

Original Article

The role of muscarinic and serotonergic-2A receptors in the antinociceptive effect of pregabalin

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Abstract

Introduction: Pregabalin (PGB) is an analog of gamma-aminobutyric acid (GABA) with antinociceptive, antihyperalgesic and antiallodynic properties which frequently used in clinical pain management. Effect of PBG in neuropathic pain, incisional-inflammatory injury, post-operational pain, chronic pain and experimental pain models have already shown. It has been already known that muscarinic and serotonergic-2A receptors have a role in pain transmission.

Methods: in this study, role of muscarinic and serotonergic-2A receptors in antinociceptive effect of pregabalin were evaluated with hot-plate and tail flick tests and effects of administered drugs on locomotor activity were measured with automated activity cage.

Results: PGB treatment (30 and 100mg/kg) caused longer latency in hot plate and tail flick tests than saline group. That antinociceptive effect of pregabalin abolished by ketanserin (1mg/kg) and atropine (1mg/kg) treatment.

Conclusion: However, there is lack of knowledge about role of nociceptive pathways underlying pregabalin mediated antinociception. Our results suggest that cholinergic and serotonergic systems have a role in antinociceptive effect of PGB which has seen in these somatic pain tests.

Keywords:

Pregabalin;
Somatic pain;
Ketanserin;
Atropine

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Introduction

Pain is the most often encountered major health issue in various of pathology and diseases. Besides, difficulty in interpreting pain objectively, main therapy is based on opioids in agonizing pain and non-steroidal anti-inflammatory drugs in mild pain (Ripamonti, 2012). But, these drugs sometimes have a lot of adverse effects and ineffective for treatment.

Accompanying complex pathological processes and interactions of classical analgesics with other drugs leading researchers to investigate new potential treatment options with fewer side effects and suitable combinations (Gore et al., 2007).

Role of cholinergic and serotonergic system in pain modulation have been already shown and receptors in dorsal horn of the spinal cord behind nociceptive effect demonstrated in experimental models (Avery and Krichmar, 2017). Intrathecal administration of

cholinergic agents have a role in nociception by cholinergic interneurons (Rashid and Ueda, 2002). Additionally, role of serotonergic receptors 5-hydroxytryptamine (5-HT)_{1A} (Bardin, 2011), 5-HT₂ (Xie et al., 2012) and 5-HT₃ in modulation of pain also demonstrated. Opioidergic and GABAergic interactions have been claimed for intricate pain modulation mechanism (Rashvand et al., 2014). Although, GABAergic system give rise to thought of pregabalin antinociceptive mechanism, existing evidence indicated that effects of pregabalin are GABA and GABAergic system independent. Pregabalin (PGB) has lack of affinity for GABAA, GABAB and benzodiazepine receptors (Marks et al., 2009).

PGB binds $\alpha_2\delta$ subunit of voltage gated calcium channels (VGCCs) in presynaptic cleft. In overstimulated neurons, PGB cause a decrease in neurotransmitter release. PGB only modulates stimulated neuron activity (Sikandar and Dickenson, 2011). VGCCs basically consist of α , $\alpha_2\delta$, β and γ subunits. PGB binds $\alpha_2\delta$ subunit potently and regulate Ca^{++} conduction and thus, cause reduction in various neurotransmitters, which have a role in pain modulation, such as glutamate, norepinephrine, serotonin, dopamine and substance P (Fehrenbacher et al., 2003). Decrease in the stimulant neurotransmitter release cause reduction in excitatory neurotransmitter release leads to a decrease in postsynaptic receptor stimulation. Along with anxiolytic properties, analgesic and anti-convulsant effects of pregabalin mainly assumed on account of decreased stimulation. Although there are several reports about PGB-induced antinociception on hot-plate and tail flick tests, there is lack of knowledge

about PGB antinociceptive mechanism (Meymandi et al., 2015). Given this background, the aim of the study is to investigate role of cholinergic and serotonergic systems in acute somatic pain model for the antinociceptive effect of pregabalin.

