Mouse Paw Edema Induced by a Novel Bradykinin Agonist and Its Inhibition by B2-Antagonists

Akinori Ueno, Hiroaki Naraba and Sachiko Ohishi*

Department of Pharmacology, School of Pharmaceutical Sciences, Kitasato University, 5–9–1 Shirokane, Minato-ku, Tokyo 108–8641, Japan

Received July 27, 1998   Accepted August 12, 1998

ABSTRACT—A novel non-peptide bradykinin B2-receptor agonist, FR190997 (8-[2,6-dichloro-3-[N-[(E)-4-[(N-methylcarbamoyl)cinnamidoacetyl]-N-methylamino]benzoyl]-2-methyl-4-(2-pyridylmethoxy)quinoline), induced dose-dependent and longer-lasting swelling than bradykinin in the mouse paw. The swelling, peaking around 30 min, was suppressed dose-dependently by intraperitoneal administration of FR173657, a novel non-peptide B2-receptor antagonist. A known B2-agonist, Hoe 140, also significantly suppressed this edema. The result indicates that the novel B2-agonist FR190997, being more stable than bradykinin, could induce plasma exudation locally in mice via the B2-receptor as a substitute for bradykinin.

Keywords: Bradykinin B2-receptor agonist, FR190997, Mouse paw edema

Bradykinin is known as a mediator of edema formation or plasma exudation, and its role has been reviewed by several authors (1). Receptors for bradykinin have been classified and pharmacologically characterized as B1- and B2-subtypes (2), and they were recently cloned as bradykinin B1- and B2-receptors. As bradykinin has been reported to be an unstable peptide in the biological fluids, it is extremely difficult to detect this peptide and prove its biological role in local inflammatory edema. To determine the possible role of bradykinin in inflammation, animal experiments with various species are necessary. There are many reports showing the involvement of bradykinin in rat paw edema, including our paper using kininogen-deficient rats in which we proved the involvement of intrinsic bradykinin in the swelling or eduate formation of carrageenin-induced edema (3). Although mouse paw edema has also been used for screening of anti-inflammatory agents, a precise study to identify its mediators is lacking. Therefore we tried to investigate if a bradykinin B2-receptor agonist could induce paw edema in mice.

FR190997 (8-[2,6-dichloro-3-[N-[(E)-4-[(N-methylcarbamoyl)cinnamidoacetyl]-N-methylamino]benzoyl]-2-methyl-4-(2-pyridylmethoxy)quinoline) (a gift from Fujisawa Pharmaceutical Co., Tsukuba) (4) was recently developed as a non-peptide B2-receptor-specific, stable agonist. To evaluate the role of the B2-receptor in vivo, this stable agonist may serve as a useful substitute for bradykinin. Therefore, we examined whether this agonist could show pro-inflammatory action in vivo in mice. Recently a B2-receptor-selective antagonist, FR173657 ((E)-3-(6-acetamido-3-pyridyl)-N-[N-2,4-dichloro-3-[2-methyl-8-quinolinyl]oxymethyl]phenyl]-N-methylamino carbonylmethyl)acrylamide) (a gift from Fujisawa Pharmaceutical Co.) has been developed (5), and this antagonist was examined in studies employing rats and dogs (5, 6).

In this communication, we describe the use of both FR190997 and FR173657 to explore the role of bradykinin B2-receptor in vivo in mice. Paw edema was induced in male 5-week-old ICR mice (SLC Japan, Hamamatsu) by subcutaneous injection of 30 μl of saline solution containing 0.3, 0.6 and 1.2 nmol of bradykinin, and 30 μl of saline solution containing 0.1, 0.3 and 0.9 nmol of FR190997 into one of the hind paw of mice. Paw volume was measured by a handheld plethysmograph, previously described (3), that was modified for mice. Swelling volume was calculated as the difference in volume between the stimulant-injected paw and the control paw. FR173657 (1 or 3 mg/ml) and indomethacin (1 mg/ml) were prepared as a suspension in 1% sodium carboxymethyl-cellulose and injected intraperitoneally 30 min before the induction of edema. Hoe 140 (a gift from Hoechst AG, Frankfurt, Germany (7)) (0.1 mg/ml) was
injected in a saline solution intraperitoneally
30 min before. All data were expressed as means ± S.E.M. The data

were analyzed by ANOVA followed by Dunnett’s t-test;

a P value less than 0.05 was considered as significant.

