THE EFFECTS OF PREGABALIN ON PSYCHO-MOTOR ABILITIES AND COGNITIVE PROCESSES IN MICE

Liliana Mititelu–Tartau¹, Lacramioara Ochiuz², Catalina Elena Lupusoru¹, Andra Sabina Neculai-Valeanu³, Gabriela Rusu¹, Gratiela Popa²
University of Medicine and Pharmacy “Grigore T. Popa” - Iasi
1. Department of Morpho-Functional Sciences
   Faculty of Pharmacy
2. Department of Pharmaceutical Technology
3. University of Agricultural Sciences and Veterinary Medicine ”Ion Ionescu de la Brad”, Iasi
Corresponding author: email: gabi_rusu11@yahoo.com

THE EFFECTS OF PREGABALIN ON PSYCHO-MOTOR ABILITIES AND COGNITIVE PROCESSES IN MICE (Abstract): The purpose of our study was the experimental research on the effects of pregabalin in two behavioural models in mice. **Material and methods:** The experiment was carried out with white Swiss mice treated intraperitoneally as follows: Group I (Control): distilled water 0.1ml/10g body weight; Group II (PGB 10): 10mg/kbw pregabalin; Group III (PGB 20): 20mg/kbw pregabalin. The psychomotor abilities of pregabalin were tested on a LE-8811 Actimeter device (Panlab), in order to investigate both global motor behaviour and number of escape attempts during an eight-minute interval session. The exploration of memory processes performance was assessed using the Y-maze model, based on the natural tendency of mice to explore new environment. Data were analyzed using SPSS 13.0 for Windows software. The experimental protocols were implemented according the guidelines of “Grigore T. Popa” University Committee for Research and Ethical Issues. **Results:** The administration of pregabalin resulted in a dose-dependent reduction of mice horizontal and vertical movements, statistically significant compared to the Control group. The administration of both PGB10 and PGB20 induced no modifications of the spontaneous alternation percent; also, it did not influence the arm entries number compared to Control group in Y-maze test. **Conclusions:** The results reflect a significant dose-dependent diminution of number of escape attempts, exploratory and self-maintenance spontaneous behavior after pregabalin treatment, which could be correlated with an anxiolytic effect. Moreover, the study proved that pregabalin did not modify the animal cognitive processes performance or influence short-term memory of mice in the Y-maze test. **Keywords:** PREGABALIN, MICE, PSYCHO-MOTOR, Y-MAZE.

The (S-[+]3-isobutylgaba) derivative pregabalin is a novel gamma-aminobutyric acid analogue, structurally related to the antiepileptic drug gabapentin, with a high efficacy as an adjunctive treatment for epilepsy and neuropathic pain (1). It is a potent ligand for the alpha-2-delta subunit of P/Q type voltage-gated calcium channels that reduce synaptic release of neurotransmitters in the central nervous
system. Pregabalin-mediated reduction in calcium currents has also been proved to produce a significant inhibition of the release of glutamate and monoamine neurotransmitters involved in the modulation of anxiety pathways (2).

Pregabalin is structural related to the inhibitory neurotransmitter gamma aminobutyric acid (GABA), but lacks affinity on GABA_A, GABA_B, or benzodiazepine receptors. It does not influence the functionality of sodium channels or the activity of cyclooxygenase enzyme subtypes. It is also inactive on the opioid, serotonin and dopamine receptors (3).

This drug, with a high solubility and good permeability is rapidly and nearly completely absorbed after oral administration, without binding to serum proteins. The maximum plasma concentration is reached approximately after 1 hour. After single or multiple dose administration maximum plasma concentration and area under the curve concentration-time curve values are linearly augmented (4). The half-life time ranges from 5,5 to 6,7 hours depending on the administration route. Usually, the major way of excretion for pregabalin is urinary as unchanged drug, with a renal clearance of 73 ml/minute and an elimination half-life of about 6 hours. (5)

Some experimental researches are consistent with a mechanism that may entail reduction of abnormal neuronal excitability through reduced different neurotransmitters (glutamate, norepinephrine, substance P, calcitonin gene-related peptide) release (3, 4).

