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H_1-receptor stimulation induces hyperalgesia through activation of the phospholipase C-PKC pathway

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Abstract

The supraspinal cellular events involved in H_1-mediated hyperalgesia were investigated in a condition of acute thermal pain by means of the mouse hot-plate test. I.c.v. administration of the phospholipase C (PLC) inhibitors U-73122 and neomycin antagonized the hyperalgesia induced by the selective H_1 agonist FMPH. By contrast, U-73343, an analogue of U-73122 used as negative control, was unable to modify the reduction of the pain threshold induced by FMPH. In mice undergoing treatment with LiCl, which impairs phosphatidylinositol synthesis, or treatment with heparin, an IP_3-receptor antagonist, the hyperalgesia induced by the H_1-receptor agonist remained unchanged. Similarly, pretreatment with D-myo inositol did not alter the H_1-induced hypernociceptive response. Neither i.c.v. pretreatment with TMB-8, a blocker of Ca^{2+} release from intracellular stores, nor pretreatment with thapsigargin, a depletor of Ca^{2+} intracellular stores, prevented the decrease of pain threshold induced by FMPH. On the other hand, i.c.v. pretreatment with the selective protein kinase C (PKC) inhibitors calphostin C and chelerytrine resulted in a dose-dependent prevention of the H_1-receptor agonist-induced hyperalgesia. The administration of PKC activators, such as PMA and PDBu, did not produce any effect on FMPH effect. The pharmacological treatments employed did not produce any behavioral impairment of mice as revealed by the rota-rod and hole-board tests. These results indicate a role for the PLC–PKC pathway in central H_1-induced hyperalgesia in mice. Furthermore, activation of PLC–IP_3 did not appear to play a major role in the modulation of pain perception by H_1-receptor agonists.

Keywords: Histamine H1-receptor; Hyperalgesia; Phospholipase C; Inositol-1,4,5-trisphosphate; Protein kinase C; Ca^{2+}

1. Introduction

Histamine is a monoamine neurotransmitter so far known to activate three G-protein coupled receptors, the H_1-, H_2-, and H_3-receptors (Hill et al., 1997). The first two histamine receptor genes cloned were H_1 (Yamashita et al., 1991) and H_2 (Ganz et al., 1991). The identification of the H_3 receptor came nearly a decade later (Lovenberg et al., 1999). A novel histamine receptor subtype, named H_4, has recently been discovered (Oda et al., 2000). The histamine receptors belong to the superfamily of G-protein coupled receptor. H_1 receptors are coupled, via the α subunits of the $G_{q/11}$ family, to phospholipase C, and their activation leads to formation of inositol-1,4,5-trisphosphate (IP_3) and diacylglycerol from phospholipids in the cell membrane; IP_3 cause a rapid release of Ca^{2+} from the endoplasmic reticulum. Diacylglycerol activates protein kinase C, while Ca^{2+} activates Ca^{2+}/calmodulin-dependent protein kinases and phospholipase A_2 in the target cell. H_2-receptors are linked, via the α subunits of the G_s family, to the stimulation of adenylyl cyclase and thus to the activation of cyclic AMP-dependent protein kinase in the target cell. H_3 and H_4-receptors are coupled via G_i-proteins to the inhibition of adenylyl cyclase (Hill et al., 1997; Leurs et al., 2002).

A large number of studies have examined the role of the central histamine system in modulating nociception.

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Whilst peripheral histamine is involved in stimulation of nociceptive fibers, it has emerged that central histamine plays an important role in antinociception (Hough, 1988). Histamine itself has been described to produce an increase of the pain threshold of central origin. When administered into the cerebral ventricles or periaqueductal gray, it has been reported to have antinociceptive effects in various analgesic tests in mice and rats (Glick and Crane, 1978; Chung et al., 1984; Malmberg-Aiello et al., 1994). Subsequently, it has been demonstrated that substances that increase histamine brain levels, such as the precursor L-histidine, the H_3-receptor antagonist thioperamide, and the catabolism inhibitor metoprine, are able to induce antinociception as well (Malmberg-Aiello et al., 1994). Conversely, reductions in brain histamine concentrations by administration of α-fluoromethylhistidine (α-FMH) or H_3-receptor agonist have a pronociceptive action or block the effects of the histamine increasing agents (Malmberg-Aiello et al., 1994, 1997).

However, not only the H_3-receptor subtype, but also the H_2 and H_1-receptors appear to be involved in the modulation of pain perception induced by the histaminergic system. Several evidences indicate that the H_2 subtype is involved in the histamine-mediated antinociception (Brown et al., 2001). An important role in the modulation of pain perception is also played by the H_1-receptor subtype. The H_1-receptor antagonists are widely used as adjuvants in preoperative analgesia as well as in postoperative pain. H_1-receptor antagonists are also able to potentiate opioid analgesia in laboratory animals (Sun et al., 1985). It has also been observed that some antihistamines, such as hydroxyzine, diphenhydramine, pyrilamine and promethazine, are endowed with analgesic properties in both laboratory animals (Rumore and Schlichting, 1985; Sun et al., 1985; Rumore and Schlichting, 1986) and humans (Rumore and Schlichting, 1986). More recently, it has been reported that the activation of H_1-receptors by using selective agonists not only prevents the antinociception induced by H_1-receptor antagonists, but also increases sensitivity to noxious stimuli in rodents (Malmberg-Aiello et al., 1998).

