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Oral anti-inflammatory action of NPC 18884, a novel bradykinin B_2 receptor antagonist

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Abstract

This study describes the anti-inflammatory actions of NPC 18884, a non-peptide bradykinin B_2 receptor antagonist in bradykinin and carrageenan-induced inflammation in the mouse model of pleurisy. The selectivity of NPC 18884 was assessed in the pleurisy caused by histamine, substance P and des-Arg^9-bradykinin. NPC 18884 given intraperitoneally or orally inhibited bradykinin-induced leukocytes influx (ID_{50} value of 63 nmol/kg and 141 nmol/kg, respectively). The NPC 18884 also inhibited the exudation induced by bradykinin ($P < 0.05$). NPC 18884 given either intraperitoneally or orally caused dose-dependent inhibition of the exudation and total and differential cell content caused by intrapleural injection of carrageenan (1%, assessed 4 h after), with mean ID_{50} values of 132 and 295 nmol/kg, respectively. The NPC 18884 actions installs rapidly (0.5 h), lasted for up to 4 h and were selective for the bradykinin B_2 receptors; at similar doses it had no significant effect against the inflammatory responses induced by des-Arg^9-bradykinin, histamine or substance P. These results indicate that the novel non-peptide bradykinin B_2 receptor antagonist, NPC 18884, exhibited selective intraperitoneal and oral anti-inflammatory properties when assessed in the inflammatory reaction induced by bradykinin and carrageenan in the mice model of pleurisy.

Keywords: NPC 18884; Anti-inflammatory; Bradykinin; Bradykinin B_2 receptor antagonist, non-peptide; Pleurisy model, mouse

1. Introduction

Kinins are endogenous peptides generated in plasma and peripheral tissues, following tissue trauma and infection from high- and low-molecular kininogens, by the action of kallikrein enzymes (Regoli and Barabé, 1980; Bhoola et al., 1992).

Kinins have multiple biological actions, including, among others increase in venular dilation and vascular permeability, and excitation of Aδ and C fibres from sensory neurones, causing pain and hyperalgesia. Apart from these effects, a great amount of evidence now suggests that kinins participate in many pathological processes, such as shock, allergy, asthma, some of the cardiovascular diseases, and in many inflammatory process (for review, see Regoli and Barabé, 1980; Proud and Kaplan, 1988; Dray and Perkins, 1993).

So far, both functional and biochemical studies have proposed two kinds of membrane kinin receptors, B_1 and B_2. Both kinin receptors have been cloned in many animal species and also in humans. They are members of seven transmembrane G-protein-coupled receptors (McEachern et al., 1991; Powell et al., 1993; Menke et al., 1994; Pesquero et al., 1996). The B_1 receptors are rarely expressed in non-traumatised tissues, but their expression is dramatically increased in traumatised tissue or after infection. The B_1 receptor presents higher affinity for the metabolites resulting from the action of carboxypeptides on kinins, des-Arg^9-bradykinin, and des-Arg^{10}-kallidin, than by bradykinin itself. On the other hand, B_2 receptors are normally constitutive and exhibit higher affinity for bradykinin than for des-Arg^9-bradykinin or des-Arg^{10}-kallidin, and are largely distributed in peripheral and central

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tissues. It has been demonstrated that B_2 receptors are responsible for most of the physiological actions of kinins (Regoli and Barabé, 1980; Farmer and Burch, 1992; Hall, 1992; Dray and Perkins, 1993; Marceau, 1995).

In spite of great interest in this field, the characterisation of kinin receptors and knowledge of most physiological and pathological roles played by kinin remained unclear until a few years ago, because potent specific and competitive antagonists were not available. After the development of the first bradykinin B_2 receptor antagonist (Stewart, 1995), a second generation of highly potent and selective peptide and pseudopeptide bradykinin B_2 receptor antagonists such as Hoe 140 (D-Arg-[Hyp^3,Thi^5,D-Tic^7,Oic^8]bradykinin) (Wirth et al., 1991), NPC 17731 (D-Arg^0-[Hyp^3,D-HypE(transpropyl)^{7}Oic^8]bradykinin), NPC 18688 (D-Arg^0-[1,3,8-triazapirol-[4.5]decan-4-one-3-acetic acids D-Tic^7,Oic^8]bradykinin) and NPC 18521 (D-Arg-Arg-[1,3-phenyl,8-triazaspiro[4,5]-decane-4-one-3-acetyl]-Ser-D-tetrahydroisoqui-nolinyl-octahydroindolinyl-Arg) (Kyle et al., 1991; Burch and Kyle, 1992; Corrêa and Calixto, 1993; Corrêa et al., 1996; De Campos et al., 1996) have been reported. More recently, some competitive non-peptide bradykinin B_2 receptor antagonists, such as Win 64338 [[4-[[2-[[bis(cyclohexylamino)methylene]amino]-3-(2-naphthnyl)-1-oxo-propyl]amino]-phenyl]-methyl]tributylphosphonium chroride, monohydrochloride) and FR 173657 ((E-3-(6-acetamido-3-pyridyl)-*N*-[*N*-[2,4-dichloro-3-[(2-methyl-8-quinolinyl)oxymethyl]-phenyl]-*N*-methylaminocarbonylmethyl]acrylamide), have been developed (Sawutz et al., 1994; Asano et al., 1997; Griesbacher and Legat, 1997; Griesbacher et al., 1997). While Win 64338 has been shown to competitively antagonise bradykinin action in vitro, FR 167344 (*N*-[*N*-[3-[(3-bromo-2-methylimidazol[1,2-a]pyridin-8-yl)oxymethyl]-2,4-dichlorophenyl]-*N*-methyl-aminocarbonylmethyl]-4-(dimethylamino-carbonyl) cinnamylamide hydrochloride), revealed in both in vitro and oral long-lasting bradykinin antagonistic properties (Asano et al., 1997; Griesbacher and Legat, 1997; Griesbacher et al., 1997; Sawutz et al., 1994).

