Available online at www.sciencedirect.com

SCIENCE DIRECT®

Pharmacology, Biochemistry and Behavior 78 (2004) 719–725

PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR

www.elsevier.com/locate/pharmbiochembeh

Pentacyclic triterpenoids, α,β-amyrins, suppress the scratching behavior in a mouse model of pruritus

Francisco A. Oliveira[a], Roberto C.P. Lima-Junior[a], Wilcare M. Cordeiro[a], Gerardo M. Vieira-Júnior[b], Mariana H. Chaves[b], Fernanda R.C. Almeida[b], Regilane M. Silva[a], Flavia A. Santos[c], Vietla S.N. Rao[a,*]

[a]*Departamento de Fisiologia e Farmacologia, Universidade Federal do Ceara, Rua Cel Nunes de Melo-1127, Caixa Postal-3157, 60430-270 Fortaleza, CE, Brazil*

[b]*Departamento de Química Organica, Universidade Federal do Piauí, Teresina, PI, Brazil*

[c]*Departamento de Farmacia, Universidade Federal do Ceará, C.P. 3157, 60430-270, Fortaleza, CE, Brazil*

Received 18 December 2003; received in revised form 10 March 2004; accepted 4 May 2004
Available online 26 June 2004

Abstract

In the search for natural compounds useful against pruritus, α,β-amyrins, the pentacyclic triterpenes isolated from the resin of popular medicinal plant *Protium heptaphyllum* were examined on scratching behavior induced by dextran T40 and compound 48/80 in mice. The animals were pretreated orally with α,β-amyrins (50, 100 and 200 mg/kg) or cyproheptadine (10 mg/kg), an antagonist of histamine and serotonin receptors and 2 h later, they were given subcutaneous injections of dextran T40 (75 mg/kg) or compound 48/80 (3 mg/kg) into the rostral back, and scratching was quantified for 20 min. The scratching behavior induced by dextran T40 and compound 48/80 was significantly inhibited in mice pretreated with α,β-amyrins (100 and 200 mg/kg) or cyproheptadine (10 mg/kg), In addition, the compound 48/80-elicited degranulation of rat peritoneal mast cells (ex vivo) was also markedly reduced in animals pretreated with α,β-amyrins (100 mg/kg) or ketotifen (1 mg/kg), a known mast cell stabilizer. In the open-field test, α,β-amyrins (100 and 200 mg/kg)-pretreated mice showed no impairment of spontaneous locomotion, suggesting that these triterpenoids possess no sedative activity that could account for suppression of scratching behavior. These results clearly indicate the antipruritic effect of α,β-amyrins and suggest that this effect may be related to a stabilizing action on mast cell membrane.

Keywords: α,β-amyrins; Scratching behavior; Compound 48/80; Dextran T40; Mast cell; Mouse

1. Introduction

Pruritus (itch) is an unpleasant cutaneous sensation, which provokes the desire to scratch, and it may be local or widespread associated with atopic dermatitis, urticaria or systemic disorders (cholestasis, uraemia). Many endogenous chemical agents like amines, proteases, growth factors, neuropeptides, opioids, eicosanoids and cytokines can act as pruritogens by causing histamine release from local mast cells and/or by sensitizing the relevant C-fibers (Lerner, 1994; Hagermark, 1995; Schmelz et al., 1997). Scratching can cause skin lesions and contribute to severe psychological disturbances (Raiford, 1995) and therefore inhibition of this response is consistently beneficial for improving the quality of life. As there is no specific remedy available for this common symptom, the search for an ideal antipruritic agent continues.

Pentacyclic triterpene compounds, in general, exhibit a wide range of pharmacological activities that include antioxidant, anti-allergic, anti-inflammatory, antitumor, antibacterial, gastroprotective and hepatoprotective effects (Ravokatra et al., 1974; Tabata et al., 1993; Yun et al., 1999; Ryu et al., 2000; Sunitha et al., 2001; Ukiva et al., 2002; Katerere et al., 2003). The resinous exudate (resin) collected from the trunk wood of *Protium heptaphyllum* is a folk remedy used in Brazil to treat inflammatory conditions and to hasten wound repair (Siani et al., 1999). This resin is rich in pentacyclic triterpenoids, α,β-amyrins, maniladiol and brein that possess

* Corresponding author. Tel./fax: +55-85-288-8333.
E-mail address: vietrao@ufc.br (V.S.N. Rao).

