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Blockade of nociceptive sensory afferent activity of the rat knee joint by the bradykinin B_2 receptor antagonist fasitibant

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SUMMARY

Objective: The aim of this study was to determine in intact and inflamed knee joints of the rat, the effect of the bradykinin (BK) B_2 receptor antagonist fasitibant (MEN16132) on nociceptor mechanosensitivity and hyperalgesia.
Methods: Joint afferent sensory fibers of the medial articular nerve of anesthetized animals were electrophysiologically recorded, measuring nerve impulse activity evoked by passive innocuous and noxious movements of the joint, in intact and kaolin and carrageenan-injected joints. Knee joints of rats were also acutely inflamed by intra-articular injection of carrageenan alone. Long term duration of fasitibant antinociceptive effects were behaviorally evaluated using the incapacitance test.
Results: BK (100 μM) injected into the saphenous artery, induced excitation and sensitization of multi- and single unit recordings. Fasitibant (300 μM) injected prior to BK, reduced its excitatory effects as well as the overall increase of movement-evoked activity resulting from repeated injections of BK. Fasitibant did not affect movement-evoked activity of sensory fibers of intact, non-inflamed knee joints.
Intra-articular fasitibant (100 μg/knee) significantly reduced the carrageenan-induced inflammatory hyperalgesia measured with the incapacitance test up to four days after treatment. This antinociceptive effect was not obtained with systemic endovenous injection of the drug.
Conclusions: Fasitibant prevents B_2 receptor-mediated activation and sensitization of peripheral joint afferents and the ensuing inflammatory hyperalgesia, and may be a useful, novel drug for arthritis pain treatment.

Introduction

Bradykinin (BK) is a small peptide (Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg) produced by proteolytic kallikreins following tissue damage[1]. Its contribution to pain and inflammation has been extensively analyzed in several experimental models[2,3]. Two kinin receptors, belonging to the rhodopsin family of G protein-coupled receptors, have been described: the inducible B_1 receptor and the constitutive B_2 receptor. BK possesses a 10,000-fold higher affinity for the B_2 receptor as compared with the B_1, which is instead preferably activated by kinin metabolites lacking of the C-terminal arginine residue[4]. Once bound to B_2 receptors, BK induces phospholipase C (PLC) activation, ensuing a cascade of secondary events that include protein kinase C (PKC) and phospholipase A_2 phosphorylation, nitric oxide synthase activation, and subsequent intracellular downstream signaling[4,5]. Different mechanisms mainly linked to PLC and PKC activation, can account for the algogenic action of BK, characterized by sensitization and/or direct activation of nociceptive afferent nerve terminals. BK-mediated activation of different transducing ion channels such as transient receptor potential channels (TRP) TRPV1, TRPV4 and TRPA1 has been reported in polymodal nociceptors[6–9]. Also, BK inhibition of M-type K^+ currents and simultaneous opening of Ca^{2+}-activated Cl^- channels have been reported[10].

Osteoarthritis (OA) is a multifactorial disease and the involvement of an inflammatory component, which is marked by symptoms such as joint pain, swelling and stiffness, is now well recognized[11,12]. Carrageenan-induced experimental inflammation model, besides to be used to investigate different mechanisms

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linked to synovitis[13,14], has been used to show the contribution of kinin receptors to the development of peripheral inflammatory signs, such as increased vascular permeability, endothelial cell proliferation and hyperalgesia[15–19]. As a confirm, it has been shown that carrageenan injection in mice deficient of B_2 receptor does not cause nociception[20]. It has been also reported that ongoing and movement-evoked impulse activity in afferent nociceptive nerve fibers of rat knee joints inflamed through intra-articular injection of carrageenan, are augmented[21–23] thus providing the electrophysiological correlate of carrageenan-induced hyperalgesia. However, the contribution of BK receptors to this enhanced joint nociceptor activity has not been explored so far.

Intra-articular injection of icatibant, a metabolically resistant peptide and selective B_2 receptor antagonist currently used for the therapy of hereditary angioedema attacks, has been shown to reduce pain intensity at rest and during activity in patients with symptomatic OA[24]. This speaks in favor of a contribution of B_2 receptor activation to the augmented nerve impulse activity at nociceptive fibers during joint inflammation and opens the possibility of using BK antagonists as new drugs for the treatment of arthritic pain.

Fasitibant (previously known as MEN16132) is a nonpeptide compound endowed with high affinity and selectivity for the kinin B_2 receptor, and appears to be more potent than icatibant in blocking BK effects mediated through B_2 receptors in several *in vitro* and *in vivo* models[25–30]. Moreover fasitibant is capable to block the hyperalgesia experimentally induced by intra-articular knee joint injection of monosodium iodoacetate in rats, as a model of chemically-evoked degenerative OA[31], as well as to partially inhibit both the hyperalgesia and the inflammatory effects produced by the intra-articular administration of carrageenan as a model of rat inflammatory arthritis[32,33]. Fasitibant is currently under clinical development and evaluated as symptomatic intra-articular treatment of knee OA (clinical trial NCT01091116 registered).

