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Effect of Capsaicin and Its Novel Derivative on the Isolated Guinea Pig Bronchi

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캡사이신과 그 합성유도체의 기니픽 기관지 평활근에 대한 작용

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ABSTRACT—In the present study we investigated the peripheral function of capsaicin and KR-25018, a newly synthesized capsaicin derivative, which was demonstrated to have a potent analgesic activity through different mechanism from morphine and nonsteroidal antiinflammatory drugs. Capsaicin (10^{-8}~10^{-5} M) and KR-25018 (10^{-8}~10^{-5} M) produced concentration-dependent contractions of the isolated guinea pig bronchi. There were no significant differences in the maximum response and the EC_{50} values (EC_{50}: 0.137± 0.025 μM and 0.097± 0.031 μM for capsaicin and KR-25018, respectively, $P>0.05$). Phosphoramidon (10 μM) and indomethacin (10 μM) had no significant effect on contractile response to the submaximal concentration range of capsaicin and KR-25018 (3×10^{-9}~3×10^{-7} M). The response to KR-25018, like that to capsaicin, was significantly inhibited by ruthenium red with reduction in the maximum response, which is indicative of non-competitive antagonism. A further common feature of the responses to capsaicin and KR-25018 in the guinea pig bronchi was their sensitivity to capsazepine. Capsazepine caused a rightward parallel shift in concentration-response curves obtained by capsaicin and KR-25018. The pA_2 values of capsazepine were 5.90 and 5.99 against capsaicin and KR-25018 response, respectively. In conclusion, KR-25018 and capsaicin exert their contractile effects in the isolated guinea pig bronchial muscle by common mechanisms, probably via the activation of a specific receptor.

Keywords □ Capsaicin, KR-25018, guinea pig, bronchi, ruthenium red, capsazepine

Introduction

Capsaicin (N-methyl-N-vanillyl-6-nonenamide), a pungent ingredient of red peppers and related plants of the *Capsicum* family,[1] has a wide spectrum of biological actions including the effects on the cardiovascular and respiratory systems.[2,3] It has been well known that the capsaicin evokes the release of neuropeptides from peripheral nerve terminals of primary afferent neurons in a Ca^{2+}-dependent manner.[4,5,6] In guinea pig bronchi, those nerves can be stimulated with capsaicin to produce a contractile response via the release of sensory neuropeptides such as substance P and neurokinin A.[7,8]

The capsaicin-induced influx of Ca^{2+} in afferent neuron, which triggers release of neuropeptides, is apparently not secondary to the opening of voltage-

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sensitive Ca^{2+} channels , but is mediated by cation channels which are activated by the capsaicin-specific receptor.[6,9-11] Studies from various laboratories have indicated that stimulation of the capsaicin, or 'vanilloid' receptor on the membrane of primary afferent neurones is followed by the opening of a novel type of receptor-operated ion channel, which admits both sodium and calcium ions.[12]

It has recently been proposed that two different substances act as capsaicin antagonists, the inorganic dye ruthenium red which does not interact with the vanilloid receptor[13] and the benzazepine derivative, capsazepine,[12,14,15] which was proposed as the first competitive antagonist of the capsaicin or vanilloid receptor. Ruthenium red, $[(NH_3)_5\text{-Ru-O-Ru}(NH_3)_4\text{-O-Ru}(NH_3)_5]Cl_6$, a water soluble inorganic dye, has been shown to act as a selective capsaicin antagonist at the level of plasma membrane by preventing the opening of cation-selective ion channels by capsaicin and potently inhibiting the capsaicin-evoked release of neuropeptides in neural tissues.[16-19]

Other studies in different models have shown that prostaglandins modulate the release of substance P from sensory nerves.[20] The same authors suggested that the antiinflammatory action of indomethacin, a classic prostaglandin synthesis inhibitor, might be due to the fact that the drug reduces the release of substance P from sensory nerves.

Previous studies demonstrated that KR-25018 (N-[3-(3,4-Dimethylphenyl) propyl]-4-(2-aminoethoxy)-3-methoxyphenyl acetamide), a newly synthesized capsaicin derivative, had a potent analgesic activity through different mechanism from morphine and nonsteroidal antiinflammatory drugs.[21] But the peripheral function of KR-25018 had not been studied. In the present study, we investigated the effect of capsaicin and KR-25018 and antagonistic activity of capsazepine and ruthenium red against capsaicin- and KR-25018-induced responses in isolated guinea pig bronchi in order to determine whether the peripheral function of KR-25018 is mediated via vanilloid receptor. We also examined the effect of phosphoramidon and indomethacin on contractile responses to capsaicin and KR-25018 in the same preparations.

