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Muscarinic and α_1-adrenergic mechanisms contribute to the spinal mediation of stimulation-induced antinociception from the pedunculopontine tegmental nucleus in the rat

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ABSTRACT

The effects of intraperitoneal (i.p.) or intrathecal (i.t.) injection of antagonists of acetylcholine, noradrenaline, serotonin, dopamine, opioids and GABA on stimulation-produced antinociception (SPA) from the pedunculopontine tegmental nucleus (PPTg) of rats were studied using the tail-flick test. The electrical stimulation of the PPTg produced a strong and long-lasting increase in tail-flick latency. The intensity and duration of the effect were significantly reduced in rats pretreated with i.p. or i.t. atropine (a non-selective muscarinic cholinergic antagonist), or i.t. phenoxybenzamine or WB 4101 (non-selective and selective α_1-adrenergic antagonists, respectively). Intraperitoneal phenoxybenzamine, i.p. or i.t. methysergide or naloxone (non-selective serotonin and opioid antagonists, respectively), or i.t. idazoxan (a selective α_2-adrenergic antagonist) only reduced the duration of the effect. The duration of SPA from the PPTg was increased by i.t. phaclofen (a $GABA_B$ antagonist). The effect from the nucleus was not altered following i.t. bicuculline (a $GABA_A$ antagonist), or i.p. or i.t. mecamylamine, propranolol or haloperidol (non-selective nicotinic cholinergic, β-adrenergic and dopaminergic antagonists, respectively). Thus, SPA from the PPTg involves the spinal activation of muscarinic and α_1-adrenergic but not nicotinic cholinergic, β-adrenergic and dopaminergic mechanisms. Serotonergic, endogenous opioid and α_2-adrenergic mechanisms are involved in the duration but not in the intensity of the effect.

1. Introduction

Stimulation-produced antinociception (SPA) has been demonstrated from several brain structures such as the mesencephalic periaqueductal gray (PAG), the anterior pretectal (APtN) and pedunculopontine tegmental (PPTg) nuclei, and the raphe magnus (NRM) and reticular gigantocellular pars α (GiA) nuclei in the rostral ventral medulla (Millan, 1999).

The PPTg is located bilaterally in the mesopontine tegmentum in close association with the superior cerebellar peduncle (Inglis and Winn, 1995). The nucleus forms the rostral portion of the cholinergic pontine parabrachial region and contains cholinergic and non-cholinergic cells (Rye et al., 1988). The PPTg is involved in Parkinson's disease (Winn, 2008) and participates in several behavioral functions including the control of locomotion, reinforcement and learning, sensation, and attention (Winn, 2006).

The participation of PPTg in antinociception has been proposed in studies showing strong antinociceptive effects in rats and cats following the injection of agonists of nicotinic cholinergic receptors such as carbachol (Katayama et al., 1984b), nicotine (Iwamoto, 1989), or N-methylcarbachol (Iwamoto and Marion, 1993) into the nucleus. Carbachol-induced antinociception from the PPTg resists local injection of naloxone and involves a local muscarinic cholinergic mechanism (Katayama et al., 1984a). In addition, nociceptive excited and inhibited neurons have been demonstrated within the pedunculopontine tegmental nucleus (Carlson et al., 2004).

Nicotinic agonist-induced antinociception from the PPTg depends on the activation of descending pathways which inhibit noxious inputs in the spinal cord. Actually, carbachol-induced antinociception from the rostral pontine parabrachial region of cats is significantly reduced by lesion of the dorsolateral funiculus (Hayes et al., 1984), through which most of the descending pain-inhibitory pathways travel (Fields and Basbaum, 1978). Also, antinociception induced by N-methylcarbachol from the PPTg depends on the activation of spinal α_2-adrenergic, serotonergic, and muscarinic cholinergic mechanisms (Iwamoto and Marion, 1993).

Carbachol-induced antinociception from the PPTg is probably mediated by polysynaptic pathways, given that a very small number of cholinergic PPTg cells project to the spinal cord (Rye et al., 1987). Alternatively, there are extensive connections from the PPTg to the NRM and GiA, which project directly to the spinal cord and belong to the descending pain-inhibitory pathways (Grofova and Keane, 1991; Rye et al., 1988; Skinner et al., 1990).

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On the other hand, substantial projections from non-cholinergic PPTg cells to the medulla and spinal cord have already been demonstrated (Inglis and Winn, 1995). Therefore, a local cholinergic mechanism may not be the only one involved in antinociception evoked from the PPTg. SPA from the PPTg has already been demonstrated in cats (Carstens et al., 1980; DeSalles et al., 1985) and rats (Rosa et al., 1998; Terenzi et al., 1992), but no study has been conducted on the mechanisms involved in this effect.

SPA from the NRM is mediated by serotonergic and endogenous opioid mechanisms (Fields and Basbaum, 1978), while noradrenaline mediates the effect from the GiA (Kuraishi et al., 1979). $GABA_B$ receptors may also control descending modulation from the rostro-ventromedial medulla (Pinto et al., 2008). The PPTg is a relay station for a descending pathway from the APtN (Terenzi et al., 1992), a brain structure from which electrical stimulation produces a strong and long-lasting antinociception which is mediated by opioid, α-adrenergic and muscarinic cholinergic mechanisms (Rees et al., 1987).

