Biomedicine & Pharmacotherapy 107 (2018) 1674–1681

Contents lists available at ScienceDirect

Biomedicine & Pharmacotherapy

journal homepage: www.elsevier.com/locate/biopha

Evaluation of the role of different neurotransmission systems in the anticonvulsant action of sildenafil in the 6 Hz-induced psychomotor seizure threshold test in mice

Dorota Nieoczym*, Katarzyna Socała, Piotr Wlaź

Department of Animal Physiology, Institute of Biology and Biochemistry, Faculty of Biology and Biotechnology, Maria Curie-Skłodowska University, Akademicka 19, PL 20-033, Lublin, Poland

ARTICLE INFO

Keywords:
Psychomotor seizures
Sildenafil
Neurotransmitter systems
Mice

ABSTRACT

Sildenafil influences seizure activity in animal seizure models, and its both proconvulsant and anticonvulsant effects were reported. We previously found that this PDE5 inhibitor significantly increased seizure threshold for the 6 Hz-induced psychomotor seizures in mice and therefore we aimed to investigate the influence of some modulators of neurotransmitter receptors, i.e., diazepam (GABA/benzodiazepine receptor agonist), flumazenil (GABA/benzodiazepine receptor antagonist), N-methyl-D-aspartic acid (NMDA glutamate receptor agonist), CGP 37849 (NMDA receptor antagonist), metergoline (serotonin receptor antagonist), 8-cyclopentyl-1,3-dipropylxanthine (adenosine A_1 receptor antagonist) and β-funaltrexamine (μ opioid receptor antagonist), on the anticonvulsant effect of sildenafil in this test. Additionally, we estimated influence of the studied compounds and their combinations with sildenafil on the muscular strength (assessed in the grip strength test) and motor coordination (assessed in the chimney test) in mice. Our results indicate that anticonvulsant properties of sildenafil in the 6 Hz test in mice might be related to its interactions with the GABAergic, glutamatergic, serotonergic and adenosinergic neurotransmission. We did not find interactions between sildenafil and μ opioid receptors. Neither the studied ligands nor their combinations with sildenafil impaired muscular strength and motor coordination. In conclusion, sildenafil has complex and extensive influence on neurotransmission and seizure generation in the CNS.

1. Introduction

Sildenafil, a selective and competitive phosphodiesterase 5 (PDE5) inhibitor, is the first oral drug for the treatment of erectile dysfunction of various etiologies. Pharmacological activity of sildenafil is strictly connected to nitric oxide (NO)/cyclic guanosine monophosphate (cGMP)/PDE5 cell signaling pathway which participates in numerous physiological and pathological processes. High level of NO, which is synthesized by different isoforms of NO synthase (NOS), stimulates guanylate cyclase (GC) to production of cGMP which is then hydrolyzed by phosphodiesterases (PDEs) to guanosine monophosphate (GMP). Among twelve isoforms of PDEs, which hydrolyze both cGMP and cyclic adenosine monophosphate (cAMP), only three of them, i.e., PDE5, PDE6 and PDE9, are selective for cGMP. They are found in different tissues, i.e., lungs, smooth and skeletal muscles, heart, placenta, liver and in several brain structures [1–3].

The presence of PDE5 in the brain and the ability of sildenafil to cross the blood-brain barrier [4] enable sildenafil to influence many CNS-related functions [3]. Sildenafil shows antinociceptive properties in a wide range of experimental models of pain [5–10], displays antidepressant activity [11–13] and influences the activity of some antidepressant drugs [14–20]. Interestingly, sildenafil showed both anxiogenic [21,22] and anxiolytic [4] effects in animal models. Inhibition of PDE5 by sildenafil may be a useful strategy in the treatment of stroke and Alzheimer's disease because cGMP improves neurogenesis and memory, reduces neurological deficits, enhances consideration and reconsideration processes in animals [23–26].

Sildenafil was also widely investigated in animal models of seizures

Abbreviations: β-FNA, β-funaltrexamine; cAMP, cyclic adenosine monophosphate; cGMP, cyclic guanosine monophosphate; CS_{50}, current strength required to induce seizure response in 50% of mice; DMSO, dimethyl sulfoxide; DPCPX, 8-cyclopentyl-1,3-dipropylxanthine; GABA, γ-aminobutyric acid; 5-HT, serotonin; *icv*, intracerebroventricularly; *ip*, intraperitoneally; NMDA, *N*-methyl-D-aspartic acid; NO, nitric oxide; PDE5, phosphodiesterase 5; PTZ, pentylenetetrazole; SEM, standard error of the mean

* Corresponding author.
E-mail address: dorota.nieoczym@poczta.umcs.lublin.pl (D. Nieoczym).

https://doi.org/10.1016/j.biopha.2018.08.163
Received 30 June 2018; Received in revised form 28 August 2018; Accepted 31 August 2018

Table 1
Effect of sildenafil in different animal seizure/epilepsy models.

Model of seizures/epilepsy	Animal	Studied doses of sildenafil and route of its administration	Effect of sildenafil	References
iv PTZ-induced seizure test	Swiss mice	1–40 mg/kg; *sc*	Proconvulsant	[39]
	Swiss mice	5–10 mg/kg; *sc*	Proconvulsant	[27]
	Swiss mice	5–40 mg/kg; *ip*	Proconvulsant	[32]
	NMRI mice	1–20 mg/kg; *ip*	No effect	[40]
	NMRI mice	1–20 mg/kg; *ip*	Proconvulsant	[28]
	NMRI mice	5–40 mg/kg; *ip*	Proconvulsant	[41]
sc PTZ test	Swiss mice	5–40 mg/kg; *ip*	No effect	[34]
PTZ kindling model	Swiss mice	20 mg/kg; *ip*	Anticonvulsant	[42]
iv bicuculine test	Swiss mice	2.5–20 mg/kg; *sc*	Proconvulsant	[39]
Cocaine-induced seizures	Swiss mice	5–20 mg/kg; *ip*	No effect	[33]
Maximal electroshock seizure threshold (MEST) test	Swiss mice	5–20 mg/kg; *ip*	Anticonvulsant	[31]
6 Hz electroshock-induced psychomotor seizure threshold test	Swiss mice	5 mg/kg; *ip*	Anticonvulsant	[30]
Amygdala-kindling model	Wistar rats	5–40 mg/kg; *ip*	Anticonvulsant	[32]

and it showed both pro- and anticonvulsant properties (Table 1). It decreased seizure threshold in the timed intravenous pentylenetetrazole (*iv* PTZ) infusion test in mice [27–29] while in the models of seizures induced by electrical stimulation, i.e., in the 6 Hz-induced psychomotor seizure threshold [30] and maximal electroshock seizure threshold (MEST) [31] tests, it had anticonvulsant activity. Furthermore, sildenafil decreased the duration of behavioral seizures and afterdischarges in the amygdala kindling model in rats [32]. It did not affect cocaine-induced seizures in mice [33]. Of note, sildenafil affected the activity of some antiepileptic drugs in experimental models of seizures in mice, and both pharmacodynamic and pharmacokinetic interactions were observed [29–31,34].

Sexual dysfunctions are very common in patients with epilepsy and they may be attributed both to the disease and to antiepileptic treatment. According to Atif et al. [35] about 30–66% of epileptic men suffer from sexual dysfunctions which include decrease in libido, erectile and ejaculation problems. Sildenafil is recommended as one of the available medications for combatting erectile dysfunctions in men with epileptic disorders [35].

