Anti-Inflammatory and Antinociceptive Properties of BP 2-94, a Histamine H₃-Receptor Agonist Prodrug

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Accepted for publication May 11, 2000 This paper is available online at http://www.jpet.org

ABSTRACT

BP 2-94 is an azomethine prodrug of (R)-α-methylhistamine ([R]-α-MeHA), a potent and selective histamine H₃-receptor agonist. When administered orally to mice BP 2-94 was distributed to various peripheral tissues where it released the active drug. BP 2-94 displayed anti-inflammatory and antinociceptive properties in mice. It dose-dependently inhibited carrageenan-induced paw edema with an ED₅₀ value of 0.17 ± 0.05 μmol/kg (p.o.) and a maximal effect of 47%. It also reduced Freund’s complete adjuvant-induced paw edema in preventive as well as in curative fashion. Repeated oral administrations of BP 2-94 reduced the pre-established Freund’s complete adjuvant-induced edema with an ED₅₀ value of 5 ± 2 μmol/kg (p.o.) and a maximal effect of 47%. The antiedema effects of BP 2-94 and indomethacin were additive. BP 2-94 was also efficient in reducing cyclophosphamide-induced cystitis in mice: it decreased leukocyte infiltration by 62% and plasma protein extravasation by 73% in urinary bladder. In addition, BP 2-94 displayed antinociceptive activity in the capsaicin-induced licking test via H₃-receptor stimulation. Its antinociceptive effect was dose dependent, occurring with an ED₅₀ value of 0.4 ± 0.1 μmol/kg (p.o.) and a maximal reduction of licking duration by 69%. No tolerance to the antinociceptive effect was observed after repeated administration of BP 2-94 for 3 days. These observations with BP 2-94 suggest that H₃-receptor agonists might represent a novel class of anti-inflammatory and antinociceptive agents.

Histamine (HA) mediates its action via three distinct molecularly and/or pharmacologically well-defined receptor subtypes H₁, H₂, and H₃ (for review, see Schwartz et al., 1991, 1995; Hill et al., 1997). The H₃ receptor has been characterized in brain as a widely distributed presynaptic autoreceptor inhibiting the synthesis and/or release of HA itself (Arrang et al., 1983) as well as of neurotransmitters in adrenergic, cholinergic, nonadrenergic noncholinergic, dopaminergic, and serotoninergic fibers (for review, see Schlicker et al., 1994). The H₃ receptor also plays an inhibition modulatory role in peripheral neurotransmission. Its stimulation inhibits vagal cholinergic transmission in the ileum (Trzeciakowski, 1987; Hew et al., 1990) and the airways (for review, see Barnes, 1992) and reduces plasma protein extravasation induced by sensory C fibers stimulated either electrically or by capsaicin (for review, see Leurs et al., 1998). In addition, H₃ receptor-mediated inhibitions of gastric acid secretion induced either by gastrin or vagal stimulation (Bertaccini and Coruzzi, 1995; Soldani et al., 1996), inhibitions of gastric mucosal injury induced by nonsteroidal anti-inflammatory drugs, cold/restraint stress or ethanol (Belcheva et al., 1997, Morini et al., 1996, 1997), and indirect inhibition of mast cell activity (Dimitriadou et al., 1994, 1997) were reported.

Recently the prototypical agent (R)-α-methylhistamine ([R]-α-MeHA), a selective and potent H₃-receptor agonist (Arrang et al., 1987), was shown to be extensively methylated by histamine N-methyltransferase, rapidly leading to an inactive metabolite (Rouleau et al., 1997). This inactivation process is particularly important in human in which higher hepatic histamine-N-methyltransferase activity was detected compared with rat (Brown et al., 1959; Hesterberg et al., 1984). The design of BP 2-94, an azomethine prodrug of (R)-α-MeHA (Krause et al., 1995), allowed to minimize the methylation of the imidazole ring of the agonist and thereby markedly improve its oral bioavailability and kinetics in human (Rouleau et al., 1997).

