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Prochlorperazine induces central antinociception mediated by the muscarinic system

Carla Ghelardini [a,*], Nicoletta Galeotti [a], Carla Uslenghi [b], Irene Grazioli [b], Alessandro Bartolini [a]

[a] *Department of Preclinical and Clinical Pharmacology, University of Florence, Viale G. Pieraccini 6, I-50139 Florence, Italy*
[b] *Solvay Pharma S.p.A., Via della Libertà 30, I-10095 Grugliasco, Turin, Italy*

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Abstract

The antinociceptive effect of the D_2 antagonist prochlorperazine was examined in the mouse hot-plate and abdominal constriction tests. Prochlorperazine (1–2 mg kg^{-1} s.c./i.p.) produced an increase of the pain threshold in the mouse hot-plate test. The antinociception produced by prochlorperazine was prevented by the D_2 selective agonist quinpirole, the unselective muscarinic antagonist atropine, the M_1 selective antagonist pirenzepine, and by the choline uptake inhibitor hemicholinium-3 hydrobromide (HC-3). Moreover, prochlorperazine antinociception was abolished by pretreatment with an aODN against the M_1 receptor subtype, administered at the dose of 2 nmol per single i.c.v. injection. By contrast the analgesic effect of prochlorperazine was not prevented by the opioid antagonist naloxone and the $GABA_B$ antagonist CGP-35348. Prochlorperazine also elicited a dose-dependent increase in ACh release from rat cerebral cortex. In the antinociceptive dose-range, prochlorperazine did not impair mouse performance evaluated by the rota-rod and hole-board tests. On the basis of the above data, it can be postulated that prochlorperazine exerted an antinociceptive effect mediated by a central cholinergic mechanism.

Keywords: Prochlorperazine; Acetylcholine; M_1 muscarinic receptor subtype; Analgesia; Pain

1. Introduction

Prochlorperazine is a dopamine D_2 receptor antagonist [1] clinically widely used for preventing nausea and vomiting of different origin, such as those induced by cyclophosphamide- and cisplatin-based chemotherapy [2,3] by radiotherapy [4] or by surgery [5–7]. Prochlorperazine is also effective, after intravenous administration, in the rapid control of vomiting in the emergency department [8]. Furthermore, several antiemetic preparations contain prochlorperazine in association with granisetron, dexamethason, lorazepam, scopolamine, or nabilone [9–12]. Another therapeutic use of prochlorperazine is the relief of migraine attacks. Migraine is a very common pain syndrome. It is a multifaceted disorder, of which the head pain is only one component. A migraine attack is often accompanied by nausea, vomiting, diarrhoea, photophobia, phonophobia, etc. [13]. Prochlorperazine is used, alone or in combination, in different forms of headache. In particular, positive therapeutic results were obtained in migraine attacks or tension-type and vascular headache [14–16]. Prochlorperazine is able not only to abolish nausea and vomiting, but also to relief pain occurring during a migraine attack better than metoclopramide [17].

Prochlorperazine, therefore, is endowed with antiemetic properties. Furthermore, it seems able to exert an antialgesic activity at least in headache attacks. Taking into account these observations, the aim of the present study was to first investigate the analgesic properties of prochlorperazine in laboratory animals by using different nociceptive stimuli, such as thermal and chemical. Then, we also investigated the neurotransmitter system and receptor subtypes involved in the increase of pain threshold induced by prochlorperazine in order to elucidate its mechanism of action.

In order to exclude that the effects produced by prochlorperazine treatment were due to the induction of side effects, some additional behavioural tests, able to assess motor co-ordination (rota rod), spontaneous motility and inspection activity (hole board), were performed.

Abbreviations: aODN, antisense oligodeoxynucleotide; dODN, degenerate oligodeoxynucleotide; HC-3, hemicolinium-3 hydrobromide; i.c.v., intracerebroventricular; i.p., intraperitoneal; s.c., subcutaneous

* Corresponding author. Tel.: +39-055-427-1312; fax: +39-055-427-1280.
E-mail address: carla.ghelardini@unifi.it (C. Ghelardini).

