THE CHANGES OF SEROTONIN-MODULATING ANTICONSOLIDATION PROTEIN AND DIHYDROPYRIMIDINASE-RELATED PROTEIN 2 IN THE AMYGDALA AND BLOOD OF DEPRESSIVE RATS

L.F. Hasanova

Academician Abdulla Garayev Institute of Physiology, 78 Sharifzadeh Street, AZ1100, Baku, Azerbaijan
E-mail: arifmekht@yahoo.com

Nowadays, depression is referred to psychiatric disease and is widely spread throughout the world. The pathogenesis of depression in humans is characterized by significant disturbances of serotonin turnover and its downregulation in brain structures. The objective of the present study was the definition of the levels of serotonin-modulating anticonsolidation protein (SMAP) and dihydropyrimidinase-related protein 2 (DRP2) in the brain amygdala, platelets, and to anti-SMAP natural autoantibodies in the depression Wistar rats of both sexes. A depression state in the rats was formed in the dominant model and its successful formation was confirmed in the forced swimming test by the decreased timeframe of active swimming. Through the application of an indirect ELISA test in the depressive male and female rats, a downregulation of SMAP and DRP2 in the amygdala, an upregulation of SMAP in the platelets of depressive male rats, a noticeable downregulation of DRP2 in the platelets of depressive female rats, and a sharp downregulation of anti-SMAP natural autoantibodies in the serum of depressive male rats were observed.

Keywords: depression, male and female rats, indirect ELISA test, amygdala, platelets, SMAP, DRP2, anti-SMAP natural autoantibodies.

INTRODUCTION

Depression pathology in humans is characterized by significant disturbances of serotonin turnover in brain structures. Especially such prominent changes are revealed in depressive patients who end their lives with suicide [3]. Along with it, different researchers demonstrated that serotonin turnover in the platelets of depressive patients, including serotonin level, types of serotonin-synthesizing enzymes, and correspondent serotonergic receptors, have a high level of similarity with its turnover in their brain cortex [2, 3]. Besides, natural autoantibodies (NAA) are elaborated to each protein and peptide synthesized in the organisms of animals and humans. Hence, measuring the levels of NAA to the protein under study gives grounds for the evaluation of its level in the organism’s tissues [1, 7].

The objective of the present study was the evaluation of the levels of serotonin-modulating protein (SMAP) [7] and of dihydropyrimidinase-related protein 2 (DRP2) [4, 5] in the amygdala and platelets and of the anti-DRP2 NAA in the serum of the rats demonstrating depressive-like behaviors.

MATERIALS AND METHODS

The study was carried out on Wistar male rats with a body mass of 180–220 g. All rats were grouped into pairs, and through the application of the dominant model [6] in each pair of rats, dominant and submissive (depressive) rats were defined.
The pairs of rats were food deprived for 48 h before being put into the dominant model box. In a box, two compartments were connected by a narrow passage, in the center of which a small round container with sweet milk was placed. The measure of this passage was so narrow that only one rat was able to reach it and drink from the milk container.

After revealing the depressive rats, the time of their active swimming during 5 min after their placing in the round container (diameter 60, height 50 cm) filled to 2/3 of its height with warm water (27°C) was measured. Then the depressive rats were sacrificed, the amygdala was removed from the brain, and blood samples were taken into sample tubes containing 500 µL of 5% EDTA as an anticoagulant. Blood samples were centrifuged at 700 g for 6 min; plasma was saved, transferred into Eppendorf sample tubes, and centrifuged at 9000 g for 15 min. Pellets were saved as platelets, whereas the supernatant was saved as serum.

Water-soluble proteins were extracted from the amygdala and platelets; they were used as antigens in the indirect ELISA test at a concentration of 20 µg/mL in 0.1 M Tris-HCl buffer (pH 8.6). Anti-DRP2 polyclonal rabbit immunoglobulins, diluted 40 times in the buffer for antibodies (pH 7.3), were used as the first antibodies, while conjugates of anti-rabbit goat immunoglobulins with horseradish peroxidase, diluted 20000 times in the buffer for antibodies (pH 7.3), were used as the second antibodies in the ELISA-test. Visualization of the reaction was realized with the application of a substrate of horseradish peroxidase: 0.05% orthophenylenediamine in 0.05 M citrate-phosphate buffer (pH 4.5). The reaction was stopped 30 min after the addition of substrate by adding a 3 M NaOH solution. The results of the reaction were recorded in the photometer for the ELISA-test "Spectra Max 250" (Molecular Devices Co., USA) at the wavelength of 492 nm.

