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European Journal of Pharmacology 331 (1997) 43–52

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Pro-inflammatory effects induced by bradykinin in a murine model of pleurisy

Tânia S.F. Saleh, João B. Calixto, Yara S. Medeiros *

Department of Pharmacology, Centre of Biological Sciences, Federal University of Santa Catarina, 88015-420 Florianópolis, SC, Brazil

Received 21 March 1997; revised 6 May 1997; accepted 12 May 1997

Abstract

Bradykinin caused a dose-related increase in cell influx 4 h after its administration into the mouse pleural cavity (ED_{50} = 3.2 nmol/cav., 95% confidence limits = 0.6–15.5). Cell influx peaked at 4 h and remained elevated for up to 72 h, whereas exudation was detected between 2 and 6 h after bradykinin administration. Both HOE 140 (D-Arg–[Hyp^3,Thi^5,D-Tic^7, Oic^8]bradykinin) and NPC 17731 (D-Arg^0–[Hyp^3D–Hyp^E(transpropyl^7)Oic^8]bradykinin) inhibited bradykinin-induced cell influx (ID_{50} 0.028 (0.05–0.16) and 0.4 (0.3–0.7) pmol/cav., respectively). Des–Arg^9–[Leu^8]bradykinin (0.1 and 3.0 nmol/cav., 30 min before) did not inhibit the effects of bradykinin. Pre-treatment of animals with either indomethacin, terfenadine, dexamethasone, N^{ω}-nitro-L-arginine benzyl ester, cromolyn, theophylline, salbutamol, FK 888 (N^2-[(4*R*)-4-hydroxy-1-(1-methyl-1H-indol-3-yl)carbonyl-L-propyl]*N*-metyl-*N*-phenyl-methyl-3-(2-naphthyl)-L-alaninamide) or SR 142801 ((*N*)-(1-{3-[1-benzoyl-3-(3,4-dichloro-phenyl)-piperidin-3-yl]propyl}-4-phenyl-piperidin-4-yl)-*N*-methyl-acetamide) significantly inhibited cell migration ($P < 0.01$). These results indicate that bradykinin had a significant pro-inflammatory effect on the pleural cavity of the mice. This effect seems to be primarily mediated via activation bradykinin B_2 receptors which trigger the release of other mediators. © 1997 Elsevier Science B.V.

Keywords: Bradykinin; Pleurisy; Anti-inflammatory drugs; Bradykinin receptor antagonist; Inflammatory mediator

1. Introduction

The role of kinins (bradykinin and des-Arg^9–bradykinin) in many inflammatory states triggered by injury, trauma, coagulation pathways and/or immune complexes is well established (Regoli and Barabé, 1980; Bathon and Proud, 1991; Perkins and Kelly, 1993; Wirth et al., 1993; Marceau, 1995). It has been reported that bradykinin is able to induce either cell influx or plasma extravasation (Damas et al., 1990; Hall and Geppetti, 1995). Once released, kinins are able to activate B_1 and/or B_2 receptors, resulting also in the release of other mediators of inflammation such as prostaglandins, leukotrienes, histamine, nitric oxide, platelet-activating factor (PAF) and cytokines, among others, derived mainly from polymorphonuclear leukocytes, mast cells, macrophages and endothelial cells (Bathon and Proud, 1991; Cruwys et al., 1994). Furthermore, accumulated evidence indicates that cells at sites of inflammation are more responsive to the kinins than normal cells (Dray and Perkins, 1993; Perkins and Kelly, 1993; Dray et al., 1994).

Until recently, however, the participation of kinins in models of experimental inflammation has been difficult to assess because first-generation bradykinin B_2 receptor antagonists, although reasonably selective, have a short half-life (Griesbacher and Lembeck, 1987; Griesbacher et al., 1989). Additionally, these antagonists possess residual agonistic properties and commonly induce non-specific mast cell degranulation (Rhaleb et al., 1991; Hall, 1992). The development of the second generation of bradykinin B_2 receptor antagonists, such as HOE 140 and NPC 17731, has provided new and potent tools for exploring the role of kinins in many physiological and pathological processes (Hall, 1992; Wirth et al., 1995). These antagonists are almost devoid of agonist and degranulating properties (Lembeck et al., 1991, 1992), besides being very potent and long-acting, even in vivo (Kyle et al., 1991; Wirth et al., 1991). In addition, the effects of bradykinin and des-Arg^9–bradykinin in both chronic pain and inflammation via B_1 receptor activation have also been demonstrated

* Corresponding author. Tel.: (55-48) 231-9491; Fax: (55-48) 222-4164.

PII S0014-2999(97)01005-4

(Farmer and Burch, 1992; Corrêa and Calixto, 1993; Dray and Perkins, 1993; Perkins and Kelly, 1993; Dray et al., 1994; Campos and Calixto, 1995; Campos et al., 1995). This latter evidence of kinin effects has recently been reinforced by the finding that in pathological conditions the B_1 receptor is induced, whereas it is not commonly expressed under physiological conditions (Regoli and Barabé, 1980; Deblois et al., 1988; Bathon and Proud, 1991; Davis and Perkins, 1994a,b).

Data reported in the literature also suggest that kinins are implicated in the pathogenesis of the neurogenic inflammation that occurs in several diseases such as asthma, arthritis, rhinitis, bronchitis and migraine (Moskowitz and MacFarlane, 1993; Geppetti et al., 1995; Shelhamer et al., 1995). Sensory nerves are stimulated by several non-specific and specific agents including kinins, which in turn release other mediators such as tachykinins, vasoactive intestinal peptide (VIP) and calcitonin gene-related peptide (CGRP), contributing to the amplification and perpetuation of the inflammatory process (Geppetti, 1993; Ricciardolo et al., 1994; Geppetti et al., 1995).

The aim of this study was to analyse the pro-inflammatory properties of kinins in a closed site such as the pleural cavity of mice. Thus, initially, we evaluated the effects caused by bradykinin administered directly into the pleural cavity, and attempted to characterise the types of kinin receptors involved by using selective B_2 (HOE 140 and NPC 17731) and B_1 receptor (des-Arg^9–[Leu^8]bradykinin) antagonists. In another series of experiments, we also evaluated whether the pro-inflammatory effects induced by bradykinin result from the release of other mediators that are triggered by this kinin, such as arachidonic acid pathway products, histamine and nitric oxide, among others. To this end, we selected different classes of drugs known for their potential effects on the synthesis of different mediators of the inflammatory process or for their actions at the level of these receptors for these mediators. Finally, to test the hypothesis that also in this model bradykinin might act through the activation of sensory nerve fibres, triggering the release of tachykinins, we examined whether selective tachykinin receptor antagonists of the NK_1, NK_2 and NK_3 receptors were able to modify the pro-inflammatory effects of bradykinin.

