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Research Article

Role of K_v7 Potassium Channels in The Morphine-Induced Antinociception in Acute and Neuropathic Pain

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Summary

Objective: We aimed to investigate the role of K_v7 potassium channels in the morphine-induced antinociception in both acute and neuropathic pain models.
Methods: Neuropathic pain was induced by partial ligation of the right sciatic nerve. For intracerebroventricular (i.c.v.) injections, each rat was equipped with a permanent cannula. The response to painful thermal stimuli was assessed by the tail-flick test. In sciatic nerve-ligated rats, thermal hyperalgesia was assessed by paw withdrawal latencies in the plantar test and the mechanical hyperalgesia was determined by rigid von Frey filaments.
Results: Rats received morphine (2, 5, 20 µg/10 µl; i.c.v.) or saline (10 µl; i.c.v.) 15 min before the tests. In all tests, morphine produced significant antinociceptive effect. When a K_v7 potassium channel blocker, linopirdine (0.1, 1, 10 µg/10 µl; i.c.v.) was administered 15 min before morphine, the effect of i.c.v. morphine in the tail-flick test and plantar test but not in the test with von Frey filaments were prevented.
Conclusions: K_v7 potassium channels contribute to the effect of i.c.v. morphine on acute pain induced by thermal stimulation. In the sciatic nerve-ligated rats, these channels play role in the effect of morphine on thermal hyperalgesia, but not on mechanical hyperalgesia.

Key words: Antinociception, K_v7 potassium channels, morphine, linopirdine, tail-flick test, plantar test, von Frey filaments, rat

Akut ve Nöropatik Ağrıda Morfine Bağlı Antinosisepsiyonda K_v7 Potasyum Kanallarının Rolü

Özet

Amaç: Akut ve nöropatik ağrı modellerinde morfine bağlı antinosisepsiyonda K_v7 potasyum kanallarının rolünü araştırmayı amaçladık.
Materyal ve Metod: Nöropatik ağrı oluşturmak için sağ siyatik sinire parsiyel ligasyon uygulandı. Intraserebroventriküler (i.c.v.) enjeksiyonlar için, her sıçana kalıcı kanül takıldı. Ağrılı termal uyarana yanıt tail-flick testi ile değerlendirildi. Siyatik sinir ligasyonu yapılan sıçanlarda, termal hiperaljezi plantar testte pençe çekme latansı ile, mekanik hiperaljezi ise rijid von Frey filamanları ile değerlendirildi.
Bulgular: Sıçanlara testlerden 15 dakika önce morfin (2, 5, 20 µg/10 µl; i.c.v.) veya salin (10 µl; i.c.v.) uygulandı.Tüm testlerde morfin anlamlı antinosiseptif etki gösterdi. Bir K_v7 potasyum kanal blokeri olan linopirdin (0.1, 1, 10 µg/10 µl; i.c.v.) morfinden 15 dakika önce uygulandığında, i.c.v. morfinin tail-flick ve plantar testte görülen etkilerini önlerken, von Frey filamanları ile yapılan test üzerine etkilerini değiştirmedi.

Sonuç: K_v7 potasyum kanalları termal stimülasyona bağlı akut ağrıda i.c.v. morfinin etkisine katılmaktadırlar. Siyatik sinir ligasyonlu sıçanlarda, bu kanallar, morfinin termal hiperaljeziye etkisinde rol oynarken, mekanik hiperaljezi üzerine etkisinde rolü yoktur.

Anahtar Kelimeler: Antinosisepsiyon, K_v7 potasyum kanalları, morfin, linopirdin, tail-flick testi, plantar test, von Frey filamanları, sıçan

INTRODUCTION

Morphine is an opioid which is widely used for the treatment of severe pain, such as pain caused by terminal cancer and myocardial infarction. Although it is quite effective in pain control, adverse effects such as constipation, nausea, respiratory depression, psychological and physical dependence, as well as development of tolerance limit its use[14]. Morphine mediates its actions by binding and activating receptors both in the peripheral nervous system and those that are found in inhibitory pain circuits that descend from the midbrain to the spinal cord dorsal horn[24].

The antinociceptive effect of morphine seems to be modulated by K^+ channels. It has been shown that, the selective blockers of ATP-sensitive K^+ channels glibenclamide and tolbutamide antagonize the peripheral antinociception induced by morphine[25,34], while the K^+ channel opener pinacidil significantly increases and prolongs the effect of morphine[40]. Ikeda et al have shown that, G-protein-activated inwardly rectifying K^+ (GIRK) channel activation is important in the induction of analgesia by morphine[13]. In addition, K^+ channels per se appear to be involved in the modulation of pain perception[3,9,23]. Particularly, voltage-activated K_v7 channels deserve more attention, since these channels play a key role in controlling the excitability of neurons within the nervous system[15]. K_v7 channels are a family of six transmembrane domain voltage-gated K^+ channels consisting of five family members ($K_v7.1$-$K_v7.5$), which are mainly expressed in heart muscle ($K_v7.1$), the central nervous system, ($K_v7.2$-5), the peripheral neurons (M channels) and the inner ear ($K_v7.1$, $K_v7.4$)[8,2,15].

