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# Modulation of defensive responses and anxiety-like behaviors by group I metabotropic glutamate receptors located in the dorsolateral periaqueductal gray

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**Abstract**

Glutamatergic neurotransmission in the dorsolateral periaqueductal gray (dlPAG) is related to defensive responses. However, the role of group I glutamate metabotropic receptors (mGluR) in these responses has been poorly investigated. The objective of the present study, therefore, was to test the hypothesis that interference with group I mGluR-mediated neurotransmission in dlPAG could modulate defensive responses. Male Wistar rats with cannulae aimed at the dlPAG were submitted to the following experiments: 1. intra dlPAG injections of vehicle (veh, 0.2 μL) or (RS)1-aminoindan-1,5-dicarboxylic acid (AIDA, 30–100 nmol, an mGluR1 receptor competitive antagonist) followed, 5 min later, by veh or *trans*-(+)-1-amino-1,3-ciclopentanedicarboxylic acid (tACPD, a group I and II mGluR agonist, 30 nmol); 2. intra-dlPAG injections of veh, AIDA (30 nmol) or 2-methyl-6-(phenylethynyl)-pyridine (MPEP, an mGluR5 receptor non-competitive antagonist, 50 nmol) followed by *trans*-azetidine-2,4-dicarboxylic acid (tADA, a group I mGluR agonist, 10 nmol); 3. and 4. intra-dlPAG injections of vehicle, AIDA (10–30 nmol) or MPEP (10–50 nmol) before the elevated plus maze (EPM) test; 5. intra-dlPAG injections of vehicle, AIDA (30 nmol) or MPEP (50 nmol) before the Vogel punished licking test. tACPD induced defensive responses characterized by jumps and an increased number of crossings in the observation box. These responses were attenuated by AIDA (30 nmol). tADA produced similar responses, although of lower intensity. tADA effects were prevented by AIDA and MPEP. Both drugs also produced anxiolytic-like effects in the EPM and Vogel tests when injected alone. The results suggest that group I metabotropic glutamate receptors in the dlPAG facilitate defensive responses and may also be involved in regulating anxiety-like behavior.


*Keywords:* AIDA; Anxiety; Central gray; MPEP; Panic; tACPD

## 1. Introduction

Glutamate is recognized as the main excitatory neurotransmitter of the central nervous system of mammals (Ozawa et al., 1998). It acts in two main classes of receptors, ionotropic and metabotropic. Based on preferential agonist compounds the ionotropic receptors are now classified as *N*-methyl-D-aspartate (NMDA) and non-NMDA receptors, the latter being composed by kainate and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) subtypes (Ozawa et al., 1998). Eight glutamate metabotropic receptor subtypes (mGluR1-8) have been identified so far. They can be divided into three classes. Group I includes mGluR1 and mGluR5 receptors, which are positively coupled to phospholipase C via Gq (Ferraguti and Shigemoto, 2006). Groups II, comprising mGluR2 and mGluR3, and III, including mGluR4, mGluR6, mGluR7 and mGluR8, are negatively coupled to adenylate cyclase (for review, see Ferraguti and Shigemoto, 2006).

Several studies suggest that glutamate metabotropic receptors can modulate defensive behaviors. For example, systemic administration of group II/III mGluR agonists produces anxiolytic-

*Abbreviations:* AIDA, (RS)1-aminoindan-1,5-dicarboxylic acid; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate; ANOVA, Analysis of Variance; dlPAG, dorsolateral periaqueductal gray; EPM, elevated plus-maze; mGluR, glutamate metabotropic receptor; MPEP, 2-methyl-6-(phenylethynyl)-pyridine; NMDA, *N*-methyl-D-aspartate; tACPD, *trans*-(+)-1-amino-1,3-ciclopentanedicarboxylic acid; tADA, *trans*-azetidine-2,4-dicarboxylic acid.

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like effects in several animal models (see Millan, 2003, for review). Similar results have also been reported for group I mGluR antagonists (Varty et al., 2005; Klodzinska et al., 2004; Iijima and Chaki, 2005; Pietraszek et al., 2005; Ballard et al., 2005; Spooren and Gasparini, 2004; Pilc et al., 2002; Tatarczynska et al., 2001a). The brain sites that mediate these effects are not completely known. Studies using intra-cerebral drug injections suggest that the amygdala (Perez de la Mora et al., 2006) and the hippocampus (Tatarczynska et al., 2001b) could be possible candidates.