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2. Methods

2.1. Animals

Male Swiss albino mice (23–25 g) and Wistar rats (150–200 g) from the Morini (San Polo d'Enza, Italy) breeding farm were used. Fifteen mice or five rats were housed per cage (26 cm × 41 cm). The cages were placed in the experimental room 24 h before the test for acclimatisation. The animals were fed a standard laboratory diet and tap water ad libitum and kept at 23 ± 1 °C with a 12-h light:12-h dark cycle, light on at 07.00 a.m. All experiments were carried out in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC) for experimental animal care. All efforts were made to minimise the number of animals used and their suffering.

2.2. Intracerebroventricular injection technique

Intracerebroventricular (i.c.v.) administration was performed under ether anaesthesia, according to the method described by Haley and McCormick [18]. Briefly, during anaesthesia, mice were grasped firmly by the loose skin behind the head. A 0.4 mm external diameter, hypodermic needle 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 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 into the right or left ventricle randomly. To ascertain that the drugs were administered exactly into the cerebral ventricle, some mice (20%) were injected with 5 μl of diluted 1:10 India ink and their brains examined macroscopically after sectioning. The accuracy of the injection technique was evaluated and the percentage of correct injections was 95.

2.3. Hot-plate test

The method adopted was described by O'Callaghan and Holtzman [19]. Mice were placed inside a stainless steel container, thermostatically set at 52.5 ± 0.1 °C in a precision water-bath from KW Mechanical Workshop, Siena, Italy. Reaction times (s) of each animal were measured with a stop-watch before and at regular intervals up to a maximum of 60 min after treatment. The endpoint used was the licking of the fore or hind paws. Before pretreating animals, a pretest was performed: those mice scoring below 12 and over 18 s in the pretest were rejected (30%). An arbitrary cut-off time of 45 s was adopted. At least seven animals per group were used.

2.4. Abdominal constriction test

Mice were injected i.p. with a 0.6% solution of acetic acid (10 ml kg^{-1}), according to Koster et al. [20]. The number of stretching movements was counted for 10 min, starting 5 min after acetic acid injection. At least 10 animals per group were used.

2.5. 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 × 4 in an equidistant, grid-like manner. Mice were placed on the centre of the board one by one and allowed to move about freely for a period of 10 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 signalled the movement of the animal (counts in 5 min) on the surface of the plane (locomotor activity). Miniature photoelectric cells, in each of the 16 holes, recorded (counts in 5 min) the exploration of the holes (exploratory activity) by the mice.

2.6. 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 rpm. The integrity of motor co-ordination was assessed on the basis of the number of falls from the rod in 30 s according to Vaught et al. [21]. Before pretreating animals, a pretest was performed: mice scoring less than 3 and more than six falls in the pretest were rejected (20%). The performance time was measured before (pretest) and 15, 30 and 45 min after s.c. treatment.

2.7. Antisense oligonucleotides

Low cell permeability and the high degradation of natural phosphodiester oligomers are considerable drawbacks in the application of aODNs both in vitro and in vivo. To overcome these drawbacks, phosphorothioate-capped phosphorodiester oligonucleotides were used. The above-mentioned compounds are a class of ODN derivatives shown to maintain more stable and effective concentrations in the brain when compared with their unmodified counterpart [22]. Phosphodiester oligonucleotides (ODNs) protected by terminal phosphorothioate double substitution (capped ODNs) against possible exonuclease-mediated degradation were purchased from Genosys (Cambridge, England, UK) and purified by high-performance liquid chromatography (HPLC). The 18-mer antisense ODN (aODN) 5′-CXAXC TGA GGT GTT CAT TXGXC-3′ (X: phosphorothioate residues) complementary to the residues 112–129 of the published mouse M_1 cDNA sequence [23] and the 18-mer fully degenerated ODN (dODN) 5′ NNN NNN NNN NNN NNN-3′ (where N is G, or C, or A, or T and

phosphorothioate residues are underlined) were vehiculated intracellularly by an artificial cationic lipid (DOTAP, Boehringer-Mannheim, Germany) to enhance both uptake and stability. aODN or dODN (100–400 μM) were preincubated at 37 °C for 30 min with 13 μM DOTAP, sterilised through a 0.2 μm filter and supplied to mice by i.c.v. injection of a 5 μl solution as described in the next section.