0091-3057/$ – see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.pbb.2004.05.013

anti-inflammatory and gastroprotective properties (Yasukawa et al., 1996; Susunaga et al., 2001; Oliveira et al., 2004). An anti-allergic activity of pentacyclic triterpene derivatives belonging to both the α,β-amyrin series has been reported (Tanaka et al., 1991). Histamine and serotonin are important mediators of inflammation and in allergy-associated pruritus. Compound 48/80 and dextran T40 are the widely used pruritogens in experimental settings to induce scratching behavior that has features similar to that of pruritus in humans (Shuttleworth et al., 1988; Ballantyne et al., 1988). There have been reports that describe the anti-allergic but not the antipruritic activity of pentacyclic triterpenes. Antihistamines and steroidal agents remain the treatment of first choice for pruritus without known causes. Since the pentacyclic amyrins possess hydro-aromatic ring systems similar to that of steroids, compounds like α,β-amyrins may present antipruritus effect. Therefore, the present study is aimed to examine whether α,β-amyrins would inhibit the scratching response in murine models of pruritus induced by dextran T40 and compound 48/80, and if so, to verify the role of endogenous opioids, and their effects on compound 48/80-induced degranulation of rat peritoneal mast cells.

2. Materials and methods

2.1. Materials

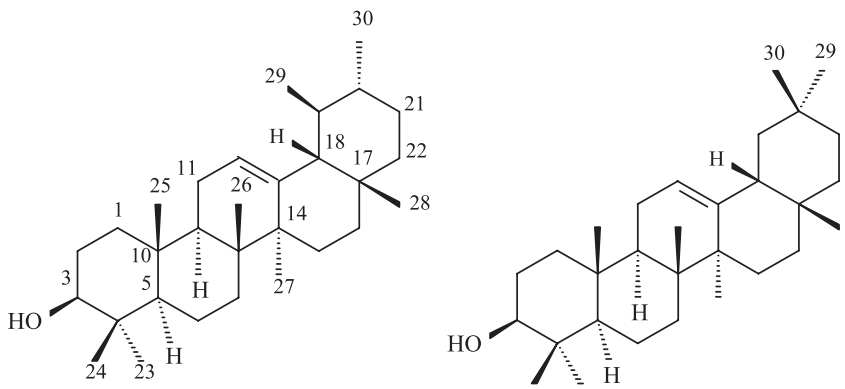
Compound 48/80, ketotifen and naloxone (Sigma), cyproheptadine (Merck) and dextran T40 (Pharmacia Biotech), were dissolved in or diluted with physiological saline. The triterpenes, α,β-amyrins, were isolated as a mixture (α and β in the proportion of 2:1; yield, 42.25%) from the crude resin, collected from the trunk wood of *P. heptaphyllum* (Viera-Junior et al., 2003) and their structures (Fig. 1) elucidated by spectroscopy, using ^{1}H and ^{13}C NMR and mass spectrum in comparison with known standards (Olea and Roque, 1990). The α,β-amyrins were dissolved in 3% Tween 80 in saline. All other reagents used were of analytical grade.

2.2. Animals

The experiments were performed using 8–10 week-old female Swiss mice (23–30 g) and female Wistar rats of 3 months old (150–180 g). The animals were housed at 24 ± 2 °C and 55 ± 5% relative humidity with food and water available ad libitum. Institutional Committee on the Care and Use of Animals for Experimentation approved the experimental protocols, which were in accordance with the guidelines of National Institute of Health, Bethesda, USA.

2.3. Scratching behavior induced by dextran T40 or compound 48/80

Scratching behavior was studied in groups of mice ($n = 8$ per group) and a day before the experiments the rostral part of the skin on the back of each mouse was clipped. On the test day, they were first habituated for about 10 min in plastic observation cages (10 × 15 × 30 cm) and then the pruritogens, dextran T40 (75 mg/kg) or compound 48/80 (3 mg/kg), were injected subcutanously into the rostral part of the back in a volume of 50 μl, using a 27-gauge needle. Control mice received a similar quantity of normal saline injection instead. Immediately after the injection, mice were returned to the observation cages, one mouse per cage. The scratching behavior was observed for 20 min by observers who were unaware of the treatments (Chakravarty, 1978; Kuraishi et al., 1995; Ishiguro and Oku, 1994) and expressed as the time in seconds (s) that each animal had spent scratching during a 20-min period. This period was chosen based on our pilot studies that showed high intensity of scratching in the time-course response. Only scratching of nose by fore- or hind paws and the injection site by hind paws



α-Amyrin β-Amyrin

Fig. 1. Chemical structures of α-amyrin and β-amyrin.

