응용약물학회지, The Journal of Applied Pharmacology 5, 211-214(1997)

The Mechanism of Thermoregulatory Action of Capsaicin Is Different from That of Its Antinociceptive Effect in Guinea Pig

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(Received April 1, 1997; accepted April 16, 1997)

Abstract – In the present study, we investigated the mechanisms of antinociceptive effect and thermoregulatory action of capsaicin in guinea pigs. The administration of capsaicin (5 mg/kg, s.c.) caused a significant decrease in frequency of eye wiping, an indicative of nociceptive threshold. This antinociceptive effect of capsaicin was abolished by co-administration of capsazepine (30 mg/kg, s.c.) with capsaicin, suggesting the involvement of a vanilloid receptor in the antinociceptive action of capsaicin. The administration of capsaicin (1 mg/kg, s.c.) produced a significant decrease in body temperature of guinea pigs. The maximum decrease in body temperature by 2 degrees was shown 1 hour after the treatment, and this decrease was not reversed by co-administration of capsazepine. In conclusion, it is suggested that the mechanism of action of capsaicin-induced thermoregulation involves different pathways from that of capsaicin-induced antinociception.

Keywords □ capsaicin, guinea pig, nociception, body temperature, capsazepine

Capsaicin (8-methyl-N-vanillyl-6-nonenamide), a pungent ingredient of red peppers, has a wide spectrum of biological actions. On local or systemic administration, capsaicin causes initially an intense short-lasting irritation, possibly due to local and/or central release of substance P from primary afferent pain fibres (Gamse and Lemberk, 1979; Yaksh *et al.*, 1980). This is followed by a later phase in which the treated animal displays insensitivity to noxious stimuli. In our previous report, systemic administration of capsaicin was shown to increase the nociceptive threshold (Jung *et al.*, 1994). It is recently reported that small single doses of capsaicin have a transient antinociceptive effect lasting for several hours (Hayes *et al.*, 1981) and this is accompanied by a profound fall in body temperature (Jancso-Gabor *et al.*, 1970). Repeated large doses of capsaicin cause a much more enduring antinociceptive effect lasting up to several months (Hayes and Tyers, 1980). This subacute effect is accompanied by a depletion of substance P and somatostatin from primary afferent fibres, whereas peptide levels are unchanged or even increased following small single doses (Jessell *et al.*, 1978; Hayes *et al.*, 1980; Gamse *et al.*, 1981).

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Capsazepine is the first competitive antagonist for capsaicin (Bevan *et al.*, 1992). It antagonizes the excitatory actions of capsaicin in vitro in a variety of preparations including guinea pig bronchi and rat urinary bladder via acting on a specific capsaicin or 'vanilloid' receptor. However, there is little information about the in vivo effect of capsazepine on the antinociceptive and/or thermoregulatory action of capsaicin in guinea pigs.

The first goal of the present study was to examine the extent of the effect of capsazepine on the antinociceptive action of capsaicin. Frequency of eye wiping was determined as an indicative of nociceptive threshold. The second goal of this study was to investigate whether co-administration of capsazepine influences the hypothermic action of capsaicin, in order to demonstrate the involvement of vanilloid receptor in these actions of capsaicin.

MATERIALS AND METHOD

All experiments were performed on male Hartley-outbred guinea pigs (350-550 g) supplied by Samyook Laboratory Animal Inc. (Osan, Korea). The animals were housed in storage room under the condition of constant temperature, relative humidity and illumination (12 hr light, 12 hr dark cycle) until the day of experiment with

free access to food and tap water.

Pretreatment of capsaicin

Guinea pigs were pretreated with terbutaline (50 μg/kg, s.c., 20 min before capsaicin pretreatment) and theophylline (100 mg/kg, i.p., 15 min before capsaicin pretreatment) to ensure respiratory function (Papka *et al.*, 1984). Ketalar (50 mg/kg, i.m., 5 min before capsaicin pretreatment) was given as an analgesic during the capsaicin pretreatment. Then one single injection of capsaicin (5.0 mg/kg, s.c. in each group) was performed on animals.

