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Stimulation of the occipital or retrosplenial cortex reduces incision pain in rats

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ABSTRACT

The electrical stimulation of the occipital (OC) or retrosplenial (RSC) cortex produces antinociception in the rat tail-flick and formalin tests. This study examined the antinociceptive effects of stimulating the OC or RSC in a rat model of post-incision pain. The involvement of the anterior pretectal nucleus (APtN) as intermediary for the effect of OC or RSC stimulation was also evaluated because the OC and RSC send inputs to the APtN, which is implicated in antinociception and nociception. It is shown that a 15-s period of electrical stimulation of the OC or RSC significantly reduced post-incision pain for less than 10 min and at least 15 min, respectively. The injection of 2% lidocaine (0.25 μl), naloxone (10 ng/0.25 μl), methysergide (40 pg/0.25 μl), or atropine (100 ng/0.25 μl) into the APtN produced a further increase in post-incision pain. The effect of RSC stimulation was shorter and less intense in rats pretreated with lidocaine, methysergide or naloxone. The effect of OC stimulation was shorter and less intense in lidocaine-treated rats, but remained unchanged in rats pretreated with methysergide or naloxone in the APtN. The effects of stimulating the OC or RSC were not changed in rats treated with atropine. We conclude that stimulation-induced antinociception from the RSC or OC in rat post-incision pain activates distinct descending pain inhibitory pathways. The pathway activated from the RSC utilizes serotonergic and opioid mediation in the APtN, whereas stimulation of the OC utilizes a non-serotonergic, non-cholinergic and non-opioid mediation in the same nucleus.

1. Introduction

The electrical stimulation of the occipital (OC) or retrosplenial (RSC) cortex induces antinociception in the rat tail-flick and formalin tests (Reis et al., 2010). Very little information exists on the contribution of the OC and RSC to nociceptive processing: activation of RSC (Hess et al., 2007) and visual cortex (Baciu et al., 1999; Craig et al., 1996) during noxious stimulation in human subjects, and bilateral activation of OC following electroacupuncture but not sham-treatment in human volunteers (Zhang et al., 2007) were reported in a functional magnetic resonance imaging (fMRI) study.

The OC and RSC project to the ipsilateral anterior pretectal nucleus (APtN) (Foster et al., 1989; Cadusseau and Roger, 1991), a structure implicated in antinociception (Roberts and Rees, 1986) and nociception (Neto et al., 1999; Porro et al., 1999; Villarreal et al., 2003). Neuroimaging studies have shown that noxious stimulation of the rat paw delineates BOLD contrasts in the APtN (Lowe et al., 2007). Stimulation-produced antinociception (SPA) from the APtN involves local muscarinic cholinergic (Rees et al., 1992), and opioid and serotonergic receptors (Rosa and Prado, 1997). Additionally, incision pain in rats is more intense after pharmacological blockade of serotonergic, muscarinic cholinergic or opioid receptors in the APtN (Villarreal and Prado, 2007).

We have recently shown that the APtN works as an intermediary for separate pathways activated from the OC and RSC to modulate nociception in the rat tail-flick test, utilizing at least serotonin and endogenous opioid as mediators in the nucleus (Reis et al., 2011). In addition, stimulation of the APtN produces antinociceptive effects of different intensities and duration depending on the pain model used (Villarreal et al., 2004a). This study was then undertaken to further examine whether stimulation of the OC or RSC induces antinociception in a model of post-incision pain. In addition, the involvement of APtN as intermediary for the effects of OC or RSC stimulation was evaluated in the same model as well. In this case, the changes induced by the injection of lidocaine, atropine, methysergide or naloxone into the APtN on the effects of cortical stimulation were examined to determine whether these drugs are effective in blocking the antinociception induced by cortical stimulation in a rat model of incision pain.

2. Materials and methods

2.1. Subjects and surgery

The experiments were conducted with 120 male Wistar rats (140–160 g), housed two to a cage with free access to food and water and maintained at an average ambient temperature of 23 ± 1 °C with a 12-h light–dark cycle, before and after surgery. The experiments were

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approved by the Commission of Ethics in Animal Research, Faculty of Medicine of Ribeirão Preto, University of São Paulo (Number 240/2005). The guidelines of the Committee for Research and Ethical Issues of IASP (Zimmermann, 1983) were followed throughout the experiments.

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. Rats were stimulated at the left OC or RSC, using the following coordinates (in mm): AP = 2.8 (from interaural line); L = 3.0 (from the midline), and H = −1.5 (from the skull surface) for the OC, and AP = 3.0 (from interaural line); L = 1.8 (from the midline) and H = −1.9 (from the skull surface), for the RSC. A 12-mm length 23-gauge stainless steel guide cannula was also stereotaxically implanted in the skull until its tip layed 3 mm above the left APtN, using the coordinates AP = +3.1 (from interaural line); L = 2.0 (from the midline) and H = −3.2 (from the skull surface). The electrode and guide cannula were then fixed to the skull with two screws and dental cement. One of these screws was used as the reference electrode. A 12-mm length sterile stainless steel wire was introduced into the guide cannula to reduce the risk of obstruction and so maintained until the time of drug administration. The animal was then given penicillin (50 mg/kg, i.m.) and allowed to recover for at least one week before the experiment. Each experimental group had 6 rats.