2. Materials and methods

2.1. Animals

Non-fasted adult Swiss mice of both sexes (18–25 g) were used, aged 2 months. The mice were maintained in an environment with a controlled temperature (21 ± 2°C), illuminated by daylight supplemented by electric light, from 06.00 to 18.00 h, with free access to food and water. All animals were pre-treated with captopril (5 mg/kg, i.p.) 1 h prior to any given experiment to prevent kinin degradation (Corrêa and Calixto, 1993).

2.2. Drugs and solutions

The following drugs were used: bradykinin, cromolyn (disodium cromoglycate), dexamethasone, indomethacin, N^{ω}-nitro-L-arginine benzyl ester (L-NOARG), salbutamol, theophylline, terfenadine (Sigma, St. Louis, MO, USA), des-Arg^9–bradykinin, des-Arg^9–[Leu^8]bradykinin (Peninsula, USA), HOE 140 (D-Arg–[Hyp^3,Thi^5,D-Tic^7,Oic^8]bradykinin) (Hoechst, Frankfurt, Germany), NPC 17731 (D-Arg^0–[Hyp^3,D-Hyp^E(transpropyl^7)Oic^8]bradykinin) (Scios Nova Pharmaceutical, USA), FK 888 (N^2-[(4*R*)-4-hydroxy-1-(1-methyl-1H-indol-3-yl)carbonyl-L-propyl]*N*-methyl-*N*-phenyl-methyl-3-(2-naphthyl)-L-alaninamide) (Fujisawa Pharmaceutical, Japan), SR 48968 ((*S*)-*N*-[4-[4-(acetylamino-4-phenyl-1-piperidinyl]-2-(3,4-dicholoro-phenyl) butyl]*N*-methylbenzamide) and SR 142801 ((*N*)-(1-{3-[1-benzoyl-3-(3,4-dichloro-phenyl)piperidin-3-yl]propyl}-4-phenyl-piperidin-4-yl)-*N*-methyl-acetamide) (Sanofi Recherche, France), heparin (Liquemine®, Roche, Brazil), Evans blue dye (Merck, Brazil), sterile saline solution (0.9%), Türk solution and May–Grunwald–Giemsa dye from different commercial sources. Phosphate-buffered saline (PBS (pH 7.6), composition mmol: NaCl 137, KCl 2.7 and phosphate buffer salts 10) was prepared and kept in a refrigerator. FK 888 (1 mM), SR 48968 (1 mM), SR 142801 (1 mM) and dexamethasone were prepared by diluting them in absolute ethanol, indomethacin in $NaHCO_3$ solution (5%), and the other drugs in PBS. All drugs were kept in siliconised plastic tubes at −20°C. On the day of the experiments, the drugs were diluted to the desired concentration with sterile saline solution at room temperature, except theophylline, which was first heated to 37°C (5 min).

2.3. Induction of pleurisy and measurement of the parameters studied

On the day of the experiments, animals were lightly anaesthetised with ether, and either bradykinin or saline solution was injected into the right pleural space through the chest skin (final volume of 0.1 ml). According to the experimental protocol, the animals were killed at different periods of time with an overdose of ether, and immediately after the thorax was opened, the pleural cavity was washed with 1 ml of PBS plus heparin (20 IU per ml) and the fluid was collected with automatic pipettes. All animals were previously challenged (24 h) with a solution of Evans blue dye (25.0 mg/kg, 0.2 ml, i.v.) in order to evaluate the extent of exudation in the pleural space (Saleh et al., 1996). Total leukocyte counts were determined with a Neubauer chamber by means of optical microscopy after diluting a sample of the pleural fluid with Türk solution (1:200). Cellular smears were stained with May–Grenwald–Giemsa for differential analysis performed under an immersion objective. A sample (500 μl) of the fluid collected from the pleural space was separated and stored in the freezer (−20°C) to further determine the concentra-

tion of Evans blue dye. On the day of the experiments, a batch of samples was defrosted at room temperature, and the amount of dye was estimated by colorimetry (Compu-Espectro Spectrometer, Brazil) at 600 nm by interpolation from a standard curve of Evans blue dye in the range of 0.01–50 μg/ml.

Throughout the experiments, the animals were managed in accordance with the principles and guidelines for the care of laboratory animals provided by Zimmermann (1983) with the approval of the local committee.

2.4. Experimental procedure

In preliminary studies (results not shown), several doses of bradykinin and different pre-treatment intervals were tested to determine the best duration of pre-treatment for analysis of effects in this model. According to the results of this protocol, separate groups of animals received, by intra-pleural route, different doses of bradykinin (5, 10, 20 and 40 nmol), and indices of inflammation (total and differential cell content and exudation) were analysed after 4 h. In other experiments, the temporal profile of the inflammatory reaction induced by bradykinin (10 nmol/cav., 1–100 h) was determined.

To evaluate the participation of B_1 and B_2 receptors in bradykinin action, another series of experiments was carried out by pre-treating (30 min) the animals with selective antagonists, the studied parameters were analysed 4 h after pleurisy induction. Thus, in a first set of experiments, different groups of animals were pre-treated (30 min) by intra-peritoneal (i.p.), intra-pleural (cav.) or intravenous (i.v.) routes, with a chosen dose of one of the bradykinin B_2 receptor peptide antagonists HOE 140 or NPC 17731. In other experiments, distinct doses of these antagonists (0.01–1.0 pmol/cav.) were injected (30 min) into the pleural space before pleurisy was triggered with bradykinin (10 nmol/cav.). The temporal profile of inhibition caused by these antagonists when administered directly into the pleural cavity was also indirectly evaluated by pre-treating the animals, at different periods of time, before induction of pleurisy with bradykinin (10 nmol), with a dose used in the above protocol. Other groups of animals were pre-treated (5 or 30 min) with 0.1 or 3.0 nmol/cav. of the bradykinin B_1 receptor antagonist, des-Arg^9–[Leu^8]bradykinin, before bradykinin (10 nmol/cav.) was injected to induce pleurisy.