The midbrain periaqueductal gray is another brain region that has been closely related to defensive responses (for review see Graeff, 1994). It is divided into dorsomedial, dorsolateral (dlPAG), lateral and ventrolateral columns (Carrive, 1993). Electrical or chemical stimulation of the dlPAG (Krieger and Graeff, 1985; Bandler and Carrive, 1988) results in a repertory of aversive behaviors (escape reactions) which are likely to reflect unconditioned fear responses to proximal danger. Based on these pieces of evidence the dlPAG has been implicated in the neuronal circuit that mediates panic-like behavioral and related cardiovascular responses (Deakin and Graeff, 1991; Jenck et al., 1995; Schenberg et al., 2001).

Glutamate receptors are widely expressed in the PAG (Albin et al., 1990). Injections of NMDA and non-NMDA-receptor antagonists into the dorsal PAG induce anxiolytic effects in animal models such as the elevated plus maze and the Vogel punished licking test (Guimarães et al., 1991; Molchanov and Guimarães, 2002; Matheus and Guimarães, 1997). Glutamate metabotropic receptors in the dlPAG could also play a role on anxiety modulation. For example, intra-dlPAG administration of tACPD, a group I and II mGluR receptor agonist, induced flight reactions characterized by jumps and wild running episodes (Molchanov and Guimarães, 1999). Moreover, MPEP, an mGluR5 antagonist, was able to prevent the antinociceptive effect of intra-PAG injection of NMDA, suggesting that these receptors modulate NMDA-mediated activity in the PAG (Berrino et al., 2001).

Considering that group I mGluR are present in cell bodies and dendritic processes in the dorsolateral areas of the PAG (Azkue et al., 1997; de Novellis et al., 2003), the objective of the present study was to test the hypothesis that group I mGluR-mediated neurotransmission are involved in defensive responses mediated by the dlPAG.

## 2. Methods

### 2.1. Animals

Male Wistar rats weighing 220–240 g at the beginning of each experiment were housed in pairs in a temperature-controlled room (24±1 °C) under standard laboratory conditions with free access to food and water and a 12 h light/12 h dark cycle (lights on at 06:30 h a.m.). Procedures were conducted in conformity with the Brazilian Society of Neuroscience and Behavior guidelines for the care and use of laboratory animals, which are in compliance with international laws and policies. All efforts were made to minimize animal suffering.

### 2.2. Apparatus

The experiments took place in a sound-attenuated, temperature-controlled (25±1 °C) room, illuminated with three 40 W fluorescent bulbs placed 4 m above the apparatus. Experiments with the mGluR agonists were carried out in a Plexiglas box (length: 29 cm, width: 19 cm, high: 34 cm). The rats were videotaped inside the box and the behavioral changes were analyzed by a trained observer (M.L.M.). The elevated plus-maze (EPM) consisted of two opposite open arms (50×10 cm), crossed at a right angle by two arms of the same dimensions enclosed by 40-cm high walls with no roof. The maze was located 50 cm above the floor and a 1-cm high edge made of Plexiglas surrounded the open arms to prevent falls. Rodents naturally avoid the open arms of the EPM and anxiolytic compounds typically increase the exploration of these arms without changing the number of enclosed arm entries (File, 1992; Carobrez and Bertoglio, 2005). The Ethovision software (V. 1.9, Noldus, Netherlands) was employed for behavioral analysis in the EPM. It detects the position of the animal in the maze and calculates the number of entries and time spent in open and enclosed arms. For these calculations, a 6-cm large exclusion zone was added between the center of the maze and each arm so that most of the animal's body should be in the open or enclosed arm for an entry to be registered. The Vogel conflict test was performed in a Plexiglas box (length: 42 cm, width: 25 cm, high: 20 cm) with a stainless grid floor. The metallic spout of a drinking bottle containing water projected into the box. The contact of the animal with the spout and the grid floor closed an electrical circuit controlled by a sensor (Anxio-Meter model 102, Columbus, USA), which produced 7 pulses per second whenever the animal was in contact with both components. Each pulse was considered as a lick, and every 20th lick produced a 0.5 mA shock for 2 s. The sensor recorded the total number of licks and shocks delivered during the test period. The whole apparatus was located inside a sound-attenuated cage (Jardim et al., 2005). To control for possible antinociceptive drug effects in the dlPAG that could interfere in the Vogel test the animals were also submitted to the tail-flick test. The apparatus consisted of an acrylic platform with a nichrome wire coil (EFF 300, Insight Instruments, Ribeirão Preto, Brazil) maintained at room temperature (24–26 °C). The coil temperature can be raised at 9 °C/s by the passage of electric current. The system had a cut-off time of 6 s to prevent tissue damage when the coil temperature approached 80 °C.

