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KR-25018: A Novel, Orally Active Analgesic with Non-narcotic Properties

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Among the new series of phenylacetamides, one of capsaicin derivatives, KR-25018 was found to have a very potent analgesic activity. Thus, the pharmacological properties of KR-25018 were compared with those of morphine, capsaicin, and nonsteroidal antiinflammatory drugs (NSAIDs). The analgesic activities were evaluated in several animal models, using different stimuli, such as phenylbenzoquinone(PBQ)-induced writhing test, tail-flick test in mice and adjuvant arthritic flexion test in rat. The relationship of pharmacological properties of KR-25018 to that of centrally acting opioids was assessed by the blocking test using naloxone. The analgesic potency of the KR-25018 ($MPED_{50}$=0.89 p.o. in PBQ-induced writhing test, $MPED_{50}$ =0.61 s.c. in tail-flick test in mice), with different action mechanism from morphine and NSAIDs, was comparable to that of morphine.

Key words: Capsaicin, KR-25018, Morphine, NSAIDs, Analgesic activity

INTRODUCTION

Current pain treatments rely heavily on two types of analgesic agents, the opioids and the nonsteroidal anti-inflammatory agents. In an attempt to develop new analgesics, we have concentrated our efforts on the novel compounds which modulate the responses of nociceptive afferent neuron. Capsaicin is one of the prototype compounds. Capsaicin is a pungent component of chilli peppers and related plants of the *Capsicum* family (Woolfe and Macdonald, 1944). Capsaicin has a wide spectrum of biological actions including effects on the cardiovascular and respiratory systems (Virus and Gebhart, 1979; Wood *et al.*, 1988). It also causes the induction of pain on topical application to skin. This is followed by a period of desensitization, both to further applications of capsaicin and to other noxious chemical, thermal and mechanical stimuli. Administration of capsaicin to neonatal rats results in permanent loss of the majority of unmyelinated sensory neurons. This neurotoxic effect is much decreased in the adult animal (Jancso *et al.*, 1985). The effects of capsaicin seem to be mediated by its actions on a subset of peripheral sensory neurons, the polymodal nociceptors (Fitzgerald, 1983). Although the precise molecular basis for the action mechanism is not understood, the available evidence suggests that capsaicin interacts with a specific membrane receptor/ion channel complex which is peculiar to this subset of sensory neurons (Szallasi and Blumberg, 1990; Szallasi and Blumberg, 1993).

We found that several vanilloids, phenyl acetamide derivatives, showed antinociceptive activity in the adult mice. In the course of pharmacological studies on a new series of vanilloids, it has been found that KR-25018 (Fig. 1) is an interesting compound with potent analgesic activity by oral route against noxious chemical stimuli in mice (Park *et al.*, 1993a; Park *et al.*, 1993b; Park *et al.*, 1991).

The present paper is concerned with the analgesic action of KR-25018 against various noxious stimuli in several animal models. The data provided in this paper reflect the different pharmacological mechanism of KR-25018 inducing analgesic activity from that of morphine.

MATERIALS AND METHODS

Materials

All experiments were performed on female ICR albino mice, weighing 14 to 20 g for PBQ writhing test and 25 to 30 g for tail-flick test and naloxone inhibi-

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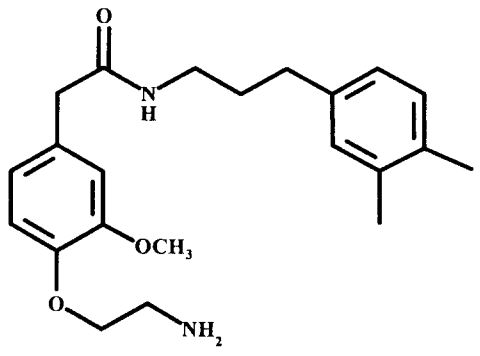

Fig. 1. Chemical structure of KR-25018: N-{3-(3,4-Dimethylphenyl)propyl}-4-(2-aminoethoxy)-3-methoxyphenylacetamide.

tion test, and female Sprague-Dawley Rats, weighing 180 to 200 g, for adjuvant arthritic flexion test, 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 conditions 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. The animals were randomly assigned to treatment groups, and the observer was unaware of the treatment of an individual animal.

Morphine·HCl was purchased from Jeil Pharmaceutical Co. (Seoul). Capsaicin, ketoprofen, aspirin, indomethacin, naproxen, and naloxone were purchased from Sigma Chemical Co. (St. Louis, MO). *M. butyricum* (desiccated) was purchased from Difco Lab. (Detroit, Michigan).

