Effect of pregabalin on fear-motivated memory and spatial learning in scopolamine-induced memory impaired mice

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Introduction

Several studies indicate that, apart from other common adverse effects, such as somnolence, sedation or dizziness, antiepileptic drugs may impair cognition. It is noteworthy that numerous patients use antiepileptic drugs for a long time and cognitive side effects of these drugs are regarded as being poorly tolerated and a cause of dose reduction or even treatment discontinuation. In this respect, the contribution of antiepileptic drugs to memory deficits are particularly troublesome.

Pregabalin, a ligand of the $\alpha_2\delta$ subunit of voltage-gated calcium channels, belongs to the so-called second generation of antiepileptic drugs called "gabapentanoids." Apart from epilepsy, it is also used as an antiallodynic and antihyperalgesic agent for the treatment of neuropathic pain in patients suffering from painful diabetic neuropathy.

In general, most of newer antiepileptic drugs are thought to have a better cognitive profile than the older drugs, but little is known about the impact of pregabalin on cognition. Two available studies indicate that pregabalin may have a lower rate of cognitive side effects than other antiepileptic drugs.

Hence, the aim of this study was to assess the effect of pregabalin on learning and memory in a mouse model of scopolamine-induced memory impairments. For this purpose a fear-motivated passive avoidance task and a spatial memory task, namely the Morris water maze, were applied to assess if this drug influences pre-existing cognitive deficits.

Materials and Methods

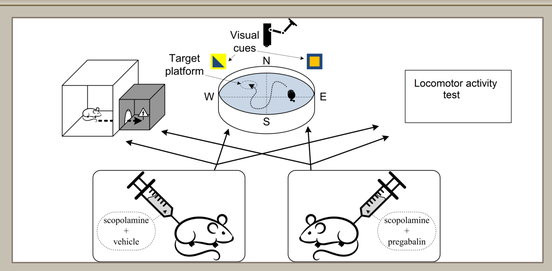
Animals

Six-week-old male Albino Swiss (CD-1) mice weighing between 18–22 g were used in the passive avoidance and locomotor activity tests, and C57BL/6J mice of the same age and body weight were used in the Morris water maze. The procedures for maintenance and treatment of laboratory animals were approved by the Local Ethics Committee of the Jagiellonian University in Krakow (101/2015) and were in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC).

Chemicals used for *in vivo* tests

Pregabalin (Tocris Bioscience, Germany) was used at the dose of 10 mg/kg. This dose had previously been proven to have a significant antiallodynic activity in mice. For behavioral experiments, it was suspended in 1% Tween 80 (Polskie Odczynniki Chemiczne, Poland) and administered intraperitoneally (i.p.) 60 min before the locomotor activity test or the acquisition phase of passive avoidance and Morris water maze tasks. Control mice were given an appropriate amount of vehicle (1% Tween 80). (−)-Scopolamine hydrobromide was purchased from Sigma-Aldrich (Poland) and, to induce memory impairments, this was dissolved in distilled water and was administered i.p. at a dose of 1 mg/kg 30 min before the tests.

In vivo tests



Passive avoidance task: acquisition trial, retention trial

- Better memory performance is indicated by longer latency before entering the black chamber in the test (retention) phase than in the conditioning (acquisition) phase

Morris water maze: acquisition trial (6 days, 4 trials/day), retention trial (drug off)

- Days 1-6: better learning skills are indicated by shorter escape latency time
- Day 7 (no platform): Latency time to the first crossing of the former platform location (target zone), the number of crossings of the target zone, time spent in the target NW quadrant, total distance, the distance spent in the NW quadrant, entries in the NW quadrant and mean speed are measured and compared

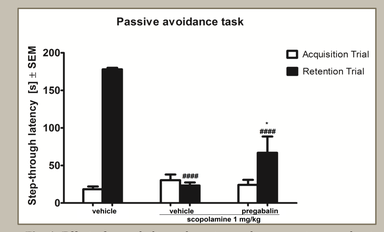
Locomotor activity test: locomotor activity of animals is measured by counting the number of light-beam crossings during the 30 min of testing

Data analysis

Numerical results from the tests are expressed as the mean ± standard error of the mean (SEM). For the statistical analysis one-way analysis of variance (ANOVA), followed by Tukey's *post-hoc* comparison or repeated measures ANOVA, followed by Bonferroni multiple comparison were used. $P < 0.05$ was considered significant.