Pregabalin has been used to treat painful diabetic neuropathy, post-herpetic neuralgia, partial seizures, fibromyalgia, but also neuropathic cancer pain (6, 7, 8). It also has proven to be effective as a sleep modulating agent and an anxiolytic drug (9, 10).

The present study aims to investigate the effects of pregabalin on spontaneous behaviour and on spatial memory performances in mice.

**MATERIAL AND METHODS**

The experiment was carried out on white male Swiss mice (20-25g), distributed into 3 groups of 6 animals each treated intraperitoneally as follows: Group I (Control): distilled water 0,1ml /10 g body weight; Group II (PGB 10): pregabalin 10mg/kbw; Group III (PGB 20): pregabalin 20mg/kbw.

The animals were housed in groups of four in Plexiglas cages maintained on a 12 hours light/dark cycle in a temperature-controlled (22 ± 1°C) colony with access to water and food *ad libitum*, except during the experimental sessions. Before the beginning of the study, animals were allowed to adapt to the environment for one week. All experiments were carried out between 08:00 a.m. and 13:00 p.m.

Pregabalin (Mesochem Technology, China) was diluted in distilled water ex tempore and injected intraperitoneally (i.p.) in a volume of 0,1ml /10 g body weight.

*Spontaneous motor activity exploration.* The psychomotor abilities induced by pregabalin were tested in the LE-8811 Ac-timeter device (Panlab) in order to investigate both global motor behavior and the number of escape attempts, during an eight-minute interval session. This device consists of three transparent cages each with eight infrared lights positioned in a frame around the cage and coupled to silent electronic counters. The apparatus is composed of a two-dimensional (X and Y axes) square frame, a frame support and a control unit.

Using this method spontaneous motor
activity was measured counting the number of times that an animal turns off a photocell beam. The system is completely modular: each frame may be used for evaluation of general activity: locomotors, stereotypic movements, rearing or exploration. Horizontal, vertical and stereotypic movements are registered simultaneously in the same experimental box.

Vertical movements were measured by number and length of bobbing. Horizontal movements indicate travel distance, and were measured based on time and count. In comparison, immobility was measured by the time that rats spent resting (11, 12).

Rats were placed individually in a cage and the spontaneous activity was measured by counting the number of light beam interruptions due to the animal's movement in the experimental area. The device is connected with a SeDaCom computer, which allows easy exportation of data in a format compatible with the Excel program for Windows.

Spatial working memory evaluation. The investigation of memory processes performance was assessed using the Y-maze model, based on the natural tendency of mice to explore novel environment (13, 14, 15).

This apparatus consists of 3 identical arms (40x9x16 cm, placed at 120 degrees from each other) and a central triangular zone. Each arm has walls with specific motifs all over the internal area allowing animals to differentiate it from the others. This experimental model was used to test if the rat could remember the arm it had just explored and would therefore enter one of the other arms of the maze (16, 17). Each animal was placed at the end of one arm and allowed to move unreservedly through the maze during an 8 min session. The short-term memory performance of mice was evaluated by counting the number of arm entries and determining the spontaneous alternation behaviour in the 8-minutes session. Alternation was defined as a consecutive entry in three different arms.

The alternation behaviour (%) was calculated using the following formula:

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\text{Alternation behaviour} = \left( \frac{\text{number of alternations}}{\text{total number of arm visits} - 2} \right) \times 100
\]

Data were analyzed using the ANOVA test implemented in SPSS 13.0 for Windows software. P-values less than 0.05 were considered statistically significant compared to those of the Control group.

The experimental protocols were approved by the University "Grigore T. Popa" Committee for Research and Ethical Issues. All procedures involving animals and their care were conducted in compliance with the international normatives and policies (19).

RESULTS
The results obtained in the Actimeter test were centralized, statistically interpreted and were represented in the following figures (fig. 1, 2).

The administration of pregabalin resulted in a dose-dependent reduction of mice horizontal movements (1313.45±1.21, respectively 1198.75±2.37), statistically significant (p<0.05) compared to the control group (1442.25±2.29) in the Actimeter test (fig. 1).

