УДК: 591.175+612.014.482

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

Vilen Hakobyan[1], Liza Balyan[1], Naira Avetisyan[2] , Kristina Ashrafyan[1]
[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^{o}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 piarcetam pyracetam injection. Behavioral measures taken during 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 experiments.

Affinity chromatography

An affinity chromatography on sorbent with immobilized γ-amino-butiric acid (GABA) [17] was carried out to isolate GABA receptor complex components. The adult rats were decapitated, brain was removed and it was used either fresh or after freezing and storage at -20°C. Immediately 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 etylenediamine-tetraacetic Acid (EDTA), 0,1mM phenylmethyl-sulfonil fluoride (PMSF), 0,8 mM ε-aminocaproic acid, 1 mM O-sulfoethanoleamine). 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 centrifuged for 15 min at 9000 rot/min. this was repeated twice. The pellet was resuspended in buffer A: 50 mM Tris HCL, pH 7,4 and 10% sodium deoxicholate in water was added dropwise 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 incubated 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 spectrophotometric analysis according to its absorption at 273 nm.

Statistic analysis was performed with the estimation of data obtained according to the Student'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 University.

RESULTS AND DISCUSSION

As spectrophotometric analysis showed, absorbance maximum of fraction A at 273 nm from the intact rats was 1,275 opt units. Injection of pyracetam (during 3 days, 20 mg/kg) into the intact animals resulted in slight increase in absorption maximum to 1,3 opt units probably 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 comparison with control more than for 83%), which suggest that amount of GABA receptor components decreases in hypokinesia conditions (Fig. 1). The content of these components is less in animals after 45-day hypokinesia. Here we observed only trace amounts of GABA-receptor proteins.

Pyracetam injection to the animals in the last three days of hypokinesia resulted in significant shifts in quantity of the investigated macromolecular combination. In animals after 15day hypokinesia the amount of GABA-receptor combinations increases by 42%. Similar regularity we observed in pyracetam treated rats after 45-day hypokinesia, but it was less expressed.

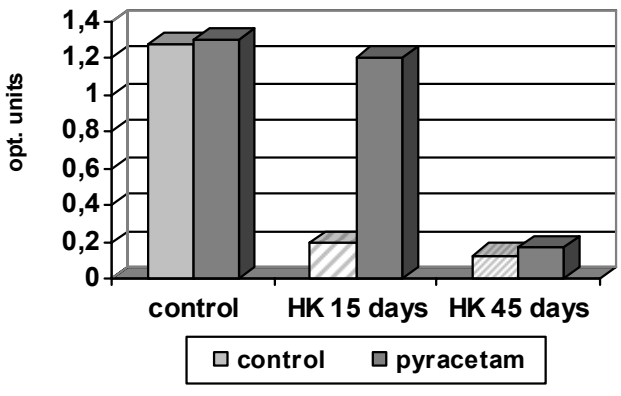
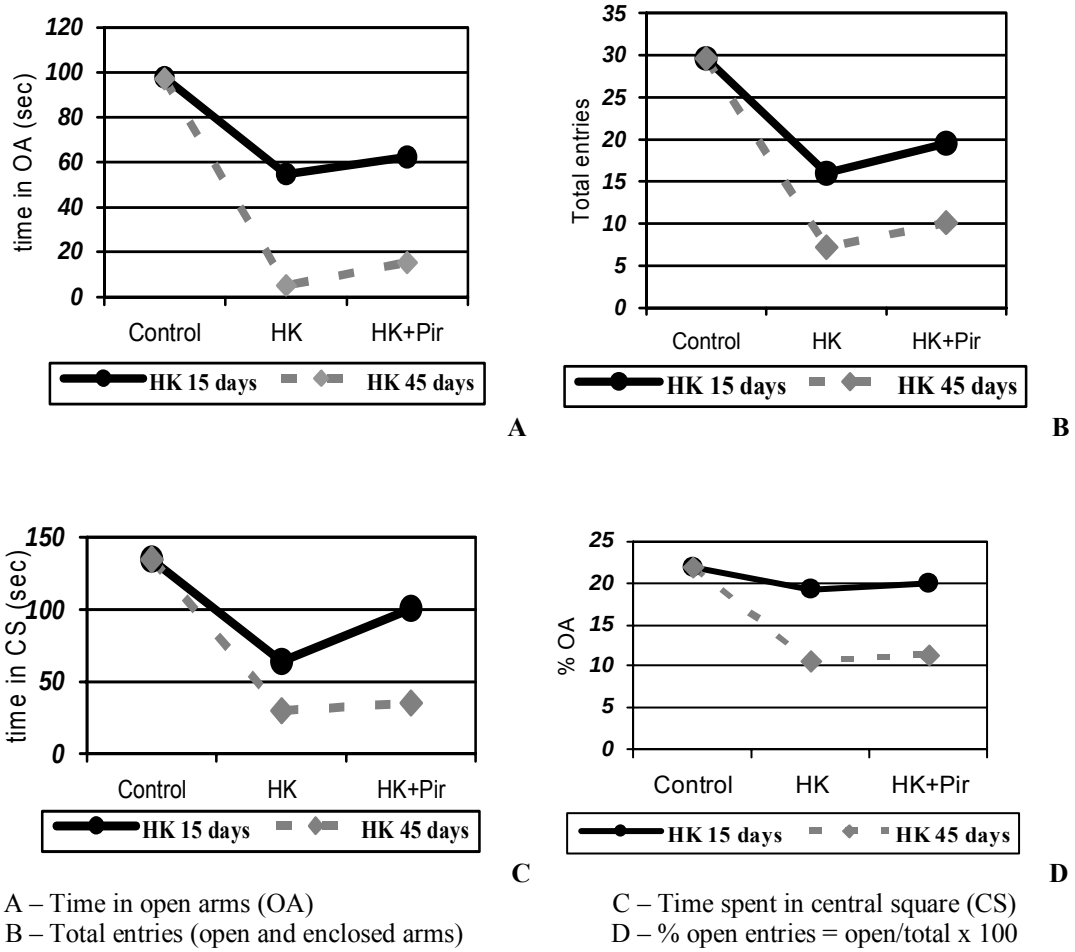