### 2.3. Drugs

*Trans*-(+)-1-amino-1,3-ciclopentanedicarboxylic acid (tACPD, TOCRIS, a group I and II mGluR agonist, 30 nmol), *trans*-azetidine-2,4-dicarboxylic acid (tADA, TOCRIS, a group I mGluR agonist, 10 nmol), (RS)1-aminoindan-1,5-dicarboxylic acid (AIDA, TOCRIS, an mGluR1 receptor competitive antagonist, 30–100 nmol), 2-methyl-6-(phenylethynyl)-pyridine (MPEP, TOCRIS, an mGluR5 receptor non-competitive antagonist, 10–50 nmol) were dissolved in buffered saline (pH

7.2–7.5) and administered in a volume of 0.2 μL. In the case of MPEP, the doses are chosen based on a previous published paper investigating its effect in the formalin test (Berrino et al., 2001). The dose of tACPD was the same that had already been shown to produce flight responses in dlPAG (Molchanov and Guimarães, 1999). The other doses were based on preliminary studies performed at the laboratory measuring the same behavioral changes (Molchanov, unpublished data).

### 2.4. Surgery

Rats were anesthetized with 2.5% 2,2,2-tribromoethanol (10 mg/kg, i.p.) and fixed in a stereotaxic frame. A stainless steel guide cannula (0.6 mm OD) was implanted unilaterally on the right side aimed at the dlPAG (coordinates: AP=0 from lambda, L=1.9 mm, D=4.0 mm. The cannula was inserted at an angle of 16° to prevent damage of the venous sinus). The cannula was attached to the bones with stainless steel screws and acrylic cement. An obturator inside the guide cannula prevented obstruction.

### 2.5. Procedure

The experiments took placed seven days after surgery. Intracerebral injections were performed with a thin dental needle (0.3 mm OD) introduced through the guide cannula until its tip was 1.0 mm below the cannula end. A volume of 0.2 μL was injected in 20 s using a microsyringe (Hamilton, USA) connected to an infusion pump (Kd Scientific, USA). A polyethylene catheter (PE 10) was interposed between the upper end of the dental needle and the microsyringe.

#### 2.5.1. Experiment 1

Rats received injections into the dlPAG of vehicle or AIDA (30–100 nmol) followed, 5 min later, by vehicle or tACPD (30 nmol). All injections were performed with the animals inside the observation box. Animal behavior was videotaped for 1 min from the beginning of the second injection and the number of jumps toward the top edges of the box and the number of midline crossings inside the box were counted by a trained observer (M.L.M.) (Aguiar et al., 2006).