The accession number of the cDNA sequence for the mouse muscarinic receptor subtype reported in this paper (M_1) is J04192.

2.8. Determination of ACh release by cerebral microdialysis

Microdialysis was performed in rat parietal cortex according to Giovannini et al. [24]. The co-ordinates used for the implantation of the microdialysis tubing (AN 69 membrane, molecular weight cut off >15 kDa, Dasco, Italy) were AP 0.5 mm and H 2.3 mm from the bregma [25]. One day after surgery the microdialysis tubing was perfused at a constant flow rate (2 μl min^{-1}) with Ringer solution (NaCl 147, KCl 4.0, $CaCl_2$ 1.2 mM) containing 7 μM physostigmine sulphate. After a 1 h settling period the perfusate was collected at 15 min intervals in test tubes containing 5 μl of 0.5 mM HCl to prevent ACh hydrolysis. The samples were then assayed for ACh content either immediately or after freezing. ACh was detected and quantified by HPLC with an electrochemical detector, as described by Damsma et al. [26] and Giovannini et al. [27].

2.9. Drugs

The following drugs were used: morphine hydrochloride (S.A.L.A.R.); hemicholinium-3 hydrobromide (HC-3), pirenzepine dihydrochloride, naloxone hydrochloride, (−)-quinpirole, hydrochloride (RBI); atropine sulphate, prochlorperazine dimaleate, amitriptyline hydrochloride, (±)-baclofen, DOTAP (Sigma); CGP-35348 (Ciba Geigy); D-amphetamine hydrochloride (De Angeli). All drugs were dissolved in isotonic (NaCl 0.9%) saline solution immediately before use. Drug concentrations were prepared in such a way that the necessary dose could be administered in a volume of 10 ml kg^{-1} by s.c. or i.p. injection or 5 μl per mouse by i.c.v. injection.

2.10. Statistical analysis

All experimental results are given as the mean ± S.E.M. 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* values of less than 0.05 were considered significant.

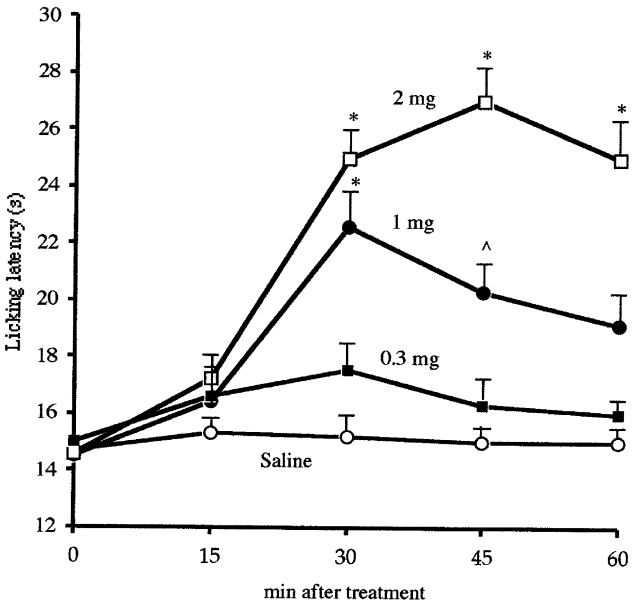

Fig. 1. Dose–response curve of prochlorperazine (i.p.) in mouse hot-plate test. Each point represents the mean of at least seven mice. $^{*}P < 0.01$; $^{\wedge}P < 0.05$ in comparison with saline-treated mice.

3. Results

3.1. Antinociceptive activity of prochlorperazine

Prochlorperazine, as shown in Fig. 1, increased the pain threshold in the mouse hot-plate test after i.p. administration (1–2 mg kg^{-1}) whereas the dose of 0.3 mg kg^{-1} i.p. was devoid of any effect. Prochlorperazine was able to induce antinociception also in the mouse abdominal constriction test. At the dose of 1 mg kg^{-1} s.c., prochlorperazine induced antinociception of intensity comparable to that exerted by well known analgesic drugs such as morphine (1 mg kg^{-1} s.c.), and amitriptyline (5 mg kg^{-1} s.c.) (Fig. 2). A dose 10-time lower of prochlorperazine as well as the above-mentioned reference compounds, was devoid of any effect (Fig. 2).

