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The Effect of Ruthenium Red on the Capsaicin-Induced Antinociception *in vivo*

Buyean LEE*, Yi-Sook JUNG, Jin-Il CHOI and Jae Yang KONG

[2]*Korea Research Institute of Chemical Technology, P.O. Box 107, Daedeog-Danji, Taejeon 305-606, Korea*

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Abstract—The effect of Ruthenium Red on the antinociceptive action of capsaicinoids was investigated using tail-flick test in mice. Capsaicin and KR-25018, when administered subcutaneously, had a potent antinociceptive effect against noxious heat stimulus. Ruthenium Red which is known to block the calcium channel coupled to the capsaicin receptor, when injected intraperitoneally more than 5 mg/kg, showed severe sedation and apparent antinociceptive effect against noxious heat stimulus. The 2.5 mg/kg Ruthenium Red, at which dose any significant sedative effect was not shown, had no effect on the antinociceptive effects of capsaicin and KR-25018. Considering this result, the antinociceptive effect of capsaicinoid may not be related to the Ruthenium Red sensitive calcium channel which is activated by capsaicin.

Keywords □ capsaicin, antinociception, Ruthenium Red, KR-25018

Capsaicin(N-methyl- N-vanillyl-6-nonenamide) is the pungent principle in hot peppers. It has the unique property of selectively activating polymodal nociceptors following local or systemic administration.

It has been well established that large systemic doses of capsaicin in the neonatal animal are neurotoxic to small unmyellinated C-fibers(Jancso *et al.*, 1977) and these high dose treatments lead to a very long lasting or even permanent increase in nociceptive thresholds (Hayes *et al.*, 1981a; Buck and Burks, 1986).

Following administration of small acute doses of capsaicin in adult rodent, however, there is a period of insensitivity to further noxious stimuli(Hayes *et al.*, 1981b) which is distinct from the toxic effects. The selectivity of capsaicin for peripheral polymodal nociceptors has made it of particular interest to those studying pain mechanism. The mechanism of action of capsaicin on peripheral polymodal nociceptors has recently been elucidated by the demonstration of the existence of a specific capsaicin receptor(Szallasi and Blumberg, 1990), the activation of which leads to the opening of cation-unselective channel which mediates the action of capsaicin on sensory nerves(O'Neill, 1991).

The inorganic dye, Ruthenium Red, at concentrations up to 10 M, acts as a specific capsaicin antagonist by blocking the opening of the cation channel coupled to the capsaicin receptor(Maggi *et al.*, 1989; Dray *et al.*, 1990). Several studies have indicated that Ruthenium Red antagonizes the acute(Amann and Lembeck, 1989; Amann *et al.*, 1989; Dray *et al.*, 1990) as well as the long term effects of capsaicin(Chahl, 1989; Maggi *et al.*, 1989; Amann *et al.*, 1990).

In the present study, we have used capsaicin and KR-25018, another capsaicinoid with potent analgesic activity(Lee *et al.*, 1994), to investigate the extent to which systemic administration of Ruthenium Red influences the capsaicinoid-induced antinociception in behavioral animal model.

Materials and methods

Materials

All experiments were performed on female ICR albino mice, weighing 25 to 31 g supplied by Animal Research Lab. of KRICT. Female animals were used as the responses obtained from various analgesic tests using female animals were more dose-dependent with smaller deviation compared to that of using male animals.

The animals were housed in a storage room under the condition of constant temperature, relative humidity and illumination(12hr light, 12hr dark cycle) until the day of experiment with free access to food and tap water. The animals were randomly assigned to treatment groups, and the observer was unaware of the treatment of an individual animal.

* To whom correspondence should be addressed.

Capsaicin and Ruthenium Red were purchased from Sigma Chemical Co.(St. Louis, MO). KR-25018 was synthesized in KRICT(Taejeon). Capsaicin and KR-25018 were suspended and administered in a vehicle consisting of 1% tween 80, 5% ethanol and 94% distilled water. Ruthenium Red was solubilized in the distilled water. This vehicle alone served as the control treatment.