Except where otherwise noted, KR-25018 and comparative standards were suspended and administered in a vehicle consisting of 1% tween 80, 5% ethanol and 94% distilled water. This vehicle alone served as the control treatment.

Routes of administration varied and are noted in the various individual studies. Concentrations of solution were varied to allow a constant injection volume of 10 ml/kg of mouse and 5 ml/kg of rat.

Tests for Analgesia

The antinociceptive action of KR-25018 was evaluated by determining the response to three types of noxious stimulation: 1) i.p. injection of a noxious chemical (writhing test), 2) radiant heat (tail-flick test) and 3) mechanical noxious stimuli (adjuvant arthritic flexion test).

PBQ-induced Writhing Test in Mice: The PBQ-induced writhing test method of the Mine and Twomey (Milne and Twomey, 1980) was used. A writhing response consisting of a slow tonic contraction of the abdominal musculature followed by extension of the hind limbs was induced by i.p. injection of PBQ. One hour after the oral administration of drugs(4 dose levels) or vehicle, animals were injected with PBQ solution and placed separately in the plastic observation box (14×20×25 cm high). The number of writhing occurring within 5 minutes from the 5th to 10th minute after PBQ injection was counted.

Tail-flick Test in Mice: 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.

Adjuvant Arthritic Flexion Test in Rat: The experiments were performed in rats rendered hyperalgesic by injection of Freund's adjuvant. According to the Winter's method (Winter *et al.*, 1979), desiccated *Mycobacterium butyricum* (Difco) was ground in a mortar, suspended in liquid paraffin, sterilized in an autoclave, and injected (0.5 mg in 0.1 ml) subcutaneously in the right hind paw. About 12 to 16 days after injection of adjuvant, a severe arthritic condition developed in the paws inducing hyperalgesia. Two hours after the administration of drugs (4 dose levels, s.c.) or vehicle (s.c.), the hypersensitivity of the hind paw was examined as follows: one experimenter held the animal comfortably with both hands and the other one extended the paw gently. The gentle extension elicited a "squeak" from the animal. The squeak could be elicited repeatedly. Thus five consecutive stimuli were given at 5-sec intervals to each animals in testing drugs. If the animal emitted no more than one squeak in five trials, it was recorded as having analgesic response and was given the rating of 1. If there was more than one squeak, the rating was 0. By measuring the "squeak" response with several dose levels, the ED_{50} of the test drug could be estimated.

Naloxone Antagonism

Separate groups of ten mice were tested using the tail-flick analgesymeter (TSE, German) after administration of vehicle, KR-25018 (0.6 mg/kg, s.c.), morphine (1 mg/kg, s.c.), and naloxone (2 mg/kg, s.c.). Analgesic effects were assessed 60 minutes after treatment of

Table I. Analgesic effect of KR-25018 compared to that of standard compounds in the PBQ-induced Writhing Test (n=8, each dose level)

Compound	$MPED_{50}$ (lower/upper 90% confidence limit, mg/kg)
Piroxicam (p.o.)	0.89 (0.50/1.60)
Ketoprofen (p.o.)	1.19 (0.23/6.34)
Naproxen (p.o.)	17.07 (10.35/28.14)
Aspirin[a] (p.o.)	31.85 (9.0/103)
KR-25018 (p.o.)	0.89 (0.61/1.3)

[a] Aspirin was suspended in 0.5% carboxymethylcellulose solution.

morphine, KR-25018 or vehicle/saline, and 15 minutes after naloxone. To see the reversing effect of naloxone upon the analgesic effect of KR-25018 or morphine, naloxone was administered 45 minutes after the treatment of KR-25018, morphine or vehicle/saline.

Statistics

For the PBQ-induced writhing test, the %MPE was calculated as follows:

$$\%MPE = \frac{\text{mean control writhings} - \text{mean test writhings}}{\text{mean control writhings}} \times 100$$

In the tail-flick test in mice, persent maximal possible effect (%MPE) was calculated as follows:

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

In either case, 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.