In rodents BP 2-94 was shown to inhibit plasma protein extravasation induced by capsaicin in a large variety of tissues, to display antinociceptive effects in the phenylbenzoquinone-induced writhing or formalin tests, and to reduce zymosan-induced paw swelling (Rouleau et al., 1997). In addition BP 2-94 reduced gastric mucosal lesions induced by ethanol, aspirin, indomethacin, or stress (Morini et al., 1996, 1997; Belcheva et al., 1997). The major aim of the present work was to further explore the anti-inflammatory and an-

Received for publication January 25, 2000.

ABBREVIATIONS: HA, histamine; (R)-α-MeHA, (R)-α-methylhistamine; FCA, Freund’s complete adjuvant; HTAB, hexadecyltrimethylammonium bromide; MPO, myeloperoxidase; NK, neurokinin.
tinociceptive profile of the $\text{H}_3$-receptor agonist by using a larger variety of tests in mice.

Materials and Methods

Animals. Male Swiss mice (25–30 g; Iffa-Credo, L’Arbresle, France) were used for all experiments. Food and water were given ad libitum.

Distribution of BP 2-94 and (R)-a-MeHA in Mouse Tissues after Oral Administration of BP 2-94. Mice received BP 2-94 (24 $\mu$mol/kg) or its vehicle orally. They were sacrificed by decapitation at various times, blood was collected, centrifuged (15,000g for 1 min), and the supernatant was brought up to a final concentration of 0.4 N HClO$_4$. The supernatant was used for assay immediately or stored at −20°C. BP 2-94 and (R)-a-MeHA levels were measured by radioimmunoassay as described (Rouleau et al., 1997). Briefly, before use one aliquot of the HClO$_4$ extracts was heated at 95°C for 30 min to allow total in vitro hydrolysis of the prodrug, and another one was used without heating. (R)-a-MeHA was derivatized and then radioimmunoassayed in the nonheated and heated extracts. The level of BP 2-94 was calculated as the difference between these two determinations. The plasma and tissues of nontreated mice also were assayed to estimate the interference of plasma and tissues in the RIA for (R)-a-MeHA. The determinations of (R)-a-MeHA for treated mice were then corrected accordingly.

Freund’s Complete Adjuvant (FCA)-Induced Paw Edema. Inflammation of one hind paw of mice was induced by intraplantar injection under ether anesthesia of 10 $\mu$l of modified FCA, containing 0.1% heat-sacrificed and dried Mycobacterium butyricum in 85% Drakel 5 NF and 15% Arlacel A (Stein et al., 1988). Control animals were anesthetized but not injected. Mice were sacrificed at various times after the injection of the inflammatory agent, both hind paws were cut off at the ankle and the difference between their weights, representing paw swelling, was calculated. Each hind paw was then put into 4 ml of 50 mM potassium phosphate buffer, pH 6.0, containing 0.5% hexadecyl-trimethylammonium bromide (HTAB), and homogenized with a Polytron. Homogenates were stored at −20°C until myeloperoxidase (MPO) activity assay. The preventive effect of an acute administration of BP 2-94 was first studied. BP 2-94 (16.4 $\mu$mol/kg) or its vehicle was orally administered 1 h before FCA injection, and the time course of its anti-inflammatory effect was determined during the next 16 h. The effect of BP 2-94 (16.4 $\mu$mol/kg) also was studied in the presence of indomethacin administered orally at increasing dosages (0.6–28 $\mu$mol/kg) (Chan et al., 1995). Next, the curative effect of repeated administrations of BP 2-94 was evaluated. Mice were injected with FCA, and 16 to 18 h thereafter, they received orally BP 2-94 or its vehicle b.i.d. during a period varying from 1 to 9 days. The effect of 3-day repeated administrations of BP 2-94 (16.4 $\mu$mol/kg p.o., b.i.d.) was then compared with the effect of 3-day repeated administrations of indomethacin (8.4 $\mu$mol/kg p.o., b.i.d.), dexamethasone (7.6 $\mu$mol/kg p.o., b.i.d.) (Gegout et al., 1995), or RP67580 (0.7 $\mu$mol/kg i.v., b.i.d.), a neurokinin (NK$_1$)-receptor antagonist (Garret et al., 1991). The effect of BP 2-94 (3-day repeated administrations) in association with indomethacin in increasing doses (0.8–28 $\mu$mol/kg p.o., b.i.d.) also was studied (Gans et al., 1990).