In other experimental group, capsazepine (30 mg/kg, s. c.) was co-administered with capsaicin (5.0 mg/kg, s.c.) under the same conditions as those in capsaicin single injection to examine the effect of capsazepine on the capsaicin-induced desensitization in guinea pig.

Test for sensory function by eye wiping test

One week after the capsaicin pretreatment, nociceptive sensitivity to chemical stimuli was assessed in the eye wiping test. A drop (20 μl) of the stock solution of capsaicin (10 mg/ml) was instilled into the right eye of guinea pig and then the frequency of protective movements (eye wiping with the forelegs) was counted for 30 sec.

Determination of body temperature

Body temperature was determined essentially as described by Obal and coworkers (de Vries and Blumberg, 1989). Prior to measurement of body temperature, the animals were treated subcutaneously with capsaicin (0.5, 1, 5 mg/kg) or capsazepine (30 mg/kg) or combination of capsaicin (1 mg/kg) and capsazepine (30 mg/kg). By using a rectal probe, body temperature was measured before (0 hr), and 0.5, 1, 2, 3, 4, 5 hr after the treatment.

Drugs

Capsaicin, terbutaline and theophylline were purchased from Sigma Chemical Co. (St. Louis, USA), ketalar from Yuhan Co. (Seoul, Korea), capsazepine from RBI (Natick, USA). Capsaicin and capsazepine was initially dissolved to a concentration of 10 mg/ml and 30 mg/ml, respectively, in a mixed solution of ethanol, Tween 80 and saline (1:1:8). Further dilutions were then made in saline to give the concentrations desired. The vehicle (ethanol:Tween 80:saline=1:1:8) alone served as the control treatment.

Statistical analysis

Statistical analysis of the data was performed by means of the nonlinear regression and Student's t test. The level of significance was taken at $p<0.05$. All data were expressed as means±S.E.M.

RESULTS AND DISCUSSION

In the weanling rat, both capsaicin and its saturated analogue, dihydrocapsaicin were known to produce, marked increases in the nociceptive pressure tehreshold and a large fall in body temperature. These effects lasted for only a few hours. The potencies and time-courses of capsaicin and dihydrocapsaicin in producing these effects were the same. Capsaicin has no therapeutic value because, as well as producing analgesia, it also causes hypothermia and irritation. The irritant effect of capsaicin could result from the release of substance P from the central terminals of primary afferent fibres in the dorsal horn of the spinal cord (Szolcsanyi and Jancso-Gabor, 1976). The hypothermia could result from peripheral vasodilatation resulting from release of substance P from the peripheral terminals of primary afferent fibres via the axon reflex collaterals. This hypothermia may be less profound in larger animals e.g. man, as is the case with other vasodilator agents like hydralazine. But it may still be a limiting side effect. The mechanism of action of the acute antinociceptive and hypothermic effect is not clear, although recent evidence suggests that capsaicin has a vanilloid receptor-mediated analgesic effect in rats (Dickenson and Dray, 1991). The purpose of the present study was to investigate the mechanism of action of capsaicin and determine whether it is possible to separate the antinociceptive from the hypothermic properties of capsaicin by using a competitive vanilloid receptor antagonist capsazepine in guinea pigs.

We first investigated the effect of capsazepine on the antinociceptive action induced by systemic treatment of capsaicin. Fig. 1 shows the decreased frequency of eye wiping by capsaicin 5 mg/kg, which indicates that systemic administration of capsaicin increase the nociceptive threshold resulting potent antinociception against chemical (capsaicin) stimulus. These data are in agreement with previous studies resulting to the analgesic effect of capsaicin (Jung *et el.*, 1994; Hayes *et al.*, 1981; Dickenson and Dray, 1991). Using capsazepine, which is known as a new competitive capsaicin receptor antagonist, we examined whether it influences the antinociceptive action of capsaicin. When co-administered with capsaicin, capsazepine completely reversed the decreased frequency of eye wiping (Fig. 1), suggesting the involvement of vanilloid receptor in the antinociceptive action of capsaicin. It has been suggested that the effect of cap-