The aim of our study was to investigate the mechanism of the anticonvulsant action of sildenafil in the psychomotor 6 Hz-induced seizure test in mice [30]. We administered sildenafil in combination with modulators of neurotransmitter systems to investigate their participation in the anticonvulsant effect of sildenafil. Diazepam (a benzodiazepine agonist on $GABA_A$ receptor) and flumazenil (a GABA/benzodiazepine antagonist) were used to study possible involvement of GABAergic system while N-methyl-D-aspartic acid (NMDA, an agonist of NMDA glutamate receptors) and CGP 37849 (an antagonist of NMDA receptors) were administered to evaluate participation of glutamatergic neurotransmission, and especially NMDA-type receptors. These two neurotransmission systems are essential in generation of seizures and are the main targets for numerous antiepileptic drugs [36–38]. Furthermore, contribution of serotonergic neurotransmission in the anticonvulsant action of sildenafil was studied by co-administration of this PDE5 inhibitor with metergoline – a potent nonselective serotonin (5-HT) receptor antagonist. We also used 8-cyclopentyl-1,3-dipropylxanthine (DPCPX; an adenosine A_1 receptor antagonist) to investigate the involvement of adenosinergic system and β-funaltrexamine (β-FNA; an irreversible μ-opioid antagonist) to determine participation of μ opioid receptors in the anticonvulsant action of sildenafil. The studied modulators of neurotransmitter receptors that have anticonvulsant potential in the 6 Hz test were combined with the subeffective dose of sildenafil, i.e., 5 mg/kg, while those with proconvulsant activity were co-administered with sildenafil at the effective dose, i.e., 20 mg/kg. In addition, the chimney test and the grip strength test in mice were used to investigate the influence of the used receptor ligands and their combinations with sildenafil on motor coordination and muscular strength, respectively.

2. Material and methods

2.1. Animals

Naïve male albino Swiss mice weighting 23–30 g were used. The animals were purchased from a licensed breeder (Laboratory Animals Breeding, Słaboszów, Poland). They were housed in groups of eight in standard polycarbonate cages (37 cm × 21 cm × 15 cm) with free access to food (Agropol S.J., Motycz, Poland) and tap water under strictly controlled laboratory conditions (ambient temperature 21–24 °C, relative humidity 45–65%) with a 12/12 h light-dark cycle (light on at 6:00 a.m.). After a 7-day period of acclimatization to laboratory conditions, the animals were randomly assigned to the experimental groups. All experiments were performed between 8:00 a.m. and 3:00 p.m. to minimize circadian influences. Control and drug experiments were always done on the same day to avoid day-to-day variations in convulsive susceptibility.

All procedures involving animals were approved by the First Local Ethics Committee at the Medical University of Lublin (49/2013) and the Local Ethical Committee in Lublin (7/2017), Poland. Both housing and experimental procedures were conducted in accordance with the European Union Directive of 22 September 2010 (2010/63/EU) and Polish legislation acts concerning animal experimentation. All efforts were made to reduce the number of animals used in the study and their suffering.

2.2. Drugs

The following drugs were used: sildenafil (as citrate, kindly provided by Polpharma S.A., Starogard Gdański, Poland), diazepam (Polfa, Warszawa, Poland), flumazenil (Selleck Chemicals, Houston, TX, USA), NMDA (Abcam Biochemicals, Cambridge, UK), CGP 37849 (Tocris Bioscience, Bristol, UK), metergoline (Sigma-Aldrich, St. Louis, MO, USA), DPCPX (Sigma-Aldrich, St. Louis, MO, USA) and β-FNA (Tocris Bioscience, Bristol, UK). Sildenafil, NMDA, CGP 37849 and metergoline were dissolved in saline, DPCPX and flumazenil were dissolved in 1% solution of dimethyl sulfoxide (DMSO; ICN Biomedicals, Inc., Aurora, OH, USA) in normal saline, β-FNA was dissolved in 100% DMSO while metergoline was suspended in a 0.5% aqueous solution of methyl cellulose. The used compounds, except for β-FNA, were injected intraperitoneally (*ip*) at a constant volume of 0.1 ml per 10 g body weight. Animals were treated with sildenafil, diazepam, flumazenil and DPCPX 30 min before the tests while CGP 37849, metergoline and NMDA were administered 60 min before the tests. β-FNA was administered intracerebroventricularly (*icv*) at a volume of 5 μl/mouse 5 min before the tests. The *icv* injection was performed according to the method described elsewhere [43,44]. Animals in the control groups received the respective vehicles at the appropriate volume and time. The pre-treatment times were taken from the literature and were confirmed in our

previous experiments.

2.3. The 6 Hz electroshock-induced seizures

Psychomotor seizures (6 Hz seizures) were induced via corneal stimulation (0.2 ms square pulse at 6 Hz for 3 s) using Grass S48 stimulator coupled with a constant current unit CCU1 (both from Grass Technologies, West Warwick, RI, USA). Prior to the stimulation, a drop of ocular anesthetic, 1% solution of tetracaine hydrochloride (Sigma-Aldrich Co., St. Louis, MO, USA), was placed on the animals' corneas. The electrodes were soaked in the 0.9% saline immediately before testing to ensure a good electrical contact. Mice were restrained manually during the stimulation and immediately thereafter they were placed in a Plexiglas box (35 cm × 20 cm × 14 cm) for observation. Six Hz electroshock-induced seizures were characterized by stun, which was often followed by rearing, forelimb clonus, twitching of vibrissae, and Straub-tail, which lasted at least 10 s from the stimulation [45]. The mouse was scored as protected if it resumed its normal activity within 10 s from the stimulation. The mice were subjected to stimuli of different current intensities according to the "up-and-down" method and the median current strength (CS_{50} in mA; the current strength of 6 Hz stimulation which induce psychomotor seizures in 50% of the tested animals) with 95% confidence limits was calculated as described elsewhere [30,46]. Each mouse was stimulated only once at any given current intensity (8–23 mA) and convulsant activity was judged as described above. If the mouse responded with convulsions, the next mouse was stimulated with current of an intensity 0.06-log step lower than the previous one. If the mouse did not have convulsions, the next one was stimulated with a current of intensity 0.06-log step higher [46]. Each experimental group consisted of 20 animals.

2.4. The grip-strength test

The influence of the studied ligands and their combinations with sildenafil on muscular strength was determined in the grip-strength test [47]. The apparatus consisted of a steel wire grid (8 × 8 cm) connected to an isometric force transducer. The mice were lifted by the tail so that they grasp the grid with their forepaws. The mice were then gently pulled backwards until they released the grid and the maximal force in newtons (N) exerted by the mouse before losing grip was measured by the apparatus. The mean of three consecutive measurements for each animal was calculated and then normalized to the body weight (mN/g). Each experimental group consisted of 10–12 animals.

2.5. The chimney test

The chimney test was used to determine the influence of the studied modulators of neurotransmitter receptors and their combinations with sildenafil on motor coordination. The mice were placed in a Plexiglas tube (3 cm, inner diameter × 30 cm, length) with rough inner surface. The inability of the mouse to climb backwards up through the tube in 60 s was an indicator of impairment of motor coordination. Each experimental group consisted of 10–12 animals.

