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Original Article

The antihyperalgesic effects of gabapentinoids, carbamazepine and its keto analogue, oxcarbazepine, in capsaicin-induced thermal hyperalgesia

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ABSTRACT

It is aimed to investigate and compare the antihyperalgesic effects of 100 mg/kg (*p.o.*) gabapentin, 30 mg/kg (*p.o.*) pregabalin, 30 mg/kg (*p.o.*) carbamazepine and 120 mg/kg (*p.o.*) oxcarbazepine in a time-dependent manner at a 1-60 minute time-interval, in capsaicine-evoked thermal hyperalgesia model of rats via Hargreave's method. Thermal thresholds were significantly increased by 100 mg/kg gabapentin, 30 mg/kg pregabalin and 30 mg/kg carbamazepine treatment at 15-30-45, 15-30-45-60and 15-30-45 minute time-points, respectively, after capsaicin injection compared to capsaicin-treated group while 120 mg/kg oxcarbazepine was ineffective at any time points. It is suggested that gabapentin, pregabalin and carbamazepine are effective in inflammatory pain and capsaicin receptor, TRPV1 channels, may contribute to antihyperalgesic effects since these antiepileptics reversed thermal hyperalgesia induced by capsaicin.

Keywords: Capsaicin; Carbamazepine; Gabapentin; Oxcarbazepine; Pregabalin

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INTRODUCTION

Antiepileptic agents are known to be effective in neuropathic pain conditions. There is also substantial preclinical evidence on their efficacy against inflammatory pain. The activation of nociceptive pathway and development of hyperexcitability of pain transmitting neurons are common features of neuropathic and inflammatory pain[1]. Increased responses to noxious stimuli, in other words hyperalgesia, is a characteristic feature of chronic pain conditions such as neuropathic and inflammatory pain. Capsaicin, an ingredient in hot peppers, produces hyperalgesia and burning pain in humans [2]. The capsaicin-induced hyperalgesia model is a human representative model imitating symptoms of pain and suitable for investigating the underlying pathophysiological mechanisms [3,4].The transient receptor potential channel subfamily V member 1 (TRPV1) is also known as the capsaicin receptor and a primary transducer molecule of nociceptors, activated by noxious thermal stimuli (with a thermal threshold >43^{o}C)[5] as well as by capsaicin [6]. The activation of TRPV1 by capsaicin triggers both mechanical and thermal hyperalgesia in rats, primates and humans [7]. TRPV1 has been stated as a potential target for inflammatory and neuropathic pain therapy because it plays a key role in nociceptive signalling;however, clinical studies with TRPV1 modulators have struggled with side effects [4,8].

Investigating of action mechanisms of agents currently used for pain relief in chronic pain conditions will shed light on research for more effective new agents, and also introduce new preclinical data that may be beneficial for therapeutic approaches. The rational drug use requires comprehension of the impacts of the available agents in distinct pain models to ideally match them to the type of pain [9]. Additionally, different types of pain models were conducted to evaluate the mechanism of action of agents in several studies [10,11]. Pregabalin and gabapentin, alias gabapentinoids, carbamazepine and its clinically approved keto analogue oxcarbazepine are antiepileptic agents which have been shown to provide clinically proven effective therapies in the management of neuropathic pain. These agents have all been shown to indicate antihyperalgesic effect in various pain models [12,13]. However, their mechanisms of action in pain have been partly discovered. Nonetheless their antihyperalgesic effects in capsaicin-induced thermal hyperalgesia model have not been studied yet although there are assertionsrelated to the link between their pharmacological effects and TRPV1 channels [14,15].

In view of these facts, and considering the importance of investigating effects of agents in different pain models, the acute antihyperalgesic effects of 100 mg/kg (*p.o.*) gabapentin, 30 mg/kg (*p.o.*) pregabalin, 30 mg/kg (*p.o.*) carbamazepine and 120 mg/kg (*p.o.*) oxcarbazepinewere investigated in the capsaicin-induced thermal hyperalgesia model of rat via Hargreave's method in a time-dependent manner at a 1-6 minute time-interval.