K_v7 channels control somatic excitability, bursting and neurotransmitter release througout the nervous system[12]. It has been reported that, a K_v7 channel opener, retigabine has antinociceptive effects in animal models of persistent and chronic pain, which were fully reversed by the selective K_v7 blocker XE-991[3]. Dost et al. have also shown the contribution of K_v7 channels to the anti-allodynic activity of retigabine[9].

Pain is a perception which constitutes an alarm that helps to protect the organism, by triggering reactions and inducing learned avoidance behaviors to limit the potentially damaging consequences[17]. Acute pain can be adequately controlled by currently used analgesics in most cases. On the other hand, neuropathic pain is generally more difficult to control and respond poorly to common medication, particularly depending on the underlying mechanisms. Neuropathic pain is defined as “pain initiated or caused by a primary lesion or dysfunction of the nervous system that, under normal conditions, transmits noxious information”(16). Peripheral neuropathic pain is a complex syndrome resulting from damage to the peripheral nervous system due to trauma, compression, neurotoxins, infection, immune and metabolic diseases, tumors, vitamin deficiencies and other causes[42]. It is often associated with the appearance of abnormal sensory signs such as allodydnia (pain as a result of a stimulus which does not normally provoke pain) or hyperalgesia (an increased response to a stimulus which is normally painful)[4]. Neuropathic pain can have delayed onset after initial nerve injury, thus pain may be

present in the absence of detectable lesion or injury[42].

One of the clinically relevant animal models for neuropathic pain is partial sciatic nerve ligation, introduced by Seltzer et al. [36]. In this model, signs of allodynia to von Frey hair stimulation and hyperalgesia to both thermal and mechano-noxious stimuli are observed within hours of ligation, and the symptoms last for over 7 months[42].

A number of animal models for both acute and neuropathic pain have been developed and widely used by researchers to explain the pathophysiology of various types of pain and to identify novel therapeutics for clinical use. Here, we used both acute and neuropathic pain models to investigate the contribution of K_v7 channels to morphine-induced antinociception.

METHODS AND RESULTS

Animals

Adult male Sprague Dawley rats, weighing 300-350 g were obtained from Uludag University Experimental Animals Breeding and Research Centre and were housed 4-6 in a cage with food and water available ad libitum. The colony room was maintained at 20-24 °C with a 12-h light-dark cycle. The surgical and experimental protocols used were approved by the Animals Care and Use Committee of Uludag University. All treatments were in accordance with the National Institutes of Health Guide of the Care and Use of Laboratory Animals.

Surgery

To induce neuropathic pain, partial ligation of the right sciatic nerve was performed, according to Seltzer et al.[36]. Under ether anesthesia, the right sciatic nerve was exposed at high thigh level through a small incision. The nerve was carefully freed from surrounding connective tissues at a site near the trochanter just distal to the point at which the posterior biceps semitendinosus nerve branches off the common sciatic nerve. Then, the dorsal 1/3-1/2 of the nerve thickness was tightly ligated with 6-0 suture. The wound was closed with stitches. In all rats the left leg and sciatic nerve were untouched.

For intracerebroventricular (i.c.v.) injections, each rat was equipped with a permanent cannula. Under ether anesthesia, rat was placed in a stereotaxic instrument (Harvard Appartus, Hollistan, MA, USA) a burr hole was drilled through the skull 1.5 mm lateral to the midline and 1-1.5 mm posterior to bregma on the right side. Through this hole, a 10 mm length of 20 gauge stainless steel hypodermic tubing was directed toward the right lateral ventricle. The cannula was lowered 4.2-4.5 mm below the surface of the skull perpendicularly and was fixed to the skull with acrylic cement. Following the surgical procedure, rats were placed individually in cages and were allowed to recover from anesthesia for at least four hours. During the recovery period, rats showed no evidence of pain.

Drugs

Morphine sulphate and linopirdine were purchased from Sigma Chemical Co. (St Louis). Morphine sulphate was dissolved in saline. Linopirdine was dissolved in a vehicle composed of 2.5% dimethyl sulfoxide, 47.5% polyethylene glycol and 50% saline. I.c.v. injections were performed using a Hamilton microsyringe.

