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ORIGINAL INVESTIGATION

Activation of CB1 cannabinoid receptors in the dorsolateral periaqueductal gray reduces the expression of contextual fear conditioning in rats

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Abstract
Rationale Conditioned fear to context causes freezing and cardiovascular changes in rodents and has been used to measure anxiety. It also activates the dorsolateral column of the periaqueductal gray (dlPAG). Microinjections of cannabinoid agonists into the dlPAG produced anxiolytic-like effects in the elevated plus maze, but the effects of these treatments on fear conditioning remains unknown.
Objective The objective of this study was to verify if intra-dlPAG injection of the CB1 cannabinoid receptor agonist anandamide (AEA) or the anandamide transport inhibitor AM404 would attenuate behavioral (freezing) and cardiovascular (increase of arterial pressure and heart rate) responses of rats submitted to a contextual fear-conditioning paradigm.
Materials and methods Male Wistar rats with cannulae aimed at the dlPAG were re-exposed to a chamber where they had received footshocks 48 h before. Fifteen minutes before the test, the animals received a first intra-dlPAG injection of vehicle or AM251, a CB1 receptor antagonist (100 pmol/200 nl), followed 5 min later by vehicle, AEA (5 pmol/200 nl) or AM404 (50 pmol/200 nl). Freezing and cardiovascular responses were recorded for 10 min.
Results Freezing and cardiovascular responses were reduced by administration of either AEA or AM404 into the dlPAG before re-exposition to the aversively conditioned context. These effects were abolished when the animals were locally pretreated with AM251. The latter drug, even at a higher dose (300 pmol), was ineffective when administered alone into the dlPAG.
Conclusion The results suggest that facilitation of endocannabinoid-mediated neurotransmission in the dlPAG, through activation of local CB1 receptors, attenuates the expression of contextual fear responses.

Keywords CB1 receptors · Anandamide · Anxiolytic-like effect · AM404 · AM251

Introduction

Re-exposure of rats to an environment where they had been previously submitted to aversive stimulation such as electrical footshocks causes cardiovascular and behavioral changes characterized by complete immobility (freezing) and mean arterial pressure (MAP) and heart rate (HR) increases (Blanchard and Blanchard 1969; Fanselow 1980; Resstel et al. 2006a).

The periaqueductal gray matter (PAG) is a midbrain structure that has been widely related to defensive responses. Although electrical or chemical stimulation of the dorsal portion of PAG is usually related to the flight reactions, it can also produce freezing responses and increase cardiovascular activity (Krieger and Graeff 1985; Bandler and Carrive 1988). Reinforcing a possible role of this region in conditioned fear, re-exposure to aversively conditioned context increases neuronal activity in the PAG (Carrive et al. 1997, 2000), and PAG lesions block freezing to aversively conditioned stimulus (LeDoux et al. 1988; Amorapanth et al. 1999).

Cannabinoid CB1 receptors and endogenous cannabinoid agonists such as anandamide (AEA) and 2-arachido-

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noylglycerol are extensively located in the brain (Devane et al. 1988, 1992; Matsuda et al. 1990; Mechoulam et al. 1995; Sugiura et al. 1995) and have been proposed to play an important role in several functions of the central nervous system (CNS; Smith et al. 1994; Adams et al. 1998). CB1 receptors are expressed in the PAG (Herkenham et al. 1991; Bandler et al. 2000; Egertova et al. 2003), and a recent study from our laboratory showed that intra-dorsolateral PAG (dlPAG) administration of AEA increases both the time spent and the number of entries into the open arms of an elevated plus-maze (EPM), indicating an anxiolytic-like effect (Moreira et al. 2007). These results suggest that the local endocannabinoid system in the dlPAG may modulate defensive responses. This hypothesis, however, has not yet been tested in other animal models such as contextual fear conditioning. The aim of the present study, therefore, was to verify the effects of intra-dlPAG administration of AEA or AM404, an AEA re-uptake inhibitor, on behavior and cardiovascular responses of rats re-exposed to an aversively conditioned context. We also tested if the effects of AEA or AM404 are being mediated by CB1 receptors by locally pretreating the animals with AM251, a CB1 receptor antagonist.

Materials and methods

Animals

Thirty-seven male Wistar rats weighing 210–230 g were used. Animals were kept in the Animal Care Unit of the Department of Pharmacology, School of Medicine of Ribeirão Preto, University of São Paulo. Rats were housed individually in plastic cages with free access to food and water and under a 12 h light/dark cycle (lights on at 0630 hours). The Institution's Animal Ethics Committee approved the housing conditions and experimental protocols.