Materials and Method

Male Hartley-outbred guinea pigs (Samyook Experimental Animal, Osan, Korea) weighing 400-600 g were stunned and bled. The trachea and main bronchi were rapidly removed and placed in Krebs solution (composition in mM: 118.3 NaCl, 4.7 KCl, 1.2 $MgSO_4$, 1.2 KH_2PO_4, 25.0 $NaHCO_3$, 2.5 $CaCl_2$, and 11.1 Glucose) at 37℃, gassed with 95% O_2 and 5% CO_2, which produced a pH of 7.4.[22,23] The main bronchi were isolated and prepared for isometric tension recording via Grass FT03 transducer and Grass 7D polygraph. Two bronchial tissues were suspended between stainless steel hooks and placed in a 20 ml organ bath under a resting tension of 500 mg, which was found to be optimal for measuring changes in tension.[24] Tissues were allowed to equilibrate for 60 min before experiments were begun. During equilibration, tissues were washed with fresh buffer at 15 min intervals. In each experiment, the response to acetylcholine (1 mM) was used as an internal standard, and responses are expressed as a percentage of the active tension obtained with acetylcholine. After control acetylcholine responses were obtained, tissues were equilibrated again for 60 min.

The agonistic effects of capsaicin ($10^{-8}\sim10^{-5}$ M) and KR-25018 ($10^{-8}\sim10^{-5}$ M) were examined. Only one concentration-response curve was obtained in each preparation because of the capsaicin desensitization. In separate experiments, the effect of phosphoramidon (10 μM), indomethacin (10 μM), capsazepine (3~30 μM) and ruthenium red (10, 30 μM) on cumulative concentration-response curves to capsaicin ($10^{-8}\sim10^{-5}$ M) or KR-25018 ($10^{-8}\sim10^{-5}$ M) was studied. Two rings were prepared from the same animal, one of which served as a control while the other received capsazepine or ruthenium red before application of the agonist. Exposure time of the rings to the capsazepine and ruthenium red was 20 min, and time to phosphoramidon and indomethacin was 15, 30 min, respectively.

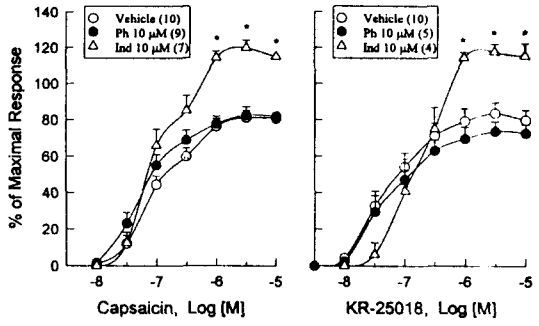

Fig. 1. Effect of phosphoramidon and indomethacin on the concentration-response curve for capsaicin and KR-25018 in the isolated guinea pig bronchi. Phosporamidon (Ph, 10 μM) and indomethacin (Ind, 10 μM) was added to the bath 15 min and 30 min before concentration-contractile response curves for capsaicin and KR-25018 were obtained. The numbers of bronchial rings used in each experiment are shown in the parentheses. Values are mean± S.E.M. expressed as % of the maximum contraction induced by acetylcholine (1 mM). * Significantly different from control value ($P<0.05$).

Statistical analysis of the data was performed by means of the Student's t test, linear regression and one-way analysis of variance. Schild analysis was performed to obtain pA_2 value. The level of significance was taken at $p<0.05$. All data were expressed as means± S.E.M.

Drugs used were phosphoramidon (Sigma), indomethacin (Sigma) capsaicin (Sigma), ruthenium red (Sigma), capsazepine (RBI) and KR-25018 (synthesized in KRICT). Stock solution of capsaicin (10 mM) was made up in 100% dimethyl sulfoxide (DMSO), then diluted with warm saline to give a final concentration of $10^{-8}\sim10^{-5}$ M. Capsazepine (10 mM)was also dissolved in DMSO and ruthenium red was dissolved in distilled water. The vehicles used to dissolve capsaicin (0.1% DMSO) or capsazepine (0.03% DMSO) had no effect on the preparations.