As nociceptive fibers are considered the fundamental source of pain in knee OA[34] the aim of the present investigation was to evaluate the attenuating effect of fasitibant on BK-induced sensory nerve activation by recording nociceptive activity of medial articular nerve fibers in inflamed vs non-inflamed knee joints in rats, and to explore the duration of its local antinociceptive effect on inflamed joints, using behavioral techniques.

Material and methods

Animals

Male adult rats (Harlan Laboratories, Udine, Italy) were housed singly in cages in sanitary ventilated animal rooms with controlled temperature (20°C), humidity (45%) and maintained on *ad libitum* food and water with a 12 h light/dark cycles. Procedures were performed in accordance to the protocols approved by the Committee on Animal Research at the University Miguel Hernández, the ethical committee of Menarini Ricerche and according to the Spanish, Italian and European Union regulations (2010/63/UE).

Recordings experiments

Experiments were performed in 42 Wistar rats (mean body weight: 331 ± 4 g) as previously described[21,22,35].

Animals were initially anesthetized with ketamine (75 mg/kg) and xylazine (10 mg/kg) (i.p.) followed by injection of 40 mg/kg (i.p.) of sodium pentobarbital for deep anesthesia. Supplementary doses of the anesthetic were injected through a venous catheter when required. Body temperature and CO_2 levels were kept at physiological levels. Heart frequency and blood pressure values were continuously monitored to evaluate the anesthesia level.

Surgery and inflammation were performed according to the procedures described previously[21,22,35] and are detailed in the Supplementary Material.

Identification and recording of knee joint afferent units

Briefly, the saphenous nerve was cut both proximally to the joint, in the inguinal region and distally from the joint. The proximal end of the saphenous nerve was placed on a black platform

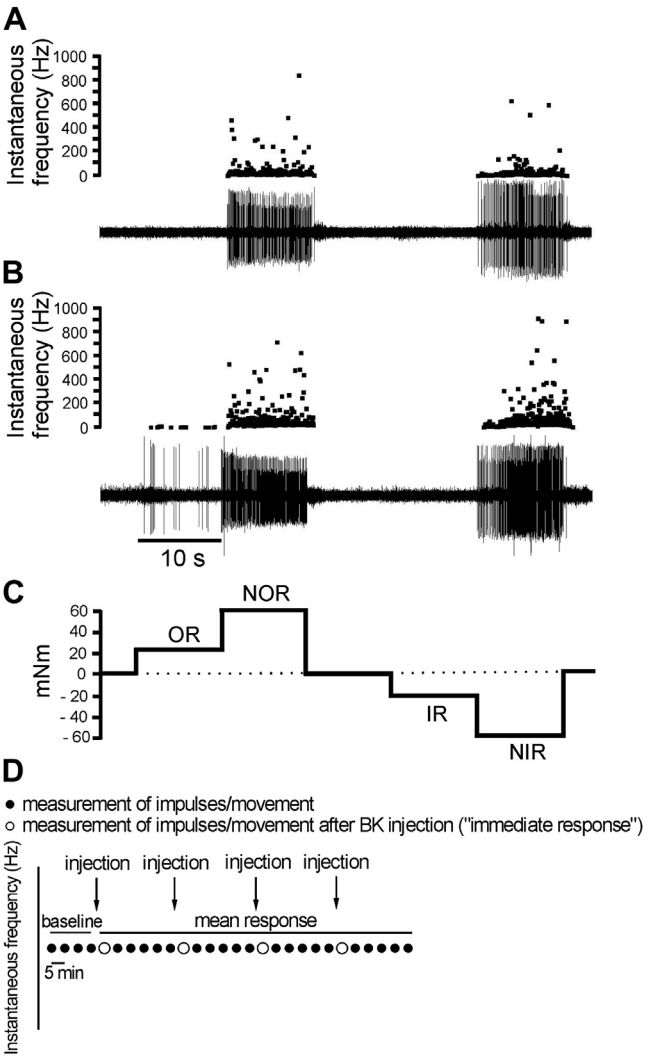

Fig. 1. Nerve impulse activity recorded from primary afferent multiunits innervating an inflamed knee joint. (A–B) Recordings of evoked impulses are displayed as instantaneous frequency (top panel) and as the original nerve impulse recordings (lower panel). A correspond to the movement performed before BK injection and B shows the increase activity after BK injection. (C) Outline of the experimental protocol. The tibia was rotated against the femur with the time course and the torque in mNm, shown in the axis. The outward rotations in two steps of 10 s each were followed by a pause of 10 s and then by two corresponding inward rotations of the same duration. OR, non-noxious outward rotation in the normal working range of the joint; NOR, noxious outward rotation over the normal working range of the joint; IR, non-noxious inward rotation; NIR, noxious inward rotation. (D) Diagram of the data analysis. Movements along the experiments are represented by circles. Baseline: movements used to calculate control values. Mean response: movements used to average the long-term effects of the injected substances. White circles represent the activity evoked by the first movement after BK injection and it is called "immediate response". The average of the four white circles gives the "mean of the immediate responses" value.