Fig. 1. The changes in macromolecular GABA-receptor combinations in rat brain under the conditions of hypokinesia and after the pyracetam administration (20 mg/kg, i/p)

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.



A – Time in open arms (OA)
B – Total entries (open and enclosed arms)
C – Time spent in central square (CS)
D – % open entries = open/total x 100

Fig. 2. The changes in rat anxiety in the elevated plus maze in different terms of HK

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 hypokinetic 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 nootrop – 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-pirrolidone), 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 and adaptive compound of cerebral hemodynamic under the conditions of HK is particularly great [5, 7, 8, 9, 13].

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

The process of aminoacide involvement into the brain tissue, phospholipids quantitative and qualitative content, neuroactive aminoacide 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 hypokinetic rats after pyracetam injection is accompanied by the increase in GABA receptor macromolecular amount in rat brain tissue.

REFERENCES

1. Балян Л.С., Аветисян Н.Г. // "Фундаментальные проблемы фармакологии". Сб. тезисов 2-го съезда РНОФ, часть 1. - Москва. - 2003. - С. 58.
2. Балян Л.С., Канаян А.С. // Журнал "Медицинская наука Армении". - Ереван. - 2002. - Т. XLII. - №1. - С. 14-18.
3. Green AR, Hainsworth AH, Jackson DM. // Neuropharmacology. - 2000. - Vol. 39. - P.1483-1494.
4. Гудашева Т.А., Сколдинов А.П. // Журнал "Эксперим. и клин. фармакология". - Москва. - 2003.- Т. 66. - № 2. - С. 15-19.
5. Гусев Е.И., Скворцова В.И. Ишемия головного мозга. - М.: "Медицина". - 2001.
6. Акопян В.П. // Журнал "Эксперим. и клин. фармакология". - Москва. - 2003. - Т. 66. - № 3. - С. 4-8.
7. Hakobyan VP. // Athens Nat. & Kapodistrian University, Athens, Greece. - 2002.
8. Акопян В.П., Балян Л.С., Мелконян К.В. // Итоги науки и техники. Серия: Фармакология. Химиотерапевтические средства. - Москва. - 1991. - Т. 26. - С. 46-62.
9. Hakobyan V.P., Balyan L.S., Kanayan A.S., et. al.//XIII Int. Congr. of Pharmac.- 1998. - Vol. 1. - P. 339.