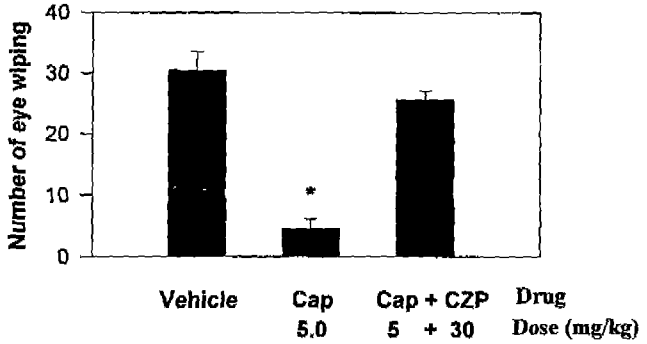

Fig. 1. Effect of capsazepine on the frequency of eye wiping one week after systemic administration (s.c.) of capsaicin. Capsazepine (CZP, 30 mg/kg) was co-administered with capsaicin (Cap, 5 mg/kg). Frequency of eye wiping, an indicative of nociceptive threshold, was measured for 30 seconds immediately after ophthalmic instillation of 20 μl of 10 mg/ml capsaicin. Values are mean±S.E.M. of determinations obtained from 4-8 animals. *Significantly different from vehicle-treated group ($P<0.01$).

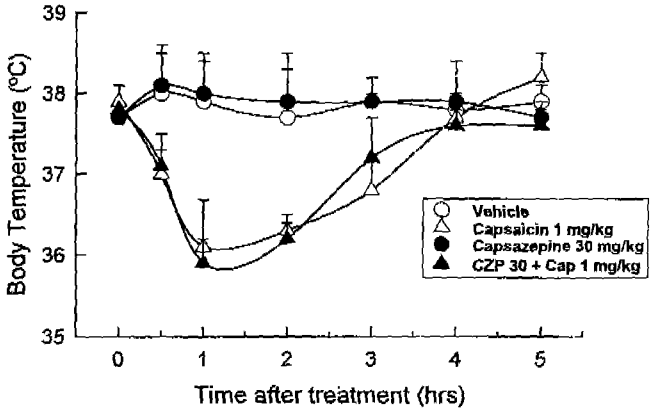

Fig. 2. Time course of the body temperature after systemic administration (s.c.) of capsaicin in guinea pig. Capsazepine (CZP, 30 mg/kg) was co-administered with capsaicin (Cap, 1 mg/kg). Values are mean±S.E.M.

saicin on mammalian sensory neurons are mediated by interacting with the specific membrane receptor (Szallasi and Blumberg, 1990). Recently, it was reported that capsazepine could reverse the antinociceptive action of capsaicin in vivo (Dickenson and Dray, 1991). Thus the antinociceptive actions of capsaicin are regarded to be caused by the consequences of the activation of capsaicin receptors by the capsaicin (Lee *et al.*, 1995), and our data from this study also support this regard.

As shown in Fig. 2, subcutaneous administration of capsaicin (1 mg/kg, s.c.) produced a significant decrease in body temperature in guinea pigs as compared to that of vehicle-treated animals ($p<0.01$), while treatment of capsazepine (30 mg/kg) alone did not have any effect on body temperature. In fact, we also treated guinea pigs with 0.5 and 5 mg/kg of capsaicin, but 0.5 mg/kg of capsaicin did not have significant effect (data not shown). In the case of 5 mg/kg capsaicin, almost animals treated with capsaicin were killed by the acute toxic effect, because we could not use terbutalin as respiratory protectives which itself has influence on body temperature. We, therefore, present the data on one dose (1 mg/kg) of capsaicin in Fig. 2. The maximum decrease in body temperature by 2 degrees was shown 1 hour after capsaicin treatment, this decrease was not reversed by co-administration of capsazepine.

In summary, capsazepine inhibits the antinociceptive action of capsaicin, but not the hypothermic effect of capsaicin. These results suggest that changes in vanilloid receptors are involved in capsaicin-induced antinociceptive desensitization, but not in hypothermic action of capsaicin in guinea pigs. In conclusion, the mechanism of action of capsaicin-induced antinociception and thermoregulation seems to be different from each other, suggesting the possibility of separation of the antinociceptive effect from the hypothermic side effect for development of a novel capsaicin-derivative as an analgesic.

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