was counted in animal groups that received 48/80 (Ishiguro and Oku, 1994), whereas in case of dextran T40, total body scratching (i.e. scratching of the nose and ears with the fore paws, and back by the hind paws) was counted, but disregarded licking of the belly and back during grooming (Ishiguro and Oku, 1994). The dosage selection of pruritogens was based on our pilot studies and at these doses, both dextran T40 (75 mg/kg) and compound 48/80 (3 mg/kg) were able to induce a consistent scratching during the first 20 min period. The test substances, α,β-amyrins (50, 100 and 200 mg/kg) and cyproheptadine (10 mg/kg) were given orally 2 h prior to the injection of pruritogens. Control mice were administered the same volume of vehicle (3% Tween 80 in distilled water, 10 ml/kg).

2.4. Effect of μ-opioid receptor antagonist naloxone on the compound 48/80-induced scratching behavior in mice pretreated with α,β-amyrins or morphine

In order to verify the possible role of endogenous opioids in the suppressive effect of α,β-amyrins against compound 48/80-induced scratching, groups of mice (eight in each) were pretreated with vehicle (3% Tween 80 in distilled water, 10 ml/kg), naloxone (2 mg/kg ip), morphine (7.5 mg/kg sc) or α,β-amyrins (100 mg/kg po) alone or in their combinations with naloxone prior to the injection of compound 48/80 (3 mg/kg sc). While α,β-amyrins were administered 2 h before, naloxone and morphine were given 30 min prior to pruritogen (Table 1).

2.5. Peritoneal mast cell degranulation

Four groups ($n=4$) of rats were included for the ex vivo study that aimed to demonstrate the effects of α,β-amyrins and ketotifen on the peritoneal mast cell degranulation. The first and second groups of animals served as normal and vehicle-treated controls and received orally, normal saline or 3% Tween 80 (vehicle for α,β-amyrins), respectively, in a volume of 10 ml/kg, whereas the third and fourth groups were treated orally with α,β-amyrins (100 mg/kg) or ketotifen (1 mg/kg), respectively. Two hours later, the animals were killed by cervical dislocation and half a centimeter pieces of mesenteric vascular plexus were collected from the respective groups into each of the glass tubes containing Ringer Locks fluid (10 ml). Mast cell degranulation was induced by incubation of tubes containing mesenteric vascular tissue collected from α,β-amyrins, ketotifen, or vehicle-treated groups with compound 48/80 (final concentration, 0.4 μg/ml) for 30 min. The concentration of 0.4 μg/ml of compound 48/80 was chosen for this study based on our preliminary studies, which caused mast cell disruption to the extent of 90%. The same volume of distilled water (instead of compound 48/80 solution) was added to tubes containing mesenteric tissue obtained from the normal control rats that received only the vehicle. After 30 min incubation, the mesenteric tissue was mounted on glass slides, allowed to dry at room temperature and stained with toluidine blue (0.1%) for the observation of mast cells by light microscopy. At least five optical fields were chosen for each tissue sample (five samples per group) and the number of total mast cells present, and the percentage of cells disrupted/degranulated were noted (Norton, 1954).

Table 1
Effect of μ-opioid receptor antagonist naloxone on the compound 48/80 (3 mg/kg sc)-induced scratching behavior in mice pretreated with α,β-amyrins or morphine

Treatment	Dose (mg/kg)	Scratching/20 min (s)
Vehicle (po)	–	64.38 ± 4.73
α,β-Amyrins (po)	100	20.09 ± 2.84[a]
Morphine (sc)	7.5	0.428 ± 0.42[a]
Naloxone (ip)	2.0	67.76 ± 5.75
Naloxone (ip) + α,β-amyrins (po)	2.0 + 100	41.50 ± 4.14[a,b]
Naloxone (ip) + morphine (sc)	2.0 + 7.5	61.71 ± 5.45[c]

Data as means ± S.E.M.; $n=8$ per group; vehicle.
[a] $P<.01$ vs. vehicle control.
[b] $P<.01$ vs. α,β-amyrins.
[c] $P<.01$ vs. morphine.

2.6. Evaluation of the spontaneous motor activity

The triterpenes, α,β-amyrins, were evaluated for their effects on spontaneous motor activity in an open-field test (Capaz et al., 1981). Briefly, mice ($n=8$ per group) were individually placed in the center of an open-field arena (40 cm of diameter) at 2 h after oral administration of α,β-amyrins (100 and 200 mg/kg) or vehicle and the locomotion frequency (the number of floor units the animal entered with all the four paws) was counted for 4 min.