2.6. Statistical analysis

The seizure thresholds in the 6 Hz test are expressed as CS_{50} values with their 95% confidence limits. These values were compared with the one-way analysis of variance (ANOVA) followed by the Tukey's post hoc test. Results from the chimney test (percent of animals with impaired motor coordination) were analyzed with the Fisher's exact probability test. The mean maximal force (± SEM) determined in the grip strength test was compared with one-way ANOVA. All statistical analyzes were carried out with GraphPad Prism 5.03 for Windows (GraphPad Software, San Diego, CA, USA).

3. Results

3.1. Influence of diazepam and flumazenil on the activity of sildenafil in the 6 Hz test in mice

Neither sildenafil administered at a dose of 5 mg/kg nor diazepam injected at a dose of 0.05 mg/kg significantly increased seizure threshold in the 6 Hz test. Combination of both significantly increased seizure threshold in comparison both to the control group ($p < 0.01$) and to the group that was treated with diazepam ($p < 0.01$). Seizure threshold in group of animals co-treated with sildenafil and diazepam was ~24% higher than in the control group and ~20% higher than in group injected with diazepam (one-way ANOVA: $F(3,32) = 7.083$, $p = 0.0009$; Fig. 1A).

Sildenafil administered at a dose of 20 mg/kg significantly increased seizure threshold in comparison to the control group ($p < 0.001$). Flumazenil at a dose of 10 mg/kg did not significantly change seizure threshold in comparison to the control group. Its co-administration with sildenafil (20 mg/kg) reduced seizure threshold by ~14% in

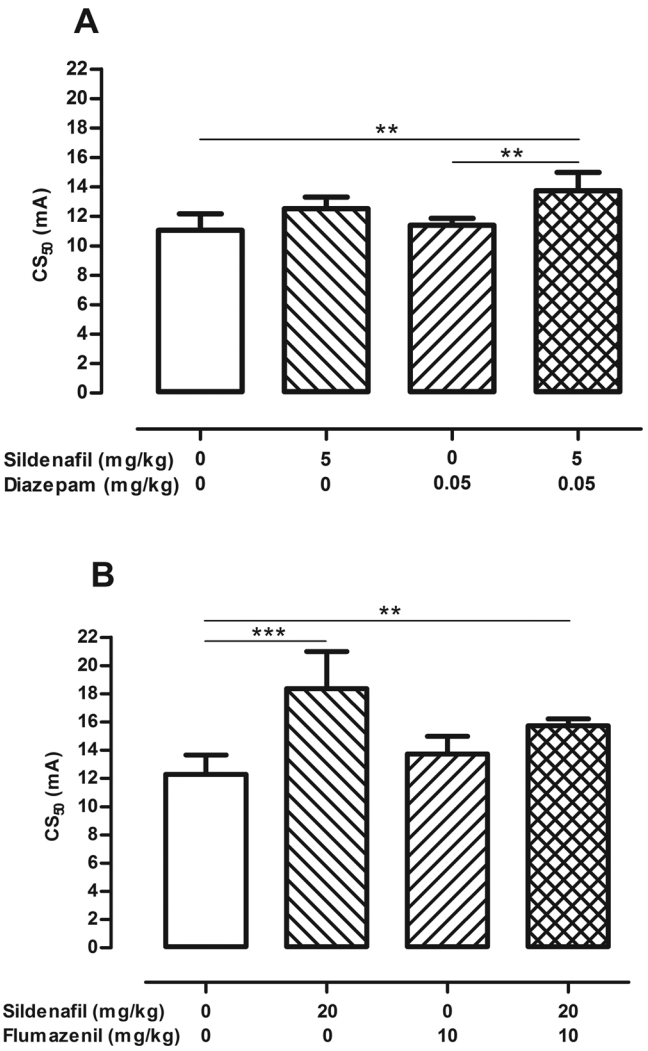

Fig. 1. Influence of diazepam and flumazenil, $GABA_A$ receptor modulators, on the activity of sildenafil in the 6 Hz test in mice. Sildenafil, diazepam and flumazenil were administered *ip* 30 min prior to the test. Animals in the control groups received the respective vehicles at the appropriate volume and time. Results are presented as median current strengths (CS_{50} in mA with their 95% confidence limits) required to produce psychomotor seizures in 50% of animal tested. ** $p < 0.01$, *** $p < 0.001$ (one-way ANOVA followed by the Tukey's post-hoc test).

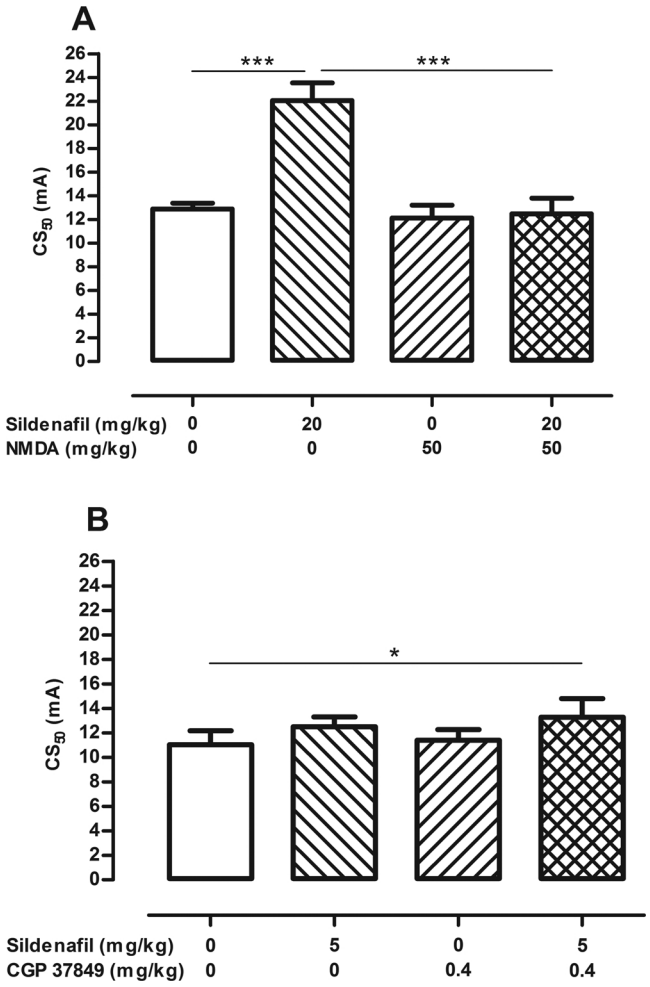

Fig. 2. Influence of NMDA and CGP 37849, glutamate NMDA receptor modulators, on the activity of sildenafil in the 6 Hz test in mice. Sildenafil was administered *ip* 30 min while NMDA and CGP 37849 were administered *ip* 60 min prior to the test. Animals in the control groups received the respective vehicles at the appropriate volume and time. Results are presented as median current strengths (CS_{50} in mA with their 95% confidence limits) required to produce psychomotor seizures in 50% of animal tested. * $p < 0.05$, *** $p < 0.001$ (one-way ANOVA followed by the Tukey's post-hoc test).

comparison to the sildenafil-treated (20 mg/kg) group, however, this change was not statistically significant. Seizure threshold in the group concomitantly treated with sildenafil and flumazenil was significantly higher than in the control group (by ~28%, $p < 0.01$). Results are presented in Fig. 1B (one-way ANOVA: $F(3,34) = 12.47$, $p < 0.0001$).

