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# Blockade of histamine $H_2$ receptors of the periaqueductal gray and inferior colliculus induces fear-like behaviors

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**Abstract**

Electrical and chemical stimulation of the dorsal periaqueductal gray matter (dPAG) and the inferior colliculus (IC) induces escape behavior, usually accompanied by autonomic responses and antinociception. Recently, we presented evidence for a tonic inhibitory control exerted by $H_2$ histamine receptors on defensive behaviors generated in these midbrain tectum sites. Since treatments of these areas that elicit the defensive behavior repertoire frequently also have anxiogenic effects, we here used the elevated plus-maze (EPM) test for assessing the effects of microinjections of histamine (5–40 nmol), dimaprit (5–10 nmol) and ranitidine (10–30 nmol) into either dPAG or IC, which have a relative abundance of histamine-containing cells and histaminergic receptors. Dimaprit is an agonist and ranitidine is an antagonist of $H_2$ histamine receptors. Immediately after the injections, the animals were submitted to the EPM test. Whereas dPAG injections of dimaprit had no behavioral effects, histamine (40 nmol) caused a significant reduction in exploratory activity. On the other hand, ranitidine alone or following saline had aversive-like effects in both structures, i.e. reduced open arm, but not closed arm, entries. This pattern is usually interpreted as representing an anxiogenic effect. These effects were more pronounced after injection into dPAG than into IC. Freezing, the most prominent effect produced by ranitidine, was significantly inhibited by histamine as well as dimaprit. Thus, $H_2$ receptor blockade has fear-like action in the midbrain tectum with predominance in the dPAG. Such an action can be understood as a concomitant of defensive behavior, which has been shown to be a consequence of $H_2$ receptor antagonism in both dPAG and IC. The functional significance of the different effects of $H_2$ receptor blockade in dPAG and IC is discussed in the light of the probable distinct roles of these structures in the organization of defensive behavior.


*Keywords:* Histamine; Midbrain tectum; Inferior colliculus; dPAG; Fear; Ranitidine; $H_2$ receptors

## 1. Introduction

Serotonin, excitatory amino acids, substance P, GABA and opioid-mediated mechanisms have all been implicated in the regulation of defense reactions that can be induced by electrical or chemical stimulation of the midbrain tectum (Brandão et al., 1994, 1999, 2001). The dorsal periaqueductal gray matter (dPAG) and inferior colliculus (IC) play important roles in the integration of fear-related behaviors (Graeff, 1990; Brandão et al., 1994, 1999). The involvement of the IC in negative emotional states has been demonstrated by behavioral, electrophysiological and immunohistochemical data (Brandão et al., 1988, 1994; Melo et al., 1992; Melo and Brandão, 1995; Silveira et al., 1993; Lamprea et al., 2002). For example, increases in the auditory evoked potentials from recording sites in the IC have been reported in threatening situations, such as the presence of conditioned aversive stimuli, ultrasounds at the frequency of 22 kHz, and fear induced by microinjections of glutamate into this structure (Brandão et al., 2001). c-*fos* immunoreactivity studies have shown that the IC is labeled along with the amygdala, hypothalamus and dPAG following either its electrical or chemical stimulation or the exposure of the animals to aversive stimulation (Silveira et al., 1993; Lamprea et al., 2002).

Many cells belonging to the vestibular and auditory systems, including the inferior colliculi, contain histamine-immunoreactive fibers (Nieuwenhuys, 1995). The IC is the

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region of the midbrain tectum most densely innervated by histaminergic cell bodies from the tuberomammillary nucleus of the hypothalamus (Panula et al., 1989; Inagaki et al., 1990). Although $H_1$ and $H_2$ receptors have a widespread expression in the brain (Taylor and Snyder, 1972; Taylor, 1975; Nieuwenhuys, 1995; Schwartz, 1979; Schwartz et al., 1980), $H_2$ receptors have high selectivity for structures of the limbic system (Hass and Butcher, 1975; Brown et al., 2001). In this context, it has been shown that microinjections of histamine into the hippocampus have both proaversive and antiaversive effects (Alvarez and Banzan, 1986; Ruarte et al., 1997). We recently found that histamine may modulate the defense reaction that is elicited at the midbrain level, since microinjection of ranitidine, an antagonist of $H_2$ receptors, into the IC elicited the defensive behavior repertory in rats (Santos et al., 2001). The aversive nature of this treatment was demonstrated by defensive behaviors, such as freezing, vigorous flight, including galloping and jumping, and autonomic responses (piloerection, micturition and defecation). Moreover, these fear-like behaviors were antagonized by dimaprit, an $H_2$ receptor agonist (Santos et al., 2002). Electrical stimulation and pharmacological treatments of the midbrain tectum, which elicits defensive-like behavior, have also been shown to have anxiogenic properties, as assessed by tests such as the elevated plus-maze (EPM).