2.7. Data analysis

All values are expressed as mean ± S.E.M. Statistical evaluation of the results was performed by one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparisons. A result with $P \leq .05$ was considered statistically significant.

3. Results

3.1. Effect on scratching behavior induced by dextran T40 and compound 48/80

Normal saline-treated control mice showed no scratching behavior whereas animal groups that received subcutaneous injections of dextran T40 or compound 48/80 into the rostral back manifested intense scratching (Figs. 2 and 3). Mice that received 100 and 200 mg/kg α,β-amyrins demonstrated a potent inhibition of compound 48/80-induced scratching, at 50 mg/kg there was no

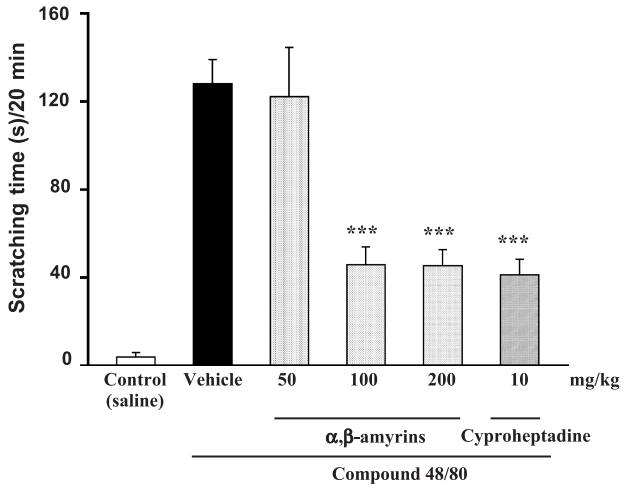


Fig. 2. The effect of oral pretreatments with α,β-amyrins (50, 100 and 200 mg/kg), cyproheptadine (10 mg/kg), vehicle (3% Tween 80; 10 ml/kg) or normal saline (10 ml/kg) on the scratching behavior induced by compound 48/80 (3 mg/kg) in mice. Normal saline injected control group of mice showed no scratching behavior. Each column represents mean ± S.E.M. ($n=8$). *** $P<.001$ vs. vehicle-treated control (ANOVA and Dunnett's test).

significant effect. The α,β-amyrins treatment was more effective in the suppression of dextran T40-induced scratching behavior (Fig. 3) and the effect was found to be significant at all doses tested. A dose–effect relationship was evident only at smaller doses. The dual histamine/serotonin receptor antagonist, cyproheptadine (10 mg/kg po) also caused marked inhibition of scratching responses elicited by both compound 48/80 as well as dextran T40. Similar to α,β-amyrins, the effect of cyproheptadine was apparently more prominent against dextran T40-induced scratching.

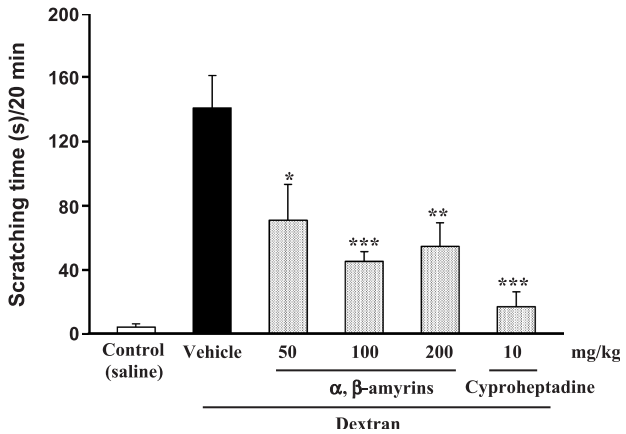


Fig. 3. The effect of oral pretreatments with α,β-amyrins (50, 100 and 200 mg/kg), cyproheptadine (10 mg/kg), vehicle (3% Tween 80; 10 ml/kg) or normal saline (10 ml/kg) on the scratching behavior induced by dextran T40 (75 mg/kg) in mice. Each column represents mean ± S.E.M. ($n=8$). * $P<.05$, ** $P<.01$, and *** $P<.001$ vs. vehicle-treated control (ANOVA and Dunnett's test).

3.2. Effect on peritoneal mast cell degranulation induced by compound 40/80 (ex vivo)

The percent numbers of degranulated mast cells in saline-treated normal rats, in vehicle-treated rats and in rats treated with α,β-amyrins (100 mg/kg po) and ketotifen were in the order of 2.5%, 90.6%, 12.6% and 13.6%, respectively (Fig. 4). A representative microphotograph showing the mast cell degranulation among various groups are shown in Fig. 5. Compared with vehicle-treated controls, treatment with α,β-amyrins and ketotifen significantly reduced the compound 48/80-induced degranulation by the extent of 86% and 85%, respectively.

