The Role of TRP and K⁺ Ion Channels in Analgesic Effect of NSAIDs

NSAİİ' ların Analjezik Etkilerinde TRP ve K⁺ İyon Kanallarının Rolü

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ABSTRACT

Objective: We aimed to clarify the possible contributions of TRP and voltage-dependent K⁺ channels to the analgesic effects of diclofenac, ketoprofen, etodolac, and dipyrone using the nonselective TRP channel blocker ruthenium red and the voltage-dependent K⁺ channel blocker (Kv7; KCNQ) XE 991, respectively.

Methods: We assessed the changes in the antinociceptive effects of diclofenac (50 mg/kg, i.p.), ketoprofen (50 mg/kg, i.p.), etodolac (70 mg/kg, i.p.), and dipyrone (500 mg/kg, i.p.) using ruthenium red (3 mg/kg, i.p.) and XE 991 (1 mg/kg, i.p.) before treatment in the hot plate, tail immersion, and writhing tests in mice.

Results: In the tail immersion test, ruthenium red administration resulted in a significant reversal in the analgesic effects of dipyrone, etodolac, and ketoprofen. In the hot plate test, a significant reversal was observed in the analgesic effect of only dipyrone. In the tail immersion test, the administration of XE 991 induced a significant reversal in the analgesic effects of dipyrone and etodolac and a relative reversal in the analgesic effects of ketoprofen and diclofenac. In the hot plate test, XE 991 produced a significant reversal in the analgesic effect of only ketoprofen, whereas it caused a relative reversal in the analgesic effects of other tested nonsteroidal anti-inflammatory drugs (NSAIDs). In the writhing test, no significant change was observed after either XE 991 or ruthenium red administration.

Concusions: Modulation of TRP and K⁺ channels may be involved in the central analgesic effects of NSAIDs. The clarification of different action mechanisms of NSAIDs will contribute to new therapeutic approaches and provide guidance for new drug development studies.

Keywords: NSAIDs, TRP channel, potassium channels, analgesia

ÖZ

Amaç: Seçici olmayan TRP kanal blokörü rutenyum kırmızısı ve voltaj bağımlı K⁺ kanal blokörü XE 991 varlığında diklofenak, ketoprofen, etodolak ve dipironun analjezik etkilerine TRP'nin ve voltaj bağımlı K⁺ kanallarının olası katkılarının incelenmesi amaçlanmıştır.

Yöntemler: Rutenyum kırmızısı (RR) (3 mg / kg, ip) ve XE 991 (1 mg / kg, ip) varlığında, diklofenak (50 mg / kg, ip), ketoprofen (50 mg / kg, ip), etodolak (70 mg / kg, ip) ve dipironun (500 mg / kg, ip) antinosiseptif etkilerindeki değişiklikler, farelerde sıcak plaka, kuyruk daldırma ve kıvranma testlerinde araştırıldı.

Bulgular: Kuyruk daldırma testinde RR uygulaması sonucu sadece dipiron, etodolak ve ketoprofen'in analjezik etkisinde anlamlı bir geri dönüş sağlanmıştır. Sıcak plaka testinde ise sadece dipiron'un analjezik etkisinde belirgin bir geri dönüş görülmektedir. XE 991 uygulanması kuyruk daldırma testinde dipiron ve etodolak'ın etkisinde anlamlı bir geri dönüş sağlarken, ketoprofen ve diklofenak'ın etkisinde göreceli bir geri dönüş sağlamaktadır. Sıcak plaka testinde ise sadece ketoprofen'in analjezik etkisinde anlamlı bir geri dönüş sağlarken, test edilen diğer non-steroidal antiinflamtuvar ilaçların (NSAİİ) etkisinde göreceli bir geri dönüş sağlamaktadır. Writhing testinde ise ne RR uygulamasında ne de XE 991 uygulaması sonucunda önemli bir değişiklik olmamıştır.