To further assess the participation of other inflammatory mediators such as arachidonic acid pathway metabolites, histamine, nitric oxide and tachykinins in this inflammatory reaction induced by bradykinin (10 nmol/cav., 4 h), different groups of animals were pre-treated (30 min) with several agents known to interfere with the synthesis of these inflammatory mediators and/or to interfere at the receptor site of these mediators. According to a previously established protocol based on data from the literature and from previous tests carried out in our laboratory, different groups of animals were pre-treated (30 min) with indomethacin (cyclooxygenase inhibitor, 5 mg/kg, i.p.), terfenadine (histamine H_1 receptor antagonist, 50 mg/kg, i.p.), dexamethasone (potent inhibitor of phospholipase A_2, of induced nitric oxide synthase and of cyclooxygenase-2, among others, 0.5 mg/kg, i.p.) and L-NOARG (inhibitor of nitric oxide synthesis pathway, 1.0 pmol/cav.). Finally, the effects of anti-asthmatic drugs such as cromolyn (0.4 μmol/cav.), theophylline (50 mg/kg, i.p.) and salbutamol (50 mg/kg, i.p.), which also has anti-inflammatory properties, were also evaluated in this model. In order to determine whether the pro-inflammatory effects of bradykinin (10 nmol/cav.) were related to the activation of sensory nerve fibres and the release of neurokinins, separate groups of animals were pre-treated (30 min) with selective tachykinin receptor antagonists. To this end, tachykinin receptor antagonists selective for tachykinin NK_1 (FK 888, 0.02–0.2 pmol/cav.), NK_2 (SR 48968, 20–100 nmol/cav.) or NK_3 (SR 142801, 0.03–100 nmol/cav.) receptors were used. The studied parameters were analysed 4 h after pleurisy induction.

Each experimental group included control animals which had received the same intra-pleural volume of sterile saline and were killed at the same time as their matched-treated group. When appropriate, control animals treated with an intra-pleural injection of either the vehicle used to dilute the agonist or one of the test antagonists were also evaluated.

2.5. Statistical analysis

Data are reported as means ± S.E.M., except for the ED_{50} or ID_{50} values in individual experiments (i.e. doses of the agonist needed to cause half maximal total cell influx, or doses of antagonists that reduced total cell migration by 50% in relation to control value), which are presented as geometric means accompanied by their respective 95% confidence limits (CL). For each group of experiments, the ED_{50} or ID_{50} values were determined by means of regression analysis. For the purpose of standardisation, in experiments with the agonists and the antagonists, the individual raw values for total cell content were subtracted from those obtained for saline matched-treated animals. Statistical differences between groups were determined by analysis of variance (ANOVA), complemented with Dunnett's test or by Student's unpaired *t*-test when indicated. $P < 0.05$ was considered as indicative of significance.

3. Results

Bradykinin (5, 10, 20 and 40 nmol) caused a small but significant dose-dependent increase in the number of cells in the mouse pleural cavity 4 h after its administration. The increase in total cell number was associated with a slight but significant increase in fluid leakage (Fig. 1). As shown,

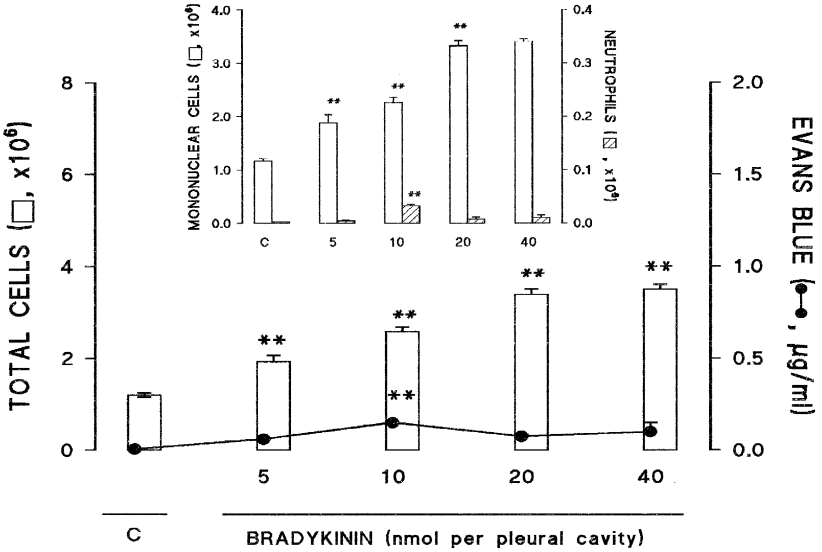

Fig. 1. Effect of bradykinin on total and differential cell content, and fluid leakage 4 h after its administration in the mouse pleural cavity. Control responses (C) obtained in animals injected only with sterile saline. The inset shows the variation in the number of mononuclear and neutrophil cells under the same experimental conditions. Each column and closed circle represents the mean for 5 to 10 animals, and the vertical bars the S.E.M. In some groups, the S.E.M. values are smaller than the symbol. ** $P < 0.01$.

the increase in cell migration caused by bradykinin was due to a marked influx of mononuclear cells ($P < 0.01$). A small but significant increase in the number of neutrophils ($P < 0.01$), which was not dose-dependent, was also observed. Furthermore, because the degree of exudation was significant but too small, the analysed pharmacological parameters (ED_{50} or ID_{50}) were only determined in relation to total cell influx into the pleural cavity. Thus, the estimated mean ED_{50} value for bradykinin was 3.2, 95% CL = 0.6–15.5 nmol/cav. Maximal cell influx (mean ± S.E.M.) was induced by 40 nmol/cav. of bradykinin (total cells = $(3.5 \pm 0.05) \times 10^6$) in comparison to saline-treated animals (total cells = $(1.3 \pm 0.08) \times 10^6$) ($P < 0.01$) (Fig. 1). No significant change in the number of eosinophils was detected (results not shown).

The time-course analysis of this effect induced by bradykinin (10 nmol/cav.) revealed that the increase in cell number (mean ± S.E.M.) peaked 4 h after bradykinin administration (total cells = $(2.6 \pm 0.1) \times 10^6$) (Fig. 2). Afterwards, a slow and gradual decrease of the total cell number was observed, but cell numbers remained significantly elevated up to 72 h after pleurisy induction (total cells = $(1.5 \pm 0.04) \times 10^6$) ($P < 0.01$) (Fig. 2). It is interesting to note that a significant influx of neutrophils was also detected between 4 and 6 h after bradykinin administration ($P < 0.01$) (Fig. 2, inset). Again, the number of eosinophils, either in saline-treated or in bradykinin-treated animals, did not vary within the period of study (results not shown). A significant increase in the exudate levels was detected between 2 and 6 h following pleurisy triggered by bradykinin ($P < 0.05$).