Behavioural tests

Tail-flick test

The response to painful thermal stimuli was assessed using the Plantar/Tail Stimulator Analgesia Meter (IITC Model 336, Woodland Hills, CA, USA)[3,5,17]. Briefly, rats (n:92) were placed inside restraining cages at least 5 min before the test. Then, each rat was placed on the apparatus and a radiant heat source was focused on 4-7 cm from the tail distal end. The time from the onset of the heat stimulus to withdrawal of the tail from the heat source (tail-flick latency) was recorded. The apparatus was calibrated to

give a tail-flick latency of approximately 2-3 s in naive rats. In order to prevent tissue damage, cut-off time was set to 10 s. Baseline measurements were obtained 2 days before the drug injection. A delay in the reaction time is interpreted as an analgesic action.

Plantar test

The Plantar/Tail Stimulator Analgesia Meter (IITC Model 336, Woodland Hills, CA, USA) was used to measure the paw withdrawal latencies in response to a thermal nociceptive stimulus in sciatic nerve-ligated rats to assess thermal hyperalgesia[17,19,20]. The rat (n: 111) was placed on a glass surface and after a 5-min acclimatization period, a light beam from a radiant heat light source below was aimed to the hind paw. Paw withdrawal latency was defined as the time of initial exposure of a thermal stimulus to the plantar surface of the hind paw to the time of withdrawal of the paw from the heat source. The apparatus was calibrated to give a paw withdrawal latency of approximately 10-12 s in naive rats. The cut-off time was set to 20 s to avoid tissue damage. Three paw withdrawal latency measurements were recorded at 3-min intervals and the average of last two measurements were calculated.

Mechanical hyperalgesia

To determine the sensitivity to noxious mechanical stimuli, a von Frey anesthesiometer (IITC Model 2290, ELECTROVONFREY, Woodland Hills, CA, USA) and rigid von Frey filaments were used[17,31,41]. The rats (n: 110) were placed in individual plexiglass boxes on a stainless steel mesh floor. Following a 15-min acclimatization period, a 0.5-mm diameter polypropylene rigid tip was used to apply a force to the plantar surface of the hind paw and the latency for the withdrawal response was recorded by the anesthesiometer. For each rat, the test was repeated three times with a minimum interval of 10 s and the average of the three measurements was calculated.

Experimental design

Experiment 1: Contribution of K_v7 channels to morphine-induced antinociception in acute pain

Effect of i.c.v. morphine on the response to painful thermal stimuli: Rats were tested on the tail-flick apparatus in order to obtain the baseline values. 2 days later, they were equipped with i.c.v. cannulas and 4-6 hours later, were injected with morphine (2, 5, 20 µg/10 µl; i.c.v.) or saline (10 µl; i.c.v.) 15 min before undergoing tail-flick test. Each group consisted of 6-7 rats.

Contribution of K_v7 channels to the effect of i.c.v. morphine on the response to painful thermal stimuli: Rats were pretreated with a selective K_v7 potassium channel blocker, linopirdine (0.1, 1, 10 µg /10 µl; i.c.v.) 15 min before morphine (5 µg/10 µl; i.c.v.) injection. Tail-flick test was performed 15 min after morphine injection. Each group consisted of 6-7 rats.

Experiment 2: Contribution of K_v7 channels to morphine-induced antinociception in neuropathic pain

Effect of i.c.v. morphine on thermal hyperalgesia induced by sciatic nerve ligation: Plantar test was performed to the rats before the sciatic nerve ligation surgery. 7 days after the ligation, the test was repeated to obtain the postoperative measurements. Then, rats were equipped with i.c.v. cannulas and 4-6 hours later, were injected with morphine (2, 5, 20 µg/10 µl; i.c.v.) or saline (10 µl; i.c.v.) 15 min before undergoing plantar test. Each group consisted of 6-7 rats.

Contribution of K_v7 channels to the effect of i.c.v. morphine on thermal hyperalgesia induced by sciatic nerve ligation: Rats were pretreated with a selective K_v7 potassium channel blocker, linopirdine (0.1, 1, 10 µg /10 µl; i.c.v.) 15 min before morphine (5 µg/10 µl; i.c.v.) injection. Plantar test was performed 15 min after morphine injection. Each group consisted of 6-7 rats.

Effect of i.c.v. morphine on mechanical hyperalgesia induced by sciatic nerve ligation: Rigid von Frey filaments were applied to the rats before the sciatic nerve ligation surgery. 7 days after the ligation, the test was repeated to obtain the postoperative measurements. Then, rats were equipped with i.c.v. cannulas and 4-6 hours later, were injected with morphine (2, 5, 20 µg/10 µl; i.c.v.) or saline (10 µl; i.c.v.) 15 min before application of von Frey filaments. Each group consisted of 6-7 rats.