At present, the involvement of the H_1-receptor subtype in pain perception has been documented. However, the intracellular mechanisms responsible for the modulation of pain threshold by H_1-receptors are not elucidated. The aim of the present study was to determine whether the stimulatory effect of H_1-receptor agonists on PLC, and on DAG- and IP_3-mediated intracellular pathways, participates in the mechanism of central hypernociception following activation of the H_1-mediated histaminergic system. To this purpose, 2-(3-trifluoromethylphenyl)histamine (FMPH), a new, potent and selective H_1-receptor agonist (Leschke et al., 1995) was employed.

2. Methods

2.1. Animals

Male Swiss albino mice (24–26 g) from Morini (San Polo d'Enza, Italy) were used. Twelve mice were housed per cage. The cages were placed in the experimental room 24 h before the test for acclimatization. The animals were fed a standard laboratory diet and tap water ad libitum and kept at 22 ± 1 °C with a 12 h light/dark cycle, light on at 7 a.m. All experiments were carried out in accordance with the NIH Guide for the Care and Use of Laboratory animals. All efforts were made to minimize animal suffering, and to reduce the number of animals used.

2.2. Hot-plate test

The method adopted was described by O'Callaghan and Holtzman (1975). Mice were placed inside a stainless steel container, which was set thermostatically at 52.5 ± 0.1 °C in a precision water-bath from KW Mechanical Workshop, Siena, Italy. Reaction times (s), were measured with a stopwatch before and 15, 30, 45 and 60 min after administration of the hyperalgesic drug. The endpoint used was the licking of the fore or hind paws. Those mice scoring less than 12 and more than 18 s in the pretest were rejected (30%). Licking latency values reported in the figures were recorded 15 min after administration of FMPH in correspondence with its maximum effect. Fourteen mice per group were tested.

2.3. Rota-rod test

The apparatus consisted of a base platform and a rotating rod with a diameter of 3 cm and a non-slippery surface. The rod was placed at a height of 15 cm from the base. The rod, 30 cm in length, was divided into five equal sections by six disks. Thus, up to five mice were tested simultaneously on the apparatus, with a rod-rotating speed of 16 r.p.m. The integrity of motor coordination was assessed on the basis of the number of falls from the rod in 30 s according to Vaught et al. (1985). Those mice scoring less than 3 and more than 6 falls in the pretest were rejected (20%). The performance time was measured before (pretest) and 15, 30 and 45 min after the beginning of the test. Ten mice per group were tested.

2.4. Hole-board test

The hole-board test consisted of a 40 cm square plane with 16 flush mounted cylindrical holes (3 cm diameter) distributed 4 by 4 in an equidistant, grid-like manner. Mice were placed on the center of the board one by one and allowed to move about freely for a per-

iod of 5 min each. Two electric eyes, crossing the plane from mid-point to mid-point of opposite sides, thus dividing the plane into four equal quadrants, automatically signaled the movement of the animal (counts in 5 min) on the surface of the plane (spontaneous motility). Miniature photoelectric cells, in each of the 16 holes, recorded (counts in 5 min) the exploration of the holes (exploratory activity) by the mice. Ten mice per group were tested.

2.5. I.c.v. injection technique

I.c.v. administration was performed under ether anesthesia with isotonic saline as solvent, according to the method described by Haley and McCormick (1957). During anesthesia, mice were grasped firmly by the loose skin behind the head. A hypodermic needle (0.4 mm external diameter) attached to a 10 μl syringe was inserted perpendicularly through the skull and no more than 2 mm into the brain of the mouse, where 5 μl solution was then administered. The injection site was 1 mm to the right or left from the midpoint on a line drawn through to the anterior base of the ears. Injections were performed randomly into the right or left ventricle. To ascertain that solutions were administered exactly into the cerebral ventricle, some mice were injected with 5 μl of diluted 1:10 India ink and their brains were examined macroscopically after sectioning. The accuracy of the injection technique was evaluated with 95% of injections being correct.

2.6. Drugs

The following drugs were used: FMPH (2-(3-trifluoromethylphenyl)histamine dihydrogenmaleate) (Institute of Pharmacy, Freie Universität, Berlin); TMB-8 (8-(*N*,*N*-diethylamino)-octyl-3,4,5-trimethoxybenzoate) hydrochloride, heparin sodium salt (mol. wt: approx. 60,000), lithium chloride, (Sigma, Milan, Italy); calphostin C, chelerytrine, U-73122 (1-[6-[[17β-3-methoxyestra-4,3,5(10)-trien-17-yl]amino]hexyl]1*H*-pyrrole-2,5-dione), U-73343 (1-[6-[[17β-3-methoxyestra-1,3,5(10)-trien-17-yl]amino]hexyl]-1*H*-pyrrolidinedione), neomycin sulphate, phorbol-12,13-dibutyrate (PDBu), phorbol-12-myristate-13-acetate (PMA), thapsigargin, D-myo inositol 1,4,5-trisphosphate hexasodium salt (Calbiochem, Milan, Italy); D-amphetamine (De Angeli, Rome, Italy). Other chemicals were of the highest quality commercially available. U-73122, U-73343, calphostin C, chelerytrine, PMA, PDBu were dissolved in 0.5% DMSO, thapsigargin was dissolved in 30% DMSO, whereas all other drugs were dissolved in isotonic (NaCl 0.9%) saline solution immediately before use. Drug concentrations were prepared so that the necessary dose could be administered in a volume of 5 μl per mouse by intracerebroventricular (i.c.v.) injection and 10 ml kg^{-1} by subcutaneous (s.c.) injection. Thapsigargin was injected 105 min before the test; chelerytrine was injected 90 min before the test; PMA, PDBu, and calphostin C were injected i.c.v. 1 h before the test; LiCl was administered s.c. 18 h before the test; U-73122, U-73343, TMB-8, heparin, D-myo inositol were injected 15 min before behavioral tests. Doses and administration schedule were chosen on the bases of time-course and dose–response experiments previously performed in our laboratory. Furthermore, literature data confirm the selectivity and efficacy of the above-mentioned treatments at time and concentration used.