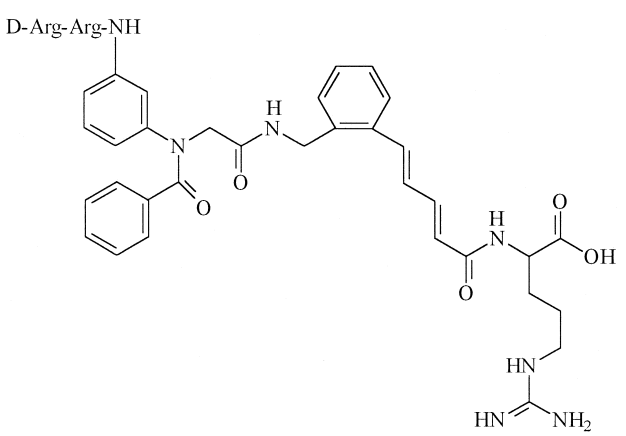

Fig. 1. Chemical structure of NPC 18884.

In the present study, we describe for the first time the systemic anti-inflammatory properties of a novel non-peptide bradykinin B_2 receptor antagonist, NPC 18884 (Fig. 1), against bradykinin and carrageenan-induced inflammation in the mouse model of pleurisy. We also assessed the selectivity of NPC 18884 by investigating its effects against the selective B_1 agonist des-Arg^9-bradykinin, histamine and substance P-induced inflammatory responses in the mouse model of pleurisy.

2. Materials and methods

2.1. Animals

Non-fasted adult Swiss mice of both sexes (18–25 g), aged 2 months, maintained in an environment of controlled temperature (21 ± 2°C), illuminated by daylight supplemented by electric light, from 06:00 to 18:00, with free access to food and water, were used. In experiments using bradykinin, animals were pretreated with captopril (5 mg/kg, i.p.) 1 h prior to any given experiment to prevent kinin degradation (Corrêa and Calixto, 1993). Throughout the experiments, the animals were managed using the principles and guidelines for the care of laboratory animals according to Zimmermann (1983).

2.2. Drugs and solutions

The following drugs and reagents were used: carrageenan λ grade IV, histamine (Sigma, St. Louis, MO, USA), des-Arg^9-bradykinin, bradykinin (Peninsula, Belmont Laboratories, CA, USA), NPC 18884 (synthesized at Scios Nova, Sunnyvale, CA, USA), heparin (Liquemine®, Roche, Brazil), Evans blue dye, Türk solution and May-Grunwald-Giemsa dye (Merck, Brazil). Phosphate-buffered saline (PBS: pH 7.6: composition mmol: NaCl 137, KCl 2.7) and phosphate buffer salts 10 were purchased from Sigma. The saline solution was previously prepared and stocked in the refrigerator. 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.

2.3. Induction of pleurisy and measurement of the parameters studied

On the day of the experiments, animals were lightly anaesthetised with ether, and bradykinin, des-Arg^9-bradykinin, carrageenan, histamine, substance P or sterile saline solution were 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 opening the thorax, the pleural cavity

was washed with 1 ml of PBS plus heparin (20 IU per ml) and the volume collected with automatic pipettes. All animals were previously injected (24 h) with a solution of Evans blue dye (25 mg/kg, 0.2 ml, i.v.) in order to evaluate further the degree of exudation in the pleural space (Saleh et al., 1996, 1997). The total leukocyte counts per pleural cavity were performed in a Neubauer chamber by means of optical microscopy after diluting a sample of the pleural fluid with Türk solution (1:20). Cellular smears were stained with May-Grunwald-Giemsa for differential analysis performed under immersion objective. A sample of the collected fluid (500 μl) from the pleural space was separated and stocked in the freezer (−20°C) to further determine the concentration of Evans blue dye. On the day of the experiments, a batch of samples was thawed at room temperature, and the amount of dye was then 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 to 50 μg/ml.

2.4. Experimental procedure

Other animals were pretreated (0.5 h) with NPC 18884, the studied parameters being analysed after 4 h of pleurisy induction. Thus, in a first set of experiments, before triggering pleurisy by bradykinin (10 nmol per pleural cavity), different groups of animals received NPC 18884 0.5 h prior given by intraperitoneal (i.p.) or oral (p.o.) routes. In another series of experiments, animals received distinct doses of the antagonist (10–100 nmol/kg, i.p.) or (100–600 nmol/kg, p.o.) before pleurisy induction by bradykinin (10 nmol per pleural cavity). The reason of employing the dose of this agonist was based on our previous work (Saleh et al., 1997).

The temporal profile of inhibition caused by NPC 18884 when administered by either i.p. or p.o. routes were also indirectly evaluated by pretreating the animals, at different periods of time (0.5–4 h), before pleurisy induction with bradykinin (10 nmol per pleural cavity).