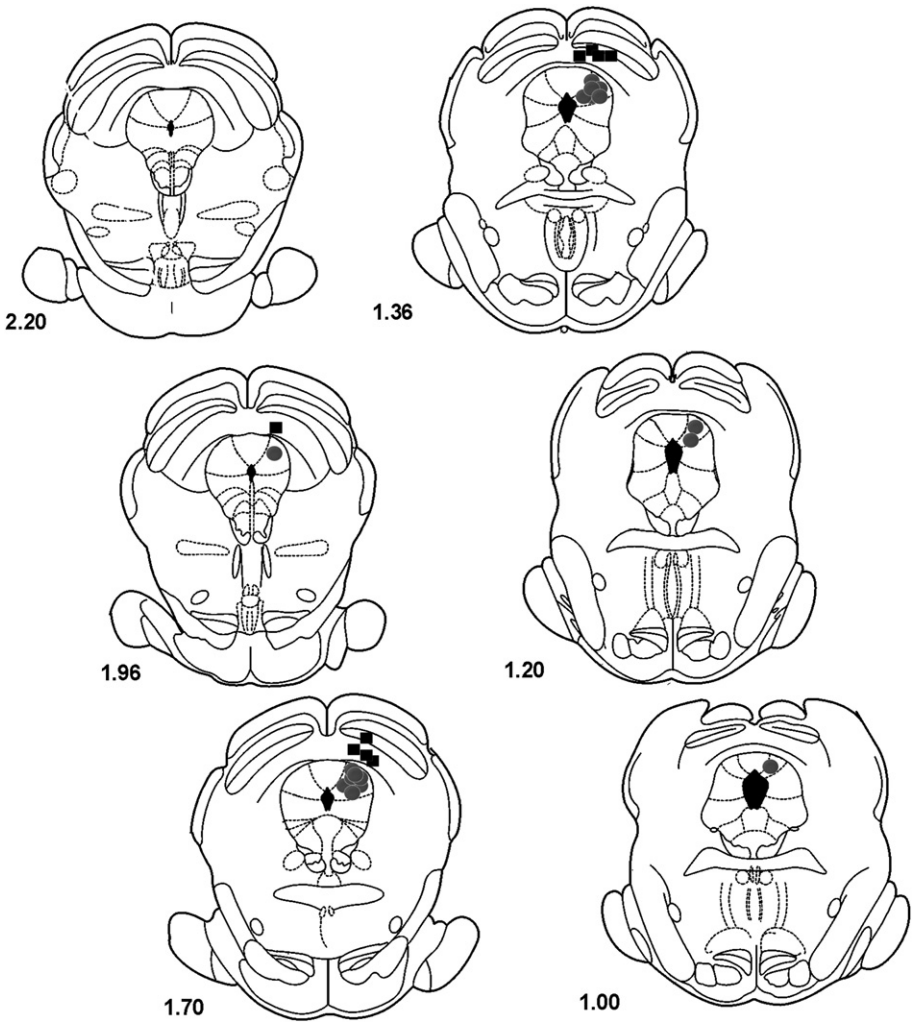


Fig. 1. Representative histological localization of injection sites (0.2 μL) into the dlPAG (circles) and deep layers of the superior colliculus (squares) in diagrams based on the atlas of Paxinos and Watson (1997).

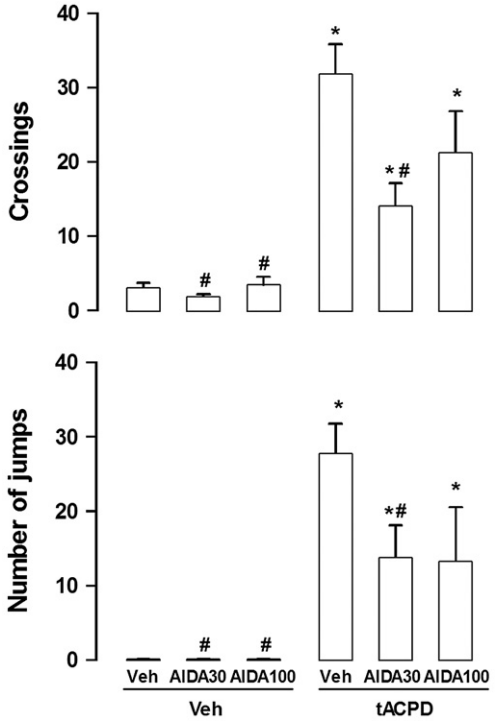


Fig. 2. Effects of tACPD injected into the dlPAG. Animals received a first microinjection of AIDA (30 or 100 nmol) or vehicle (Veh, 0.2 μL) followed, 5 min later, by a second microinjection of vehicle or tACPD (30 nmol). For all groups $n=7$ except for Veh+tACPD ($n=8$). The number of midline crossing and jumps inside a Plexiglas box were recorded for 1 min. Data are represented as mean±SEM. *Different from vehicle–vehicle, #different from and vehicle–tACPD, $p<0.05$.

*2.5.2. Experiment 2*

It was similar to experiment one, except that the animals received a first microinjection of vehicle, MPEP (50 nmol) or AIDA (30 nmol), followed 5 min later by vehicle or tADA (10–100 nmol).

*2.5.3. Experiment 3*

The animals received intra-dlPAG injections of vehicle or AIDA 10–30 nmol and were placed, 10 min later, in the center of the EPM facing an enclosed arm. The number of entries and time spent in the open and enclosed arms were recorded for 5 min.

*2.5.4. Experiment 4*

Similar to experiment 3 except that the animals received vehicle or MPEP (10–50 nmol) before being submitted to the EPM test.

*2.5.5. Experiment 5*

The animals were water deprived for 48 h before the test. After the first 24 h of deprivation they were allowed to drink freely for 3 min in the test cage in order to find the drinking bottle spout. Some animals did not find the spout and were not included in the experiment. Twenty four hours later they received intra-dlPAG injections of vehicle, AIDA (30 nmol) or MPEP (50 nmol) and 5 min later were placed into the apparatus. The test period lasted for 3 min and the animals received a 0.5 mA shock through the bottle spout every 20 licks. The number of punished licks was registered. The procedure was similar to the one already used and validated in the laboratory (Jardim et al., 2005; Moreira et al., 2006).