2.7. Statistical analysis

All experimental results are given as the mean ± SEM. An analysis of variance ANOVA, followed by Fisher's protected least significant difference procedure for post-hoc comparison were used to verify significance between two means of behavioral results. Data were analyzed with the StatView software for the Macintosh (1992). *P* values of less than 0.05 were considered significant.

3. Results

3.1. Role of PLC on FMPH-induced hyperalgesia

The H_1-receptor agonist FMPH, at the dose of 6.5 μg per mouse i.c.v., induced hyperalgesia in the mouse hot-plate test which peaked 15 min after administration and then it slowly diminished, disappearing after 60 min (data not shown). The administration of the PLC inhibitor U-73122 (0.6–5.0 μg per mouse i.c.v.) dose-dependently prevented the hypernociception induced by FMPH (6.5 μg per mouse i.c.v.) in the mouse hot-plate test. The U-73122 dose of 0.6 μg per mouse i.c.v. was devoid of any effect, whereas the maximum antagonistic effect was reached at 5.0 μg per mouse i.c.v. By contrast U-73343 (5.0 μg per mouse i.c.v.), an analogue of U-73122 inactive on PLC, used as negative control, did not alter the reduction of pain threshold produced by the H_1-receptor agonist (Fig. 1A).

The PLC inhibitor neomycin (5–20 μg per mouse i.c.v.) dose-dependently prevented the FMPH hyperalgesic effect, reaching its maximum effect at 10 μg per mouse i.c.v. (Fig. 1B).

U-73122 and neomycin, when injected alone, produced neither an hyperalgesic nor an analgesic effect (Fig. 1A,B).

3.2. Role of PKC on FMPH-induced hyperalgesia

Pretreatment with the PKC blocker calphostin C produced a dose-dependent prevention of FMPH-induced (0.6 μg per mouse i.c.v.) hyperalgesia in the

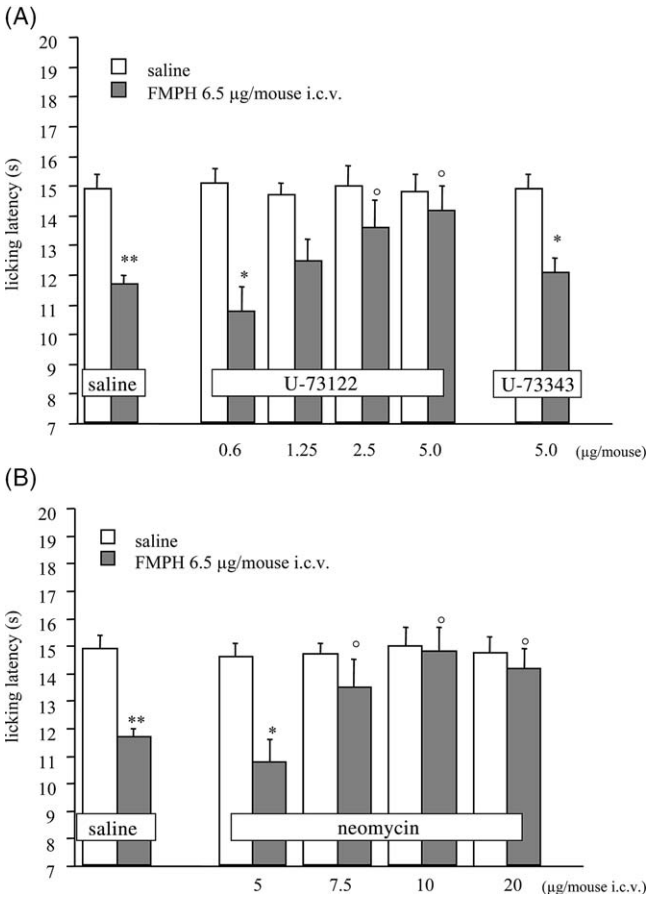

Fig. 1. (A): Prevention by pretreatment with U-73122 (0.6–5 μg per mouse i.c.v.) and lack of effect by U-73343 (5 μg per mouse i.c.v.) of FMPH-induced hyperalgesia. (B): Prevention by pretreatment with neomycin (5–20 μg per mouse i.c.v.) of FMPH-induced hyperalgesia in the mouse hot-plate test. The licking latency values were recorded 15 min after FMPH administration. Vertical lines represent s.e.m. $^{*}P < 0.05$, $^{**}P < 0.01$ in comparison with saline group. $^{\circ}P < 0.05$ in comparison with FMPH-treated group.