MPO Activity. Changes in MPO activity represent a reliable index of polymorphonuclear leukocyte infiltration in the inflamed paw (Bradley et al., 1982). Therefore, paw homogenates were freeze thawed three times and centrifuged to collect the supernatant that was used for MPO activity assay adapted to a 96-well plate format (Rao et al., 1994). Briefly, 10 $\mu$l of unknowns or human neutrophil MPO standards were added to a 96-well plate. The reaction was initiated by the addition of 200 $\mu$l of assay buffer containing 0.187 mg/ml o-dianisidine and 0.0005% hydrogen peroxide and absorption measured at 405 nm (Spectrophotometer Dynatech, MR5000). Results are expressed as the difference in MPO activity between the two hind paws.

Carrageenan-Induced Paw Edema. Paw swelling was elicited with 25 $\mu$l of 0.5% lambda carrageenan suspension in saline into the right hind paw (Drelon et al., 1994). The left hind paw was injected with 25 $\mu$l of saline. BP 2-94 at increasing doses and indomethacin (28 $\mu$mol/kg) were administered 1 h before the injection of the carrageenan suspension. Paw edema and MPO activity were measured as described above, 6 h after the induction of the inflammation.

Cyclophosphamide-Induced Cystitis. At various times after cyclophosphamide injection, plasma protein extravasation and MPO activity were evaluated. For that, one group of mice was anesthetized with pentobarbital (10 mg/kg i.p.), injected with Evans blue dye (30 mg/kg i.v.), and sacrificed 30 min thereafter. The urinary bladder was dissected out, immersed into 0.4 ml of formamide, and maintained at 45°C for 18 h. The extracted dye concentration was measured by spectrophotometry at 630 nm (Dynex, MRX). Another group of mice was sacrificed and the urinary bladder was dissected out and homogenized in 0.4 ml of phosphate buffer (50 mM, pH 6) containing 0.5% HTAB and the resulting homogenates were frozen until MPO assay. Changes in MPO activity also were evaluated 5 h after cyclophosphamide was administered in increasing doses. The H$_3$-agonist prodrg BP 2-94 (33 $\mu$mol/kg p.o.) or its vehicle was administered to mice 1 h before cyclophosphamide and blue Evans content and MPO activity were evaluated. For that, one group of mice was anesthetized with pentobarbital (10 mg/kg i.p.), injected with Evans blue dye (30 mg/kg i.v.), and sacrificed 30 min thereafter. The urinary bladder was dissected out, immersed into 0.4 ml of formamide, and maintained at 45°C for 18 h. The extracted dye concentration was measured by spectrophotometry at 630 nm (Dynex, MRX).

Capsaicin-Induced Licking. The capsaicin test was performed according to Sakurada et al. (1992). BP 2-94 or vehicle was given orally 1 h before capsaicin injection. After 30 min, mice were placed individually in transparent cages (26 × 16 × 14.5 cm) that served as the observation chambers. After a 30-min adaptation period, mice received a 20 $\mu$l-injection of capsaicin (1.5 $\mu$g in saline with 7.5% dimethyl sulfoxide) under the skin of the dorsal surface of the right hind paw. When required, ciproxifan (37 $\mu$mol/kg) (Ligneau et al., 1998) was orally administered 1 h before BP 2-94 (1 $\mu$mol/kg). The observation period started immediately after the capsaicin injection and lasted for 5 min. The time the animals spent licking the injected paw was evaluated by using a stopwatch.