Sonuç: Çalışmamızdan elde edilen sonuçlara göre NSAİİ'ın santral analjezik etkisinde TRP ve potasyum kanal modülasyonunun katkısının olabileceği düşünülmektedir. Bu projede kullanılan ve klinikte ağrı üzerine etkileri bilinen farmakolojik ajanların farklı etki mekanizmalarının aydınlatılmış olması, terapötik yaklaşımlara katkı sağlamakla birlikte yeni ilaç geliştirme çalışmalarında da yol gösterici olacağı düşünülmektedir.

Anahtar Kelimeler: NSAİİ, TRP kanalları, potasyum kanalları, analjezi

INTRODUCTION

Pain is an unpleasant sensory and auditory experience, which can develop due to various causes and is the most common complaint people present with. Pain has sensory, emotional, and cognitive components; the central modulation of the pain occurs by evaluating these three components (1, 2). Pain conduction is a mechanism that involves a complex interaction of peripheral and central structures from the skin surface to the central cerebral cortex. Pain is transmitted to the central nervous system (CNS) in two separate ways via myelinated A delta fibers and unmyelinated C fibers, following the induction of nociceptors (3). Nociception encompasses all the electrochemical events occurring between tissue damage and

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pain perception. TRP ion channels are extensively found in the C and A delta fibers, and they mediate these complex electrochemical events (4). Seven different types of TRP ion subchannels have been identified since their first discovery in the cells of eyes of Drosophila flies. Various inflammatory mediators, cytokines, and neuromediators as well as temperature and pH mediate the operation of these channels. These channels are inactive in physiological conditions; however, they become active in the presence of chemical and thermal stimuli and activate various pathways (5, 6, 7).

Another channel group that plays an important role in pain and neuronal conduction are the potassium channels. Voltage-dependent K^+ (Kv) channels play an important role in the formation of action potentials and neuronal excitability. There are five different Kv7 K^+ channel subtypes (Kv7.1–Kv7.5), and four (Kv7.2–Kv7.5) of these are located in the nervous system; Kv7.2 and Kv7.3 are associated with the slow voltage-gated M-channel (8, 9). In addition to these channels, ATP-sensitive K channels play a role in neuronal conduction and therefore in nociception (10, 11). K^+ channels play an important role in the formation of resting membrane potential and control of the excitability of nerve cells. Opening of the K^+ channels results in hyperpolarization of the cell membrane, and therefore cell excitability decreases. Thus, K^+ channels emerge as potential peripheral and central targets for the analgesic effect (9, 12, 13).

Nonsteroidal anti-inflammatory drugs (NSAIDs) are among the most frequently drugs used for relieving pain. In response to inflammation, cyclooxygenase (COX) enzymes increase the formation of prostaglandins from arachidonic acid. NSAIDs exhibit analgesic and anti-inflammatory effects by inhibiting COX enzymes. However, the inhibition of these enzymes is insufficient to explain the peripheral and central analgesic effects of NSAIDs. Therefore, different mechanisms may contribute to their effectiveness (14-16). This study aimed to clarify the possible contributions of TRP and voltage – dependent K^+ channels to the analgesic effects of diclofenac, ketoprofen, etodolac, and dipyrone (Sigma, St. Louis, USA) using the nonselective TRP channel blocker ruthenium red and the voltage – dependent K^+ channel blocker XE 991 in the hot plate, tail immersion, and writing tests.

METHODS

Animals

Swiss albino mice (30-35 g) were housed in rooms with a 12-h light/dark cycle at 23±2°C. They were provided tap water and standard mouse feed. They were brought to the laboratory 1 week before the experiment to allow adaptation to the laboratory environment. Animal care and research protocols were based on the principles and guidelines adopted by the Guide for the Care and Use of Laboratory Animals (NIH Publication no. 85-23, revised in 1985) and were approved by the local ethics committee, Eskisehir, Turkey (Decision no: 2015-13).