Materials and methods

Animals

The 12 weeks old male Balb-C mice, weighing 25-40g were used for experiments. All animals were housed four or five mice per cage at a controlled temperature and conditions (22±1°C, %60 humidity, 12/12 light-dark cycle) with free access to food and water. The study protocol was approved by Institutional Animal Care and Use Committee of the Ondokuz Mayıs University (HEK/177) and conducted in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals.

Experimental design and drug treatments

Atropine sulphate, ketanserin tartarate and pregabalin were obtained from Sigma-Aldrich Inc. (St. Louis, MO). All drugs were freshly dissolved in normal saline and injected intraperitoneally (ip) at a constant volume of 10ml/kg. Muscarinic receptor antagonist dose of atropine sulphate and 5-HT_{2a} antagonist dose of ketanserin tartarate for mice was selected from previous studies (Pinaridi et al., 2003; Santos et al., 2005). The groups were established as per the sample sizes outlined in Table 1.

Hot plate test

Hot plate test was carried out with metal platform (Model:7280, Ugo Basile, Milan, Italy). Mice were

Table 1: Description of experimental groups

Groups	Drug treatments
1 (n=8)	Saline
2 (n=8)	Pregabalin 10mg/kg
3 (n=8)	Pregabalin 30mg/kg
4 (n=8)	Pregabalin 100mg/kg
5 (n=8)	Atropine 1mg/kg
6 (n=8)	Ketanserin 1mg/kg
7 (n=8)	Atropine 1mg/kg + pregabalin 30mg/kg
8 (n=8)	Ketanserin 1mg/kg + pregabalin 30mg/kg

placed on a hot plate which was set at $54\pm 0.4^{\circ}\text{C}$. Mice were observed for discomfort reactions such as licking their forepaws, hindpaws or jumping and latency for reaction was recorded. Animals which failed to step their paws were removed from the plate.

Tail flick test

Tail flick test was employed with radiant heat supply (COMMAT TF 211-01, TURKEY). Tails of mice were placed carefully and withdrawal of tails from heat source was observed and latency time recorded with chronometer. Latency time as accepted from the onset of heat exposure to withdrawal of the tail. To avoid tissue damages heat stimulus was discontinued after 10 seconds.

Locomotor activity

In order to investigate effects of drugs on locomotor activity, the activity cage apparatus (41cm^2 with 33cm walls) was used (Ugo Basile, Biological Research Apparatus). Mice were placed and individually tracked for five minutes in the activity cage.

Statistical analysis

All data are presented as mean \pm SEM (standard error of mean). One-way analysis of variance (ANOVA) followed by Tukey's test was used to evaluate significance between groups. Statistical analysis was performed using SPSS software v21.0 (Chicago, Illinois, USA) and P values <0.5 were considered statistically significant.

Results

Effects of PGB treatment on hot plate test

In order to determine antinociceptive action of PGB, intraperitoneally administered 10mg/kg, 30mg/kg and 100mg/kg doses were compared. The 30 and 100mg/kg doses caused significant longer time compared to saline group ($P<0.001$, Fig. 1a). However, 10mg/kg PGB administration failed to show significance compared to saline group ($P>0.05$, Fig. 1A).

Ketanserin and atropine treatment reversed antinociceptive effect of PGB in hot plate test

Before administration of ketanserin to PGB administered rats, effect of ketanserin (1mg/kg) investigated separately but failed to show difference