While measuring the level of anti-DRP2 NAA, purified DRP2 was used as an antigen at a concentration of 20 µg/mL. Serum diluted 100 times with the buffer for antibodies (pH 7.3) was used as the first antibodies. The following steps were realized in the determination of DRP2 levels in the amygdala and platelets.

SMAP was purified from cow brains. The brains were homogenized in the extracting buffer containing 0.05 M phosphate buffer (pH 7.2), 0.3 M NaCl, 5 mM EDTA, and 0.1% Triton X-100 in a volume ratio of tissue and buffer of 1:4. The main stages of fractionation were as follows: 1) protein partial precipitation by ammonium sulfate under the final concentration of 40%; 2) gel chromatography on the column (3 X 60 cm) of Sephadex G-150. The process of fractionation and selection of the immune-positive protein fractions was realized under screening control by the indirect ELISA test with the application of anti-SMAP polyclonal immunoglobulins [7].

The purification of DRP2 in the first two stages was similar to SMAP purification. After elution from the Sephadex G-150 column, the protein fraction (SMAP) was exposed to the effect of 40 mM of deionized EDTA throughout the night on the end-to-end shaker. The next morning, a mixture of protein with EDTA was subjected to isoelectric focusing on the gel with the application of ampholines of a narrow pH range (pH 4-6). After ending isoelectric focusing, 1 cm-wide gel strips were collected, pH values were measured in each gel strip, and the fraction with a pH value that was equal to the pI value of DRP2, was eluted from the gel and analyzed in SDS electrophoresis with protein standards.

Anti-DRP2 polyclonal immunoglobulins were produced through 5- to 6-month immunization of the male Chincilla rabbits by subcutaneous administration of 300 µg of the purified protein per animal, always in a mixture with complete Freund adjuvant (Sigma, Germany). The first three injections were done within a timeframe of 14 days; the following injections were done once per month. Ten days after the third and following injections, blood samples were taken from the ear vein, serum was separated, and polyclonal immunoglobulins G were precipitated by adding 100% ammonium sulfate.

The data were averaged within each group and analyzed by Student’s t-criterion.
RESULTS AND DISCUSSION

After revealing the depressive animals in each pair of rats in the dominant model, we observed the following regularities while measuring the time of their active swimming. The depressive rats swam actively for 211±16.6 sec, whereas control animals swam actively for 300 sec (p<0.001; Fig. 1).

![Figure 1. Duration of active swimming in the depressive male rats. *** - p<0.001.](image)

Measuring the levels of SMAP in the amygdala and platelets of the depressive male rats revealed its significant downregulation in the amygdala and upregulation in the platelets. In particular, the level of SMAP in the amygdala of the depressive male rats was equal to 0.253±0.001 optic units of extinction (OUE), while in the control rats it reached 0.263±0.002 OUE (p<0.001; Fig. 2). The level of SMAP in the platelets of the depressive animals was 0.247±0.004 OUE, while in the controls it was 0.237±0.003 OUE (p<0.05; Fig. 3). The level of anti-SMAP NAA in the serum of the depressive rats decreased sharply and was equal to 0.0098±0.0002 OUE, whereas in the control rats its level was 0.0127±0.0005 OUE (p<0.001; Fig. 4). These data indicate that levels of anti-SMAP autoantibodies in the blood serum may serve as an informative and valid index of depression state.

![Figure 2. Changes of SMAP level in the amygdala of the depressive male rats. *** - p<0.001.](image)

The measured mean duration of active swimming in the depressive female rats relative to the control ones (153.2±14.2 sec) was sharply low and equal to 54±7.7 sec (p<0.001; Fig. 5). At the same time, evaluation of the level of DRP2 in the amygdala and platelets of the depressive female rats revealed the following regularities. The DRP2 level in the amygdala of the depressive female rats reached 0.103±0.003 OUE (p<0.001; Fig. 6). In contrast to the depressive male rats, the DRP2 level in the platelets of the depressive female rats was sharply downregulated relatively to the intact ones: 0.171±0.0023 vs. 0.192±0.0035 OUE, p<0.001; Fig. 7).
CONCLUSION

The obtained results indicate significant changes in SMAP and DRP2 levels in the brain amygdala, platelets, and anti-SMAP natural autoantibodies in the blood. The levels of these proteins bear similar character in male and female rats; they downregulate relatively compared to the controls. Along with it, the levels of these proteins in the platelets have quite different character: if the SMAP level slightly increases in the depressive male rats, its level gets significantly lower than in the controls in the depressive female rats. Character of the changes in SMAP level in the amygdala and of the anti-SMAP NAA of the depressive rats have similar tendencies, giving grounds for drawing a conclusion concerning the level of SMAP in the amygdala based on the level of anti-SMAP NAA in the serum.