To evaluate the effect of antagonist in the pleurisy induced by carrageenan, separate groups of animals were pretreatead (0.5 h) with several doses of NPC 18884 administered by i.p. (1–300 nmol/kg) or p.o. (100–1200 nmol/kg) routes, and the indices of inflammation (total and differential cell content and exudation) were analysed 4 h after. In other experiments, the temporal profile of the inhibitory effect induced by this inflammatory agent (carrageenan 1%) was determined. Animals were pretreated at different periods of time (0.5 to 8 h), but each dose employed was the same as that used in the previous experiments, and the same parameters were analysed 4 h after pleurisy induction.

We further evaluated the effect of the NPC 18884 in the mouse pleurisy induced by the selective B_1 receptor agonist des-Arg^9-bradykinin (30 nmol per pleural cavity). The dose of des-Arg^9-bradykinin was chosen based on our previous work (Vianna and Calixto, 1998). The inflammatory response induced by des-Arg^9-bradykinin in the pleural space of the mice was characterised by an increase in exudation observed after 5 min, peaking at 1 h. This effect was associated with an increase in total cells which peaked at 4 h and lasted for up to 48 h (Vianna and Calixto, 1998). Thus, these periods of time (1 and 4 h) were chosen in order to analyse the effect of NPC 18884 which was administered 0.5 h prior by different routes (100 nmol/kg, i.p. or 600 nmol/kg, p.o.).

Finally, NPC 18884 was tested against the inflammatory response induced by other mediators. In separate series of experiments, animals were pretreated (0.5 h) with NPC 18884 (1200 nmol/kg) administered by oral route, and the pleurisy was induced by histamine (0.9 nmol per pleural cavity) or substance P (20 nmol per pleural cavity). The inflammatory process was analysed 1 and 4 h after injection of histamine and substance P, respectively.

Each experimental group included control animals, which had received the same intrapleural volume of sterile saline and were killed at the same time as their matched-treated group. Control animals, treated with the vehicle used to dilute the agonist or the tested antagonist, were also evaluated.

2.5. Statistical analysis

Data is reported as mean ± S.E.M., except for the ID_{50} values in individual experiments (i.e., doses of antagonist that reduced response by 50% in relation to control value) which are presented as geometric means, accompanied by their respective 95% confidence limits (CL). The ID_{50} values were determined by means of regression analysis from individual experiments. 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

3.1. Effect of NPC 18884 on bradykinin and carrageenan-induced pleurisy in mice

As reported previously (Saleh et al., 1997), intrapleural injection of bradykinin (10 nmol per pleural cavity) caused a weak increase of fluid leakage measured 4 h after the peptide injection (0.64 ± 0.07 μg/ml). The treatment of animals with NPC 18884 (100 nmol/kg, i.p.) 0.5 to 2 h prior, inhibited significantly (42 ± 1,8 and 30 ± 1,6%) bradykinin-induced exudation, respectively. When different doses of NPC 18884 (30 to 100 nmol/kg, i.p.) was administrated 0.5 h prior, it produced a significant inhibition of bradykinin-induced Evans blue extravasation

