

EXPERIMENTAL ASSESSMENT OF CEREBRAL MONOAMINERGIC STATUS IN A MURINE MODEL OF BEHAVIOR

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Abstract

The purpose of our study was to evaluate some biochemical markers of neuronal metabolism in a classical pattern of behavior, developed by Friedman and Rosenman (1974), the adrenergic "A" type. Epidemiologic studies demonstrated an association between type A behavioral characteristics and specific psychopathologic conditions: hypersensitivity to pain, increased heart rate, high systolic blood pressure, bipolar disorders, or specific preferences for atherogenic diet, despite the fact that molecular basis of these findings have not been established, yet.

Mice were divided in two behavioral groups, according to their reactivity to painful stimulus (endogenous analgesia): type A (associated with hypersensitivity to pain) and type B ("non-A", the opposite type of behavior) which exhibits hypo reactivity to pain. Mice pain sensitivity was assessed using the hot-plate test (60°C).

Cerebral monoaminergic status was assessed by measuring the levels of noradrenaline (NA), dopamine (DA), serotonin (5-HT) using HPLC with electrochemical detection, and γ -aminobutyric acid (GABA) using a fluorimetric assay.

Rezumat

Studiul experimental și-a propus evaluarea unor parametrii biochimici ai metabolismului neuronal în cadrul unui model experimental comportamental clasic, și anume în cadrul tipologiei adrenergice, propusă și descrisă pentru prima dată de Friedman și Rosenman (1974). Studiul a fost efectuat pe animale de laborator (șoareci albi), acestea fiind grupate în trei loturi (în funcție de reactivitatea la stimuli dureroși-testul plăcii fierbinți, 60°C): tipul adrenergic (hipersensibil la durere), tipul comportamental opioid (hiposensibil la stimuli dureroși) și tipul intermediar, echilibrat.

A fost evaluat status-ul monoaminergic neuronal prin determinarea cantitativă, din omogenatele cerebrale ale animalelor luate în studiu, a următorilor neuromediatorii: noradrenalină, serotonină și dopamină (prin cromatografie de lichide de înaltă performanță cu detecție în UV) și a acidului gamma-amino butiric (printr-o metodă fluorimetrică).

Keywords: behavior, noradrenaline, serotonin, dopamine, γ -aminobutyric acid

Introduction

The adrenergic behavioral pattern (the A type) is a concept developed by Friedman and Rosenman (1974) based on their studies on patients with myocardial dysfunctions. In this regard, type A individuals display specific behavioral traits: an extreme sense of competitiveness, spirit of fight, hostility, irritability, aggressiveness, permanent alertness and timeless mood. At that time these specific behavioral dispositions were considered contributing factors in rendering type A individuals susceptible to cardiovascular diseases [7,8].

Ever since there had been made epidemiologic studies that demonstrated an association between type A behavioral pattern and specific physiopathologic conditions: hypersensitivity to pain, increased heart rate, high systolic blood pressure, bipolar disorders, or specific preferences for atherogenic diet [1].

Many clinical studies speculated that the pathophysiological hallmark of type A individuals is the hyperactivity of the sympathetic nervous system, although the molecular basis of these findings have not been established, yet [9].

The behavioral type described as being opposed to type A has been called type B (or non-A type) and there was made the hypothesis that type „B” might be an opioid type (type „O”) [12]. Experimental and clinical research showed that type O is statistically significant opposed to type A, in terms of general behavior, psychosomatic reactions to pain and stress, and response to drugs.

Materials and methods

Animals

Studies were conducted on 120 male, Albino Swiss mice, weighing 20-22 g. They were housed in a room and maintained at 25 ± 2 °C and 45-55% relative humidity, with an alternating 12h light-dark cycle. They had free access to food and water until the morning of the experiment. All animals used in this study were maintained in facilities fully accredited and the experiments described here were performed in compliance with the European Communities Council Directive of 24 November 1986 (86/609/EEC) and Ordinance No. 37 of the Romanian Government from 2nd February 2002.

Identification of the murine behavior type

For the identification of the murine behavior type we used the hot plate test (Ugo Basile apparatus). The plate was heated at 60 °C and the

animals were divided, based on their reactivity to painful stimulus (endogenous analgesia, expressed as *jumping time off the heated plate*), into three working groups: the adrenergic “A” type, the equilibrated, intermediate, “EQUI” type and the “O” type, according to Gauss normal distribution curve. The average value of the jumping time off the plate was 26.91 ± 8.25 sec. Mice that possessed a value of the jumping time of $M \pm 1SD$ were selected as intermediate, “EQUI” type. Mice that possessed the value of the jumping time less than $M - 1SD$ ($<M - 1SD$) were selected as adrenergic “A” type, while the jumping time of more than $M + 1SD$ ($>M + 1SD$) were selected as the “non-A” type (“O” type).