3.2. Influence of NMDA and CGP 37849 on the activity of sildenafil in the 6 Hz test in mice

Seizure threshold in the control group was 12.9 (13.4–12.4) mA and it increased by ~71% in the group treated with sildenafil at a dose of 20 mg/kg ($p < 0.001$). NMDA at a dose of 50 mg/kg did not change significantly seizure threshold in comparison to the control group but it entirely abolished the anticonvulsant effect of sildenafil (20 mg/kg). Results are presented in Fig. 2A (one-way ANOVA: $F(3,33) = 52.36$, $p < 0.0001$).

Neither sildenafil at a dose of 5 mg/kg nor CGP 37849 at a dose of 0.4 mg/kg changed significantly seizure threshold in comparison to the control group in the 6 Hz test. However, co-treatment with these compounds at sub-effective doses significantly increased seizure threshold (Fig. 2B, one-way ANOVA: $F(3,31) = 3.774$, $p = 0.0203$).

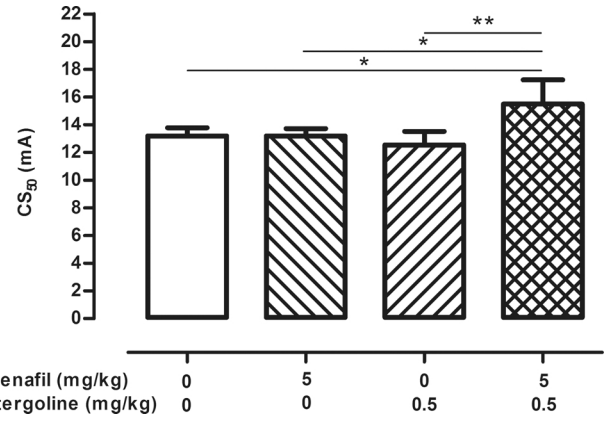

Fig. 3. Influence of metergoline, a nonselective 5-HT1, 5-HT2 and 5-HT7 receptors antagonist, on the activity of sildenafil in the 6 Hz test in mice. Sildenafil was administered *ip* 30 min while metergoline *ip* 60 min prior to the test. Animals in the control group received the respective vehicles at the appropriate volume and time. Results are presented as median current strengths (CS_{50} in mA with their 95% confidence limits) required to produce psychomotor seizures in 50% of animal tested. * $p < 0.05$, ** $p < 0.01$ (one-way ANOVA followed by the Tukey's post-hoc test).

3.3. Influence of metergoline on the activity of sildenafil in the 6 Hz test in mice

Injection of sildenafil at a dose of 5 mg/kg or metergoline at a dose of 0.5 mg/kg did not significantly influence seizure threshold in comparison to the control group while co-administration of these compounds raised the seizure threshold by ~15% ($p < 0.05$ vs. control group). Seizure threshold in the group treated with the combination of sildenafil and metergoline differed significantly from the seizure threshold in the group of animals treated with sildenafil ($p < 0.05$) and metergoline alone ($p < 0.01$). Results are presented in Fig. 3 (one-way ANOVA: $F(3,30) = 6.602$, $p = 0.0015$).

3.4. Influence of DPCPX on the activity of sildenafil in the 6 Hz test in mice

Sildenafil at a dose of 20 mg/kg significantly increased seizure threshold from 11.6 (12.1-11.1) mA in the control group to 14.8 (15.9-13.8) mA ($p < 0.001$). Although DPCPX administered at a dose of 0.5 mg/kg did not significantly influence psychomotor seizure threshold, it completely abolished the anticonvulsant effect of sildenafil. Seizure threshold in the group treated with DPCPX (0.5 mg/kg) and sildenafil (20 mg/kg) did not differ significantly from the control group but was significantly lower than in the sildenafil-treated group ($p < 0.001$). Results are presented in Fig. 4 (one-way ANOVA: $F(3,34) = 19.66$, $p < 0.0001$).

3.5. Influence of β-FNA on the activity of sildenafil in the 6 Hz test in mice

Neither sildenafil at a dose of 5 mg/kg nor β-FNA at a dose of 10 nmol/mouse caused statistically significant change in psychomotor seizure threshold in comparison to the control group. Moreover, there was also no statistically significant change in the seizure threshold in the group treated with combination of sildenafil (5 mg/kg) and β-FNA (10 nmol/mouse). Results are presented in Fig. 5 (one-way ANOVA: $F(3,34) = 1.824$, $p = 0.1613$).

3.6. Influence of combinations of sildenafil with the studied modulators of neurotransmitter receptors on the muscular strength and motor coordination in mice

Neither sildenafil and the studied ligands nor their combinations significantly influenced muscular strength and motor coordination (not

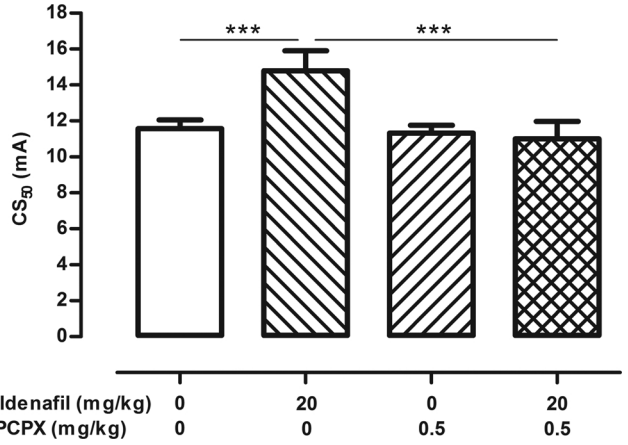

Fig. 4. Influence of DPCPX, an adenosine A1 receptor antagonist, on the activity of sildenafil in the 6 Hz test in mice. Sildenafil and DPCPX were administered *ip* 30 min prior to the test. Animals in the control group received the respective vehicles at the appropriate volume and time. Results are presented as median current strengths (CS_{50} in mA with their 95% confidence limits) required to produce psychomotor seizures in 50% of animal tested. *** $p < 0.001$ (one-way ANOVA followed by the Tukey's post-hoc test).

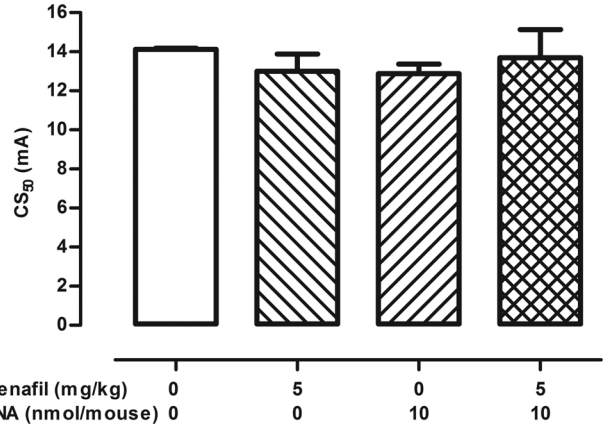

Fig. 5. Influence of β-FNA, an irreversible μ-opioid receptor antagonist, on the activity of sildenafil in the 6 Hz test in mice. Sildenafil was administered *ip* 30 min and β-FNA was administered *icv* 15 min prior to the test. Animals in the control group received the respective vehicles at the appropriate volume and time. Results are presented as median current strengths (CS_{50} in mA with their 95% confidence limits) required to produce psychomotor seizures in 50% of animal tested. One-way ANOVA was used to analyze the data.

illustrated).