mouse hot-plate test. The maximum effect was obtained at the dose of 0.2 μg per mouse i.c.v., whereas the increase of the licking latency values produced by the dose of 0.15 μg per mouse i.c.v. was not statistically significant. The dose of 0.1 μg per mouse i.c.v. was devoid of any effect (Fig. 2). Similarly, pretreatment with chelerytrine (2.5 μg per mouse i.c.v.), another PKC blocker, reverted the hypernociception produced by the H_1-receptor agonist (Fig. 2). Calphostin C (0.1–0.2 μg per mouse i.c.v.) and chelerytrine (1–2.5 μg per mouse i.c.v.) exerted their antagonistic effect without modifying the mouse pain threshold when administered alone (Fig. 2).

The PKC activators PDBu (1–40 pmol per mouse i.c.v.) and PMA (0.1–15 pmol per mouse i.c.v.) were unable to modify the hyperalgesic effect induced by FMPH (Fig. 3). At the doses employed, the two PKC activators did not modify the animals' licking latency values in comparison with control animals (Fig. 3).

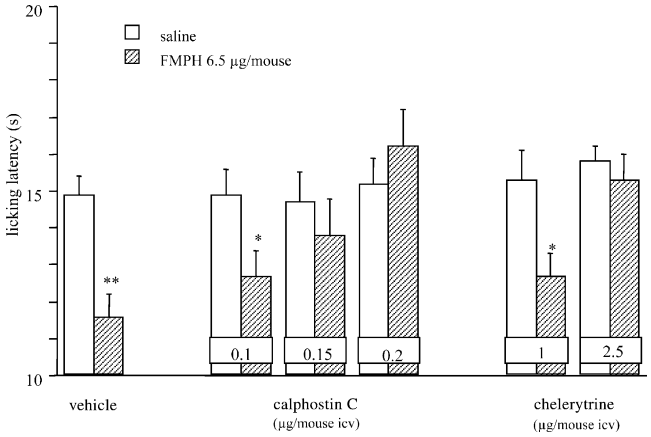

Fig. 2. Prevention by pretreatment with calphostin C (0.1–0.2 μg per mouse i.c.v.) and chelerytrine (1-2.5 μg per mouse i.c.v.) of FMPH (0.6 μg per mouse i.c.v.)-induced hypernociception in the mouse hot-plate test. The licking latency values were recorded 15 min after FMPH administration. Vertical lines represent s.e.m. $^{*}P < 0.05$ in comparison with control group.

3.3. Role of IP_3 on FMPH-induced hyperalgesia

Pretreatment with LiCl (40–200–400 mg kg^{-1} s.c.), which impairs phosphatidylinositol synthesis, 18 h before the test did not prevent the hyperalgesia induced by FMPH (6.5 μg per mouse i.c.v.) in the mouse hot-plate test. The injection of LiCl (400 mg kg^{-1} s.c.) alone, did not modify the licking latency values of mice in comparison with saline-treated animals (Fig. 4A).

Similarly, the administration of heparin (1–40 μg per mouse i.c.v.), an antagonist of IP_3-receptors, did not prevent the decrease of the pain threshold induced by FMPH (6.5 μg per mouse i.c.v.). Furthermore, pretreatment with D-myo inositol did not produce any

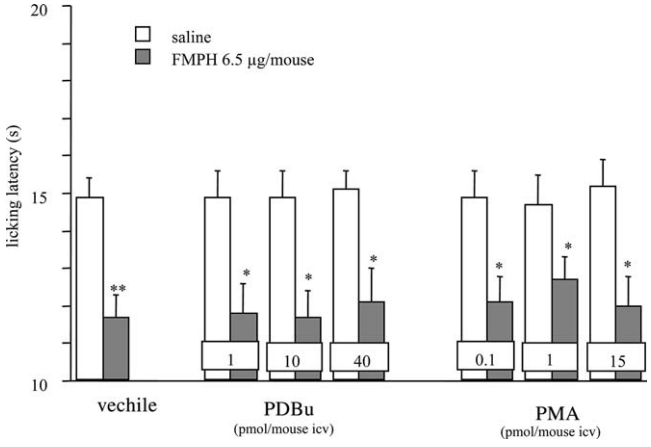

Fig. 3. Lack of effect of PDBu (1–40 pmol per mouse i.c.v) and PMA (1–15 pmol per mouse i.c.v.) of FMPH (0.6 μg per mouse i.c.v.)-induced hypernociception in the mouse hot-plate test. The licking latency values were recorded 15 min after FMPH administration. Vertical lines represent s.e.m. $^{*}P < 0.05$ in comparison with control group.