Analysis of Data. For the determination of $ED_{50}$ (dose responsible for 50% of the maximal effect) and maximal effect, inhibitory effects of drugs were analyzed by using an iterative computer least-squares method derived from that of Parker and Waud (1971), with the following nonlinear regression:

$$\text{Inhibitory effect of the drug} = \frac{\text{[maximal inhibitory effect of the drug]} \times \text{[drug dose]} - \text{[drug dose]} + \text{$ED_{50}$}}{\text{[$\text{drug dose}$]} + \text{$ED_{50}$}}$$

Statistical analyses were by one-way ANOVA followed by Student-Newman-Keuls or Tukey-Kramer’s post test.

Drugs and Drug Solutions. BP 2-94 and ciproxifan were from Laboratoire Bioprojet (Paris, France). HTAB, o-dianisidine, a-carrageenan, capsaicin, cyclophosphamide, indomethacin, and dexamethasone were from Sigma Chemical Co. (St. Louis, MO). For oral administration to animals BP 2-94, indomethacin and dexamethasone were dissolved into 1% methylcellulose plus 5% dimethyl sulfoxide. RP67580, a kind gift of C. Garret (Rhône-Poulenc Rorer, Vitry, France), was dissolved in saline. Human neutrophil MPO and FCA were from Calbiochem (La Jolla, CA). All other reagents were from commercial sources and were of the highest purity available.

Results

Distribution of BP 2-94 and (R)-a-MeHA in Mouse Tissues after Oral Administration of BP 2-94. After oral administration of BP 2-94 to mice, both the prodrug and the active drug (R)-a-MeHA were detected in plasma and various
tissues. Figure 1 shows the fits of concentration versus time profile. The levels of both compounds peaked at 1 h and then declined with a half-life of around 1 h. Similar (R)-a-MeHA levels were reached in lung and plasma, whereas levels in liver and kidney were twice as high and hardly detectable in cerebral cortex (data not shown).

Effects of BP 2-94 on FCA-Induced Paw Edema. After preliminary trials a dose of 10 μl FCA was selected and its proinflammatory effects were studied in mice. Swelling of the injected paw was apparent as soon as 1 h after treatment with FCA. The edema increased slightly until 24 h and then slowly subsided, but it was still important at the end of the 9-day observation period (Fig. 2). Changes in MPO activity followed almost the same pattern (data not shown). The FCA-induced paw swelling was reduced by about 50% during at least 16 h in mice receiving BP 2-94 (16.4 μmol/kg p.o.) 1 h before FCA (Fig. 2A). BP 2-94 also reduced paw swelling (by 34–45%) when its chronic administration (16.4 μmol/kg p.o., b.i.d.) started 16 h after the FCA injection, i.e., at a time when the inflammation was firmly established (Fig. 2B). However, BP 2-94 did not affect the FCA-induced increase in MPO activity, whatever its time of administration (data not shown).

Repeated administrations of BP 2-94 twice a day for 3 days, starting 16 h after the FCA injection, decreased in a dose-dependent manner the FCA-induced edema with an ED_{50} of 5 ± 2 μmol/kg (b.i.d.) and a maximal effect of 47% (data not shown). The maximal effect of BP 2-94 was close to that of RP67580, whereas indomethacin and dexamethasone in maximal doses elicited a higher anti-inflammatory effect, decreasing paw swelling by 70 and 64%, respectively (Fig. 3, top). Higher doses of RP67580 did not display any higher inhibitory effect (data not shown). In addition, only indomethacin and dexamethasone were efficient in reducing neutrophil infiltration evaluated by the increase in MPO activity (Fig. 3, bottom).

When BP 2-94 was coadministered with indomethacin in increasing doses, either preventively in single administration or curatively in a repeated manner, the anti-inflammatory effects of the two drugs seemed to be additive for nonmaximal doses of indomethacin. However, BP 2-94 (16.4 μmol/kg once or b.i.d. for 3 days) did not increase the maximal effect of indomethacin (28 μmol/kg once or b.i.d. for 3 days) (Fig. 4).