4. Discussion

The aim of the study was to evaluate participation of different neurotransmitter systems in the anticonvulsant effect of sildenafil in the 6 Hz test in mice [30]. Although there are some studies which clarify the proconvulsant effect of sildenafil in the PTZ tests [27,28], there are no studies aimed to identify its anticonvulsant activity. In our study we investigated participation of $GABA_A$, NMDA, 5-HT, adenosine A_1 and opioid μ receptors in its anticonvulsant effect in this test. The mentioned receptors and neurotransmission systems have been proved to play a pivotal role in seizure activity [36,38,48–51].

Briefly, seizure disturbances in the CNS are induced by an imbalance between the neuronal excitation and inhibition which are provided in substantial part by GABAergic and glutamatergic neurotransmission [36,38,52]. We noted that co-administration of sildenafil and diazepam at sub-effective doses produced significant increase in psychomotor seizure threshold in mice. However, flumazenil did not completely abolish the anticonvulsant effect of sildenafil in this test. These results suggest that sildenafil might enhance GABAergic neurotransmission. Influence of sildenafil on the GABAergic system was studied previously in other seizure [27] and neuropathic pain [6] models. Gholipour et al. [27] revealed that sildenafil decreased threshold for clonic seizures and weakened anticonvulsant effect of diazepam in the *iv* PTZ test in mice. Differences between activity of sildenafil in the *iv* PTZ test [27] and the 6 Hz test [30] might be a result of distinct mechanisms which are involved in generation of these seizures [53], however, in both cases the influence of sildenafil on GABAergic neurotransmission and $GABA_A$ receptors was confirmed. Huang et al. [6] demonstrated that antinociceptive effect of sildenafil in neuropathic pain model in rats was reversed by bicuculline – a competitive $GABA_A$ receptor antagonist, which indicated that sildenafil might also affect the activity of these receptors through GABA binding site [6]. Furthermore, high concentration of cGMP in neurons might also enhance GABA release [54].

The anticonvulsant effect of sildenafil in the 6 Hz test was abolished by NMDA administration and potentiated by the competitive NMDA antagonist CGP 37849. These results indicate that NMDA receptors and glutamatergic neurotransmission are affected by sildenafil and might participate in its anticonvulsant effect in the 6 Hz-induced psychomotor seizure test in mice. There are no other studies focusing on influence of ligands of NMDA receptor and other modulators of glutamatergic neurotransmission on the activity of sildenafil in experimental models of neurological disorders but there are experimental studies which confirmed interplay between the glutamatergic neurotransmission and NO/cGMP pathway which is modulated by sildenafil [55].

Our study revealed that anticonvulsant activity of sildenafil in the 6 Hz test in mice is also mediated by interactions with both adenosinergic and serotonergic systems. Protective role of adenosine in various pathological conditions in the CNS, i.e., ischemia, excitotoxicity or epileptic disturbances [56], result mainly from the activation of adenosine A_1 receptors [57]. Adenosinergic neurotransmission might regulate the CNS-related processes indirectly through interactions with other neurotransmitter systems because adenosine A_1 receptors are located in synapses where they might limit glutamatergic neurotransmission and hyperpolarize neurons [56]. Blocking of adenosine A_1 receptors by DPCPX abolished the anticonvulsant effect of sildenafil in the 6 Hz test, which indicates that protective properties of the studied PDE5 inhibitor might arise partly from its influence on adenosinergic system. Interactions between sildenafil and adenosinergic neurotransmission were also found in the formalin test in rats [7,8] because antinociceptive effect of sildenafil was suppressed both by the nonselective antagonist of adenosine receptors – CGS 15943 [7], and by selective antagonists of the respective adenosine receptors, i.e., A_1, A_{2A}, A_{2B} and A_3 [8].

Contribution of 5-HT receptors in convulsive processes is not clearly defined because results obtained in experimental studies varied considerably and seem to be dependent on the experimental model, type of seizures and 5-HT receptor subtypes [49,58]. In case of 5-HT_1, 5-HT_2 and 5-HT_7 receptors, which are non-selectively blocked by metergoline, both pro- and anticonvulsant effects were noted. Our pilot study revealed that metergoline increases seizure threshold in the 6 Hz test in mice (unpublished observation). It also protected DBA/2 J mice from sound-induced seizures and this effect seemed to be connected with antagonism of 5-HT_7 receptor [59]. We noted a significant synergistic effect between sildenafil and metergoline when these compounds were administered at sub-effective doses, however, our results did not determine which subtype of 5-HT receptors antagonized by metergoline had the most meaningful influence in the observed effect. Lee et al. [7] confirmed interactions between sildenafil and serotonergic neurotransmission in the formalin test in rats because antinociceptive activity of sildenafil in this test was attenuated by dihydroergocristine methanesulfonate – a nonselective antagonist of 5-HT receptors. However,

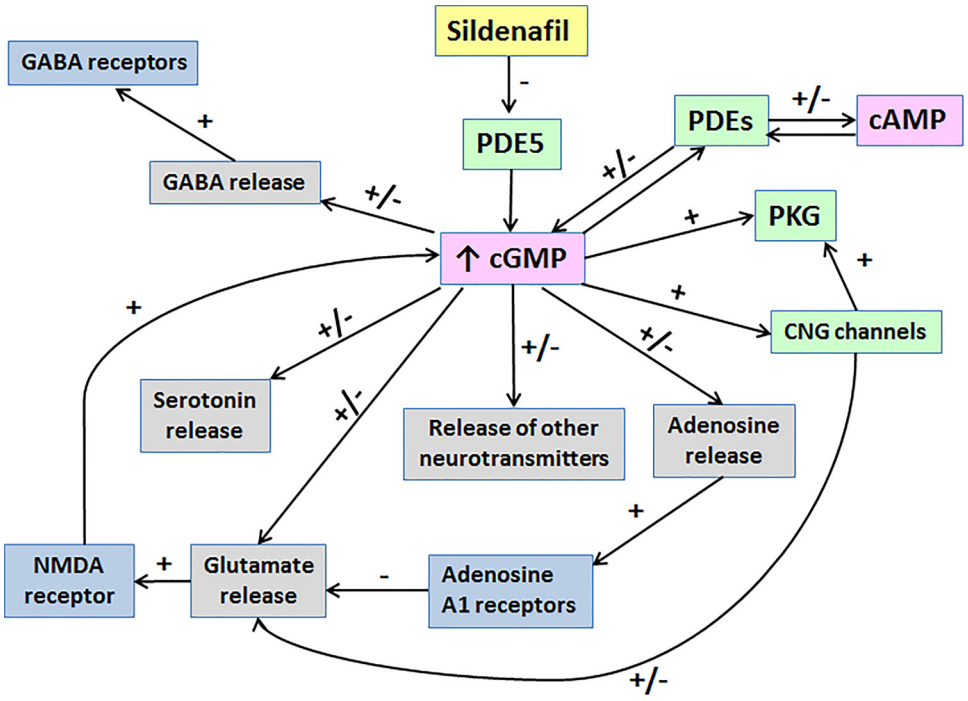

Fig. 6. Mechanism of probable interactions of sildenafil with neurotransmitter systems in the central nervous system. cAMP – cyclic adenosine monophosphate; cGMP – cyclic guanosine monophosphate; CNG – cyclic nucleotide gated, GABA – γ-aminobutyric acid; NMDA – N-methyl-D-aspartic acid; PDE5 – phosphodiesterase 5; PDEs – phosphodiesterases; PKG – protein kinases G.

this study also did not evaluate which subtypes of 5-HT receptors are involved in the interactions with sildenafil [7].