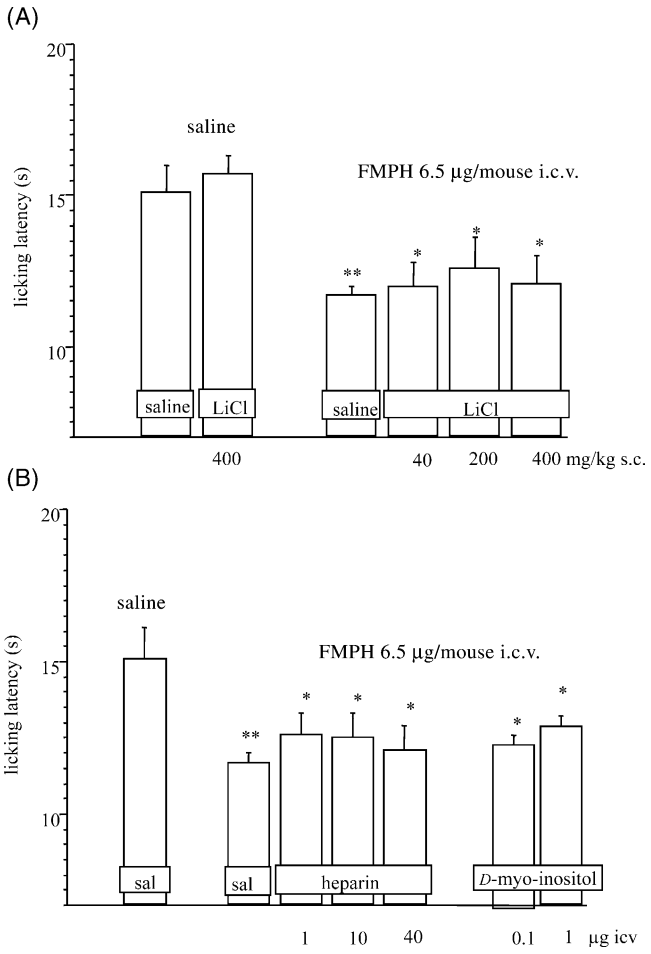

Fig. 4. (A) Lack of effect of LiCl (40–400 mg kg^{-1} s.c.) on FMPH (0.6 µg per mouse i.c.v.)-induced hypernociception. (B) Lack of effect of heparin (1–40 µg per mouse i.c.v.) on FMPH (0.6 µg per mouse i.c.v.)-induced hypernociception. The licking latency values were recorded 15 min after FMPH administration. Vertical lines represent s.e.m. $^{*}P < 0.05$, $^{**}P < 0.01$ in comparison with control group.

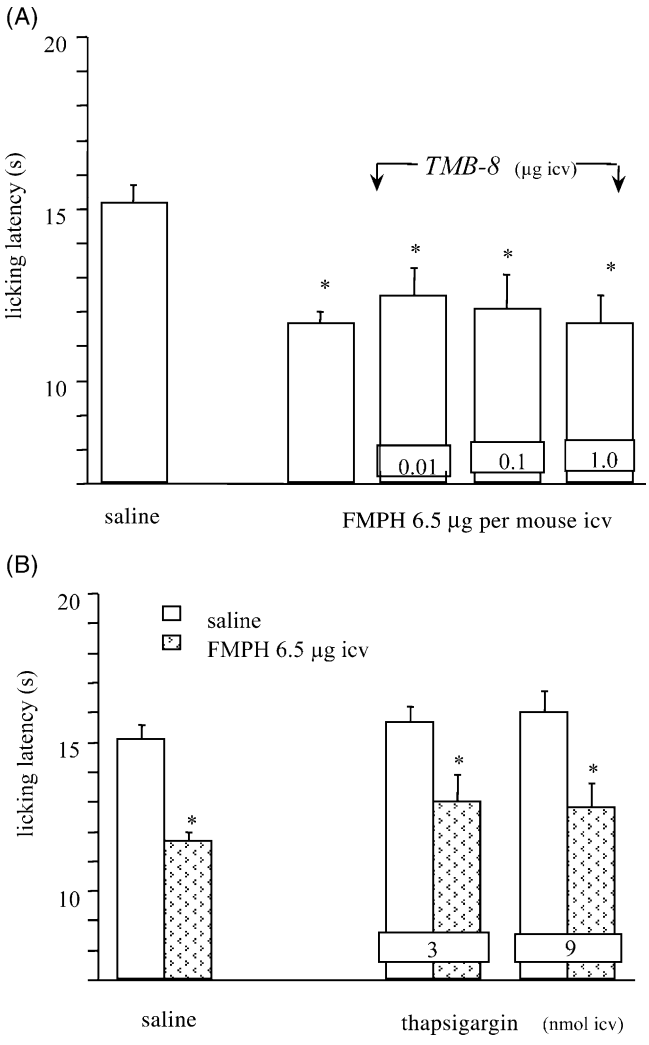

Fig. 5. (A) Lack of effect of TMB-8 (0.01–1.0 µg per mouse i.c.v.) on FMPH hypernociception in the mouse hot-plate test. (B) Lack of effect of thapsigargin (3–9 nmol per mouse i.c.v.) on FMPH hypernociception in the mouse hot-plate test. The licking latency values were recorded 15 min after FMPH administration. Vertical lines represent s.e.m. $^{*}P < 0.05$ in comparison with control group.

effect on the H_1-receptor agonist hyperalgesia (Fig. 4B). Both heparin and D-myo inositol, when injected alone, did not alter the mouse pain threshold in comparison with the control group (data not shown).

3.4. Role of intracellular Ca^{2+} on FMPH-induced hyperalgesia

TMB-8 (0.01–1 µg per mouse i.c.v.), a blocker of Ca^{2+} release from intracellular stores, administered 15 min before the beginning of the test, did not antagonize FMPH-induced hyperalgesia in the mouse hot-plate test, as illustrated in Fig. 5A.