Fig. 3A and B show the effect of HOE 140 and NPC 17731, selective antagonists of bradykinin B_2 receptor, given by different routes of administration. As shown, HOE 140 was effective in inhibiting cell migration ($P < 0.01$) only when it was administered directly into the pleural cavity (0.1 pmol) or by the intravenous route (5.0 pmol/kg) (Fig. 3A). The mean inhibition was approximately 70 and 100%, respectively. In contrast, HOE 140 (5.0 pmol/kg, i.p.) did not significantly inhibit cell influx in response to bradykinin ($P > 0.05$). Fig. 3B shows that distinct doses of NPC 17731 (i.p. = 50 pmol/kg, cav. = 1.0 pmol, i.v. = 50 pmol/kg) significantly inhibited cell mi-

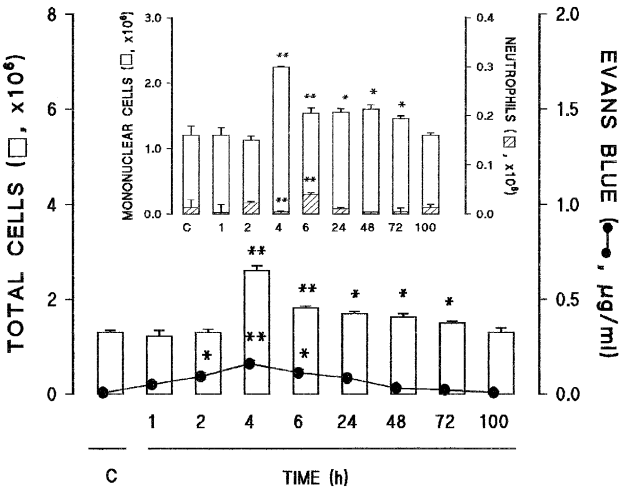

Fig. 2. Time-course profile of bradykinin (10 nmol/cav.)-induced pleurisy in mice. Control responses (C) obtained in animals injected only with sterile saline. The inset shows the variation in the number of mononuclear and neutrophil cells under the same experimental conditions. Each column and closed circle represents the mean for 4 to 10 animals, and the vertical bars the S.E.M. In some groups, the S.E.M. values are smaller than the symbol. * $P < 0.05$ and ** $P < 0.01$.

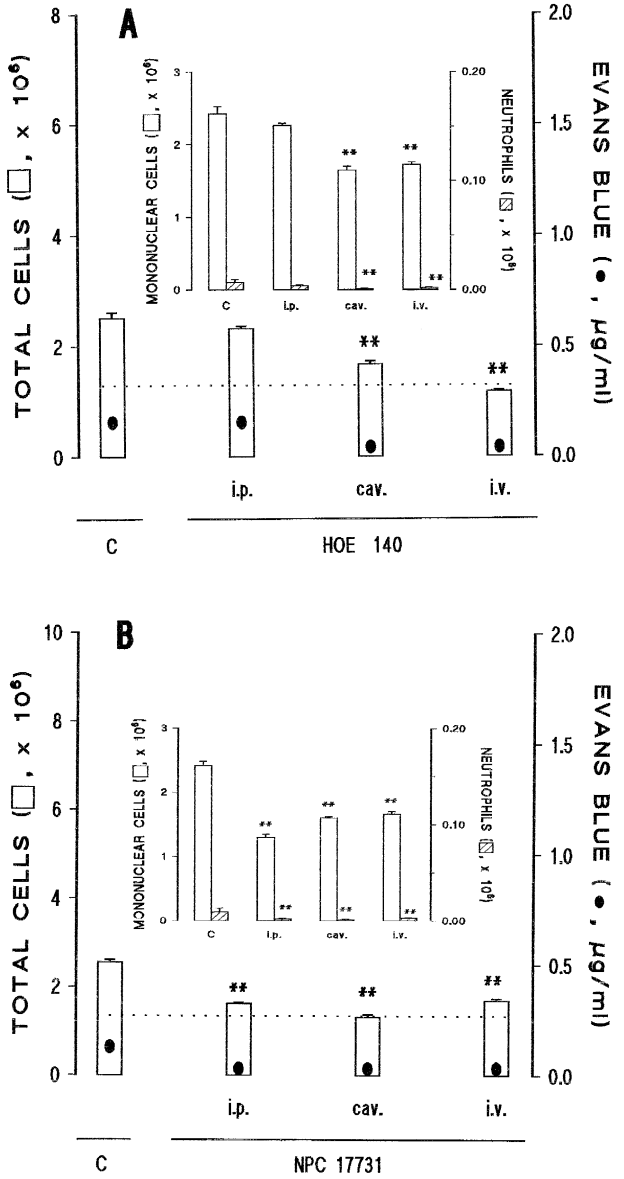

Fig. 3. Effect of bradykinin B_2 receptor antagonists given by different routes, against the pleurisy induced by bradykinin (10 nmol, 4 h). i.p. = intra-peritoneal, cav. = intra-pleural and i.v. = intravenous routes. (A) Animals pre-treated (30 min) with HOE 140: i.p. = 5 pmol/kg, cav. = 0.1 pmol, i.v. = 5 pmol/l. (B) Animals pre-treated (30 min) with NPC 17731: i.p. = 50 pmol/kg, cav. = 1 pmol, i.v. = 50 pmol/l. Control responses (C) obtained in animals injected only with bradykinin (10 nmol/cav.). The dotted line indicates the mean total cell content of the pleural cavity in saline-treated animals. The insets show the variation in the number of mononuclear and neutrophil cells under the same experimental conditions. Each column and closed circle represents the mean for 6 to 10 animals, and the vertical bars the S.E.M. In some groups, the S.E.M. values are smaller than the symbol. ** $P < 0.01$.

gration caused by bradykinin (10 nmol/cav., 4 h) ($P < 0.01$). When animals were pre-treated with this antagonist by intra-pleural, i.v. or i.p. routes, the magnitude of this inhibition was approximately 100, 68 and 62%, respectively ($P < 0.01$).

