fin des expériences, et le cerveau a été prélevé pour des déterminations biochimiques.

Les amino-acides provenus des hémisphères cérébraux ont été identifiés par chromatographie circulaire sur papier.

En ce qui concerne l'effet du stress sur les processus d'apprentissage et de mémoire les résultats ont montré des différences significatives entre les groupes étudiés; les rats témoins femelles donnent une solution correcte au problème du labyrinthe en proportion de 80%, les rats stressés non traités en proportion de 7%, et les rats stressés et traités, en proportion de 27%; chez les rats mâles, les pourcentages sont 67%, 0 et 13%.

Chez les rats stressés on a observé une augmentation du contenu d'amino-acides libres des hémisphères cérébraux, sous l'action du stress.

Chez les animaux stressés et traités à l'Aslavital, le contenu d'amino-acides étudiés a connu des valeurs diminuées par rapport aux valeurs enregistrées chez les animaux stressés et non traités, les différences étant significatives du point de vue statistique.

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