Intraperitoneal injection of pregabalin was associated with a dose-dependent diminution of vertical movements (372.25± 3.17, respectively 258.65±4.11), statistically significant (p<0.05) compared to the distilled water group (419.25±2.54), (fig. 2).

The treatment with two doses of pregabalin (PGB 10 and PGB 20) did not significantly modify the number of stereo-
typic movements (1511.25±2.32, respectively 1509.15±3.41) compared to the Control group (1514.25±1.53) in this behavioural experimental model in mice (fig. 3).

Fig. 1. Effects of pregabalin (10 mg/kbw and 20 mg/kbw) administration on horizontal movements in the Actimeter test (values expressed as mean ± SEM of horizontal movements for 6 animals; *p<0.05 vs Control).

Fig. 2. Effects of pregabalin (10 mg/kbw and 20 mg/kbw) administration on vertical movements in the Actimeter test (values expressed as mean ± SEM of vertical movements for 6 animals; *p<0.05 vs Control).

Fig. 3. Effects of pregabalin (10 mg/kbw and 20 mg/kbw) administration on stereotypic movements in Actimeter test (values expressed as mean ± SEM of vertical movements for 6 animals).
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The results obtained in the Y-maze test were collected, statistically processed and were represented in the following figures (fig. 4, 5).

Intraperitoneal injection of the both doses (10 mg/kbw or 20 mg/kbw) of pregabalin did not significantly influence the number of arm entries compared to Control group in the same time interval, in the Y-maze assay (fig. 4). These results suggest that pregabalin administration did not modify the mice performances in this behavioural test (20).

The administration of PGB 10 did not induce modification of spontaneous alternation percent, compared to the Control group (fig. 5).

The use of PGB20 tended to increase the spontaneous alternation percent, but statistically non-significant compared to the group treated with distilled water in the Y-maze test (fig. 5). These results signify the lack of effect on the spatial memory performance of pregabalin administration in mice (13).

No significant changes were observed in the latency of the first arm visit, of the same arm return number and alternate arm return number between the groups treated with both doses of pregabalin and distilled water groups in this behavioural model in mice.

Fig. 4. Effects of pregabalin (10 mg/kbw and 20 mg/kbw) administration on arm entries number in Y-maze (values expressed as mean ± SEM of arm entries number for 6 animals).

Fig. 5. Effects of pregabalin (10 mg/kbw and 20 mg/kbw) administration on spontaneous alternation percent in Y-maze (values expressed as mean ± S.E.M of spontaneous alternation percent for 6 animals).
Moreover, the exploration capacity was not modified after the treatment with both doses of pregabalin in the same test in mice.

**DISCUSSION**

Limited literature data has been published regarding the involvement of pregabalin in spontaneous motor activity and cognitive processes of laboratory animals.

An experimental research conducted in the Elevated plus maze test revealed that pregabalin demonstrated evident short term anxiolytic effect, attenuating anxiety-like behaviours in mice exposed to traumatic stress. The authors of the study suggest that pregabalin may be an option for the treatment of anxious state after traumatic stress experience, lacking long-term protective or preventive actions (21).

Other experimental investigation proved that the administration of pregabalin attenuates ketamine-induced hyperlocomotor activity in an animal model of psychosis induced by ketamine, an NMDA receptor blocker that mimics the symptoms of schizophrenia (22).

Regarding the influence on cognitive functions it has been demonstrated that the treatment with pregabalin is associated with an impairment of the memory performance in step-down passive avoidance task in mice (23).

Using the behavioral experimental test, our research study has gathered information regarding three type of animal spontaneous motor activity: horizontal, vertical and stereotypic movements. The spontaneous motor activity is an elementary component of a normal animal behavior.

In our experimental condition the experiment proved that depending on the dose administered, pregabalin decreased mice horizontal movements, effect corresponding to a reduction of the general behavior exploration of the environment (24).

Intraperitoneal administration of pregabalin was associated with a diminution of vertical movements, parameter that reveals the animal trying to climb on the transparent walls of the registration cage, and is an indicative for attempt behavior, indirectly signifying fear and anxiety manifestations (11, 25).