In the present study we used the rat tail-flick test to evaluate the antinociceptive effect produced by stimulating electrically the PPTg, consequently activating both cholinergic and non-cholinergic neurons in the nucleus. The effects of intraperitoneal or intrathecal injection of antagonists on SPA from the PPTg were also evaluated to determine whether antagonists of acetylcholine, noradrenaline, serotonin, dopamine, opioids and GABA are effective, as they are known to be against SPA from NRM, GiA, or APtN. It is shown that, in addition to spinal α_2-adrenergic, serotonergic, and muscarinic mechanisms already known to mediate the nicotinic agonist-induced antinociception from the PPTg, SPA from this nucleus involves the spinal activation of α_1-adrenergic and opioid mechanisms as well.

2. Materials and methods

2.1. Subjects and surgery

Male Wistar rats (160–180 g) were used in this study. Animals were housed two to a cage under controlled temperature (24 ± 2 °C) and on a 12-h light–dark cycle, with the dark cycle beginning at 07:00 h., and they had free access to food and water. The experiments were approved by the Commission of Ethics in Animal Research, Faculty of Medicine of Ribeirão Preto, University of São Paulo (Number 009/2004). The guidelines of the Committee for Research and Ethical Issues of IASP (Zimmermann, 1983) were followed throughout the experiments. Each rat was used on only one occasion.

2.2. Electrode implant

Each animal was anesthetized with tribromoethanol (250 mg/kg, i.p.), and a Teflon-insulated monopolar electrode (o.d. = 0.125 mm) was stereotaxically implanted into the skull to lie in the PPTg, using the following coordinates (in mm): AP = 0.2 from the ear bars; $L = 2.0$ from the midline, and $H = -6.6$ from the skull surface, as proposed elsewhere [29]. The electrode was then fixed to the skull with two screws and dental cement. One of these screws was used as the reference electrode. After receiving penicillin (50 mg/kg, i.m.), the animal was allowed to recover for at least one week before the experiment.

2.3. Stimulation procedures

Electrical stimulation (AC, 60 Hz) of 35 μA r.m.s. (root mean square) was applied for a period of 15 s to the PPTg of freely moving rats. At this intensity and duration, electrical stimulation of the PPTg has been shown to inhibit effectively the tail-flick reflex in rats (Rosa et al., 1998). During the period of stimulation, the drop in voltage across a 1-kΩ resistor in series with the electrode was continuously monitored on an oscilloscope. Control (sham) rats were submitted to identical procedures for electrode implant and its connections to the stimulator assembly, but no current was passed through the electrode.

During the period of observation, the animals were observed regarding behavioral or motor function changes which were assessed, but not quantified, by testing the animals' ability to stand and walk in a normal posture, as proposed elsewhere (Chen and Pan, 2001).

2.4. Tail-flick test

The tail-flick test was conducted as described elsewhere (Azami et al., 1982). Each animal was placed in a ventilated tube with the tail laid across a wire coil, which was at room temperature (23 ± 2 °C). The coil temperature was then raised by the passage of electric current and the latency for the tail withdrawal reflex was measured. Heat was applied to a portion of the ventral surface of the tail between 4 and 6 cm from the tip. Each trial was terminated after 6 s to minimize the probability of skin damage. Tail-flick latency was measured at 5-min intervals until a stable baseline was obtained over three consecutive trials. The latency was measured again within 30 s after drug administration and then at 5-min intervals for up to 40 or 60 min after intraperitoneal or intrathecal drug administration, respectively. Only rats showing stable baseline latency after up to 6 trials were used in each experiment.

2.5. Spinal catheterization

Each rat was anesthetized with halothane via a loose-fitting, cone-shaped mask, and catheterization of the spinal subarachnoid space was performed as described elsewhere (Prado, 2003). Briefly, a 20-gauge Weiss needle was introduced through the skin into the L5–L6 intervertebral space. The correct positioning of the needle was assured by a typical flick of the tail or hind paw. A 12-mm length of polyethylene tubing (PE tubing, o.d. = 0.4 mm, dead space = 10 μl) was then introduced through the needle to protrude 1.5 cm into the subarachnoid space in a cranial direction. The needle was then carefully removed and the tubing anchored to the back skin with a cotton thread suture. In a separate group of catheterized rats, we found no significant change in tail-flick latency before or 3 h after surgery (not shown in Results). Injections via the catheter were performed 3 h after catheter implantation to avoid any residual analgesia produced by the anesthetic procedure. In each case, only rats showing no sign of motor impairment were considered for further experimentation. Drug or saline was injected intrathecally in a volume of 5 μl over a period of 30 s, followed by 5 μl of sterile saline at the same rate to flush the catheter.