2.2. Model of incision pain

Each animal was anesthetized with 1.5% halothane in oxygen via a loose-fitting, cone-shaped mask and a 1-cm longitudinal incision was made through the skin and fascia of the plantar region of the right hind paw, starting 0.5 cm from the proximal edge of the heel, as described elsewhere (Brennan et al., 1996). The plantaris muscle was left intact during the procedure. The skin was then sutured with two 5–0 nylon stitches. Rats were placed in an elevated clear plastic cage with a nylon mesh bottom, which allowed easy access to the paw plantar surface. Before each test, the animals remained in the cage for approximately 15 min to allow behavioral acclimation. The threshold to mechanical punctate stimulation was measured with an automated electronic von Frey apparatus (IITC Electronic Equipments, CA, U.S.A.), consisting of a hand-held probe unit to which a rigid plastic tip (tip area = 0.44 mm^2) was connected. The experimenter then applied the plastic tip with an increasing force in an upward direction against sites near the heel, 1–2 mm adjacent to the medial border of the wound and to similar sites in the non-incised hind paw. The movement of the probe was interrupted when a withdrawal of the stimulated paw occurred (positive response). During this procedure the applied force was continuously recorded by a main unit connected to the probe. A single trial consisted of 3 applications of the tip, once every 5 s in each hind paw. The mean of three readings was taken as the mechanical threshold for a particular timing. In all cases, the thresholds for both hind paws of each rat were measured immediately before (BL1), and 2 h (BL2) after the incision. Drug or saline was then injected into the left APtN and the mechanical threshold for both hind paws of each animal was measured at 5-min intervals for up to 15 min. Sham or electrical stimulation of the left cortical target was performed 2 min later, and the animal was retested immediately after the stimulation (20 min after the injection) and then at 5-min intervals for a total of 40 min.

In each experiment the mechanical threshold in APtN-blocked rats is expected to be lower than in control as reported elsewhere (Villarreal and Prado, 2007). Thus, comparison of the effects of stimulating a cortical target may be somehow difficult. We tried to overcome this difficulty by comparing the difference (Δ) between BL1 and the threshold obtained 15 min after the injection into the APtN or immediately after the OC stimulation (20 min after the injection). This was done in these experiments whenever the groups of rats did not differ at BL1, stimulated- and sham stimulated-rats treated with saline were not different at 15 min after the injection, and stimulated- and sham stimulated-rats treated with drug showed no difference at 15 min after the injection as well.

2.3. Stimulation procedures

Electrical stimulation (AC, 60 Hz) was applied for 15 s to the cortical target 20 min after the intracerebral injection. The mean current thresholds required for OC and RSC to inhibit the tail flick reflex in rats were early found to be 15 and 17 μA, respectively (Reis et al., 2010). By this reason, the present study utilized current intensities of 15 and 20 μA for OC and RSC, respectively. During the period of stimulation, the rat was gently restrained by hand, and the drop in voltage across a 1-kΩ resistor in series with the electrode was continuously monitored on an oscilloscope. The threshold to mechanical stimulation was then recorded within 30 s after cortical stimulation and then at 5-min interval for up to 25 min. No attempt was made to test for the presence of antinociception during the stimulation. Control (sham) rats were submitted to identical procedures for electrode implant and their connections to the stimulator assembly. They also received saline or drug in the APtN but no current was passed through the electrode.

2.4. Intracerebral injection

Drug or vehicle was microinjected into the APtN using a glass needle (70–90 μm, o.d.) protected by a system of telescoping steel tubes (Azami et al., 1980). The assembly was inserted into the guide cannula and the needle advanced to protrude 3.0 mm beyond the guide cannula tip. The volume of microinjection was 0.25 μl, delivered at a constant rate over a period of 3 min. The needle was removed 20 s after completion of the injection. Animals were gently restrained by hand during the microinjection procedures.

2.5. Histology

At the end of the experiments, each animal was deeply anesthetized with intraperitoneal sodium thiopental and perfused through the heart with 4% paraformaldehyde in 0.1 M phosphate buffered saline. Fast green (0.25 μl) was injected through the guide cannula to label the site of intracerebral injection. The brain was removed and the electrode track or dye spot was localized from 50-μm serial coronal sections stained with neutral red, and was identified on diagrams from a rat brain atlas (Paxinos and Watson, 1986). Only animals that had the electrode or injection site confirmed by histology were considered for data analysis.

2.6. Drugs

Atropine sulfate (100 ng/0.25 μl), methysergide maleate (40 pg/0.25 μl) and naloxone hydrochloride (10 ng/0.25 μl) were purchased from Sigma (St. Louis, MO, USA). All antagonists were diluted in saline, and their doses referred to the salt. Two percent lidocaine chloride (Xylocaine®) was purchased from AstraZeneca from Brasil (São Paulo, Brazil). The doses and volume used were based in Villarreal and Prado (2007).

2.7. Data analysis

Mechanical thresholds (in grams) are reported as means ± SD. Comparisons between control (sham-stimulated rats treated with saline into the APtN) and test groups were made by analysis of variance (ANOVA) followed by Bonferroni's post-hoc test, or multivariate analysis of variance (MANOVA) with repeated measures to compare the groups over all times. The factors analyzed in MANOVA were treatments, time

and treatment × time interaction. In the case of treatment × time interaction, one-way analysis of variance also followed by Bonferroni's test was performed for each time. Since repeated observations from the same subject are not independent, we opted to perform a MANOVA of the repeated factors to avoid having to correct the degree of freedom in case the sphericity condition of the usual univariated ANOVA approaches was not satisfied (Jennen-Steinmetz, 1989). The analysis was performed using the statistical software package SPSS/PC+, version 3.0. The values of Δ of saline- and drug-treated rats at 15 min or 20 min after injection into the APtN were compared by ANOVA followed by the Tukey test. The level of significance was set at $P<0.05$ in all cases.

3. Results

The experiments were conducted in 120 rats. They all had histologically verified electrode and injector placements within the cortical and APtN targets, respectively, and were considered for data analysis. Microscope images showing the electrode track in the OC or in the RSC were similar to those shown elsewhere (Reis et al., 2010) and were not shown in figures.