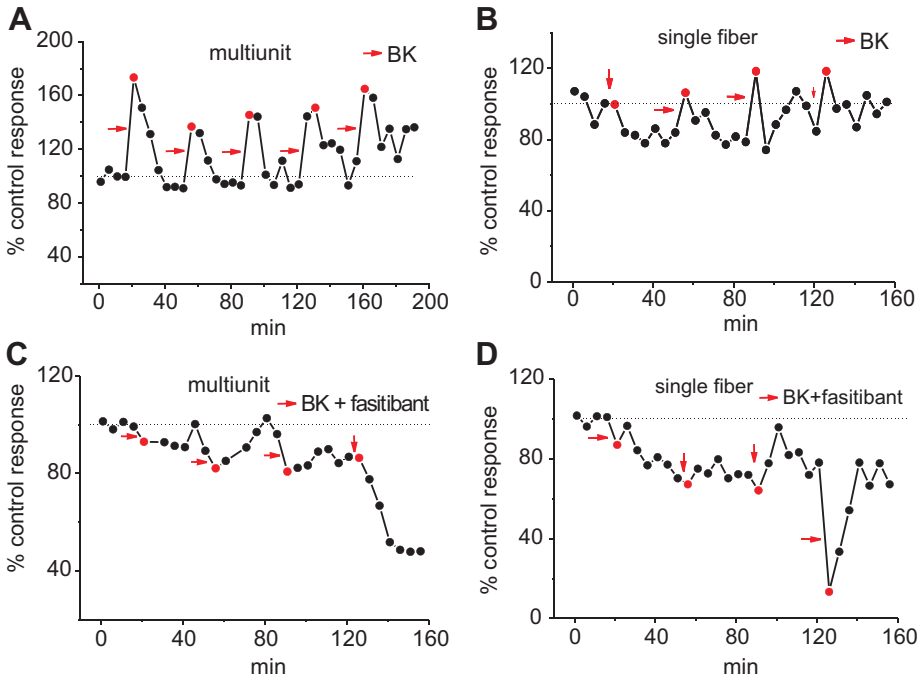

Fig. 2. Movement-evoked activity recorded from primary afferent multiunits and single fibers innervating an inflamed knee joint. Representative experiments showing the effects of BK (A ($n = 5$), B ($n = 4$)) and fasitibant plus BK (C ($n = 4$), D ($n = 5$)). Circle symbols in the graphs represent the averaged response of impulse activity evoked by non-noxious and noxious outward and inward rotations. The red arrows indicate the interval between movements at which the substances are injected. The red dots indicate the movement used for the activity analysis. Dotted lines indicate control baseline.

and fine filaments were dissected and placed over a silver wire electrode for extracellular recording.

The sensitivity to knee joint movements of articular afferent units was explored using the protocol shown in panel C of Figs. 1 and 4. The maneuver started from a resting, middle position of the joint and consisted of a 10 s outward rotation within the working range of the joint (non-noxious) continued by a 10 s duration outward rotation which overpasses the normal working

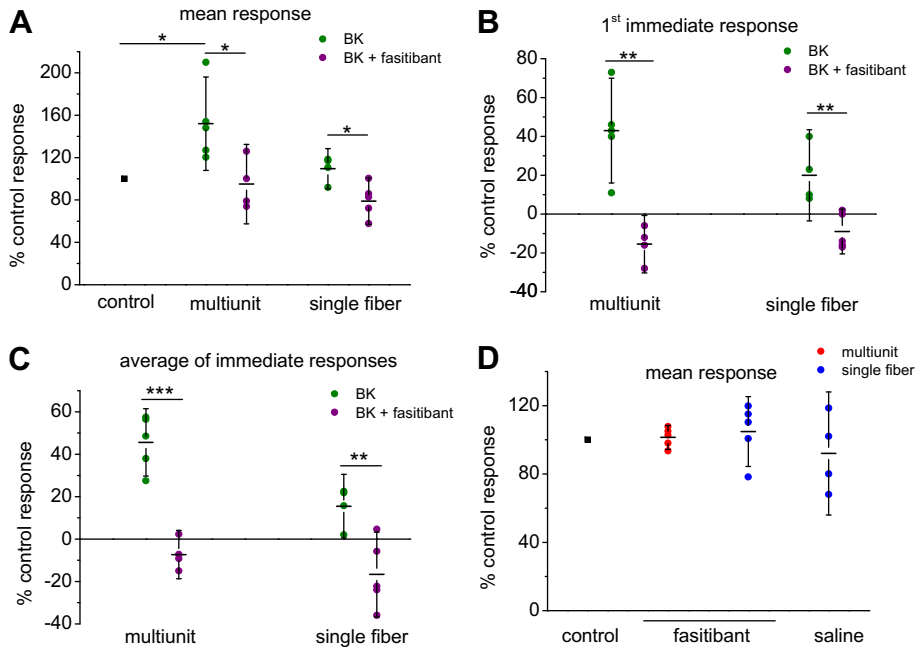

Fig. 3. Effect of BK and fasitibant on the movement-evoked impulse activity of fine primary afferences. Dot plots summarizing the nerve activity recorded from primary afferent multi and single units innervating inflamed knee joints (A–C) and non-inflamed joints (D). Black dots in figures A and D represent the averaged activity of the movements performed prior the injections. These values were set to 100% and considered as control responses. The color dots represent data from individual experiments in each experimental group i.e., multiunits ($n = 5$) and single fibers ($n = 4$) recorded from animals receiving injections of BK (green dots) (A–C), multiunits ($n = 4$) and single fibers ($n = 5$) recorded from animals receiving injections of fasitibant and BK (purple dots) (A–C), multiunits ($n = 5$) and single fibers ($n = 5$) recorded from animals receiving injections of fasitibant (red and blue dots respectively) (D) and single fibers ($n = 4$) recorded from animals receiving injections of saline (blue dots) (D). Horizontal bars represent the mean values and error bars represent 95% CI. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ denote statistical significance between treatments.