In the Y-maze assay spontaneous alternation refers to the normal tendency of the animal to spontaneously choose alternate arms. Arm exploration was defined as entering at least the first third section of an arm (20).

In this behavioural model we noticed that neither 10 mg/kbw nor 20 mg/kbw pregabalin influenced the exploration capacity of animal placed in the device area.

Moreover, both doses of pregabalin did not modify the spontaneous alternation percent in the same behavioural experimental model in mice.

All these results suggest that pregabalin may be not involved in the mediation of cognitive function and learning capacity of laboratory animals.

**CONCLUSIONS**

The results reflect a significant dose-dependent diminution of the number of escape attempts, exploratory and self-maintenance spontaneous behaviour after pregabalin treatment, which could be correlated with an evident anxiolytic effect.

The study demonstrated that pregabalin did not modify the animal cognitive process performances or influence the short-term memory in the Y-maze test in mice.
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LYMPHANGIOMA OF THE PALATINE TONSIL

Lymphangioma of the palatine tonsil is a rare, benign lesion that presents as a tonsillar outgrowth and causes symptoms related to irritation and airway obstruction. Histologically, the mass has abundant dilated lymphatic channels in a fibrous stroma with lymphoid and adipose elements. A lymphangioma is mostly a congenital lesion consisting of a benign proliferation of lymphatic channels. Approximately 50% of these lesions are apparent at birth, with 80% to 90% detected within the first 2 decades of life. In contrast to congenital hemangiomas, lymphangiomas do not involute over time. Although these lesions tend to surround and sometimes invade nearby anatomic structures, they have no malignant potential. There is also considerable debate over the etiology of lymphangiomas, which may represent true neoplasms, malformations, or hamartomas. Three morphologic types of lymphangiomas are distinguished. Capillary, or simple, lymphangiomas are usually found in the superficial skin and composed of capillariesized, thin-walled lymphatic vessels. Cavernous lymphangiomas are subcutaneous lesions composed of mildly dilated, large spaces that are larger than those seen in capillary lymphangiomas. Cystic hygromas, the third type, are most common in the neck and composed of large, dilated, cystic lymphatic spaces. Thus, these 3 types of lymphangiomas can be differentiated from one another based purely on the characteristics of the component lymphatic spaces. In addition, combinations of the 3 types can sometimes be found in a single lesion. Therefore, the 3 types of lymphangiomas represent the same pathologic process along a spectrum and should be considered as a unified concept. Moreover, differentiating lymphangiomas on the basis of histologic type appears without clinical implications. Lymphangiomas tend to occur in places where lymphatics are abundant, with more than 90% of all lymphangiomas arising in the head and neck region. Most are found in the skin and subcutaneous tissues, but they have also been described in the larynx, parotid gland, mouth, and tongue. The palatine tonsil is a far less common site for the development of lymphangioma and has rarely been reported in the literature. Regardless of the uncertain pathogenesis of tonsillar lymphangiomas, these lesions have histologic features that differ from their counterparts in other anatomic locations. Although the lymphatic spaces of tonsillar lymphangiomas are frequently dilated, they are generally not as large or as prominent as in the lymphangiomas found elsewhere. Furthermore, the lymphoid and fibrous stromal components in tonsillar lymphangiomas are frequently more abundant than the lymphatic vessels. Thus, tonsillar lymphangiomas may represent a somewhat unique lesion. Reports in the literature use different nomenclature when referring to these lesions, such as tonsillar lymphangiomatous polyp, angioma, angiofibroma, fibrolipoma, polypoid tumor containing fibroadipose tissue, hamartomatous tonsillar polyp, and lymphangiectatic fibrous polyp. Because a lymphangioma may resemble a true neoplasm of the palatine tonsil clinically, the lesion must be removed for accurate histologic diagnosis and to rule out malignancy. Lymphangioma of the palatine tonsil is treated with surgical excision and has no recurrence once completely resected (Mardekian S and Karp JK. Lymphangioma of the Palatine Tonsil. Arch Pathol Lab Med. 2013;137:1837–1842).

Doina Butcovan