3.1. Effects of electrical stimulation of the OC on post-incision pain

The time course of the effects produced by the electrical stimulation of OC on the post-incision pain is shown in Fig. 1. The groups did not differ significantly regarding the mechanical thresholds for both hind paws measured immediately before the incision (BL1). Two hours after the incision (BL2), a significant reduction in the mechanical threshold of the incised paw was observed. In contrast, no change was observed in the non-incised hind paw as compared to BL1. The mechanical thresholds of both hind paws were not changed significantly after the injection of saline (0.25 μl) into the APtN contralateral to the incised paw. The rats used in the present study did not exhibit spontaneous pain-like behavior. The stimulation of the OC significantly increased the threshold of the incised paw for less than 10 min, but did not change the threshold of the non-incised hind paw throughout the period of observation. The curves in Fig. 1 were significantly

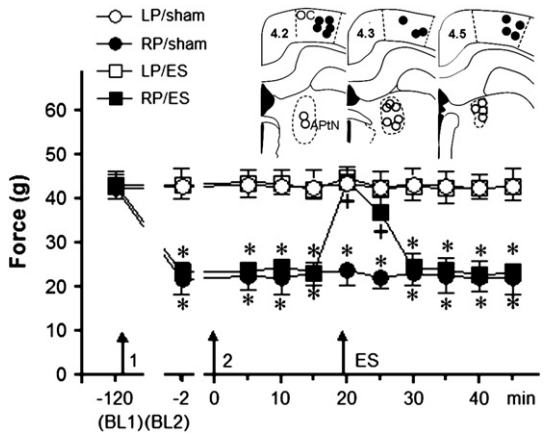

Fig. 1. Time–course of the changes produced by electrical stimulation (ES) of the occipital cortex (OC) on the paw withdrawal threshold to mechanical stimulation in a rat model of incision pain. The thresholds for both hind paws of each rat were measured immediately before (BL1), and 2 h (BL2) after a surgical incision, which was performed on the plantar aspect of the right hind paw (RP) at the moment indicated by arrow 1. Saline (0.25 μl) was injected into the left anterior pretectal nucleus (APtN) at the moment indicated by arrow 2. The left OC was stimulated (15 μA) or sham stimulated for 15 s at the moment indicated by arrow ES. The force that produced a withdrawal reflex of the RP and left hind paw (LP) was recorded in grams. The locations of the sites of stimulation in the OC and injection of saline into the APtN are illustrated on coronal sections taken from Paxinos and Watson (1986) and shown in the insert. Data are the mean ± SD of 6 sham-stimulated rats or 6 rats submitted to electrical stimulation. *Different from LP/sham and LP/ES; + Different from RP/sham ($P<0.05$).

different regarding treatment ($F_{3,20}=81.31$; $P<0.0001$) and time ($F_{10,200}=80.36$; $P<0.0001$), and had significant treatment × time interaction ($F_{30,200}=37.88$; $P<0.0001$). The locations of the electrode tip in the OC and injection site in the APtN are shown in the insert of Fig. 1. Twelve additional rats injected with saline in the APtN had electrode tip located in the parietal cortex (not shown in figures). They had a significant reduction in the mechanical threshold of the incised paw but displayed no significant change in the mechanical threshold following cortical stimulation, as shown elsewhere (Reis et al., 2010).

The results obtained from rats treated with saline in the APtN and sham or effectively stimulated in the OC were used throughout the whole testing period as control for the next experiment to reduce the number of rats used in the study.

3.2. Effects of electrical stimulation of the OC on the post-incision pain of rats treated with lidocaine, naloxone, methysergide or atropine in the APtN

The results of the experiment with lidocaine, naloxone, methysergide or atropine are shown in Figs. 2A, B, 3A and B, respectively. In each experiment, the groups were not significantly different between each other regarding thresholds for either hind paw measured immediately before (BL1) or 2 h after the incision (BL2). BL2 was significantly lower than BL1 in the incised paw, while the threshold of the non-incised paw did not change significantly throughout the period of observation (not shown in figures). In all experiments, the injection of saline (0.25 μl) into the APtN did not change the threshold as compared to BL2 of the same experimental group (groups sal/sham in Figs. 2 and 3). However, the injection of 2% lidocaine (0.25 μl), naloxone (10 ng/0.25 μl), methysergide (40 pg/0.25 μl), or atropine (100 ng/0.25 μl) into the APtN produced a further significant reduction of the threshold (Figs. 2A, B, 3A, and B, respectively). Sham-stimulated rats treated with saline or drug in the APtN did not exhibit significant changes in threshold throughout the experimental period after BL2. In all experiments, stimulation of the OC produced a significant reduction in post-incision pain in both saline- and drug-treated rats. The effect was significantly weaker and more short-lived in rats with neural block of the APtN than in control rats (Fig. 2A). In contrast, the effect of the OC stimulation in naloxone- (Fig. 2B), methysergide- (Fig. 3A), or atropine- (Fig. 3B) treated rats, was similar to the respective control in intensity and duration.

The curves shown in Figs. 2 and 3 differ significantly regarding treatment ($F_{3,20}=57.00$ in 2A, 41.92 in 2B, 41.98 in 3A, and 31.36 in 3B; $P<0.001$ in all cases) and time ($F_{10,200}=322.22$ in 2A, 389.81 in 2B, 276.19 in 3A, and 320.01 in 3B; $P<0.0001$ in all cases), and had significant treatment × time interaction ($F_{30,200}=25.24$ in 2A, 46.11 in 2B, 26.75 in 3A, and 34.46 in 3B; $P<0.0001$ in all cases). The locations of the electrode tip in the OC and injection site in the APtN are shown in the inserts of Figs. 2 and 3.

Fifteen minutes after the injection into the APtN, Δ of saline-treated rats was significantly lower than Δ of groups of rats treated with lidocaine (Fig. 2A; $t=4.224$; $P<0.01$), naloxone (Fig. 2B; $t=6.462$; $P<0.001$), methysergide (Fig. 3A; $t=3.618$; $P<0.05$), or atropine (Fig. 3B; $t=3.887$; $P<0.01$). In the electrically stimulated groups, at 20 min after the injection into the APtN, Δ was nearly absent in saline-treated but still significantly larger in lidocaine-treated rats ($t=6.121$; $P<0.001$) (Fig. 2A). On the other hand, the Δ values at 20 min after the intracerebral injection of naloxone- (Fig. 2B; $t=0.7080$; $P>0.05$), methysergide- (Fig. 3A; $t=2.553$; $P>0.05$), or atropine- (Fig. 3B; $t=0.9237$; $P>0.05$) treated rats did not differ significantly from saline-treated groups.