The assessment of cerebral monoaminergic status

Mice were sacrificed by decapitation and the whole brains were rapidly removed, weighed and kept at -80°C until analyzed.

Cerebral monoaminergic status (neuronal monoamines levels) within the behavioral model was evaluated by measuring the murine brain levels of noradrenaline (NA), dopamine (DA), serotonin (5-HT) using HPLC with UV detection, and γ -aminobutyric acid (GABA), using a fluorimetric assay[4,10,11].

Results were expressed as μg neurotransmitter/mg wet tissue.

Simultaneous, HPLC evaluation of endogenous NA, DA and 5-HT

Briefly, brain samples were homogenized and deproteinized in 0.2M perchloric acid containing $100\mu\text{M}$ EDTA_2Na . The homogenate was left for 30min. to deproteinize. Then, the homogenate was centrifuged at $10,000 \times g$ for 15min at 0°C (Janetzki K24 cooling centrifuge). After centrifugation, the supernatant was adjusted to $\text{pH}=3.35$ by adding 1M acetic acid. $20\mu\text{l}$ were injected into an HPLC reversed-phase system (VARIAN – PROSTAR) with a Chromsep-Inertsil 5 OSD2, 250×4.6 mm column. The mobile phase consisted of 0.8 mM EDTA-Na_2 , 0.12M $\text{NaH}_2\text{PO}_4 \times \text{H}_2\text{O}$, 0.646g sodium heptane sulphonate and 17% methanol. Monoamines were detected simultaneously, using an UV detector (210nm).

Determination of endogenous GABA concentrations

Brain tissue samples were separately homogenized in 10 volumes of 0.01 M HCl using a glass homogenizer.

The determination of endogenous GABA concentration is based on a fluorimetric assay that depends on the formation of a fluorescent product from the reaction between GABA and ninhydrin at alkaline pH and in the presence of glutamate. The reagents used in the assay were 0.05 M glutamic acid in 0.2 M sodium phosphate buffer, pH 6.4, 14 mM ninhydrin in 0.5M sodium carbonate buffer, pH 9.9-10 and copper tartrate reagent consisting of

1.6g Na_2CO_3 , 329 mg tartaric acid and 300 mg $\text{CuSO}_4 \times 5\text{H}_2\text{O}$, all made up in 1 liter of distilled water.

0.25 ml of homogenate was diluted with 0.25 ml of 0.01 M HCl and 0.5 ml of 10% trichloroacetic acid. This last reagent was used to precipitate the proteins. After the samples were centrifuged, 100 μl aliquots of the supernatant were added to 15 μl of glutamate solution and 200 μl of the ninhydrin solution. This mixture was incubated at 60°C for 30 minutes and allowed to cool before the addition of 5 ml copper tartrate reagent. We also prepared two internal standards by adding to the samples of homogenate known amounts of GABA (50 μg and 100 μg GABA per sample) with the trichloroacetic acid.

Statistical analysis

Results were expressed as mean values $\pm$ standard deviation. Differences between groups were assessed by use of Student's t and Anova test. Results were considered statistical significant at $p < 0.05$.

Results and discussion

The assessment of cerebral monoaminergic status

HPLC analysis revealed that type A behavioral group ($n=21$) possesses lower neuronal concentrations of NA, DA and 5-HT ($\mu\text{g}/\text{mg}$ wet tissue), compared with the the O type ($n=20$), as follows:

- noradrenaline: 1.01 ± 0.189 (type A) vs. 1.52 ± 0.26 (O type), $p < 0.05$ (fig.3);
- dopamine: 0.67 ± 0.2 (type A) vs. 1.1 ± 0.01 (type O), $p < 0.01$ (fig.4);
- serotonin: 0.12 ± 0.005 (type A) vs. 0.53 ± 0.01 (type O), $p < 0.05$ (fig.5).

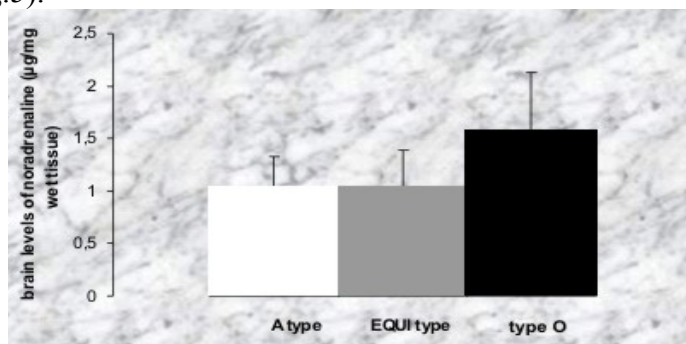


Figure 3

Dynamics of cerebral noradrenaline (NA) concentrations within the murine behavioral models studied ($p < 0.01$: A type vs. O type)