MATERIALS AND METHOD

Animals

Male Sprague Dawley rats, weighing 250-300 g, were used in this study. The animals were housed in well-ventilated and temperature-controlled (22 ± 1 ºC) cages that are set to the 12 h/12h light/dark cycle with free access to standard animal feed and water. Animal care and research protocols were based on the principles and guidelines adopted by the National Institute of Health and Welfare guidelines on the ethical use of animals. This study was approved by the Local Ethics Committee of Osmangazi University, Eskisehir, Turkey.

Chemicals

Capsaicin (Sigma, St. Louis, U.S.A.) was dissolved in 10 % Tween 80 (Merck, Darmstadt, Germany) in saline and used for inducing hyperalgesia. Gabapentin (Santa Cruz Biotechnology, Dallas, U.S.A), pregabalin (Tocris Bioscience, Ellisville, Missouri, U.S.A.), carbamazepine (Sigma) and oxcarbazepine (Sigma) were dissolved in saline.

Capsaicin-induced hyperalgesia model

Capsaicin (2g/ 1mL) was dissolved and sonicated in 10 % Tween 80 (in saline). 20 µg capcaicin was injected into the right hindpaw (i.pl.) of each rat in a volume of 30µL [16].

Experimental groups and treatment regimens

In order to carry out this study, animals were randomly divided into six groups, each group containing eight (n=8) animals. The groups were formed as follows;

Control group: The vehicle (saline) was administered per orally 45 min prior to intraplantar injection of 10 % Tween 80 solution in a volume of 30 µL.

Capsaicin group: The vehicle (saline) was administered per orally 45 min prior to intraplantar injection of capsaicin (20 µg in 30 µL).

Treatment groups: 100 mg/kg gabapentin, 30 mg/kg carbamazepine and 120 mg/kg oxcarbazepine were administered per orally 45 min, while 30 mg/kg pregabalin was administered per orally 105 min prior to intraplantar injection of 20 µg/30 µL capsaicin.

Before the administration of drugs, hind paw withdrawal latencies evoked by radiant thermal stimuli were assessed using a plantar test apparatus and recorded as the baseline values. Thermal

withdrawal thresholds were reassessed 15, 30, 45 and 60 min after capsaicin injection.

Plantar Test (Hargreaves' Method)

Thermal hyperalgesia was assessed by determining the withdrawal thresholds of the right hind paws of rats in response to thermal stimulation generated by the plantar test (Ugo-Basile, 7280, Italy) apparatus. The infrared intensity of the device was adjusted to give an average paw withdrawal latency of about 10 s in naïve rats and cut-off time was set to 20 s to avoid tissue damage [17]. Animals were placed in glass floored clear plastic cages and they were allowed to acclimate for 30 min prior to testing. Movable high intensity radiant heat source was put beneath the mid-plantar surface of the right hind paw. During the measurement, it was verified that the animal and therefore its paw are immobile. As a result of stimulus, photocell-controlled timer was activated and it stopped automatically when the light beam was interrupted because of movement of the hind paw. Animal reflex movements were measured from the onset of the thermal stimulus to withdrawal of the paw [18]. 3-4 consecutive measurements were performed and the withdrawal threshold for each rat was calculated by mean latency of these responses.

Statistical analyses

Two-way ANOVA followed by Bonferroni's post-hoc test was performed for statistical analyses by using GraphPad Prism version 5.0. The results were expressed as the mean ± SEM to show variation in experimental groups. Differences were considered significant when $P \leq 0.05$.

RESULTS and DISCUSSION

Development of hyperalgesia

Thermal hyperalgesia was developed by capsaicin injection as seen in Figure 1 and 2. The latency of paw withdrawal against to thermal stimuli were significantly decreased at 15^{th} min (P<0.001) after capsaicin injection and persisted for about 30 min (P<0.001) compared to control group. The thermal hyperalgesia induced by capsaicin disappeared at around 60^{th} min.

