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Capsaicinoids-induced Neurotoxic Desensitization in Guinea Pig: Antinociception and Loss of Substance P-like Immunoreactivity from Peripheral Sensory Nerve Endings in Bronchi

Yi-Sook JUNG*[1], Buyean LEE*, Hwa-Sup SHIN*, Jae-Yang KONG*, No-Sang PARK* and Tai-Soon CHO[1]

**Korea Research Institute of Chemical Technology, P.O. Box 107, Daedeogdanji, Taejon 305-606 and [1]College of Pharmacy, SungKyunKwan University, Suwon 441-746, Korea*

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Abstract—Antinociceptive and desensitizing effects of systemically administered capsaicinoids (capsaicin and KR25018) were investigated in guinea pig. Nociceptive sensitivity to chemical stimulus was examined to test sensory function, and the content of substance P-like immunoreactivity (SP-LI) in bronchi was determined as a peripheral marker of capsaicin-sensitive primary afferent neurons. Guinea pigs were pretreated s.c. with several doses of capsaicin (1, 2.5, 5, 10 mg/kg) or KR25018 (1, 2.5, 5, 10 mg/kg) one week prior to the experiments. Frequency of eye wiping was significantly decreased by capsaicin and KR25018 in a pretreatment dose-dependent manner. In capsaicin- or KR25018-pretreated guinea pigs, there was a significant loss of SP-LI in bronchial tissue extracts. In summary, a newly synthesized capsaicin analogue KR25018 exhibited antinociceptive effect against chemical stimulus in guinea pig, with comparable potency to capsaicin. This desensitizing activity of capsaicin or KR25018 might be related to the loss of SP-LI in peripheral afferent nerves.

Keywords □ capsaicin, KR25018, neurotoxicity, desensitization, antinociception, substance P-LI, guinea pig, bronchi

Capsaicin (N-methyl-N-vanillyl-6-nonenamide), the pungent ingredient of red peppers, exerts a selective excitatory action on a specific class of neurons i.e., sensory C-fiber afferent neurons (Jin *et al.*, 1990; Belvisi *et al.*, 1992). These neurons contain tachykinins such as substance P (SP) and neurokinin A that are thought to be involved in the perception of pain and in neurogenic inflammation (Buck *et al.*, 1986; Holzer, 1988). Capsaicin causes acute release of SP from peripheral sensory nerve endings in guinea pig bronchi as well as their central endings in spinal cord (Gamse *et al.*, 1979; Theriault *et al.*, 1979) followed by a long lasting depletion of SP from the primary afferent C-fibers (Jessell *et al.*, 1978; Nagy *et al.*, 1980; Furness *et al.*, 1982). In neonatal rats, large doses of capsaicin are neurotoxic resulting in selective degeneration of small unmyelinated C-fibers (Jancso *et al.*, 1977) and these treatments lead to a very long lasting or even permanent increase in nociceptive thresholds (Hayes *et al.*, 1981a; Buck *et al.*, 1986). Following administration of small acute doses of capsaicin in adult rodent, however, there is a period of insensitivity to further noxious stmuli (Hayes *et al.*, 1981b) which is distinct from the toxic effects. These long-term effects of capsaicin are termed 'desensitization', and capsaicin analogues have therefore been of interest as potential non-narcotic analgesics.

Recently, we have demonstrated that a newly synthesized KR25018 (N-[3- (3,4-Dimethylphenyl) propyl]-4-(2-aminoethoxy)-3-methoxyphenyl acetamide) has a potent analgesic activity with non-narcotic properties in rat and mice (Lee *et al.*, 1994; Lee *et al.*, 1995). In guinea pig bronchi, capsaicin and KR25018 produced a contractile response (Jung *et al.*, 1994), which appears to be mediated via the release of sensory neuropeptides such as substance P and neurokinin A from capsaicin-sensitive primary afferent neurons (Chahl 1982; Lundberg and Saria 1987). It has also been reported that systemic capsaicin pretreatment results irreversible changes in the respiratory tract in guinea pig (Lundberg *et al.*, 1983). However, little information is available about the antinociceptive action of capsaicin and

*[1] To whom correspondence should be addressed.

KR25018 in this species.

In the present study, we investigated whether systemic administration of capsaicin or KR25018 produce antinociceptive effect against chemical stimuli (capsaicin). In addition, we examined whether these capsaicinoids cause a reduction of SP in peripheral afferent nerve endings. The number of eye wiping was measured as an indicative of nociception, and the content of substance P-like immunoreactivity (SP-LI) in bronchi was determined as a peripheral marker of capsaicin-sensitive primary afferent nerves.