Contribution of K_v7 channels to the effect of i.c.v. morphine on mechanical hyperalgesia induced by sciatic nerve ligation: Rats were pretreated with a selective K_v7 potassium channel blocker, linopirdine (0.1, 1, 10 µg /10 µl; i.c.v.) 15 min before morphine (5 µg/10 µl; i.c.v.) injection. Von Frey filaments were applied 15 min after morphine injection. Each group consisted of 6-7 rats.

At the end of the experiments, 5 µl of a methylene blue solution was injected into the cerebral ventricle through the cannula, to verify the placement of the inner end of the cannula. After decapitation, the brains were removed and sections were observed macroscopically to ascertain whether the cannula had been correctly placed into the lateral cerebral ventricle.

Statistical analysis

Data are presented as means ± S.E.M. Analysis of variance (ANOVA) followed by Tukey's test for multiple comparisons were used to determine statistical significance. Differences were considered to be significant at $p<0.05$.

RESULTS

Experiment 1: Contribution of K_v7 channels to morphine-induced antinociception in acute pain

Effect of i.c.v. morphine on the response to painful thermal stimuli: The tail-flick latencies following saline (10 µl; i.c.v.) and morphine (2 µg/10 µl; i.c.v.) injections were not significantly different from the baseline values of the same rats. On the other hand, the tail-flick latencies of both 5 µg and 20 µg morphine-injected rats were significantly different from their baseline recordings [$F(7,48) = 22.28$, $p<0.0001$] (Figure 1). The tail-flick latencies following 5 µg and 20 µg morphine but not 2 µg morphine were significantly higher than the tail-flick latencies of the saline-treated rats [$F(3,24) = 23.29$, $p<0.0001$] (Figure 1).

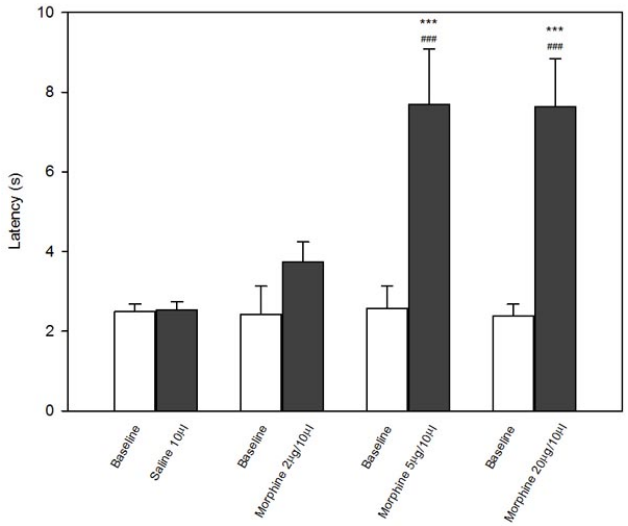

Figure 1: *Effect of i.c.v. morphine on response to painful thermal stimuli. Rats received morphine (2, 5, 20 µg/10 µl; i.c.v.) or saline (10 µl; i.c.v.) 15 min before the tail-flick test. The tail-flick latencies were measured. Results were presented as means ± S.E.M. Each group consisted of 6-7 rats.*
**** : p<0.001 with respect to their baseline values.*
: p<0.001 with respect to the saline-treated rats.

Contribution of K_v7 channels to the effect of i.c.v. morphine on the response to painful thermal stimuli: Pretreatment with a selective K_v7 potassium channel blocker, linopirdine (0.1, 1, 10 µg /10 µl; i.c.v.) 15 min before morphine (5 µg/10 µl; i.c.v.) injection significantly prevented the increase in tail-flick latencies due to morphine injection [$F(4,29) = 9.37$, $p<0.0001$] (Figure 2). Linopirdine (0.1, 1, 10 µg /10 µl; i.c.v.) alone did not have any effect on tail-flick latency.

Experiment 2: Contribution of K_v7 channels to morphine-induced antinociception in neuropathic pain

Effect of i.c.v. morphine on thermal hyperalgesia induced by sciatic nerve ligation: The paw withdrawal latencies were significiantly lower in all groups with respect to their baseline values 7 days following ligation [$F(7,50) = 18.65$, $p<0.0001$] (Figure 3). Paw withdrawal latencies of 2 µg, 5 µg and 20 µg morphine-injected rats but not saline-injected rats were significantly higher than their post-ligation recordings [$F(7,45) = 20.13$, $p<0.0001$]. Paw withdrawal latencies following all doses of morphine were significantly higher than the paw withdrawal latencies of the saline-treated rats [$F(3,19) = 17.49$, $p<0.0001$] (Figure 3).