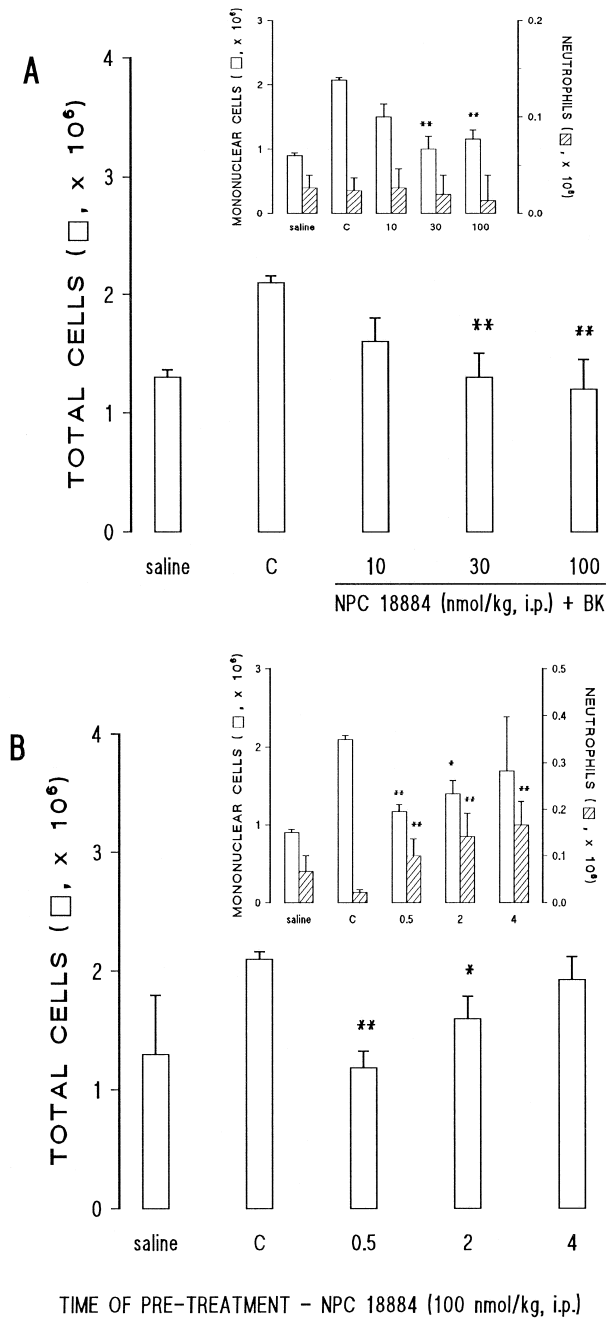

Fig. 2. Effect of NPC 18884 on the mouse pleurisy induced by bradykinin (10 nmol per pleural cavity, 4 h). (A) Effect of different doses (10–100 nmol/kg, i.p.) administered 0.5 h prior to pleurisy induction. Control responses (C) in animals treated previously with saline, i.p. and challenged with bradykinin. Asterisks indicate the statistically-significant differences of total cell content in comparison to control group. The inset shows the drug effects on both mononuclear and neutrophil cells. (B) Effect of NPC 18884 (100 nmol/kg, i.p.), administered 0.5 to 4 h prior to pleurisy induction, under the same experimental conditions. Asterisks indicate the statistically-significant differences of total cell content in comparison to control group. The inset shows the drug effects on both mononuclear and neutrophil cells. Each group represents the mean of 6 to 10 animals, and the vertical bars the S.E.M. $*\ P < 0.05$ and $**\ P < 0.01$ when compared with respective control values.

(30 nmol/kg: 0.30 ± 0.7 μg/ml and 100 nmol/kg 0.37 ± 0.5 μg/ml, when compared with animals treated previously with saline, i.p. and challenged with bradykinin (10 nmol/cavity) (0.64 ± 0.07 μg/ml, $n = 6$ to 10 animals in each group) (results not shown). The treatment of animals with NPC 18884 (600 nmol/kg, p.o.) 0.5 and 2 h prior also produced significant inhibition of bradykinin-induced fluid leakage (47 ± 1.2 and 35 ± 2.1%, respectively). NPC 18884 (300 and 600 nmol/kg, given p.o. 0.5 h prior) was also effective in preventing significantly bradykinin-induced fluid extravasation (percent of inhibition of 38 ± 1.2 and 47 ± 1.5%, respectively; $n = 6$ to 10 animals per group).

Fig. 2A shows the effect of intraperitoneal injection of NPC 18884 on the pleurisy induced by bradykinin (10 nmol per pleural cavity, 4 h). As shown, NPC 18884 caused a graded inhibition on cell migration ($P < 0.01$) when it was administered 0.5 h before (30–100 nmol/kg, i.p.). The estimated mean ID_{50} value (and 95% confidence limits) for this effect was 63 (3–115) nmol/kg (Table 1).

As shown in Fig. 2B, the inhibitory effect caused by NPC 18884 (100 nmol/kg) in cell migration induced by bradykinin (10 nmol per pleural cavity, 4 h) was still observed when the antagonist was administered up to 2 h before pleurisy induction. At the 0.5 and 2 h periods of time, the total cell content was significantly reduced in comparison to that obtained in animals treated previously with saline, i.p. and challenged with bradykinin. On the other hand, the differential cell count revealed an enhancement of neutrophil for the same period of pretreatment, with NPC 18884 (Fig. 2B, inset).

The NPC 18884 (300–600 nmol/kg) administered by oral route 0.5 h prior, produced graded inhibition of cell influx in response to intrapleural injection of bradykinin (10 nmol per pleural cavity, 4 h) (Fig. 3A). The estimated mean ID_{50} value for this antagonist was 141 (26–760) nmol/kg (Table 1). The NPC 18884 at the dose 600 nmol/kg administered by oral route 0.5–2 h before was effective in inhibiting the cell influx induced by bradykinin (10 nmol/cavity, 4 h). Thus, the pretreatment at 0.5 h with

Table 1
The estimated mean ID_{50} values of cell migration of NPC 1884 against bradykinin and carrageenan-induced pleurisy in mice

Agent	Route	Doses (nmol/kg)	ID_{50} values (nmol/kg)
Bradykinin	i.p.	10–100	63 (3–115)
Bradykinin	p.o.	100–600	141 (26–760)
Carrageenan	i.p.	1–300	132 (63–278)
Carrageenan	p.o.	100–1200	295 (117–730)

The parameters were analysed 4 h after the administration of either bradykinin, carrageenan or sterile saline solution in the pleural cavity. i.p. = treatment given by intraperitoneal route 0.5 h before; p.o. = treatment given by oral route 0.5 h before. Results are expressed as geometric means accompanied by their respective 95% confidence limits. Each group represents the mean of 6 to 10 animals.