3.3. Effect of μ-opioid receptor antagonist naloxone on the compound 48/80-induced scratching behavior in mice pretreated with α,β-amyrins or morphine

The results obtained are shown in Fig. 5. Morphine (7.5 mg/kg sc) and α,β-amyrins (100 mg/kg po) pretreatments resulted in significant suppression of compound 48/80-induced scratching behavior in mice. Although naloxone alone showed no significant influence, it could completely reverse the morphine effect on induced scratching. The suppressive effect of α,β-amyrins was only partially antagonized by naloxone.

3.4. Effect on spontaneous locomotor activity

At the tested doses of 100 and 200 mg/kg, α,β-amyrins failed to modify the locomotion frequency. The observed locomotion frequencies in control and at either dose of α,β-

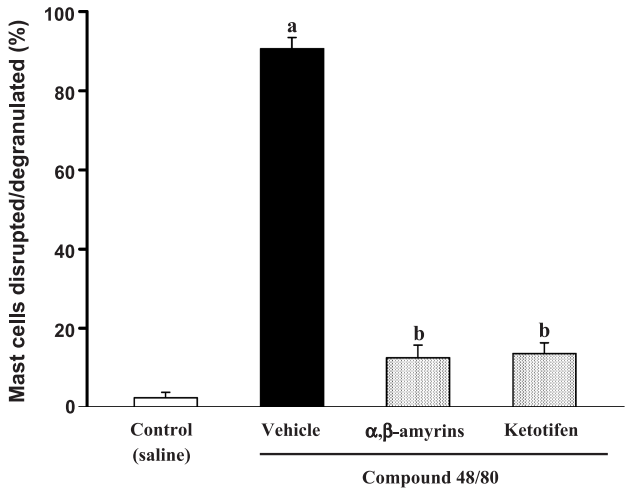


Fig. 4. The effect of oral pretreatments with α,β-amyrins (100 mg/kg), ketotifen (1 mg/kg) or vehicle (3% Tween 80; 10 ml/kg) or normal saline (10 ml/kg) on compound 48/80 (0.4 μg/ml)-induced degranulation of rat peritoneal mast cells ex vivo. Each column represents mean ± S.E.M. ($n=5$). [a] $P<.001$ vs. saline-treated control; [b] $P<.001$ vs. vehicle-treated control (ANOVA and Bonferroni test).

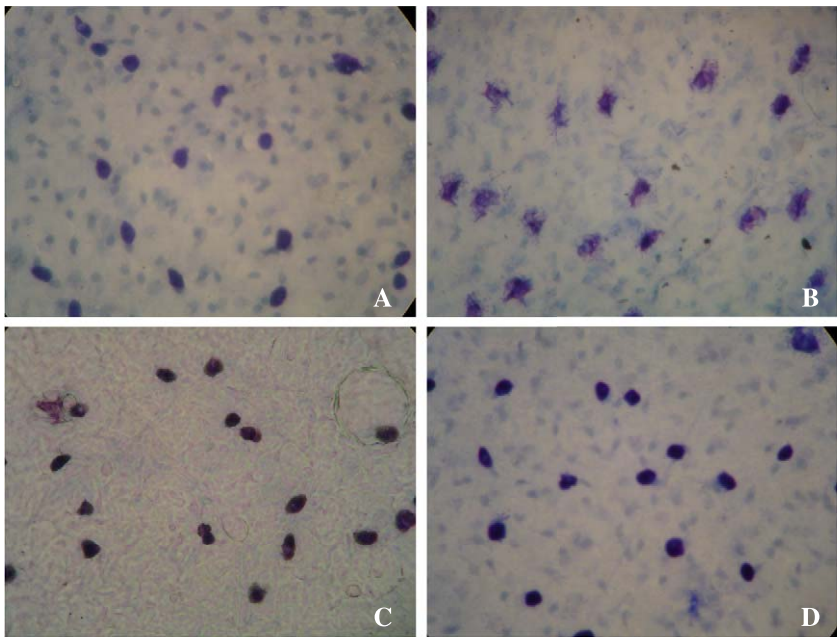


Fig. 5. The representative microphotograph of toluidine blue stained rat peritoneal mast cells subjected or not to compound 48/80 (0.4 μg/ml)-induced degranulation ex vivo. (A) Mast cells from saline-treated rats; (B) Mast cells from vehicle (3% Tween 80; 10 ml/kg)-treated rats incubated with compound 48/80, showing extensive mast cell degranulation; (C) Mast cells from α,β-amyrins (100 mg/kg) and (D) ketotifen (1 mg/kg)-treated rats incubated with compound 48/80, showing inhibition of degranulation.

amyrins-treated mice were 39.93 ± 4.53, 36.52 ± 2.81 and 37.38 ± 3.45, respectively.