Evaluation of antihyperalgesic activity of gabapentinoids

The paw withdrawal latencies against to thermal stimuli are shown in Figure1. Thermal thresholds were significantly increased by 100 mg/kg gabapentin treatmentat 15, 30 and 45 min after capsaicin injection (P<0.001, P<0.001, P<0.05, respectively) compared to capsaicin-treated group. The paw withdrawal latencies against thermal stimuli were significantly increased by 30 mg/kg pregabalin treatmentat 15, 30, 45 and 60 min after capsaicin injection (P<0.01, P<0.001, P<0.05 and P<0.05, respectively) compared to capsaicin-treated group. Additionally, pregabalin showed significant effect (P<0.05) at 60^{th} min compared to control group.

Evaluation of antihyperalgesic activity of carbamazepine and its keto analogue, oxcarbazepine

The thermal thresholds were significantly improved in 30 mg/kg carbamazepine treated group at 15, 30 and 45 min post capsaicin injection compared to capsaicin-treated group (P<0.01). However, there was no significant difference between the paw withdrawal latencies against to thermal stimuli in 120 mg/kg oxcarbazepine and capsaicin treated groups at any time points tested.

These results reveal the preclinical data that shows the effectiveness of gabapentin, pregabalin and carbamazepine in inflammatory pain as well as neuropathic pain.

Several findings have enhanced the interest in capsaicin receptor/TRPV1, as an important site of thermal hyperalgesia and pain hypersensitivitysubsequent to tissue inflammation and/or injury [5, 19, 20],and have supported the applicability of pharmacological studies of capsaicin-induced pain to evaluate the antinociceptive efficacy of available and potential analgesics [21]. Thereby, the capsaicin-induced thermal hyperalgesia model was performed for evaluating antihyperalgesic effects of the tested antiepileptic drugs with the aim of assessing the contribution of TRPV1 activity to their effects. The capsaicin-induced thermal hyperalgesia has been well documented in several studies and consistent with these studies [22,23,24,25,26] a short-term thermal hyperalgesia, lasting for 30 minutes, was observed in our experimental conditions. Antiepileptic drugs tested in this study are all pronounced to be effective in neuropathic pain [14,27]. There is also substantial preclinical evidence on their efficacy against inflammatory pain [1].However, their mechanisms of action in pain have been partly discovered. The inhibition of calcium currents via high-voltage-activated channels containing the α2δ-1 subunit is the main mechanism defined for pharmacological effects of gabapentin and pregabalin [28]. The main target of carbamazepine is voltage-dependent sodium channels although it interacts with different types of channels and receptors [29]. Oxcarbazepine blocks voltage-dependent sodium and also N-type calcium channels [30]. Nonetheless, additional mechanisms may be involved in their effects. The fact that the tested drugs in this study attenuated "*capsaicin*"-induced thermal hyperalgesia indicates the possible implication of the TRPV1 channel, capsaicin receptor, and activation at least in part in