3.3. Effects of electrical stimulation of the RSC on post-incision pain

The time course of the effects produced by the electrical stimulation of the RSC on post-incision pain is shown in Fig. 4. The groups

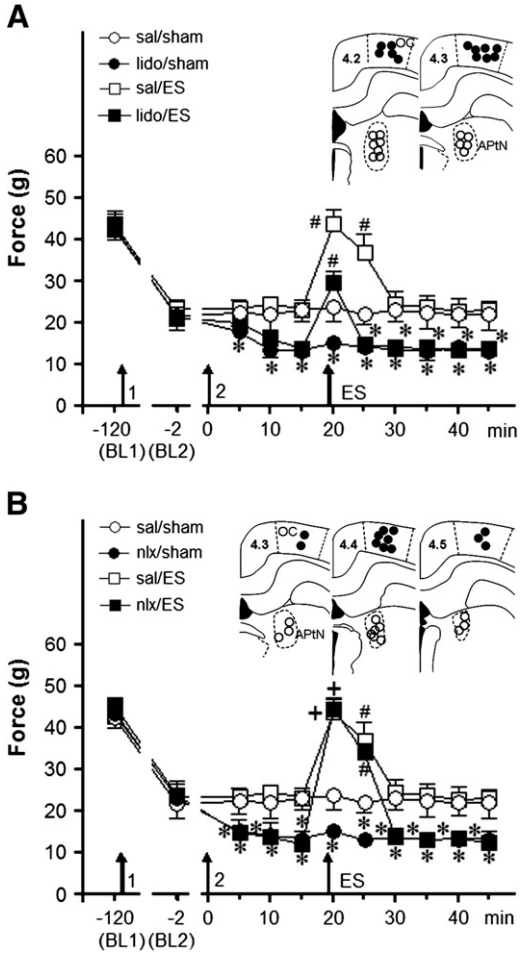

Fig. 2. Time–course of the changes produced by injection of saline, lidocaine (A) or naloxone (B) into the anterior pretectal nucleus (APtN) on the antihyperalgesic effect produced by the electrical stimulation (ES) of the occipital cortex (OC) in a rat model of incision pain. The thresholds for both hind paws of each rat were measured immediately before (BL1), and 2 h (BL2) after a surgical incision, which was performed on the plantar aspect of the right hind paw at the moment indicated by arrow 1. Saline (sal = 0.25 μl), 2% lidocaine (lido = 0.25 μl), or naloxone (nlx = 10 ng/0.25 μl) was injected into the left APtN at the moment indicated by arrow 2. The left OC was stimulated (15 μA) or sham stimulated for 15 s at the moment indicated by arrow ES. The force that produced a withdrawal reflex of the incised paw was recorded in grams. The locations of the sites of stimulation in the OC and injection of saline into the APtN are illustrated on coronal sections taken from Paxinos and Watson (1986) and shown in the inserts. Data in each graph are the mean ± SD of 6 rats per group. *Different from sal/sham; + Different from sal/sham and drug/sham; #Different from the remaining groups ($P<0.05$).

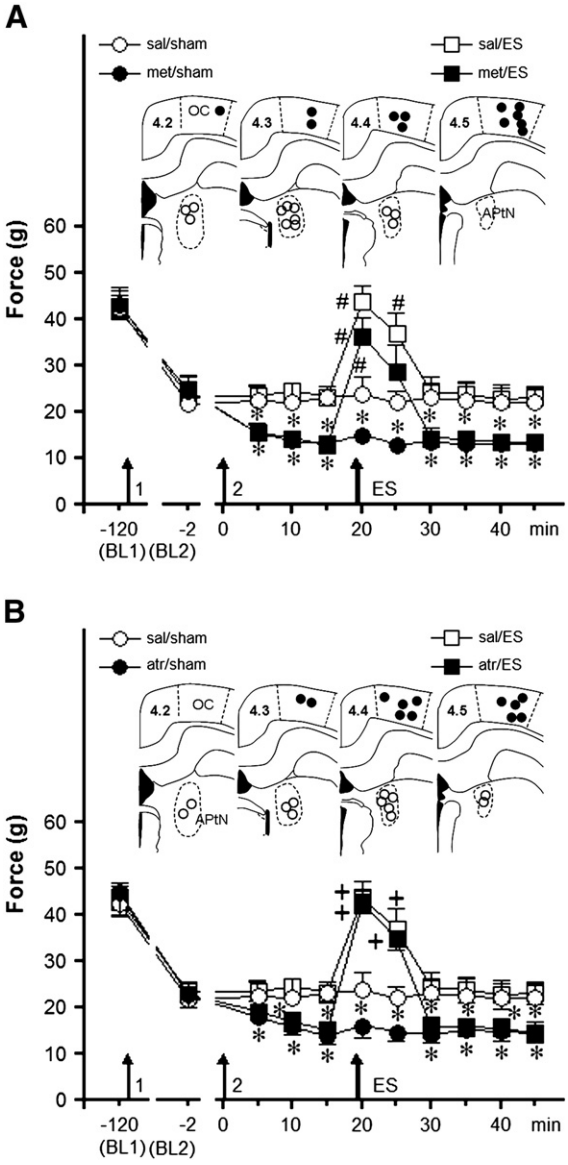

Fig. 3. Time–course of the changes produced by injection of saline, methysergide (A) or atropine (B) into the anterior pretectal nucleus (APtN) on the antihyperalgesic effect produced by the electrical stimulation (ES) of the occipital cortex (OC) in a rat model of incision pain. The thresholds for both hind paws of each rat were measured immediately before (BL1), and 2 h (BL2) after a surgical incision, which was performed on the plantar aspect of the right hind paw at the moment indicated by arrow 1. Saline (sal = 0.25 μl), methysergide (met = 40 pg/0.25 μl), or atropine (atr = 100 ng/0.25 μl) was injected into the left APtN at the moment indicated by arrow 2. The left OC was stimulated (15 μA) or sham stimulated for 15 s at the moment indicated by arrow ES. The force that produced a withdrawal reflex of the incised paw was recorded in grams. The locations of the sites of stimulation in the OC and injection of saline into the APtN are illustrated on coronal sections taken from Paxinos and Watson (1986) and shown in the inserts. Data in each graph are the mean ± SD of 6 rats per group. *Different from sal/sham; #Different from the remaining groups ($P<0.05$).