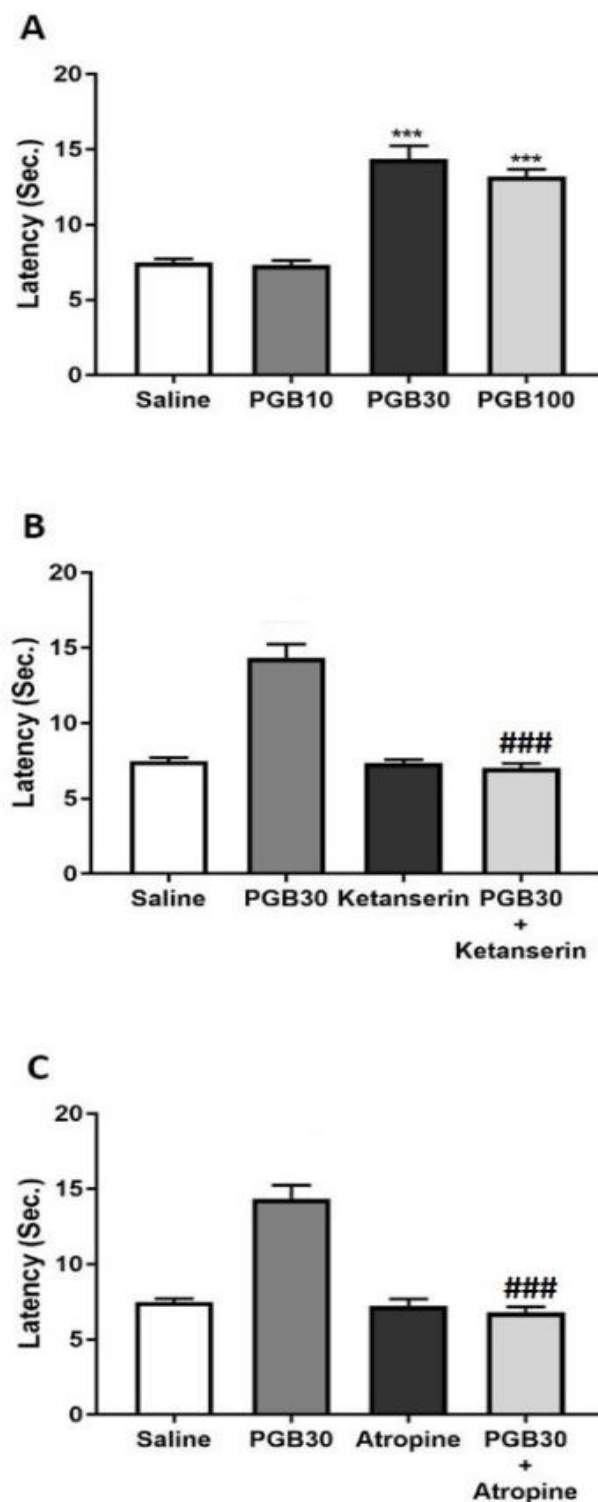


Fig.1. (A) Antinociceptive effect of pregabalin at 30 minutes (10, 30 and 100mg/kg, ip) in hot-plate test. Effect of ketanserin (B) and atropine (C) on the antinociceptive effect of pregabalin (30mg/kg). *** $P<0.001$ compared to saline, ### $P<0.001$ compared to PGB30 group.

between saline group ($P>0.05$, Fig. 1B). Later on, ketanserin administration to PGB (30mg/kg) treated rats, ketanserin reversed antinociceptive effect of