Although opioidergic neurotransmission participates in seizure activity, opioids and modulators of opioid receptors showed both pro- and anticonvulsant effects. Furthermore, these effects may be biphasic and dependent on the used dose [28,51,60,61]. Although β-FNA increased seizure threshold in the 6 Hz test in mice [62] it did not influence the activity of sildenafil in our study. We could eliminate participation of the μ opioid receptors in the effect of sildenafil but we could not exclude the contribution of other components of opioidergic neurotransmission, for example κ or δ opioid receptors. Montaser-Kouhsari et al. [28] demonstrated that co-administration of sildenafil and morphine (a μ opioid receptor agonist) at sub-effective doses significantly decreased threshold for clonic seizures in the *iv* PTZ test in mice. In addition, the proconvulsant effect of sildenafil was blocked by naltrexone – an antagonist of opioid receptors. These results indicate that proconvulsant effect of sildenafil in the *iv* PTZ test is also connected with the opioidergic neurotransmission [28].

Additionally, observed effects may result from the cross-talk between cGMP- and cAMP-dependent signaling pathways. Integral elements involved in the cross-talk between these pathways are PDEs which activity is directly and indirectly regulated by level of cyclic nucleotides. Therefore, cGMP concentration might affect cAMP level and vice versa. For example, PDE2 is allosterically stimulated by cGMP to hydrolyze cAMP while in case of PDE3, cGMP competitively inhibits hydrolysis of cAMP [63]. The interplay between cAMP and cGMP pathways with participation of PDE2 was revealed in the central nervous system structures [64,65].

In conclusion, both results of our study and previous studies revealed interactions of sildenafil with different neurotransmission systems. These interactions might not be the result of its indirect influence on receptors in the CNS but might rather be mediated by changes in the activity of NO/cGMP pathway and changes in cGMP level. The main effect of activity of sildenafil is the growth of cGMP level but, due to the cross-talk processes, changes in concentration of cAMP cannot be excluded. Cyclic nucleotides are widely distributed second messengers in the brain and are responsible for regulating numerous processes in the neurons. Their classical targets are cyclic nucleotide dependent kinases, ion channels and PDEs. It was previously revealed that cGMP-dependent mechanism is involved in the release of glutamate, which then influences the release of other neurotransmitters in various brain regions, i.e., in the hippocampus, striatum and hypothalamus. Moreover, effects mediated by $GABA_A$ receptors are depressed by cGMP and NO donors [66]. Likewise, release of 5-HT in the hypothalamus is also mediated by cGMP [67]. Interactions between cGMP- and cAMP-dependent signal transduction pathways and different neurotransmission systems are in many cases reciprocal and changes in neurotransmitters concentration in the CNS might have a wide range of effects and might also influence seizure activity. Some likely interactions between cGMP and neurotransmission systems have been presented in Fig. 6. Complexity of these interactions makes understanding of the influence of sildenafil on neuronal activity and seizure processes difficult, it needs further and more precise studies with using another chemicals.

Funding

This study was supported by Funds for Statutory Activity of Maria Curie-Skłodowska University, Lublin, Poland.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

We thank Mateusz Pieróg and Nina Kowalczyk for skillful technical assistance.

References

[1] A.T. Bender, J.A. Beavo, Cyclic nucleotide phosphodiesterases: molecular regulation to clinical use, Pharmacol. Rev. 58 (2006) 488–520.
[2] K. Domek-Łopacinska, J.B. Strosznajder, Cyclic GMP metabolism and its role in brain physiology, J. Physiol. Pharmacol. 56 (Suppl. 2) (2005) 15–34.
[3] S. Uthayathas, S.S. Karuppagounder, B.M. Thrash, K. Parameshwaran, V. Suppiramaniam, M. Dhanasekaran, Versatile effects of sildenafil: recent pharmacological applications, Pharmacol. Rep. 59 (2007) 150–163.
[4] N. Liebenberg, B.H. Harvey, L. Brand, G. Wegener, C.B. Brink, Chronic treatment with the phosphodiesterase type 5 inhibitors sildenafil and tadalafil display

