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Antinociception induced by stimulation of ventrolateral periaqueductal gray at the freezing threshold is regulated by opioid and 5-HT_{2A} receptors as assessed by the tail-flick and formalin tests

Maria Cecília Zanoto de Luca[a], Marcus Lira Brandão[a], Vítor Augusto Motta[b], J. Landeira-Fernandez[c,d,*]

[a]*Laboratório de Psicobiologia, Departamento de Psicologia, Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto-Universidade de São Paulo, Avenida Bandeirantes 3900, 14049-900 Ribeirão Preto, Brazil*
[b]*Laboratório de Psicobiologia, Departamento de Processos Psicológicos Básicos, IP-Universidade de Brasília, Campus Darcy Ribeiro, 70910-900 Brasília, Brazil*
[c]*Departamento de Psicologia, Pontificia Universidade Católica do Rio de Janeiro, Rua Marquês de São Vicente, 225 Gávea, 22453-900, Rio de Janeiro, Brazil*
[d]*Laboratório de Psicologia Comparada, Universidade Estácio de Sá, Campus Akxe, Avenida Prefeito Dulcílio Cardoso 2900, 22631-021 Rio de Janeiro, Brazil*

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Abstract

It has been suggested that antinociception is part of the animal's defensive reaction to threatening situations. Chemical or electrical stimulation of the ventrolateral portion of the periaqueductal gray (vlPAG) produces both defensive freezing behavior and antinociception, supporting the view that the vlPAG is a critical structure in the coordination of the defensive reaction. The present study indicated that electrical stimulation of the vlPAG, at a current intensity sufficient to induce defensive freezing, caused a decrease in reactivity to a phasic escapable noxious stimulus (as measured in the tail-flick test) and to a tonic, inescapable noxious stimulus (as measured in the formalin test). These antinociceptive effects were reversed by microinjections of the opioid antagonist naltrexone or the specific 5-HT_{2A} receptor antagonist ketanserin into the stimulation sites. These results suggest that (a) activation of neural circuits of the vlPAG, responsible for the production of freezing behavior, reduces the reactivity to nociceptive stimuli (as evaluated by the tail-flick and formalin tests) and that (b) opioid- and 5-HT_{2A}-mediated mechanisms are called into action for regulating the antinociceptive response that accompanies the freezing behavior induced by vlPAG stimulation.

Keywords: Antinociception; vlPAG; Opioid receptors; 5-HT_{2A} receptors; Naltrexone; Ketanserin

1. Introduction

Electrical stimulation of several brain areas elicits antinociceptive processes through activation of pathways that inhibit sensitive neurons in the spinal cord (Basbaum and Fields, 1984). These antinociceptive systems can be activated by innate (Fanselow and Sigmundi, 1986) or learned (Fanselow et al., 1994) danger stimuli, suggesting that a reduction in nociceptive sensitivity allows a threatened animal to engage in necessary defensive reaction without being disturbed by competing reactions triggered by the noxious stimulus (Bolles and Fanselow, 1980). The ventrolateral portion of the periaqueductal gray (vlPAG) seems to be a critical structure in the coordination of this defensive process because its electrical or chemical stimulation produces defensive freezing behavior (Morgan and Carrive, 2001; Vianna et al., 2001a,b) and selectively inhibits responses to noxious stimuli in a variety of pain test conditions (Coimbra et al., 1992; Giesler and Liebeskind, 1976; Mayer et al., 1971; Reynolds, 1969).

It is well established that opioid receptors located in the vlPAG play an active role on pain inhibition. Several reports

* Corresponding author. Laboratório de Neurociências e Comportamento, Departamento de Psicologia, Pontificia Universidade Católica do Rio de Janeiro, Rua Marquês de São Vicente, 225 Gávea, 22543-900, Rio de Janeiro, RJ, Brazil. Tel.: +55-21-3114-1186; fax: +55-21-3114-1187.
E-mail address: landeira@psi.puc-rio.br (J. Landeira-Fernandez).

