The role of potassium channels in antihistamine analgesia

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Abstract

The effect of the administration of pertussis toxin as well as modulators of different subtypes of K+ channels on the antinociception induced by the H1-antihistamines pyrilamine, diphenhydramine and promethazine was evaluated in the mouse hot plate test. Pretreatment with pertussis toxin (0.25 µg/mouse i.c.v.) prevented pyrilamine, diphenhydramine and promethazine antinociception. The K_ATP channel openers minoxidil and pinacidil potentiated the antinociception produced by the H1-antihistamines whereas the K_ATP channel blocker gliquidone prevented the anti H1-induced analgesia. The Ca2+-gated K+ channel blocker apamin antagonized pyrilamine, diphenhydramine and promethazine analgesia. Pretreatment with an antisense oligonucleotide (aODN) to mKv1.1, a voltage-gated K+ channel, at the dose of 3.0 nmol/single i.c.v. injection, never modified the antinociception induced by the H1-antihistamines in comparison with degenerate oligonucleotide (dODN)-treated mice. At the highest effective doses, none of the drugs used modified animals’ gross behaviour nor impaired motor coordination, as revealed by the rota rod test. The present data demonstrate that both K_ATP and Ca2+-gated K+ channels, contrary to voltage-gated K+ channel Kv1.1, represent an important step in the transduction mechanism underlying central antinociception induced by H1-antihistamines. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: K+ channel; Antinociception; H1-antihistamines; KATP channel; Ca2+-gated K+ channel; mKv1.1; Pertussis toxin

1. Introduction

The H1-receptor antagonists are among the most widely used medications in the world. These compounds produce inhibition of the effects of histamine mediated by H1 receptors such as smooth-muscle contraction in the respiratory and gastrointestinal tracts, pruritus, sneezing by sensory-nerve stimulation and vasodilation (Simons and Simons, 1994). Therefore, their most common use is as antiallergic drugs. In addition to these well known peripheral effects, H1-receptor antagonists produce various central inhibitory actions (Simons and Simons, 1994). Antihistamines have been shown to be analgesic adjuvants in both animal and human studies. They are widely used as adjuvants in preoperative analgesia as well as in postoperative pain (Hupert et al., 1980; Sunshine et al., 1989). Clinically, hydroxyzine decreases the amount of narcotic that is necessary, provides sedating, and other antihistaminic effects that are helpful in certain clinical situations. Furthermore, hydroxyzine itself has some analgesic effect (Stambaugh and Lane, 1983; Rumore and Schlichting, 1986). It has also been observed that some other antihistaminics, such as diphenhydramine, pyrilamine and promethazine, are endowed with analgesic properties in both laboratory animals (Rumore and Schlichting, 1985) and humans (Campos and Solis, 1980; Rumore and Schlichting, 1986).

Central K+ channels appear to be involved in the modulation of pain perception. The central administration of K+ channel openers, such as diazoxide, minoxidil, lemakalim and cromakalim, has been reported to produce antinociception in laboratory animals (Welch and Dunlow, 1993; Narita et al., 1993) and to potentiate the enhancement of the pain threshold produced by opioid and α2-adrenoceptor agonists (Vergoni et al., 1992; Ocaña et al., 1996). Furthermore, treatment with K+ channel blockers, such as sulphonyleureas, 4-aminopyridine and tetraethylammonium, prevented the antinociception induced by α2-adrenoceptor, GABA and opioid receptor agonists (Ocaña and Baeyens, 1993; Raffa and Martinez, 1995).
Histamine exerts multiple effects on neurones including modulation of \( K^+ \) currents. The \( Ca^{2+} \)-activated \( K^+ \) current in rat hippocampal neurones was suppressed by histamine (Haas and Konnerth, 1983). Histamine decreased a resting or ‘leak’ \( K^+ \) current and shifted the voltage dependency of a hyperpolarization-activated current in the lateral geniculate nucleus of the guinea-pig (Mc Cormick and Williamson, 1991) as well as reduced \( K^+ \) currents in neostriatal interneurones (Munakata and Akaike, 1994). Furthermore, the involvement of the \( H_1 \) receptor subtype in the block of \( K^+ \) currents produced by histamine has been reported (Munakata and Akaike, 1994; Jafri et al., 1997). We, therefore, thought it worthwhile to investigate the role of \( K^+ \) channels in the antinociception induced by \( H_1 \)-receptor antagonists. Since several kinds of \( K^+ \) channels with different electrophysiological characteristics and pharmacological sensitivities have been described in neurones (Halliwell, 1990; Aronson, 1992), in the present work we employed different \( K^+ \) channel modulators. Apamin has been reported to specifically block currents through \( Ca^{2+} \)-activated \( K^+ \) channels (Cook, 1988). Sulphonylureas such as gliclazide block \( K_{ATP} \) channels in neurones whereas minoxidil and pinacidil open the same type \( (K_{ATP}) \) of \( K^+ \) channel (Edwards and Weston, 1993). So far, the blockers of neuronal voltage-dependent \( K^+ \) channels are not selective (Cook and Quast, 1990; Halliwell, 1990). An antisense oligonucleotide \( (aODN) \) was, therefore, used as a selective blocker of \( mKv1.1 \), a voltage-gated \( K^+ \) channel (Wang et al., 1994). \( aODNs \) are short synthetic DNA segments complementary to sequences of an mRNA target. By forming DNA/mRNA heteroduplexes, \( aODNs \) can transiently inactivate single genes.