Effects of BP 2-94 on Carrageenan-Induced Paw Edema. After preliminary trials the rat test (Drelon et al., 2000) was used to determine the effect of BP 2-94 on carrageenan-induced paw edema in rats. Fig. 1 shows the time course of paw swelling after intraplantar injection of carrageenan. The paw swelling reached a maximum at 2 h and then slowly subsided, but it was still important at the end of the 9-day observation period (Fig. 2A). Changes in MPO activity followed almost the same pattern (data not shown).

Fig. 1. Tissue and plasma levels of BP 2-94 and (R)-a-MeHA immunoreactivities in mice receiving BP 2-94. Groups of four mice were sacrificed at various times after oral administration of BP 2-94 (24 μmol/kg). (R)-a-MeHA was radioimmunoassayed in tissue and plasma extracts before and after hydrolysis of BP 2-94 into (R)-a-MeHA. BP 2-94 level was calculated as the difference between these two values.
1994) was adapted to mice. Administration of 25 μl of 0.5% carrageenan was selected out and found to induce a moderate inflammation with a maximal edema peaking at 6 h (data not shown). Administered orally 1 h before carrageenan, BP 2-94 decreased in a dose-dependent manner the carrageenan-induced paw swelling measured at 6 h with an ED50 of 0.17 ± 0.05 μmol/kg and a maximal reduction of 47%, similar to that elicited by a maximal dose of indomethacin (28 μmol/kg) (Fig. 5), but BP 2-94 had no effect on MPO activity (data not shown).

Effects of BP 2-94 on Cyclophosphamide-Induced Cystitis in Mice. The rat test (Lantéri-Minet et al., 1995) was adapted to mice. Cyclophosphamide (100 mg/kg i.p.) induced plasma protein extravasation in urinary bladder as well as leukocytes infiltration evidenced by an increase in MPO activity, starting 3 h after injection and still present 8 h thereafter. In contrast significant plasma protein extravasation was detected as soon as 1 h after the injection and persisted for at least 5 h thereafter (Ahluwalia et al., 1994) (Fig. 6). From these observations, the times of 3 and 5 h after cyclophosphamide injection were chosen for measurement of plasma protein extravasation and MPO activity, respectively. The effect of cyclophosphamide-induced enhancement of MPO activity was dose related, a maximal effect being obtained with a dose of 200 mg/kg (data not shown). BP 2-94 (33 μmol/kg p.o.) administered 1 h before cyclophosphamide (200 mg/kg) reduced MPO activity by 62% (485 ± 114 versus 186 ± 56 milliunits/bladder for vehicle and BP 2-94-treated mice, respectively) (Fig. 7). The amount of Evans blue dye in urinary bladder was increased by 186% 3 h after cyclophosphamide injection, and pretreatment with BP 2-94 (33 μmol/kg p.o.) reduced this response by 73% (Fig. 7).

Antinociceptive Activity of BP 2-94. The duration of licking induced by capsaicin was significantly reduced in mice receiving BP 2-94 orally. The antinociceptive activity of the compound was dose related and occurred with an ED50 of 0.4 ± 0.1 μmol/kg and a maximal reduction of the licking duration by 69% (Fig. 8).

The antinociceptive effect of BP 2-94 (1 μmol/kg) was abolished by previous administration of ciproxifan (37 μmol/kg),
a selective H₃ receptor antagonist (Table 1). Ciproxifan alone did not change the duration of licking. BP 2-94 (1 μmol/kg) administered 1 h before capsaicin elicited a similar effect in mice pretreated for 3 days with BP2–94 (1 μmol/kg p.o., b.i.d.) or with its vehicle (Table 1).