The administration of different doses of HOE 140 caused a dose-dependent inhibition of cell migration (ID_{50} = 0.028 (0.05–0.16) pmol/cav.). The mean inhibition of total cell migration caused by HOE 140 (0.01–1.0 pmol/cav.) was 27, 56, 69, 89 and 89%, respectively (Fig. 4A). At the doses of 0.1 and 0.3 pmol of this antagonist, the total cell content of the pleural cavity did not differ from that obtained in saline-treated animals ($P > 0.05$). In relation to NPC 17731, the estimated mean ID_{50} value of this antago-

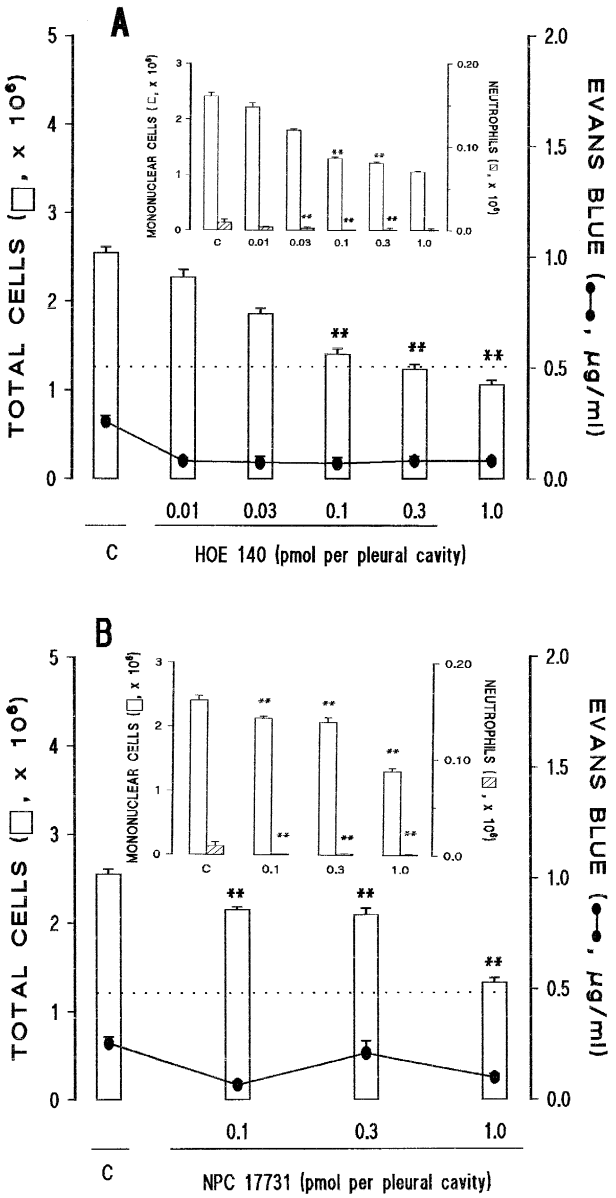

Fig. 4. Effect of different doses of HOE 140 (A) and NPC 17731 (B) administered 30 min prior to pleurisy induced by bradykinin (10 nmol/cav., 4 h). Control responses (C) obtained in animals injected only with bradykinin. The dotted line indicates the mean total cell content of the pleural cavity obtained in saline-treated animals. The insets show the variation in the number of mononuclear and neutrophil cells under the same experimental conditions. Each column and closed circle represents the mean for 4 to 10 animals, and the vertical bars the S.E.M. In some groups, the S.E.M. values are smaller than the symbol. ** $P < 0.01$.

nist against pleurisy induced by bradykinin (10 nmol, 4 h) was 0.4 (0.3–0.7 pmol/cav.). Doses of 0.1 and 0.3 pmol of NPC 17731, 30 min before bradykinin, were equieffective in reducing the cell influx (27–32%) induced by bradykinin (Fig. 4B) ($P < 0.01$), whereas at 1 pmol/cav. this antagonist was able to restore the cell content of the pleural cavity to within the normal range ($P < 0.01$).

As shown in Fig. 5A, the inhibitory effect caused by HOE 140 (0.1 pmol/cav.) was long-lasting, since a signif-

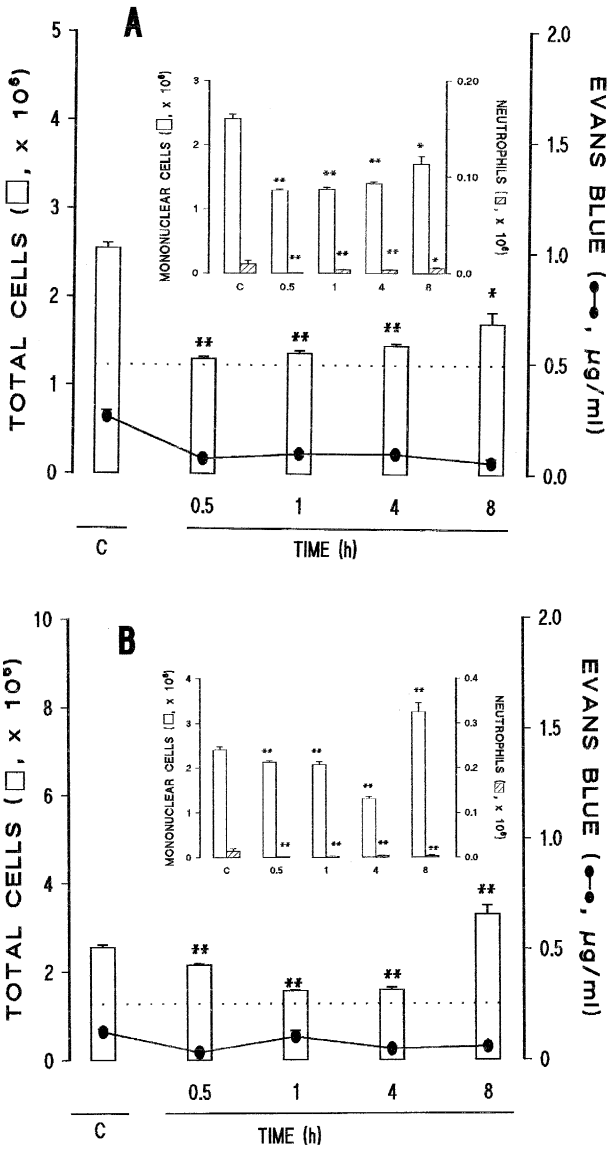

Fig. 5. Time-course profile of the effects induced by HOE 140 (0.1 pmol) (A) and NPC 17731 (1.0 pmol) (B) administered intra-pleurally at different periods of time before pleurisy induction by bradykinin (10 nmol/cav., 4 h). Control responses (C) obtained in animals injected only with bradykinin. The dotted line indicates the mean total cell content of the pleural cavity in saline-treated animals. The insets show the variation in the number of mononuclear and neutrophil cells under the same experimental conditions. Each column and closed circle represents the mean for 6 to 10 animals, and the vertical bars the S.E.M. In some groups, the S.E.M. values are smaller than the symbol. * $P < 0.05$ and ** $P < 0.01$.