Contribution of K_v7 channels to the effect of i.c.v. morphine on thermal hyperalgesia induced by sciatic nerve ligation: Pretreatment with a selective K_v7 potassium channel blocker, linopirdine (0.1 µg/10 µl; i.c.v.) 15 min before morphine (5 µg/10 µl; i.c.v.) injection significantly prevented the increase in paw withdrawal latency due to morphine injection, while higher doses of linopirdine (1 and 10 µg/10 µl; i.c.v.) were ineffective [$F(4,32) = 20.22$, $p<0.0001$] (Figure 4). Linopirdine (0.1 µg/10 µl; i.c.v.) alone did not have any effect on paw withdrawal latency.

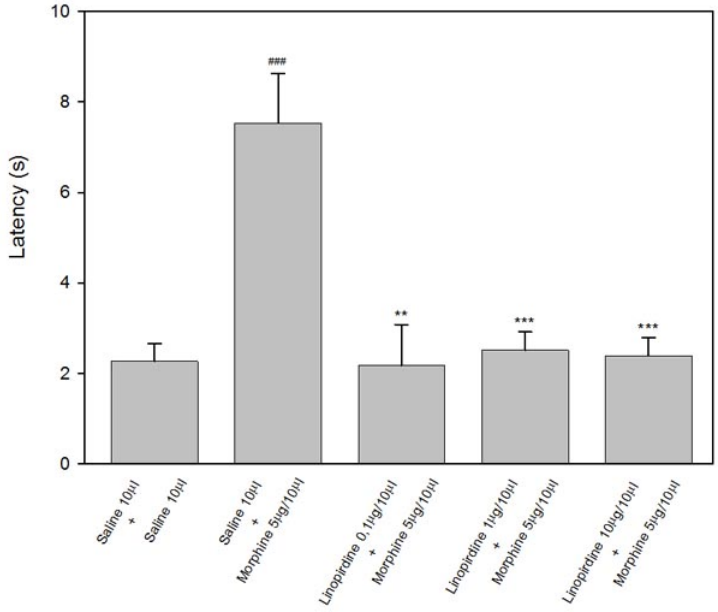

Figure 2: *Contribution of K_v7 channels to the effect of i.c.v. morphine on the response to painful thermal stimuli. Rats received linopirdine, a K_v7 potassium channel blocker (0.1,1,10 µg/10 µl; i.c.v.) 15 min prior to morphine (5 µg/10 µl; i.c.v.) injection. Tail-flick test was performed 15 min after morphine injection. The tail-flick latencies were measured. Results were presented as means ± S.E.M. Each group consisted of 6-7 rats.*
: $p<0.001$ with respect to the saline + saline group.
*** : $p<0.01$ and *** :$p<0.001$ with respect to the saline + morphine group.*

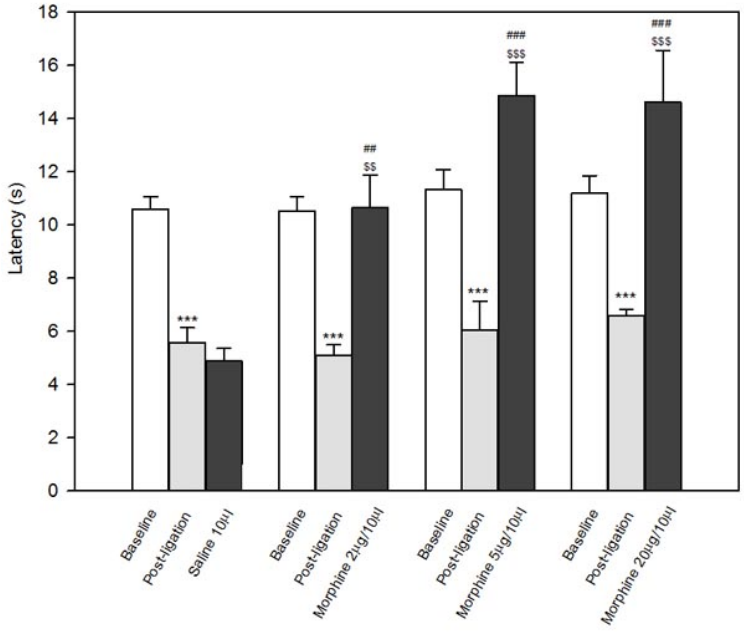

Figure 3: *Effect of i.c.v. morphine on thermal hyperalgesia induced by sciatic nerve ligation. Rats received morphine (2, 5, 20 µg/10 µl; i.c.v.) or saline (10 µl; i.c.v.) 15 min before the plantar test. The paw withdrawal latencies were measured. Results were presented as means ± S.E.M. Each group consisted of 6-7 rats.*
**** : p<0.001 with respect to their baseline values.*
: p<0.01 and ### : p<0.001 with respect to their post-ligation values.
$$: p<0.01 and $$$: p<0.001 with respect to the saline-treated rats.