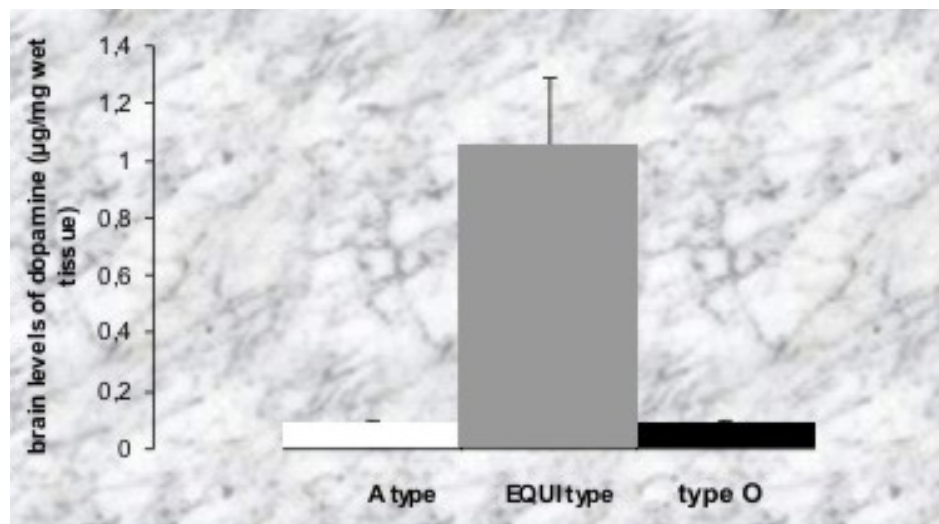


Figure 4

Dynamics of cerebral dopamine (DA) concentrations within the murine behavioral models studied ($p < 0.001$: A type vs. O type)

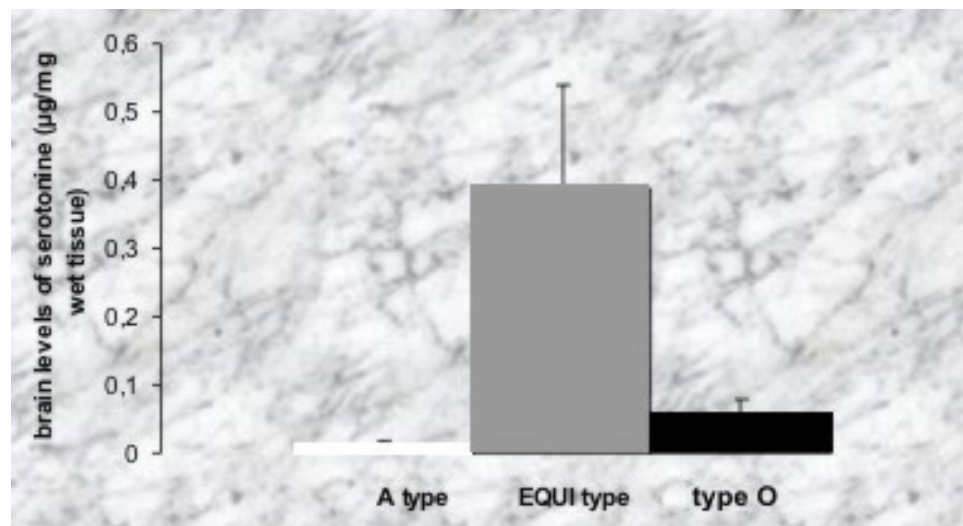
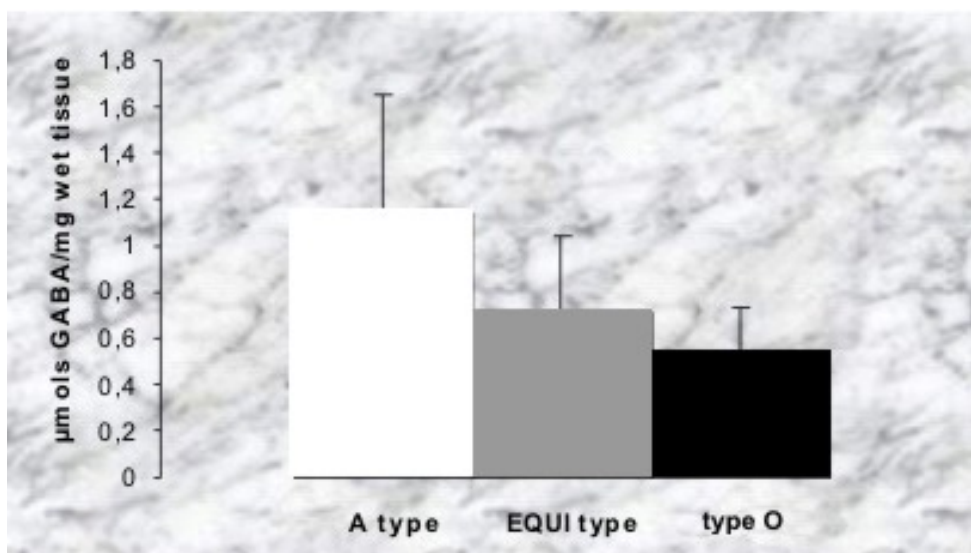