3.2. Antagonism of prochlorperazine antinociception

In the mouse hot-plate test, the antinociceptive effect of prochlorperazine (2 mg kg^{-1} i.p.) was antagonized by the unselective muscarinic antagonist atropine (5 mg kg^{-1} i.p.), the selective M_1 muscarinic antagonist pirenzepine (0.1 μg per mouse i.c.v.), the choline uptake inhibitor HC-3 (1 μg per mouse i.c.v.) and the D_2 agonist quinpirole (0.5 μg per mouse i.c.v.) (Table 1). Otherwise, the opioid antagonist naloxone (1 mg kg^{-1} i.p.) and the $GABA_B$ antagonist CGP-35348 (100 mg kg^{-1} i.p.) were unable to prevent prochlorperazine antinociception (Table 1). The minimal dose of quinpirole able to prevent prochlorperazine antinociception was 0.5 μg per mouse i.c.v., a concentration unable to modify morphine (7 mg kg^{-1} s.c.) and the $GABA_B$ agonist baclofen (4 mg kg^{-1} i.p.)-induced antinociception. By

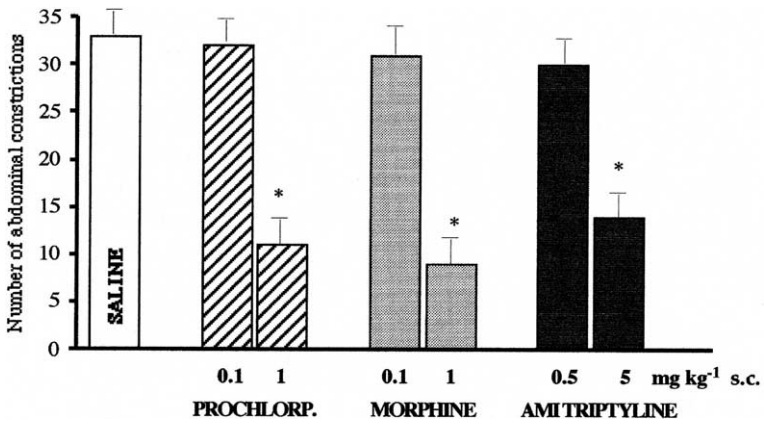

Fig. 2. Antinociceptive effect of prochlorperazine in comparison with morphine and amitriptyline in mouse abdominal constriction test. Nociceptive response was recorded 30 min after drug administration (s.c.). * $P < 0.01$ in comparison with saline-treated mice. Each column represents the mean of at least 10 mice.

contrast, the dose of 0.1 μg per mouse i.c.v. of quinpirole was ineffective (Fig. 3).

All antagonists were injected 15 min before the test, with the exception of HC-3 injected 5 h before the test.

Pretreatment with a single (2 nmol per mouse i.c.v.) injection of antisense ODN (aODN) to M_1 gene on days 1, 4 and 7, prevented prochlorperazine-induced increase of pain threshold in the mouse hot-plate 24 h after the last i.c.v. injection (Fig. 4). This antagonistic effect was not detected in the dODN-treated group, used as control ODN (Fig. 4). Forty-eight hours after the end of the treatment, a partial reversion of prochlorperazine antinociception was detected, whereas after 96 h, prochlorperazine induced an antinociceptive effect of the same intensity in aODN-, dODN- and vehicle-treated mice indicating the loss of antagonistic activity by the anti-M_1 aODN (Fig. 4). The aODN pretreatment (2.0 nmol per i.c.v. injection) did not reduce the pain threshold in mice showing a lack of any hyperalgesic effect (Fig. 4). The pretreatment with the dODN did not modify prochlorperazine-induced antinociception in comparison with mice injected with vehicle as shown in Fig. 4.