2.6. Examination of catheter and electrode position

At the end of the experiments, fast green (10 μl) was injected through the catheter. The animal was deeply anesthetized with intraperitoneal sodium thiopental and perfused through the heart with 4% paraformaldehyde in 0.1 M phosphate buffered saline. The spinal cord was cut through the L4–L5 intervertebral disk to locate the catheter tip under a dissecting microscope. Only rats showing the catheter tip positioned dorsally to the spinal cord were considered for further analysis. The brain was removed and the electrode track localized from 100-μm serial coronal sections stained with neutral red, and identified on diagrams from the atlas of Paxinos and Watson (1986). The sites of stimulation in each experiment are shown on the right side of the corresponding figure.

2.7. Drugs

Phenoxybenzamine hydrochloride (a nonselective antagonist of α-adrenergic receptors), propranolol hydrochloride (a nonselective antagonist of β-adrenergic receptors), WB4101 hydrochloride and idazoxan hydrochloride (selective antagonist of α_1- and α_2-

adrenergic receptors, respectively), mecamylamine hydrochloride, methysergide maleate and naloxone hydrochloride (nonselective antagonists of nicotinic cholinergic, serotonergic and opioid receptors, respectively), and bicuculline methiodide (an antagonist of $GABA_A$ receptors) were from RBI; haloperidol (a non selective antagonist of dopaminergic receptors), atropine sulfate (a nonselective antagonist of muscarinic cholinergic receptors) and phaclofen (an antagonist of $GABA_B$ receptors) were purchased from Sigma. Phaclofen was dissolved in a dimethylsulfoxide (DMSO) solution (5%). The other drugs were dissolved in saline. All doses refer to the salt. Electrical stimulation of the PPTg was performed 3 h after intraperitoneal or intrathecal phenoxybenzamine or 15 min after intraperitoneal or intrathecal administration of the other antagonists.