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employing a phasic and escapable nociceptive stimulation procedure, such as the tail-flick test, or a long-lasting tonic pain test, such as that induced by subcutaneous injections of formalin, have clearly indicated that antinociception induced by electrical stimulation of the vlPAG could be reversed by opioid antagonists in both acute and tonic pain tests (Cannon et al., 1982; Morgan et al., 1991). Moreover, morphine microinjection into the vlPAG decreased pain sensitivity to tail-flick (Yaksh et al., 1976; Schul and Frenk, 1991) and formalin tests (Manning et al., 1994). Finally, it has been reported that the progressive loss of the antinociceptive effect induced by morphine (tolerance) is mediated by opioid receptors within the vlPAG (Tortorici et al., 1999).

Besides opioid mechanisms, 5-hydroxytryptamine (5-HT, serotonin) receptors within the vlPAG are also involved in pain inhibitory mechanisms. For example, studies employing the tail-flick test indicated that administration of the nonspecific 5-HT antagonist methysergide into the vlPAG inhibited the antinociceptive effect produced by electrical stimulation of the vlPAG (Nichols et al., 1989) or by microinjection of morphine into the vlPAG (Schul and Frenk, 1991). However, it remains to be shown which 5-HT receptor type within the vlPAG is responsible for the occurrence of this form of antinociception. Moreover, it is still unclear whether 5-HT receptors located in the vlPAG also play a role on inhibitory mechanism in a long-lasting tonic pain test such as that induced by subcutaneous injection of formalin. This is an important issue since the type of pain test is an important variable in assessing nociception and its inhibitory mechanisms (Dennis and Melzack, 1979).

The purpose of the present study was twofold: (i) to examine whether vlPAG electrical stimulation at freezing threshold triggers antinociception assessed by the tail-flick and formalin tests, which use two different kinds of nociceptive stimuli, and (ii) whether opioid and serotoninergic mechanisms are involved in the antinociception induced by aversive stimulation of the vlPAG. The participation of vlPAG opioid and 5-HT_{2A} receptors on antinociception resulting from the vlPAG electrical stimulation at defensive freezing threshold was investigated by microinjecting naltrexone or the selective 5-HT_{2A} antagonist ketanserin (Kristiansen et al., 1993) at the electrical stimulation sites. Doses and latencies for action of ketanserin and naltrexone were chosen based on similar studies from our laboratory aimed at studying the antinociceptive effects elicited by aversive stimulation of the dorsal portion of the periaqueductal gray (dPAG) (Coimbra and Brandão, 1997; Coimbra et al., 1992; Castilho and Brandão, 2001).

2. Methods

2.1. Animals

Experiments were performed on male Wistar rats weighing 250–300 g from the animal house of the Campus of Ribeirão Preto of the University of São Paulo. Animals were housed in groups of four to five in Plexiglas-wall cage and given free access to food and water. Room temperature was controlled (23 ± 1 °C) and light/dark cycle was maintained on a 12:12-h cycle (lights on at 07:00 h). The experiments were conducted during the light phase of the cycle. The experimental protocols described below were in conformity with the regulations for care and use of laboratory animals of the Brazilian Society for Neuroscience.

2.2. Surgery

The animals were anaesthetized with tribromoethanol (250 mg/kg ip) and secured in a David-Kopf stereotaxic frame with skull oriented in the horizontal plane between bregma and lambda for the implant of a chemitrode in the vlPAG. The chemitrode was made of a stainless steel guide cannula (OD 0.6 mm; ID 0.4 mm) glued to a brain electrode made of stainless steel wire (diameter 160 μm), insulated except at the cross section of the tip extending 1 mm below the lower end of the cannula. The electrode wire was connected to a male pin, parallel to the outer end of the cannula that could be plugged into an Amphenol socket at the end of a flexible electrical cable and used for brain stimulation. The chemitrode was introduced at a 12° angle using the following coordinates with the lambda serving as the reference for each plane according to the Paxinos and Watson atlas (1997): anteroposterior 0.7 mm, mediolateral 1.7 mm, and dorsoventral 6.1 mm. The chemitrode was attached to the skull by means of autopolymerizing resin and two stainless steel screws. At the end of the surgery, the guide cannula was sealed with a stainless steel wire to protect it from congestion.