*2.5.6. Experiment 6*

Independent groups of animals were gently handled and their tails were laid across the coil. The heating was applied to a portion of the ventral surface of the tail between 4 and 6 cm from its end. The time to withdraw the tail was recorded as tail-flick latency. The electric current was calibrated to provoke this reflex within 2.5–3.5 s in non-treated animals (Molchanov and Guimarães, 2002). The tail flick latency was measured at 5-min intervals until a stable baseline (BL) was obtained over three consecutive trials. The tail withdrawal latency was measured at 10-min intervals after drug administration for up to 40 min (Molchanov and Guimarães, 2002).

*2.6. Histology*

After the behavioral tests the rats were sacrificed under deep urethane anesthesia and perfused through the left ventricle of the heart with isotonic saline followed by 10% formalin solution. The brains were removed and, after a minimum period of 5 days immersed in a 10% formalin solution, 50 μm sections

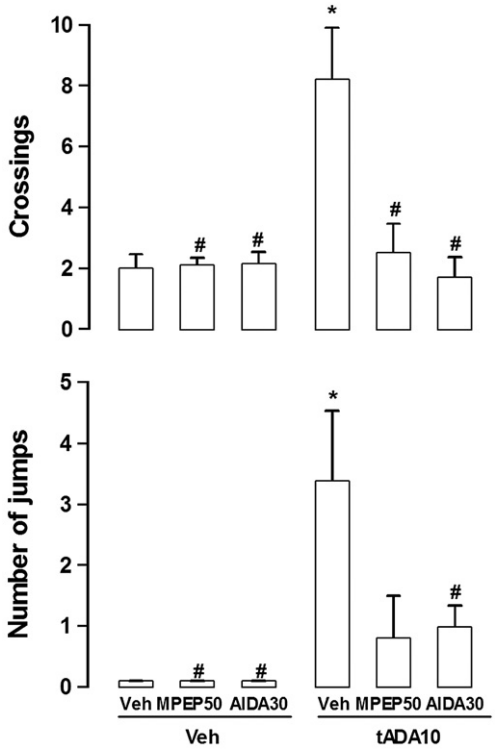


Fig. 3. Effects of tADA injected into the dlPAG. Animals received a first microinjection of MPEP (50 nmol), AIDA (30 nmol) or vehicle (Veh, 0.2 μL) followed, 5 min later, by a second microinjection of vehicle or tADA (10 nmol). The number of midline crossing and jumps inside a Plexiglas box were recorded for 1 min. Data are represented as mean±SEM. The number of animals in each group was as follows: Veh+Veh=13, Veh+tADA=13, AIDA+Veh=8, AIDA+tADA=10, MPEP+Veh=10, MPEP+AIDA=8. *Different from Vehicle–Vehicle, #different from vehicle–tADA, $p<0.05$.

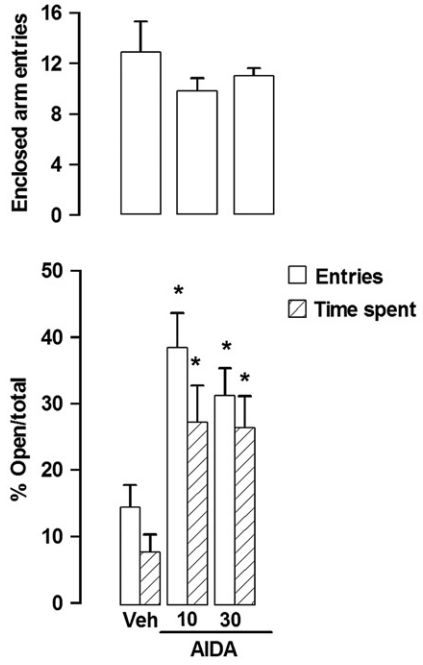


Fig. 4. Effects of AIDA (10–30 nmol, $n=11$ and 12, respectively) injected into the dlPAG of rats tested in the EPM. The upper panel shows the number of entries into the enclosed arms whereas the lower panel shows the percentage of entries (open bars) and the time spent (hatched bars) in the open arms. Bars represent the mean±SEM. *Different from vehicle (Veh, 0.2 μL, $n=8$), $p<0.05$.