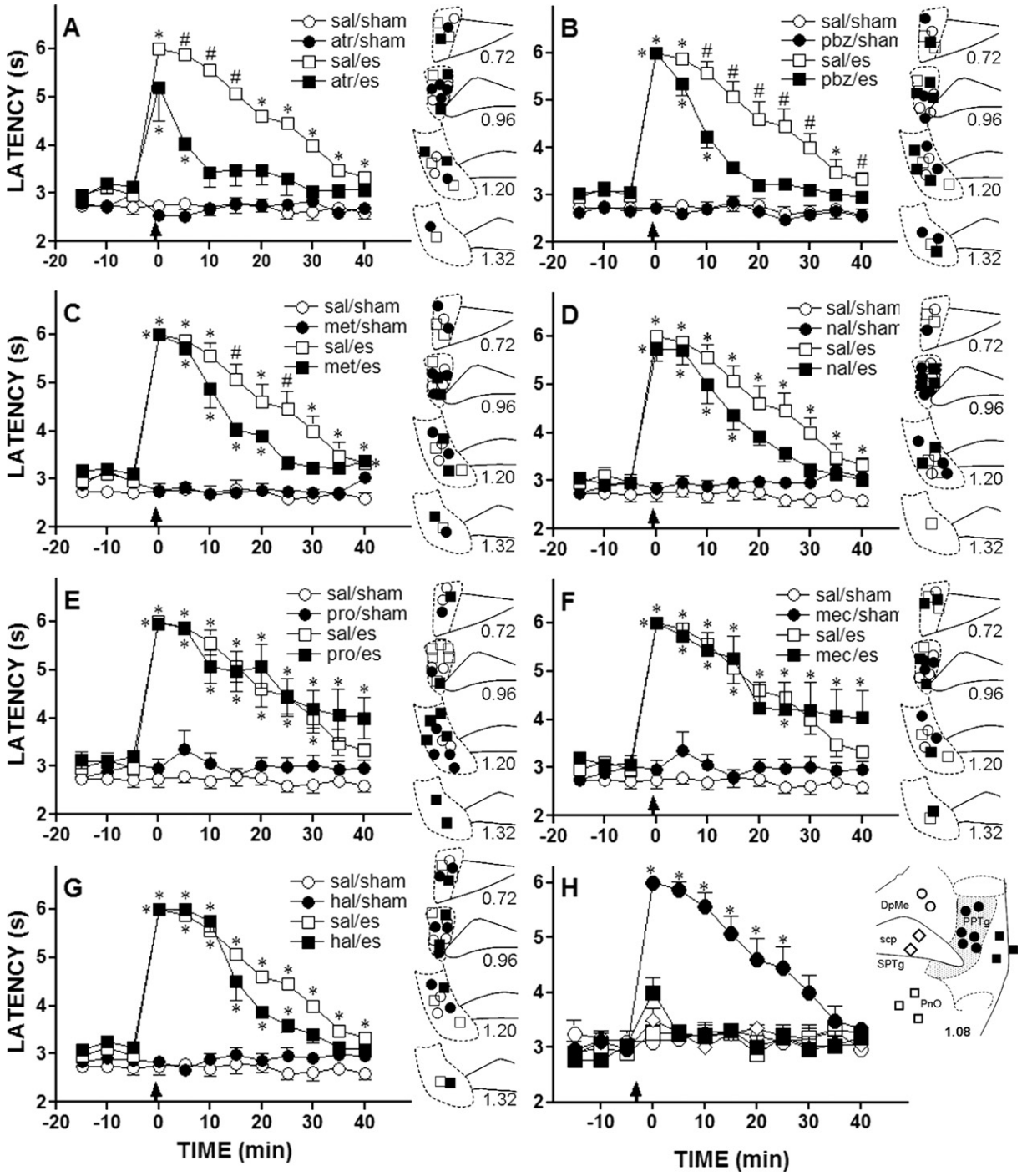

Fig. 1. Effects of the intraperitoneal injection of atropine (atr) (A), phenoxybenzamine (pbz) (B), methysergide (met) (C), naloxone (nal) (D), propranolol (pro) (E), mecamylamine (mec) (F), or haloperidol (hal) (G) on the increase in tail-flick latency caused by electrical stimulation (es) of the pedunculopontine tegmental nucleus. The es (arrow) was applied 3 h after phenoxybenzamine or 15 min after the other antagonists. The antagonists were all administered at a dose of 1 mg/kg, and saline (sal) was given at a dose of 0.1 ml/kg. (H) Time course of effects on tail-flick latency upon stimulation of the pedunculopontine tegmental nucleus (PPTg, shaded area) and adjacent structures (DpMe = deep mesencephalic nucleus; scp = superior cerebellar peduncle; SPTg = subpeduncular tegmental nucleus; PnO = pontine reticular nucleus, oral part). Each rat used in this experiment is represented by one symbol which was also used to represent mean ± S.E.M. in the graph. The location of the electrode tips in each experiment is shown on the right side of each graph on coronal sections taken from the Paxinos and Watson atlas (1986) at the indicated plane anterior to the interaural line. Points in A–G are means ± S.E.M., $n = 5–7$ for all groups. *Different from the sal/sham group (A–G) or baseline value (H). #Different from the other groups. $P < 0.05$ in all cases.

2.8. Statistics

The tail-flick latencies are reported as means ± S.E.M. Comparisons between control and test groups were made by multivariate analysis of variance (MANOVA) with repeated measures to compare the groups over all times. The factors analyzed were treatments, time and treatment × time interaction. In the case of a significant treatment × time interaction, one-way analysis of variance followed by the Bonferroni post-hoc test was performed for each time. The analysis was performed using the statistical software package SPSS/PC+, version 6.0.

3. Results

3.1. Intraperitoneal injection of saline or antagonists

The results from these experiments are shown in Fig. 1A–G. The groups did not differ significantly regarding baseline latencies ($F \leq 3$; $P \geq 0.05$). A strong and long-lasting increase in tail-flick latency was obtained following electrical stimulation of the PPTg in saline-treated rats, the effect remaining significantly above control throughout the period of observation (40 min). Sham-stimulated rats treated with