Pretreatment with thapsigargin (3–9 nmol per mouse i.c.v.), an inhibitor of Ca^{2+} uptake into the endoplasmic reticulum by inhibiting the sarco-endoplasmatic reticulum Ca^{2+}-ATPases, was unable to modify the reduction of pain threshold induced by the investigated H_1-receptor agonist (Fig. 5B).

3.5. Effect of treatments on mouse behavior

The compounds used in the present study, at the highest doses investigated, were tested in order to assess their effect on mouse behavior. Mice pretreated with LiCl (400 mg kg^{-1} s.c.), TMB-8 (1 µg per mouse i.c.v.), neomycin (20 µg per mouse i.c.v.), heparin (40 µg per mouse i.c.v.), D-myo inositol (1 µg per mouse i.c.v.), U-73122 (5 µg per mouse i.c.v.), U-73343 (5 µg per mouse i.c.v.), calphostin C (0.2 µg per mouse i.c.v.), chelerytrine (2.5 µg per mouse i.c.v.), PMA (15 pmol per mouse i.c.v.), PDBu (40 pmol per mouse i.c.v.), thapsigargin (9 nmol per mouse i.c.v.) were evaluated for motor coordination by use of the rota-rod test, and for spontaneous motility and inspection activity by use of the hole-board test.

The endurance time on the rotating rod, evaluated before and 15, 30 and 45 min after the beginning of the rota-rod test, indicated the lack of any impairment in the motor coordination of animals pretreated with LiCl, TMB-8, neomycin, D-myo inositol, heparin or pretreated with U-73122, U-73343, alphostin C, chelerytrine, PMA, PDBu, thapsigargin in comparison, respectively, with saline (Fig. 6A) or vehicle group (Fig. 6B).

The spontaneous motility (Fig. 7) as well as the inspection activity (data not shown) of mice, expressed as counts in 10 min, was unmodified by the above-mentioned compounds in comparison with the corresponding control group.

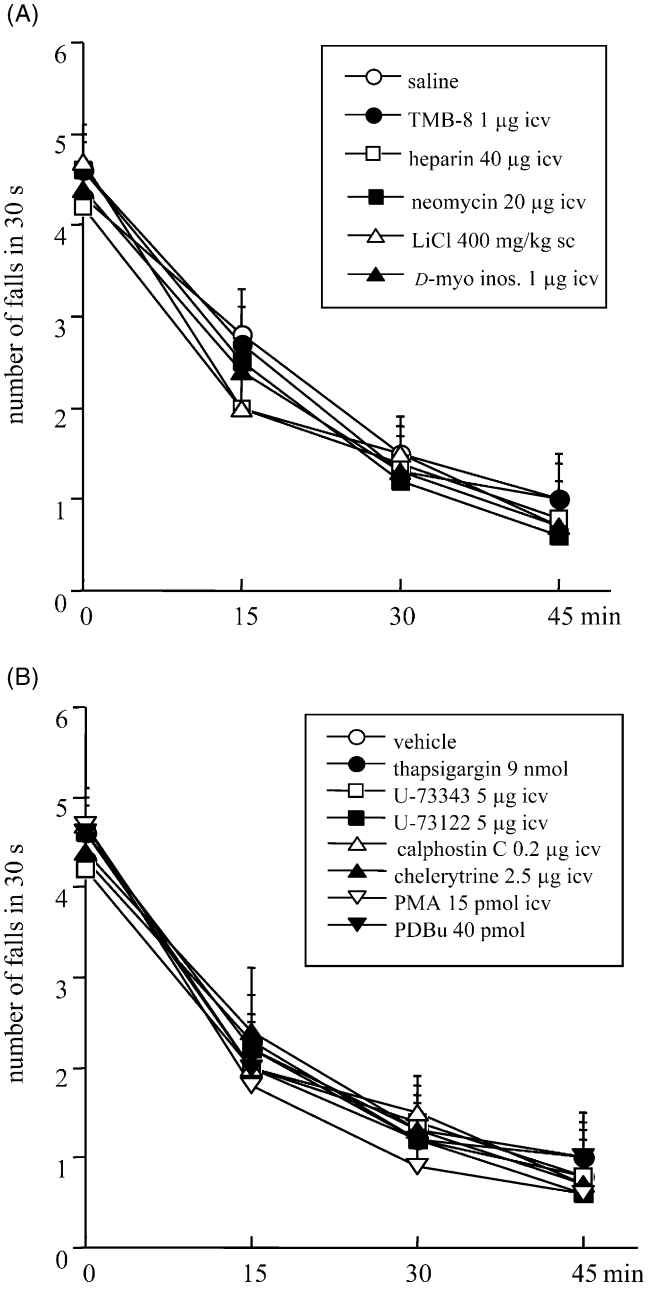

Fig. 6. Lack of effect of intracellular pharmacological modulators on motor coordination in the mouse rota-rod test in comparison with saline (panel A) or vehicle (panel B). Vertical lines represent s.e.m.