The EPM has been one of the most useful tests for detecting anxiolytic and anxiogenic drug effects and for disclosing their mechanisms of action (Pellow et al., 1985; File, 1992, 1992; Handley and McBlane, 1993; Motta and Brandão, 1993; Cruz et al., 1994; Anseloni and Brandão, 1997). Standard anxiolytic drugs, such as diazepam, increase the percentage of entries into and the time spent in the open arms of the maze. Ethological analysis has been an important tool in the analysis of behavioral responses to innate and acquired anxiety-inducing stimuli. Behavior acts representing risk assessment, such as stretched attend posture and peeping-out, have been taken as measures of anxiety (Blanchard et al., 1991; Cole and Rodgers, 1993; Anseloni and Brandão, 1997). On the other hand, immobility has been taken as an indirect measure of fear (Cole and Rodgers, 1993; Rodgers and Johnson, 1995; Cruz-Morales et al., 2002). Previous reports have described freezing, defecation and increases in plasma corticosteroids as behavioral and physiological expressions of fear when the animals are restricted to the open arms (Pellow et al., 1985; Treit et al., 1993). Thus, we analyzed the behavioral effects of microinjections of histamine, the $H_2$ receptor agonist dimaprit (Eriks et al., 1992; Nakamura et al., 1997; Paquay et al., 1999) and the $H_2$ receptor antagonist ranitidine (Bradshaw et al., 1979; Hill et al., 1997; Sakurada et al., 2002), into the dPAG and IC of rats submitted to the EPM test using an ethopharmacological approach to obtain a broad behavioral profile (Blanchard et al., 1991; File, 1992; Anseloni and Brandão, 1997; Cole and Rodgers, 1993).

## 2. Material and methods

### 2.1. Animals and surgery

Male rats ($n = 214$; Wistar) weighing 230–260 g were used. The animals were housed in a colony room with food and water ad libitum. They were maintained on a 12:12 L/D cycle (lights on at 0700) at $23 \pm 1$ °C and tested during the light phase of the cycle. Each rat was implanted with a unilateral stainless steel guide cannula (0.6 mm o.d., 0.4 mm i.d.) under tribromoethanol anesthesia (250 mg/kg ip). The cannula was directed to the IC at the following coordinates (using λ as the reference for each plane): 0.9 mm posterior, 1.5 mm lateral and 4.5 mm ventral. The coordinates used for the dPAG were 0.1 mm anterior, 1.9 mm lateral (with angle of 16°) and 4.0 mm ventral to λ (Paxinos and Watson, 1997). Each cannula was fixed with polyacrylic cement anchored to the skull with three stainless steel screws and was plugged with stainless steel stylets. The experiments reported in this article were performed in compliance with the recommendation of Brazilian Society of Neuroscience and Behavior (SBNeC), which are based on the U.S. National Institute of Health Guide for Care and Use of Laboratory Animals.

### 2.2. Apparatus

The EPM was made of wood with two open arms ($50 \times 10$ cm) and two enclosed arms of the same size, with 50 cm high walls made of transparent sheets of Plexiglas. The level of illumination was 20 lx on the floor level of the closed arms of the maze. The maze was configured such that arms of the same type were opposite each other, and the whole maze was raised 50 cm from the floor. A raised edge (0.5 cm) on the open arms provided additional grip for the rats.

All tests were conducted during the light phase of the L/D cycle, between 1300 and 1700. Rats were placed individually in the center of the maze facing a closed arm and allowed 5 min of free exploration. The behavior of the animals was recorded by a video camera positioned above the maze, allowing the discrimination of all behaviors, with the signal relayed to a monitor in another room via a closed circuit TV camera. The maze was cleaned thoroughly after each test using damp and dry cloths.

The behavioral categories were scored from videotapes using ethological analysis software (Observer, Noldus), which allows measurement of the number of entries into both arms and the time spent in different parts of the maze as well as the recording of duration and frequency of behaviors such as grooming, rearing, etc. Behaviors scored from videotape included traditional and nonstandard plus-maze parameters as outlined below.

### 2.3. Ethological analysis

The performance of each animal in the maze was analyzed, taking the standard measurements recorded in each

section of the maze into account (closed and open arms, central platform), comprising the frequency of open and closed arm entries (an arm entry or exit being defined as all four paws inside or outside of an arm, respectively), the total arm entries and the amount of time spent in each section of the maze. In addition, these data were used to calculate percentages of open arm entries, time spent in open arms, time spent in closed arms, and time spent on the central platform.