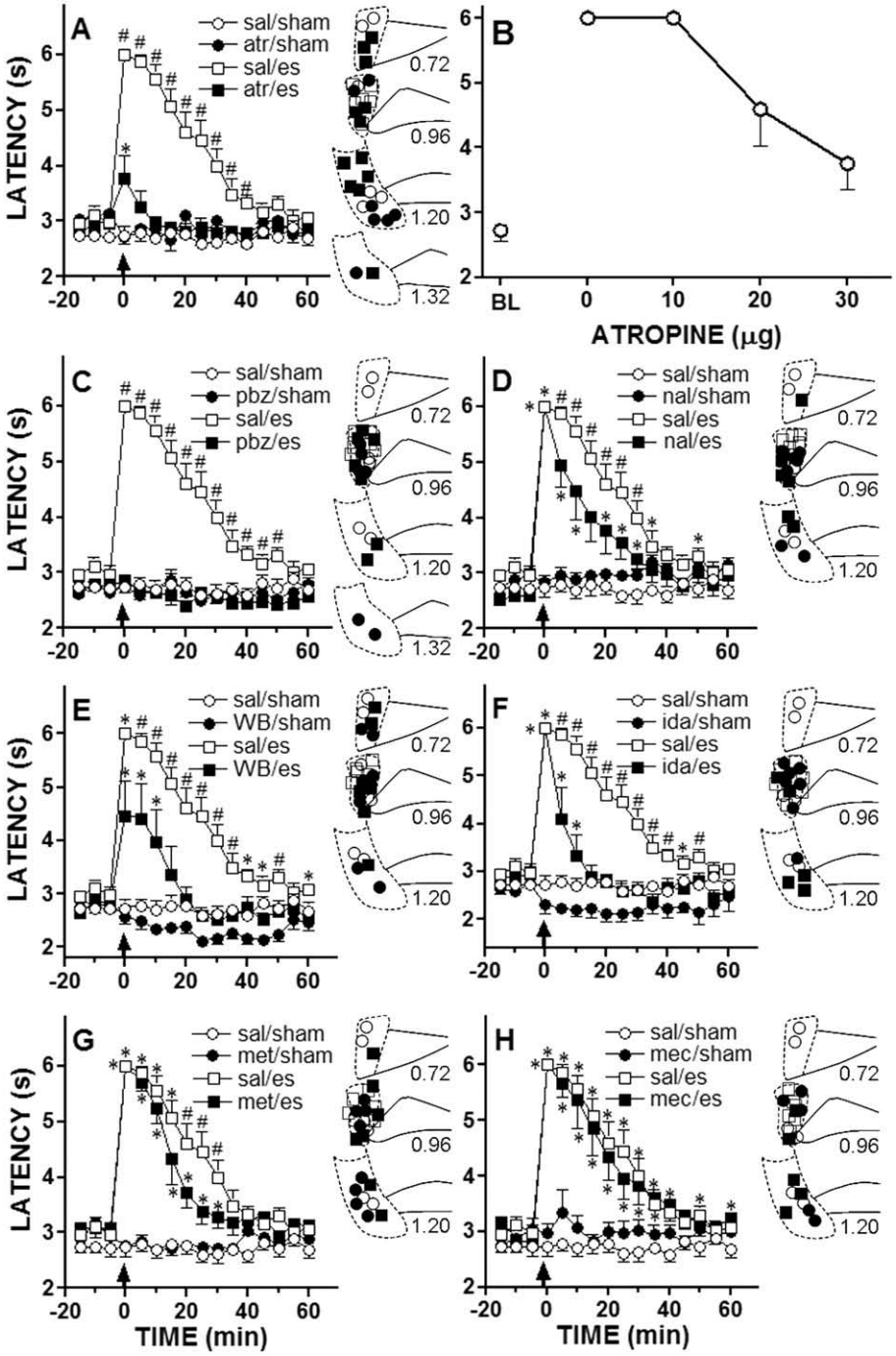

Fig. 2. Effects of the intrathecal injection of atropine (atr), (A and B), phenoxybenzamine (pbz = 20 μg) (C), naloxone (nal = 20 μg) (D), WB 4101 (WB = 20 μg) (E), idazoxan (ida = 50 μg) (F), methysergide (met = 30 μg) (G), or mecamylamine (mec = 40 μg) (H), on the increase in tail-flick latency caused by electrical stimulation (es) of the pedunculopontine tegmental nucleus. The es (arrow) was applied 3 h after phenoxybenzamine or 15 min after the other antagonists. Atropine (atr = 30 μg) inhibited the effect of PPTg stimulation (A). The changes in the peak effects of electrical stimulation in the presence of different doses of atropine are shown in B. Saline (sal) was given a dose of 5 μl. The location of the electrode tips is indicated as in Fig. 1. Points are means ± S.E.M., $n = 5$–7 for all groups. *Different from the sal/sham group. #Different from the other groups. $P < 0.05$ in all cases.

intraperitoneal atropine (Fig. 1A), phenoxybenzamine (Fig. 1B), methysergide (Fig. 1C), naloxone (Fig. 1D), propranolol (Fig. 1E), mecamylamine (Fig. 1F), or haloperidol (Fig. 1G), all at a dose of 1 mg/kg, had latencies nonsignificantly different from sham-stimulated rats injected with saline (1 ml/kg).

SPA from the PPTg was reduced in intensity and duration by atropine (Fig. 1A), and had the same intensity but shorter duration in phenoxybenzamine-treated rats (Fig. 1B) than in saline-treated rats. The duration, but not the intensity of the SPA from the PPTg was reduced also by intraperitoneal methysergide (Fig. 1C) or naloxone (Fig. 1D). However, SPA from the PPTg was not significantly altered in rats treated intraperitoneally with propranolol (Fig. 1E), mecamylamine (Fig. 1F) or haloperidol (Fig. 1G). The curves in Fig. 1A–G were significantly different regarding treatment ($F \geq 19$; $P<0.0001$) and time ($F \geq 27.6$; $P<0.0001$), and showed a significant treatment × time interaction ($F \geq 9.8$; $P<0.0001$).

During brain stimulation, several rats showed muscle contractions sometimes accompanied by vocalization and jumping. No animal showed gross disturbance of motor coordination after the period of brain stimulation. The animals walked normally and responded to non-noxious stimulation throughout the period of observation. The stimulation sites were all localized in the PPTg in an area surrounding the lateral half of the superior cerebellar peduncle, as shown on the right side of each graph of Fig. 1A–G.

Some rats used in this study had stimulation sites near to but outside the PPTg. The results obtained from these animals were pooled and compared to the effects obtained from rats stimulated in the PPTg. A strong and long-lasting increase in tail-flick latency was obtained following electrical stimulation in sites within the PPTg, but not in sites near the PPTg (Fig. 1H).

3.2. Intrathecal injection of saline or antagonists

The results from these experiments are shown in Fig. 2A–G. The groups did not show significant differences regarding baseline latencies ($F \leq 2.1$; $P \geq 0.055$). Saline-treated rats had a strong ($F_{1,10}=52$; $P<0.0001$) and long-lasting increase ($F_{15,150}=30.17$; $P<0.0001$) in tail-flick latency following electrical stimulation of the PPTg, as compared to that obtained from sham-stimulated rats. These groups also showed a significant treatment × time interaction ($F_{15,150}=29.98$; $P<0.0001$). The tail-flick latency of saline-treated rats stimulated in the PPTg remained above control throughout the period of observation (60 min).