REFERENCES


ИЗМЕНЕНИЕ УРОВНЕЙ СЕРОТОНИН-МОДУЛИРУЕМОГО АНТИКОНСОЛИДАЦИОННОГО БЕЛКА И ДИГИДРОПИРИМИДИНАЗА-ПОДОБНОГО БЕЛКА 2 В АМИГДАЛЕ И КРОВИ У ДЕПРЕССИВНЫХ КРЫС

Ламия Фазиль гызы Гасанова

Институт физиологии им. академика Абдуллы Гараева, Баку, Азербайджан

В настоящее время депрессия относится к психическим заболеваниям и широко распространена во всём мире. Патогенез депрессии у человека характеризуется значительным нарушением обмена серотонина и снижением его уровня в структурах головного мозга. Целью настоящего исследования было определение уровней серотонин-модулируемого антиконсолидационного белка (СМАБ) и дигидропириmidiназа-подобного белка 2 (ДПБ2) в амигдале головного мозга, тромбоцитах и уровней естественных аутоантител к СМАБ у депрессивных крыс линии Вистар обоего пола. Состояние депрессии у крыс вырабатывали в домinantной модели и его формирование подтверждалось в teste насильственного плавания по уменьшению продолжительности активного плавания. С помощью непрямого иммуноферментного анализа у депрессивных самцов и самок крыс было выявлено снижение уровней СМАБ и ДПБ2 в амигдале, тромбоцитах и уровнях естественных аутоантител к СМАБ у депрессивных крыс. С помощью непрямого иммуноферментного анализа у депрессивных самцов и самок крыс было выявлено снижение уровней СМАБ и ДПБ2 в амигдале, тромбоцитах и уровнях естественных аутоантител к СМАБ у депрессивных крыс.

Ключевые слова: депрессия, крысы-самцы и самки, непрямой иммуноферментный анализ, амигдала, тромбоциты, СМАБ, ДПБ2, естественные аутоантитела к СМАБ.
DEPRESSİV SIÇOVULLARIN AMİQDALASINDA VƏ QANINDA SEROTONİN-MODULLU ANTIKONSOLİDASIYA ZÜLALININ VƏ DIHİDROPİRİMİDİNAZAYA BƏNZƏR ZÜLAL 2-NİN DƏYİŞİKLİKLƏRİ

Lamiyə Fazil qızı Həsənova

Akademik Abdulla Qarayev adına Fiziologiya İnstitutu, Baki, Azərbaycan

Hazırda depressiya psixiatriya xastəliklərinə aid edilir və dünya üzrə geniş şəkildə yayılıb. İnsanlarda depressiya patogenezi beyn nahiylərinədə serotonin mübadiləsinin pozulması və onun tənəzzülü ilə səciyənir. Bu təqdiqatın məqsədi har iki cinsinin Vistar xəttlə siçovulların beynin amiqdalasında, trombositlərdən serotonin-modullu antikonsolidasiya zülalinin (SMAZ) və dihidropirimidinazaya bənzər zülal 2-nin (DBZ2) səviyyəsindən və SMAZ-a qarşı təbii autoanticisimlərin səviyyəsinin müəyyən edilərdən ibarət idi. Siçovullarda depressiya vəziyyətə dominant modelində yaradılmışdır və onun uğurlu yaradılması məbərdən müəyyən vaxtın azalması ilə təşdiqənilib. Dolaylı immuno-enzim analizinin tətbiqsi ilə depressiv erkek və dişi siçovulların amiqdalasında SMAZ-n və DBZ2-nin səviyyələrinin azalması, depressiv erkek trombositlərdə SMAZ-n səviyyəsinin artırılması və depressiv dişi siçovullarda DBZ2-nin səviyyəsinin azalması və depressiv erkek siçovullarda SMAZ-a qarşı təbii autoanticisimlərin azalması müşahidə edilir.

Açar sözləri: depressiya, erkek və dişi siçovullar, dolaylı immun-enzim analizi üsulu, trombositlər, SMAZ, DBZ2, SMAZ-a qarşı təbii autoanticisimlər.

Çapa təqdim etmişdir: Xuraman Mirhəsən qızı Miryusifova, b.ü.f.d.