RESULTS AND DISCUSSIONS

Analgesic Activity in Mice

In mice, KR-25018 at a dose of 0.89 mg/kg (p.o.) produced a 50% reduction in PBQ-induced writhing frequency (Table I). The KR-25018 was as potent as piroxicam and ketoprofen. The similar subcutaneous dose of KR-25018 ($MPED_{50}$=0.61 mg/kg) exhibited potent antinociceptive effect against the thermal stimulus in the tail-flick test in mice (Table II). Morphine and capsaicin were also active in the tail-flick test in mice with the $MPED_{50}$ of 1.02 mg/kg (s.c.) and 2.87 mg/kg (s.c.), respectively. However nonsteroidal antiinflammatory drugs (NSAIDs) such as indomethacin, aspirin and piroxicam, were inactive in the tail-flick test in mice (Table II). In this regard, analgesic effect of KR-25018 seems to be mediated via central mechanism, differently from NSAIDs. According to Sietsema *et al.* (1988), capsaicin and olvanil, another capsaicin analog, have very weak oral analgesic activities in mice. However KR-25018 was as potent as morphine in mice when administered orally ($MPED_{50}$=2.61 mg/kg).

Table II. Analgesic effect of KR-25018 compared to that of standard compounds in the tail-flick test in mice (n=10, each dose level)

Compound	$MPED_{50}$ (lower/upper90% confidence limit, mg/kg)
Morphine·HCl[a] (s.c.)	1.02 (0.7/1.6)
Capsaicin (s.c.)	2.87 (1.5/5.5)
KR-25018 (p.o.)	2.60 (2.0/3.4)
KR-25018 (s.c.)	0.61 (04./0.9)
Aspirin[b] (s.c.)	no effect
Piroxicam (s.c.)	no effect
Indomethacin (s.c.)	no effect

[a] Morphine·HCl was dissolved in saline.
[b] Aspirin was suspended in 0.5% carboxymethylcellulose solution.

Analgesic Activity in Adjuvant Arthritic Flexion Test in Rat

Groups of 10 rats received injections of adjuvant containing 0.5 mg of *M. butyricum*. The thickness of the hindpaw measured by clipers changes biphasically (data not shown). About 12-16 days after the injection of the adjuvant, the secondary arthritic edema of the hindpaw was developed. Hypersensitivity against foot flexion was observed in those animals showing gross signs of the arthritic condition. The KR-25018, morphine and capsaicin showed dose related analgesic activities in this test with ED_{50} (probit 5) values of 2.61, 0.95 and 6.31 mg/kg, respectively (Fig. 2).

As shown in Fig. 2, the KR-25018 was less potent than morphine and more potent than capsaicin in this rat model. That result indicates that KR-25018 and capsaicin would be less potent in the arthritic pain compared to morphine.

This study was the first time to seek dose-response curve using adjuvant arthritis model to compare the analgesic potencies of capsaicin and morphine.

Animal models commonly employed to assay narcotic and non-narcotic analgesics require the use of artificial provocations such as heat, mechanical or chemical insult. The PBQ writhing test in mice and the Randall-Selitto test in rats are used extensively for the experimental evaluation of analgesic agents and pro-

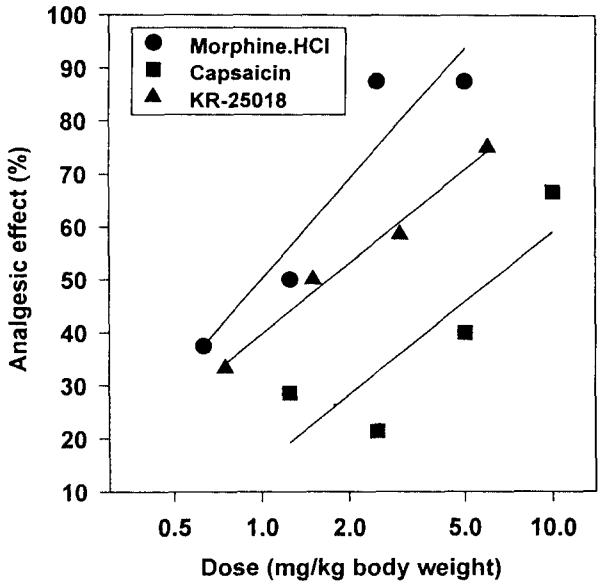

Fig. 2. Analgesic effects of KR-25018(s.c.) compared to that of morphine(s.c.) and capsaicin(s.c.) in the Adjuvant Arthritic Flexion Test in rat (n=10, each dose leves). Regression lines, morphine: response(probits)=1.8458Log(dose)+5.0390; capsaicin: response(probits)=1.1717Log(dose)+4.6063; KR-250 18: response (probits)=1.0810Log(dose)+4.5494. ED_{50} (probit 5) values of morphine, capsaicin and KR-25018 were 0.95, 6.31 and 2.61 mg/kg, respectively. Morphine was dissolved in saline.