the antihyperalgesic activity of the tested drugs, except for oxcarbazepine. The antihyperalgesia induced by gabapentin was found to be similar to pregabalin, but the effect of pregabalin lasted longer as well as it had later onset of action. Ourpreliminary studies introduced the fact that pregabalin was effective only 45 min after capsaicin injection (data not shown), when the reversal of the thermal hyperalgesia began, suggests that the effects of pregabalin developed later than the anticipated onset of action. Thereby, unlike other drug injections, pregabalin injection was performed 105 min prior to capsaicin injection. The antihyperalgesic effect of gabapentin and pregabalin were weakened at 45^{th} min, the point where the capsaicin-induced hyperalgesia started to reverse. Thereby, it can be inferred that the activation of TRPV1 channels plays an evident role in the antihyperalgesic effects of these agents. Similar to results of gabapentin, carbamazepine was also effective at 15-45 minutes time interval. Therefore, it may be indicated that the antihyperalgesic effect of carbamazepine via TRPV1 channels is similar to that of gabapentin. But its analogue, oxcarbazepine, was not found to be effective at any time interval. Oxcarbazepine is a keto-analogue, which distinguishes it from carbamazepine in terms of adverse effects, auto-induction or drug interaction properties, but also probably changes its mode of action [31]. It is known that TRPV1 channels sensitization alter in pathologic conditions as neuropathic pain [32].Through the guidance of the data introduced byBiggset. al. [14], showingthat capsaicin increase gabapentin effectiveness since gabapentin entry via the open pore of capsaicin-activated TRPV1 channel was 500× more rapid than usual, we hypothesized that effectiveness of the drugs tested in this study can be related to the open state of capsaicin-activated TRPV1 channels. This hypothesis may explain why the antihyperalgesic effect of drugs tested in this study was attenuated after the impact of the capsaicin disappeared. Additionally, effect of gabapentin and carbamazepine totally disappeared at 60^{th} min whereas effect of pregabalin was persistent slightly at this time point. The residual effect of pregabalin at around 60^{th} minute may be due to the differences in pharmacokinetic and pharmacodynamic profiles of these anticonvulsant agents [33,34,35,36].Ineffectiveness of oxcarbazepine in TRPV1 related hyperalgesia may indicate that its mechanisms of action are irrelevant to TRPV1 or its effects could not started because it could not enter from this channel efficiently. These results may also help explain why these drugs are effective in the treatment of neuropathic pain, but not equally effective in acute pain [37]. In addition these claims, since phosphorylation is closely related to TRPV1 sensitisation, the anticonvulsants found effective in this study may be interacting with serine/threonine kinases, protein kinase A / protein kinase C / Ca^{2+} / calmodulin-dependent kinase 2 / cyclin-dependent kinase 5. Indeed, the association between serine/threonine kinases with pregabalin, gabapentin and carbamazepine was reported in various studies [38,39,40].

CONCLUSION

This study is the preclinical evidence about the effectiveness of gabapentin, pregabalin and carbamazepine in inflammatory pain as well as neuropathic pain since they demonstrate the antihyperalgesic activitiesin capsaicin-induced thermal hyperalgesia model. The fact that the tested drugs in this study attenuated "*capsaicin*"-induced thermal hyperalgesia indicates the possible implication of the TRPV1channel, capsaicin receptor,activation at least in part in the antihyperalgesic activity of the tested drugs, except for oxcarbazepine. It is hypothesized that effectiveness of the drugs tested in this study can be related to the open state of capsaicin-activated TRPV1 channels. The evaluation of action mechanisms of drugs will provide guide findings for novel drug development studies, and also may introduce beneficial findings that concerns clinical approach. TRPV1 mediated antihyperalgesic effects shown in this study points out the need for research areas related to TRPV1 activation in further studies for pain relief. The mechanistic studies concerning the link between endogenous pain related systems, TRPV1 channels and tested drugs, are currently under investigation by our team.

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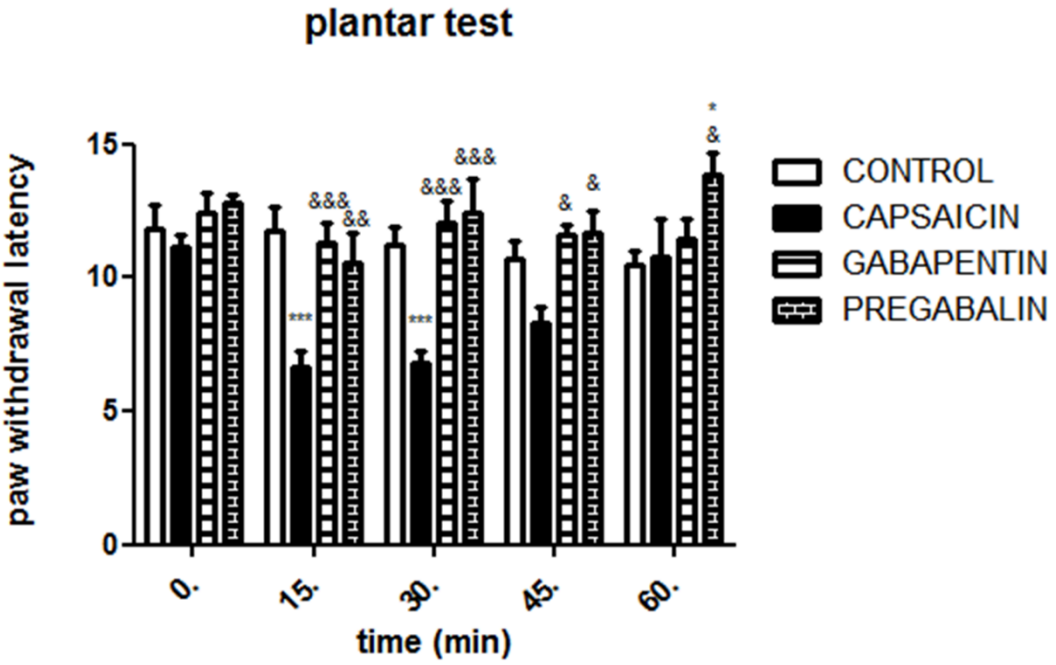