Drug Application

We assessed the changes in the analgesic effects of diclofenac (50 mg/kg, i.p.), ketoprofen (50 mg/kg, i.p.), etodolac (70 mg/kg, i.p.), and dipyrone (500 mg/kg, i.p.) (Sigma, St. Louis, USA) using

the nonselective TRP channel antagonist ruthenium red (3 mg/kg) and the voltage-dependent K^+ channel (Kv7; KCNQ) blocker XE 991 (1 mg/kg). A vehicle (SF) was given to the control group. The mice response times to painful stimuli in analgesia tests were measured before and 40 min after NSAID test substance administration. The animals were given NSAIDs 30 min following the injection of ruthenium red and 15 min following that of XE 991. All drugs were administered intraperitoneally. The experimental protocols were performed between 10:00 and 16:00 (17, 18).

Analgesia Tests Hot plate test

The hot plate test is one of the most commonly used thermal and supraspinal analgesia measurement methods. The plate (Ugo-Basile, 7280, Italy) was heated to 55±1°C and surrounded by a plexiglass cylinder. The time between the animal's release onto the hot plate and its reaction (pulling or licking its hind legs, ascending over its legs, or jumping) was measured (19). The ending time for each measurement was set at 20 s to prevent animals feet from damage due to the hot plate (20).

Tail immersion test

The tail immersion test is another thermal method used for the evaluation of spinal analgesia. From the end of the animal's tail, 3 cm was submerged in 52.5±0.2°C water in a beaker. The time from tail submersion in the water until the animal pulled its tail out of the water was measured using a chronometer. The ending time for each measurement was set at 15 s to prevent damage to the animal's tail from hot water (21, 22).

Acetic acid-induced writhing test

The acetic acid-induced writhing test is a method of analgesia measurement wherein an acetic acid solution is used to produce strong visceral pain in animals. After intraperitoneal administration of acetic acid, writhing occurs in the animals, which is characterized by contraction in the abdominal muscles and then stretching the hind legs back and rubbing the abdomen (20, 23). Forty minutes after the injection of an NSAID test substance, 0.6% acetic acid solution was intraperitoneally administered to the animals. At the end of a 5 min-waiting period, the abovementioned writhing movements in each animal were observed for 10 min.

Statistical Analyses

All values obtained in the study were expressed as the mean ± standard error of the mean to show variation in groups. A one-way ANOVA Tukey's post-hoc test was used to assess the statistical differences between the animals in the control and experimental groups. GraphPad Prism version 5.0 statistical program was used to perform statistical analysis of all data. The values obtained in the hot plate and tail immersion tests were converted to the percent maximum possible effect (MPE%) using the following formula:

% maximum possible effect=(post-drug reaction time−predrug reaction time)/(test cutoff time−predrug reaction time)×100

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RESULTS

Hot Plate Test

The hot plate test results for NSAIDs in the presence of ruthenium red (3 mg/kg) and XE 991 (1 mg/kg) are presented as MPE% in Figures 1 and 2. All the NSAIDs (diclofenac, dipyrone, etodolac, and ketoprofen) produced significant analgesic effects in the hot plate test ($p < 0.01$ and $p < 0.001$). When the effects of NSAIDs were examined in the presence of ruthenium red, a significant reversal was observed in the analgesic effect of only dipyrone (Fig. 1). When the effects of NSAIDs were examined in the presence of XE 991, a relative reversal was found in the analgesic effects of all the NSAIDs used in the test, whereas a significant reversal was found in the analgesic effect of only ketoprofen ($p < 0.05$; Fig. 2).
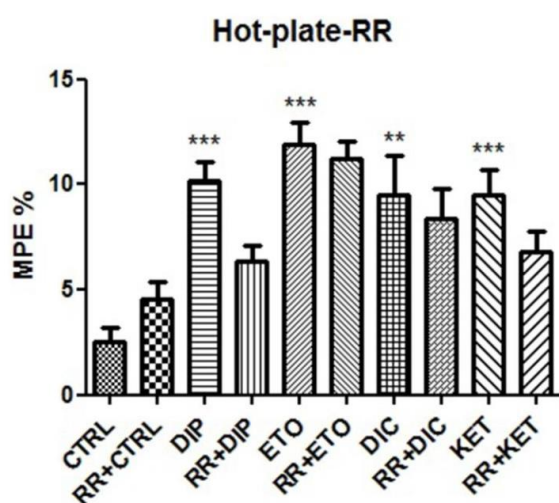
Figure 1. Hot plate test results for NSAIDs in the presence of ruthenium red (n=7–8). Compared with control group, *** $p < 0.001$, ** $p < 0.01$.