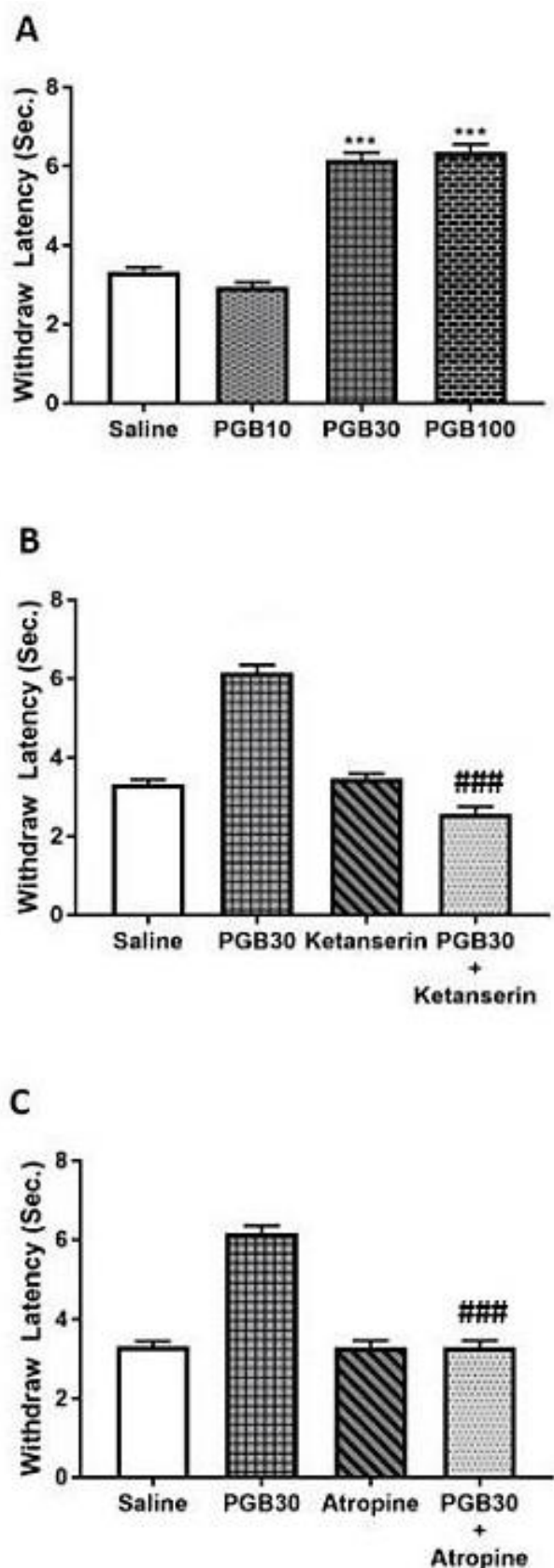


Fig.2. (A) Antinociceptive effect of pregabalin at 30 minutes (10, 30, 100 mg/kg, ip) in tail-flick test. Effect of ketanserin (B) and atropine (C) on the antinociceptive effect of pregabalin (30mg/kg). ^{***} $P < 0.001$ compared to saline, ^{###} $P < 0.001$ compared to PGB30 group.

PGB significantly ($P < 0.001$, Fig. 1B). Single administration of atropine (1mg/kg) was failed to show significance compared to saline group ($P > 0.05$, Fig. 1C); but, atropine (1mg/kg) administration to PGB (30mg/kg) treated mice, caused significant decrease in antinociceptive effect of PGB ($P < 0.001$, Fig. 1C).

Effects of pregabalin on tail flick test

Although, like hot-plate test results, PGB (10mg/kg) had no difference in tail withdrawal latency ($P > 0.05$), PGB (30 and 100mg/kg) administration caused significant antinociception compared to saline group ($P < 0.001$, Fig. 2A).

Ketanserin and atropine treatment on antinociceptive effect of PGB in tail flick test

In order to clarify to the effect of ketanserin treatment on tail flick test, single administration of ketanserin caused no difference in tail withdrawal time. But, ketanserin (1mg/kg) treatment caused significant decrease in withdrawal time in PGB (30mg/kg) administered mice ($P < 0.001$, Fig. 2B). Atropine (1mg/kg) treatment failed to showed difference with saline group ($P > 0.05$, Fig. 2C). However, atropine (1mg/kg) treatment to PGB (30mg/kg) group reversed the antinociceptive effect of PGB in tail flick test, significantly ($P < 0.001$, Fig. 2C).

Locomotor activity results of drug treatments

Among all the treatment groups, only PGB (100mg/kg) caused significant decrease in locomotor activity ($P < 0.001$, Fig. 3). Serotonin antagonism (5-HT_{2A} receptors) and cholinergic system antagonism (muscarinic receptors) did not effect locomotor activity of mice. Additionally PGB30 and PGB10 groups failed to show difference compared to saline group ($P > 0.05$).

Discussion

In this study, administration of 30 and 100mg/kg PGB shows antinociceptive effect and this effect was reduced by administration of muscarinic receptor antagonist atropine and 5-HT₂ receptor antagonist ketanserin.