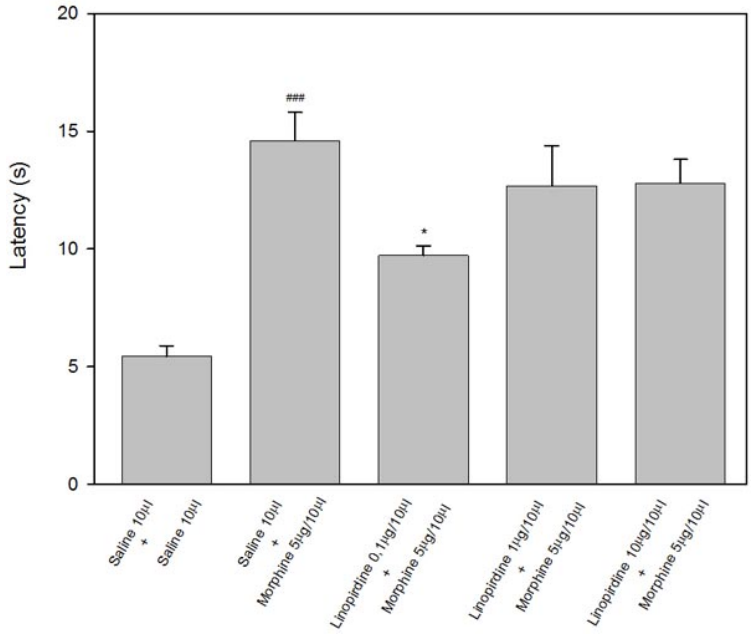

Figure 4: *Contribution of K_v7 channels to the effect of i.c.v. morphine on thermal hyperalgesia induced by sciatic nerve ligation. Rats received linopirdine, a K_v7 potassium channel blocker (0.1,1,10 µg/10 µl; i.c.v.) 15 min prior to morphine (5 µg/10 µl; i.c.v.) injection on the 7th day of ligation. Plantar test was performed 15 min after morphine injection. The paw withdrawal latencies were measured.Results were presented as means ± S.E.M. Each group consisted of 6-7 rats.*
: p<0.001 with respect to the saline + saline group.
** : p<0.05 with respect to the saline + morphine group.*

Effect of i.c.v. morphine on mechanical hyperalgesia induced by sciatic nerve ligation: The paw withdrawal latencies in response to stimulation with rigid von Frey filaments were significantly lower in all groups with respect to their baseline values 7 days following ligation [$F(7,32) = 12.19$, $p<0.0001$] (Figure 5). The paw withdrawal latencies following saline (10 µl; i.c.v.) and morphine (2 µg/10 µl; i.c.v.) injections were not significantly different from the post-ligation values of the same rats. On the other hand, the paw withdrawal latencies of both 5 µg and 20 µg morphine-injected rats were significantly higher than their post-ligation recordings [$F(7,33)= 7.82$, $p<0.0001$] (Figure 5). The paw withdrawal latencies following 5 µg and 20 µg morphine but not 2 µg morphine were significantly higher than the paw withdrawal latencies of the saline-treated rats [$F(3,17)= 7.61$, $p<0.0001$] (Figure 5).

Contribution of K_v7 channels to the effect of ic.v. morphine on mechanical hyperalgesia induced by sciatic nerve ligation: Pretreatment with a selective K_v7 potassium channel blocker, linopirdine (0.1, 1, 10 µg/10 µl; i.c.v.) 15 min before morphine (5 µg/10 µl; i.c.v.) injection did not affect the increase in paw withdrawal threshold due to morphine injection (Figure 6).