Surgical procedures

Seven days before the experiment, rats were anesthetized with tribromoethanol (250 mg/kg i.p.). After scalp anesthesia with 2% lidocaine, 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 at an angle of 16°, D=4.0 mm) according to the atlas published by Paxinos and Watson (1997). The cannula was fixed to the skull with dental cement and a metal screw. One day before the test, rats were anesthetized with tribromoethanol and a catheter (a 4-cm PE-10 segment heat-bound to a 13-cm PE-50 segment, Clay Adams, USA) was inserted into the abdominal aorta through the femoral artery for blood pressure recording. Catheters were tunneled under the skin and exteriorized on the animal's dorsum.

Drugs

The endogenous cannabinoid anandamide (TOCRIS) and the anandamide transporter inhibitor 4-hydroxyphenylarachidonylamide (AM404; TOCRIS) were dissolved in TocrisolveTM 100 (a solvent that contains a 1:4 ratio of soya oil/water, emulsified with the block co-polymer Pluronic F68). The CB1 cannabinoid receptor antagonist *N*-(piperidin-1yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-Hpyrazole-3-arboxamide(AM251; TOCRIS, USA) was dissolved in dimethyl sulfoxide 10% in saline (0.9% NaCl). The solutions were prepared immediately before use and were kept on ice and protected from the light during the experimental sessions.

Fear conditioning and testing

Preconditioning, conditioning, and testing were carried out in 25×22×22 cm experimental chamber. The chamber had a grid floor composed of 18 stainless steel rods (2 mm in diameter), spaced 1.5 cm apart and wired to a shock generator (Automatic Reflex Conditioner, model 8572, Ugo Basile, Italy). The experimental chamber was cleaned with 70% ethanol before and after use. The preconditioning (habituation) session took place 1 week after surgery. The animals were exposed, during the morning period, to the experimental chamber for 10 min and were placed back into their home cages. No shock was delivered during preconditioning session. The conditioning session took place in the afternoon of the same day 6 h after the preconditioning. The animals were placed again into the experimental chamber, and after 3 min, they received six 1.5 mA/3 s electric footshocks (Resstel et al. 2006b) delivered at 20-s to 1-min intervals. Twenty-four hours after the conditioning session, a catheter was implanted into the femoral artery for blood pressure and heart rate recording.

The test session started 24 h after the catheter implantation and consisted of a 10-min-long re-exposure to the experimental chamber without shock delivery. Animals were initially transferred from the animal room to the experimental room (a different room was used for conditioning) in their home box. MAP and HR were recorded using an HP-7754A amplifier (Hewlett Packard, USA) connected to a signal acquisition board (Biopac M-100, USA) and a computer. The cardiovascular recordings began after an adaptation period of 1 h to the acoustically isolated experimental room in the laboratory. Rats were tested only once. Freezing was evaluated continuously during the 10-min test. It was manually recorded by an experimenter blind to the treatment groups who sat 30 cm away from the footshock chamber. Freezing was defined as the complete absence of movement other than respiration while the animal assumed a characteristic tense posture (Fanselow 1980).

Experimental design

Each animal received two 200-nl unilateral injections into the dlPAG of the drugs or their respective vehicles. The experimental groups were: vehicle + vehicle (n=7 per group), vehicle + AEA (5 pmol, n=6), vehicle + AM404 (50 pmol, n=6), AM251 (100 pmol) + AEA (5 pmol, n=6), AM251 (100 pmol) + AM404 (50 pmol, n=6), or AM251 (100 pmol) + vehicle (n=6). The dose of AEA was similar to the one that produced anxiolytic effects in the EPM after dlPAG injection in a previous study (Moreira et al. 2007). The doses of AM404 and AM251 were based on those that potentiated or blocked, respectively, these effects in the same study. To confirm the lack of AM251 effect in the dlPAG observed in this previous study (Moreira et al. 2007), an additional group received a single microinjection of AM251 300 pmol (n=8). A 33G needle (Small Parts, Miami Lakes, FL, USA) 1 mm longer than the guide cannula and connected to a 10 μl syringe (7001 KH, Hamilton, USA) through a PE-10 tubing was used. The needles were carefully inserted into the guide cannulae, and the solutions were infused over a 30-s period with a rate of 400 nl/min. They remained in place for an additional 20-s period to prevent reflux.

The interval between the first and second microinjection was 5 min, and the animals were tested 10 min after the last microinjection. In all animals, the cardiovascular as well as behavioral responses were recorded during the re-exposition to experimental chamber where they had received footshocks 48 h before.