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 or KR25018.

Guinea pigs were pretreated with terbutaline (50 μg/kg, s.c., 20 min before capsaicinoids pretreatment) and theophylline (100 mg/kg, i.p., 15 min before capsaicinoids pretreatment) to ensure respiratory function (Franco-Cereceda and Hughes 1988). Ketalar (50 mg /kg, i.m., 5 min before capsaicinoids pretreatment) was given as an analgesic during the capsaicinoids pretreatment. Then one single injection of capsaicin or KR 25018 (1.0, 2.5, 5.0, 10 mg/kg, s.c. in each group) was made on animals one week prior to the experiments.

Test for sensory function.

Nociceptive sensitivity to chemical stimuli was assessed in the eye wiping test. One week after pretreatment, 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 foreleg) was counted for 30 sec.

Radioimmunoassay for SP-LI.

One week after pretreatment, guinea pigs were stunned and bled. The main bronchi were rapidly dissected out, weighed and frozen on dry ice. After storage at −70℃ the specimens were transferred to at least 20 volumes of 1.0 M acetic acid at 95℃ in Dri-Block (Teche, USA) (Lundberg *et al.*, 1983). After 10 min, the biopsies were homogenized using a Polytron®. Subsequently, the homogenates were centrifuged at 1000 ×g at 4℃ for 10 min and the supernatants were collected and lyophilized using a speed-vac. concentrator (Savant, USA). Lyophilized pellet was resuspended in distilled water and spun at 1000×g at 4℃ for 10 min. The final supernatants were used for protein assay by Bradford method and for quantitative determination of substance P-like immunoreactivity by using ^{125}I-SP RIA Kit (Incstar Co., USA). Each extract was assayed in duplicate.

Drugs.

KR25018 was synthesized in Korea Research Institute of Chemical Technology (Taejon, Korea). Capsaicin, terbutaline and theophylline were purchased from Sigma Chemical Co. (St. Louis, USA), Ketalar from Yuhan Co. (Seoul, Korea), capsazepine from RBI (Natick, USA) and protein assay kit from Bio-Rad Lab. Inc. (Hercules, USA). ^{125}I-substance P RIA kit purchased from Incstar Co. (Stillwater, USA) was used within one month after being radiolabeled. KR25018 was dissolved in saline. Capsaicin was initially dissolved to a concentration of 10 mg/ml 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 (saline for KR25108, or the above mixed solution for capsaicin) 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 & Discussion

In this study, we first investigated the extent to which systemic treatment of capsaicin or KR25018 causes antinociception. Fig. 1 shows the decreased frequency of eye wiping by capsaicin or KR25018 in the dose range of 2.5~10 mg/kg, which indicates that systemic administration of capsaicin or KR25018 increase the nociceptive threshold resulting in a potent antinociception against chemical (capsaicin) stimuli. These are in agreement with previous studies referring to the analgesic effect of capsaicinoids (Hayes *et al.*, 1981 b; Dickenson and Dray, 1991). However, the relative potency of KR25018 was somewhat different from that in previous studies. Comparing the antinociceptive effect, KR25018 and capsaicin was almost epuipotent (ED_{50} : 2.3, 2.7 mg/kg, respectively), whereas in the previous studies KR25018 was 4-fold more potent than capsaicin in mouse tail-flick test (Lee *et al.*, 1995) and 2.5-fold more potent in rat adjuvant arthritic flexion test (Lee *et al.*, 1994). These differences in the relative potency could be explained by the difference in assay system