To this purpose we have evaluated the effects produced by the \( K^+ \) channel blockers apamin and gliclazide, the \( K^+ \) channel openers minoxidil and pinacidil, as well as an \( aODN \) to \( mKv1.1 \) in the antinociception induced by \( H_1 \)-receptor antagonists in the mouse hot-plate test.

We also investigated whether antinociception induced by pyrilamine and promethazine, like that produced by diphenhydramine (Galeotti et al., 1996), was prevented by intracerebroventricular administration of pertussis toxin.

2. Methods

2.1. Animals

Male Swiss albino mice (23–30 g) from the Morini (San Polo d’Enza, Italy) breeding farm were used. Fifteen 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 23 ± 1°C with a 12 h light–dark cycle, light on at 07:00.
Fig. 2. Prevention by pertussis toxin (PTX) pretreatment of antinociception induced by pyrilamine (15 mg kg s.c.), diphenhydramine (20 mg/kg s.c.) and promethazine (6 mg/kg s.c.) in the mouse hot-plate test. The test was performed 7 days after a single i.c.v. injection of vehicle or PTX (0.25 mg mouse i.c.v.). Vertical lines represent SEM; between 14 and 18 mice were tested. * \( P < 0.05 \), ** \( P < 0.01 \) in comparison with corresponding analgesic-treated mice.

2.3. 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 pyrilamine, diphenhydramine and promethazine. 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%). An arbitrary cut-off time of 45 s was adopted.

The licking latency values reported in all figures were evaluated in relation to the maximum analgesic effect of pyrilamine, diphenhydramine and promethazine which was reached 15 min after administration.

2.4. Rota-rod test

The test was performed according to the method described by Kuribara et al. (1977). Briefly, the apparatus consisted of a base platform and a rotating rod of 3 cm diameter with 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 endurance time of the animals on the rotating rod. One day before the test, the animals were trained twice. On the day of the test only the mice that were able to stay balanced on the rotating rod between 90 and 120 s (cut-off time) were selected for testing. The performance time was measured before and 15, 30, 45 and 60 min after treatment.

2.5. Antisense oligonucleotides

The 24mer phosphodiester oligonucleotides were capped by a terminal phosphorothioate double substitution and purified by chromatography (Genosys, The Woodlands, USA). The antisense ODN (5’-CGA CAT CAC CGT CAT GAT GAA AGC-3’) was designed to target the 5’ portion of the murine Kv1.1 (mKv1.1) mRNA, residues 575-598 of the published cDNA sequence (Chandy et al., 1990). A fully degenerate 24mer ODN was used as control.

Mice were randomly assigned to antisense oligodeoxyribonucleotide (mKv1.1 aODN), degenerate oligodeoxyribonucleotide (mKv1.1 dODN) or vector groups. A total of 600 \( \mu \)M ODNs were preincubated at 37°C for 30 min with 13 \( \mu \)M DOTAP (N-[1-(2,3-dioleyloxy)propyl]-N,N,N-trimethylammonium methyl sulphate), used as the vector. Each group received a single intracerebroventricular (i.c.v.) injection on days 1, 4 and 7.
2.6. Drugs

The following drugs were used: pyrilamine maleate, promethazine hydrochloride, minoxidil, pinacidil, apamin, pertussis toxin (RBI); diphenhydramine hydrochloride (De Angeli); gliclidone (Boehringer Ingelheim).

Drugs were dissolved in isotonic (NaCl 0.9%) saline solution, with the exception of pinacidil, that was dissolved in a water and dimethyl sulfoxide (DMSO) (3:1) vehicle. Drug concentrations were prepared in such a way that the necessary dose could be administered in a vol. of 5 ml/mouse by i.c.v. injection and 10 ml/kg by subcutaneous (s.c.) injection.