Changes in Na⁺ channels expression in nociceptors and sensory neurons have been claimed to be the possible mechanism for neuropathic pain. Thus,

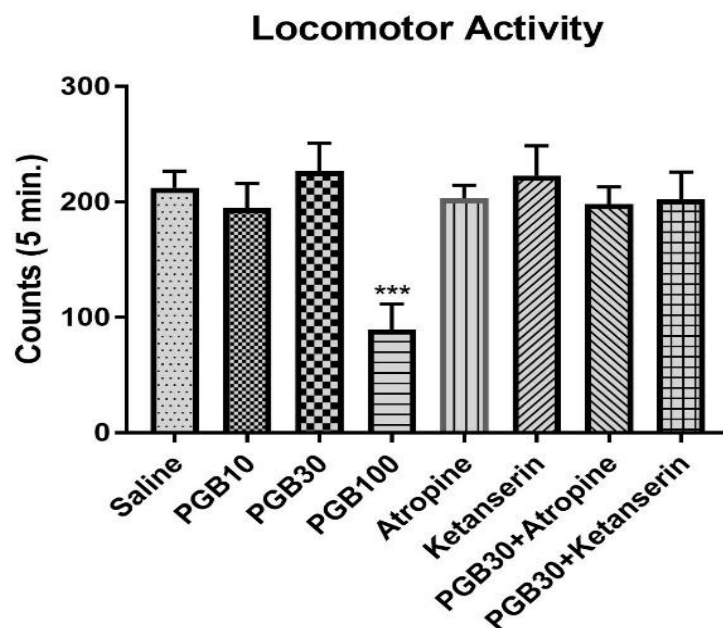


Fig.3. Ketanserin and atropine treatment did not cause any impairment in locomotor activity test when administered separately or concomitantly with PGB30. Only pregabalin 100mg/kg caused significant difference compared to saline group (** $p < 0.001$).

effects of anti-epileptic drugs in neuropathic pain related with that Na^+ channel activity. Also, importance of T type- Ca^{+2} channels was proposed antiepileptic drugs (ethosuximide and zonisamid) mechanism of action on neuropathic pain which mediated by Ca^{+2} channel blockage (Rogawski and Löscher, 2004). Antinociceptive effect of PGB has been shown in various experimental pain models. However, that effect mostly observed in chemical stimulated-visceral pain models such as formalin and writhing test, it was investigated in tail clip (Kaygisiz et al., 2015) and tail flick (Meymandi et al., 2015) tests which were based on mechanic and thermal stimulation. The dose dependent antinociceptive effect of PGB was also observed in acetic acid induced-visceral pain model (Shamsi Meymandi and Keyhanfar, 2013). Additionally, PGB decreased otonomic response of pain related visceromotor response which employed with mechanical stimulation of colon in rat (Ravnefjord et al., 2008). Luszczi (2010) also observed dose-dependent antinociceptive effect of PGB in hot plate test somatic pain study. Furthermore, PGB-induced antinociception has been showed in acetic acid-induced writhing test which is used for evaluation of peripheral nociception (Kaygisiz et al., 2015). Although, PGB (100mg/kg) increased withdraw latency, administration of NMDA receptor antagonist

MK801 had no effect on antinociceptive effect of PGB (Meymandi et al., 2015).

The 5-HT has complex role in pain modulation. It has been suggested that serotonin receptors, depending on nociceptive stimulant, receptors subtype and dose of serotonergic agonist and antagonist, modulate nociceptor reflexes and could be inhibitors in nociceptive responses (Kurihara et al., 2003). Because of intricate mechanism behind serotonin modulation on nociception, several studies investigated subtype of serotonin receptors (Sandrini et al., 2002). In a few of these studies ketanserin antagonized antinociceptive effect of paracetamol (Ruggieri et al., 2008). Contrary to our study Kaygisiz et al. investigated antinociceptive effect of PGB with 5-HT₂ receptor antagonist siproheptadin and assert no difference between PGB and combination with siproheptadin (Kaygisiz et al., 2015; Dolphin, 2013). But, high dose and selectivity of ketanserin on serotonin receptors blocked antinociceptive effect of PGB which was observed in our study and suggested that possible interactions between serotonin receptors and Ca^{+2} channels.