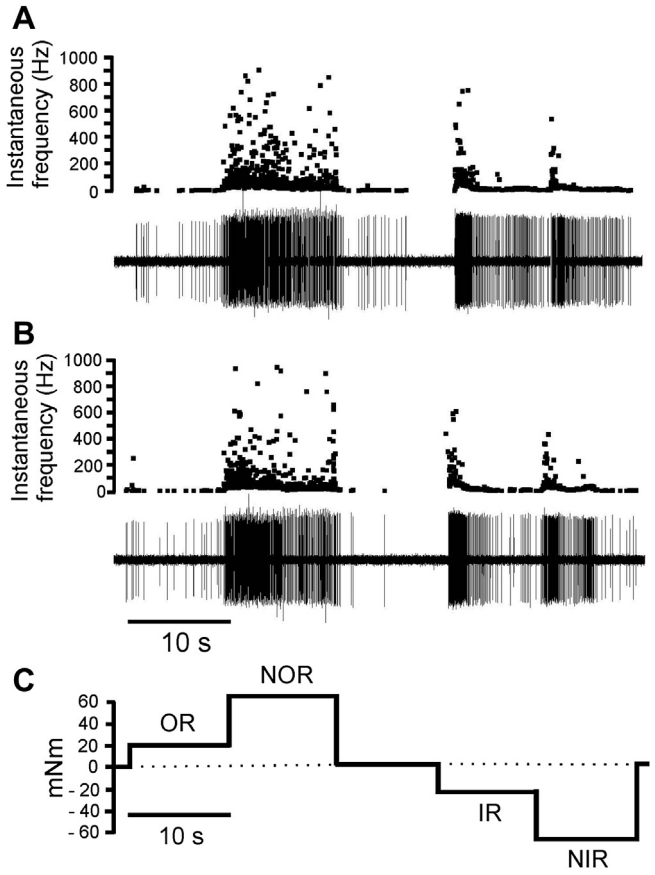

Fig. 4. Nerve impulse activity recorded from primary afferent multiunits innervating an inflamed knee joint. (A–B) Recordings of evoked impulses are displayed as instantaneous frequency (top panel) and as the original nerve impulse recordings (lower panel). A, correspond to the movement performed before substances injection and B shows the activity after injection of 200 μl of fasitibant 300 μM, followed by the injection of 200 μl of BK 100 μM 30 s afterward. (C) Shows an outline of the experimental protocol [for details see Fig. 1(C)].

range of the joint (noxious). Thereafter, the joint was returned to the resting position for 10 s and the same maneuvers were performed using inward rotations[21,22,35,36]. The discharges recorded during the complete movement cycle, were analyzed by counting the total number of impulses evoked by the four rotations [OR + NOR + IR + NIR; represented by circles in Fig. 1(D)]. After four movements, a 200 μl intra-arterial injection of BK 100 μM, fasitibant 300 μM, of both substances or saline was performed and the effect assessed in next movement following the injection [white circles, Fig. 1(D)]. The injection of the test substance was repeated up to five times at 30–35 min intervals. Movement cycles were repeated every 5 min.

Experimental groups in recording experiments

The animals were divided in four groups. Animals that received intra-arterial injections of BK alone (group 1); animals receiving injections of fasitibant 30 s previous to the injection of BK (group 2); animals receiving injections of fasitibant alone (group 3), and animals receiving injections of saline (group 4).

Analysis of data

The numbers of impulses/cycle evoked by the four movement cycles, preceding the first injection of the test substance were averaged. This value was taken as the "control response" and served as 100%. Accordingly, the number of impulses during the movement cycles performed along the post-injection period was expressed as percentage of the control response.

The numbers of impulses of all movement cycles of the post-injection period were averaged and this value was called "mean response" and served to evaluate the overall sensitization to repeated injections of BK and its modification by fasitibant [Fig. 1(D)].

We called "immediate response" of BK the activity evoked by the first movement that followed the injection of the drug [first white circle in Fig. 1(D)]. The values of all the immediate responses evoked by repeated injections of BK were also averaged to obtain a "mean value of the immediate response" in each experiment [Fig. 1(D)].

Behavioral experiments

Experiments were performed in 42 Wistar rats (250–300 g) as previously described[28]. Inflammation was induced in the right knee joint by intra-articular injection (25 μl) of 2% λ-carrageenan whereas an equal volume of sterile saline was administered in the left knee joint. Animals belonging to the control group were intra-articularly administered with saline.