The items recorded were grooming, rearing, peeping-out, stretched attend posture, scanning, head-dipping, freezing and end-arm exploration based on previous studies (Blanchard et al., 1991; Cruz et al., 1994; Rodgers and Johnson, 1995; Anseloni and Brandão, 1997): *Grooming:* species-typical sequences beginning with snout, progressing to ears and ending with whole body groom, including scratching. *Rearing:* partial or total rising onto the hind limbs. *Scanning:* olfactory and vibrissal exploration of maze floor and walls, including sniffing. *Head-dipping:* exploratory-like movement of head/shoulders over sides of the maze and towards the floor. *End-arm exploration:* number of times the rat reached the end of an open arm. *Peeping-out:* stretching of the head/shoulders from the closed arms towards the central platform. *Stretched attend posture (SAP):* when the animal stretches to its full length and returns to its initial position without any forward motion of the hind legs. *Freezing:* arrest of movement for more than 10 s at any arm of the maze.

### 2.4. Microinjection procedures

The animals were gently wrapped in a cloth, and an injection cannula (0.3 mm o.d.) was introduced through the guide cannula until its lower end protruded 2.0 mm. The injection needle was linked to a 5-μl Hamilton syringe by means of polyethylene tubing. A volume of 0.2 μl was injected during 30 s with the aid of an infusion pump (Harvard Apparatus, USA). The displacement of an air bubble inside the polyethylene (PE-10) catheter connecting the syringe to the intracerebral needle was used to monitor the microinjection. Following the end of the injection, the needle was held in place for 30 s and then withdrawn.

### 2.5. Drugs

Histamine dihydrochloride (Sigma, USA), dimaprit (Sigma) and ranitidine (Sigma) were each dissolved in physiological saline (0.9%) shortly before use and injected (0.2 μl) into one of the two midbrain structures. Independent groups of animals were used for evaluating the effects of each dose of histamine, dimaprit, ranitidine and saline on the behavioral items recorded. Thus, for each structure studied (IC or dPAG), the animals were assigned to the following treatment groups: (a) saline, (b) histamine (5 and 40 nmol), (c) dimaprit (5 and 10 nmol) and (d) ranitidine (10 and 30 nmol). In an additional experiment, the effects of ranitidine (30 nmol) were challenged with saline, histamine (40 nmol) and dimaprit (10 nmol) injected 10 min before the antagonist into each structure. As controls for these treatments, saline, histamine and dimaprit were also injected 10 min before saline. Each animal received only one treatment. The animals were tested immediately after the second injections. The doses of drugs were chosen on the basis of previous studies (Santos et al., 2001, 2002). The doses of ranitidine used here (10–30 nmol) were taken to produce only aversive states measurable by the EPM test without the behavioral activation (running, rearing and jumping) characteristically caused by higher doses injected into the midbrain tectum of rats submitted to the open-field test (Santos et al., 2001). The number of animals used in each treatment for each structure is shown in Table 1.

Table 1
Number of animals used in each treatment for each structure

| Treatments | dPAG | IC |
|---|---|---|
| Saline | 8 | 8 |
| Histamine, 5 nmol | 8 | 6 |
| Histamine, 40 nmol | 8 | 8 |
| Dimaprit, 5 nmol | 7 | 8 |
| Dimaprit, 10 nmol | 8 | 8 |
| Ranitidine, 10 nmol | 8 | 8 |
| Ranitidine, 30 nmol | 10 | 9 |
| Saline + saline | 9 | 9 |
| Histamine + saline | 8 | 8 |
| Dimaprit + saline | 8 | 9 |
| Saline + ranitidine | 10 | 9 |
| Histamine + ranitidine | 8 | 7 |
| Dimaprit + ranitidine | 9 | 8 |

Histamine or dimaprit was injected 10 min before and ranitidine was injected immediately before the test. In the combined treatments, the doses used were 40 nmol for histamine, 10 nmol for dimaprit and 30 nmol for ranitidine.

### 2.6. Analysis of results

The data obtained are expressed as means ± S.E.M. Differences between groups were analyzed by an analysis of variance (ANOVA). Dunnett's or Newman–Keuls' post hoc comparisons when appropriate were carried out if significant overall $F$-values were obtained ($P < .05$).

### 2.7. Histology

On completion of the experiments, all rats were sacrificed with an overdose of urethane and then perfused intracardially with saline followed by 10% formalin. Serial 50-μm brain sections were stained with neutral red, and the locations of the injection sites were determined using the corresponding planes of the Paxinos and Watson (1997) stereotaxic atlas.

## 3. Results

The sites of drug injections in the midbrain tectum were located in the dPAG and central nucleus of the IC, as in-

