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Short communication

Evidence for bradykinin mediation of carrageenin-induced inflammatory pain: a study using kininogen-deficient Brown Norway Katholiek rats

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Abstract

Inflammatory pain was induced following an intradermal injection of carrageenin into rat paws, and the hyperalgesia was measured in terms of withdrawal time following thermal pain stimulation of the inflamed paw. This hyperalgesia was significantly less in kininogen-deficient Brown Norway (B/N)-Katholiek rats, which also showed less swelling in carrageenin-induced paw edema, than in normal B/N-Kitasato rats at 1~4 hr after the carrageenin injection (at the early phase). However, 24 hr after the injection, hyperalgesia and the swelling volume of the kininogen-deficient rats were almost the same as those in normal rats. The bradykinin B_2 receptor antagonist FR173657, (*E*)-3-(6-acetamido-3-pyridyl)-*N*-[*N*-[2,4-dichloro-3-[(2-methyl-8-quinolinyl)oxymethyl]phenyl]-*N*-methylaminocarbonylmethyl]acrylamide, attenuated the carrageenin-induced swelling and hyperalgesia of the normal rats at the early phase to almost the levels of the B/N-Katholiek rats. Pretreatment with indomethacin, a cyclooxygenase inhibitor, also inhibited the carrageenin-induced responses significantly in normal rats. These results indicate that bradykinin, acting on the B_2 receptor, is the main mediator at the early phase of inflammatory pain of carrageenin edema and that prostaglandins, produced by cyclooxygenase, potentiate the effects of bradykinin.

Keywords: Bradykinin; Thermal pain; Kininogen-deficient rat; Carrageenin paw edema; Prostaglandins

1. Introduction

Bradykinin is known as a pro-inflammatory peptide that possesses the ability to induce hyperalgesia, vasodilation, and increased vascular permeability, and its role has been reviewed by several authors [1–3]. It can be released from kininogen by the action of kallikrein. The role of bradykinin in the development of inflammatory processes has long been proposed, by the pharmacological approach to experimental inflammation. The approach has been based mainly on pharmacological strategies using agents that affect bradykinin production or metabolism, or act as antagonists of bradykinin receptors. However, interpretation of the evidence is not definitive because of the short half-life of bradykinin in biological fluids, and the poor specificity of currently available bradykinin receptor antagonists.

We have reported that kininogen-deficient B/N-Katholiek rats [4] show less swelling and less exudation in carrageenin-induced paw edema [5] and pleurisy [3], respectively, and these results indicate that kinin is a major mediator of plasma exudation in these inflammatory models. Furthermore, kinin can activate the arachidonate cascade to release prostaglandins to modulate inflammation, as in the case of PGI_2 enhancing the paw edema induced by bradykinin or carrageenin [6]. Bradykinin B_2 receptor knockout mice were produced by gene-targeting [7], and these mice showed less activity for carrageenin- or bradykinin-induced paw nociception [8], as well as weaker bradykinin-induced contractions of the ileum or uterus [7].

Therefore, to confirm the previous results, and to validate the historical view that bradykinin is a major substance causing pain receptor sensitization [1], we examined kinin involvement in inflammatory pain by using kininogen-deficient animals and demonstrated that kinin may be a main mediator of the initial hyperalgesia in rat paw edema induced by carrageenin.

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Abbreviations: B/N, Brown Norway; PGI_2, prostaglandin I_2; HMW, high molecular weight; LMW, low molecular weight; COX, cyclooxygenase; and PGE_2, prostaglandin E_2.

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2. Materials and methods

Brown Norway (B/N)-Katholiek and B/N-Kitasato rats were bred in a room controlled for temperature (22 ± 2°) and illumination (12 hr on and 12 hr off) at the Experimental Animal Laboratory of Kitasato University, School of Pharmaceutical Sciences [4]. Adult female rats were used for this experiment, although there was no significant difference in edema formation or algesic response between male and female rats.

Carrageenin and indomethacin were purchased from the Sigma Chemical Co., and FR173657, (*E*)-3-(6-acetamido-3-pyridyl)-*N*-[*N*-[2,4-dichloro-3-[(2-methyl-8-quinolinyl)-oxymethyl]phenyl]-*N*-methylaminocarbonylmethyl]acrylamide [9], was a gift from the Fujisawa Pharmaceutical Co. These agents were prepared for injection as previously reported [3,10].

Hind-paw edema and hyperalgesia were induced by the subplantar injection of 0.1 mL of 2% carrageenin solution in sterile saline into a hind footpad. Paw volumes were determined by measuring water displacement before and at selected times after the carrageenin injection. Hind-paw swelling was calculated as a percent increase from initial volume of the paw measured prior to the carrageenin injection.

A hyperalgesic response to thermal stimulation was determined by using a model 390 analgesia meter (IITC Inc.). Rats were placed on a transparent plastic box, their hind paws were exposed to a light beam emitted from a high intensity projection bulb before and at selected times after the injection of carrageenin, and the time lapse before withdrawal of the paw from the light beam was measured. The intensity of the light beam was adjusted so that most rats expressed around 15 sec of withdrawal time when a non-inflamed paw was subjected to the beam prior to the carrageenin injection. Hyperalgesia was defined by subtracting the initial withdrawal latency for each paw from its subsequent post-carrageenin measurements. All animals used in the investigation of hyperalgesia were also used for examining edema.