Experimental groups in behavioral experiments

Animals were randomized into six experimental groups. Animals that received intra-articular saline (group 1, $n = 6$, control); animals that received intra-articular carrageenan (group 2, $n = 12$); animals receiving intra-articularly different doses (10, 30, 100 μg) of fasitibant 30 min previous carrageenan treatment (group 3, 4, 5, $n = 6$ each); animals receiving intravenously 100 μg of fasitibant 30 min previous carrageenan treatment (group 6, $n = 6$).

Treatment and weight bearing measurements

Fasitibant intravenous administration was performed in the tail vein (0.3 ml). Intra-articular injection (25 μl) was made into the right knee joint of anaesthetized rats (pentobarbital, 40 mg kg^{-1} i.p.), that received 25 μl of sterile saline into the contralateral (left) knee. The antagonist was administered 30 min before the induction of inflammation. After each intra-articular injection[37], the knee joint was repeatedly flexed and extended to allow the dispersion of both drugs and carrageenan. The animals recovered from anesthesia within 60–90 min.

Pain related to knee joint inflammation was assessed by an incapacitance tester MkV (Linton Instrumentation, Norfolk, UK) to measure carrageenan-treated (right) and saline-treated (left) limb weight distribution. Rats were placed in an angled plexiglass chamber positioned so that each hind paw rested on a horizontal force-transducing plate. The weight (g) borne by each hind limb was averaged over 5 s; eight readings were taken and mean values calculated. Animals hind limb weight bearing behavior was measured on the same animals repeatedly over the course of the study. Results are right hind limb weight bearing as a percentage of total weight borne by both hind limbs (no change around 50%).

Statistical analysis

In nerve recording experiments, statistical comparisons were generated using SigmaStat (Systat Software, Inc., CA). We used the paired *t*-test to compare changes in the animals before and after treatments and the Student's *t*-test for comparison between animals groups, as indicated.

Analysis of data from behavioral experiments was performed using GraphPad Prism 4.0 (San Diego, CA). Raw data were analyzed by the one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test. The percentage of antinociceptive effect was calculated against the value obtained in the carrageenan group.

Data are reported as mean and 95% confidence interval (CI) in parentheses. The significance level was set at $P < 0.05$.

Drugs

Fasitibant (chloride dihydrochloride salt, batch number 42758P, Menarini Ricerche, Chemistry Development Department, Pisa, Italy)[25].

Fasitibant was dissolved in saline and sterilized by filtration (MillexGV 0.22 μm, Millipore, Billerica, MA). λ-carrageenan, kaolin and BK were from Sigma–Aldrich (St. Louis, MO).

Results

Electrophysiological recordings

Successful recordings of duration longer than 120 min were obtained from 35 nerve filaments of the saphenous nerve of separate animals (one filament per animal, $n = 35$). 21 recordings correspond to filaments containing a single active unit and 14 to filaments exhibiting multiunit activity. Most of the saphenous nerve fibers of rats responding to movements of the knee joint had conduction velocities (CV) that included them either into the thin myelinated, A-delta group (CV = 2.5–20 m/s) or in the unmyelinated C-fiber group (CV≤2.5 m/s; Table I).

Effect of BK on joint nerve activity

As shown in the example of a multiunit recording illustrated in Fig. 1, joint movements of the inflamed joint in the working range (non-noxious OR and IR) did not generate impulse activity or at best evoked a very low firing response; however rotations that exceeded the working range of the joint (noxious rotation, NOR, NIR) yield a vigorous firing response in all phases of the cycle [Fig. 1(A)]. After BK injection, movements of the joint in the working range (OR movement) already evoked a burst of impulses, and impulse activity during noxious rotations was considerably increased, i.e., the units became sensitized [Fig. 1(B)].

Repeated intra-arterial injection of 200 μl BK 100 μM evoked on the average, an increase in movement-evoked sensory activity although individual responses in the same animal were rather variable. As illustrated in the representative experiment of Fig. 2A and B, the maximal increase in impulse activity (marked with a red dot) usually occurred at the first movement performed after BK injection, but the effect of BK was in some cases less immediate and the maximal rise in impulse frequency appeared during the second movement [Fig. 2(A)]. For this reason we selected for the analysis of BK effects, the movement that evoked the highest frequency value among the movements performed after injection of BK (marked with a red dot in Fig. 2). On average, the increase of the mean response to BK in multiunit recordings (152% (108–196) $n = 5$) was statistically significant when compared with control movements [100%; Figs. 2(A)–3(A), Table I; $P = 0.031$, Paired t-test]. The first immediate response increased a 43% (16–70) and the average of immediate responses a 46% (30–62) respect to control movements [Fig. 3(B–C); Table I].

The fact that the average of the first responses is similar to the average of the repeated injections indicates a very limited tachyphylaxis, i.e., decrease in response caused by repeated doses of BK.

BK injection was also tested on seven single units in seven different animals. In three of them, BK did not have any effect on movement-evoked impulse response, suggesting that they were insensitive to the drug (see supplementary data). Thus, they were not included in the analysis of BK effects. In the remaining units, the mean responses after repeated intra-arterial injection of 200 μl BK 100 μM did not reach significant differences when was compared with control [109.5% (90.5–128.5) $n = 4$, Figs. 2(B)–3(A), Table I; $P = 0.217$, Paired t-test]. The first immediate response was augmented with respect to the movement activity prior BK injections (20% (−3.5 to 43.5)) as also occurred with the average of the immediate responses [15.5% (0.5–30.5) Figs. 2(B), 3(B–C), Table I].