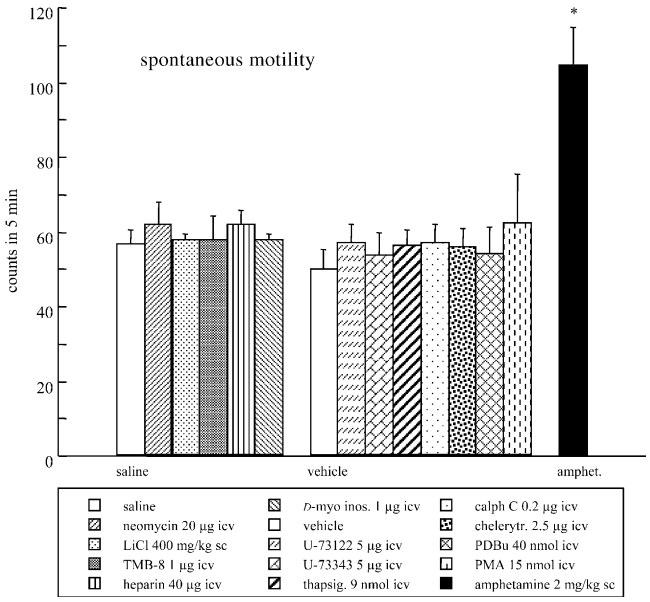

Fig. 7. Lack of effect of intracellular pharmacological modulators on spontaneous motility in the mouse hole-board test in comparison with saline or vehicle. Vertical lines represent s.e.m.

4. Discussion

The present study investigated and elucidated the intracellular mechanisms involved in central hyperalgesia induced by H_1-receptor activation in a condition of acute thermal nociception in normal animals. The importance of the receptor-mediated activation of the PLC–PKC pathway to obtain a decrease of the pain threshold by histamine H_1-receptor stimulation was demonstrated.

Several PLC inhibitors are currently available. U-73122, an aminosteroid, has been found to be a potent inhibitor of aggregation of human platelets induced by a variety of agonists, and this compound has been further characterized as an inhibitor of G-protein-mediated PLC (Wakdo et al., 1983; Yule et al., 1992). Neomycin has been reported to inhibit hormone-stimulated IP_3 production through the blockade of PLC (Phillippe, 1994). The administration of U-73122 and neomycin dose-dependently prevented the hypernociception induced by 2-(3-trifluoromethylphenyl)-histamine (FMPH), a new, potent and selective H_1-receptor agonist (Leschke et al., 1995). By contrast, U-73343, a succinimido analogue used as negative control for U-73122 being a weak inhibitor of PLC (Bleasdale et al., 1990; Smith et al., 1990), did not modify the hyperalgesia induced by FMPH. These results are in agreement with a previous study which illustrated the

prevention by U-73122, but not U-73343, of the nociceptive response induced by i.pl. administration of histamine in mice (Ueda and Inoue, 2000). It has been reported that U-73122 and U-73343 bind to the histamine H_1-receptor in the concentration range in which U-73122 acts as an inhibitor of PLC. This effect could compromise both the use of U-73122 to provide evidence that an H_1 agonist action is mediated via PLC and the use of U-73343 as negative control (Hughes et al., 2000). The lack of any antagonistic effect by U-73343 observed in the present study suggests that the prevention of FMPH-induced hyperalgesia by U-73122 is mediated by PLC activation. This hypothesis is also supported by the reversal of FMPH hypernociception produced by neomycin. These results indicate that the stimulation of H_1-receptors by i.c.v administration of FMPH activates the PLC pathway and provides evidence that PI-specific PLC (PLCβ) is involved in the H_1-mediated central hyperalgesia.

PLC isozymes hydrolyze the highly phosphorylated lipid phosphatidylinositol 4,5-biphosphate generating two intracellular products: DAG, an activator of PKC, and IP_3, a universal calcium-mobilizing second messenger. The involvement of PKC in the mechanisms that underlie the development of acute and persistent pain has been widely reported (Basbaum, 1999; Malmberg, 2000; Zimmermann, 2001; Kamei et al., 2001). In the present study, pretreatment with calphostin C and chelerytrine, selective, potent and membrane-permeable PKC inhibitors (Kobayashi et al., 1989; Herbert et al., 1990), dose-dependently reversed the hyperalgesia induced by the H_1-receptor agonist FMPH. Furthermore, activation of PKC by phorbol esters, such as PMA and PDBu (Nishizuka, 1992), did not modify the decrease of pain threshold induced by FMPH. These data clearly indicate that activation of PKC constitutes a significant pathway involved in the central nociceptive response induced by H_1-receptor agonists. Stimulation of histamine H_1-receptors has been shown to induce the expression of the proto-oncogene c-fos, a neural marker of pain (Harris, 1998), in human airway (Panettieri et al., 1990), vascular smooth muscle cells (Satoh et al., 1994), hypothalamic neurons (Kjaer et al., 1994), human T lymphocytes (Kitamura et al., 1996). Recently, it has been reported that the PKC inhibitor Ro-31-8220 was able to inhibit completely the c-fos response to histamine in CHO-H1 cells expressing the H_1-receptor (Megson et al., 2001), confirming a role of PKC in mediating the nociceptive response induced by H_1-receptor activation.