There are several reports which claim role of cholinergic system in antinociception (Bartolini et al., 2011). Muscarinic receptors in the dorsal horn of spinal cord have strong innervation with GABAergic, opioidergic, serotonergic and adrenergic systems

which are possibly related with antinociception (Honda et al., 2003; Kommalage and Höglund, 2005). Additionally, subtypes of muscarinic receptors (M_1 , M_2 , M_3 and M_4) contributes that antinociception (Bartolini et al., 2011) and supraspinal administration of selective M_1 and M_4 receptor toxins (MT-7, MT-3) abolished antinociceptive effect of cholinergic agonist xanomeline (Chen and Pan, 2004; Martino et al., 2011). Moreover, longer latency in tail withdraw which has been seen with morphine administration reversed with muscarinic receptor antagonist atropine (Foroud and Vesal, 2015). In our study reduction of effect of PGB with atropine administration could be in consequence of sole atropine effect on pain pathways. However, alone administration of atropine lack of antinociceptive effect in our study. Furthermore, PGB binding $\alpha 2\delta$ subunit of Ca^{+2} channels caused cholinergic activation and up-regulated inhibitor type muscarinic receptors in sensory afferents (Hayashida et al., 2006).

Conclusion

In our study, PGB showed antinociceptive action in hot plate and tail flick somatic pain models. Antinociceptive effect of PGB reversed with 5-HT₂ and muscarinic receptor antagonists. Possible mechanism underlying antinociceptive effect of PGB could be related with Ca^{+2} channel binding and release of neurotransmitters but further studies needed. We hope our study provide basic aspects of different mechanism in antinociceptive action of PGB for investigation in future studies.

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Conflict of interest

The authors or any individual reported conflict of interest statement about current manuscript.

References

- Avery MC, Krichmar JL. Neuromodulatory systems and their interactions: a review of models, theories, and experiments. *Front Neural Circuits* 2017; 11: 108.
- Bardin L. The complex role of serotonin and 5-HT receptors in chronic pain. *Behav Pharmacol* 2011; 22: 390-404.
- Bartolini A, Di Cesare Mannelli L, Ghelardini C. Analgesic and antineuropathic drugs acting through central cholinergic mechanisms. *Recent Pat CNS Drug Discov* 2011; 6: p. 119-40.
- Chen SR, Pan HL. Activation of muscarinic receptors inhibits spinal dorsal horn projection neurons: role of GABAB receptors. *Neuroscience* 2004; 125: 141-8.
- Dolphin AC. The $\alpha 2\delta$ subunits of voltage-gated calcium channels. *Biochim Biophys Acta* 2013; 1828: 1541-9.
- Fehrenbacher JC, Taylor CP, Vasko MR. Pregabalin and gabapentin reduce release of substance P and CGRP from rat spinal tissues only after inflammation or activation of protein kinase C. *Pain* 2003; 105: 133-41.
- Foroud M, Vesal N. Evaluation of the anti-nociceptive effects of morphine, tramadol, meloxicam and their combinations using the tail-flick test in rats. *Vet Res Forum* 2015; 6: 313-8.
- Gore M, Sadosky A, Tai KS, Stacey B. A retrospective evaluation of the use of gabapentin and pregabalin in patients with postherpetic neuralgia in usual-care settings. *Clin Ther* 2007; 29: 1655-70.
- Hayashida KI, Bynum T, Vincler M, Eisenach JC. Inhibitory M2 muscarinic receptors are upregulated in both axotomized and intact small diameter dorsal root ganglion cells after peripheral nerve injury. *Neuroscience* 2006; 140: 259-68.
- Honda K, Murao N, Ibuki T, Kamiya HO, Takano Y. The role of spinal muscarinic acetylcholine receptors in clonidine-induced anti-nociceptive effects in rats. *Biol Pharm Bull* 2003; 26: 1178-80.
- Kaygisiz B, Kilic FS, Senguleroglu N, Baydemir C, Erol K. The antinociceptive effect and mechanisms of action of pregabalin in mice. *Pharmacol Rep* 2015; 67: 129-33.
- Kommalage M, Höglund AU. Involvement of spinal serotonin receptors in the regulation of intraspinal acetylcholine release. *Eur J Pharmacol* 2005; 509: 127-34.
- Kurihara T, Nonaka T, Tanabe T. Acetic acid conditioning stimulus induces long-lasting antinociception of somatic inflammatory pain. *Pharmacol Biochem Behav* 2003; 74: 841-9.
- Luszczki JJ. Dose-response relationship analysis of pregabalin doses and their antinociceptive effects in hot-plate test in mice. *Pharmacol Rep* 2010; 62: 942-8.
- Marks DM, Patkar AA, Masand PS, Pae CU. Does pregabalin have neuropsychotropic effects?: a short perspective. *Psychiatry Investig* 2009; 6: 55-8.
- Martino G, Puma C, Yu XH, Gilbert AK, Coupal M, Markoglou N, et al. The M1/M4 preferring agonist xanomeline is analgesic in rodent models of chronic inflammatory and neuropathic pain via central site of action. *Pain* 2011; 152: 2852-60.
- Meymandi MS, Keyhanfar F, Yazdanpanah O, Heravi G. The role of NMDARs ligands on antinociceptive effects of pregabalin in the tail flick test. *Anesth Pain Med* 2015; 5: e28968.
- Pinardi G, Sierralta F, Miranda HF. Atropine reverses the antinociception of nonsteroidal anti-inflammatory drugs in the tail-flick test of mice. *Pharmacol Biochem Behav*