Results

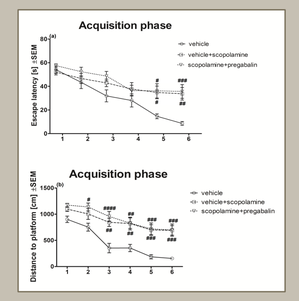
Passive avoidance task



In the retention phase, the step-through latencies of the two scopolamine-treated groups were significantly shorter than in the vehicle-treated control animals ($P < 0.0001$). There was also a statistically significant ($P < 0.05$) difference in the step-through latency between scopolamine-treated control mice and mice that received combined scopolamine+pregabalin, which indicates that pregabalin partially reversed memory deficits induced by scopolamine (Fig. 1).

Fig. 1 Effect of pregabalin on learning and memory in scopolamine-induced memory-impaired mice in the passive avoidance task. Results are shown as the mean step-through latency (±SEM) to enter the dark compartment in the acquisition trial (white bars), and in the retention trial (black bars). Statistical analysis: repeated-measures analysis of variance (ANOVA), followed by Bonferroni multiple comparison. Significance vs. vehicle-treated mice in the retention phase: #### $P < 0.0001$, or vs. scopolamine-treated control in the retention phase: * $P < 0.05$.

Morris water maze task



Scopolamine-treated control and combined scopolamine+pregabalin-treated mice performed the acquisition phase of the Morris water maze task with longer escape latencies than the vehicle-treated mice ($P < 0.05$ on day 5, and $P < 0.01$ on day 6). There were no differences in this parameter between scopolamine-treated control mice and mice treated with combined scopolamine and pregabalin indicating that pregabalin did not influence spatial learning in this task (Fig. 2).

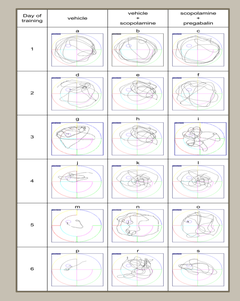
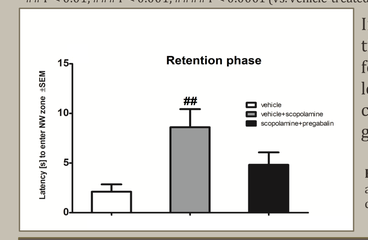


Fig. 2 Learning curves showing escape latencies (Fig. 2a) and distance traveled to reach the platform (Fig. 2b) during the acquisition phase of the Morris water maze task in vehicle-treated mice, scopolamine-treated control and mice treated with combined scopolamine+pregabalin. Results are shown as mean ±SEM. Statistical analysis: repeated-measures ANOVA, followed by Bonferroni *post hoc* comparison. Significance: # $P < 0.05$, ## $P < 0.01$, ### $P < 0.001$, #### $P < 0.0001$ (vs. vehicle-treated mice).



In the retention phase the latency time to the first crossing of the former platform location was longer in scopolamine-treated controls than in the two other groups ($P < 0.01$, Fig. 3).

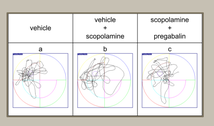
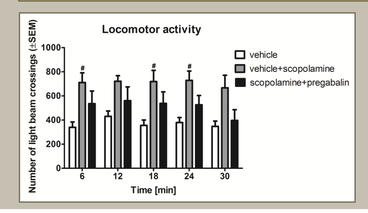


Fig. 3 Latency to the first crossing of the former platform location. Results are expressed as mean ±SEM. Statistical analysis: one-way ANOVA, followed by Tukey's *post hoc* comparison. Significance: ## $P < 0.01$ (vs. vehicle-treated mice).

Locomotor activity test



Compared to vehicle-treated controls, scopolamine increased locomotor activity of mice ($P < 0.05$ in the following test periods: 0–6 min, 12–24 mins). The injection of pregabalin partially reversed scopolamine-induced hyperlocomotion, but this difference was not statistically significant (Fig. 4).

Fig. 4 Effect of scopolamine alone or in combination with pregabalin on animals' locomotor activity. Results are shown as mean ±SEM. Statistical analysis: repeated-measures ANOVA, followed by Bonferroni *post hoc* comparison. Significance: # $P < 0.05$ (vs. vehicle-treated mice at the respective time-point).

Acknowledgements

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Key findings

Pregabalin attenuated or partially reversed fear-motivated and spatial memory deficits induced by scopolamine

These results are relevant for patients who use pregabalin for various therapeutic indications (e.g., epilepsy, neuropathic pain, anxiety)