CTRL: Control; RR + CTRL: Ruthenium red + control; DIP: Dipyrone; RR + DIP: Ruthenium red + Dipyrone; ETO: Etodolac; RR + ETO: Ruthenium red + Etodolac; DIC: Diclofenac; RR + DIC: Ruthenium red + Diclofenac; KET: Ketoprofen; RR + KET: Ruthenium red + Ketoprofen
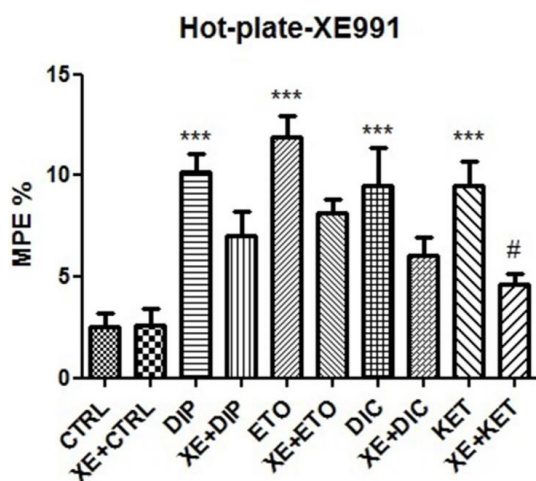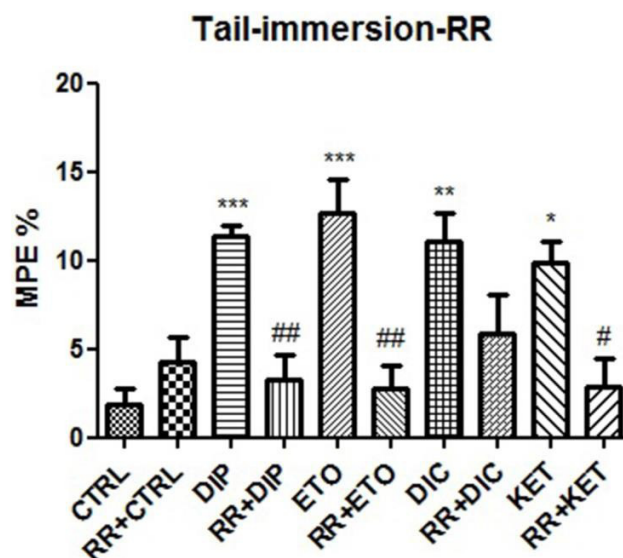

Figure 2. Hot plate test results for NSAIDs in the presence of XE 991 (n=7–8). Compared with control group, ***$p < 0.001$. Comparison

of NSAID with XE 991 + NSAID: #$p < 0.05$. CTRL: Control; XE 991 + CTRL: XE 991 + control; DIP: Dipyrone; XE 991 + DIP: XE 991 + Dipyrone; ETO: Etodolac; XE 991 + ETO: XE 991 +Etodolac; DIC: Diclofenac; XE 991 + DIC; KET: Ketoprofen; XE 991 + KET: XE 991 + Ketoprofen
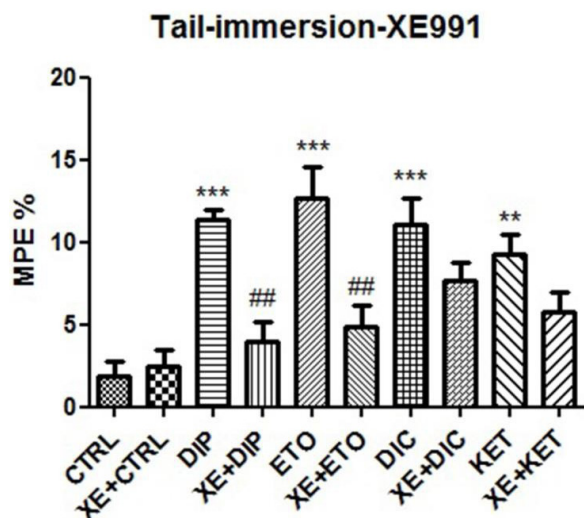
Tail Immersion Test