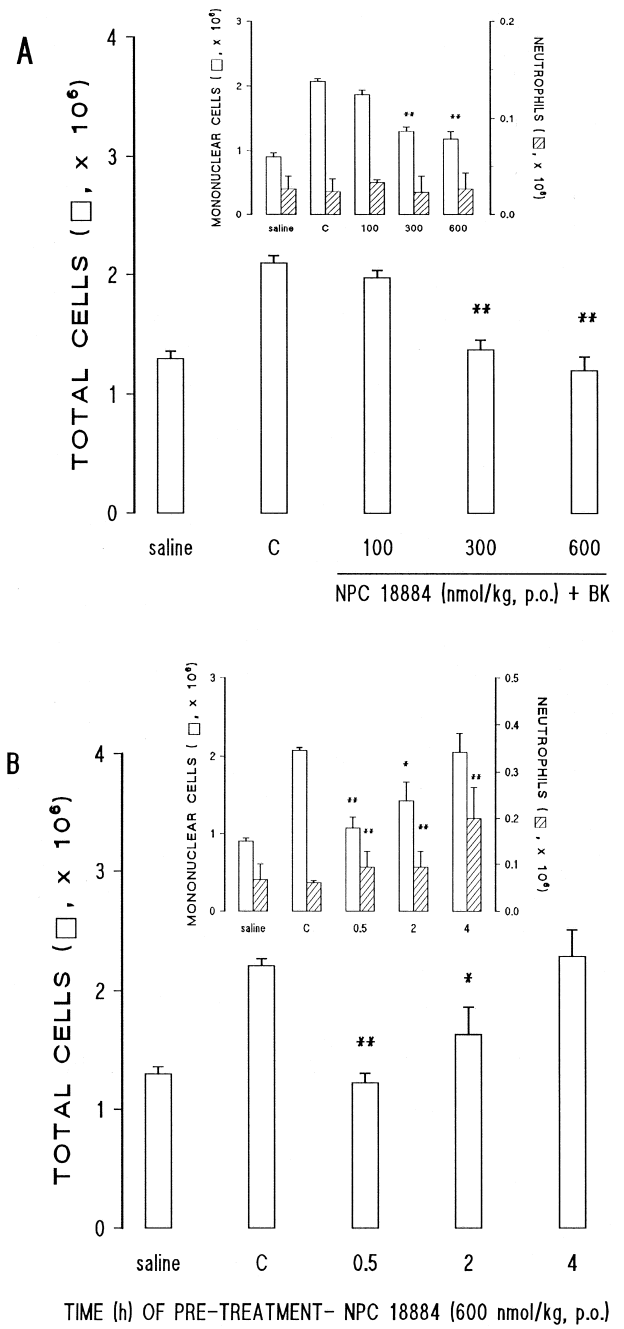

Fig. 3. Effect of NPC 18884 on the mouse pleurisy induced by bradykinin (10 nmol per pleural cavity, 4 h). (A) Effect of different doses (100 and 600 nmol/kg, p.o.) administered 0.5 h prior to pleurisy induction. Control responses (C) in animals treated previously with saline, p.o. and challenged with bradykinin. Asterisks indicate the statistically-significant differences of total cell content in comparison to control group. The inset shows the drug effects on both mononuclear and neutrophil cells. (B) Effect of NPC 18884 (600 nmol/kg, p.o.), administered 0.5 to 4 h prior to pleurisy induction, under the same experimental conditions. Asterisks indicate the statistically-significant differences of total cell content in comparison to control group. The inset shows the drug effects on both mononuclear and neutrophil cells. Each group represents the mean of 6 to 10 animals, and the vertical bars the S.E.M. $*P < 0.05$ and $**P < 0.01$ when compared with respective control values.

