CEREBRAL GABA-RECEPTOR MACROMOLECULAR COMBINATIONS IN RAT UNDER THE CONDITIONS OF HYPOKINESIA

Vilen Hakobyan1, Liza Balyan1, Naira Avetisyan2, Kristina Ashrafyan1

1 Department of Pharmacology, Yerevan State Medical University, Yerevan, Armenia
2 Institute of Biochemistry of after H. Buniatyan, NAS RA, Yerevan, Armenia

SUMMARY

Inactive style of life leads to many pathologic changes in an organism as well as cerebral blood flow disturbances. The depth of pathologic shifts depends on terms of hypokinesia (HK) and these shifts involve both cellular and subcellular levels. As GABA-ergic system relates to stress-limiting system, the objective of our investigation is to study the changes in GABA-ergic macromolecular combination under the conditions of HK in experimental animals with simultaneous exploration of pathologic anxiety development and its correction by well-known nootrops – Pyracetam. It was established, that in 15-day HK the amount of GABA-receptor macromolecular combination decrease is observed, and it continues to decrease up to 45-days of HK. Study of animal behavioral reaction in plus-maze test has shown that in animals under the conditions of HK anxiety develops. Pyracetam administration during the last three days results to GABA-receptor macromolecular combination amount increase and anxiety elimination both in 15-day and 45-day HK.

KEY WORDS: anxiety, brain, GABA-receptor, hypokinesia, macromolecular combination

INTRODUCTION

The discovery of principal GABA system key in cerebral hemodynamics regulation lets us to consider not only cerebral discirculation problem caused by inactive style of life or monotone muscle activity (Hypokinesia – HK) [7], but also to find out the level of this system involvement in mechanisms of cerebral blood flow disorder compensation especially if to take into consideration early aging and psychoneurological disorders after HK [5, 6, 7, 10, 11, 15]. On the other hand, identification of different types of GABA-receptors according to their pharmacological and biochemical character are linked not only to these receptors agonists and antagonist creation, but also with study of intimate mechanisms of used medicines [1, 2, 8, 9, 16]. It is known, that GABA receptors are complicated oligomer macromolecules with specific link-sites not only for GABA, its agonists and antagonist, but also for other compound junction. The realization of their activity follows the GABA-receptor function modulation [3, 4, 10, 12]. Quantitative and qualitative changes of their macromolecular combination parameters can be the indices of GABA system involvement in some processes.

The aim of investigations is to study the influence of both early (15 days) and late (45 days) terms of HK on the changes in state of receptor macromolecular combinations which bind GABA in experimental animal brain and on the anxiety development and its prevention by well-known nootrop-medicine–Pyracetam.

MATERIALS AND METHODS

Rats

White male inbred rats weighing approximately 200-220g were used in experiments. They housed under standard conditions of 22°C, water and food available ad libitum. As a model of hypokinesia we housed rats in individual narrow cages which were constructed from Plexiglas. Anxiety related behavior was measured by the elevated plus-maze test. The GABA receptor macromolecular combinations were separated from rat brain after 15- and 45-days of HK. All experimental rats were divided into the following groups: 1 – control; 2 – rats received injection of pyracetam (20 mg/kg, i/p); 3 – hypokinetic rats with appropriate terms of HK; 4 – hypokinetic rats that were treated with pyracetam for the last three days of 15- and 45-days of HK; 5 – untreated hypokinetic rats with following passive readaptation.

Elevated plus maze test

The anxiety condition was investigated according to well-known method elevated plus-maze test [14] EPM. The plus-maze was made of wood, with two opposite open arms, 50x10 cm without any walls and two of the same size with 40-cm-high side walls and an end wall. The arms were connected by a central platform 10x10 cm. Both open arms were divided into three parts of equal size by lines which also separated the central platform from all arms. The central platform and open arms formed the ‘open part’ of the apparatus. The maze was elevated to a height of 50 cm from the floor. An entry into open arms was counted when the rat crossed the line between the central arena and an open arm with all four paws. The rat was considered to explore the open part of the apparatus when it had clearly crossed the line between a closed arm and the central arena with both its forepaws. The rat was placed on the central platform of the maze facing an open arm...
before and after 30 min after piacetaem pyra-
cetam injection. Behavioral measures taken dur-
ing 5 min included: a) the total number of arm
entries; b) the number of open arm entries; c)
time spent in the open arms of the apparatus; d)
time spent in the central square.