2.3. Determination of the electrical stimulation threshold for the occurrence of defensive freezing

Five days after surgery, defensive freezing threshold was determined by gradually increasing the current intensity of the electrical stimulation (AC 60 Hz, 15 s) applied through the chemitrode. Fifteen minutes after the animal's placement in an acrylic box (30 × 30 × 50 cm), electrical brainstem stimulation, monitored by measuring the voltage drop across 1-kΩ resistor, was presented for 15 s at 1-min interval. Current intensity was increased by steps of 5 μA until freezing behavior occurred. Freezing threshold was operationally defined as the lowest current intensity that produced a complete immobility in two consecutive series of electrical stimulation accompanied by at least two of the following autonomic reactions: urination, defecation, piloerection, or exophthalmia. Animals with freezing threshold above of 200 μA (peak to peak) were discarded from the experiments.

2.4. Drugs

Naltrexone hydrochloride (Sigma, USA) was dissolved in saline (0.9%) to a dose of 26 nmol in 0.2 μl volume.

Ketanserin tartrate (RBI, USA) was dissolved in saline (0.9%) to a dose of 5 nmol in 0.2 μl volume. The naltrexone and ketanserin doses as well as the waiting time for testing were chosen according to previous reports from our laboratory (Coimbra and Brandão, 1997; Castilho et al., 1999; Castilho and Brandão, 2001). To perform the formalin test, formaldehyde (38%, Labsynth, Brazil) was dissolved in saline (0.9%) to a 1.5% solution.

2.5. Drug microinjections

Drug microinfusion was made by an internal cannula, which was introduced and lowered 1.0 mm below the guide cannula. The internal cannula was connected to a Hamilton syringe via polyethylene tube (PE-10). A volume of 0.2 μl was microinjected during a 20-s period and the displacement of an air bubble inside the PE tube was used to monitor the microinjection. At the end of injection, the internal cannula was held inside the neural tissue for 30 s to prevent drug backup and allow absorption.

2.6. Experimental design

Animals were randomly assigned to one of four groups. Three of these groups, SAL(+), NTX(+), and KET(+), were given brainstem electrical stimulation, whereas no electrical stimulation was delivered to the fourth group NO STIM. The groups SAL(+) and NO STIM were microinjected with saline vehicle. Group NTX(+) was microinjected with naltrexone whereas group KET(+) was microinjected with ketanserin. Each group was comprised of at least eight animals and each animal served as subjects in both Experiments 1 and 2, as described below.

2.6.1. Experiment 1: Tail-flick test

The tail-flick test was conducted using a tail-flick device (Ugo Basile, Italy) and consisted in placing the animal into an acrylic tube and presenting a radiant heat to the distal third portion of the animal's tail. The latency of the first vigorous tail reflex, usually a flicking or a lashing, was recorded. The heat was adjusted to elicit tail-flick latencies (TFLs) between 2.5 and 3.5 s. A cutoff time of 6.0 s was used for termination of the heat to prevent tissue damage. Each TFL was normalized by an antinociception index (AI) according to the formula

$$\mathrm{AI} = \frac{(\mathrm{TFL\ test}) - (\mathrm{TFL\ control})}{6 - (\mathrm{TFL\ control})}$$

TFL control indicates the average tail-flick baseline latencies taken at 5 min intervals before the drug or control saline microinjections into the vlPAG. TFL test indicates the TFLs taken after the vlPAG sham or electrical stimulation.