4. Discussion

The present data demonstrate that the pentacyclic triterpenes, α,β-amyrins, are able to suppress experimental pruritus as evidenced from their inhibitory effect on scratching behavior induced by dextran T40 or compound 48/80 in mice. At the doses employed, the pruritogens did not show local edema or any other behavioral sign concurrent with the scratching. Although there is no clear dose–response relationship, more so against compound 48/80-induced scratching, the amyrins produced a suppressive effect in these models, valid for testing antipruritic agents (Shuttleworth et al., 1988; Ballantyne et al., 1988). There are several reports that describe the effectiveness of natural compounds (e.g. flavonols, 1,4-naphthoquinones, saponins; monoterpenes and capsaicin) useful against histamine-dependent and histamine-independent pruritus (Ishiguro and Oku, 1994; Oku et al., 2002; Fu et al., 2003). To our knowledge, this is the first study that demonstrates the mast cell-mediated inhibition of scratching response by pentacyclic triterpenes. The pruritogenic effect of compound 48/80 is attributed to its histamine releasing activity from mast cells (Inagaki et al., 1999) and some studies suggest that the membrane permeability increase and activation of mast cell phospholipase D via heterotrimeric GTP-binding proteins may be an essential trigger for the release of mediators from mast cells (Tasaka et al., 1986; Chadi et al., 2000). Compound 48/80, however, can induce scratching behavior in mice independent of histamine (Sugimoto et al., 1998) and serotonin (Inagaki et al., 2002). This might be the reason why both cyproheptadine, a dual histamine/serotonin receptor antagonist as well as α,β-amyrins show more efficacy in mitigating the scratching response induced by dextran T40 than by compound 48/80.

Similar to compound 48/80, dextran T40 also releases mediators from the skin mast cells (Chakravarty, 1978) and produces scratching in mice (Ishiguro and Oku, 1994). In studies of Kuraishi et al. (1995), subcutaneous injections of histamine did not elicit the scratching behavior in ddy mice, whereas studies of Maekawa et al. (2000) have shown an increased scratching in ICR mice. In another study, mice were found to be more sensitive to serotonin-induced scratching than rats (Dai et al., 2002). Apparently, there are species and strain differences in the scratching responses to histamine and serotonin. Therefore, one of the conceivable mechanisms for the antipruritic activity of α,β-amyrins is the inhibition of degranulation of mast cells. In the study carried out ex vivo, α,β-amyrins were able to prevent mast cell degranulation, which was almost comparable to that caused by ketotifen, a known mast cell stabilizer. Furthermore, α,β-amyrins could inhibit the paw edema response, induced by compound 48/80 and dextran T40 in mice (unpublished observations). Taken together, these observations suggest that this class of pentacyclic triterpenes suppress the function of mast cell secretogogues.

It was reported that μ-opioid receptor antagonists like naloxone and naltrexone inhibit the scratching behavior associated with cutaneous passive anaphylaxis, subcutaneous compound 48/80 and intradermal substance P injections, suggesting an opioidergic mechanism in the scratching behavior (Inagaki et al., 1999; Umeuchi et al., 2003). In the present work, we verified the role of endogenous opioids in the scratching response induced by compound 48/80 and its suppression by amyrins. The μ-opioid receptor antagonist naloxone produced no discernible effect either on basal scratching activity (data not shown) or on compound 48/80-induced scratching behavior in mice (Fig. 5). However, naloxone was found to antagonize the suppressive effect of morphine more completely and of amyrins only partially the scratching induced by compound 48/80. This suggests that naloxone-sensitive endogenous opioids are at least in part involved in the suppressive effect of amyrins on compound 48/80-induced scratching in Swiss mice.

It is known that drugs with sedative activity may also inhibit scratching behavior (Watanabe et al., 1999). However, at the doses used in the present experiments, α,β-amyrins failed to impair spontaneous locomotor activity in mice. Therefore, the sedative action of amyrins may not be involved in the inhibitory mechanism of scratching behavior.