Figure 5

Dynamics of cerebral serotonin (5-HT) concentrations within the murine behavioral models studied ($p < 0.001$: A type vs. O type)

The quantification of neuronal GABA concentrations (μmol s GABA/mg wet tissue) showed that the adrenergic type of behavior ($n=19$) develops high amounts of this major inhibitory neurotransmitter of the nervous system, compared to the B type ($n=20$): 1.14 ± 0.5 (type A) vs. 0.54 ± 0.18 (type O), $p < 0.01$ (fig.6)

**Figure 6**

Dynamics of cerebral γ -aminobutyric acid (GABA) concentrations within the murine behavioral models studied ($p < 0.05$: A type vs. EQUI type; $p < 0.01$: A type vs. O type)

HPLC analysis revealed low cerebral concentrations of NA, DA and 5-HT for the adrenergic type of behavior, while GABA was detected in higher amounts (expressed as $\mu\text{g}/\text{mg}$ wet tissue): noradrenaline: 1.01 ± 0.189 (type A) vs. 1.52 ± 0.26 (O type), $p < 0.05$; dopamine: 0.67 ± 0.2 (type A) vs. 1.1 ± 0.01 (type O), $p < 0.01$; serotonin: 0.12 ± 0.005 (type A) vs. 0.53 ± 0.01 (type O), $p < 0.05$; GABA: 1.14 ± 0.5 (type A) vs. 0.54 ± 0.18 (type O), $p < 0.01$.

The alert and hostile type A evoked an interesting neuronal pattern: low concentrations of catecholamines and serotonin, together with high amounts of GABA.

Conclusions

In order to investigate the cellular mechanisms that may link behavioral characteristics to the susceptibility of developing certain diseases (cardiovascular and psychiatric dysfunctions), our study assessed, in a murine experimental model, some biochemical markers of the neuronal metabolism. The cerebral monoaminergic status was analyzed as specific neuromediators concentrations: noradrenalin (NA), dopamine (DA), serotonin (5HT) and gamma-amino butyric acid (GABA).

Epidemiologic studies demonstrated an association between individuals' behavior pattern and the risk for developing certain diseases. In this regard type A behavior is conceptualized as a risk factor for developing

coronary heart disease and/or bipolar disorder, due to an emotion-action complex of time urgency, hostility, irritability and aggressiveness. In contrast, type O behavior pattern („non-A”) does not show any of these traits. Type A and O also possess different dispositions for responding to pain: while type A shows hyperreactivity to pain (extreme basal pain sensitivity), type O exhibits hyposensitivity to pain (significantly opposed to type A).

Endogenous monoamines are fundamental components of organisms closely related to stress and emotions. On the other hand monoaminergic imbalance ends with multiple pathologic conditions (endocrine, cardiovascular or psychiatric). In this regard, their evaluation may provide important information regarding the evolution of a certain disease and also, regarding an appropriate (targeted) pharmacotherapy). Noradrenaline, dopamine and serotonin are the most important activating monoamines within the nervous system, while gamma-amino butyric acid is the major inhibitory neuromediator. It acts like an inhibitory–modulator of the activating neurotransmissions (adrenergic, serotonergic, dopaminergic, glutamatergic, etc) and significantly diminishes cerebral excitability, inhibits anxiety responses and modulates the thymic mood.

The adrenergic behavioral pattern (type A of behavior) revealed low brain levels of NA, DA and 5-HT together with high concentrations of GABA. These results indicate that the adrenergic–like behavior is highly equilibrated from this point of view. This observation is experimentally sustained by our previous research in which we investigated the enzymatic activity of monoaminoxidase (MAO), a biochemical parameter involved in catecholamines’ metabolism [2]. MAO (monoamine: O₂ – oxidoreductase, EC: 1.4.3.4.) is an iron containing enzyme which is localized on the outer mitochondrial membrane. Its major biological role is caused by its affinity for various bioactive amines (noradrenalin, adrenaline, dopamine, serotonin, tyramine, etc.) The activity of MAO reflects in the regulation (modulation) of catecholamines’ metabolism at neuronal level as well as in other tissues [5]. In this regard, type A of behavior showed, at neuronal level, low activity of this enzyme which may help the adrenergic organism to use endogenous catecholamines with maximum efficiency. Our study also suggests different behavioral approaches in respect to MAO substrates like drugs and food bioactive amine containing [1].

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