were obtained in a Cryostat (Cryocut 1800). The injection sites were identified in diagrams from the Paxinos and Watson's atlas (Paxinos and Watson, 1997) and are illustrated in Fig. 1. Rats

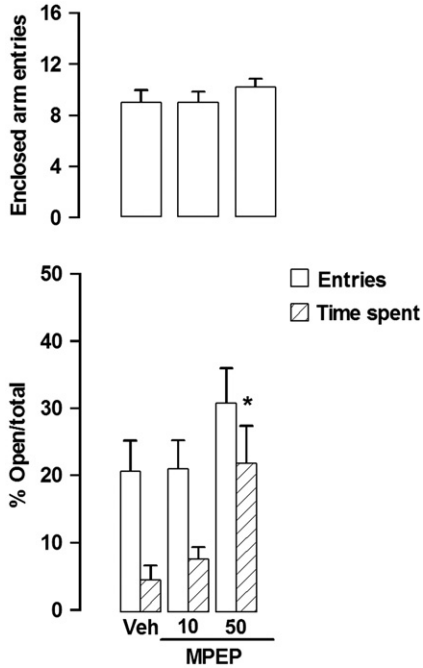


Fig. 5. Effects of MPEP (10–50 nmol, $n=11$/group) injected into the dlPAG of rats tested in the EPM. The upper panel shows the number of entries into the enclosed arms whereas the lower panel shows the percentage of entries (open bars) and the time spent (hatched bars) in the open arms. Bars represent the mean±SEM. *Different from vehicle (Veh, 0.2 μL, $n=8$), $p<0.05$.

Table 1
Effects of vehicle, AIDA (30 nmol) and MPEP (50 nmol) injected into the superior colliculus (see Fig. 1) of rats submitted to the EPM

| | Vehicle ($n=2$) | AIDA ($n=4$) | MPEP ($n=4$) |
|---|---|---|---|
| Percentage of entries onto open arms | 30.6±10.5 | 29.3±11.1 | 26.5±6.6 |
| Percentage of time spent in open arms | 16.3±4.9 | 9.5±2.6 | 13.6±9.1 |
| Number of enclosed arm entries | 13.0±3.0 | 9.7±2.4 | 10.2±1.2 |

Data represent the mean±SEM.

receiving injections outside the aimed area were not included in the analysis. In an additional control experiment rats were submitted to the EPM test 10 min after receiving microinjections of vehicle ($n=2$), AIDA (30 nmol, $n=4$) or MPEP (50 nmol, $n=4$) outside the dlPAG, into the deep layers of the superior colliculus.

### 2.7. Statistical analysis

The total number of crossing was analyzed by one-way Analysis of Variance (ANOVA). Since the control groups showed no variance, the number of jumps was analyzed by Kruskal–Wallis followed by the Mann–Whitney test. The percentages of entries and time spent in the open arms (100×open/open+enclosed) during the 5-minute sessions in the EPM were calculated for each animal. These results plus the number of enclosed arm entries and the number of licks in the Vogel test were also analyzed by one-way ANOVA. Data from the tail flick experiment were analyzed by a repeated measure ANOVA followed by one-way ANOVAs at each time. The Duncan test was employed for multiple comparisons. Differences were considered significant at $p<0.05$ level.

## 3. Results

### 3.1. Experiment 1

Representative injections sites into the dlPAG can be seen in Fig. 1. Animals receiving tACPD preceded by vehicle showed an increased number of midline crossings ($F_{5,37}=3.64$, $p<0.01$,

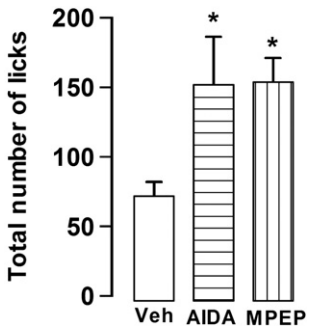


Fig. 6. Effects of AIDA (30 nmol, $n=7$) and MPEP (50 nmol, $n=7$) injected into the dlPAG of rats submitted to the Vogel test. Bars represent the mean±SEM total number of punished licks in the 3 min session. *Different from vehicle (Veh, 0.2 μL, $n=5$).

Duncan, $p<0.5$) and jumps (Mann–Whitney, $p<0.05$) as compared to controls (vehicle+vehicle, Fig. 2). Pretreatment with AIDA 30 nmol, but not 100 nmol, was able to attenuate these effects.