also did not differ significantly regarding the mechanical thresholds for both hind paws measured immediately before the incision (BL1). Two hours after the incision (BL2), a significant reduction in the mechanical threshold of the incised paw was also observed, but no change was observed in the non-incised hind paw as compared to BL1. The mechanical thresholds of both hind paws were not changed significantly after the injection of saline (0.25 μl) into the APtN contralateral to the incised paw. The stimulation of the RSC significantly increased the threshold of the incised paw for at least 15 min, but did not change the threshold of the non-incised hind paw throughout the period of observation. The threshold recorded soon after RSC stimulation was significantly higher than that recorded before surgery.

The curves in Fig. 4 are significantly different regarding treatment ($F_{3,20} = 164.36$; $P<0.0001$) and time ($F_{10,200} = 115.47$; $P<0.0001$), and had significant treatment × time interaction ($F_{30,200} = 63.74$; $P<0.0001$). The locations of the electrode tips and sites of injection into the APtN in each group are shown in the insert of Fig. 4. Eleven additional rats injected with saline in the APtN had electrode tip located at sites in frontal cortex (not shown in figures). They had a significant reduction in the mechanical threshold of the incised paw but displayed no significant change in the mechanical threshold following cortical stimulation, as shown elsewhere (Reis et al., 2010).

The results obtained from rats treated with saline in the APtN and sham or effectively stimulated in the RSC were used throughout the whole testing period as control for the next experiment to reduce the number of rats used in the study.

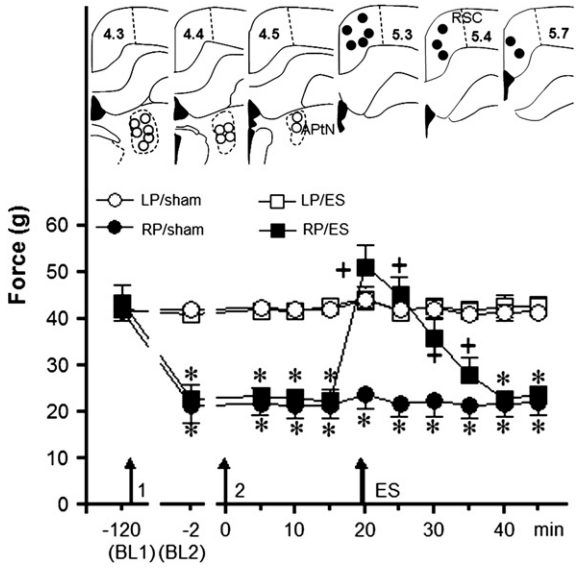

Fig. 4. Time–course of the changes produced by electrical stimulation (ES) of the retrosplenial cortex (RSC) on the paw withdrawal threshold to mechanical stimulation in a rat model of incision pain. The thresholds for both hind paws of each rat were measured immediately before (BL1), and 2 h (BL2) after a surgical incision, which was performed on the plantar aspect of the right hind paw (RP) at the moment indicated by arrow 1. Saline (0.25 μl) was injected into the left anterior pretectal nucleus (APtN) at the moment indicated by arrow 2. The left RSC was stimulated (20 μA) or sham stimulated for 15 s at the moment indicated by arrow ES. The force that produced a withdrawal reflex of the RP and left hind paw (LP) was recorded in grams. The locations of the sites of stimulation in the RSC and injection of saline into the APtN are illustrated on coronal sections taken from Paxinos and Watson (1986) and shown in the insert. Data are the mean ± SD of 6 sham-stimulated rats or 6 rats submitted to electrical stimulation (ES). *Different from LP/sham and LP/ES; + Different from RP/sham ($P<0.05$).

3.4. Effects of electrical stimulation of the RSC on post-incision pain of rats treated with lidocaine, naloxone, methysergide or atropine in the APtN

The results of the experiments with lidocaine, naloxone, methysergide or atropine are shown in Figs. 5A, B, 6A, and B, respectively. In each experiment, the groups did not differ significantly between each other regarding BL1 or BL2. BL1 was significantly higher than BL2 in the incised paw, whereas the threshold of the non-incised paw did not significantly change throughout the period of observation (not shown in Figures). In all experiments, the injection of saline (0.25 μl) into the APtN did not change the threshold as compared to BL2 of the same experimental group (groups sal/sham and sal/ES in Figs. 5 and 6). However, a further significant reduction of the threshold was obtained following the injection of 2% lidocaine (0.25 μl), naloxone (10 ng/0.25 μl), methysergide (40 pg/0.25 μl), or atropine (100 ng/0.25 μl) into the APtN (Figs. 5A, B, 6A, and B respectively). Sham stimulated rats treated with saline or drug in the APtN did not exhibit significant changes of the thresholds throughout the period of observation after BL2. A significant reduction in post-incision pain in all experiments occurred in saline-treated (control) rats after RSC stimulation. The effect of RSC stimulation was significantly weaker than in control and lasted less than 5 min in rats treated with lidocaine (Fig. 5A), naloxone (Fig. 5B), or methysergide (Fig. 6A) in the APtN. Stimulation of the RSC in atropine-treated rats significantly reduced post-incision pain, but the effect had intensity similar to that of control and lasted less than 10 min (Fig. 6B). The effect of RSC stimulation in atropine-treated rats was stronger than in saline-treated rats. The significant difference between the thresholds of these groups at the moment before the RSC stimulation may account for the apparent stronger efficacy of cortical stimulation in atropine-treated rats.