In addition to causing the activation of PKC, PLC induces IP_3 formation. To investigate the IP_3-mediated pathway, LiCl, an uncompetitive inhibitor of inositol monophosphatase, which regenerates inositol from inositol monophosphate, was used. The LiCl-induced inhibition depletes inositol and prevents the formation of IP_3 (Kennedy et al., 1990; Phiel and Klein, 2001). Animals pretreated with LiCl did not show any alteration of the hypernociceptive response to administration of FMPH. The lack of involvement of IP_3 in H_1-induced hyperalgesia was also confirmed by the lack of effect of low molecular weight heparin on FMPH-induced decrease of pain threshold. Heparin is an IP_3-receptor antagonist (Jonas et al., 1997). This compound must be injected into cells or perfused onto permeabilized cells because of its high molecular weight (12,000–13,000 Da) and lack of membrane permeability. Some evidence indicates that the low molecular weight heparin (6000 Da) used in this study is membrane permeable. Perfusion of low molecular weight heparin over a non-permeabilized cerebellar slice preparation attenuated glutamate-stimulated increases in free intracellular Ca^{2+} (Jonas et al., 1997). Present results indicate that IP_3 production does not play a major role in the H_1-mediated hypernociceptive response. IP_3, through the interaction with specific receptors located on the endoplasmic reticulum, causes release of Ca^{2+} from intracellular stores into the cytoplasm (Mignery and Sudhof, 1990; Ferris et al., 1992). Since numerous central and peripheral effects induced by H_1-receptor agonists are mediated through variation of the intracellular Ca^{2+} levels (Hill et al., 1997; Brown et al., 2001), we thought it worthwhile to investigate the role played by the intracellular Ca^{2+} in the mechanism of action of FMPH. To this purpose, TMB-8, an agent that antagonizes the mobilization of Ca^{2+} from intracellular stores (Malagodi and Chiou, 1974), and thapsigargin, an inhibitor of Ca^{2+} uptake into the endoplasmic reticulum by inhibiting the sarco-endoplasmatic reticulum Ca^{2+}-ATPases (Treiman et al., 1998) were used. Pretreatment with TMB-8, as well as with thapsigargin, did not antagonize the decrease of pain threshold induced by the investigated H_1-receptor agonist suggesting that the release of Ca^{2+} from intracellular stores is not necessary to produce histaminergic hyperalgesia. These results are in agreement with a reference in which the lack of effect of thapsigargin on preventing histamine hyperalgesia was observed (Ueda and Inoue, 2000). However, in the same study, the prevention of histamine hyperalgesia by the i.pl. administration of the IP_3 antagonist xestospongin C was reported. The discrepancy between the literature data and the lack of effect produced by the IP_3 antagonist heparin that we observed might let us hypothesize that the central and peripheral histaminergic nociceptive responses are mediated by different intracellular pathways. Furthermore, it should also be considered that in the study of Ueda and Inoue the activation of the histaminergic system was made by i.pl. administration of histamine and we cannot exclude the involvement of a receptor subtype different from the H_1 subtype able to, directly or indirectly, activate the IP_3-mediated

intracellular pathway. As confirmation of the lack of involvement of the PLC–IP_3 intracellular signaling pathway in the hyperalgesia induced by FMPH, we observed no effect by pretreatment with D-myo inositol 1,4,5-trisphosphate on the reduction of the licking latency produced by the administration of the H_1 agonist.

The lack of effect produced by LiCl, TMB-8, heparin, thapsigargin and D-myo inositol-1,4,5-trisphosphate cannot be imputable to the administration of inactive concentrations. At the highest doses used, LiCl, TMB-8 and heparin were able to prevent analgesia induced by cholinomimetic drugs (Galeotti et al., 2002), μ- and δ-opioid agonists (Ohsawa et al., 1998; Narita et al., 2000), thapsigargin was able to potentiate analgesia induced by δ-opioid agonists (Ohsawa et al., 1998) and nicotine (Damaj et al., 1993), and D-myo inositol-1,4,5-trisphosphate restored opioid analgesia in LiCl-pretreated mice (Narita et al., 2000).

The highest doses used of U-73122, neomycin, LiCl, heparin, TMB-8, thapsigargin, calphostin C, chelerytrine, PMA and PDBu, in the absence of co-administration of FMPH, did not modify the licking latency values of mice in comparison with control groups excluding that the prevention of FMPH hyperalgesic effect origins from antinociceptive properties of the compounds employed.

The receptor-mediated activation of the histaminergic system, as well as the modulation of the intracellular events promoted by H_1 agonists, can induce several side effects. It is known that the PKC activator PMA and PDBu are convulsant (Smith and Meldrum, 1992) and, similarly, LiCl can induce neurological toxicity characterized by tremors, convulsion, ataxia (Kores and Lader, 1997). All the compounds, at the highest active doses employed in the present study, did not cause any detectable modification in mouse gross behavior. At the same doses, all treatments did not impair motor coordination nor modify spontaneous motility nor inspection activity in comparison with control groups excluding that the results obtained were due to animals' altered viability. It should be noted that higher doses of the intracellular modulators could not be investigated since they induced toxicity (tremors, convulsions, etc.) in animals. The induction of toxicity can also be considered as an indication not only of the diffusion of these compounds in the brain, but also of the consequent reaching of key targets by using the administration schedule employed in the present study.

Seen as a whole, our data evidence the role of PLC–PKC, through DAG generation, in the induction of the central nociceptive response by H_1-receptor activation in a condition of acute thermal pain in mice. By contrast, the concomitant activation of the PLC–IP_3 pathway does not appear to play a major role in FMPH hyperalgesia.

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