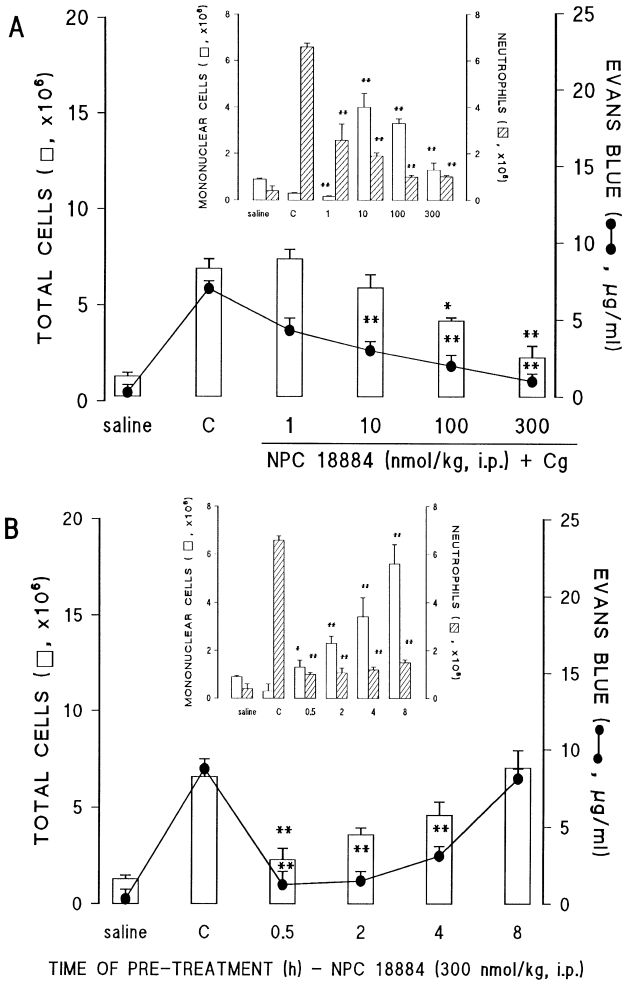

Fig. 4. Effect of NPC 18884 on the mouse pleurisy induced by carrageenan (1%) (4 h). A) Effect of different doses (1–300 nmol/kg, i.p.) administered 0.5 h prior to pleurisy induction. Control responses (C) in animals treated previously with saline, i.p. and challenged with carrageenan. Asterisks outside and inside the boxes indicate the statistically-significant differences of both total cell content and exudate levels in comparison to control group. The inset shows the drug effects on both mononuclear and neutrophil cells. (B) Effect of NPC 18884 (300 nmol/kg, i.p.), administered 0.5 to 8 h prior to pleurisy induction, under the same experimental conditions. Asterisks next to the experimental values indicate the statistically-significant differences of both total cell content and exudate levels in comparison to control group. The inset shows the drug effects on both mononuclear and neutrophil cells. Each column and closed circle represents the mean of 6 to 10 animals, and the vertical bars the S.E.M. $*P < 0.05$ and $**P < 0.01$ when compared with respective control values.

this antagonist gave a total leukocyte number (mean ± S.E.M.) which was within or almost near to normal values (0.5 h: $[1.3 \pm 0.02] \times 10^6$ cells) which did not differ from values obtained in animals treated only with saline ($[1.3 \pm 0.08] \times 10^6$ cells) (Fig. 3B). It is interesting to note that the differential cell count revealed an significant increase in neutrophils for the same period of pretreatment (Fig. 3B, inset).

Fig. 4A shows the effect of NPC 18884 (1–300 nmol/kg, i.p.) on the first phase of the inflammatory

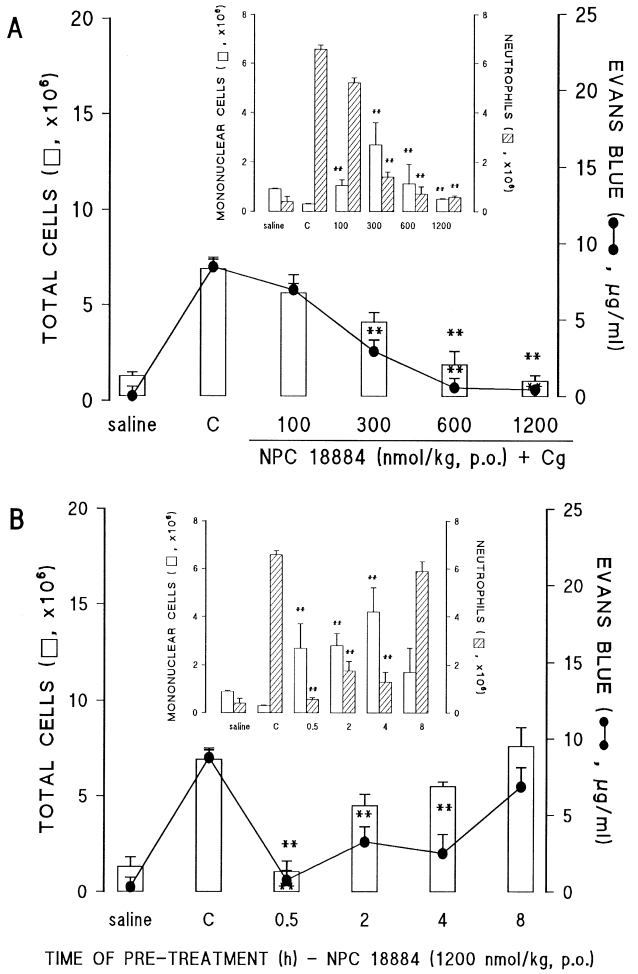

Fig. 5. Effect of NPC 18884 on the first phase (4 h) of mouse pleurisy induced by carrageenan (1%). (A) Effect of different doses (100–1200 nmol/kg, p.o.) administered 0.5 h prior to pleurisy induction. Control responses (C) in animals treated previously with saline, p.o. and challenged with carrageenan. Asterisks outside and inside the boxes indicate the statistically-significant differences of both total cell content and exudate levels in comparison to control group. The inset shows the drug effects on both mononuclear and neutrophil cells. (B) Effect of NPC 18884 (1200 nmol/kg, p.o.), administered 0.5 to 8 h prior to pleurisy induction, under the same experimental conditions. Asterisks next to the experimental values indicate the statistically-significant differences of both total cell content and exudate levels in comparison to control group. The inset shows the drug effects on both mononuclear and neutrophil cells. Each column and closed circle represents the mean of 6 to 10 animals, and the vertical bars the S.E.M. $**P < 0.01$ when compared with respective control values.

reaction (4 h) induced by carrageenan. Doses between 100 and 300 nmol/kg, i.p., significantly inhibited the total cell migration, which was primarily composed of neutrophils ($P < 0.05$). This effect was dose-dependent, with a mean ID_{50} value of 132 (63–278) nmol/kg (Table 1). In addition, NPC 18884, at doses between 10–300 nmol/kg, given i.p., caused a significant reduction of exudation ($P < 0.01$). Pretreatment of animals (0.5–4 h) with NPC 18884 (300 nmol/kg, i.p.) revealed that this drug caused an inhibitory effect on fluid leakage in the first phase of the inflammatory reaction induced by carrageenan (Fig. 4B). In addition, the cell migration was reduced only when animals were treated at 0.5 h before pleurisy induction.