The curves shown in Figs. 5 and 6 differ significantly regarding treatment ($F_{3,20}=92.60$ in 5A, 66.41 in 5B, 74.89 in 6A, and 49.83 in 6B; $P<0.001$ in all cases) and time ($F_{10,200}=332.34$ in 5A, 339.17 in 5B, 376.56 in 6A, and 411.80 in 6B; $P<0.0001$ in all cases), and had significant treatment × time interaction ($F_{30,200}=46.37$ in 5A, 42.34 in 5B, 52.69 in 6A, and 62.97 in 6B; $P<0.0001$ in all cases). The locations of the electrode tip in the RSC and injection site in the APtN are shown in the inserts of Figs. 5 and 6.

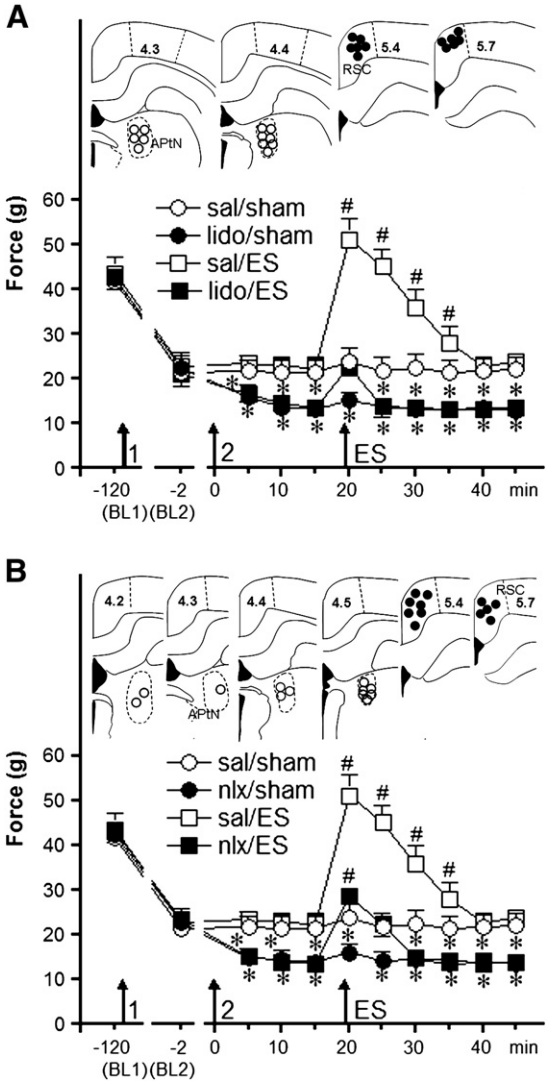

Fig. 5. Time–course of the changes produced by injection of saline, lidocaine (A) or naloxone (B) into the anterior pretectal nucleus (APtN) on the antihyperalgesic effect produced by the electrical stimulation (ES) of the retrosplenial cortex (RSC) in a rat model of incision pain. The thresholds for both hind paws of each rat were measured immediately before (BL1), and 2 h (BL2) after a surgical incision, which was performed on the plantar aspect of the right hind paw at the moment indicated by arrow 1. Saline (sal = 0.25 μl), 2% lidocaine (lido = 0.25 μl) or naloxone (nlx = 10 ng/0.25 μl) was injected into the left APtN at the moment indicated by arrow 2. The left RSC was stimulated (20 μA) or sham stimulated for 15 s at the moment indicated by arrow ES. The force that produced a withdrawal reflex of the incised paw was recorded in grams. The locations of the sites of stimulation in the RSC and injection of saline into the APtN are illustrated on coronal sections taken from Paxinos and Watson (1986) and shown in the inserts. Data in each graph are the mean ± SD of 6 rats per group. *Different from sal/sham; #Different from the remaining groups ($P<0.05$).

Fifteen minutes after the injection into the APtN, Δ of saline-treated rats was significantly lower than Δ of groups of rats treated with lidocaine (Fig. 5A; $t=4.16$; $P<0.01$), naloxone (Fig. 5B; $t=4.07$; $P<0.01$), methysergide (Fig. 6A; $t=4.89$; $P<0.001$), or atropine (Fig. 6B; $t=3.80$; $P<0.01$). In the electrically stimulated groups, 20 min after the injection into the APtN, Δ was nearly absent in