Apamin, gliclidone, minoxidil and pinacidil were injected i.c.v 15 min before the test. The drug administration schedule was chosen on the basis of preliminary experiments in which the time-course and the dose-response curves for every compound were determined.

Concerning pertussis toxin treatment, mice were randomly assigned to a vehicle (water solution containing 0.01 M sodium phosphate buffer, pH 7.0, with 0.05 M sodium chloride) or a pertussis toxin group (0.25 μg/mouse) which received a single i.c.v. injection on day 0. The hot-plate test was performed 7 days after pretreatment.

Following the pretreatment schedule with saline, vehicle, aODN or dODN mentioned in the above section, the antinociceptive effect of pyrilamine, diphenhydramine and promethazine was tested 72 h after the last i.c.v. injection. The administration schedule of ODNs employed was chosen on the basis of preliminary experiments in which the dose-response and time-course curves for aODN to mKv1.1 were determined (Galeotti et al., 1997a).

2.7. Statistical analysis

All experimental results are given as the mean ± SEM. Analysis of variance (ANOVA), followed by Fisher’s Protected Least Significant Difference (PLSD) procedure for post-hoc comparison, was used to verify significance between two means. Data were analysed with the StatView software for the Macintosh (1992). P < 0.05 were considered significant.
Fig. 4. Enhancement of pyrilamine (15 mg/kg s.c.), diphenhydramine (20 mg/kg s.c.) and promethazine (6 mg/kg s.c.) antinociception by the K<sub>ATP</sub> openers minoxidil (10 μg/mouse i.c.v.) and pinacidil (25 μg/mouse i.c.v.) in the mouse hot plate test. Vertical lines represent SEM; between 14 and 19 mice were tested. * P < 0.05 in comparison with corresponding analgesic-treated mice.

Fig. 5. Prevention by the Ca<sup>2+ </sup>-gated K<sup>+</sup> channel blocker apamin (0.1–1.0 ng/mouse i.c.v.) of pyrilamine (15 mg/kg s.c.), diphenhydramine (20 mg/kg s.c.) and promethazine (6 mg/kg s.c.) antinociception in the mouse hot-plate test. Vertical lines give SEM; there were 15 mice/group. * P < 0.05, ** P < 0.01 in comparison with saline-treated mice.
3. Results

3.1. Effect of pertussis toxin on H₁-antihistamine antinociception

Pyrilamine (5–15 mg/kg s.c.), diphenhydramine (10–20 mg/kg s.c.) and promethazine (3–6 mg/kg s.c.) produced a dose-dependent antinociception in the mouse hot-plate test (Fig. 1).

Pertussis toxin (PTX), administered at the dose of 0.25 μg/mouse i.c.v. 7 days prior to the test, led to a prevention of the antinociceptive effect of pyrilamine (15 mg/kg s.c.), diphenhydramine (20 mg/kg s.c.) and promethazine (6 mg/kg s.c.) as illustrated in Fig. 2.

3.2. Effect of K<sub>ATP</sub> channels modulators on H₁-antihistamine antinociception

The effect produced by the blocker (gliquidone) and the openers (minoxidil, pinacidil) of K<sub>ATP</sub> channels on the enhancement of the pain threshold produced by pyrilamine (15 mg/kg s.c.), diphenhydramine (20 mg/kg s.c.) and promethazine (6 mg/kg s.c.) was investigated in the mouse hot-plate test.

The administration of the K<sub>ATP</sub> channel blocker gliquidone (5–6 μg/mouse i.c.v.), 15 min before the mouse hot-plate test, prevented the antinociception induced by pyrilamine, diphenhydramine and promethazine (Fig. 3). A lower dose of gliquidone (3 μg/mouse i.c.v.) was ineffective (Fig. 3).

Both K<sub>ATP</sub> channel openers minoxidil (10 μg/mouse i.c.v.) and pinacidil (25 μg/mouse i.c.v.), injected 15 min before the test, potentiated the antinociceptive activity of all the H₁-antagonists investigated (Fig. 4).

In the same experimental conditions, neither gliquidone (Fig. 3) nor minoxidil and pinacidil (Fig. 4) modified the licking latency values of mice when given alone.

3.3. Effect of the Ca<sup>2+</sup>-activated K<sup>+</sup> channel blocker apamin on H₁-antihistamine antinociception

The effect produced by the Ca<sup>2+</sup>-activated K<sup>+</sup> channel blocker apamin on the enhancement of the pain threshold induced by pyrilamine (15 mg/kg s.c.), diphenhydramine (20 mg/kg s.c.) and promethazine (6 mg/kg s.c.) was investigated in the mouse hot-plate test.