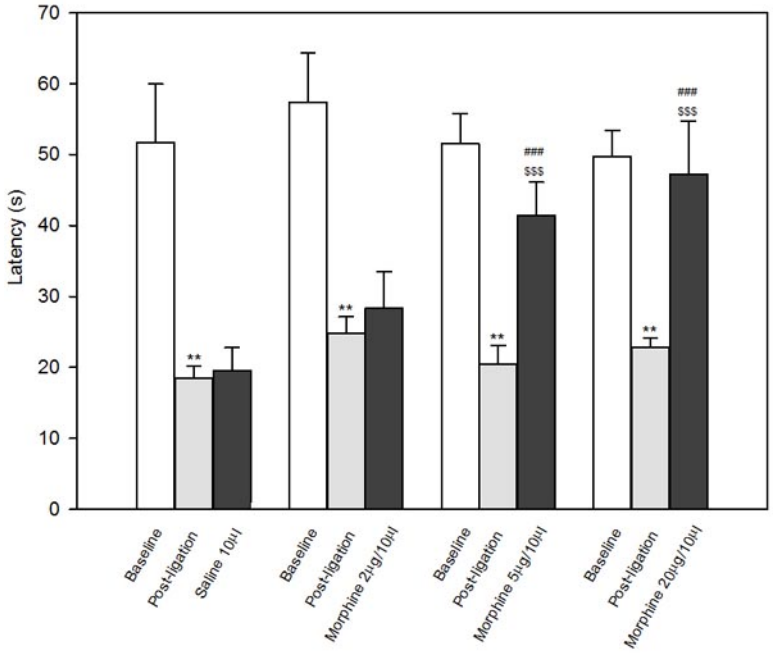

Figure 5: *Effect of i.c.v. morphine on mechanical allodynia induced by sciatic nerve ligation. Rats received morphine (2, 5, 20 µg/10 µl; i.c.v.) or saline (10 µl; i.c.v.) 15 min before the application of von Frey filaments. The paw withdrawal thresholds were measured. Results were presented as means ± S.E.M. Each group consisted of 6-7 rats.*
*** : p<0.01 with respect to their baseline values.*
: p<0.01 and ### : p<0.001 with respect to their post-ligation values.
$$$: p<0.001 with respect to the saline-treated rats.

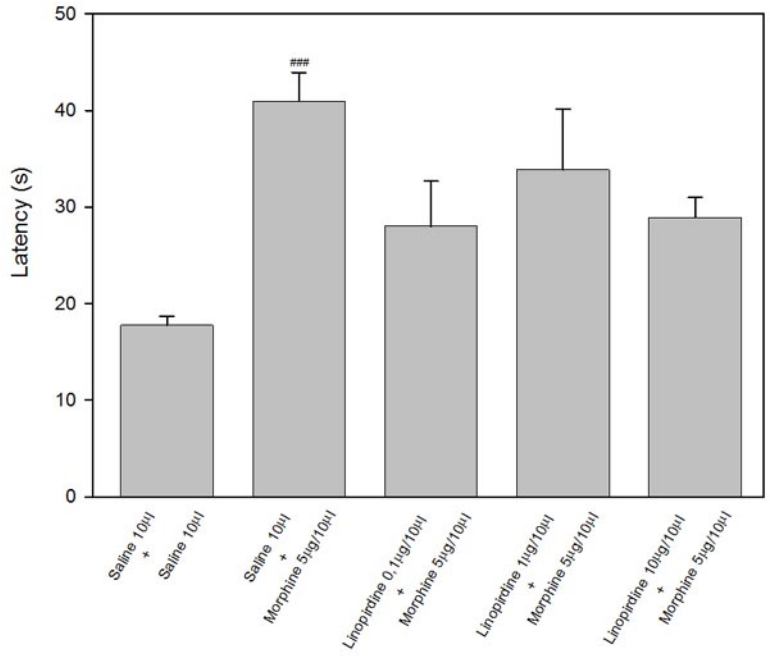

Figure 6: *Contribution of K_v7 channels to the effect of i.c.v. morphine on mechanical allodynia induced by sciatic nerve ligation. Rats received linopirdine, a K_v7 potassium channel blocker (0.1,1,10 µg/10 µl; i.c.v.) 15 min prior to morphine (5 µg/10 µl; i.c.v.) injection on the 7th day of ligation. Von Frey filaments were applied 15 min after morphine injection. The paw withdrawal thresholds were measured. Results were presented as means ± S.E.M. Each group consisted of 6-7 rats.*
: $p<0.001$ with respect to the saline + saline group.

DISCUSSION

The present findings demonstrated that, K_v7 potassium channels contribute to the antinociceptive effect of i.c.v. morphine against acute pain observed in the tail-flick test. These channels have also a role, at least in part, in the morphine-induced decrease in thermal hyperalgesia in sciatic nerve-ligated rats, measured by the plantar test. On the other hand, testing with the von Frey filaments revealed that K_v7 potassium channels do not modulate the effect of morphine on mechanical hyperalgesia.

Tail-flick test was used to assess the effect of i.c.v. morphine on acute pain and the contribution of K_v7 potassium channels in this effect. The tail-flick is a spinal reflex that is influenced by the activity of supraspinal structures and does no require the integrative action of higher brain centers[30]. The advantages of this method are its simplicity and the small interanimal variability in reaction time measurements under a given set of controlled conditions[17].

We found that, 5 µg and 20 µg i.c.v. morphine significantly increased the tail-flick latencies, indicating that centrally-injected morphine has an antinociceptive effect against acute pain. This finding is in accordance with those of He L et al., suggesting that i.c.v. morphine is potently antinociceptive in both rats and mice[11]. The antinociceptive activity of i.c.v. morphine on response to thermal stimulus seems to be mediated by µ1-opioid receptor subtype[35]. Sweeney et al. have shown that, administration of 40 nmol of morphine (i.c.v.) acutely to rats increases both tail-flick and hot-plate nociceptive latencies[39]. A component of supraspinal antinociception by morphine in these tests has been thought to be due to a net activation of descending noradrenergic and

serotonergic systems terminating in the spinal cord[1,10]. De Lander and Hopkins have demonstrated that antinociceptive effect of i.c.v. morphine is antagonized by i.t. injection of adenosine receptor antagonists and potentiated by spinal administration of adenosine analogs and adenosine uptake inhibitors, suggesting the role of adenosine in the i.c.v. morphine-induced antinociception[6,7].