The effect of NPC 18884, when administered 0.5 h before by oral route (100–1200 nmol/kg), on mouse pleurisy induced by carrageenan, is shown in Fig. 5A. Both cell influx and exudation were significantly inhibited at the doses between 600 and 1200 nmol/kg, p.o. ($P < 0.01$) (Fig. 5A and Table 1). The estimated mean ID_{50} value for this effect was 295 (117–730) nmol/kg. At 300 nmol/kg, given p.o., NPC 18884 was effective in inhibiting the exudation ($P < 0.01$). The NPC 18884 (1200 nmol/kg, p.o.), given 0.5–4 h (but not 8 h) before carrageenan, caused a significant reduction of exudation (Fig. 5B and Table 1). Under this condition, cell migration was abolished only in animals treated with the same dose of this bradykinin B_2 receptor antagonist 0.5 h prior to carrageenan administration ($P < 0.01$) (Fig. 5B).

3.2. Effect of NPC 18884 on des-Arg^9-bradykinin, histamine and substance P-induced pleurisy in mice

To evaluate the selectivity of NPC 18884 towards the bradykinin B_2 receptor, another groups of animals were pretreated with NPC 18884 (100–600 nmol/kg, p.o.) 0.5 h before pleurisy induction by des-Arg^9-bradykinin

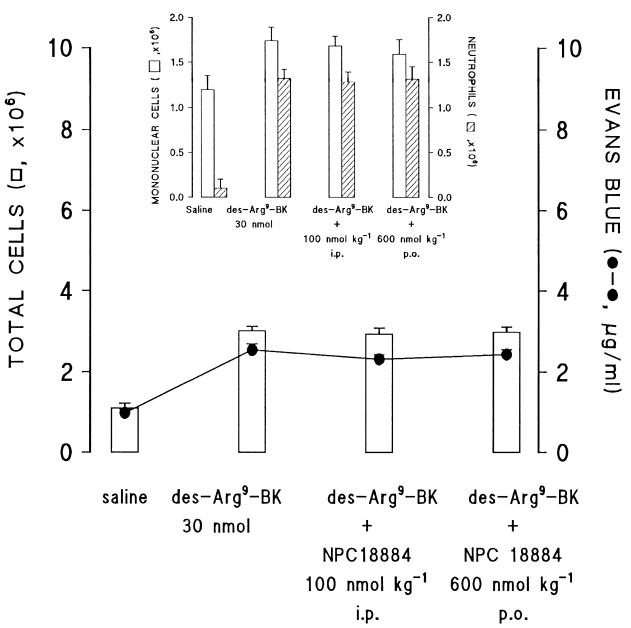

Fig. 6. Effect of NPC 18884 on the mouse pleurisy induced by des-Arg^9-bradykinin (30 nmol per pleural cavity). The exudation was analysed 1 h after and the total cell content 4 h after injection of agonist. Effect of NPC 18884 administered by different routes (100 nmol/kg, i.p. and 600 nmol/kg, p.o.) 0.5 h prior to pleurisy induction. Control responses (C) in animals treated previously with saline, p.o. and challenged with des-Arg^9-bradykinin. The inset shows the drug effects on both mononuclear and neutrophil cells. Each column and closed circle represents the mean of 6 to 10 animals, and the vertical bars the S.E.M.

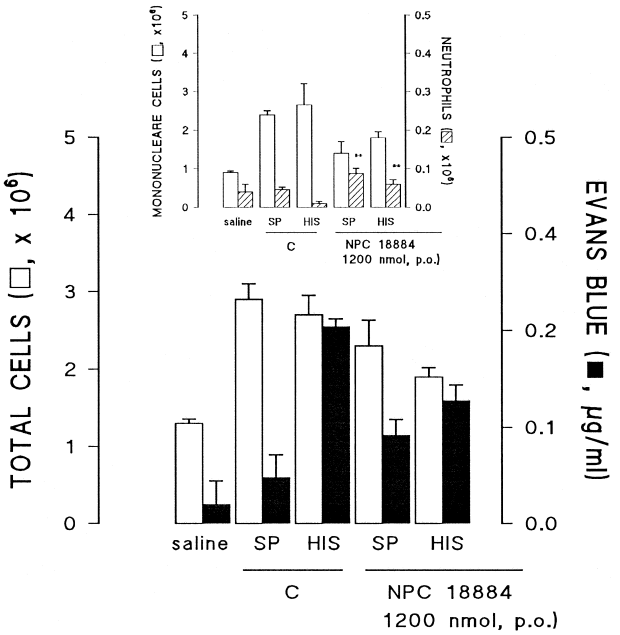

Fig. 7. Effect of NPC 18884 administered by oral route (1200 nmol/kg) on the mouse pleurisy induced by histamine (0.9 nmol per pleural cavity) or substance P (20 nmol per pleural cavity). Control responses (C) in animals treated with saline, p.o. and challenged with histamine or substance P. The inset shows the drug effects on both mononuclear and neutrophil cells. Each column and closed circle represents the mean of 6 to 10 animals, and the vertical bars the S.E.M.

(30 nmol per pleural cavity), histamine (0.9 nmol per pleural cavity) or substance P (20 nmol per pleural cavity).

Intrapleural injection of substance P and histamine produced a weak fluid leakage (0.52 ± 0.03 μg/ml and 2.5 ± 0.1 μg/ml, $n = 6$ to 10 animals per group, respectively). NPC 18884 even at higher dose (1200 nmol/kg, p.o., 0.5 h prior) had no significant effect on pleurisy induced by des-Arg^9-bradykinin (Fig. 6), histamine or substance P-induced exudation (Fig. 7).