Table 1
Effect of pre-treatment (30 min) of animals with various drugs on the development of pleurisy induced by bradykinin

Groups	Dose	Leukocytes ($\times 10^6$)	Exudate [a] (µg/ml)
Saline-treated	—	1.2 ± 0.3	0.002 ± 0.1
Bradykinin-treated	—	2.50 ± 0.13	0.40 ± 0.08
des-Arg^9–[Leu^8]BK [c]	0.1 nmol	2.3 ± 0.11	0.35 ± 0.10
	3.0 nmol	1.90 ± 0.12	0.60 ± 0.10
Indomethacin [b]	5.0 mg/kg	1.40 ± 0.05 [d]	0.50 ± 0.17
Terfenadine [b]	50.0 mg/kg	1.0 ± 0.04 [d]	0.44 ± 0.05
Dexamethasone [b]	0.5 mg/kg	1.0 ± 0.04 [d]	0.36 ± 0.04
L-NOARG [c]	1.0 pmol	1.40 ± 0.05 [d]	0.50 ± 0.05
Cromolyn [c]	0.4 µmol	1.6 ± 0.18 [d]	0.29 ± 0.08
Theophylline [b]	50.0 mg/kg	1.50 ± 0.09 [d]	0.32 ± 0.04
Salbutamol [b]	50.0 mg/kg	1.10 ± 0.05 [d]	0.48 ± 0.03
SR 48968 [c]	20 nmol	2.75 ± 0.2	0.30 ± 0.40
	100 nmol	1.57 ± 0.05 [d]	0.30 ± 0.05

All animals, except the saline-treated group were injected with bradykinin (10 nmol/cav.) and the parameters analysed 4 h later. Values represent the means ± S.E.M. ($n = 6$–10 animals per group).
[a] Evaluated by means of Evans blue dye extravasation.
[b] Treatment given by i.p. route.
[c] Drug administered into the pleural cavity, des-Arg^9–[Leu^8]BK = des-Arg^9–[Leu^8]bradykinin, L-NOARG = ω-nitro-L-arginine benzyl ester.
[d] Statistically significant differences between treated and control values, $P < 0.01$.

icant decrease in cell migration induced by bradykinin (10 nmol, 4 h) was observed even when the antagonist was administered 8 h before pleurisy induction. At all time periods tested, the total cell content was significantly reduced in comparison to that obtained in bradykinin-treated animals. Thus, pre-treatment for 0.5 and 4 h with this antagonist gave a total leukocyte number (mean ± S.E.M.) which was near to normal values (0.5 h, $(1.3 \pm 0.02) \times 10^6$; 4 h, $(1.37 \pm 0.03) \times 10^6$) and did not differ from values obtained in animals treated only with saline ($(1.3 \pm 0.08) \times 10^6$). A less marked but significant temporal inhibition was obtained in animals treated with NPC 17731 (1 pmol/cav.), since its maximal inhibitory effect lasted only about 4 h ($P < 0.01$) (Fig. 5B). Administration of NPC 17731 (1 pmol/cav.), 8 h before pleurisy induction with bradykinin, resulted in a significant influx of leukocytes into the pleural cavity due to mononuclear cell migration (total cells = $(3.3 \pm 0.2) \times 10^6$) ($P < 0.01$).

The administration of the B_1 receptor antagonist des-Arg^9–[Leu^8]bradykinin (0.1 and 3.0 nmol/cav.), 30 min before bradykinin (10 nmol, 4 h), did not significantly modify the pattern of cell influx (Table 1). Also, no significant effect was observed when the same doses of this antagonist were given 5 min prior to pleurisy induction with bradykinin (results not shown).

Table 1 shows that indomethacin, terfenadine, dexamethasone and L-NOARG significantly inhibited the recruitment of leukocytes induced by bradykinin (10 nmol, 4 h) into the mouse pleural cavity. As shown, prior treatment (30 min) of the animals with one of these drugs before

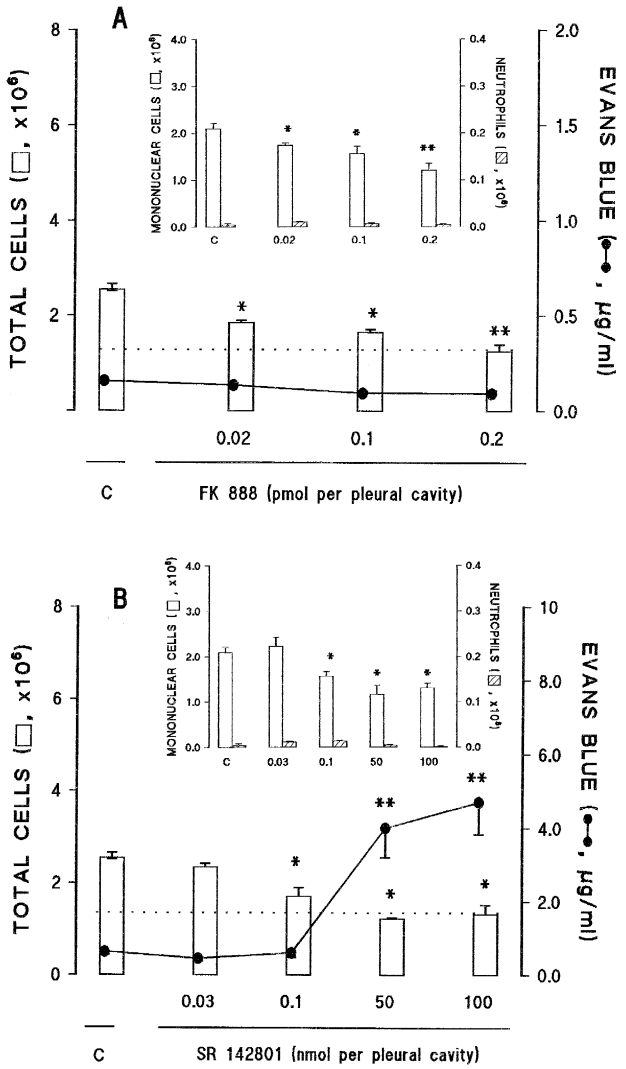

Fig. 6. Effect of different doses of the selective tachykinin NK_1 (FK 888, 0.02–0.2 pmol/cav.) (A) and NK_3 (SR 142801, 0.03–100 nmol/cav.) (B) receptor antagonists administered 30 min prior to pleurisy induced by bradykinin (10 nmol/cav., 4 h). Control responses (C) obtained in animals injected only with bradykinin. The dotted line indicates the mean total cell content of the pleural cavity in saline treated animals. The insets show the variation in the number of mononuclear and neutrophil cells under the same experimental conditions. Each column and closed circle represents the mean for 6 to 10 animals, and the vertical bars the S.E.M. In some groups, the S.E.M. values are smaller than the symbol. * $P < 0.05$ and ** $P < 0.01$.