Discussion

The present report confirms and extends to several novel tests our initial report that a potent histamine H₃-receptor agonist exerts both anti-inflammatory and antinociceptive effects. The compound studied, BP 2-94, is a prodrug of (R)-α-MeHA, which releases it by nonenzymatic hydrolysis (Rouleau et al., 1997). With a radioimmunoassay to measure (R)-α-MeHA levels in tissues before and after heat-induced hydrolysis in vitro, both the prodrug and drug levels could be reliably detected in all tissues tested. After oral administration of BP 2-94, levels of both compounds in mouse tissues were generally higher than in plasma, being maximal after 1 h but still detectable after 9 to 16 h. In brain, however, neither the prodrug nor (R)-α-MeHA could be detected.

We show herein that administration of the histamine H₃-receptor agonist significantly reduced the paw edema induced by intraplantar administration of either FCA or carrageenan, a similar edema-preventing effect having been previously shown to occur against zymosan (Rouleau et al., 1997). In all three tests, the anti-inflammatory activity of BP 2-94 occurred at low oral dosage (≈1 μmol/kg), and the reduction of FCA-induced edema occurred when BP 2-94 was administered in either a preventive or a curative manner.

Studies of the mediators involved in the edema induced by carrageenan have suggested that there are distinct phases in the inflammatory effects of this agent. The first ones, taking place during the first 2 h, involve histamine and kinins as mediators, whereas from 2.5 to 6 h after the injection, prostaglandins seem to be involved (Di Rosa, 1972). The edema-preventing effect of the H₃-receptor agonist was observed during this prostaglandin-mediated phase along which the vascular response and polymorphonuclear cell infiltration reach their maximum. However, the edema-preventing effect of BP 2-94 in the carrageenan, zymosan, and FCA tests was not accompanied by any significant attenuation of polymorphonuclear cell infiltration, as assessed by changes in myeloperoxidase activity. In contrast, steroidal and nonsteroidal

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**Table 1**

Antinociceptive activity of single or repeated BP 2-94 administration on capsaicin-induced licking

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Licking(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A) Saline</td>
<td>71.0 ± 4.6</td>
</tr>
<tr>
<td>BP 2-94</td>
<td>26.9 ± 5.1**</td>
</tr>
<tr>
<td>Ciproxifan</td>
<td>64.3 ± 8.5</td>
</tr>
<tr>
<td>BP 2-94 + ciproxifan</td>
<td>65.5 ± 3.8*</td>
</tr>
<tr>
<td>B) Saline</td>
<td>56.8 ± 7.5</td>
</tr>
<tr>
<td>BP 2-94 (single administration)</td>
<td>28.7 ± 4.4*</td>
</tr>
<tr>
<td>BP 2-94 (repeated administration)</td>
<td>20.2 ± 2.9**</td>
</tr>
</tbody>
</table>

* P < .01; ** P < .001 compared with saline; + P < .05 compared with BP 2-94 alone.

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**Fig. 6.** Time course of cyclophosphamide-induced plasma protein extravasation and MPO activity in urinary bladder. Cyclophosphamide (100 mg/kg i.p.) was injected to groups of mice. One group of animals was anesthetized with pentobarbital (10 mg/kg i.p.), received an injection of Evans blue dye (30 mg/kg i.v.), and was sacrificed 30 min later; the urinary bladder was dissected out and dye extracted and measured. The other group was sacrificed at indicated times, the urinary bladder dissected out, and MPO activity determined.

**Fig. 7.** Effects of BP 2-94 on cyclophosphamide-induced cystitis. Cyclophosphamide (200 mg/kg i.p.) was injected 1 h after BP 2-94 (p.o.) or its vehicle. One group of mice was injected with Evans blue dye (30 mg/kg i.v.) 2.5 h after cyclophosphamide injection and plasma protein extravasation in urinary bladder was evaluated 30 min later. A second group was sacrificed 5 h after cyclophosphamide injection, the urinary bladder dissected out for MPO activity assay. *** P < .001 with control; ** P < .01 with cyclophosphamide. Mean ± S.E. of 9 to 29 values.