3.3. Effect of prochlorperazine on cerebral ACh levels

As shown in Fig. 5, prochlorperazine (2 mg kg^{-1} i.p.) significantly increased the ACh release from cerebral cortex

Table 1
Effect of atropine, pirenzepine, HC-3, quinpirole, naloxone and CGP-35348 on antinociception induced by prochlorperazine (2 mg kg^{-1} i.p.) in the mouse hot-plate test

Pretreatment	Treatment	Licking latency (s)		
		Before		After treatment
		30 min	45 min	60 min
Saline-prochlorper.	Saline	13.6 ± 0.7	14.4 ± 1.0	13.9 ± 0.9	14.3 ± 0.8
		14.6 ± 0.9	25.1 ± 1.8*	27.2 ± 1.6*	23.8 ± 1.6*
Atropine (5 mg kg^{-1} i.p.)	Saline-prochlorper.	14.0 ± 0.7	13.3 ± 1.3	13.1 ± 1.1	14.2 ± 1.3
		13.9 ± 1.0	17.2 ± 1.7***	17.8 ± 2.0***	15.1 ± 1.7***
Pirenzepine (0.1 μg i.c.v.)	Saline-prochlorper.	14.1 ± 1.2	14.2 ± 1.5	13.8 ± 1.4	14.5 ± 1.6
		14.2 ± 1.3	16.2 ± 1.8***	15.4 ± 1.9***	16.0 ± 2.1***
HC-3 (1 μg i.c.v.)	Saline-prochlorper.	14.4 ± 0.9	13.8 ± 1.5	15.5 ± 1.3	15.4 ± 1.2
		14.4 ± 1.1	18.0 ± 2.1***	16.5 ± 1.7***	15.3 ± 1.5***
Quinpirole (0.5 μg i.c.v.)	Saline-prochlorper.	14.4 ± 0.9	14.8 ± 1.5	15.5 ± 1.3	15.4 ± 1.2
		15.0 ± 1.1	17.3 ± 13***	16.9 ± 1.6***	14.2 ± 1.5***
Naloxone (1 mg kg^{-1} i.p.)	Saline-prochlorper.	13.5 ± 0.8	14.0 ± 1.5	13.9 ± 1.6	14.3 ± 1.7
		13.5 ± 0.6	27.2 ± 2.4*	25.6 ± 2.0*	21.7 ± 2.4*
CGP-35348 (100 mg kg^{-1} i.p.)	Saline-prochlorper.	13.5 ± 0.7	11.4 ± 1.3**	12.5 ± 2.0	12.7 ± 1.5
		13.5 ± 1.2	23.2 ± 1.7*	25.7 ± 2.5*	21.9 ± 1.7**

* $P < 0.01$ in comparison with saline.
** $P < 0.05$ in comparison with saline.
*** $P < 0.01$ vs. saline-prochlorperazine treated mice. The number of mice ranged from 8 to 26.

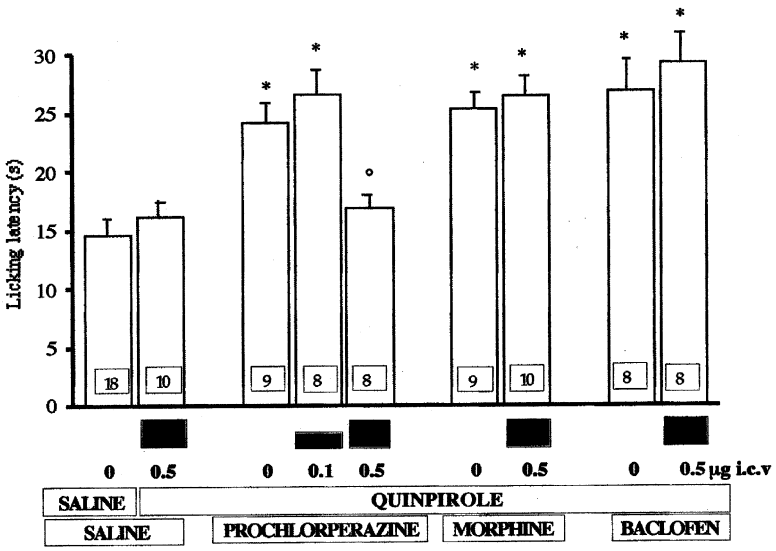

Fig. 3. Effect of quinpirole on prochlorperazine-induced antinociception in mouse hot-plate test. Quinpirole was administered 5 min before the other drugs. Nociceptive response was recorded 15 min after prochlorperazine (2 mg kg^{-1} i.p.) and 30 min after morphine (7 mg kg^{-1} i.p.) and baclofen (4 mg kg^{-1} s.c.) injection. Number of mice is reported inside the columns. $^{*}P < 0.01$ vs. saline-treated mice. $^{\circ}P < 0.01$ vs. prochlorperazine-treated mice.