Atropine (20 and 30 μg) produced a significant inhibition of SPA from the PPTg (Fig. 2A and B). A smaller dose of atropine (10 μg) produced nonsignificant changes in this effect. The time course of the effects of atropine (30 μg) in sham or stimulated rats was considered in the statistical analysis but was not included in Fig. 2A to avoid data overcrowding. Under this condition, the data differed significantly regarding treatment ($F_{7,48}=16.02$; $P<0.0001$) and time ($F_{15,720}=32.4$; $P<0.0001$), and showed a significant treatment × time interaction ($F_{105,720}=9.33$; $P<0.0001$). A dose–response curve was constructed using the peak effects of electrical stimulation in the presence of different doses of atropine to show a dose-dependent effect of this antagonist against SPA from the PPTg (Fig. 2B).

SPA from the PPTg was completely inhibited by phenoxybenzamine (20 μg) (Fig. 2C) and had a slightly shorter duration after naloxone (20 μg) (Fig. 2D). The higher dose of naloxone (30 μg) did not produce additional changes (not shown in the figures). A lower dose of phenoxybenzamine (10 μg) was ineffective against SPA from the PPTg (not shown in the figures). The curves in Fig. 2C and D were significantly different ($P<0.0001$ in all cases) regarding treatment ($F_{3,21}=42.6$; $F_{3,22}=21.5$, respectively) and time ($F_{15,315}=27.1$; $F_{15,315}=45.8$, respectively), and showed a significant treatment × time interaction ($F_{45,315}=22.4$; $F_{45,330}=17$, respectively). Atropine, phenoxybenzamine or naloxone did not produce significant changes in the tail-flick latencies of sham-stimulated rats throughout the period of observation.

SPA from the PPTg was significantly reduced in intensity and duration by WB 4101 (20 μg) (Fig. 2E) and had shorter duration after idazoxan (50 μg) (Fig. 2F). Lower doses of WB 4101 (10 μg) or idazoxan (25 μg) were ineffective, but were considered in the

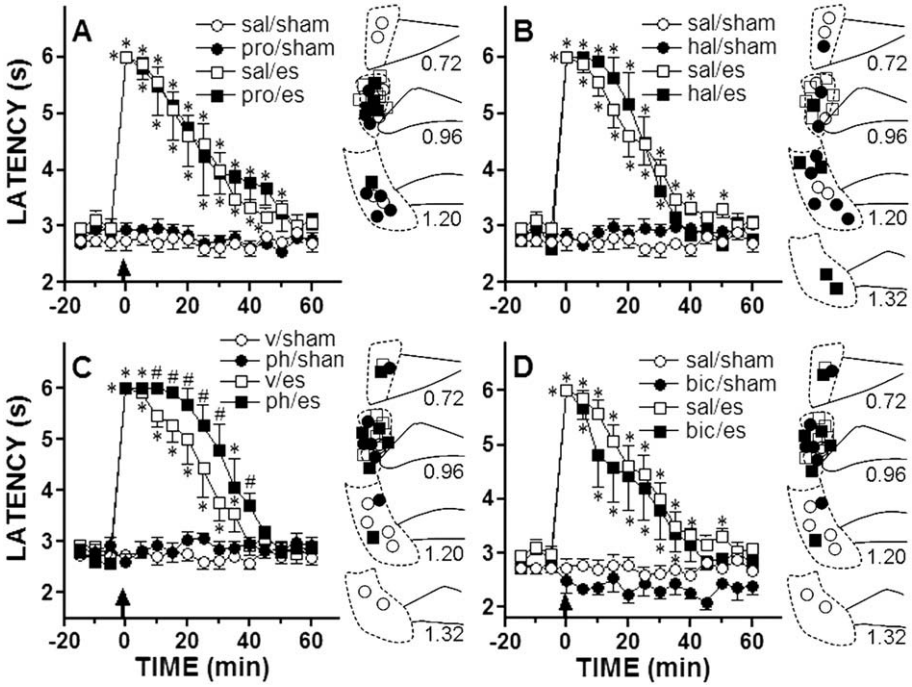

Fig. 3. Effects of intrathecal injection of propranolol (pro = 20 μg) (A), haloperidol (hal = 25 μg) (B), phaclofen (ph = 20 μg) (C), or bicuculline (bic = 0.3 μg) (D) on the increase in tail-flick latency caused by electrical stimulation (es) of the pedunculopontine tegmental nucleus (PPTg). The es (arrow) was applied 15 min after each antagonist. Saline (sal) was given at a dose of 5 μl. The location of the electrode tips is indicated as in Fig. 1. Points are means ± S.E.M., $n = 5–7$ for all groups. *Different from the sal/sham group. #Different from the other groups. $P<0.05$ in all cases.

statistical analysis of the data in Fig. 2E and F, respectively. Under this condition, the curves in Fig. 2E and F were significantly different ($P<0.0001$ in all cases) regarding treatment ($F_{5,33}=35.4$; $F_{6,28}=33.5$, respectively) and time ($F_{15,495}=44.1$; $F_{15,435}=33.5$, respectively), and showed a significant treatment×time interaction ($F_{75,495}=11.1$; $F_{75,435}=2.2$, respectively). Sham-stimulated rats had a progressive but nonsignificant reduction of tail-flick latency following intrathecal administration of WB 4101 (20 μg) or idazoxan.