One-way analysis of variance (ANOVA)
was used to compare the data from all experi-
ments.

**Affinity chromatography**

An affinity chromatography on sorbent with
immobilized γ-amino-butiric acid (GABA) [17]
was carried out to isolate GABA receptor com-
plex components. The adult rats were decapi-
tated, brain was removed and it was used either
fresh or after freezing and storage at -20°C. Im-
mediately before isolation it was defrozed to
+4°C, all the following procedures were made at
the same temperature. All the buffers contained
protease and transaminase inhibitors (1mM ety-
lenediamine-tetraacetic Acid (EDTA), 0,1mM
phenylmethyl-sulfonil fluoride (PMSF), 0,8 mM 
ε-aminocaproic acid, 1 mM O-sulfoethanolae-
mine). Brains were homogenized in 150 ml 0,
32 M sucrose with glass/Teflon homogenizer.
The homogenate was centrifuged at 8000 rot/
min (K-24 centrifuge, Germany). The pellet was
osmotically shocked by suspension in ice-cold
water (200 ml, simultaneously membranes were
washed out of endogenous GABA), then centri-
fuged for 15 min at 9000 rot/min. this was re-
peated twice. The pellet was resuspended in
buffer A: 50 mM Tris HCL, pH 7,4 and 10%
sodium deoxicholate in water was added drop-
wise to give a final concentration of 1% (w/v).
This was gently stirred for 60 min, then mixture
was centrifuged at 20 000 rot/min for 40 min.
The supernatant was carefully decanted and in-
cubated immediately with CPG-GABA sorbent,
equilibrated with buffer A overnight, butch-
procedure. Then sorbent was washed with
buffer A, GABA receptor complex components
we desorbed with buffer A, containing 50 mM
GABA, 0,1% sodium deoxicholate during 60
minute at butch-procedure (fraction A). Affinity
sorbent controlled pore glasses (CPG)-GABA
was synthesized according to E.V. Grishin's
modified method [17]. The concentration of
protein in preparations was evaluated by spec-
trophotometric analysis according to its absorp-
tion at 273 nm.

Statistic analysis was performed with the es-
timation of data obtained according to the Stud-
ent’s t-parameter.

**Acknowledgements**

We sincerely thank E.V. Grishin and G.I.
Kovalev for helpful advices. We thank N.R.
Nazaretyan for a critical reading. This work was
supported by the Yerevan State Medical Uni-
versity.

**RESULTS AND DISCUSSION**

As spectrophotometric analysis showed, ab-
sorbance maximum of fraction A at 273 nm
from the intact rats was 1,275 opt units. Injec-
tion of pyracetam (during 3 days, 20 mg/kg)
into the intact animals resulted in slight increase
in absorption maximum to 1,3 opt units proba-
bly because of activation of protein synthesis.
Isolation of GABA receptor components from
the brains of animals after 15-day hypokinesia
showed that here the absorption maximum of
fraction is decreased to 0,2 opt units (in com-
parison with control more than for 83%), which
suggest that amount of GABA receptor compo-
nents decreases in hypokinesia conditions (Fig.
1). The content of these components is less in
animals after 45-day hypokinesia. Here we ob-
served only trace amounts of GABA-receptor
proteins.

Pyracetam injection to the animals in the last
days of hypokinesia resulted in significant
shifts in quantity of the investigated macromo-
lecular combination. In animals after 15-day hy-
pokinesia the amount of GABA-receptor com-
binations increases by 42%. Similar regularity
we observed in pyracetam treated rats after 45-
day hypokinesia, but it was less expressed.
During the experiments the development of anxiety caused by HK has been investigated in the elevated plus maze in rats (Fig. 2). The results showed that the percentage of entries and time spent on open arms as well as in the central square reliably decreased after 15-day HK. The number of entries into enclosed arms increased as compared to control groups of rats which may be interpreted as an anxiogenic effect of restricted movement activity. 45-day hypokinesia results in a significant decrease in the number of entry into the open arms and lowering of investigation activity as compared to the control group and prolongation of time spent in enclosed arms.

Administration of pyracetam results in investigation activity increase, the evidence of which is prolongation of time spent in the central square, whereas an increase in the number of entries into the open arms demonstrates pyracetam anxiolytic activity increase which agrees with data of other authors [16].