Test for antinociceptive effect of capsaicin and KR-25018

The tail-flick assay method of Smith and D'Amour (Smith *et al.*, 1943) was used with mice. Radiant heat was applied on a tail spot using a focused beam of high-intensity light. The response time, defined as the interval between the onset of the stimulus and the tail-flick, was measured electronically(to the nearest 0.1 second) one hour after the subcutaneous administration of drugs(4 dose levels) or vehicle. The beam intensity was set at the level giving a mean control reaction time of 4.93 ± 0.53 seconds(S.E.M., n=20). Animals that did not flick their tails within 15 seconds were assigned a 15-second(maximum possible value) response latency.

Effect of Ruthenium Red on the antinociceptive effect of capsaicin and KR-25018

Separate groups of ten mice were tested by the tail-flick assay method using the analgesymeter(TSE, German) after administration of vehicle, capsaicin(3 mg/kg, s.c.) and KR-25018(0.6 mg/kg, s.c.). Analgesic effects were assessed 60 minutes after treatment of capsaicin, KR-25018 or vehicle.

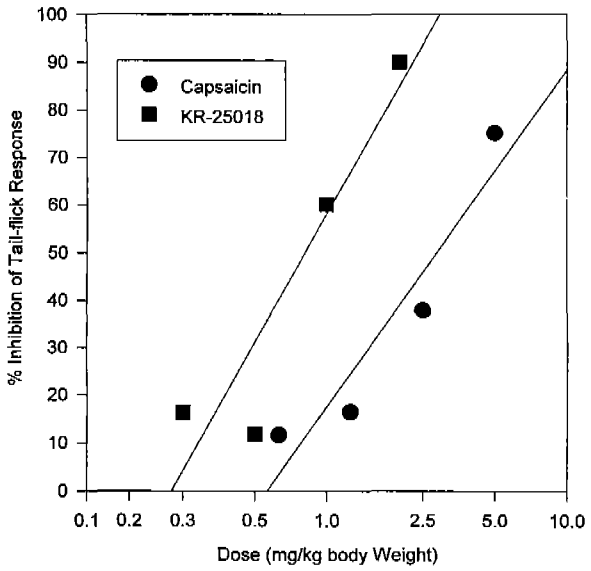

Fig. 1. Antinociceptive effect of capsaicin and KR-25018 in the tail-flick test in mice(n=10 in each group).

To see the reversing effect of Ruthenium Red upon the analgesic effect of capsaicin and KR-25018, Ruthenium Red was administered 30 minutes before the treatment of capsaicin, KR-25018 or vehicle.

Statistics

The %MPE(% maximal possible effect) was calculated as follows:

$$\%MPE = \frac{\text{mean test value} - \text{mean control value}}{\text{maximum possible value} - \text{mean control value}} \times 100$$

The %MPE could be interpreted as the mean degree of analgesic effect on a given test. The 100 %MPE indicates that the drug produced the maximum possible effect. The 0 %MPE means that the drug produced no effect. Mean %MPE data were subjected to a linear least squares regression analysis to determine "$MPED_{50}$". $MPED_{50}$ could be interpreted as the best estimate of the dose level of a test drug to obtain the 50% of maximum possible effect on a given test.

Statistical significance of differences between groups was determined by unpaired Student's t test and ANOVA test.

Results and discussion

In mice, subcutaneous dose of capsaicin($MPED_{50}$=3 mg/kg) and KR-25018($MPED_{50}$=0.61 mg/kg) exhibited potent antinociceptive effect against the thermal stimu-

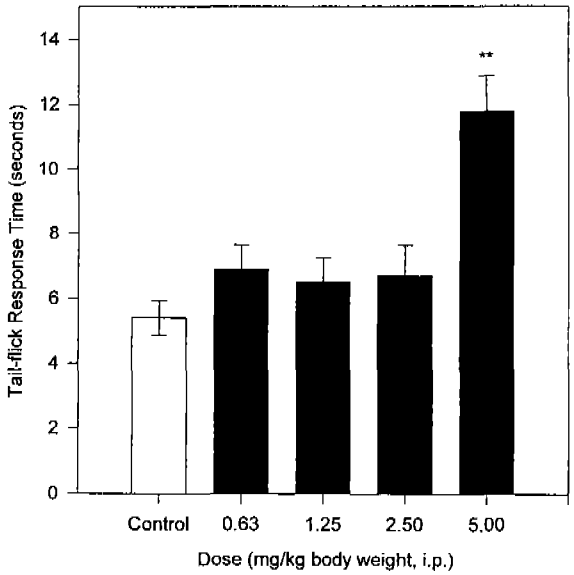

Fig. 2 Changes of tail-flick response latency after intraperitoneal administration of Ruthenium Red(n=10 in each group). ** : statistically different from distilled water treatment group ($p<0.01$).