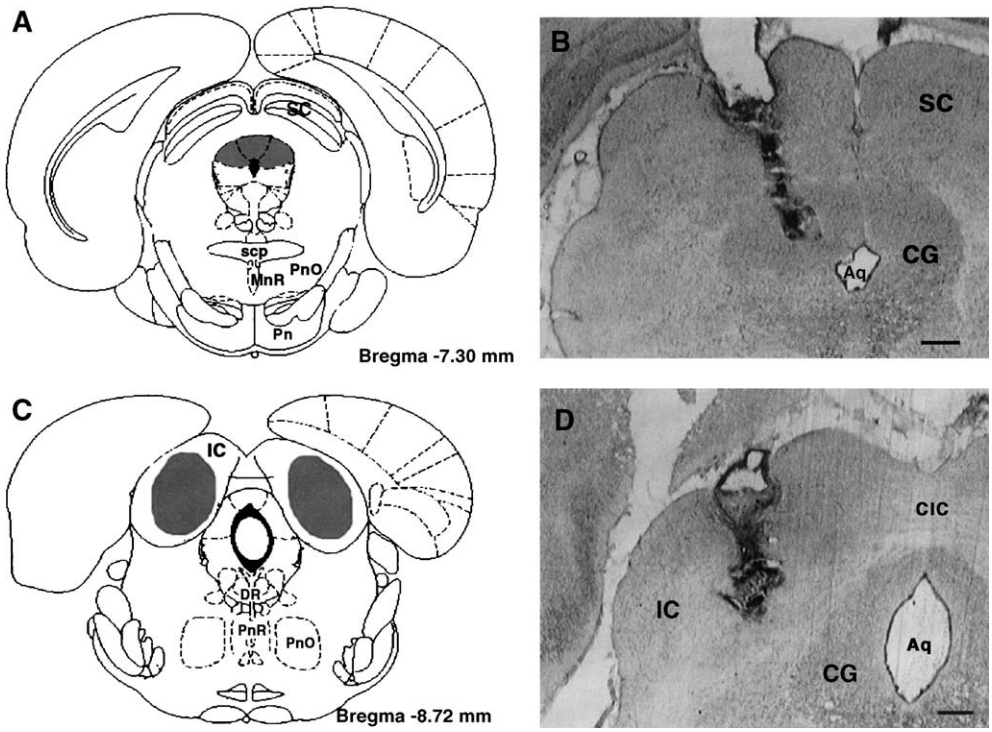


Fig. 1. Location of cannula placements (shaded area) into the dPAG (A) and the IC (C) on cross sections from the Paxinos and Watson (1997) atlas. Figures represent the atlas coordinates (in mm) posterior to bregma. (B and D) Photomicrographs showing typical examples of cannula placements into the dPAG and into the IC, respectively. Aq = aqueduct; CG = central gray; scp = superior cerebellar peduncle; MnR = median raphe nucleus; SC: superior colliculus; DR: dorsal raphe nucleus; PnO = pontine reticular nucleus, pars oralis; PnR = pontine raphe nucleus; CIC = commissure of the IC. Bar = 500 μm.

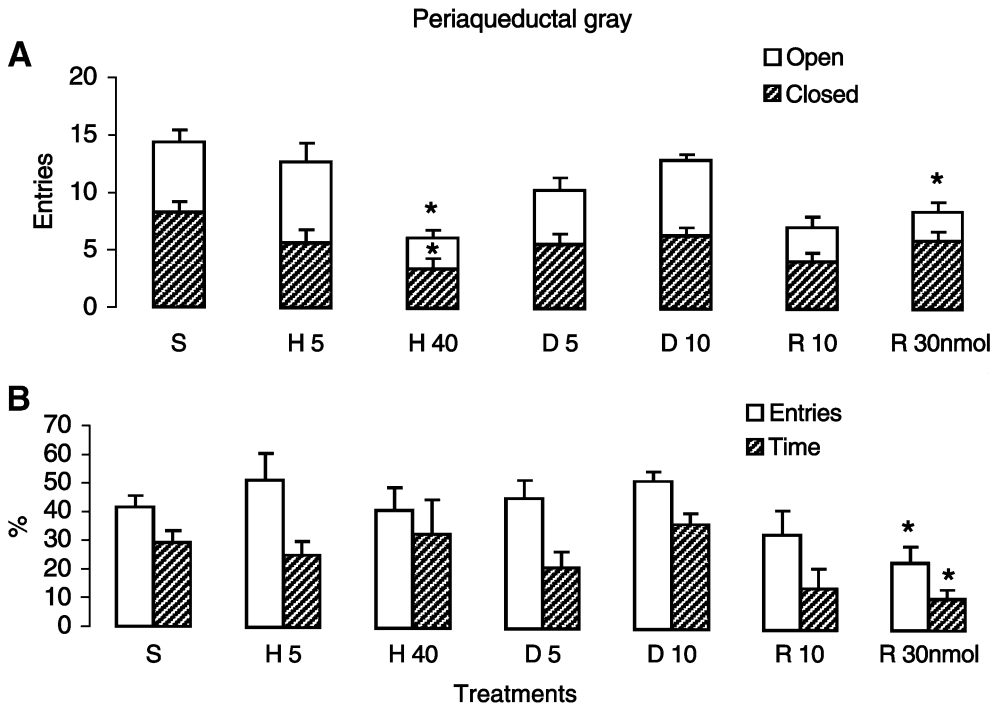


Fig. 2. Effects of microinjections of histamine (5 and 40 nmol), dimaprit (5 and 10 nmol) and ranitidine (10 and 30 nmol) into the dPAG on behavior of rats (mean ± S.E.M.) in the EPM. (A) number of entries into both types of arms. (B) entries and time spent in the open arms expressed as percentage of totals. S = saline, H = histamine, D = dimaprit, R = ranitidine. $n = 8$ for each group, with the exception of dimaprit (5 nmol) with $n = 7$ and ranitidine (30 nmol) with $n = 10$. * $P < .05$, different from the control group, Dunnett's test.