Figure 1. The paw withdrawal latencies against thermal stimuli of gabapentin and pregabalin-treated rats. $^{*}P<0.05$,$^{***}P<0.001$; Significant difference based on the control group. $^{\&}P<0.05$,$^{\&\&}P<0.01$, $^{\&\&\&}P<0.001$; Significant difference based on the capsaicin group. Two-way ANOVA followed by Bonferroni post-test was performed. Values expressed as mean ± SEM. (n=8)

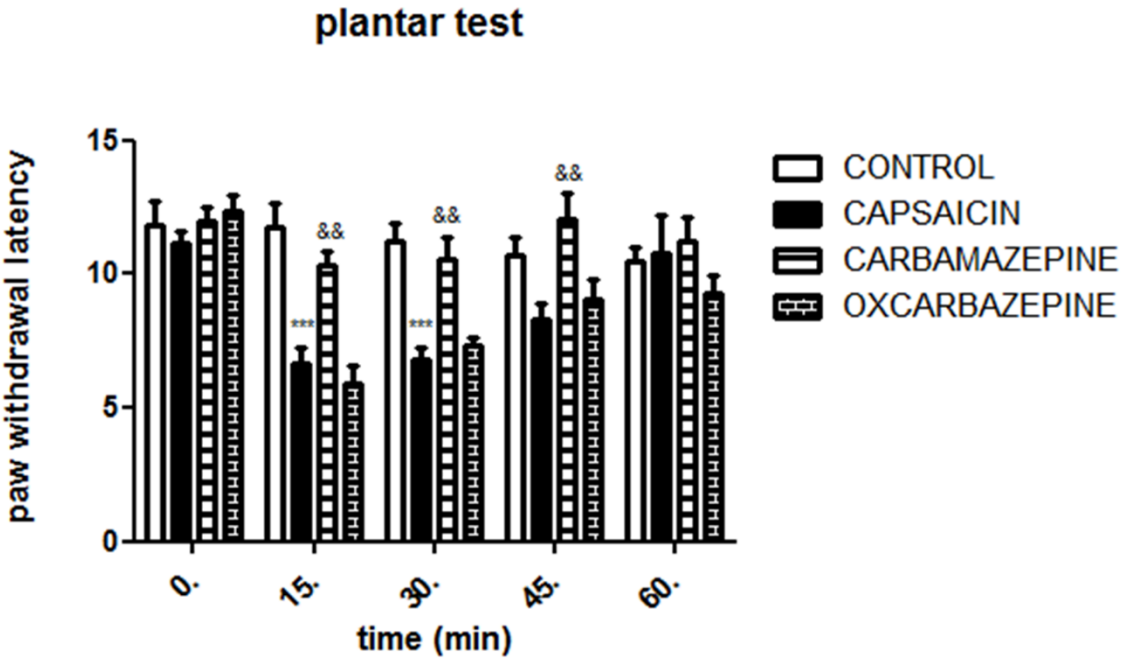

Figure 2. The paw withdrawal latencies against thermal stimuli of carbamazepine and oxcarbazepine-treated rats. $^{***}P<0.001$; Significant difference based on the control group. $^{\&}P<0.05$,$^{\&\&}P<0.01$, $^{\&\&\&}P<0.001$; Significant difference based on the capsaicin group. Two-way ANOVA followed by Bonferroni post-test was performed. Values expressed as mean ± SEM. (n=8)

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