All the NSAIDs (diclofenac, dipyrone, etodolac, and ketoprofen) produced significant analgesic effects in the tail immersion test ($p < 0.01$, $p < 0.001$, and $p < 0.05$; Fig. 3). When the effects of NSAIDs in the presence of ruthenium red were examined, a significant reversal in the analgesic effects of dipyrone, etodolac, and ketoprofen was observed ($p < 0.01$, $p < 0.001$, and $p < 0.05$, respectively; Fig. 3). When the effects of NSAIDs in the presence of the potassium channel blocker XE 991 were examined, a significant reversal was found in the analgesic effects of dipyrone and etodolac ($p < 0.01$), whereas no significant reversal was found in the analgesic effects of diclofenac and ketoprofen (Fig. 4).

Figure 3. Tail immersion test results for NSAIDs in the presence of ruthenium red (n=7–8). Compared with control group: ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$. Comparison of NSAID with RR + NSAID: ##$p < 0.01$, #$p < 0.05$.

CTRL: Control; RR + CTRL: Ruthenium red + control; DIP: Dipyrone; RR + DIP: Ruthenium red + Dipyrone; ETO: Etodolac; RR + ETO: Ruthenium red + Etodolac; DIC: Diclofenac; RR + DIC: Ruthenium red + Diclofenac; KET: Ketoprofen; RR + KET: Ruthenium red + Ketoprofen

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Tail-immersion-XE991

Figure 4. Tail immersion test results for NSAIDs in the presence of ruthenium red (n=7–8). Compared with control group: ***p<0.001, **p<0.01. Comparison of NSAID with XE 991 + NSAID: ##p<0.01.

CTRL: Control; XE 991 + CTRL: XE 991 + control; DIP: Dipyrone; XE 991 + DIP: XE 991 + Dipyrone; ETO: Etodolac; XE 991 + ETO: XE 991 + Etodolac; DIC: Diclofenac; XE 991 + DIC: XE 991 + Diclofenac; KET: Ketoprofen; XE 991 + KET: XE 991 + Ketoprofen
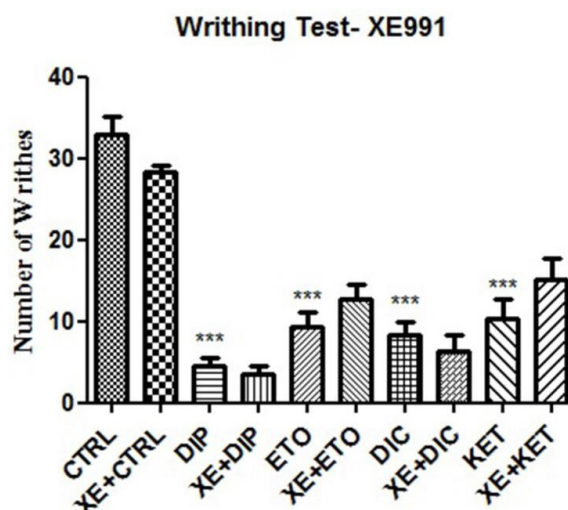
Acetic Acid Writhing Test

According to the acetic acid writhing test results wherein the peripheral analgesic effect was evaluated by measuring the writhing numbers of mice after chemical stimulus, the analgesic effects of all the NSAIDs were highly significant (p<0.001). No reversal was found in their analgesic effects in the presence of either ruthenium red or XE 991 (Fig. 5 and 6).
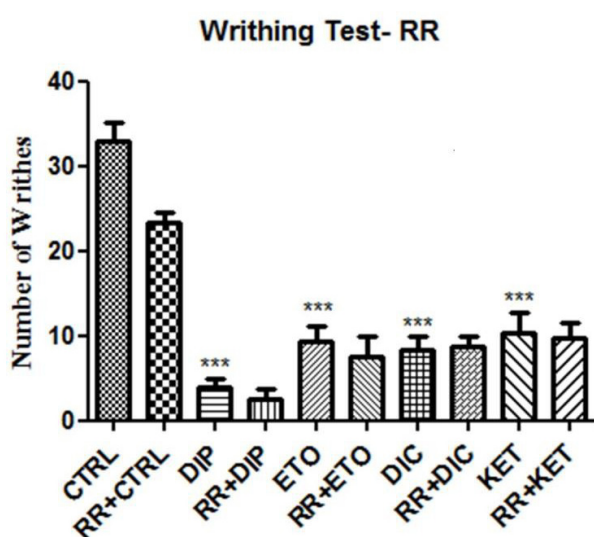
Writing Test- RR