After recording three tail-flick baseline latencies with 5-min intervals, saline, ketanserin, or naltrexone were microinjected into the vlPAG according to each group assignment. Five (naltrexone), 10 (saline), or 15 min (ketanserin) after the drug microinjection, animals in the SAL(+), NTX(+), and KET(+) groups were electrically stimulated for 15 s with the current intensity previously determined to trigger the defensive freezing response. Animals from the NO STIM group received sham electrical stimulation. At the end of the electrical stimulation procedure, each animal was immediately replaced to the tail-flick device and new TFL was recorded. Six additional TFLs were recorded during the experiment with a 5-min interval.

2.6.2. Experiment 2: Formalin test

Forty-eight hours after the tail-flick experiment, all the animals with the same group assignment were submitted to the same procedure as in the Experiment 1 with the exception that formalin, instead of the tail-flick test, was performed. The formalin test consisted of injecting 0.05 ml of 1.5% formalin subcutaneously into the ventral surface of the rat's right hind-paw immediately after the 15-s sham or brain electrical stimulation. The animal was then placed into an acrylic box (30 × 30 × 50 cm) and nociceptive responses were recorded utilizing a time sample procedure. For each 2 s, the experimenter rated the animal's behavior according to a pain scoring scale developed by Dubuisson and Dennis (1977): The scoring was as follows: 0 = normal weight-bearing on the formalin-injected paw, grooming, and rearing; 1 = limping during locomotion, favoring the formalin-injected paw; 2 = total elevation the formalin-injected paw; 3 = chewing, licking, shaking, or biting the injected paw. The behavior of each animal was scored continuously for a 60-min test period, which was divided into twelve 5-min time-bin periods. The nociceptive index (NI) was calculated for every 5-min time-bin period according to the formula:

$$\mathrm{NI} = \frac{R(0) + R(1) + R(2) + R(3)}{150}$$

R(0), R(1), R(2), and R(3) indicate the occurrence of the respective behavioral category of a maximum of 150 possible scoring opportunities during the 5-min time-bin scored every 2 s.

2.7. Histology

After the experiments, animals were deeply anaesthetized with urethane (250 g/l; Sigma) and perfused intracardially with phosphate-buffered saline (0.2 M, pH 7.6) followed by a paraformaldehyde in phosphate buffer (PB; 0.2 M pH 7.2). The brain was removed and postfixed in PB for approximately 2 days. The brains were then placed in a 10% (24 h) and then a 20% (24 h) sucrose solution in PB. Serial 45-μm midbrain sections were cut using a cryostat (Reichert-Jung, USA) and stained with methylene blue to localize the chemitrode tract according to the Paxinos and Watson (1997) atlas. Data from animals with electrode tips outside the vlPAG were not included in the statistical analysis.

2.8. Statistical analysis

Results from both experiments are graphically expressed as means ± S.E.M. Data obtained from each experiment were evaluated using a two-way analysis of variance (ANOVA) with repeated measures. Factor conditions refer to each of the four independent groups across the time course of the nociceptive test. Whenever ANOVA was significant, the Bonferroni post hoc test was employed for pairwise comparison. The level of statistical significance was $P < .05$.

3. Results

3.1. Histological analysis

Histological examination of the midbrain slices indicated that all electrode and cannula tips were located within the vlPAG. Fig. 1A presents a composite of the cannula placements and electrode tips aimed at the vlPAG. A photomicrography of a representative histological section showing the location of the chemitrode tract is presented in Fig. 1B.