anxiolytic effects in Flinders Sensitive Line rats, Metab. Brain Dis. 27 (2012) 337–340.
[5] M.M. Bezerra, V. Lima, V.C. Girao, R.C. Teixeira, J.R. Graca, Antinociceptive activity of sildenafil and adrenergic agents in the writhing test in mice, Pharmacol. Rep. 60 (2008) 339–344.
[6] L.J. Huang, M.H. Yoon, J.I. Choi, W.M. Kim, H.G. Lee, Y.O. Kim, Effect of sildenafil on neuropathic pain and hemodynamics in rats, Yonsei Med. J. 51 (2010) 82–87.
[7] H.G. Lee, W.M. Kim, C.H. Park, M.H. Yoon, Roles of adenosine and serotonin receptors on the antinociception of sildenafil in the spinal cord of rats, Yonsei Med. J. 51 (2010) 960–964.
[8] H.G. Lee, W.M. Kim, J.I. Choi, M.H. Yoon, Roles of adenosine receptor subtypes on the antinociceptive effect of sildenafil in rat spinal cord, Neurosci. Lett. 480 (2010) 182–185.
[9] C.H. Park, H.G. Lee, S.H. Lee, C.W. Chung, M.H. Yoon, The role of adrenergic and cholinergic receptors on the antinociception of sildenafil in the spinal cord of rats, Neurosci. Lett. 502 (2011) 99–102.
[10] C.S. Patil, N.K. Jain, V.P. Singh, S.K. Kulkarni, Cholinergic-NO-cGMP mediation of sildenafil-induced antinociception, Indian J. Exp. Biol. 42 (2004) 361–367.
[11] H. Matsushita, M. Matsuzaki, X.J. Han, T.I. Nishiki, I. Ohmori, H. Michiue, H. Matsui, K. Tomizawa, Antidepressant-like effect of sildenafil through oxytocin-dependent cyclic AMP response element-binding protein phosphorylation, Neuroscience 200 (2012) 13–18.
[12] V.S. Tomaz, R.C. Cordeiro, A.M. Costa, D.F. de Lucena, H.V. Nobre Junior, F.C. de Sousa, S.M. Vasconcelos, M.L. Vale, J. Quevedo, D. Macedo, Antidepressant-like effect of nitric oxide synthase inhibitors and sildenafil against lipopolysaccharide-induced depressive-like behavior in mice, Neuroscience 268 (2014) 236–246.
[13] C. Wang, J. Zhang, Y. Lu, P. Lin, T. Pan, X. Zhao, A. Liu, Q. Wang, W. Zhou, H.T. Zhang, Antidepressant-like effects of the phosphodiesterase-4 inhibitor etazolate and phosphodiesterase-5 inhibitor sildenafil via cyclic AMP or cyclic GMP signaling in mice, Metab. Brain Dis. 29 (2014) 673–682.
[14] R.C. Almeida, C.S. Felisbino, M.G. Lopez, A.L. Rodrigues, N.H. Gabilan, Evidence for the involvement of L-arginine-nitric oxide-cyclic guanosine monophosphate pathway in the antidepressant-like effect of memantine in mice, Behav. Brain Res. 168 (2006) 318–322.
[15] C.B. Brink, J.D. Clapton, B.E. Eagar, B.H. Harvey, Appearance of antidepressant-like effect by sildenafil in rats after central muscarinic receptor blockade: evidence from behavioural and neuro-receptor studies, J. Neural Transm. 115 (2008) 117–125.
[16] A. Dhir, S.K. Kulkarni, Involvement of nitric oxide (NO) signaling pathway in the antidepressant action of bupropion, a dopamine reuptake inhibitor, Eur. J. Pharmacol. 568 (2007) 177–185.
[17] K. Socała, D. Nieoczym, E. Wyska, E. Poleszak, P. Wlaź, Sildenafil, a phosphodiesterase type 5 inhibitor, enhances the antidepressant activity of amitriptyline but not desipramine, in the forced swim test in mice, J. Neural Transm. 119 (2012) 645–652.
[18] K. Socała, D. Nieoczym, E. Wyska, E. Poleszak, P. Wlaź, Sildenafil, a phosphodiesterase type 5 inhibitor, reduces antidepressant-like activity of paroxetine in the forced swim test in mice, Pharmacol. Rep. 64 (2012) 1259–1266.
[19] K. Socała, D. Nieoczym, E. Wyska, E. Poleszak, P. Wlaź, Influence of sildenafil on the antidepressant activity of bupropion and venlafaxine in the forced swim test in mice, Pharmacol. Biochem. Behav. 103 (2012) 273–278.
[20] K. Socała, D. Nieoczym, E. Wyska, E. Poleszak, P. Wlaź, Sildenafil, a phosphodiesterase type 5 inhibitor, enhances the activity of two atypical antidepressant drugs, mianserin and tianeptine, in the forced swim test in mice, Prog. Neuropsychopharmacol. Biol. Psychiatry 38 (2012) 121–126.
[21] D. Demirci, O. Mutlu, F. Akar, C.I. Komsuoglu, G. Ulak, Sildenafil enhances locomotor activity in young mice and exerts anxiogenic effects in both young and aged mice, Med. Sci. Monit. Basic Res. 20 (2014) 15–21.
[22] M. Kurt, S.S. Bilge, E. Aksoz, O. Kukula, S. Celik, Y. Kesim, Effect of sildenafil on anxiety in the plus-maze test in mice, Pol. J. Pharmacol. 56 (2004) 353–357.
[23] M. Cuadrado-Tejedor, I. Hervias, A. Ricobaraza, E. Puerta, J.M. Perez-Roldan, C. Garcia-Barroso, R. Franco, N. Aguirre, A. Garcia-Osta, Sildenafil restores cognitive function without affecting beta-amyloid burden in a mouse model of Alzheimer's disease, Br. J. Pharmacol. 164 (2011) 2029–2041.
[24] J.N.E. Olmestig, I.R. Marlet, A.H. Hainsworth, C. Kruuse, Phosphodiesterase 5 inhibition as a therapeutic target for ischemic stroke: a systematic review of preclinical studies, Cell. Signal. 38 (2017) 39–48.
[25] J. Zhang, J. Guo, X. Zhao, Z. Chen, G. Wang, A. Liu, Q. Wang, W. Zhou, Y. Xu, C. Wang, Phosphodiesterase-5 inhibitor sildenafil prevents neuroinflammation, lowers beta-amyloid levels and improves cognitive performance in APP/PS1 transgenic mice, Behav. Brain Res. 250 (2013) 230–237.
[26] R. Zhang, Y. Wang, L. Zhang, Z. Zhang, W. Tsang, M. Lu, L. Zhang, M. Chopp, Sildenafil (Viagra) induces neurogenesis and promotes functional recovery after stroke in rats, Stroke 33 (2002) 2675–2680.
[27] T. Gholipour, A. Rasouli, A. Jabbarzadeh, B.G. Nezami, K. Riazi, M. Sharifzadeh, A.R. Dehpour, The interaction of sildenafil with the anticonvulsant effect of diazepam, Eur. J. Pharmacol. 617 (2009) 79–83.
[28] L. Montaser-Kouhsari, B. Payandemehr, T. Gholipour, P. Ziai, P. Nabavizadeh, A. Ghasemi, A. Bahremand, M. Ghasemi, A.R. Dehpour, A role for opioid system in the proconvulsant effects of sildenafil on the pentylenetetrazole-induced clonic seizure in mice, Seizure 20 (2011) 409–413.
[29] D. Nieoczym, K. Socała, J.J. Łuszczki, S.J. Czuczwar, P. Wlaź, Sildenafil influences the anticonvulsant activity of vigabatrin and gabapentin in the timed pentylenetetrazole infusion test in mice, Prog. Neuropsychopharmacol. Biol. Psychiatry 39 (2012) 129–135.
[30] D. Nieoczym, K. Socała, P. Jedziniak, M. Olejnik, P. Wlaź, Effect of sildenafil, a selective phosphodiesterase 5 inhibitor, on the anticonvulsant action of some antiepileptic drugs in the mouse 6-Hz psychomotor seizure model, Prog. Neuropsychopharmacol. Biol. Psychiatry 47 (2013) 104–110.
[31] D. Nieoczym, J.J. Łuszczki, S.J. Czuczwar, P. Wlaź, Effect of sildenafil on the anticonvulsant action of classical and second-generation antiepileptic drugs in maximal electroshock-induced seizures in mice, Epilepsia 51 (2010) 1552–1559.
[32] D. Nieoczym, K. Socała, C. Rundfeldt, P. Wlaź, Effects of sildenafil on pentylenetetrazol-induced convulsions in mice and amygdala-kindled seizures in rats, Pharmacol. Rep. 62 (2010) 383–391.
[33] D. Nieoczym, K. Socała, P. Wlaź, Lack of effect of sildenafil on cocaine-induced convulsions in mice, Pharmacol. Rep. 61 (2009) 930–934.
[34] D. Nieoczym, K. Socała, J.J. Łuszczki, S.J. Czuczwar, P. Wlaź, Influence of sildenafil on the anticonvulsant action of selected antiepileptic drugs against pentylenetetrazole-induced clonic seizures in mice, J. Neural Transm. 119 (2012) 923–931.
[35] M. Atif, M.R. Sarwar, S. Scahill, The relationship between epilepsy and sexual dysfunction: a review of the literature, Springerplus 5 (2016) 2070.
[36] M. Barker-Haliski, H.S. White, Glutamatergic mechanisms associated with seizures and epilepsy, Cold Spring Harb. Perspect. Med. 5 (2015) a022863.
[37] W. Lasoń, M. Dudra-Jastrzębska, K. Rejdak, S.J. Czuczwar, Basic mechanisms of antiepileptic drugs and their pharmacokinetic/pharmacodynamic interactions: an update, Pharmacol. Rep. 63 (2011) 271–292.
[38] D.M. Treiman, GABAergic mechanisms in epilepsy, Epilepsia 42 (Suppl. 3) (2001) 8–12.
[39] K. Riazi, M. Roshanpour, N. Rafiei-Tabatabaei, H. Homayoun, F. Ebrahimi, A.R. Dehpour, The proconvulsant effect of sildenafil in mice: role of nitric oxide-cGMP pathway, Br. J. Pharmacol. 147 (2006) 935–943.
[40] A. Bahremand, S.E. Nasrabady, P. Ziai, R. Rahimian, T. Hedayat, B. Payandemehr, A.R. Dehpour, Involvement of nitric oxide-cGMP pathway in the anticonvulsant effects of lithium chloride on PTZ-induced seizure in mice, Epilepsy Res. 89 (2010) 295–302.
[41] M. Khoshneviszadeh, R. Rahimian, G. Fakhfouri, B. Payandemehr, F. Khodagholi, M.S. Ejtemaei, A.R. Dehpour, Oxytocin is involved in the proconvulsant effects of Sildenafil: possible role of CREB, Toxicol. Lett. 256 (2016) 44–52.
[42] K.M. Tawfik, Y.M. Moustafa, M.F. El-Azab, Neuroprotective mechanisms of sildenafil and selenium in PTZ-kindling model: implications in epilepsy, Eur. J. Pharmacol. 833 (2018) 131–144.
[43] J.J. Lipman, P.S. Spencer, Rapid intracerebroventricular injection assisted by an automatic syringe, J. Pharmacol. Methods 4 (1980) 327–333.
[44] K. Socała, D. Nieoczym, M. Pieróg, P. Wlaź, Role of the adenosine system and glucose restriction in the acute anticonvulsant effect of caprylic acid in the 6 Hz psychomotor seizure test in mice, Prog. Neuropsychopharmacol. Biol. Psychiatry 57 (2015) 44–51.
[45] M.E. Barton, B.D. Klein, H.H. Wolf, H.S. White, Pharmacological characterization of the 6 Hz psychomotor seizure model of partial epilepsy, Epilepsy Res. 47 (2001) 217–227.
[46] A.W. Kimball, W.T. Burnett Jr., D.G. Doherty, Chemical protection against ionizing radiation. I. Sampling methods for screening compounds in radiation protection studies with mice, Radiat. Res. 7 (1957) 1–12.
[47] O.A. Meyer, H.A. Tilson, W.C. Byrd, M.T. Riley, A method for the routine assessment of fore- and hindlimb grip strength of rats and mice, Neurobehav. Toxicol. 1 (1979) 233–236.
[48] D. Boison, Adenosinergic signaling in epilepsy, Neuropharmacology 104 (2016) 131–139.
[49] M.H. Gharedaghi, M. Seyedabadi, J.E. Ghia, A.R. Dehpour, R. Rahimian, The role of different serotonin receptor subtypes in seizure susceptibility, Exp. Brain Res. 232 (2014) 347–367.
[50] J.S. Hong, Hippocampal opioid peptides and seizures, Epilepsy Res. Suppl. 7 (1992) 187–195.
[51] G.R. Lauretti, I. Ahmad, B.J. Pleuvry, The activity of opioid analgesics in seizure models utilizing N-methyl-DL-aspartic acid, kainic acid, bicuculline and pentylenetetrazole, Neuropharmacology 33 (1994) 155–160.
[52] R.W. Olsen, GABAA receptor: positive and negative allosteric modulators, Neuropharmacology (2018) 10.
[53] S.N. Mandhane, K. Aavula, T. Rajamannar, Timed pentylenetetrazol infusion test: a comparative analysis with s.c.PTZ and MES models of anticonvulsant screening in mice, Seizure 16 (2007) 636–644.
[54] P. Saransaari, S.S. Oja, Characteristics of GABA release induced by free radicals in mouse hippocampal slices, Neurochem. Res. 33 (2008) 384–393.
[55] R. Feil, T. Kleppisch, NO/cGMP-dependent modulation of synaptic transmission, Handb. Exp. Pharmacol. 184 (2008) 529–560.
[56] K. Varani, F. Vincenzi, S. Merighi, S. Gessi, P.A. Borea, Biochemical and pharmacological role of A1 adenosine receptors and their modulation as novel therapeutic strategy, Adv. Exp. Med. Biol. 1051 (2017) 193–232.
[57] M.J. Świader, J. Kotowski, J.J. Łuszczki, Modulation of adenosinergic system and its application for the treatment of epilepsy, Pharmacol. Rep. 66 (2014) 335–342.
[58] J. Szyndler, P. Maciejak, D. Turzyńska, A. Sobolewska, A. Bidziński, A. Płaźnik, Time course of changes in the concentrations of monoamines in the brain structures of pentylenetetrazole-kindled rats, J. Neural Transm. 117 (2010) 707–718.
[59] A. Bourson, V.Ă. Kapps, C. Zwingelstein, A. Rudler, F.G. Boess, A.J. Sleight, Correlation between 5-HT7 receptor affinity and protection against sound-induced seizures in DBA/2J mice, Schmiedebergs Arch. Pharmacol 356 (1997) 820–826.
[60] M. Ghasemi, H. Shafaroodi, S. Nazarbeiki, H. Meskar, A. Ghasemi, A. Bahremand, P. Ziai, A.R. Dehpour, Inhibition of NMDA receptor/NO signaling blocked tolerance to the anticonvulsant effect of morphine on pentylenetetrazole-induced seizures in mice, Epilepsy Res. 91 (2010) 39–48.
[61] M. Gholami, E. Saboory, S. Roshan-Milani, Proconvulsant effects of tramadol and morphine on pentylenetetrazol-induced seizures in adult rats using different routes