SPA from the PPTg had a shorter duration also in rats treated with methysergide (30 μg) (Fig. 2G), but was not altered significantly in intensity or duration by mecamylamine (40 μg) (Fig. 2H), propranolol (20 μg) (Fig. 3A), or haloperidol (25 μg) (Fig. 3B). In contrast, SPA from the PPTg had a longer duration in phaclofen-treated (20 μg) rats (Fig. 3C), but was not altered in bicuculline-treated (0.3 μg) rats (Fig. 3D). The curves in Figs. 2G and F and 3A–D were significantly different ($P<0.0001$ in all cases) regarding treatment ($F_{3,22}=37.5$; $F_{3,18}=20.7$; $F_{3,20}=23$; $F_{3,21}=35.9$; $F_{3,20}=22.2$; $F_{3,20}=22.3$, respectively) and time ($F_{15,435}=52.4$; $F_{15,270}=42.7$; $F_{15,300}=48.4$; $F_{15,315}=62.9$; $F_{15,300}=32.3$; $F_{15,300}=32.3$, respectively), and showed a significant treatment×time interaction ($F_{75,435}=2.2$; $F_{45,270}=13.3$; $F_{45,300}=14.6$; $F_{45,315}=23$; $F_{45,300}=12$; $F_{45,300}=12$, respectively). Sham-stimulated rats had a nonsignificant reduction in tail-flick latency following bicuculline. The other drugs shown in Fig. 3 did not produce significant changes in the tail flick latencies of sham-stimulated rats. SPA from the PPTg was also nonsignificantly altered by metergoline, a 5-$HT_{1/2/6/7}$ receptor antagonist (not shown in the figures). A higher dose of bicuculline produced a strong agitation in the animals, thus precluding any observation regarding nociceptive changes in the test.

4. Discussion

The present study confirmed that the electrical stimulation of the PPTg, but not of sites in its close vicinity, produces a strong and long-lasting antinociceptive effect in the rat tail-flick test, as demonstrated earlier in cats and rats (see Introduction). No animal showed gross disturbance of motor coordination after the stimulation period, but several rats had muscle contractions during stimulation, occasionally accompanied by vocalization and jumping. Locomotor changes following electrical stimulation of or injection of glutamate agonist into the PPTg have already been shown in rats (Garcia-Rill et al., 1990; Milner and Mogenson, 1988). The PPTg connections to brain regions involved in the control of muscle tonus (Inglis and Winn, 1995) may account for the changes in animal behaviors observed during the stimulation period.

The threshold for tail withdrawal was significantly increased for periods of up to 60 min following stimulation of the PPTg, as compared to sham-stimulated rats. This long-lasting effect may hypothetically result from the changes produced by endogenous factors released during the stimulation period, or the activation from the PPTg of supraspinal reverberating circuitry that acts to inhibit the spinal transmission of nociceptive inputs (Melzack and Melinkoff, 1974). A spinal reverberating circuit is also possible, since specific nociceptive afferents from the spinal cord lamina I ascending via the dorsolateral funiculus to the parabrachial pontine region (which includes the PPTg) have already been demonstrated (Berkley and Scofield, 1990).

SPA from the PPTg was weaker and shorter in rats treated intraperitoneally with atropine (a nonselective antagonist of muscarinic cholinergic receptors) than in saline-treated rats. Intraperitoneal naloxone, methysergide or phenoxybenzamine (nonselective antagonists of opioid, serotonergic and α-adrenergic receptors, respectively) only reduced the duration of the effect. In contrast, intraperitoneal propranolol, mecamylamine or haloperidol (nonselective antagonists of β-adrenergic, nicotinic cholinergic and dopaminergic receptors, respectively) was ineffective against SPA from the PPTg, even though used at doses equal to or higher than those shown to be effective against chemically or electrically induced antinociception (Drago et al., 1984; Rees et al., 1987; Sahley and Berntson, 1979). Therefore, at least muscarinic cholinergic, α-adrenergic, serotonergic and endogenous opioid mechanisms but not β-adrenergic, nicotinic cholinergic or dopaminergic mechanisms seem to be involved in the modulation of the PPTg stimulation-produced inhibition of the tail-flick reflex. However, we cannot make conclusions about the particular site of action for each of the antagonists while using systemic antagonists.

SPA from the PPTg was dose-dependently reduced by intrathecal atropine, but remained unchanged following intrathecal mecamylamine. Thus, spinal muscarinic but not nicotinic cholinergic receptors are involved in the modulation of SPA from the PPTg. The lack of effectiveness of mecamylamine confirms earlier studies showing that systemic, intrathecal or local mecamylamine does not alter analgesia induced by the injection of carbachol (Katayama et al., 1984b) or N-methylcarbachol (Iwamoto and Marion, 1993) into the PPTg. Corroborating this view, intrinsic spinal cholinergic neurons are present in the deep dorsal horn, and cholinergic receptors are located in the superficial and deep dorsal horn (Barber et al., 1984), which are known as areas of nociceptive modulation. In addition, several lines of evidence point to a spinal muscarinic-mediated antinociception involved in the descending modulation of pain (Eisenach, 1999).