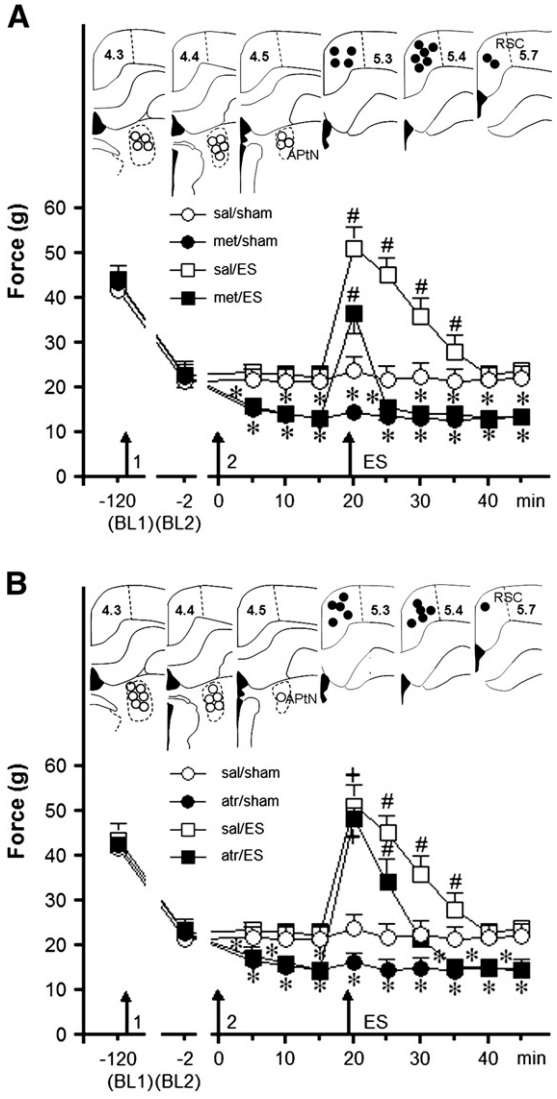

Fig. 6. Time–course of the changes produced by injection of saline, methysergide (A) or atropine (B) into the anterior pretectal nucleus (APtN) on the antihyperalgesic effect produced by the electrical stimulation (ES) of the retrosplenial cortex (RSC) in a rat model of incision pain. The thresholds for both hind paws of each rat were measured immediately before (BL1), and 2 h (BL2) after a surgical incision, which was performed on the plantar aspect of the right hind paw at the moment indicated by arrow 1. Saline (sal = 0.25 μl), methysergide (met = 40 pg/0.25 μl), or atropine (atr = 100 ng/0.25 μl) was injected into the left APtN at the moment indicated by arrow 2. The left RSC was stimulated (20 μA) or sham stimulated for 15 s at the moment indicated by arrow ES. The force that produced a withdrawal reflex of the incised paw was recorded in grams. The locations of the sites of stimulation in the RSC and injection of saline into the APtN are illustrated on coronal sections taken from Paxinos and Watson (1986) and shown in the inserts. Data in each graph are the mean ± SD of 6 rats per group. *Different from sal/sham; + Different from sal/sham and drug/sham; #Different from the remaining groups ($P<0.05$).

saline-treated rats, but was still significantly larger in lidocaine- (Fig. 5A; $t=9.17$; $P<0.001$), naloxone- (Fig. 5B; $t=6.19$; $P<0.001$), and methysergide- (Fig. 6A; $t=3.61$; $P<0.05$) treated rats. Values of Δ in atropine-treated rats, however, were not significantly different from saline-treated group (Fig. 6B; $t=2.35$; $P>0.05$).

4. Discussion

The results of the present study confirm earlier findings that the paw withdrawal threshold to mechanical punctate stimulation in rats decreases following plantar incision, thus characterizing a post-incision hyperalgesia, as reported elsewhere (Zahn and Brennan, 1990). In addition, it was shown that a 15-s period of electrical stimulation of the OC or RSC significantly reduces post-incision hyperalgesia for less than 10 min and at least 15 min, respectively. The stimulation of the RSC significantly increased the threshold of the incised paw for at least 15 min, but did not change the threshold of the non-incised hind paw throughout the period of observation. The threshold of the incised paw recorded soon after RSC stimulation was significantly higher than that recorded before surgery. Therefore, the effect of RSC stimulation was not only antihyperalgesic (as was the effect of OC stimulation) but was also analgesic in the test. Withdrawal threshold to mechanical stimulation was earlier used to characterize the effect of electrical stimulation to reduce incision pain (Villarreal et al., 2004a). An antinociceptive effect of shorter duration following stimulation of the OC or RSC (5 and 10 min, respectively) was first demonstrated using the rat tail-flick and formalin tests (Reis et al., 2010).

The results are in agreement with data from the literature showing an increase in regional cerebral blood flow (Paulson et al., 2002) and decreased volume correlated with mechanical hyperalgesia (Seminowicz et al., 2009) in the rat RSC following injury of the sciatic nerve. Activation of the rat RSC during application of noxious stimulation (Hess et al., 2007) or persistent noxious pancreatic inflammation (Westlund et al., 2009) was also reported in fMRI studies. Patients with ongoing visceral pain displayed increased fMRI signals in the RSC as well (Dunckley et al., 2005; Iannetti et al., 2005). The regional cerebral blood flow of fibromyalgic patients under resting conditions is higher in the RSC than in control patients in a fMRI study (Wik et al., 2003), while RSC deactivation was observed during painful stimulation of fibromyalgic patients (Wik et al., 2006). According to Seminowicz et al. (2009) the rat RSC (which is equivalent to the human posterior cingulate cortex) may play an important role in pain perception. Indeed, the RSC seems to be a part of the medial pain system involved in cortical planning of responses to pain (Vogt et al., 1996). More recent studies suggest that the RSC may be involved in memory and visuospatial processing (Maddock, 1999). A decreased activity of the posterior cingulated cortex has been reported in MRI studies during noxious heat stimulus in man (Coghill et al., 1994) or when the allodynic state of neuropathic patients was compared to the non-painful conditions (Petrovic et al., 1999). In contrast, a fMRI study from Pogatzki-Zahn et al. (2010) showed activation in the anterior cingulate cortex, insular cortex, thalamus, frontal cortex, and somatosensory cortex, but did not report changes in the posterior cingulate cortex of volunteers that received an experimental incision within the right forearm. However, bilateral increases in the regional cerebral blood flow during ongoing post-surgical pain due to extraction of third molars were identified in posterior cingulate gyrus (Howard et al., 2011). The time between lesion and scanning used in each study may account for the differences. In fact, Pogatzki-Zahn et al. (2010) observed that peak brain activation occurred about 2 min after incision and decreased subsequently, whereas Howard et al. (2011) submitted their patients to postsurgical scan only when pain intensity was scored to be greater than 30/100 mm.

Reports on the involvement of OC and antinociception are sparser: the regional cerebral blood flow of fibromyalgic patients under resting conditions was lower in OC, compared to control patients in a fMRI study (Wik et al., 2003). During central sensitization, nociceptive stimulation of the area of secondary hyperalgesia induced stronger deactivations in a larger set of brain regions including the occipital cortex (Iannetti et al., 2005). Activation of several cortical regions, including the OC was detected following rectal pain in healthy subjects (Baciu et al., 1999). However, the activation of visual cortex may be related to visual imagery during the painful stimulation. In contrast, stimulation of the OC with train of 1 ms strain waves at 10 Hz was ineffective in the rat tail-flick and hot-plate tests (Hardy, 1985). The different pattern of stimulation used in each study may account for the discrepancy.