of freely moving rats. The prochlorperazine curve represents the time-course of the increase expressed as percentage change from the means of the three collection periods before prochlorperazine administration, taken as control. Prochlorperazine ACh release peaked 30 min after administration and returned to basal values within 120 min.

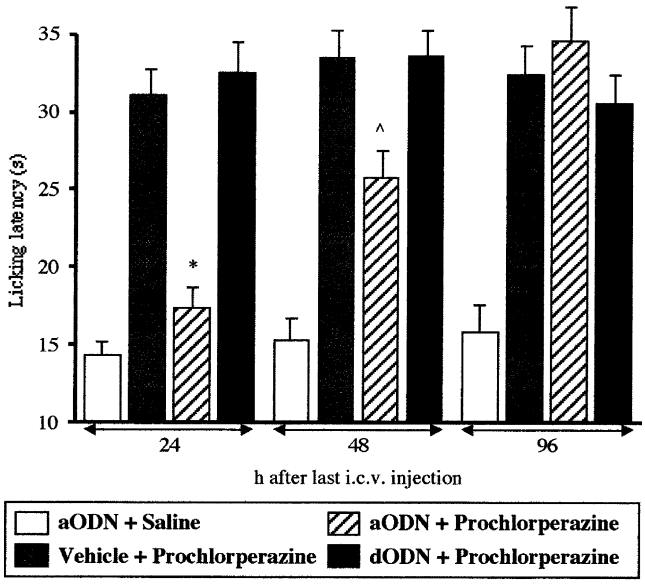

Fig. 4. Effect of antisense ODN (aODN) to M1 gene on prochlorperazine (2 mg kg^{-1} i.p.)-induced antinociception 24, 48 and 96 h after the end of the aODN treatment. Mice were i.c.v. injected with vehicle, aODN or degenerated ODN (dODN) at the dose of 2.0 nmol per single i.c.v. injection on days 1, 4 and 7. Modification of pain threshold was evaluated by using the mouse hot-plate test. The licking latency was detected 30 min after prochlorperazine administration. Vertical lines give SEM. Each column represents the mean of 10–14 mice. $^{\wedge}P < 0.05$; $^{*}P < 0.01$ in comparison with vehicle-prochlorperazine-treated mice.

3.4. Effect of prochlorperazine on mouse behaviour

The spontaneous motility and exploratory behaviour of mice was not modified by treatment with prochlorperazine (2–3 mg kg^{-1} i.p.) as revealed by the hole-board test (Fig. 6). In the same experimental conditions D-amphetamine (2 mg kg^{-1} s.c.), used as the reference drug, increased both parameters evaluated.

The motor co-ordination of mice treated with prochlorperazine was evaluated by using the rota-rod test (Fig. 7). The rota-rod performance of mice treated with prochlorperazine

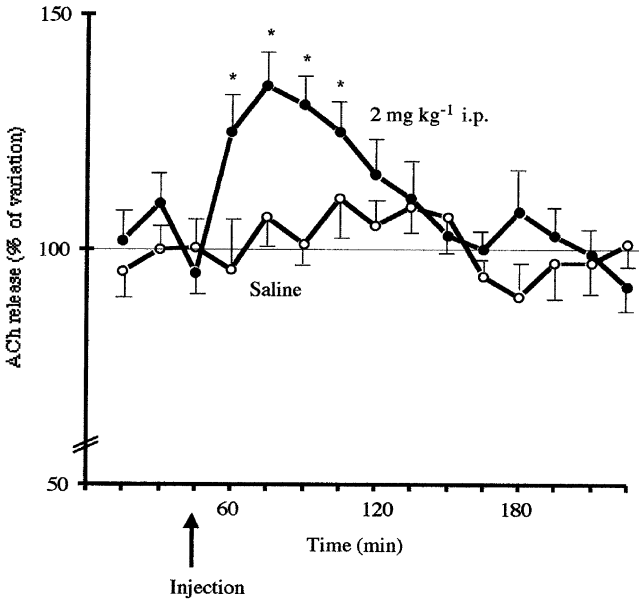

Fig. 5. Effect of prochlorperazine on ACh release from rat cerebral cortex. $^{*}P < 0.01$ in comparison with saline treated rats. Each point represents the mean of three rats.