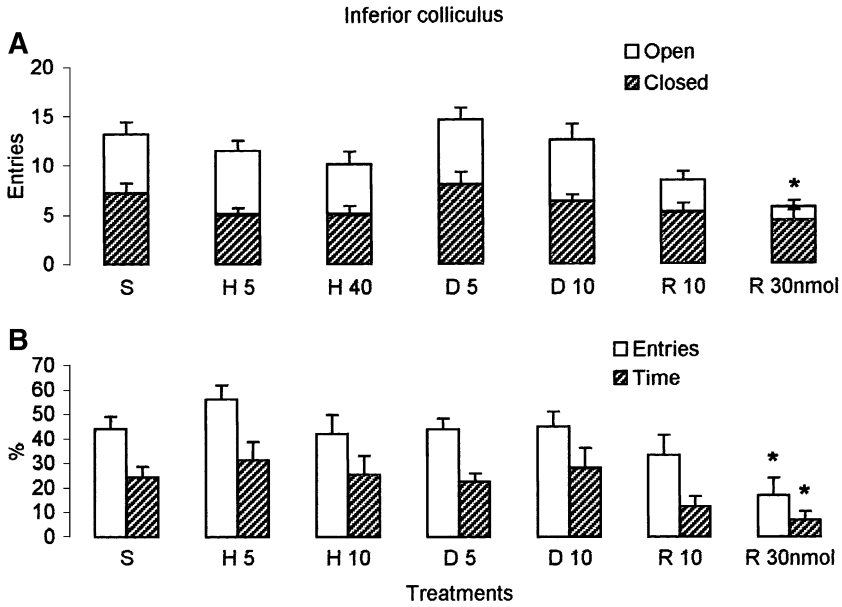


Fig. 3. Effects of microinjections of histamine (5 and 40 nmol), dimaprit (5 and 10 nmol) and ranitidine (10 and 30 nmol) into the IC on exploratory behavior of rats (mean ± S.E.M.) in the EPM. (A) number of entries into both types of arms. (B) percentage of entries and time spent in the open arms in relation to totals. S = saline, H = histamine, D = dimaprit, R = ranitidine. $n = 8$ for each group, with the exception of histamine (5 nmol) with $n = 6$ and ranitidine (30 nmol) with $n = 9$. * $P < .05$, different from the control group, Dunnett's test.