In summary, we showed that α,β-amyrins from *P. heptaphylum* resin inhibits scratching in mice. Mast cells and mast cell-derived mediators may play an essential role in this response. Allergic skin disorders, such as atopic dermatitis, contact dermatitis and urticaria, are accompanied by severe pruritis wherein mast cell mediators play a definitive role (Lorette and Vaillant, 1990). The major mechanism of the antipruritus activity of amyrins is the inhibition of degranulation of mast cells. Given the reason that scratching in animal model is a valid and reliable model for studying human pruritus (Fjellner and Hagermark, 1981; Kuraishi et al., 1995), the present data suggest that α,β-amyrins may have a clinical significance in the treatment of pruritus associated with atopic allergy, contact dermatitis and eczema.

Acknowledgements

This work was financially supported by grants from Brazilian National Agencies, CAPES (PROCAD, Proc. No. 0014/091-0) and CNPq (Proc. No. 472717/03-0).

References

Ballantyne JC, Loach AB, Carr DB. Itching after epidural and spinal opiates. Pain 1988;33:149–60.

Capaz FR, Vanconcellos L, De Moraes S, Palermo-Neto J. The open-field: a simple method to show ethanol withdrawal symptoms. Arch Int Pharmacodyn 1981;251:228–36.

Chadi A, Fraundorfer PF, Beaven MA. Compound 48/80 activates mast cell phospholipase D via heterotrimeric GTP-binding proteins. J Pharmacol Exp Ther 2000;292:122–30.

Chakravarty N. Metabolic changes in mast cells associated with histamine release. In: Mauricio RS, editor. Histamine and histamine antagonist. New York: Springer Verlag; 1978. p. 93–129.

Dai Y, Ye WC, Wang ZT, Matsuda H, Kubo M, But PPH. Antipruritic and antinociceptive effects of *Chenapodium album* L. in mice. J Ethnopharmacol 2002;81:245–50.

Fjellner B, Hagermark O. Studies on pruritogenic and histamine-releasing effects of some putative peptide neurotransmitters. Acta Dermatovener 1981;61:245–51.

Fu L-J, Dai Y, Wang Z-T, Zhang M. Inhibition of experimental allergic rhinitis by the *n*-butanol fraction from the anomalous fruits of *Gleditsia sinensis*. Biol Pharm Bull 2003;26:974–7.

Hagermark O. Itch mediators. Semin Dermatol 1995;14:271–6.

Inagaki N, Nakamura N, Nagao M, Musoh K, Kawasaki H, Nagai H. Participation of histamine H1 and H2 receptors in passive cutaneous anaphylaxis-induced scratching behavior in ICR mice. Eur J Pharmacol 1999;367:361–71.

Inagaki N, Igeta K, Kim JF, Nagao M, Shiraishi N, Nakamura N, Nagai H. Involvement of unique mechanisms in the induction of scratching behavior in BALB/c mice by compound 48/80. Eur J Pharmacol 2002; 448:175–83.

Ishiguro K, Oku H. Antipruritic effect of flavonol and 1,4-naphthoquinone derivatives from *Impatiens balsamina* L. Chemical mediators of itching. In: Berhard J, editor. Itch: mechanisms and management of pruritis. New York: McGraw-Hill; 1994. p. 23–5.

Katerere DR, Grev AI, Nash RJ, Waigh RD. Antimicrobial activity of pentacyclic triterpenes isolated from African Combretaceae. Phytochemistry 2003;63:81–8.

Kuraishi Y, Nagasawa T, Hayashi K, Satoh M. Scratching behavior induced by pruritogenic but not algesiogenic agents in mice. Eur J Pharmacol 1995;275:229–33.

Lerner EA. Chemical mediators of itching. In: Berhard J, editor. Itch: mechanisms and management of pruritis. New York: McGraw-Hill; 1994. p. 23–5.

Lorette G, Vaillant L. Pruritis: current concepts in pathogenesis and treatment. Drugs 1990;39:218–23.

Maekawa T, Nojima H, Kuraishi Y. Itch-associated responses of afferent nerve innervating the murine skin: different effects of histamine and serotonin in ICR and ddy mice. Jpn J Pharmacol 2000;84:462–6.

Norton S. Quantitative determination of mast cell fragmentation by compound 48/80. Br J Pharmacol 1954;9:494–7.

Oku H, Kato T, Ishiguro K. Antipruritic effects of 1,4-naphthoquinones and related compounds. Biol Pharm Bull 2002;25:137–9.

Olea RSG, Roque NF. Análise de Misturas de Triterpenos por ^{13}C NMR. Quimica Nova 1990;13:278–81.

Oliveira FA, Viera-Junior GM, Chaves MH, Almeida FRC, Florencio MG, Lima RCP, et al. Gastroprotective and anti-inflammatory effects of resin from *Protium heptaphyllum* in mice and rats. Pharmacol Res 2004; 49:105–11.