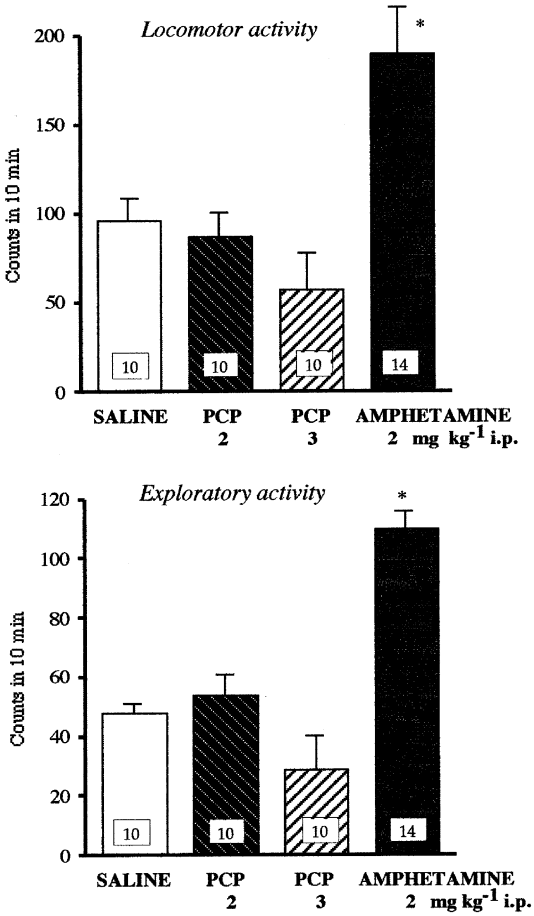

Fig. 6. Lack of effect of prochlorperazine (PCP) in mouse hole-board test in comparison with amphetamine. Test was performed 30 min after PCP and amphetamine administration. $^{*}P < 0.01$ in comparison with saline-treated mice.

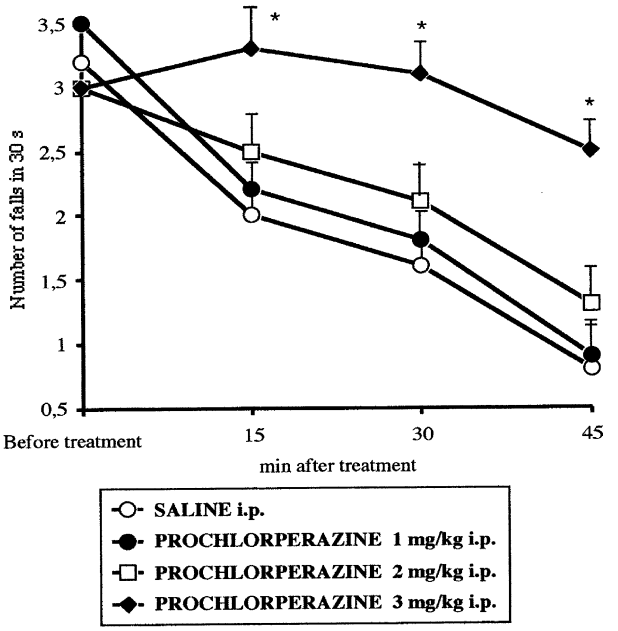

Fig. 7. Dose–response curve of prochlorperazine in mouse rota-rod test. $^{*}P < 0.01$ in comparison with saline controls. Each point represents the mean of 10 mice.

at the dose of 1–2 mg kg^{-1} i.p. was not impaired in comparison with controls as indicated by a progressive reduction of the number of falls. The number of falls by control animals progressively decreased at every measurement since the mice learnt how to balance on the rotating rod. By contrast, no reduction of the number of falls was observed after administration of prochlorperazine at the dose of 3 mg kg^{-1} i.p. indicating the induction of motor incoordination (Fig. 7).