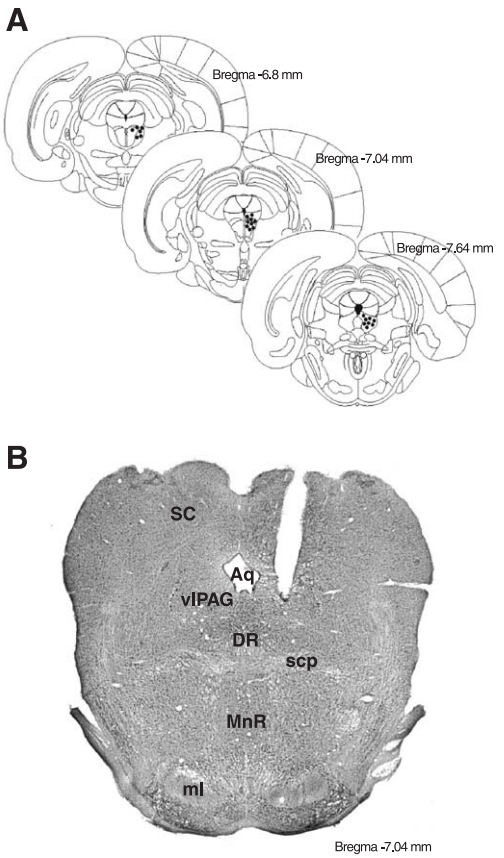

Fig. 1. (A) Composite of electrode and cannula tip (closed circles) locations on cross sections from Paxinos and Watson atlas (1997). Figures represent the atlas coordinates in millimeters posterior to bregma. The points indicated in the figure are less than the total number of animals used because of several overlaps. (B) Photomicrograph showing a typical chemitrode tract aimed at the vlPAG. Aq = aqueduct; scp = superior cerebellar peduncle; ml = medial lemniscus; MnR = median raphe nucleus; SC = superior colliculus; DR = dorsal raphe nucleus.

3.2. Experiment 1: Time course of antinociceptive effect of vlPAG electrical stimulation in the tail-flick test: role of opioid and 5-HT_{2A} receptors within the stimulation sites

Fig. 2 presents the mean (± S.E.M.) of the tail-flick AI during the three tail-flick baseline latencies recorded with a 5-min intervals before drug administration (−15, −10, and −5 min) along with the TFLs recorded with a 5-min interval immediately after the vlPAG sham or electrical stimulation. As can be seen from the figure, there are no differences among groups during the three tail-flick baseline latencies. On the other hand, a two-way ANOVA revealed an interaction among the four independent groups across the seven 5-min tail-flick tests [$F(30,320) = 3.43$, $P < .001$]. Post hoc pairwise comparison indicated that groups SAL(+) and NO STIM showed significantly different responses at 0, 5, and 10 min. This result indicates that antinociception was detected immediately after electrical stimulation of the vlPAG and lasted for at least 10 min (all P's $< .01$). Therefore, vlPAG electrical stimulation at current intensities able to induce a defensive freezing response also inhibited the tail-flick reflex. Moreover, this antinociceptive effect was reduced by both opioid and 5-HT_{2A} antagonists since groups NTX(+) and KET(+) displayed significantly lower AI than SAL(+) group at the intervals where antinociception was observed (all P's $< .01$). Finally, naltrexone promoted a partial blockade of the antinociception produced just after the vlPAG electric stimulation, since the group NTX(+) displayed a statistically higher AI than the sham stimulated group NO STIM immediately after electrical stimulation ($P < .01$).

3.3. Experiment 2: Time course of antinociceptive effect of vlPAG electrical stimulation in the formalin test: role of opioid and 5-HT_{2A} receptors within the stimulation sites

Fig. 3 illustrates the mean (± S.E.M.) of the formalin NI during twelve 5-min time-bin periods immediately after the vlPAG electrical stimulation. A two-way ANOVA revealed an interaction among the four independent groups across the 12 measurements of the formalin test [$F(36,396) = 1.51$, $P < .05$]. Inspection of the figure indicated that formalin injections yielded the biphasic nociceptive response normally observed in rats (Dubuisson and Dennis, 1977). Nociceptive behavior in the early phase of the test (0–5 min after the formalin injection) indicated that vlPAG electrical stimulation at the freezing threshold was not able to cause a change in pain sensitivity during this phase of the test. Although the group SAL(+) presented a lower NI in comparison to NO STIM group, this difference did not reach statistical significance ($P > .05$). Further analysis revealed that groups SAL(+) and NO STIM were signifi-