pleurisy induction resulted in a normal cell content of the pleural cavity. A similar inhibition of cell influx induced by bradykinin was also observed when the animals were pre-treated with either theophylline, cromolyn or salbutamol ($P < 0.01$) (Table 1). Again, both total and differential cell contents were restored to normal values when the animals were pre-treated with one of these agents. In all groups studied, the test drugs were not able to modify significantly the amount of exudation.

Pre-treatment of the animals with tachykinin receptor antagonists revealed that both tachykinin NK_1 (FK 888, 0.02–0.2 pmol/cav.) and NK_3 (SR 142801, 0.03–100 nmol/cav.) receptor antagonists inhibited, in a dose-dependent fashion, the cell influx caused by bradykinin (Fig. 6A,B). The estimated mean ID_{50} values for these antagonists were 0.1 (0.02–0.5) pmol/cav. and 21.5 (8.9–51.9) nmol/cav., respectively. The inhibitory effect on cell migration caused by SR 142801 was associated with a parallel increase in exudation ($P < 0.01$) when doses of 50 and 100 nmol/cav. of this antagonist were used (Fig. 6B). In contrast, the intra-pleural administration of the tachykinin NK_2 receptor antagonist (SR 48968, 20 and 100 nmol) resulted in opposite effects. Whereas the dose of 20 nmol/cav. of this antagonist followed by bradykinin injection did not modify the cell influx elicited by bradykinin, higher doses of 100 nmol/cav. were associated with a significant decrease in cell influx ($P < 0.01$) (Table 1). At this latter dose, the experimental protocol was not completed because of the toxic effects of this antagonist, which caused death in some animals (3 out of 8). It is worth noting that doses of 50 nmol/cav. of this antagonist also caused the same inhibitory effect observed with the dose of 100 nmol ($n = 3$, results not shown).

Both bradykinin B_2 receptor antagonists, at all doses tested when injected alone into the pleural cavity, induced a significant increase in total cell migration (HOE 140 = $(2.3 \pm 0.01) \times 10^6$ and NPC 17731 = $(2.1 \pm 0.2) \times 10^6$, $n = 4$) ($P < 0.01$). A similar effect was also observed in relation to FK 888 (total cell = $(2.2 \pm 0.3) \times 10^6$, $n = 3$), SR 48968 at the dose of 20 nmol/cav. ($(1.8 \pm 0.2) \times 10^6$, $n = 4$) and SR 142801 at the dose of 50 nmol/cav. (1.7 ± 0.4, $n = 3$). Higher doses of this latter antagonist (100 nmol/cav.) did not cause any change in the total cell content of the pleural cavity.

4. Discussion

Our study shows that bradykinin caused a significant influx of leukocytes into the mouse pleural cavity. Furthermore, analysis of the time-course profile of this effect elicited by bradykinin indicated that it peaked at 4 h and was long-lasting (up to 72 h). Under our conditions, cell migration induced by bradykinin was inhibited by antagonists of the bradykinin B_2 receptor (HOE 140 and NPC 17731), whereas the same effect evoked by bradykinin was not blocked by the tested doses of the B_1 selective receptor antagonist (des-Arg^9–[Leu^8]bradykinin). These findings indicate that the pro-inflammatory effects exerted by bradykinin in this model may be mediated primarily by activation of the B_2 receptor.

According to our data, in the mouse pleural cavity, bradykinin caused significant cell migration, due mainly to mononuclear and neutrophil cell influxes, whereas in the rat pleural cavity this agonist promoted initially an increase in neutrophil migration (6 h) that was later (24 h)

followed by eosinophil migration (Pasquale et al., 1991; Martins et al., 1992). Although it is not possible to compare these results, both experimental models (mouse and rat) show that bradykinin is able to elicit a long-lasting increase in cell influx. These data reinforce the hypothesis that differences among animal species and/or experimental designs result in distinct types of cell recruitment and/or release of various inflammatory mediators (Paegelow et al., 1995). Furthermore, although the effect caused by bradykinin seems to be less pronounced than that evoked by non-specific agents (carrageenin and zymosan, for instance) (Brito, 1989), these results provide evidence that bradykinin contributes to the initiation and maintenance of the inflammatory response.

The fact that cell migration induced by bradykinin was fully inhibited, in a dose-dependent manner, by HOE 140 and NPC 17731, but not by des-Arg^9–[Leu^8]bradykinin, suggests that this effect may be primarily mediated via activation of the bradykinin B_2 receptor. These findings are in agreement with the evidence indicating that most of the acute inflammatory effects elicited by bradykinin, including exudation, cell influx and production of pain, are mediated via bradykinin B_2 receptor activation (Steranka and Burch, 1991; Wirth et al., 1991; Farmer and Burch, 1992; Hall, 1992). In addition, it has also been shown that several peptide bradykinin B_2 antagonists are effective in reducing indices of inflammation both in the model of paw oedema (Burch and De Haas, 1990) and the model of pleurisy (Burch et al., 1990) induced by carrageenan, in addition to having anti-hyperalgesic effects in urate crystal, formalin and acetic acid writhing models (Steranka and Burch, 1991; Bhoola et al., 1992; Corrêa and Calixto, 1993; Campos et al., 1995). However, although several studies in the literature have demonstrated that bradykinin B_2 receptor antagonists consistently inhibit bradykinin-induced hyperalgesic or pro-inflammatory effects in vivo, few of these studied have used three or more different doses of the antagonists, which permits estimation of their ID_{50} values. For instance, in relation to HOE 140, the majority of studies have used only one dose of the antagonist that varied from pmol (Wirth et al., 1993; Davis and Perkins, 1994b) to μmol (Bertrand et al., 1993; Pethö et al., 1994; Ricciardolo et al., 1994) per site or per kilogram of body weight. In this context, Wirth et al. (1993) have shown that HOE 140 is significantly more potent in inhibiting bradykinin-induced bronchoconstriction in guinea-pigs by the i.v. route (ID_{50} = 13.4 pmol/kg) than by the inhalation route (ID_{50} = 1.34 nmol/kg). In addition, Campos and Calixto (1995) have shown that the inhibitory potencies of both HOE 140 and NPC 17731 against bradykinin-induced rat paw oedema, when co-administered with other mediators, range from 0.7 to 1.5 nmol/paw. The results of our study show that the potencies of the tested bradykinin B_2 receptor antagonists, when applied directly into the pleural cavity, are very much higher (pmol/site) than the values obtained with other experimental protocols (Wirth et al., 1993; Campos and Calixto, 1995; Campos et al., 1995). HOE 140 was 14 times more potent than NPC 17731 in inhibiting leukocyte influx. Based on these findings, we again cannot discard the possibility that both animal species and the site of the inflammatory process might have contributed to these differences. In addition, it is less probable in our study that bradykinin was metabolised to des-Arg^9–bradykinin, since both HOE 140 and NPC 17731 completely inhibited cell influx 4 h after bradykinin administration.