Figure 5. Acetic acid-induced writhing test results for NSAIDs in the presence of XE 991 (n=7–8). Compared with control group: ***p<0.001, **p<0.01. Comparison of NSAID with RR + NSAID: #

CTRL: Control; RR + CTRL: Ruthenium red + control; DIP: Dipyrone; RR + DIP: Ruthenium red + Dipyrone; ETO: Etodolac; RR + ETO: Ruthenium red + Etodolac; DIC: Diclofenac; RR + DIC: Ruthenium red + Diclofenac; KET: Ketoprofen; RR + KET: Ruthenium red + Ketoprofen

Writhing Test- XE991

Figure 6. Acetic acid-induced writhing test results for NSAIDs in the presence of XE 991 (n=7–8). Compared with control group: ***p<0.001, **p<0.01. Comparison of NSAID with XE 991 + NSAID: #

CTRL: Control; XE 991 + CTRL: XE 991 + control; DIP: Dipyrone; XE 991 + DIP: XE 991 + Dipyrone; ETO: Etodolac; XE 991 + ETO: XE 991 + Etodolac; DIC: Diclofenac; XE 991 + DIC: XE 991 + Diclofenac; KET: Ketoprofen; XE 991 + KET: XE 991 + Ketoprofen

DISCUSSION

Using central and peripheral pain experiments, this study examined the role of TRP and potassium channels in the analgesic effects of dipyrone, etodolac, diclofenac, and ketoprofen, commonly prescribed NSAIDs for reducing pain. The results show that the analgesic effects of some NSAIDs in the mice administered with ruthenium red and XE 991 before administration of NSAIDs were reversed in the hot plate and tail immersion tests, whereas no reversal of effects was observed in the writhing test.

In the hot plate and tail immersion tests, pain is generated by thermal stimuli; these tests are frequently used in central analgesic effect studies to measure supraspinal and spinal responses, respectively (24). The present study used these methods to examine the central analgesic effect of a selected group of NSAIDs and showed that they were effective in both tests.

Several investigators have demonstrated that NSAIDs could have central analgesic effects. However, COX enzyme inhibition is not sufficient to explain the effects of these drugs on CNS, despite the presence of both COX types in neurons and glial cells (14, 15, 25, 26). Although the principal analgesic mechanisms of NSAIDs include COX enzyme inhibition, this is not sufficient information to explain the complete central effect mechanisms of these drugs. Recent studies have indicated that many different mechanisms can influence the

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central analgesic effects of NSAIDs (27, 28). Various subtypes of potassium and TRP channels play a role in the antinociceptive effect. Potassium channel structures are classified as voltage-dependent (Kv), calcium-dependent, inward rectifier, and two-pore according to their functions and pharmacological effects (29, 30). These channels act as inhibitors to regulate neuronal excitability and action potential due to their features of low threshold, slow activation, slow deactivation, and non-inactivating K^+ currents. Potassium channels are principally associated with neuronal processes, and Kv7 (or KCNQ) is one of the voltage-gated potassium channels found in both the central and peripheral nervous system (31). Kv7.2, 7.3, and 7.5 channels and functional Kv7-M currents are found in the sensory nerves' peripheral terminals, cell bodies, axons, and central terminals. The presence of these channels in CNS and dorsal root ganglion sensory neurons suggests that they also play a role in the regulation of neuronal conduction and thus in pain control (13, 32, 33). Passmore et al. (12) showed that retigabine had antinociceptive effects in the carrageenan-induced hyperalgesia model and that XE 991 reversed this effect. It was shown that meclofenamic acid and diclofenac are KCNQ channel openers, and these channels could be targeted for migraine and neuropathic pain (34). In addition, ICA-27243 (a KV7.2/7.3 selective activator), which was developed as a potassium channel activator, showed a significant antinociceptive activity in the animal models of inflammatory, chronic, and neuropathic pain. Many different KV7.2–7.5 activators developed by Bristol Myers Squibb are effective in diabetic neuropathy and other rodent neuropathic pain models following intravenous administration (35). XE 991 reversed the analgesic effects obtained in studies on various types of inflammatory, neuropathic, and chronic pain (12, 36, 37).