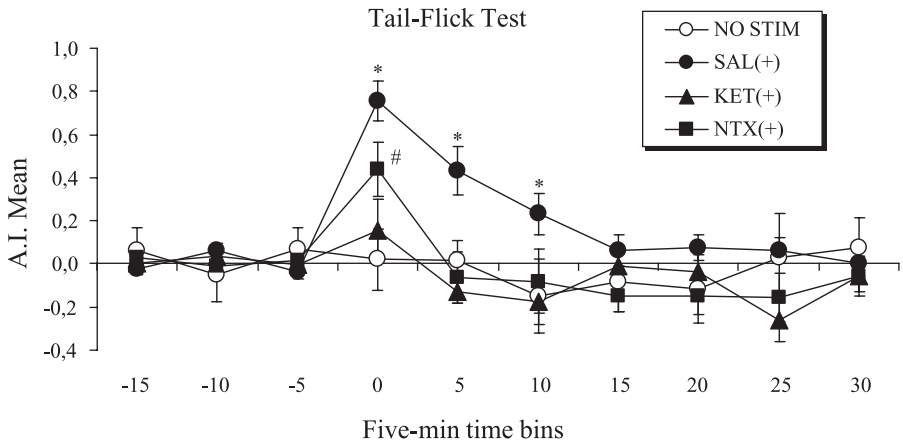

Fig. 2. Time course of the AI during the tail-flick test for each 5-min time-bin period across 15-min baseline period (− 15, − 10, and − 5 min) and the 30 min after the sham or vlPAG electrical stimulation. Rats in groups SAL(+), KET(+), and NTX(+) received an intra-vlPAG microinjection of saline, ketanserin, or naltrexone, respectively, and were electrically stimulated at the microinjection site. Animals from NO STIM group were microinjected with saline but were not electrically stimulated. * $P < .01$ SAL(+) from all other groups. # $P < .01$ NTX(+) × NO STIM.

cantly different during the late phase of the test (20–30 min after the formalin injection; all P's < .01) indicating an antinociceptive effect. Therefore, vlPAG electrical stimulation at freezing threshold was able to reduce formalin-induced behaviors exclusively during the late phase of the test. Naltrexone and ketanserin inhibited this antinociceptive effect since NTX(+) and KET(+) groups displayed significantly higher NI than SAL(+) group at the intervals where antinociception was observed (all P's < .01). Finally, it is noteworthy to mention that, although vlPAG electrical stimulation did not lead to an antinociception during the early phase of the formalin test, microinjection of naltrexone and ketanserin into the vlPAG increased nociceptive behavior during this phase. For example during the 0- to 5-min interval the NTX(+) and KET(+) groups showed significantly higher NI than the SAL(+) group (all P's < .01).

4. Discussion

Electrical or chemical stimulation of the vlPAG produces clear-cut defensive behavior, such as freezing (Morgan and Carrive, 2001; Vianna et al., 2001a,b,c), as well as a reduction in pain sensitivity in several pain tests (Coimbra et al., 1992; Giesler and Liebeskind, 1976; Mayer et al., 1971; Reynolds, 1969). It has been suggested that the role of this antinociceptive mechanism is to allow the animal to engage in the defensive behavior in spite of the pain associated with injuries it might have received (Bolles and Fanselow, 1980). According to this view, activation of vlPAG neurons might be able to produce both defensive freezing behavior and antinociception. Results from the present study indicated that sensitivity to phasic escapable nociceptive stimulus, as measured in the tail-flick test, and tonic inescapable nociceptive stimulus, as measured in the