Thus data obtained have shown the reduction of GABA receptor component synthesis under the conditions of hypokinesia, which may be caused by anxiety development. The tendencies to restoration of the researched data are observed after following pyracetam injection. It shows that pyracetam injection to hypokinet c rats leads to activation of restoration processes especially in early terms of HK. Probably, anxiety elimination (well-known anxiolytic effect of pyracetam) under the pyracetam influence in rat with HK [1] may be also explained by changes in macromolecular GABA-receptor combination amount.

The action mechanism of such classic nootropic – pyracetam is doubtful elucidated and now many hypotheses are suggested.

On the one hand, concerning the data of Gudasheva [4] based on structural similarity between pyracetam and dipeptide, particularly, dipeptide cyclo-prolylglycine, pyracetam is presented as exogenous ligand of endogenous cyclopropylglycine, which activate cognitive functions of brain and as the authors suppose its action, is intermediated by hypothetic nootrop receptors.

On the other hand the structural basis of pyracetam is cyclic form of GABA (2-pirroldone), which is not a source of metabolically
active GABA [5, 12, 13]. However, metabolic effects of pyracetam are expressed enough and intermediated by both influence upon oxidative-recovering processes and energetic exchange and regenerative-reparation processes especially under the conditions of disturbances of cerebral blood flow. The most important, fast developing energetic effects of pyracetam connected with ATP rotation increase and creatinphosphate synthesis activation; activation of anaerobic glucose metabolism without lactate formation, acceleration of DNA and RNA synthesis, protein, phospholipid synthesis. The literature data concerning pyracetam influence upon the GABA system show the following: pyracetam brakes the GABA production and utilization increase due to GABA mimetic postsynaptic action with compensatory function of GABA shunt increase which causes the alternative mechanism of α-ketoglutarate transformation to succinate [5, 11, 12, 13].

The role of GABA system as the protector mechanism of action with compensatory function of GABA brakes the GABA production and utilization GABA system show the following: pyracetam concerning pyracetam influence upon the action with compensatory function of GABA brakes the GABA production and utilization. The literature data concerning pyracetam influence upon the GABA system show the following: pyracetam brakes the GABA production and utilization increase due to GABA mimetic postsynaptic action with compensatory function of GABA shunt increase which causes the alternative mechanism of α-ketoglutarate transformation to succinate [5, 11, 12, 13].

Our early obtained data show that a lot of pathologic processes particularly cerebral blood flow disorders [1, 2, 6, 7] occur under the conditions of HK.

The process of aminoacid involvement into the brain tissue, phospholipids quantitative and qualitative content, neuroactive aminoacid content in brain tissue, energetic exchange regenerative-reparative processes, especially in the late term of HK [6, 7] are disturbed. GABA mimetics administration, particularly pyracetam, in cerebral hemodynamic disturbances caused by HK leads to expressed positive morphofunctional changes in neurocytes and microcirculatory bed of brain cortex [1, 2, 6, 7, 8, 9] in experimental animal.

Concerning interaction between pyracetam and receptors there is an option that nootrop compounds, particularly pyracetam, don’t influence upon the connection of radioligand, which characterize receptors to specific neuromediators of CNS since affinity between DA, 5-HT, NA, Ach, opiates and benzodiazepines and receptors is absent whereas for glutamate receptors pyracetam increase quantity of glutamate connection sites in uncompetitive manner. It is interesting, that among nootrop-acetams nefiracetam has the expressed affinity to GABA-A receptors [11]. It is important to note, that pyracetam possesses membrane-stabilizing effect, improves the fluidic property of neuronal membrane, probably by receptor affinity increase to GABA. It modulates both activity of neurotransmitter processes and plastic processes of brain [5, 11].

Cerebral hemodynamics disturbances caused by HK leads to chronic ischemization of brain tissue, changes in endogenous GABA contents [5]. Besides, a significant increase of GABA content in brain tissue doesn’t always have protective character. In this aspect the role of GABA as stress limiting system modulator, which can be activated by pyracetam has a specific meaning.

All the above mentioned shows that pyracetam influence upon the macromolecular GABA-receptor complexes under the condition of HK may be explained either by activation of reparative processes, which are induced more easily in early HK, than in last HK, or affinity GABA to GABA-A receptors increase.

Since the response of brain tissue and hemodynamics under the condition of HK straightly depend on the terms of HK, we must take into consideration the fact that for preservation of viability of an individual transition from active resistant strategy to passive tolerance is possible and in this process of viability the pyracetam administration can help.