We found that pretreatment with a selective K_v7 potassium channel blocker, linopirdine (0.1, 1, 10 µg /10 µl; i.c.v.) 15 min before morphine (5 µg/10 µl; i.c.v.) injection significantly prevented the increase in tail-flick latencies due to morphine injection. Indeed, the activation of K+ flow through the neuronal cytoplasmic membranes is suggested as the final molecular mechanism of the opioid morphine[25,34]. Stimulation of µ-, δ- and κ-opioid receptors decreases neuronal Ca^{2+} influx and Ca^{2+}-dependent action potentials and induces antinociception[44,46,32]. The effect of µ and δ stimulants on Ca^{2+} fluxes is secondary to the opening of neuronal K^+ channels[45].

We tested the effect of i.c.v. morphine on thermal hyperalgesia in sciatic nerve-ligated rats by the plantar test. This test is widely used to assess thermal hyperalgesia, with the advantage of being applicable on the freely moving animal. We found that i.c.v. morphine significantly increases the paw withdrawal latency in response to thermal stimulation, thus decreases the thermal hyperalgesia due to sciatic nerve ligation. We also determined the effect of i.c.v. morphine on mechanical hyperalgesia using rigid von Frey filaments and observed that morphine alleviates the mechanical hyperalgesia induced by sciatic nerve ligation.

There is a debate over the efficacy of opiates in treating neuropathic pain. The antinociceptive efficacy of intrathecally-administered morphine decreases in rats with peripheral nerve injury, such as chronic constriction injury or L5/L6 nerve ligation[27,33,2]. It has been shown that, intrathechal morphine dose-dependently inhibits thermal hyperalgesia[21,43], while it has no effect on tactile allodynia in rats with L5/L6 nerve ligation[2,18]. On the other hand, morphine administered i.p. or i.c.v. produced dose-dependent antiallodynia, suggesting that i.p. and i.c.v. morphine may reduce tactile allodynia via supraspinal µ opioid receptors and subsequent activation of descending modulatory systems. On the contrary, the failure of intrathechal morphine to produce antiallodynic effects may be due, in part, to the lack of available functional spinal opioid µ receptors which may occur following nerve injury[2,18]. Thus, it seems that opiate sensitivity in neuropathic pain depends on the models used, modalities of nociceptive stimuli and route of administration.

When administered 15 min before morphine injection, low dose of linopirdine (0.1 µg/10 µl; i.c.v.), but not the higher doses (1 and 10 µg/10 µl; i.c.v.) significantly inhibited the effect of morphine on thermal hyperalgesia measured by the plantar test. This can be explained by the possibility that i.c.v. linopirdine might interact with two different receptor–effector systems that mediate opposite effects. On the other hand, no dose of linopirdine changed the effect of morphine on mechanical hyperalgesia tested by the rigid von Frey filaments. Our finding that linopirdine was effective in preventing the effect of morphine on thermal hyperalgesia, but not on mechanical hyperalgesia may be due to the different fibers involved in conducting pain sensation (Pascual et al., 2010), since mechanical hyperalgesia has been suggested to be mediated through large diameter, Aß-afferent fibers, whereas thermal hyperalgesia is likely to be mediated through small diameter, unmyelinated, high threshold C-fibers (Ossipov et al, 1999; Shir & Seltzer, 1998). Although there is evidence about the contribution of K_v7 channels to

antinociception (Blackburn-Munro & Jensen, 2003; Dost et al., 2004; Passmore et al., 2003), the role of these channels in the morphine-induced antinociception is not known. Thus, more studies using both K_v7 channel openers and blockers may provide evidence for their roles in this process. Analysis of opioid receptor knockout mice have shown that μ opioid receptors play a central role in opioid-induced analgesia (Matthes et al., 1996; Sora et al., 1997). These receptors couple wıth various effectors, such as G-protein-activated inwardly rectifying potassium (GIRK) channels, voltage-gated Ca^{2+} channels and others. Demonstration of a coupling of opioid receptors with K_v7 potassium channels would be a key evidence to elucidate the mechanism underlying this effect.

We conclude that, K_v7 potassium channels contribute to the effect of i.c.v. morphine on acute pain induced by thermal stimulation. In the sciatic nerve-ligated rats, these channels play role in the effect of morphine on thermal hyperalgesia, but not on mechanical hyperalgesia.

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