4. Discussion

Prochlorperazine was showed to be able to induce antinociception in mice. Antinociception was elicited regardless of which noxious stimulus was used: thermal (hot-plate test) and chemical (abdominal constriction test). Prochlorperazine antinociception was obtained without producing changes of animals' gross behaviour. Moreover, prochlorperazine-treated mice showed a complete integrity of motor co-ordination on the rota-rod test, normal spontaneous motility, as well as exploratory behaviour as revealed by the hole-board test.

The antinociceptive effect of prochlorperazine appears to be due to the antagonism of D_2 receptors since the increase of the pain threshold induced by the investigated compound was prevented by pretreatment with the D_2 agonist quinpirole. Present data extend and support previous results in which the capability of D_2 antagonists to induce analgesia was suggested. Sulpiride was able to exert a potentiating effect of morphine analgesia in diabetic mice [28] and the D_2 blocker risperidone induced a potent increase of the pain threshold in the mouse tail flick test [29]. Furthermore, present results indicate that prochlorperazine antinociception is dependent on central cholinergic activation. It should be noted that several D_2 antagonists, such as sulpiride, pipotiazine and domperidone, increase K^+ evoked ACh release [30], whereas the D_2 agonist quinpirole inhibit ACh efflux [31,32] from rat striatal preparations. The increase of the pain threshold induced by the investigated compound was prevented by the non-selective muscarinic antagonist atropine, the selective M_1-antagonist pirenzepine, the ACh depletor HC-3 and an aODN to the M_1 receptor subtype. The aODN treatment induces a transient prevention of muscarinic antinociception since the inhibition of prochlorperazine effect disappeared 96 h after the last i.c.v. injection of the aODN. This return to normal sensitivity to analgesic treatments implies both the total reversal of aODN-induced specific inhibition of M_1 gene expression and a lack of damage or toxicity associated with aODN treatment.

Cholinergic antinociception in mice is mediated by M_1 receptor stimulation. By using selective and unselective

muscarinic agonists and antagonists, the involvement of M_1 receptors in muscarinic analgesia was evidenced [33,34]. Furthermore, cholinergic antinociception induced both directly, through muscarinic agonists, and indirectly, by enhancing ACh extracellular levels through cholinesterase inhibitors, is prevented by i.c.v. administration of an antisense to the M_1 gene coding for the mouse M_1 receptor [35].

Taking into account that HC-3 and pirenzepine were able to antagonise prochlorperazine antinociception after i.c.v. injection, this indicates that the analgesic site of action of the investigated compound is localised in the CNS.

A presynaptic mechanism facilitating cholinergic transmission is involved in prochlorperazine antinociception as revealed by the antagonism by HC-3 postsynaptic mechanism of action can be ruled out since, as reported by Bartolini et al. [33,36], HC-3 was not able to antagonise antinociception induced by agonists of postsynaptic muscarinic receptors such as oxotremorine, McN-A-343 and AF-102B. The hypothesis of a presynaptic cholinergic mechanism for prochlorperazine is further supported by microdialysis studies, in which an increase in ACh release from rat cerebral cortex was induced by prochlorperazine administration. This effect occurred at the same doses ($2\,mg\,kg^{-1}$ i.p.) in which the investigated D_2 antagonist exerted its antinociceptive activity. The observed facilitation of the cholinergic transmission induced by prochlorperazine appears to be a consequence of a blockade of D_2 receptors.

Other neurotransmission systems able to modulate pain threshold, such as opioid and GABAergic, are not involved in prochlorperazine antinociception since the opioid antagonist naloxone and the $GABA_B$ antagonist CGP-35348 were unable to prevent the effect of prochlorperazine. The doses and administration schedules of the above-mentioned drugs were ideal for preventing antinociception induced respectively by morphine and the $GABA_B$ agonist baclofen.

In summary, our results have shown that prochlorperazine is able to produce antinociception without inducing behavioural side effects, by potentiating endogenous cholinergic activity through antagonism of D_2 receptors.

Acknowledgements

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