Thus, we can do the following conclusion

1. The synthesis of GABA-receptor complex compound decreases under the condition of HK, especially after prolonged HK.
2. Administration of pyracetam for the last 3 days of HK promotes more rapid recovery of the GABA receptor compound amount.
3. The anxiety elimination in hypokinetical rats after pyracetam injection is accompanied by the increase in GABA receptor macromolecular amount in rat brain tissue.

REFERENCES

ГАМК-РЕCEPTОРНІ МАКРОМОЛЕКУЛЯРНІ КОМПЛЕКСИ
ГОЛОВНОГО МОЗКУ ПАЦІЮКІВ В УМОВАХ ГІПОКІНЕЗІЇ

В.П. Акопян1, Л.С. Балаян1, Н.Г. Аветисян2, К.Б. Ашираян1
1 Кафедра фармакології ЕрГМУ ім. М. Гераці, Ереван, Вірменія
2 Інститут біохімії ім. Г. Буніятяна НАН РА, Ереван, Вірменія

РЕЗЮМЕ
Малоактивний спосіб життя є причиною виникнення багатьох патологічних змін в організмі, у тому числі і порушення мозкового кровотоку. Глибина цих порушень, що розвиваються як на клітинному, так і на субклітинному рівнях, знаходиться в прямій залежності від тривалості і гостроти гипокінезії (ГК). Оскільки до стрес-лімітуючих систем відносяться ГАМК-ергічна, метою нашого дослідження стало вивчення змін у ГАМК-ергічних макромолекулярних комплексах головного мозку експериментальних тварин в умовах ГК із рівномірним дослідженням виникнення тривожності внаслідок ГК. Окрім того, у нашій запланий входила і фармакологічна корекція зрушень, що спостерігаються, добре відомим представником класу ноотропів – Пірацетамом. Було встановлено, що на 15-й день ГК спостерігається зменшення кількості макромолекулярних комплексів ГАМК-рецепторів головного мозку, що продовжується до 45-ї доби ГК, коли вона виявляється вже у відсутність дослідників. Дослідження повідомлює, що реакція тварин у тесті піднятого хрестоподібного лабіринту показало, що ГК приводить до збільшення кількості макромолекулярних комплексів ГАМК-рецепторів і одночасного зменшення тривожності на 15-день ГК.

КЛЮЧОВІ СЛОВА: тривожність, мозок, ГАМК-рецептор, гипокінезія, макромолекулярні рецепторні комплекси

ГАМК-РЕCEPTОРНІ МАКРОМОЛЕКУЛЯРНІ КОМПЛЕКСИ
ГОЛОВНОГО МОЗКА КРІСІ В УМОВАХ ГІПОКІНЕЗІЇ

В.П. Акопян1, Л.С. Балаян1, Н.Г. Аветисян2, К.Б. Ашираян1
1 Кафедра фармакології ЕрГМУ ім. М. Гераці, Ереван, Вірменія
2 Інститут біохімії ім. Г. Буніятяна НАН РА, Ереван, Вірменія

РЕЗЮМЕ
Малоактивний образ жизни является причиной возникновения многих патологических изменений в организме, в том числе и нарушения мозгового кровотока. Глубина этих нарушений, которые развиваются как на клеточном, так и на субклеточном уровнях, находится в прямой зависимости от длительности и жесткости гипокинезии (ГК). Поскольку к стресс-лимитирующим системам относится и ГАМК-ергическая, целью нашего исследования является изучение изменений в ГАМК-ергических макромолекулярных комплексах головного мозга экспериментальных животных в условиях ГК с параллельным исследованием возникновения тревожности вследствие ГК. Кроме того, в нашу задачу входила и фармакологическая коррекция наблюдаемых сдвигов хорошо известным представителем класса ноотропов – Пирacetamen. Было установлено, что на 15-й день ГК наблюдается уменьшение количества макромолекулярных комплексов ГАМК-рецепторов головного мозга, продолжающееся до 45-х суток ГК, когда они выявляются уже в виде следов. Исследование поведенческой реакции животных в тесте приподнятого крестообразного лабиринта показало, что ГК приводит к развитию тревожности. Введение Пирacetama в последние три дня ГК приводит к увеличению количества макромолекулярных комплексов ГАМК-рецепторов и одновременному уменьшению тревожности на 15-день ГК.

КЛЮЧЕВЫЕ СЛОВА: тревожность, мозг, ГАМК-рецептор, гипокинезия, макромолекулярные рецепторные комплексы