Raiford DS. Pruritis of chronic cholestasis. Q J Med 1995;88:603–7.

Ravokatra A, Nigeon-Dureuil M, Ratsimamanga AR. Action of a pentacyclic triterpenoid, asiaticoside, isolated from *Hydrocotyle masagascariensis* or *Centella asiatica*, against gastric ulceration in the Wistar rat exposed to cold at 2°C. CR Acad Sci Hebd Seances Acad Sci D 1974;278:1743–6.

Ryu SY, Oak MH, Yoon SK, Cho DI, Yoo GS, Kim TS, et al. Antiallergic and anti-inflammatory triterpenes from the herb of *Prunella vulgaris*. Planta Med 2000;66:358–60.

Siani AC, Ramos MF, Menezes de Lima Jr O, Ribeiro dos Santos R, Fernandez-Ferreira E, Soares RO, et al. Evaluation of anti-inflammatory-related activity of essential oils from the leaves and resin of species of Protium. J Ethnopharmacol 1999;66:57–69.

Schmelz M, Schmidt R, Bickel A, Handwerker HO, Torebjork HE. Specific C-receptors for itch in human skin. Neuroscience 1997;17:8003–8.

Shuttleworth D, Hill S, Marks R, Connelly MD. Relief of experimentally-induced pruritis with a novel eutectic mixture of local anaesthetic agents. Br J Dermatol 1988;119:535–40.

Sugimoto Y, Umakoshi K, Nojiri N, Kamei C. Effects of histamine H1 receptor antagonists on compound 48/80-induced scratching behavior in mice. Eur J Pharmacol 1998;35:1–5.

Sunitha S, Nagaraj M, Varalakshmi P. Hepatoprotective effect of lupeol and lupeol linoleate on tissue antioxidant defence system in cadmium-induced hepatotoxicity in rats. Fitoterapia 2001;72:516–23.

Susunaga GS, Siani AC, Pizzolatti MG, Yunes RA, Delle Monache F. Triterpenes from the resin *Protium heptaphyllum*. Fitoterapia 2001; 72:709–11.

Tabata M, Tanaka S, Cho HJ, Uno C, Shimakura J, Ito M, et al. Production of an antiallergic triterpene bryonolic acid, by plant cell cultures. J Nat Prod 1993;56:165–74.

Tanaka S, Uno C, Akimoto M, Tabata M, Honda C, Kamisako W. Anti-allergic effect of bryonolic acid from *Luffa cylindrica* cell suspension culture. Planta Med 1991;57:527–30.

Tasaka K, Mio M, Okamoto M. Intracellular calcium release induced by histamine releasers and its inhibition by some antiallergic drugs. Ann Allergy 1986;56:464–9.

Ukiva M, Akihisa T, Tokuda H, Suzuki H, Mukainaka T, Ichiishi E, et al. Antitumor promoting effects and cytotoxic activity against human cancer lines of triterpene diols and triols from edible Chrysanthemum flowers. Cancer Lett 2002;177:7–12.

Umeuchi H, Togashi Y, Honda T, Nakao K, Okano K, Tanaka T, et al. Involvement of central μ-opioid system in the scratching behavior in mice, and the suppression of it by the activation of k-opioid system. Eur J Pharmacol 2003;477:29–35.

Viera-Junior JB, Souza CML, Chaves MH. Estudo das propriedades físico-químicas da resina de almécega (*Protium heptaphyllum*). 55 a Reunião da Sociedade Brasileira para o Progresso da Ciência, Recife-PE, Julho/ 2003; 1999.

Watanabe C, Satoh T, Tahara E, Murakami K, Hayashi K, Hase K, et al. Inhibitory mechanism of glycoprotein fraction derived from *Miscanthus sinensis* for the immediate phase response of an IgE-mediated cutaneous reaction. Biol Pharm Bull 1999;22:26–30.

Yasukawa K, Akihisa T, Oinuma H, Kasahara Y, Kimura Y, Yamanouchi S, et al. Inhibitory effect of di- and trihydroxy triterpenes from the flowers of compositae on 12-*O*-tetradecanoylphorbol-13-acetate-induced inflammation in mice. Biol Pharm Bull 1996;19:1329–31.

Yun BS, Ryoo IJ, Lee IK, Park KH, Choung DH, Han KH, et al. Two bioactive pentacyclic triterpene esters from the root bark of *Hibiscus syriacus*. J Nat Prod 1999;62:764–6.