Administered alone by different routes NPC 1884 (10 to 100 nmol/kg, i.p, or 100 to 1200 nmol/kg, p.o) had any detectable agonistic activity in relation of either exudation or cell migration (results not shown).

4. Discussion

There is considerable evidence to support the involvement of kinins, acting through either B_1 or B_2 receptors, in the modulation of inflammatory and pain responses (see for review: Hall, 1992; Marceau, 1995; Hall and Morton, 1997). These findings led us to examine, in the present study, whether or not the recently-developed non-peptide bradykinin B_2 receptor antagonist NPC 18884 (Chakravarty et al., 1996) might antagonise bradykinin and carrageenan-induced acute inflammatory responses in a murine model of pleurisy (Saleh et al., 1996, 1997). Attempts have also been made to assess the selectivity of NPC 18884 towards the bradykinin B_2 receptor-mediated responses.

Recently Chakravarty et al. (1996) reported that the novel non-peptide bradykinin B_2 receptor antagonist NPC 18884 inhibited [^{3}H]bradykinin binding ($K_d = 80 \pm 26$ nmol) in CHO cell lines that express the human B_2 receptor. However, the pharmacological properties of this compound seem to vary according to the animal species used. Thus, NPC 18884 failed to block either bradykinin-induced hypotension in rabbit or contraction in guinea pig ileum in vitro (Chakravarty et al., 1996). Results of the present study extend these previous observations described by Chakravarty et al. (1996) and show that both intraperitoneal and oral administration of NPC 18884 to mice did not result in any detectable residual agonistic activity, but causing instead a dose and time-dependent inhibition of bradykinin-induced acute inflammatory response in the mouse model of pleurisy. The anti-inflammatory action of NPC 18884 was rapidly effective (0.5 h) and lasted for up to 2 h when it was given by i.p. or p.o. routes. However, when compared with the selective and peptide bradykinin B_2 receptor antagonists, Hoe 140 or NPC 17731 (Saleh et al., 1997), NPC 18884 was less potent in antagonising bradykinin-induced pleurisy in mice, depending on the route of administration used. When compared with the available non-peptide bradykinin B_2 receptor antagonist FR 167344, NPC 18884 was about 8-fold more potent (mg/kg) in inhibiting carrageenan-induced inflammation. Furthermore, the duration of the anti-inflammatory properties of NPC 18884 (about 2 h) was lower than FR 167344 (about 4 h) (Asano et al., 1997). In addition, the anti-inflammatory action of NPC 18884 was quite selective towards bradykinin effect through B_2 receptors, evident by the fact that at the same dose where this compound significantly antagonised bradykinin-mediated inflammatory response, it had no significant effect on pleurisy induced by intrapleural injection of the selective B_1 receptor agonist, des-Arg^9-bradykinin histamine or SP. We have recently reported that intrapleural injection of des-Arg^9-bradykinin into mice resulted in a dose- and time-related inflammatory response, characterised by cell migration, mainly neutrophils and exudation, peaking at 4 and 1 h, respectively (Vianna and Calixto, 1998). Molecular and pharmacological studies have now demonstrated that B_1 receptors are constitutively present in mice (Mass et al., 1995; Pesquero et al., 1996; Hess et al., 1996). As des-Arg^9-bradykinin-mediated pleurisy in mice was selectively inhibited by B_1, but not by the selective B_2 receptor antagonists, we suggest that the inflammatory responses induced by des-Arg^9-bradykinin might be mediated by activation of constitutive B_1 receptors (Vianna and Calixto, 1998).

There are several reports in the literature demonstrating that kinins play an important modulatory role in the carrageenan-induced acute inflammatory responses (Costello and Hargreaves, 1989; Burch and Dehaas, 1990; Damas et al., 1990; Wirth et al., 1991; De Campos et al., 1996). Results of the current study confirm and extend such

previous data and show that the non-peptide bradykinin B_2 receptor antagonist NPC 18884, administered either intraperitoneally or by oral route, dose-dependently antagonise both exudation and cell migration in response to intrapleural injection of carrageenan. As reported for bradykinin-induced pleurisy, the antagonistic action of NPC 18884 against carrageenan-induced inflammation occurred rapidly (0.5 h, i.p. and p.o.) and lasted for up to 4 h (fluid leakage). Interestingly, although NPC 18884 was found to be less potent when it was given orally, its exhibits very similar efficacy in preventing either cell migration or exudation in response to intrapleural injection of carrageenan.

In summary, the novel non-peptide bradykinin B_2 receptor antagonist NPC 18884 revealed a rapid onset systemic anti-inflammatory properties when administered by intraperitoneal or oral routes and assessed in bradykinin and carrageenan-mediated inflammatory responses in the murine model of pleurisy. The anti-inflammatory property of NPC 18884 was found to be quite selective against kinin B_2 receptors, as it has no significant effect against des-Arg^9-bradykinin, histamine or substance P-induced pleurisy. Thus, the novel non-peptide B_2 kinin receptor antagonist NPC 18884 may be important as a pharmacological tool for investigating the role of kinins acting through B_2 receptors in physiological and pathological processes.

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