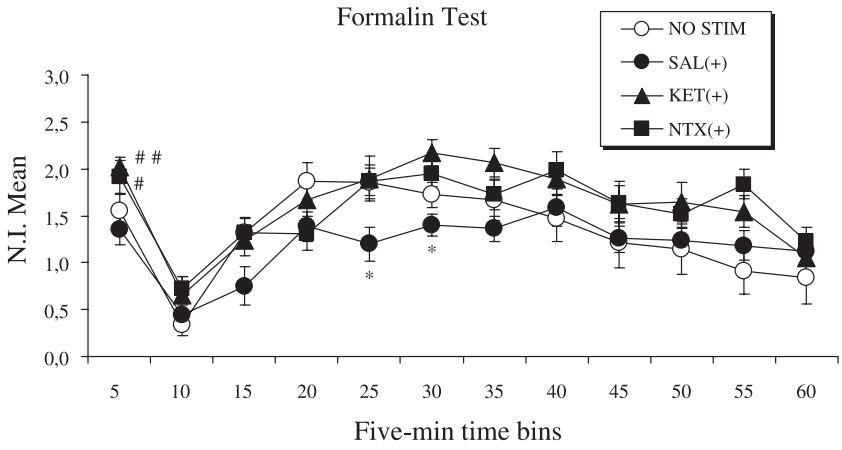

Fig. 3. Time course of the NI mean after subcutaneous formalin (1.5%, 0.05 ml) into the right hind-paw for each 5-min time-bin period across the 60-min test period. Rats in groups SAL(+), KET(+), and NTX(+) received an intra-vlPAG microinjection of saline, ketanserin, or naltrexone, respectively, and were electrically stimulated at the microinjection site. Animals from NO STIM group were microinjected with saline but were not electrically stimulated. * $P < .01$ SAL(+) from all other groups. # $P < .01$ SAL(+) x KET(+). ## $P < .01$ SAL(+) × NTX(+).

formalin test, could be inhibited by electrical stimulation of the vlPAG at current intensity able to elicit defensive freezing behavior. Therefore, these results are consistent with the hypothesis that antinociception is part of the animal's defensive reaction to threatening situations. It must be noted that while defensive freezing behavior and antinociception might share common gross anatomical substrate at the vlPAG level, they probably have different neurochemical substrates. In fact, microinjection of the opioid receptor antagonist naltrexone into the vlPAG reduced tonic antinociception in the formalin test induced by contextual cues previously associated with electrical footshocks but had no effect on the amount of defensive freezing behavior (Helmstetter and Landeira-Fernandez, 1990).

The antinociceptive effect observed in the tail-flick test lasted for 10 min after the vlPAG electrical stimulation and was blocked by microinjections of ketanserin into the same site of stimulation. An involvement of 5-HT_{2A} receptors in the modulation for these effects is suggested based on the affinity of ketanserin to these receptors (Leysen et al., 1981). The time course of this effect is compatible with an action on 5-HT_{2A} receptors already demonstrated in electrophysiological studies using microinjection of ketanserin into the periaqueductal gray (Brandão et al., 1991). Naltrexone caused a clear-cut blockade of the antinociception produced by vlPAG electrical stimulation during the entire period of the pain inhibition (immediately and 5 and 10 min after the stimulation). The occurrence of a partial blockade soon after vlPAG stimulation was probably due to the fact that the drug had not yet occupied all receptors since the antagonism was complete at 5 and 10 min following this stimulation. An opioid modulation for the effects of this compound is suggested bases of the affinity of this agent for μ-opioid receptors (Martin, 1983; Tempel et al., 1985). Although it is known that vlPAG opioid and 5-HT receptors within the vlPAG are involved in the modulation of pain sensitivity to a phasic escapable noxious stimulus, as measured in the tail-flick test, our results adds new information about the type of 5-HT receptors involved in this pain-inhibiting mechanism.