Our findings also confirm previous results which show that both bradykinin B_2 receptor antagonists exert a sustained inhibitory effect (Kyle et al., 1991; Bhoola et al., 1992). However, the duration of the inhibitory effect caused by HOE 140 (8 h), when administered directly into the pleural cavity, was greater than that induced by NPC 17731 (4 h). In relation to the administration of these antagonists by different routes (i.p., cav., i.v.), although differences in doses and routes of administration restrict comparisons among protocols, these results show that both drugs are effective either when applied into the pleural cavity or when given i.v. Taken together, these findings are relevant, since it is proposed that these agents may have potential therapeutic applications (Wirth et al., 1995).

Our data also confirm the evidence that the pro-inflammatory actions evoked by bradykinin are the result of the release of other inflammatory mediators. It is well known that, depending on the tissue and/or cell type, kinins may stimulate the release of products of the arachidonic acid pathways, histamine and/or nitric oxide among others (Hall, 1992; Burch et al., 1993). This is because in nearly all tissues, activation of the bradykinin B_2 receptor can lead to activation of different pathways of second-messenger systems (Burch et al., 1993). In our study, we also observed that agents which classically inhibit some steps of the arachidonic acid pathway (indomethacin and dexamethasone), nitric oxide synthesis (L-NOARG), and interact with histamine receptors (terfenadine) were effective in inhibiting bradykinin-induced pro-inflammatory effects. These findings provide indirect evidence that the pro-inflammatory effects induced by bradykinin in the mouse pleural cavity are, at least in part, mediated by the release of these mediators. However, it is not possible to determine the hierarchical importance of each of these released mediators in this inflammatory process induced by bradykinin, because at doses used, each test drug restored the cell content of the pleural cavity to within the normal range. This may be, in part, due to the fact that the dose of each agent used was maximal. Thus, complementary experiments measuring the concentrations of these mediators in the exudate will certainly throw light on this point. In addition, drugs like cromolyn, theophylline and salbutamol, which inhibit neurogenic inflammation such as that which occurs in asthma (Crescioli et al., 1991; Erjefalt and Persson, 1991; Becker and Bierman, 1994), also inhibited the cell influx induced by bradykinin.

It is interesting to mention that the increase in cell migration caused by bradykinin was also inhibited, in a dose-dependent manner, by both tachykinin NK_1- and NK_3-tested receptor antagonists. Indeed, although both tachykinin NK_1 and NK_3 receptor antagonists showed the same efficacy, the tachykinin NK_1 receptor antagonist was significantly more potent than the tachykinin NK_3 receptor antagonist. The activation of tachykinin NK_2 receptors following tachykinin release by bradykinin in this model seems to be less probable, since only a higher dose of the selective antagonist (SR 48968, 100 nmol/cav.) was able to inhibit bradykinin-induced cell migration. These results support the working hypothesis that, in our model, bradykinin is able to release tachykinins from sensory nerves as described in other models of inflammation (Geppetti, 1993; Ricciardolo et al., 1994), in addition to its action on other types of cells such as macrophages, leukocytes, endothelial and mast cells. From our results, it is also suggested that tachykinin-induced cell migration in this model occurs primarily via tachykinin NK_1 receptor activation. The findings that tachykinin NK_3 receptor antagonist (SR 142801) exerted an important inhibitory effect against bradykinin-induced pleurisy, and the apparent lack of inhibitory effect induced by the tachykinin NK_2 receptor antagonist tested (SR 48968), are not per se an indication that events mediated through the activation of these receptors have or have not a role in this model. Regarding the existence of animal species differences (Belvisi et al., 1994; Chung et al., 1995; Patacchini and Maggi, 1995) and possible intra-species heterogeneity of tachykinin receptors (Patacchini et al., 1991) associated with the fact that tachykinin NK_3 receptors have not been detected in sensory C-fibres (Belvisi et al., 1994; Canning and Undem, 1994; Bai et al., 1995), additional research is necessary to clarify these points.

In summary, the intra-pleural injection of bradykinin caused a dose-related and long-lasting inflammatory reaction in the pleural cavity of mice. This effect was primarily mediated via bradykinin B_2 receptor activation, since it was fully inhibited by both HOE 140 and NPC 17731. Our results also suggest that pleurisy induced by bradykinin is indirectly mediated by the release of several inflammatory mediators, i.e. histamine, nitric oxide and tachykinins, in addition to products derived from the arachidonic acid pathway. Taken together, these findings are relevant to the understanding of the contribution of kinins to the inflammatory process. Further studies, including biochemical assays, to validate these findings, and the extension of this model to other animal species, are necessary in order to ascertain the role of bradykinin in inflammatory reactions.

Acknowledgements

The authors wish to thank Fujisawa Pharmaceutical Co., Dr. X. Edmonds-Alt (Sanofi Recherche, France), Dr. K.J. Wirth (Hoechst AG, Germany) and Dr. Donald J. Kyle (Scios Nova Pharmaceutical Corporation, USA) for donations of FK 888, SR 48968, SR 142801, HOE 140 and NPC 17731, respectively. This study was supported by the Conselho Nacional de Pesquisa (CNPq) and Financiadora de Estudos e Projetos (FINEP).

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