- 2003; 74: 603-8.
- Rashid MH, Ueda H. Neuropathy-specific analgesic action of intrathecal nicotinic agonists and its spinal GABA-mediated mechanism. *Brain Res* 2002; 953: 53-62.
- Rashvand M, Khajavai A, Parviz M, Hasanein P, Keshavarz M. GABAA receptors are involved in the analgesic effects of morphine microinjected into the central nucleus of the amygdala. *Clin Exp Pharmacol Physiol* 2014; 41: 338-44.
- Ravnefjord A, Brusberg M, Larsson H, Lindström E, Martínez V. Effects of pregabalin on visceral pain responses and colonic compliance in rats. *Br J Pharmacol* 2008; 155: 407-16.
- Ripamonti CI. Pain management. *Ann Oncol* 2012; 10: x294-301.
- Rogawski MA, Löscher W. The neurobiology of antiepileptic drugs for the treatment of nonepileptic conditions. *Nat Med* 2004; 10: 685-92.
- Ruggieri V, Vitale G, Pini LA, Sandrini M. Differential involvement of opioidergic and serotonergic systems in the antinociceptive activity of N-arachidonoyl-phenolamine (AM404) in the rat: comparison with paracetamol. *Naunyn Schmiedebergs Arch Pharmacol* 2008; 377: 219-29.
- Sandrini M, Vitale G, Pini LA. Central antinociceptive activity of acetylsalicylic acid is modulated by brain serotonin receptor subtypes. *Pharmacology* 2002; 65: 193-7.
- Santos AR, Gadotti VM, Oliveira GL, Tibola D, Paszcuk AF, Neto A, et al. Mechanisms involved in the antinociception caused by agmatine in mice. *Neuropharmacology* 2005; 48: 1021-34.
- Shamsi Meymandi M, Keyhanfar F. Relative potency of pregabalin, gabapentin, and morphine in a mouse model of visceral pain. *Can J Anaesth* 2013; 60: 44-9.
- Sikandar S, Dickenson AH. Pregabalin modulation of spinal and brainstem visceral nociceptive processing. *Pain* 2011; 152: 2312-22.
- Xie DJ, Uta D, Feng PY, Wakita M, Shin MC, Furue H, et al. Identification of 5-HT receptor subtypes enhancing inhibitory transmission in the rat spinal dorsal horn in vitro. *Mol Pain* 2012; 8: 58.