The present results also provide evidence that activation of defensive freezing response through electrical stimulation of the vlPAG can inhibit the occurrence of nociceptive behavior observed during the late or chronic phase of the formalin test. The inhibition of these formalin-induced behaviors during the late phase of the test lasted for 10 min and was also inhibited by microinjections of both opioid and 5-HT_{2A} antagonists into the vlPAG. These results are consistent with previous reports that implicate the participation of vlPAG opioid receptors in the pain reactivity to a long-lasting tonic and inescapable noxious stimulus (Morgan et al., 1991). Furthermore, they also implicate 5-HT_{2A} receptor-mediated mechanisms of the vlPAG in pain inhibition triggered by activation of the neural substrates of fear in this region. Therefore, aversive stimulation of the vlPAG can trigger mutually exclusive antinociceptive systems with different neurochemical mechanisms, which coexist within the vlPAG (Harris, 1996; Watkins and Mayer, 1986).

The temporal course of the effects of naltrexone in both tests is in agreement with other studies showing a similar duration of action for naltrexone when injected into the midbrain (Coimbra and Brandão, 1997; Coimbra et al., 2000; Monassi et al., 1999). Similarly, the time course of the effect of ketanserin is compatible with an action on 5-HT_{2A} receptors, as already demonstrated in electrophysiological studies using microinjection of ketanserin into the periaqueductal gray (Brandão et al., 1991).

The antinociception triggered by the electrical stimulation of the vlPAG is mediated through descending projections that relay in the rostral ventromedial medulla and inhibit nociceptive neurons in the dorsal horn of the spinal cord (Basbaum and Fields, 1984). The vlPAG receives opioid projections from the amygdaloid complex (Helmstetter et al., 1998; Oliveira and Prado, 2001) whereas 5-HT inputs to the vlPAG arise from the dorsal raphe nucleus (Beitz et al., 1986; Kwiat and Basbaum, 1990). Since our results demonstrated that antinociception induced by electrical stimulation of the vlPAG at freezing threshold was reversed by either opioid or 5-HT_{2A} antagonists microinjected into the same stimulation site, it might be possible that both opioid and 5-HT projections to the vlPAG converge onto a common site containing both opioid and 5-HT_{2A} receptors. In accordance with this view, it has been reported that antinociception induced by microinjection of morphine into the vlPAG was reduced by microinjection of methysergide into the same site (Schul and Frenk, 1991), indicating that 5-HT receptors within the vlPAG can modulate opioid-mediated antinociception in the vlPAG.

The present data confirm recent findings showing that vlPAG and dPAG mediate distinct types of defense reaction (Vianna et al., 2001c) and brings new evidence for distinct antinociceptive mechanisms accompanying the defensive behavior generated in both regions. Indeed, this study provided additional information about the differential involvement of opioid mechanisms in the antinociception induced by electrical stimulation of dPAG and vlPAG at the freezing threshold. While opioid antagonists clearly inhibit the antinociception elicited by vlPAG stimulation, as shown here, they did not affect that elicited by electrical stimulation of the dPAG (Castilho and Brandão, 2001). On the other hand, reduction of pain sensitivity accompanying freezing generated in both structures seems to be regulated by similar 5-HT-mediated mechanisms since, as shown in the present study, 5-HT_{2A} antagonists also inhibited the antinociception elicited by dPAG stimulation (Castilho and Brandão, 2001; Coimbra and Brandão, 1997; Coimbra et al., 1992). Interestingly, other reports have shown that the conditioned antinociception obtained following exposure to stimuli associated with footshocks can be blocked by opioid antagonists (Bellgowan and Helmstetter, 1998; Helmstetter and Landeira-Fernandez, 1990). In view of the

present findings, it is likely that these opioid mechanisms are associated with the vlPAG neural circuits rather than those of the dPAG. In contrast to other findings indicating that nonopioid mechanisms regulate the antinociception derived from activation of the neural substrates of fear in the dPAG, the antinociception that accompanies freezing behavior, triggered by aversive stimulation of the vlPAG, is of opioid nature. Moreover, 5-HT_{2A} receptor-mediated mechanisms are also called into action to modulate the antinociception induced by aversive stimulation of the vlPAG.

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