Apamin (0.5–1 ng/mouse i.c.v.), injected 15 min before the test, prevented the antinociception induced by the three H₁-antihistamines investigated as illustrated in Fig. 5. A lower dose of apamin (0.1 ng/mouse i.c.v.) did not produce any effect on pyrilamine, diphenhydramine and promethazine antinociception (Fig. 5).
Furthermore, apamin, when given alone, did not show any hyperalgesic activity (Fig. 5).

3.4. Effect of an aODN to mKv1.1 on H1-antihistamine antinociception

The effect produced by repeated administration of an antisense oligonucleotide (aODN) to the mKv1.1 gene on the antinociception induced by H1-antihistamines was evaluated by using the mouse hot-plate test. The experiments were performed 72 h after the end of the aODN administration. aODN, at the concentration of 3 nmol/i.c.v. injection, did not modify the antinociception induced by pyrilamine (15 mg/kg s.c.), diphenhydramine (20/mg kg s.c.) and promethazine (6 mg/kg s.c.) in comparison with the mice pretreated with the degenerate oligonucleotide (dODN) as illustrated in Fig. 6.

The pretreatment with the dODN, used as a reference oligonucleotide, never modified H1-antihistamine antinociception in comparison with vector i.c.v.-injected mice (data not shown).

The aODN pretreatment did not modify the pain threshold in mice, having no hyperalgesic or analgesic effect (Fig. 6).

3.5. Effect of H1-antihistamines on mouse behaviour

Pyrilamine, diphenhydramine and promethazine, at the doses used in the present work, elicited their antinociceptive effect without changing the gross behaviour of the mice.

The three compounds investigated did not alter the mice motor coordination as revealed by the rotarod test (Fig. 7). The rotarod endurance time of mice treated with pyrilamine (15 mg/kg s.c.), diphenhydramine (20 mg/kg s.c.) and promethazine (6 mg/kg s.c.) was not modified in comparison with saline-treated mice (Fig. 7). On the contrary, pyrilamine, diphenhydramine and promethazine, administered at higher doses (25, 30 and 10 mg/kg s.c., respectively), produced a significant impairment of the rotarod performance by reducing the endurance time on the rotating rod (Fig. 7).

Furthermore, pyrilamine (15 mg/kg s.c.), diphenhydramine (20 mg/kg s.c.) and promethazine (6 mg/kg s.c.) did not modify the spontaneous motility of the mice as revealed by the Animex apparatus (data not shown).

4. Discussion

Present results indicate that neuronal K+ channels play an important role in the mechanism of analgesic action of the H1-receptor antagonists.

The administration of gliquidone, a potent blocker of ATP-dependent K+ channels (\(K_{\text{ATP}}\)) (Amoroso et al., 1990), prevented the antinociception produced by all the H1-antagonists investigated. Pretreatment with minoxidil and pinacidil, openers of neuronal KATP channels (Longman and Hamilton, 1992), potentiated the enhancement of the pain threshold produced by pyrilamine, diphenhydramine and promethazine. The functionality of \(K_{\text{ATP}}\) channels appears, therefore, fundamental in the antinociception induced by blockade of the H1 receptors. It has been reported that stimulation of histamine H1 receptors inhibited \(K_{\text{ATP}}\) currents (Boney and Nelson, 1996). On this basis we can suppose that this potentiation may occur because minoxidil and pinacidil facilitate the opening of \(K_{\text{ATP}}\) channels induced by the H1-antagonists.

The intracellular mechanism of the analgesic action of H1-antihistamines involves the activation of a pertussis toxin (PTX)-sensitive G-protein, since not only diphenhydramine, as previously reported (Galeotti et al., 1996), but also pyrilamine and promethazine analgesia is prevented by the i.c.v. administration of PTX. PTX-sensitive G-proteins represent the most widespread modulatory signaling pathway in neurones (Holz et al., 1986) and are responsible for modulation of ionic conductance through a direct interaction with the ion channel and/or by lowering intracellular cyclic AMP levels (Hille, 1994). \(K_{\text{ATP}}\) channels can be opened through a mechanism mediated by G-proteins. It has been reported that the interaction between the \(\alpha\)-GTP subunit and the \(K_{\text{ATP}}\) channel produces a conformational change that stimulates the opening of the channel (Edwards and Weston, 1993). In the presence of GTP\(\gamma\)S, a non hydrolyzable GTP analogue, an irreversible activation of \(K_{\text{ATP}}\) is obtained. Furthermore, the \(\alpha\) subunits involved in the modulation of \(K_{\text{ATP}}\) channel function have been identified as belonging to the \(\alpha_2\) and \(\alpha_3\) subtypes (Edwards and Weston, 1993). From this evidence it is plausible to suppose that \(K_{\text{ATP}}\) channels are involved in the analgesia induced by H1-antagonists as an intracellular effector underlying the activation of a \(G_{i,0}\) protein.