Result and Discussion

In this study we investigated the peripheral function of a newly developed capsaicin derivative, KR-25018. This compound has been demonstrated to act as a potent analgesic with different action mechanism from morphine and nonsteroidal antiinflammatory drugs (NSAIDS).[21]

KR-25018 ($10^{-8}\sim10^{-5}$ M) induced concentration-dependent contractile response in the bronchial rings (Fig. 1). The maximum contractile responses to KR-25018 were similar to those of capsaicin and significant differences were not observed in EC_{50} values. The EC_{50} value for KR-25018 and capsaicin was 0.097± 0.031, 0.137± 0.025 μM, respectively ($p>0.05$). EC_{50} value of capsaicin obtained in this study is consistent with that determined by others (0.11± 0.029 μM).[22]

Addition of phosphoramidon, an endopeptidase inhibitor, had no significant inhibitory effects on capsaicin- and KR-25018-induced contractions ($n=4-10$, $p>0.05$) . In control rings, the maximum capsaicin and KR-25018 contractions were 89.29± 3.81% and 83.53± 5.87%, respectively. In the presence of phosphoramidon, those were 82.59± 4.57% and 73.74± 5.25%, respectively. These findings suggest that the influence of endogenous peptidase activity can be excluded in this experimental condition. Thus, phosphoramidon was not added throughout the whole experiment.

Indomethacin (10 μM) had no significant effect on contractile responses to capsaicin at submaximal concentration range ($3\times10^{-9}\sim3\times10^{-7}$ M, $n=4-7$, $P>0.05$). This is in accordance with the result from Mapp *et al.*[25] However, above the maximal concentration of capsaicin (10^{-6}, 3×10^{-6} M), contractile responses were potentiated by indomethacin pretreatment, suggesting the modulatory effect of cyclooxygenase products on bronchocontriction induced by high concentration of capsaicin ($n=4-7$, $p>0.05$). Indomethacin had a similar dual effects on contractile responses to KR-25018 depending on the concentration of KR-25018. The contractle response induced by 3×10^{-6} M capsaicin was 81.29± 3.81% in the absence and 119.76± 4.14% in the presence of indomethacin, and that by 3×10^{-6} M KR-25018 was 83.53± 5.87% in the absence and

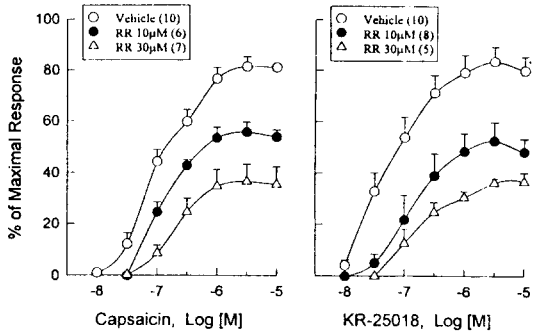

Fig. 2. Effect of ruthenium red (RR) on the contractile responses to capsaicin and KR-25018 in the isolated gunea pig bronchi.

The numbers of rings used (given in parentheses) for each concentration-response curve are shown. Values are means± S.E.M., and data are expressed as % of the maximum contraction to acetylcholine (1 mM).

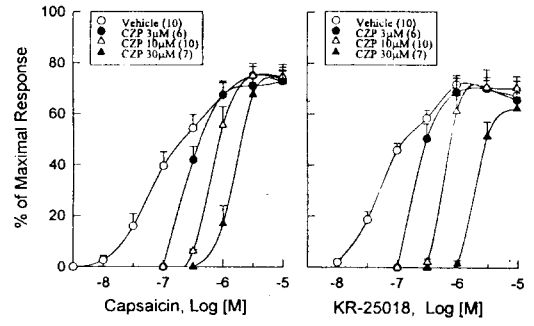

Fig. 3. Effect of capsazepine (CZP) on capsaicin- or KR-25018-induced contractile response in the isolated guinea pig bronchi.

The numbers of rings used for each concentration-response curve are shown in the parentheses. Each value is mean± S.E.M. expressed as % of the maximum contraction to acetylcholine (1 mM).

117.67± 4.31% in the presence of indomethacin. In contrast, other studies have shown that prostaglandins act presynaptically to enhance the release of substance P from trigeminal nerve terminals, and that capsaicin-sensitive fibers are stimulated indirectly by stimuli that generate bioactive substances such as prostanoids.[20] Moreover, it has been recently shown that the effect of indomethacin and capsaicin pretreatment on bladder activity are non-additive, indicating a commom site of action at this level.[25] Although data from this study does not support those results, further studies should be done to assess the role of prostaglandins and indomethacin in modulating the effects of capsaicin and KR-25018.