All results are expressed as means ± SEM. Statistical significance was evaluated by two-way ANOVA followed by Dunnett's test.

3. Results and discussion

Using B/N-Katholiek rats, in which the gene encoding kininogen had been mutated [11] and the plasma levels of HMW-kininogen and LMW-kininogen were about 3% of those of normal rats [12], we examined the nociceptive responses to thermal stimuli in inflammatory edema in order to further elucidate the role of bradykinin in inflammatory hyperalgesia. Hind-paw swelling induced by injection of 2% carrageenin was significantly less in the kininogen-deficient B/N-Katholiek rats than in the normal B/N-Kitasato rats, the latter expressing 2-fold greater paw swelling at 4 hr (Fig. 1A and B). This result is consistent with our previous study using a 1% carrageenin injection [3], but the time course of the swelling lasted longer in the case of 2% carrageenin.

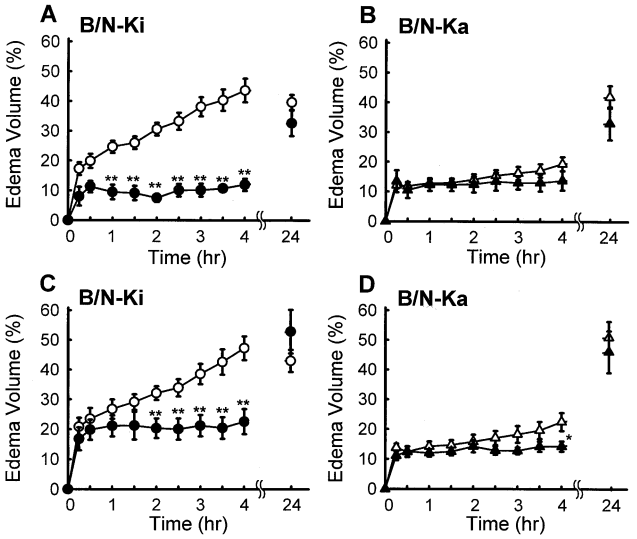

Fig. 1. Effects of a bradykinin B_2 receptor antagonist, FR173657, and a COX inhibitor, indomethacin, on paw edema induced by carrageenin. Paw edema was induced by injection of 2% carrageenin in sterile saline solution [0.1 mL into the hind-paws of normal B/N-Kitasato rats (A and C, open circles) and of kininogen-deficient B/N-Katholiek rats (B and D, open triangles)]. FR173657, 30 mg/kg, in 1% CMC suspension (A and B, closed symbols), or indomethacin, 10 mg/kg, in 1% CMC suspension (C and D, closed symbols), was injected intraperitoneally 30 min before the carrageenin injection. Values are means ± SEM (A and B: $N = 6–7$; C and D: $N = 8–13$). Key: (*) and (**) indicate that the values are significantly different from each control value at $P < 0.05$ and $P < 0.01$, respectively.

Pretreatment with FR173657, a non-peptide kinin B_2 receptor antagonist [10], suppressed the carrageenin-induced swelling in the paws of B/N-Kitasato rats to the level seen in the B/N-Katholiek rats, but the antagonist did not further attenuate the swelling in B/N-Katholiek rats, indicating that in carrageenin-induced paw swelling, endogenous bradykinin may cause exudate formation at the site through the B_2 receptor. However, in this experiment, we found that the swelling of the paw at 24 hr after the carrageenin injection in B/N-Katholiek animals was not significantly different from that in B/N-Kitasato rats and that the paws of both rats were still edematous. These results indicate that during the early phase of carrageenin-induced edema (up to 4 hr) bradykinin may be involved, but that at the later phase (around 24 hr) mediators other than bradykinin may be involved in the swelling.

Thermal hyperalgesia, evidenced by a reduction in the paw withdrawal latency, developed in the normal rats during edema formation at 1, 4, and even 24 hr after carrageenin injection, but the extent did not parallel the swelling volume (Fig. 2A). In contrast, B/N-Katholiek rats experienced significantly less algesia at 1 and 4 hr, indicating the