XE 991, a potent and selective inhibitor of voltage-gated Kv7 (or KCNQ) potassium channels, was used in the present study (38). At the spinal level, the administration of XE 991 on the central analgesic effects of NSAIDs created a significant reversal in the analgesic effects of dipyrone and etodolac ($p < 0.01$), while providing a relative reversal in the analgesic effects of ketoprofen and diclofenac. At the supraspinal level, a significant reversal was noted only in the analgesic effect of ketoprofen, while relative reversal was seen in the analgesic effects of other tested NSAIDs. Accordingly, Kv7/KCNQ channels may contribute to the analgesic effects of the tested NSAIDs at the spinal and supraspinal level. These results show that Kv7 channels may contribute to the analgesic effects of NSAIDs, particularly in the tail immersion test. Kv7/KCNQ channels are located in the dorsal horn, spinal cord, and important locations in the brain, such as the cortex and thalamus, and function as inhibitors in pain pathways because of their activation (39). For different types of pain suppression, the activation of Kv7/KCNQ channels and neuronal M currents results in a decrease in the neuronal excitability of nociceptive neurons and C-type nerve fibers (17).

TRP channels are specialized detectors that play a role in responding to thermal and mechanical perception and painful stimuli. These channels serve as a cellular sensor for a broad spectrum of physical and chemical stimuli, such as temperature, cyclic nucleotides, phosphorylation potential, osmotic pressure, and environmental inputs. TRP channels are involved with the pain-sensing neurons and primer afferent nociceptors. NSAIDs have effects, such as inhibition, activation, or expression, on channels, which may be voltage-gated Na+, Ca^{2+}, or K^+ channels; ligand-gated K^+ channels; or TRP cation channels (7, 40). A study on the effects of NSAIDs about TRPA1 and TRPV1 channels using mechanical and thermal paw withdrawal tests on mice suggested that NSAIDs produce an effect by inactivating or desensitizing these channels (41). Moreover, some NSAIDs, such as flufenamic acid, may regulate various channel activities and exhibit agonistic activity on TRPA1s via allosteric binding in addition to covalent modification of channel proteins (42). In the present study, the analgesic effects of dipyrone, etodolac, and ketoprofen were significantly reversed ($p<0.01$, $p<0.01$, and $p<0.05$, respectively) in the tail immersion test after ruthenium red administration. However, in the hot plate test, a dramatic reversal was observed only in the analgesic effect of dipyrone. In a study by Biggs et al. (43), gabapentin added into the cell through open TRPV1 channels produced an analgesic effect via intracellular targets. It is thought that TRP channels provide a pathway to the cell for various molecules, and different TRP channels may mediate this pathway in different cell types. Therefore, it may be possible that NSAIDs exhibit activity in the cell by passing through these channels into the cell. Additional detailed studies are needed for clarifying this.

In conclusion, the modulation of TRP and potassium channels may contribute to the central analgesic effects of NSAIDs, particularly at spinal level, in this study. The demonstration of the TRP and potassium channels' role in the formation of analgesic effects of NSAIDs may mediate the determination of targets for new analgesic and anti-inflammatory drug development studies. Additionally, the establishment of different action mechanisms of NSAIDs will also contribute to therapeutic approaches and provide guiding data for new drug development studies.

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