To exclude a direct, unspecific stimulatory effect of the injection itself, we tested the effect of an injection of saline on movement-evoked activity, in single unit recordings. The mean responses after repeated injection of saline alone [92% (56–128) $n = 4$, Fig. 3(D), Table I], was not significantly different from control movements ($P = 0.54$, Paired t-test). We also found no significant differences in the first immediate response (−4.75% (−16.5 to 7)) and in the average of immediate responses (−0.67% (−5.5 to 4.2)) with respect to the control movements ($P = 0.288$ and $P = 0.68$ respectively, Paired t-test; Table I).

Effect of fasitibant on BK sensitization

Fig. 4 shows a representative example of the effect of fasitibant on a multiunit nerve firing recording of BK-sensitized movement-evoked activity before (A) and after (B) close intra-arterial injection of fasitibant. The impulse activity evoked by the injection of BK was reduced when fasitibant was injected 30 s earlier, indicating that the drug blocks the sensitizing effect of BK. The time course of this type of experiment in multi- and single unit recordings is shown in Fig. 2C and D. Previous injection of fasitibant (red arrows) consistently reduced the number of movement-evoked impulses seen under BK alone. The mean response in multiunit recordings following BK plus fasitibant injection was 95% (57–132, $n = 4$). This activity was significantly lower when compared with the equivalent activity in animals that received intra-arterial injections of BK but were not treated with fasitibant [152% (108–196) Fig. 3(A);

Table I
Summary of the results for the different analysis performed in each experimental group. The numbers of animals (n) used in each experimental group are indicated. The last column indicates the type of fiber identified in single units recordings. Values represent mean and 95% CI

Experimental groups		% Of mean response (95% CI)	% Of first immediate response (95% CI)	% Of average immediate response (95% CI)	Type of fibers
Group 1 BK	Multiunit	152 (108–196) $n = 5$	43 (16–70) $n = 5$	46 (30–62) $n = 5$	
	Single unit	109.5 (90.5–128.5) $n = 4$	20 (−3.5 to 43.5) $n = 4$	15.5 (0.5–30.5) $n = 4$	C4
Group 2 BK+ fasitibant	Multiunit	95 (57–132) $n = 4$	−15.5 (−30.3 to −0.7) $n = 4$	−7.3 (−18.7 to 4) $n = 4$	
	Single unit	79 (57–101) $n = 5$	−9 (−20.5 to 2.5) $n = 5$	−16.7 (−36.6 to 3.2) $n = 5$	4C, 1Aδ
Group 3 fasitibant	Multiunit	101.4 (94–108) $n = 5$		4.3 (−11 to 20) $n = 5$	
	Single unit	104.8 (84–125) $n = 5$		−3.4 (−9.1 to 2.3) $n = 5$	2Aδ, 3C
Group 4 saline	Single unit	92 (56–128) $n = 4$	−4.75 (−16.47 to 7) $n = 4$	−0.67 (5.5–4.2) $n = 4$	3C

$P = 0.028$, Student's *t*-test]. In single unit recordings the averaged activity in rats treated with fasitibant was also significantly lower (79% (57–101) $n = 5$) than in non-treated animals [109.5% (90.5–128.5) Fig. 3(A), Table I; $P = 0.022$, Student's *t*-test].

We also analyzed the first immediate response after BK plus fasitibant. This response was on average −15.5% (−30.3 to −0.7) and −9% (−20.5 to 2.5) with respect to control movements previous the injections, in multi- and single unit recordings, respectively. These decreases were statistically significant compared to animals not treated with fasitibant [Fig. 3(B); $P = 0.002$ and $P = 0.008$ respectively, Student's *t*-test].

Finally, inhibition of BK sensitization by fasitibant was also evident when we measured the mean value of the immediate responses after BK plus fasitibant treatment, in both multi- and single unit experiments. A mean reduction of −7.3% (−18.7 to 4) in multiunit recordings and −16.7% (−36.6 to 3.2) in single unit recordings were measured. These decreases were statistically significant when compared with the increases in activity recorded after BK injection alone [Fig. 3(C), Table I; $P < 0.001$ and $P = 0.01$ respectively, Student's *t*-test].

Direct effects of fasitibant

To rule out the possibility of a direct effect of fasitibant on knee joint impulse responses to mechanical stimuli, we recorded the nerve activity in non-inflamed knee joints after fasitibant injection, in which BK levels are expectedly lower in comparison with inflamed knee joints.

As shown in Fig. 3D, the mean response after intra-arterial injection of fasitibant 300 μM alone was not statistically significant compared to control in multi- and in single unit recordings (101.4% (94–108) $n = 5$ and 104.8% (84–125), $n = 5$; $P = 0.612$ and $P = 0.550$ respectively, Paired *t*-test; Table I). Likewise, the first immediate responses after fasitibant were 4.3% (−11 to 20) and −3.4% (−9.1 to 2.3) in multi- and single units respectively, and were not different from the previous immediate movement (data not shown; $P = 0.063$ and $P = 0.181$ respectively, Paired *t*-test; Table I).