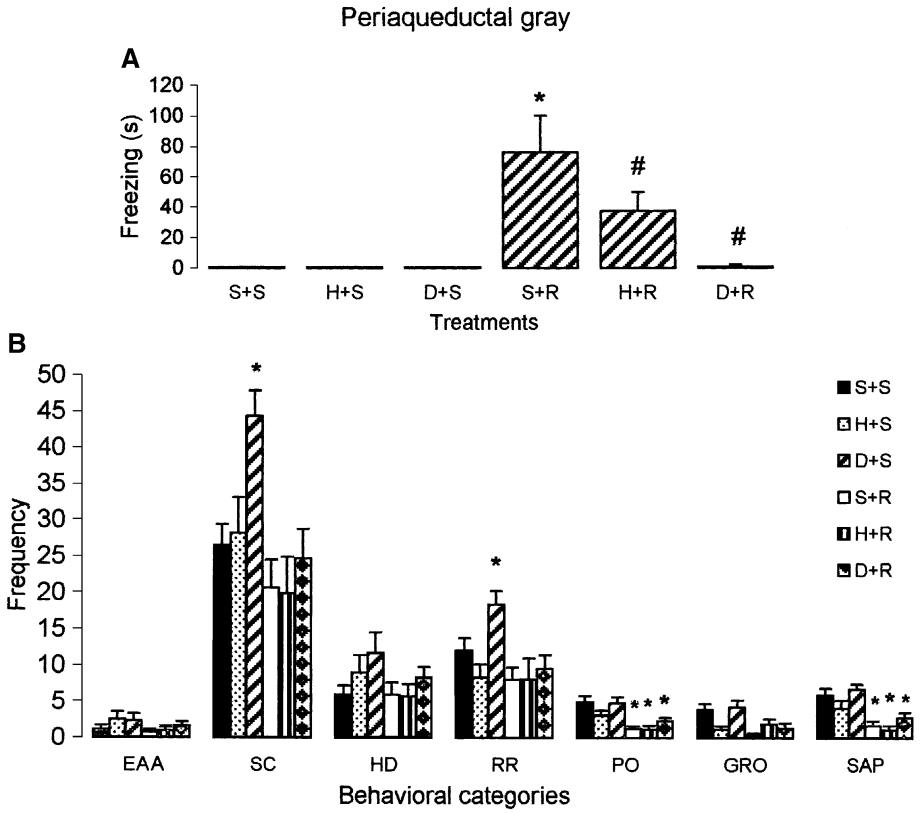


Fig. 4. Inhibition by histamine (40 nmol) and dimaprit (10 nmol) of the effects of ranitidine (30 nmol) injected into the dPAG on freezing (A) and exploratory behaviors (B) of rats exposed to the EPM test. EAA = end-arm activity; SC = scanning; HD = head-dipping; RR = rearing; PO = peeping out; GRO = grooming; SAP = stretched attend posture. S = saline, H = histamine, D = dimaprit, R = ranitidine. Data are presented as means ± S.E.M. $n = 8$ for H + S, D + S and H + R, $n = 9$ for S + S and D + R and $n = 10$ for S + R. The two injections were separated by a 10-min interval and the second injection was made immediately before the test. * Different from the control group. #Different from saline + ranitidine group ( $P < .05$, Newman–Keuls' test).

dicated in Fig. 1A and C, respectively. Representative cannula placements into the dPAG and IC can be seen in Fig. 1B and D, respectively.

ANOVA revealed significant effects of drug injections into the dPAG upon the number of entries into the open arms ($F_{6,50}=3.78$, $P<.01$) and also on the number of entries into the closed arms ($F_{6,50}=2.86$, $P<.05$) of the maze (Fig. 2A). Fig. 2B shows that these drug treatments caused a significant reduction in the percent of entries and time spent on the open arms of the maze ($F_{6,50}=2.42$ and 2.30, respectively, $P<.05$ in both cases). Post hoc analysis revealed that the group differences were mainly due to the group treated with ranitidine compared to the control group ($P<.05$). Histamine injected into the dPAG also caused a reduction in entries into both arms ($P<.05$).

Injections of histaminergic drugs into the IC had significant effects upon the number of entries into the open arms ($F_{6,48}=2.80$, $P<.05$) but not into the closed arms ($F_{6,48}=1.96$) (Fig. 3A). This analysis also revealed significant effects on the percent of entries into the open arms ($F_{6,48}=3.52$, $P<.01$) and percent of time spent on the arms ($F_{6,48}=2.35$, $P<.05$) (Fig. 3B). Post hoc comparisons revealed that all these effects were due to the dose of 30 nmol of ranitidine ($P<.05$).

Fig. 4 illustrates the effects of histaminergic drugs injected into the dPAG on the "ethological" parameters. The most noticeable effect was the occurrence of freezing caused by ranitidine ($F_{5,46}=7.12$, $P<.001$), which was significantly reduced by both histamine and dimaprit (Fig. 4A). The effects of these treatments on the remaining variables are shown in Fig. 4B. ANOVA detected significant effects on scanning ($F_{5,46}=4.48$, $P<.01$), rearing ($F_{5,46}=3.82$, $P<.01$), peeping-out ($F_{5,46}=6.41$, $P<.001$) and stretched attend postures ($F_{5,46}=6.56$, $P<.001$). Newman–Keuls' post hoc analysis ($P<.05$) showed that dimaprit increased scanning and rearing and that ranitidine decreased pepping-out and stretched attend postures, independent of the previous injections of either saline, histamine or dimaprit ($P<.05$). No significant effects were obtained on end-arm activity, head-dipping and grooming ($F_{5,46}=0.98$, 1.36, and 2.86, respectively). Statistical analysis on flat-back approach is not presented because of the low level of this activity.

Fig. 5 depicts the effects of histaminergic drug injections into the IC on the "ethological" parameters. Again, freezing