The OC and RSC in rats project to the ipsilateral APtN (Foster et al., 1989; Cadusseau and Roger, 1991), a diencephalic structure known to be implicated in antinociception (Reis et al., 2011) and nociception (Neto et al., 1999; Porro et al., 1999). We found that the injection of lidocaine into the APtN reduced both the intensity and duration of the antihyperalgesic effect of the OC or RSC stimulation. Therefore, APtN integrity is necessary for the effect of OC or RSC stimulation against post-incision hyperalgesia. It is noteworthy that the neural block of the APtN almost abolished the effect of RSC stimulation while only reducing the effect of OC stimulation. A possibility then remains that the antihyperalgesic effect of stimulating the OC needs the participation of an intermediary other than the APtN.

The post-incision hyperalgesia was significantly intensified after the injection of lidocaine into the APtN as described elsewhere (Villarreal et al., 2003). The APtN participates in a descending pain control pathway, which is tonically involved in the suppression of persistent spinal nociceptive inputs (Rees and Roberts, 1987). In contrast, post-incision hyperalgesia were not changed in rats after bilateral lesions of the rostral medial medulla (Pogatzki et al., 2002). The rostral medial medulla contains the nucleus raphe magnus and the nucleus reticullaris gigantocellularis–pars α (Urban and Gebhart, 1997) and contributes with descending influences that act to enhance nociceptive inputs in the spinal dorsal horn (Urban and Gebhart, 1999). A dense innervation from the APtN to the gigantocellular reticular nucleus pars α and a relatively lower density of APtN efferents was noted in the medullary raphe nuclei (Itoh et al., 1983; Zagon et al., 1995). However, the integrity of the rostral medial medulla has very little effect on the analgesic effects induced by stimulation of the APtN (Terenzi et al., 1991), although it is crucial for opiate- and stimulation-induced analgesia from the periaqueductal gray (Azami et al., 1982; Fields et al., 1988). The tonic descending control exerted by the APtN against incision pain may involve brain structures other than those found in the rostral medial medulla. The APtN send connections onto the lateral paragigantocellularis nucleus (Zagon et al., 1995), where cells projections into the dorsal horn of the lumbar spinal cord were already demonstrated (Siddall et al., 1994). In fact, bilateral lesions in the rostral ventrolateral medulla reduce the antinociceptive effects of APtN stimulation by up to 70% (Terenzi et al., 1995). Fibers from the APtN were observed also in the region of the contralateral pedunculopontine tegmental nucleus (Terenzi et al., 1995), a mesopontine structure from which glutamate reduces the incisional pain (Villarreal et al., 2004b), electrical stimulation produces analgesia in the tail flick test (Rosa et al., 1998), inhibits the nociceptive inputs in the spinal dorsal horn (Carstens et al., 1980) and mediates some of the descending influences from the APtN (Terenzi et al., 1992).

Intrinsic muscarinic, opioid and serotonergic mechanisms are implicated with the SPA from the APtN in the rat tail-flick test (Prado, 1989; Rees et al., 1992). The injection of naloxone, atropine or methysergide into the APtN increased post-incision hyperalgesia, thus confirming the involvement of APtN intrinsic muscarinic, opioid and serotonergic mechanisms in the modulation of persistent pain as reported elsewhere (Villarreal and Prado, 2007).

The previous injection of methysergide or naloxone into the APtN reduced both the intensity and duration of the effect of stimulating the RSC, whereas atropine was ineffective. In contrast, injection of methysergide, naloxone or atropine into the APtN was ineffective against the antihyperalgesic effect of stimulating the OC. The dose of atropine used here was twice that shown earlier to be effective in the APtN (Villarreal and Prado, 2007). In addition, a significant increase in post-incision hyperalgesia was observed soon after its administration into the APtN. Consequently, the lack of effect of atropine in this study is unlikely the result of an inadequate dose of this antagonist.

Overall, studies point to a role of APtN in the antinociceptive effect of stimulating the OC or RSC in models of phasic and persistent inflammatory (Reis et al., 2010) and post-incision pain (present study). The antihyperalgesic effect of stimulating the OC or RSC is likely to result from the activation of a descending pain inhibitory mechanism that utilizes the APtN as an intermediary. Supporting this view, the SPA from the OC or RSC (Reis et al., 2010) or APtN (Rees and Roberts, 1987) in the tail-flick test did not occur in rats with lesion of the dorsolateral funiculus, which is the main route through which pain modulator pathways descend to the spinal cord (Millan, 1999). The stimulation of the RSC reduces post-incision hyperalgesia, activating at least serotonergic and opioid but not cholinergic terminals in the APtN. In contrast, the stimulation of the OC also reduces persistent post-incision hyperalgesia, but utilizing a mechanism in the APtN that is not serotonergic, cholinergic or opioid. Stimulation of the OC inhibits the tail-flick reflex, by activating serotonergic terminals in the APtN, while stimulation of the RSC inhibits the same reflex, by activating opioid terminals in the APtN (Reis et al., 2011). As a consequence, the effect of stimulating the OC or RSC depends on the integrity of the APtN, but the mechanism used in the nucleus differs depending on the type of noxious stimulus utilized in the test.

The heterogeneity of neurons in the APtN (Bokor et al., 2005) may account for these differences. In fact, stimulating dorsal APtN is more effective at producing antinociceptive effects on tail flick test, whereas stimulating ventral APtN is more effective at producing antinociception to surgical incision, as well as inhibiting ventral APtN caused a greater increase in incisional pain than did inhibiting dorsal APtN (Villarreal et al., 2004a). It was earlier proposed that pain models utilizing different types of noxious stimuli activate different pain suppression mechanisms (Ryan et al., 1985). A possibility remains that the processing of different types of stimuli at the OC or RSC involves distinct neurochemical systems in APtN.

5. Conclusions

The results presented demonstrate the involvement of the APtN in the antihyperalgesic effect of stimulating the OC or RSC in a rat model of postoperative pain. Serotonergic and opioid mechanisms in the APtN participate in the effect from the RSC, whereas a mechanism that is not cholinergic, serotonergic or opioidergic in the APtN participates in the effect from the OC.

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