Effect of fasitibant on joint pain-associated behavior

In non-inflamed rats body weight distribution between the two hind limbs was similar, whereas 6 h after carrageenan administration, animals maintained only 24.4 ± 4.1% of the weight on the inflamed (right) hind limb, shifting the remaining onto the contralateral leg. This value returned gradually to control, increasing the weight on the treated limb to 30.3% (28.2–32.5), 36.1% (32.4–39.8), 40.1% (38.3–41.9), and 42.2% (39.0–44.8) of control at 1, 2, 3, and 4 days after carrageenan injection, respectively, presumably reflecting a gradual decrease in the joint pain [Fig. 5(A)], whereas no more differences could be observed after 7 days from carrageenan treatment (data not shown).

Intra-articular injection of fasitibant produced a dose-related antinociceptive effect on the carrageenan-induced incapacitation. Data obtained in animals treated with 10 μg of fasitibant (11.4 nmol/rat) overlapped to those obtained with carrageenan injection alone [Fig. 5(A)]. However, 30 μg (34.2 nmol/rat) fasitibant produced a significant antinociceptive effect both at 6 h and 1 day after carrageenan treatment [Fig. 5(B)]. The antinociceptive effect of fasitibant was greater and longer lasting in animals treated intra-articularly with 100 μg (114 nmol/rat). At this dose, fasitibant significantly inhibited the carrageenan-induced incapacitation after 6 h and persisted up to 4 days after carrageenan treatment [Fig. 5(C)]. No differences were observed between antagonist-treated and vehicle-treated groups after 7 days after carrageenan treatment, when nociceptive response were not more evident (data not shown).

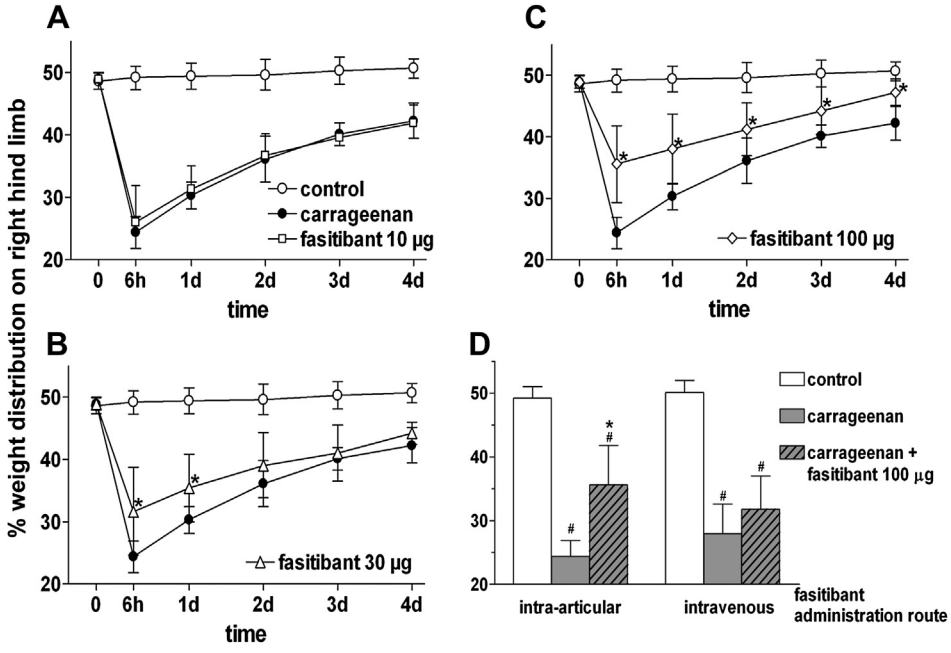

Fig. 5. Dose-dependent effect of the intra-articular administration of the kinin B_2 receptor antagonist fasitibant on inflammatory pain in the time (6 h–4 days) (A, B, C) and comparison of intra-articular and intravenous antagonist administration route (D). Fasitibant was administered at different doses 30 min before carrageenan-induced inflammation into the right knee joint. The knee joint incapacitation was measured at different times (*x*-axis) from carrageenan administration (A–C) or 6 h later (D). Data points represent the mean values of right hind limb weight bearing as a percentage of total weight borne by both hind limbs. Error bars represent 95% CIs. *$P < 0.05$ vs the carrageenan-treated animals, #$P < 0.05$ vs the control group (one-way ANOVA followed by Dunnett post-test).

The effect of fasitibant administered intra-articularly or intravenously was compared at 6 h after carrageenan treatment. As shown in Fig. 5D, contrary to the local treatment (intra-articular), the intravenous fasitibant administration was ineffective in decreasing the carrageenan-induced joint incapacitation.