vide reasonable correlations to clinical efficay and potency. However, some centrally and peripherally acting non-analgeic agents are active in PBQ writhing test, and a high degree of variation has been demonstrated in Randall-Selitto test (Capetola *et al.*, 1980; Robert *et al.*, 1980). A major complaint of rheumtoid arthritic patients is the pain associated with an affected joint. Adjuvant arthritis in rats resembles the human condition with respect to both inflammation and hyperalgesia upon joint movement. In this regard, the adjuvant arthritis flexion test in rat represents a unique model of pathologically induced pain.

Naloxone Antagonism

The relationship between the pharmacological properties of KR-25018 and that of centrally acting opioids was assessed by using opioid receptor antagonist, naloxone. The ability of naloxone to reverse the analgesic effect of morphine or KR-25018 was assessed in the tail-flick test in mice.

As shown in Fig. 3, naloxone (2 mg/kg) administered subcutaneously in the nape of the neck had no significant analgesic effect by itself. Analgesic effect of morphine exerted by the subcutaneous dose of 1 mg/kg was completely reversed by naloxone. In contrast, naloxone had no reversing effect upon the analgesic effect of KR-25018.

From this finding, it could be demonstrated that the action mechanism of KR-25018 is not based on the interaction with opioid receptors.

In summary, the KR-25018 showed very potent analgesic activities in writhing test, tail-flick test and adjuvant arthritic flexion test. The analgesic potency of KR-25018 administered orally in tail-flick test ($MPED_{50}$ =2.60 mg/kg) was comparable to that of morphine injected subcutaneously ($MPED_{50}$=1.02 mg/kg), indicating its potent analgesic activity via central mechanism. However the action mechanism of KR-25018 is different from that of morphine considering its analgesic effect that is not antagonized by naloxone and pre-

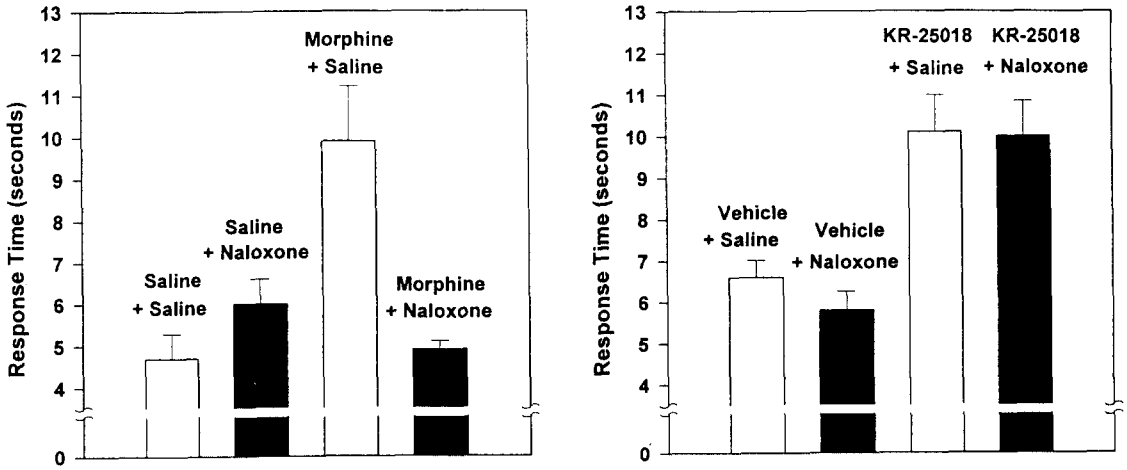

Fig. 3. Failure of naloxone to antagonize the analgesic action of KR-25018 (n=10, each group). The heights of the bars represent the mean response latency± S.E.M. (vertical lines). Morphine and naloxone were dissolved in saline. (Left panel) Naloxone reversed the analgesic effect of morphine with statistical significance ($p<0.01$; Saline+Saline vs. Morphine+Saline, $p<0.01$ Morphine+Saline vs. Morphine+Naloxone)(Right panel) Naolxone had no reversing effect upon the analgesic effect of KR-25018 ($p<0.01$; Vehicle+Saline vs. KR-25018+Saline, not significant; KR-25018+Saline vs. KR-25018+Naloxone).

vious studies showing its low binding affinity to the opioid receptors (Kong *et al.*, 1993).

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