### 3.2. Experiment 2

tADA injection into the dlPAG induced a lower, as compared to tACPD, but nevertheless significant increase in the number of crossings ($F_{5,55}=4.87$, $p<0001$, Duncan, $p<0.05$) and jumps (Mann–Whitney, $p<0.05$) as compared to controls (Fig. 3). These effects were prevented by pretreatment with AIDA (30 nmol) and MPEP (50 nmol). Higher doses of tADA (30–100 nmol) did not produced larger effects when compared to the 10 nmol dose (jumps, vehicle=0, tADA 10 nmol=4.2±1.4, 30 nmol=1.0±0.7, 100 nmol=2.7±1.5; crossing, vehicle=2.9±0.5, 10 nmol=8.3±1.9, 30 nmol=7.0±1.0, 100 nmol=8.18±2.0).

### 3.3. Experiment 3

AIDA (10 and 30 nmol) injection into the dlPAG increased the percentage of entries ($F_{2,36}=6.45$, $p=0.004$, Fig. 4) and time spent ($F_{2,36}=4.37$, $p=0.02$) in the open arms as compared to controls (Duncan test, $p<0.05$). No effect was found in the number of enclosed arm entries ($F_{2,36}=0.28$, $p>0.10$).

### 3.4. Experiment 4

MPEP (50 nmol) increased the percentage of time spent in the open arms ($F_{2,27}=6.34$, $p<0.05$, Duncan, $p<0.05$, Fig. 5). No other significant effect was found. The histological localization of injection sites into the superior colliculus can be seen in Fig. 1. No difference was found between animals that received vehicle, AIDA or MPEP into these sites (Table 1).

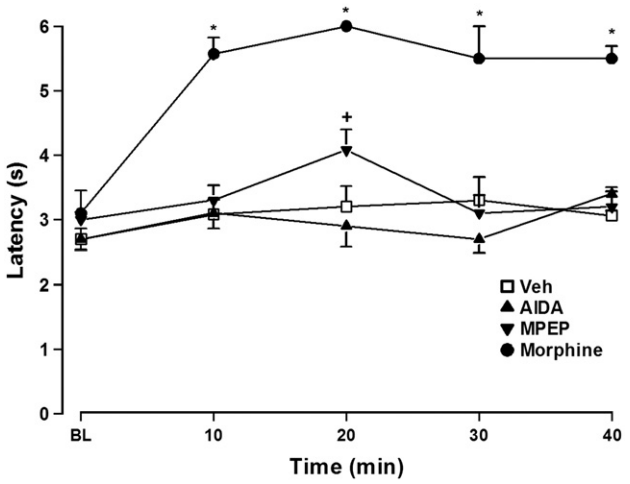


Fig. 7. Time course of the effects of vehicle (Veh, $n=5$), AIDA (30 nmol, $n=3$), MPEP (50 nmol, $n=6$) or morphine 5 mg/kg ($n=4$) on the tail flick test. Each point represents the mean±SEM. for the latency of tail withdrawal. *Different from all other groups, +different from vehicle, $p<0.05$.

### 3.5. Experiment 5

Both AIDA (30 nmol) and MPEP (50 nmol) increased in the number of punished licks ($F_{2,16}=3.00$, $p=0.078$, Duncan, $p<0.05$) as compared to controls (Fig. 6).

### 3.6. Experiment 6

Morphine increased withdrawal latency in the tail-flick test as compared to all other groups (Drug factor, $F_{3,14}=38,0$, $p<0.001$, Duncan test, $p<0.05$). Except for a small increase in withdrawal latency 20 min after MPEP injection, no other difference against vehicle was found (drug×time interaction, $F_{12,56}=3.6$, Duncan, $p<0.05$, Fig. 7).

## 4. Discussion

Our results agree with a previous report that tACPD, a group I and II mGluR agonist (Pin and Duvoisin, 1995), produces flight responses characterized by jumps and wild running episodes when injected into the dlPAG (Molchanov and Guimarães, 1999). These effects were attenuated by pretreatment with AIDA, an mGluR1 antagonist (Schoepp et al., 1999). However, only the lower dose (30 nmol) was effective. The reasons for the lack of effect of the higher dose are unknown. Since group I mglu receptors in the periaqueductal grey have been suggested to modulate the release of glutamate and GABA, a differential change in GABA and glutamate levels could be involved (de Novellis et al., 2003, see bellow). In addition, a non-selective action of high doses can not be ruled out. In fact, a weak agonist effect of these doses in group II mGluR has already been previously reported (Moroni et al., 1997).