Intrathecal phenoxybenzamine or WB4101 (an antagonist of α_1-adrenergic receptors) reduced both the intensity and duration of SPA from PPTg, while idazoxan (an antagonist of α_2-adrenergic receptors) reduced only its duration. Therefore, SPA from the PPTg depends on the activation of descending pathways that utilize both α_1-adrenergic receptors (to modulate both intensity and duration of the effect) and α_2-adrenergic receptors (to modulate the duration of the effect). The antinociceptive effect of stimulating noradrenergic nuclei in the rostral ventral medulla or injecting noradrenaline intrathecally has already been demonstrated (Millan, 2002). Intrathecal methoxamine or ST-91 (α_1- and α_2-adrenergic agonists, respectively) produces antinociception in the rat-tail flick and hot-plate tests (Howe et al., 1983), thus revealing that α_1- and α_2-adrenergic receptors are both involved in the spinal modulation of nociceptive inputs.

Iwamoto and Marion (1993) showed that carbachol-induced antinociception from the PPTg in rats is reduced by intrathecal idazoxan but enhanced by prazosin, another α_1-adrenergic receptor antagonist. The high affinity of prazosin for all α_1-adrenergic receptor subtypes contrasts with the higher affinity of WB4101 for the α_{1A}- and α_{1C}-adrenergic receptor subtypes (Bylund et al., 1994), and may be the reason for the discrepant results. Carbachol activates local cholinergic cells only, whereas electrical stimulation modifies the activity of non-cholinergic cells and fibers of passage as well (Randich et al., 1988). Thus, the different mode of stimulation used in each study may also account for the controversial results.

SPA from the PPTg was not altered by intrathecal propranolol or haloperidol. These antagonists were also ineffective against the N-methylcarbachol-induced antinociception from the PPTg (Iwamoto and Marion, 1993) or SPA from the APtN (Rees et al., 1987). Therefore, spinal β-adrenergic or dopaminergic mechanisms are unlikely to be involved in the modulation of SPA from the PPTg.

Intrathecal naloxone or methysergide reduced only the duration of SPA from PPTg, as described above for systemic antagonists, and indicate that spinal opioid- and serotonergic-mechanisms mediate the duration but do not contribute to the intensity of SPA from the PPTg. The change induced by the antagonists in the duration, but not in the intensity of the SPA may alternatively be due to kinetic factors. However, the effects of naloxone given intrathecally at the dose of 20 or 30 μg were not different. These results contrast with reports showing the effectiveness of 5-$HT_{1C/2}$ and 5-HT_3 receptor antagonists in reducing the intensity of the N-methylcarbachol-induced antinociception from the PPTg (Iwamoto and Marion, 1993). Therefore, we postulate that both cholinergic and non-cholinergic cells in the PPTg contribute to SPA from the nucleus. On the other hand, our results are in agreement with the earlier demonstration that nicotine-induced

analgesia from the PPTg is not affected by systemic naloxone (Iwamoto, 1991), while the carbachol-induced analgesia from the PPTg may be prolonged by intrathecal naloxone (Iwamoto and Marion, 1993).

Intrathecal phaclofen (an antagonist of $GABA_B$ receptors) but not bicuculline (an antagonist of $GABA_A$ receptors) prolonged SPA from the PPTg. Thus, the activation of spinal $GABA_B$, but not $GABA_A$ receptors modulates negatively the inhibitory influence exerted by pathways descending from the PPTg against nociceptive inputs. Sham-stimulated animals treated intrathecally with bicuculline had a non-significant reduction of the nociceptive threshold. Intrathecal bicuculline produces strong agitation, autonomic responses to tactile stimulation and increased withdrawal flexion responses (Sivilotti and Woolf, 1994), which are behaviors indicative of a high degree of GABAergic tonic spinal inhibition (Cronin et al., 2004). It is possible that phaclofen interferes with GABAergic terminals controlling the release of neurotransmitters from descending pathways during the activation of the PPTg.

In summary, the SPA from the PPTg involves the spinal activation of cholinergic muscarinic and α_1-adrenergic mechanisms, but it is unlikely that nicotinic cholinergic, β-adrenergic and dopaminergic mechanisms participate in the effect. Serotonergic, endogenous opioid and α_2-adrenergic mechanisms are somewhat involved in the duration but not in the intensity of the effect. These results differ from those obtained following stimulation of the PAG or APtN, which are very sensitive to naloxone, or stimulation of the NRM, which is sensitive to antagonists of serotonin receptors (Sorkin et al., 1993). They are similar to the findings of studies of SPA from the APtN regarding the effectiveness of antagonists of α-adrenergic receptors, but differ regarding the effectiveness of naloxone only against the effects of APtN stimulation (Rees et al., 1987). Finally, the effects from the PPTg resemble those with GiA stimulation, which depends on the activation of α-adrenergic mechanisms (Besson and Chaouch, 1987) to inhibit spinal nociceptive inputs directly or via intrinsic cholinergic neurons (Eisenach, 1999).

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