**Fig. 8.** Antinociceptive effect of BP 2-94 in the capsaicin test. Capsaicin (1.5 μg in 20 μl) was injected under the skin of the dorsal surface of the right hind paw 1 h after BP 2-94 (p.o.) or its vehicle. The duration of licking was measured for 5 min immediately after capsaicin injection. * P < .01; ** P < .001. Means ± S.E. of 4 to 36 values.
inflammatory drugs appear to affect both parameters of inflammation in these three models, as shown herein in the case of the FCA model, whereas in this respect, an NK1-receptor antagonist displayed a pattern similar to that of BP 2-94 (Fig. 3). Interestingly the maximal-preventing effects of the H3-receptor agonist on edema were similar to those of indomethacin in the carrageenan model, but somewhat lower in the FCA model. In addition, in the latter model the anti-inflammatory effects of the two drugs appeared additive, whatever the dose of indomethacin, consistent with their distinct action mechanisms. Because the anti-inflammatory efficacy of cyclooxygenase inhibitors in either experimental or in human therapeutics is limited, presumably as a result of the participation of multiple mediators in inflammatory responses, combination of drugs having distinct mechanisms of actions seems a rational approach as long as tolerance is not compromised.

It seems likely that these anti-inflammatory responses to BP 2-94, characterized by a predominant effect upon local edema, are related to the prevention of plasma protein extravasation resulting from the inhibition of tachykinin release. In agreement, stimulation of presynaptic H3-heteroreceptors on capsaicin-sensitive sensory nerves inhibits tachykinin release and neurogenic plasma leakage in a variety of tissues (Ichinose et al., 1990; Ohkubo et al., 1995; Rouleau et al., 1997).

The marked anti-inflammatory and antinociceptive effects of BP 2-94 on the two other tests used in the present study are also in agreement with such a postulated mechanism. In the cyclophosphamide-induced cestisus, a mouse model that we have adapted from the rat model (Lantéri-Minet et al., 1995), the H3-receptor agonist significantly attenuated not only plasma protein extravasation but also the increase in myeloperoxidase activity in the inflamed bladder. The existence of a major capsaicin-sensitive component in the plasma protein extravasation induced by acrolein, the active metabolite of cyclophosphamide, was demonstrated, namely, via the use of capsaicin-induced desensitization and an NK1-receptor antagonist (Ahlawalia et al., 1994). The reasons for which BP 2-94 reduced cyclophosphamide-induced polymorphonuclear influx into bladder, whereas it does not affect this parameter in the inflamed paw in the other models, are not known.

In the capsaicin-induced licking the antinociceptive activity of BP 2-94 can obviously be ascribed to an H3-receptor-mediated attenuation of primary sensory C-fiber excitation by the pungent principle of hot peppers. Indeed, in this test the nociceptive response is inhibited by intrathecal administration of Substance P antagonists or antibodies (Sakurada et al., 1992). However, the fact that BP 2-94 hardly enters the brain and displays activity in “peripheral” but not “central” tests of nociception suggests that it acts via peripheral H3-receptors depressing the afferent activity of primary sensory neurons.

All together, the present observations confirm that BP 2-94 exerts clear anti-inflammatory and antinociceptive activities in animal models. As potential anti-inflammatory and analgesic drugs, H3-receptor agonists theoretically display their advantage over NK1 (or NK2) antagonists of reducing the release not only of tachykinins (acting upon various receptor subtypes) but also of other proinflammatory mediators of the sensory fibers such as calcitonin-gene-related peptide, hence a potentially wider therapeutic spectrum. As compared with nonselective cyclooxygenase inhibitors, they display the advantage of lacking ulcerogenic activity and, even more, of displaying gastric mucosa protective ability (Morini et al., 1996, 1997; Belcheva et al., 1997), but they appear to have a more restricted anti-inflammatory spectrum. Compared with selective cyclooxygenase-2 inhibitors, only the more restricted anti-inflammatory spectrum can be invoked, but additive effects of potential therapeutic interest may be anticipated from the combination of the two classes of drugs.

Acknowledgment

We thank A. Galtier for processing this manuscript.

References


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