10. Kram M.L., Kramer L.G., Steciuk M., et. al. // Neuroscience Research. - 2000. - Vol. 38. - P. 193-198.
11. Ковалев Г.И., Воробьев В.В., Ахметова Е.Р., и др. // Журнал "Эксперим. и клин. фармакология". - Москва. - 2000. - Т. 63. - № 1. - С. 3-6.
12. Машковский М.Д. Лекарственные средства. -М.: "Новая волна". - 2003. - Т. 1. - С. 111.
13. Мирзоян Р.С.//Журнал "Эксперим. и клин.фармакология".- Москва. - 2003. - Т. 66. - № 2.- С. 53-57.
14. Pellow S, Chopin P, File S.E., et. al. // J. of Neuroscience Methods. -1985. - V. 14. - № 3. - P.149-167.
15. Сергеев П.В., Шимановский Н.Л., Петров В.И. Рецепторы физиологической активности веществ.- Москва–Волгоград: "Семь ветров". - 1999.
16. Степанян З.В. // Сб. научн. трудов, посвящ. 70-летию ЕрГМУ. - Ереван. - 2000. - С. 525-527.
17. Volkova T.M., Avetisayn N.A., Galkina T.G., et. al. // J. of Protein Chemistry. - 1989. - Vol. 8. - P. 322-324.

ГАМК-РЕЦЕПТОРНІ МАКРОМОЛЕКУЛЯРНІ КОМПЛЕКСИ ГОЛОВНОГО МОЗКУ ПАЦЮКІВ В УМОВАХ ГІПОКІНЕЗІЇ

В.П. Акопян[1], Л.С.Балян[1], Н.Г. Аветісян[2], К.Б. Ашрафян[1]
[1] Кафедра фармакології ЄрДМУ ім. М. Гераці, Єреван, Вірменія
[2] Інститут біохімії ім. Г. Буніятяна НАН РА, Єреван, 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 субклітинному рівнях, знаходиться в прямій залежності від тривалості і твердості гіпокінезії (ГК). Оскільки до стрес-лімітуючих систем відноситься і ГАМК-ергічна, метою нашого дослідження стало вивчення змін у ГАМК-ергічних макромолекулярних комплексах головного мозку експериментальних тварин в умовах ГК із рівнобіжним дослідженням виникнення тривожності внаслідок ГК. Окрім того, у наше завдання 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-х доби ГК, коли вона виявляються вже у виді слідів. Дослідження поведінкової реакції тварин у тесті піднятого хрестоподібного лабіринту показало, що ГК приводить до розвитку тривожності. Введення пірацетаму в останні три дні ГК призводить до збільшення кількості макромолекулярних комплексів ГАМК-рецепторів і одночасного зменшення тривожності на 15-день ГК.

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

ГАМК-РЕЦЕПТОРНЫЕ МАКРОМОЛЕКУЛЯРНЫЕ КОМПЛЕКСЫ ГОЛОВНОГО МОЗГА КРЫС В УСЛОВИЯХ ГИПОКИНЕЗИИ

В.П. Акопян[1], Л.С.Балян[1], Н.Г. Аветисян[2], К.Б. Ашрафян[1]
[1] Кафедра фармакологии ЕрГМУ им. М. Гераци , Ереван, Армения
[2] Институт биохимии им. Г. Буниятяна НАН РА, Ереван, Армения

РЕЗЮМЕ

Малоактивный 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 субклеточном уровнях, находится в прямой зависимости от длительности и жесткости гипокинезии (ГК). Поскольку к стресс-лимитирующим системам относится и ГАМК-эргическая, целью нашего исследования является изучение изменений в ГАМК-эргических 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 – Пирацетамом. Было установлено, что на 15-й день ГК наблюдается уменьшение количества макромолекулярных комплексов ГАМК-рецепторов головного мозга, продолжающееся до 45-х суток ГК, когда она выявляются уже в виде следов. Исследование поведенческой реакции животных в тесте приподнятого крестообразного лабиринта показало, что ГК приводит к развитию тревожности. Введение Пирацетама 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