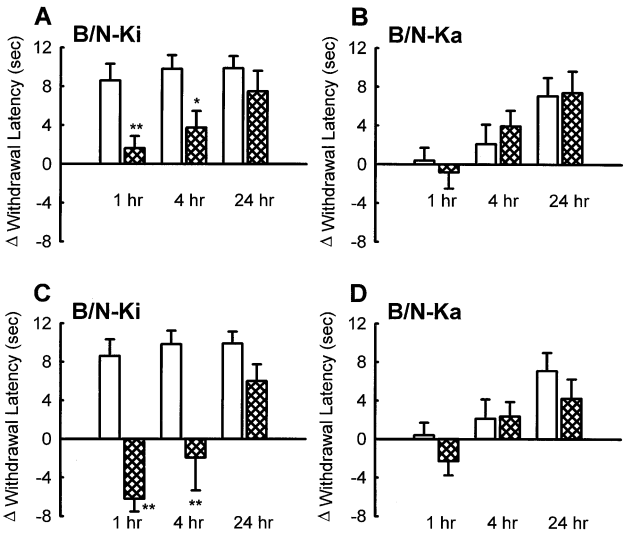

Fig. 2. Effects of a bradykinin B_2 receptor antagonist, FR173657, and a COX inhibitor, indomethacin, on hyperalgesia induced in the inflamed paw. The ordinate indicates the difference in withdrawal latency, which is expressed as the difference in the time lapse (sec) of withdrawal between the response of the paw before carrageenin injection and that of the inflamed paw. Both normal B/N-Kitasato (A and C) and kininogen-deficient B/N-Katholiek (B and D) rats were subjected to measurement of paw volume and then placed on the pain stimulator at 1, 4, and 24 hr after the carrageenin injection. FR173657 (A and B) and indomethacin (C and D) were injected (cross-hatched columns) in the same way as described in the legend of Fig. 1. Values are means ± SEM of 6–8 animals. Key: (*) and (**) indicate that the values are significantly different from each control value at $P < 0.05$ and $P < 0.01$, respectively.

involvement of bradykinin in the sensitization of pain receptors in this inflammatory model. However, the kininogen-deficient rats showed the same extent of hyperalgesia as normal rats at 24 hr, suggesting no involvement of bradykinin at 24 hr. The involvement of B_2 receptors in the acute phase of inflammation was also confirmed by the use of FR173657, which suppressed the carrageenin-induced hyperalgesia in normal rats at 1 and 4 hr to almost the same level as that in kininogen-deficient rats. Our findings are broadly consistent with pharmacological studies showing that the B_2 receptor antagonist HOE 140 exhibits activity in some, but not all, acute nociception screens [13] and has little effect in assays of persistent hyperalgesia [14]. In some prolonged inflammation models, such as lipopolysaccharide-pretreated rats [15], induction of the bradykinin B_1 receptor has been demonstrated to contribute to the edematogenic response [16]. In our present study, the paw swelling and hyperalgesia at 24 hr in the kininogen-deficient rats were almost the same as those in normal rats, suggesting that at the later phase (around 24 hr) there may not be involvement of either the B_2 or the B_1 receptor, because the kininogen-deficient B/N-Katholiek rats cannot produce bradykinin [4], and, hence, cannot produce des-Arg^9-bradykinin either.

Initial paw withdrawal latency, before the injection of carrageenin, was not different in normal rats (15.7 ± 0.6 sec) and kininogen-deficient rats (14.7 ± 0.5 sec), indicating that bradykinin is not involved in perception of the noxious thermal stimulus itself. The nociceptive stimulation used here elicits a moderate thermal pain, and the use of this pain stimulant clearly demonstrated that carrageenin-induced paw inflammation causes hyperalgesia in normal rats. Therefore, the difference of withdrawal latency between B/N-Katholiek and B/N-Kitasato rats demonstrates the involvement of bradykinin in carrageenin-induced inflammatory hyperalgesia. Our findings are consistent with a recent study using B_2 receptor-deficient mice, which suggests the involvement of bradykinin in carrageenin-induced hyperalgesia but not in thermal algesia [8].

Pretreatment with indomethacin, a COX inhibitor, also suppressed carrageenin-induced paw edema and hyperalgesia in B/N-Kitasato rats, but almost not at all in the B/N-Katholiek ones (Fig. 1C and D, and Fig. 2C and D). Suppression of carrageenin-induced hyperalgesia by indomethacin was very strong at 1 hr, and the withdrawal latency was even longer than in the pre-carrageenin measurements. This suggests that prostaglandins are involved in both inflammatory hyperalgesia and noxious thermal algesia.

Indomethacin is a non-selective COX inhibitor, as it inhibits both COX-1 and COX-2 isozymes. The involvement of COX-2 and PGE_2 in carrageenin-induced paw edema and hyperalgesia was demonstrated earlier by using selective COX-2 inhibitors or anti-PGE_2 antibody and by the measurement of the carrageenin-induced increase in the expression of COX-2 or the production of PGE_2 [17,18]. We previously reported, based on a study using PGI_2-receptor-deficient mice, that intrinsic prostacyclin contributes to carrageenin-induced paw edema [6]. Furthermore, edema formation and hyperalgesia induced by bradykinin are known to be potentiated by prostaglandins [19,20]. Therefore, the inhibitory effect of indomethacin demonstrated in this study may be due to inhibition of COX-1 and/or COX-2 and subsequent reduced production of PGI_2 and/or PGE_2, resulting in suppression of the enhancement effects of prostaglandins.

Thus, the current study using the inflammatory model of carrageenin paw edema and kininogen-deficient rats demonstrates that bradykinin is an important nociceptive mediator in inflammation and confirms previous pharmacological studies [9,14].

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