Fig. 1. Induction of paw edema in mice by bradykinin (A) and the bradykinin B2-receptor agonist FR190997 (B). Paw edema

was induced by subcutaneous injection of 30 μl of physiological saline containing 1.2 (open circles, n=6), 0.6 (closed circles,

n=6) or 0.3 (open triangles, n=5) nmol of bradykinin or vehicle alone (closed triangles, n=5) (A) or subcutaneous injection of

30 μl physiological saline containing 0.9 (open circles, n=6), 0.3 (closed diamonds, n=6) or 0.1 (open squares, n=7) nmol of

the bradykinin agonist FR190997 (B) into the right hind paw of ICR mice. Increases in paw volume (edema volume) were

measured at the indicated time after injection of stimulants. Values are the means with S.E.M. for each group.

Fig. 2. Effects of bradykinin B2-receptor antagonists on the FR190997-induced paw edema. In panel A, effects of the

bradykinin B2-antagonist Hoe 140 (1 mg/kg, closed diamonds, n=6) and the novel antagonist FR173657 (30 mg/kg, closed

circles, n=6) on the bradykinin-induced edema (1.2 nmol/site) are shown as time courses of means with S.E.M. The control
group was pretreated with the vehicle solution (open squares, n=9). All agents were intraperitoneally administered 30 min prior

to the edema induction. In panel B, the effect of the same antagonists and indomethacin on the edema induced by the B2-

receptor agonist FR190997 (0.9 nmol/site) was examined. FR173657, at a dose of 30 mg/kg (closed circles, n=7) or 10 mg/kg

(closed triangles, n=10); Hoe 140 (1 mg/kg, closed diamonds, n=7); and indomethacin (10 mg/kg, closed squares, n=9) were

intraperitoneally injected 30 min before the agonist injection. The control group was pretreated with the vehicle solution (open

squares, n=10). * and ** indicate that the values were statistically significantly different from the values of the control groups

at corresponding times at P < 0.05 and P < 0.01, respectively, when calculated by ANOVA followed by Dunnett’s comparison.
As shown in Fig. 1A, bradykinin injection produced a dose-dependent swelling in the mouse paw, and the same volume of saline only caused a transient and small swelling. FR190997, the bradykinin B2-receptor-specific agonist, caused dose-related swelling similar to that induced by bradykinin. The swelling induced by FR190997 peaked around 30 min, and those induced by lower doses disappeared by 180 min (Fig. 1B). However, the effect was more potent than that of bradykinin on a molar basis, and the time course of the swelling was longer than that achieved with bradykinin; i.e., 0.9 nmol of FR190997 caused larger and more sustained swelling than that of 1.2 nmol of bradykinin, which showed a peak at 15 min and disappeared by 150 min. This could be explained by the fact that bradykinin is so rapidly digested into inactive peptides in biological fluid (8).

The effect of bradykinin B2-antagonists on the agonist-induced swelling was examined (Fig. 2). Hoe 140 significantly suppressed the edema induced by bradykinin as well that by FR190997. FR173657, at 10 and 30 μg/kg, suppressed the FR190997-induced swelling dose-dependently. The latter dose of the antagonist reduced the swelling almost to the level that was present with the vehicle alone and also significantly suppressed bradykinin-induced paw swelling (Fig.1A).

Bradykinin is also known as a releaser of prostaglandins, and we previously found evidence that prostaglandin is involved in bradykinin-mediated inflammation (9). In the present study indomethacin, 10 mg/kg, suppressed the swelling induced by FR190997 significantly (Fig. 2B). Therefore, cyclooxygenase products could also be involved in this bradykinin B2 agonist-mediated swelling.

The fact that the swelling induced by FR190997 was suppressed by two B2-receptor-specific antagonists, Hoe 140 and FR173657, may prove that a major portion of the swelling may be mediated by B2-receptors in vivo. A previous study showed that FR190997 bound specifically to B2-receptors expressed in cells in vitro (4), and this specificity appears to hold true in vivo in the present study using B2-agonists. Thus, FR 190997 may be a better B2-agonist and serve to replace the specific action of bradykinin via B2-receptor in vivo, because bradykinin may also act on B1-receptors after cleaved into des-Arg9-bradykinin in biological fluids or in the animal body.

Acknowledgments
The authors are grateful to Fujisawa Pharmaceutical Co. (Tsuuka- ba), for the gift of FR190997 and FR173657 and to Hoechst Co. (Frankfurt, Germany) for donating Hoe 140. The authors are also grateful to Ms Taeko Yamaguchi for her technical assistance. This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Sports and Culture of Japan (No. 10672155).

REFERENCES