Confirming the involvement of group I mGluR in the flight-inducing effect of tACPD, tADA, tADA, a selective group I mGluR agonist, also provoked defensive reactions (jumps and crossings) similar to those described in previous studies employing electrical or chemical stimulation of the dlPAG (Aguiar et al., 2006; Schenberg et al., 2001). When performed in larger places than the injection box used in the present study, these reactions also include changes such as wild running and galloping that resemble normal behavioral responses to predators (see Schenberg et al., 2001, for review). They probably reflect, therefore, fight–flight responses to proximal threats. The lack of such threats in our experiment could explain the lack of effect of MPEP and AIDA in the injection box of animals not injected with the mGluR agonists.

The effects of tADA were less intense then those observed with tACPD. Even higher doses (30–100 nmol) were unable to significantly increase the effects observed with the 10 nmol doses. One possibility to explain this results lies on the apparent lack of efficacy of tADA in mGluR1b and mGluR5a, two group I mGluR subtypes (Schoepp et al., 1999). The defensive reactions produced by tADA, however, were prevented by AIDA and MPEP, competitive and non-competitive antagonists of mGluR1 and mGluR5, respectively (Schoepp et al., 1999). Therefore, the results suggest that activation of mGluR1 and mGluR5 in the dlPAG promotes aversive responses.

Intra-dlPAG administration of AIDA and MPEP, at a dose range capable of antagonizing flight reactions induced by local injection of mGluR agonists, was able to increase open arm exploration of the EPM without changing the number of enclosed arm entries. This effect is usually interpreted as indicating an anxiolytic action (File, 1992). In agreement with this possibility, both compounds were also able to increase the number of punished licks in the Vogel conflict anxiety model. Except for a small increase in withdrawal latency induced by MPEP, the anxiolytic-doses of MPEP and AIDA produced no effect in the tail-flick test. In addition, the effect of MPEP was observed only 20 min after injection. Since the anxiety tests were performed 10 min after drug administration, the anxiolytic-like effects observed with the mGluR antagonists in the Vogel test can not be attributed to antinociceptive effects.

These results are compatible with the anxiolytic effects reported for these antagonists after systemic or intra-cerebral administration (Varty et al., 2005; Klodzinska et al., 2004; Iijima and Chaki, 2005; Pietraszek et al., 2005; Ballard et al., 2005; Spooren and Gasparini, 2004; Pilc et al., 2002; Perez de la Mora et al., 2006; Tatarczynska et al., 2001a,b). Together they suggest that activation of group I mGluR exerts a tonic modulation on defensive responses in the dlPAG.

Immunohistochemical and in situ hybridization studies have demonstrated the presence of group I (mGluR1/5) receptors in cell bodies and dendritic processes within the PAG, particularly in the dorsolateral areas (Azkue et al., 1997; de Novellis et al., 2003). Axon terminals making synaptic contact with mGluR I receptors contain round clear vesicles and exhibited asymmetric synaptic densities, suggesting an excitatory nature (Azkue et al., 1997; de Novellis et al., 2003). These receptors are located mainly at the periphery of the postsynaptic densities of asymmetrical synapses. They are linked to postsynaptic NMDA receptors by synapse-associating proteins, facilitating NMDA currents (Ferraguti and Shigemoto, 2006; Heidinger et al., 2002). Antagonism of NMDA-mediated neurotransmission in the dlPAG produces anxiolytic effects in the EPM and Vogel conflict tests (Guimarães et al., 1991; Molchanov and Guimarães, 2002). Therefore, group I mGluR antagonists could be exerting their anxiolytic effects in the dlPAG by blocking a possible facilitatory effect on NMDA activity. However, although there are contradictory results, they could also be interfering with glutamate and GABA release. For example, an electrophysiological study suggested that all classes of mGluR inhibit GABAergic synaptic transmission in the PAG, possibly by decreasing the probability of transmitter release from GABAergic terminals (Drew and Vaughan, 2004). On the other hand, an in vivo microdyalisis study showed that mGluR I agonists increase dialysate glutamate and GABA concentrations (de Novellis et al., 2003). Additional studies using, alone or in combination, drugs that interact with specific mGluR subtypes, could help to elucidate the role of these receptors in the dlPAG.

In conclusion, even if the mechanisms involved are still not completely understood, the present results suggest that group I metabotropic glutamate receptors can facilitate defensive responses in the dlPAG.

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