The present study also provides evidence for the involvement of \(Ca^{2+}\)-gated K+ channels in the antinociception induced by H1-antihistamines. The i.c.v. administration of the bee venom apamin, a blocker of small (low) conductance \(Ca^{2+}\)-gated K+ channels (Rudy, 1988), prevented the enhancement of the pain threshold produced by pyrilamine, diphenhydramine and promethazine indicating the important role of \(Ca^{2+}\)-gated K+ channels in modulation of the pain threshold produced by blockade of histamine H1 receptors. These results are supported by electrophysiological studies in which histamine decreased the post-spike slow after-hyperpolarization, a long-duration outward \(Ca^{2+}\)-dependent K+ current which hyperpolarizes the membrane potential (Weinreich, 1986; Weinreich and...
Wonderlin, 1987), in hippocampal pyramidal cells (Haas and Konnerth, 1983) and in vagal afferent neurones (Jafri et al., 1997).

By using an antisense ODN (aODN) to the mKv1.1 gene coding for the mouse Shaker-like Kv1.1, the involvement of this voltage-gated K⁺ channels in central antinociception induced by H₁-antihistamines can also be excluded. mKv1.1 is a K⁺ channel of the Shaker-like subfamily that, when expressed in Xenopus oocytes, gives rise to a fast activating, slowly inactivating K⁺ current (Hopkins and Tempel, 1992). The investigation into the involvement of mKv1.1 in central analgesia was carried out on the basis of its wide distribution in the mammalian brain including areas involved in the modulation of the pain threshold (Wang et al., 1994). Repeated i.c.v. administration of aODN to mKv1.1 did not modify pyrilamine, diphenhydramine and promethazine antinociception. We can exclude the possibility that the lack of prevention exerted by anti-mKv1.1 could be due to the employment of inadequate concentrations since, at the dose used in the present study, aODN was able to prevent antinociception induced by morphine, baclofen (Galeotti et al., 1997a), tricyclic antidepressants (Galeotti et al., 1997b) as well as to modulate different neuronal functions such as learning and memory (Meiri et al., 1997) and food consumption (Ghelardini et al., 1997). Furthermore, the specificity for mKv1.1 channels of the aODN used in the present investigation has been confirmed by results obtained from a quantitative RT-PCR which indicated a lowering of mKv1.1 mRNA brain levels specifically in the anti-mKv1.1 aODN-treated mice (Galeotti et al., 1997a,b).

In these experimental conditions, neither the K⁺ channel blockers (gliquidone, apamin), nor the K⁺ channel openers (minoxidil, pinacidil) used modified the licking latency values of mice in comparison with control groups. The lack of effect of both gliquidone and apamin agrees with results of studies in which these compounds did not modify the nociceptive threshold against thermal noxious stimuli (Welch and Dunlow, 1993). We can, therefore, rule out that the potentiation of H₁-antihistamine antinociception could be subsequent to an enhancement of the pain threshold produced by minoxidil and pinacidil.

Pyrilamine, diphenhydramine and promethazine exerted their antinociceptive activity altering the motor coordination of the mice as revealed by the rota-rod test. Furthermore, the antihistamines did not modify the spontaneous motility as revealed by the Animex apparatus (data not shown). The administration of histamine H₁ receptor antagonists produces various inhibitory effects including sedation (Simons and Simons, 1994) whose appearance could lead to a modification of the licking latency values observed in the hot-plate test. It has been, therefore, necessary to choose doses of pyrilamine, diphenhydramine and promethazine at which these compounds showed antinociceptive properties without any behavioural side effects. Similarly, the K⁺ channel modulators and the aODN to mKv1.1 were used at doses which did not modify the animals' behaviour as revealed by the rota rod and hole board tests (Galeotti et al., 1997a; Ghelardini et al., 1997, 1998).

In conclusion, the present data demonstrate that both Kₐ,ATP and Ca²⁺-gated K⁺ channels are an important intracellular effector in the antinociceptive activity of H₁-antihistamines.

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