of administration, Epilepsy Behav. 36 (2014) 90–96.
[62] J. Fichna, K. Socała, D. Nieoczym, K. Gach, R. Perlikowska, A. Janecka, P. Wlaź, The mu-opioid receptor-selective peptide antagonists, antanal-1 and antanal-2, produce anticonvulsant effects in mice, Prog. Neuropsychopharmacol. Biol. Psychiatry 40 (2013) 126–131.
[63] M. Zaccolo, M.A. Movsesian, cAMP and cGMP signaling cross-talk: role of phosphodiesterases and implications for cardiac pathophysiology, Circ. Res. 100 (2007) 1569–1578.
[64] L. Gomez, J.G. Breitenbucher, PDE2 inhibition: potential for the treatment of cognitive disorders, Bioorg. Med. Chem. Lett. 23 (2013) 6522–6527.
[65] M. Polito, J. Klarenbeek, K. Jalink, D. Paupardin-Tritsch, P. Vincent, L.R. Castro, The NO/cGMP pathway inhibits transient cAMP signals through the activation of PDE2 in striatal neurons, Front. Cell. Neurosci. 7 (2013) 211.
[66] G.P. Ahern, V.A. Klyachko, M.B. Jackson, cGMP and S-nitrosylation: two routes for modulation of neuronal excitability by NO, Trends Neurosci. 25 (2002) 510–517.
[67] H. Prast, A. Philippu, Nitric oxide as modulator of neuronal function, Prog. Neurobiol. 64 (2001) 51–68.