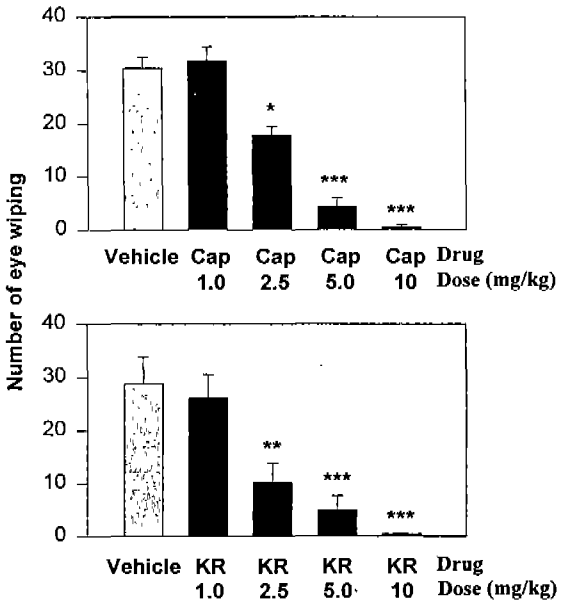

Fig. 1. Frequency of eye wiping measured one week after systemic administration (s.c.) of capsaicin (Cap) or KR25018 (KR). Frequency of eye wiping was measured for 30 seconds immediately after ophthalmic instillation of 20 μl capsaicin (10 mg/ml). Values are means± S.E.M. of determinations obtained from 4~8 animals. *p<0.05 as compared to control vehicle-treated group. **p<0.01, ***p<0.001 *vs* vehicle.

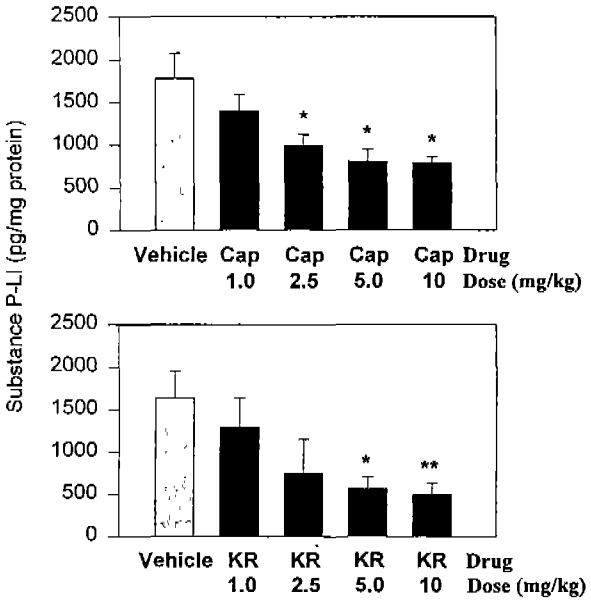

Fig. 2. SP-LI in guinea pig bronchi measured by radioimmunoassay one week after subcutaneous administration of capsaicin (Cap) or KR25018 (KR). Each experiment was carried out in duplicate in each tissue from different animals. Values are means± S.E.M. of determinations obtained from 4~6 animals. *p<0.05 as compared to control vehicle-treated group, **p<0.01 *vs* vehicle.

and/or species.

As shown in Fig. 2, subcutaneous administration of capsaicin or KR25018 significantly decreased the content of SP-LI in guinea pig bronchi as compared to that of control animals ($p<0.01$). SP was first described by von Euler and Gaddum in 1931 as having a wide distribution in the central and peripheral nervous system innervating guinea pig bronchus, rat heart and uterus (Wharton *et al.*, 1981; Weihe *et al.*, 1984). Substance P-immunoreactive (SP-IR) fibers are of sensory afferent origin since they are depleted of SP-LI by systemic capsaicin pretreatment, which results in degeneration of a population of sensory nerves followed by antinociception against further mechanical or chemical stimuli (Murphy *et al.*, 1982; Dalsgaard et al., 1983; Papka *et al.*, 1984). Although there are some evidences that antinociceptive activities of capsaicinoids are related to depletion of neuropeptides such as SP from the central sensory nerve endings in spinal cord (Szolcsanyi *et al.*, 1975; Bucsics *et al.*, 1981; Buck *et al.*, 1983; Jancso *et al.*, 1987), the exact mechanism of antinociception is not known. In the present study, we compared antinociceptive activity with SP-LI-reducing activity of capsaicin or KR25018 in view of their potencies. A significant reduction of SP-LI was shown in guinea pig peripheral afferent-innervated bronchi by capsaicin or KR25018 pretreatment (2.5~10 mg/kg) in the range of dose exhibiting the antinociceptive effect. This result suggests that antinociceptive activity of capsaicin or KR25018 may involve the loss of substance P in peripheral sensory nerve endings as well as central endings. It still remains to be studied whether this phenomenon is shown in all the other types of peripheral sensory nerve endings including urinary bladder and ileum.

In conclusion, KR25018 was comparable to capsaicin in its potency for antinociceptive effect and SP-LI-reducing effect in peripheral sensory nerves.

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