Discussion

The present study aimed at defining the contribution of kinin B_2 receptors to inflammatory hyperalgesia and activation of sensory afferent fibers in the rat knee joint, as well as to ascertain the efficacy of the kinin B_2 receptor antagonist fasitibant in preventing these phenomena. Previous reports indicated that intra-articular application of BK into the rat knee joint increases the firing rate of its primary afferents, and that this sensitization appeared both in normal and inflamed joints[23,38]. However, the blockade of these BK effects by B_2 receptor antagonists had not been explored so far. The results of this study confirm that in multiunits recordings of sensory nerves innervating inflamed knee joints of rats, intra-articular BK injection causes an acute and significant increase of the impulse firing evoked by joint movements. Moreover, our work shows that this stimulatory action of BK is still obtained after repeated injections. In fact, the mean response to repeated BK injection is, on the average, similar to the first injection response, indicating that desensitization did not play a critical role in our experimental conditions. The average movement-evoked activity during the complete time period of BK injections was significantly higher than during the control period, further suggesting that despite variability, BK had a sustained and long lasting sensitizing effect on movement-evoked activity. The acute stimulatory effect of BK injection on multiunit movement-evoked discharges was confirmed in single unit recordings, where an overall increase in impulse activity during the period of repeated BK injections was also observed, although the significance levels obtained in multiunit recordings were not reached. These results contrast with previous observations made in the rat ankle joint[39,40], where tachyphylaxis induced by repeated intra-arterial injection of BK was reported. Differences with our results could rely on the experimental model used: non-inflamed joints in one case[39] and joints inflamed with Freund's complete adjuvant injection in the other[40]. Furthermore, in both studies, comparatively higher concentrations of BK (2–4 fold those employed in the current protocol) were used.

The main finding of the present work was that administration of fasitibant prior to BK injection reduced the excitatory effects of BK, including those evoked by repeated injections, thus leading to an inhibition of the overall increase in movement-evoked activity resulting of repeated exposure to BK. The effects of fasitibant were observed both in multiunit and in single unit recordings. The reduced movement-evoked activity in single units noticed after injection of fasitibant plus BK could be attributed to a direct inhibitory effect of the drug on movement-evoked impulse activity, independent of its blocking action on BK effects. However, this possibility can be excluded because repeated injections of fasitibant into non-inflamed joints did not cause a significant decrease of movement-evoked activity. Therefore, the most likely explanation to the reduction by fasitibant of impulse activity in inflamed in contrast with non-inflamed knee joints, is that the drug not only attenuated impulse discharge evoked by exogenous BK but also the sensitizing effects on nerve endings of the endogenous BK released in inflamed knee joints. In favor of this interpretation is the observation that in the synovial lavage fluid of carrageenan-treated rats, the content of BK is higher when compared with controls[32]. Thus, present findings indicate that B_2 receptor blockade prevents sensory neurons activation induced by BK, besides that induced by the activation of proteinase-activated receptors[41].

We recently reported that fasitibant can partially block the hyperalgesia and other inflammatory effects (after 6 h from carrageenan injection)[32], and that different other mediators are involved, such as leukotrienes, prostaglandins, and catecholamines[33]. In agreement with previous reports, B_1 receptor activation was found to be involved in the carrageenan-induced hyperalgesia, because this was partially inhibited by the B_1 receptor antagonist [DesArg^{9}Leu^8]-BK[33]. Furthermore, the present results indicate that although fasitibant effectively blocked BK-induced sensitization of joint afferents and decreased nociceptor firing rate consecutive to chemically induced joint inflammation, the drug did not entirely eliminate hyperalgesia. This is in line with the additional contribution of inflammatory agents other than BK to the inflammation and sensitization developed in this model of arthritis. In such context, it is worth pointing out that, with the same experimental preparation, a combination of fasitibant with the glucocorticoid dexamethasone completely reversed the signs of both inflammation and hyperalgesia[32].

The present data highlight the remarkable long duration of the antinociceptive action of fasitibant, that at the highest concentration tested (100 μg/knee) reduced the carrageenan-induced inflammatory hyperalgesia up to 4 days after antagonist administration. This long lasting effect is in agreement with the pharmacodynamic profile of the drug. In fact, the slow dissociation profile of fasitibant from the B_2 receptor compartment, and the docking site of the drug, that is buried into the transmembrane portion of the receptor protein[42] can account for the long residence time of the drug on the B_2 receptor and explain the long duration of its action *in vivo*[43].

Finally, our data show that fasitibant antinociceptive effect was obtained only with intra-articular injection whereas the intravenous route was ineffective, indicating a local rather than systemic action. This result is in line with previous observations in different experimental models where fasitibant delivered by intratracheal instillation or aerosol prevented bronchoconstriction and recruitment of inflammatory cells but not the hypotension induced by systemic administration of BK in anaesthetized guinea pigs[29,44].

In conclusion, the present data show that fasitibant prevents B_2 receptor-mediated activation and sensitization of joint nociceptors. In spite of the limitations imposed by the differences between animal models of joint pain and pain in human OA, this drug appears as a novel potential pharmacologic and symptomatic treatment of pain in OA.

Author contributions

AG, SM, SG, CB and CAM contributed to conception and design of the study. AG, AM and CV contributed to acquisition and analysis of data. AG, SM, SG, CB and CAM contributed in interpretation of data. AG and SM contributed in writing the manuscript. CB and CAM contributed in revising the manuscript critically. All authors approved the final version before submission.

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Conflict of interest

The authors have no conflicts of interest.

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Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.joca.2013.03.013.

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