The response to KR-25018, like that to capsaicin, was significantly inhibited by ruthenium red (Fig. 2). There was significant reduction in the maximum response to KR-25018 (83.5± 5.87% in the absence and 52.69± 7.09% , 36.48± 3.61 in the presence of 10, 30 μM ruthenium red, respectively, $p<0.05$), which is indicative of non-competitive antagonism. Ruthenium red has been recently characterized as a selective capsaicin antagonist in a variety of tissue preparations in which it blocked prejunctionally peptide release from sensory nerves when they are activated by capsaicin but not by other stimuli, such as bradykinin.[16,26] Apparently ruthenium red blocks, in some way, a nonselective cation channel which is opened by activation of the vanilloid receptor.[26,27] Thus, similar pattern of inhibitory effects by ruthenium red on contractile response to both KR-25018 and capsaicin appears to indicate a common post-receptor mechanism of action for these agonists.

A further common feature of the response to capsaicin and KR-25018 in the guinea pig bronchi is their sensitivity to capsazepine, a selective capsaicin antagonist, which has provided direct pharmacological evidence for the localization of capsaicin receptor on the mammalian sensory neural membrane.[22] Capsazepine (3~30 μM) caused a rightward parallel shift in both concentration-response curves obtained with KR-25018 and capsaicin (Fig. 3). In addition, capsazepine did not reduce the maximum response produced by capsaicin and KR-25018. The pA_2 values obtained from Schild plot analysis of data on capsaicin and KR-25018 responses were 5.90 and 5.99, respectively, with no significant difference noted ($P>0.05$). The slopes of the plot were 0.86 and 1.11 respectively. These results suggest

that capsazepine is acting as a competitive antagonist of both capsaicin- and KR-25018 induced contractile responses in the guinea pig bronchi. Overall, the present results are in good agreement with the competitive antagonist character of capsazepine action at the vanilloid receptor, as demonstrated in other test systems.[12,24,28]

In conclusion, the results from this study suggest that KR-25018 and capsaicin both induce contractile response in guinea pig bronchial muscle by common mechanisms, probably acting on a specific receptor.

국문요약

새로이 합성된 capsaicin 유도체 KR-25018은 강력한 진통작용을 가지고 있으며 그 기전은 morphine이나 비마약성진통제와 다른것으로 보고된 바 있다. 그러나 말초기관에 대한 KR-25018의 작용 및 기전은 알려지지 않았기에 본 실험에서 기니픽의 기관지 평활근에 대한 capsaicin과 KR-25018의 직접적인 작용을 관찰하였고 기전실험의 일환으로서 indomethacin의 효과를 검색하여 capsaicin 및 KR-25018 반응과 prostaglandin 합성경로와의 상관성을 관찰하고자 하였다. Capsaicin과 KR-25018 모두 기니픽 기관지 평활근에서 농도 의존적으로 수축반응을 일으켰으며, Capsaicin의 EC_{50}는 $(1.367\pm 0.253)\times 10^{-7}$ M, KR-25018의 EC_{50}값은 $(0.965\pm 0.311)\times 10^{-7}$ M로서 이들간의 차이는 유의성이 없었다($P>0.05$). 최고농도 이하의 capsaicin과 KR-25018에 의한 수축반응에 대하여 indomethacin(10 μM)은 유의성 있는 영향을 미치지 않았다. 이는 capsaicin과 KR-25018의 수축반응에는 prostaglandin이 크게 관여하고 있지 않음을 시사한다. Capsaicin 경우와 마찬가지로 KR-25018의 수축반응에 대해서도 ruthenium red (10 μM)는 최대반응을 저하시키는 비경쟁적 길항작용을 나타내었다. Capsazepine은 capsaicin 및 KR-25018에 의한 수축반응 곡선을 평행하게 right-shift 시켰으며 이로써 capsaicin과 KR-25018의 반응이 capsazepine에 의해 경쟁적으로 길항됨을 알 수 있었다. 결론적으로, capsaicin과 KR-25018은 기니픽 기관지 평활근에서 농도 의존적으로 수축반응을 일으키며, 특이적 수용체를 매개로 하여 수축반응을 일으킨다는 것을 시사한다.

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