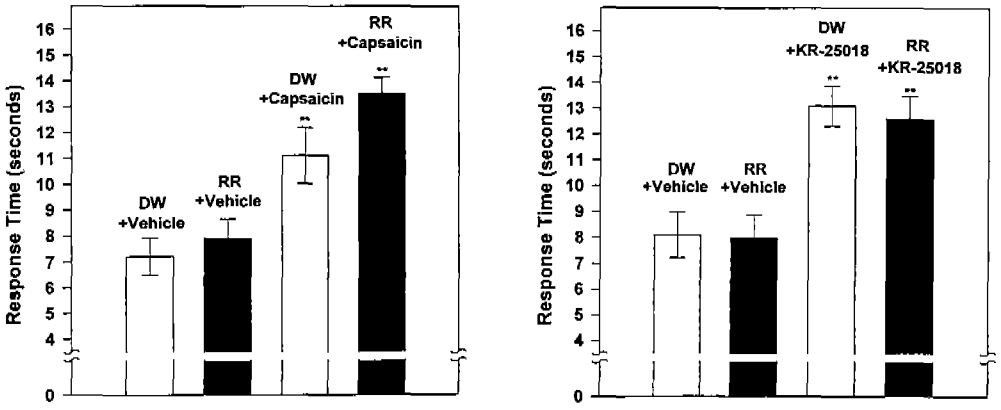

Fig. 3 Failure of Ruthenium Red to antagonize the antinociceptive activity of capsaicin(n=10, each group) and KR-25018(n=10, each group). The heights of the bars represent the mean response latency± S.E.M.(vertical lines). DW means distilled water. **: Statistically different from vehicle+distilled water treatment group($p<0.01$).

lus in the tail-flick test(Fig. 1). This data is in agreement with previous studies relating to the acute analgesic actions of capsaicin(Dickenson and Dray, 1991; Hayes *et al.*, 1981b). Ruthenium Red at the dose of 2.5 mg/kg(i.p.) had no obvious effect on behaviour of the mice. Within 5 min after the administration of Ruthenium Red, the urine was pink indicating fast absorption of the dye. At this dose there was no significant change in the response time to the noxious heat stimulus. Administration of larger dose of Ruthenium Red(5 mg/kg, i.p.) resulted in severe sedation and apparent antinociception in the tail-flick test in mice(Fig. 2). At the dose of 8 mg/kg(i.p.), none of the ten animals survived with the treatment. According to these results, 2.5 mg/kg(i.p.) of Ruthenium Red was used in the following experiment.

It has been suggested that the effects of capsaicin on mammalian sensory neurons are mediated by interacting with the specific membrane receptor(Dickenson and Dray, 1991; Szallasi and Blumberg, 1990). Recently, it was reported that Capsazepine, a new competitive capsaicin receptor antagonist, could reverse the antinociceptive action of capsaicin *in vivo*(Perkins and Campbell, 1992). Thus the antinociceptive actions of capsaicin are regarded to be caused by the consequences of the activation of capsaicin receptor by the capsaicin.

Using Ruthenium Red, which is known as the capsaicin-activated calcium channel blocker, we studied whether it blocks the antinociceptive action of capsaicinoid in behavioral animal model.

Capsaicin(3 mg/kg, s.c.) and KR-25018(0.6 mg/kg, s.c.) significantly increased tail flick latency in mice. Fig. 3 shows that Ruthenium Red by itself at 2.5 mg/kg, i.p., in mice, did not significantly change tail flick latency and unexpectedly had no effect on the antinociception of capsaicin and KR-25018.

In conclusion, our data suggest that systemic Ruthenium Red cannot reverse the antinociceptive action of capsaicinoid. Considering this result, the antinociceptive action of capsaicinoid may not be related to the Ruthenium Red sensitive calcium channel which is activated by capsaicinoid.

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