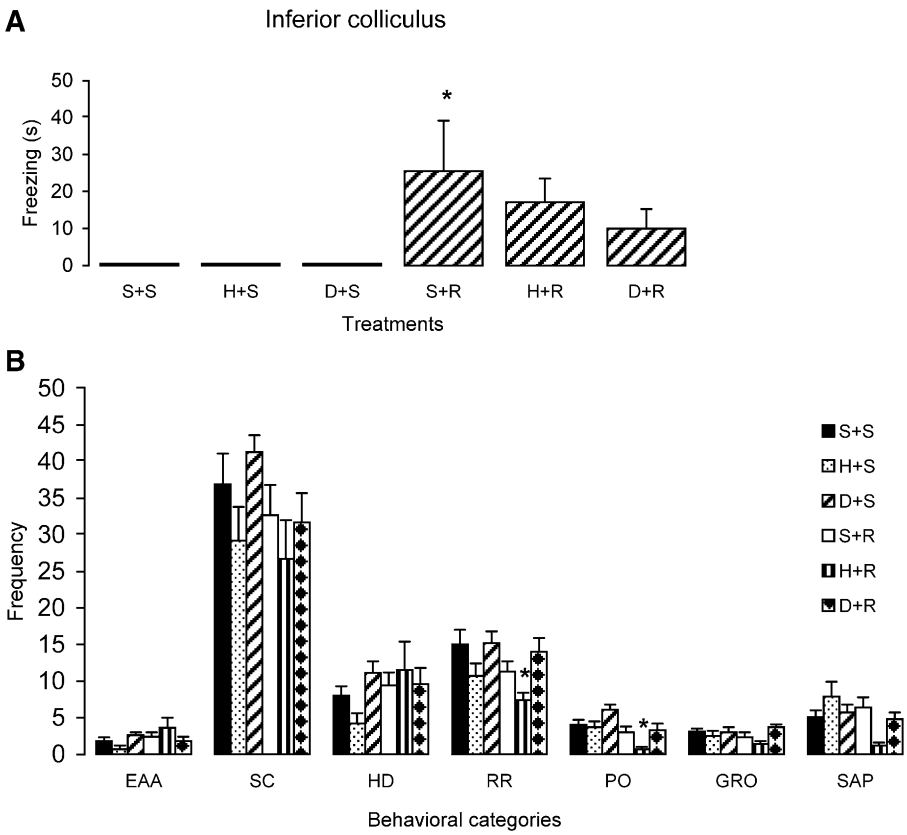


Fig. 5. Inhibition by histamine (40 nmol) and dimaprit (10 nmol) of the effects of ranitidine (30 nmol) injected into the IC on freezing (A) and exploratory behaviors (B) of rats exposed to the EPM test. EAA = end-arm activity; SC = scanning; HD = head-dipping; RR = rearing; PO = peeping out; GRO = grooming; SAP = stretched attend posture. S = saline, H = histamine, D = dimaprit, R = ranitidine. Data are presented as means ± S.E.M. $n=8$ for H + S and D + R, $n=9$ for S + S, D + S and S + R and $n=7$ for H + R. The two injections were separated by a 10-min interval and the second injection was made immediately before the test. * $P<.05$, different from the control group.

was the most pronounced effect caused by saline + ranitidine in relation to the control group (saline + saline) ($F_{5,44} = 2.61$, $P < .05$). Histamine + ranitidine and dimaprit + ranitidine did not produce significant effects (Fig. 5A). The effects of these treatments on the remaining variables are shown in Fig. 5B. Overall, ANOVA detected significant effects only on rearing ($F_{5,44} = 3.04$, $P < .05$) and peeping-out ($F_{5,44} = 3.30$, $P < .05$). Newman–Keuls' post hoc analysis showed that these effects were due to the combined treatment (histamine + ranitidine) ($P < .05$), but saline + ranitidine did not significantly change any of these behavioral categories.

## 4. Discussion

The neural substrates for defensive behavior integrated in the dPAG and IC are under modulatory influences of several neurotransmitters, including histamine (for a review, see Brandão et al., 1999, 2003). Indeed, ranitidine injected into these structures caused an intense behavioral activation interspersed with freezing behavior in the open-field test, which was inhibited by histamine and dimaprit, a $H_2$ receptor agonist (Santos et al., 2001, 2002). In the present study, we have gone one step further and present evidence for an involvement of $H_2$ receptors in the control of defensive behavior through analysis of behavior of rats in the EPM test of the effects of local injections into these structures of the selective $H_2$ receptor antagonist ranitidine.

The results of the present study show that injections of ranitidine into the midbrain tectum decreased the percentage of open arm entries and caused a decrease in the percentage of time spent in the open arms, whereas no significant effect could be detected for closed arm entries. Regarding the ''ethological parameters,'' injections of ranitidine into the dPAG caused a marked induction of freezing behavior with concomitant reductions in peeping-out and stretched attend postures, indicating a tendency to reduce exploration of potentially dangerous areas (Cole and Rodgers, 1993). These results are consistent with the notion that ranitidine promoted a clear fear-like effect in this study, increasing avoidance of the open arms. These effects could not be attributed to a motor deficit, because this drug did not influence the overall activity of the animals. Furthermore, injections of still higher doses (50–100 nmol) of ranitidine into the dPAG or the IC produced a behavioral activation in the open-field test (Santos et al., 2001). The reduction in the exploratory activity measures of the EPM test was concomitant with an increase in freezing behavior. The aversive effects of ranitidine were clearly inhibited by prior injections into the dPAG of histamine itself and by dimaprit, a selective agonist of $H_2$ receptors.

Dimaprit and histamine caused different effects on some behavioral categories recorded in the EPM test. Indeed, an increase in scanning and rearing was observed following injections of dimaprit into the dPAG, while histamine reduced the entries in the closed arms of rats when injected alone into this structure. However, these latter effects disappeared when saline preceded the dPAG injections of histamine. These distinct effects produced by histamine and dimaprit could be due to the selective action of dimaprit on the $H_2$ receptors (Eriks et al., 1992; Nakamura et al., 1997; Paquay et al., 1999). In agreement with this prediction, these effects of dimaprit were antagonized by ranitidine. On the other hand, histamine has a nonspecific action on $H_1$, $H_2$ and $H_3$ receptors.

The present data may disclose important functional differences between neural substrates of aversion in the dPAG and in the IC. Indeed, ranitidine injected into the dPAG caused more freezing with the concomitant reduction in exploratory behaviors than that seen after injections into the IC. When the open-field test was used for quantification of the behavioral effects (freezing, rearing, jumps and crossings) of 50 nmol of ranitidine injected in the midbrain tectum, it was also found that freezing was also more evident following injections of the drug into the dPAG than after injections into IC (Santos et al., 2001, 2002). More antinociception was also found upon electrical stimulation of the dPAG than of the IC (Castilho and Brandão, 2001).

The inhibitory control exerted by $H_2$ mechanisms on the neural substrates of aversion in the dPAG seems to be weaker in the dPAG than in the IC. These differential effects may be related to the fact that histamine content and histaminergic cells in the dPAG seem to be lower than those reported for the IC (Nieuwenhuys, 1995; Steinbusch and Mulder, 1984; Watanabe et al., 1984; Inagaki et al., 1990). A dense bundle of histaminergic neurons from the tuberomammillary nucleus project to the IC (Inagaki et al., 1990). Histaminergic neurons from the tuberomammillary nucleus have an inhibitory impact on the content of IC dopamine, a neurotransmitter implicated in the processing of somatosensory information at the level of the IC (Maisonnette et al., 1998). Dopaminergic mechanisms have clear modulatory influence over defensive responses generated at the level of the IC, whereas they do not seem to be involved in those generated at the level of the dPAG (Cuadra et al., 2000; Troncoso et al., 2003).

A variety of neurotransmitters, such as GABA, excitatory amino acids, serotonin and opioids, act in the regulation of the transmission of aversive information to higher brain structures (Cardoso et al., 1994; Brandão et al., 1994, 1999; Melo and Brandão, 1995). The tuberomammillary nucleus, the major source of brain histamine, which is located in the posterior hypothalamic region, projects through a dense plexus of histamine-positive axons to the midbrain tectum (Steinbusch and Mulder, 1984; Watanabe et al., 1984). Furthermore, several cell masses belonging to the auditory system contain histamine-immunoreactive fibers (Nieuwenhuys, 1995). These

findings, along with the fact that $H_2$ receptors are present in the brain (Schwartz, 1979; Schwartz et al., 1980; Brown et al., 2001), support the hypothesis that histamine exerts an inhibitory influence on the neural substrates of aversion in the dPAG and IC through $H_2$ receptors. A tonic inhibitory control over the neural substrates of aversion has primarily been attributed to GABA (Brandão et al., 1994, 1999), since the reduction and activation of physiological and behavioral indices of aversion have been reported with microinjections of GABA agonists and antagonists, respectively, into the dPAG, medial hypothalamus, amygdala, superior colliculus, and IC (Graeff, 1990; Brandão et al., 1994). On the other hand, a phasic modulatory role has been proposed for serotonin, acting through the activation of 5-$HT_{2/1C}$ receptors in the midbrain tectum (Graeff, 1990). Now, we suggest that histamine also plays a role in the control of the neural substrates of aversion at the midbrain level. $H_2$ receptors are mainly postsynaptically located and are coupled positively to adenylyl cyclase (Brown et al., 2001). Thus, activation of $H_2$ receptors leads to enhanced production of the second messenger molecule cyclic AMP that has the phosphorylase kinase (PKA) as its prominent target, which phosphorylates cell membrane proteins responsible for the calcium-dependent potassium conductance. This cascade of events commonly results in neuronal excitation (Hill et al., 1997; Brown et al., 2001). However, other reports have also indicated inhibition of firing in the nerve cells (Hass and Butcher, 1975; Jahn et al., 1995).

This functional role of histamine in emotional behavior has also been examined in forebrain structures. Indeed, local application of $H_1$ and $H_2$ antagonists into the nucleus basalis magnocellularis produces anxiolytic-like effects, as measured in the EPM test (Privou et al., 1998). Furthermore, antiaversive effects have also been reported after injection of histamine into the ventral hippocampus of rats submitted to the EPM (Ruarte et al., 1997).

Thus, in addition to the known influences of GABA, serotonin, opioids, neuropeptides and excitatory amino acids on the neural substrates of fear in mesencephalic structures, the present data provide evidence for a tonic inhibitory control of these systems by $H_2$ receptors. This presumed tonic inhibitory control of mechanisms mediated by $H_2$ receptors is stronger in the IC than in the dPAG. Indeed, histamine-containing cells and histaminergic receptors have been shown to predominate in the IC over other midbrain structures. The functional significance of such differences may be probably due to the distinct roles played by these structures in the regulation and expression of defensive behavior.

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