Histological control

To control for the specificity of the injection site, additional groups of animals received injections of AEA (5 pmol) or AM404 (50 pmol) into the following dlPAG surrounding structures: dorsomedial PAG (dmPAG, n=3 per group), lateral PAG (lPAG, n=3 per group) and superior colliculus (SC, n=3–4 per group).

Histological procedure

At the end of the experiments, the rats were anesthetized with urethane (1.25 g/kg, i.p.), and 200 nl of 1% Evan's blue dye was unilaterally injected in the dlPAG as a site marker. The chest was surgically opened, the descending aorta occluded, the right atrium severed, and the brain perfused with 10% formalin through the left ventricle. Brains were post-fixed in 10% formalin for 24 h at 4°C, and 40 μm sections were cut using a cryostat (CM-1900, Leica, Germany). Serial brain sections were stained with 1% neutral red and injection sites determined using the rat brain atlas of Paxinos and Watson (1997) as reference (Fig. 1).

Data analysis

The MAP and HR values were continuously recorded in the 5-min period before and during the 10-min test period. For statistical purpose, the continuous recordings were averaged over 60-s intervals. Data were expressed as means±SEM of MAP or HR changes (respectively, ΔMAP and ΔHR). The average of the points sampled during the 300 s before exposure was used as control baseline value. MAP and HR changes were analyzed using a two-way repeated measure analysis of variance (ANOVA) with group treatment as main independent factor and time as a repeated measurement.

Freezing was expressed as percentage of the total test period (10 min). Freezing was analyzed using one-way ANOVA. In all cases, post hoc comparisons were performed with t tests with a Bonferroni adjustment. $p<0.05$ was considered significant.

Results

A representative photomicrograph and a diagrammatic representation indicating the injection sites in the dlPAG can be seen in Fig. 1.

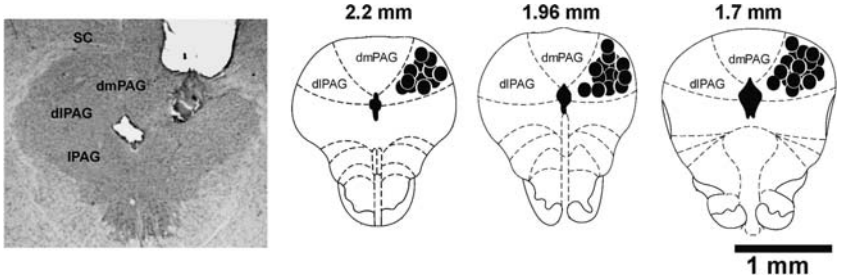

Fig. 1 Photomicrograph of a coronal brain section showing a unilateral microinjection site in the dlPAG and a diagrammatic representation based on the rat brain atlas of Paxinos and Watson (1997) indicating injections sites of vehicle, AEA, AM404, and AM251 into the dlPAG of conditioned animals. *dmPAG* Dorsomedial periaqueductal gray; *dlPAG* dorsolateral periaqueductal gray; *lPAG* lateral periaqueductal gray; *SC* superior colliculus

Behavioral responses to fear conditioning

Intra-dlPAG injections of either AEA ($p<0.001$) or AM404 ($p<0.001$) induce a significant reduction in the percentage of freezing when compared to vehicle-treated animals ($F_{6,39}=39.1$, $p<0.001$; Fig. 2). Pretreatment with AM251 blocked the effects of AEA and AM404 (Fig. 2). No effect was observed when AM251 100 or 300 pmol (Fig. 2) was injected alone into the dlPAG.

Cardiovascular responses to fear conditioning

No drug differences were observed in basal values of MAP or HR. During the test, there were significant effects of treatment (MAP: $F_{5,31}=21.0$, $p<0.001$ and HR: $F_{5,31}=27.01$, $p<0.001$) and time (MAP: $F_{9,279}=61.3$, $p<0.001$ and HR: $F_{9,279}=11.0$, $p<0.001$; Fig. 3). No significant interaction between treatment and time was found. Post hoc analysis showed that injections of AEA or AM404 into the dlPAG attenuated the increase in MAP and HR induced by re-exposure to the aversively conditioned chamber ($p<0.001$). This effect was blocked by pretreatment with AM251 (Fig. 3).

Histological control

Compared to animals that received vehicle injection into the dlPAG, administration of AEA and AM404 into dlPAG surrounding structures did not change freezing behavior or cardiovascular responses induced by re-exposure to the aversively conditioned chamber (Fig. 4).

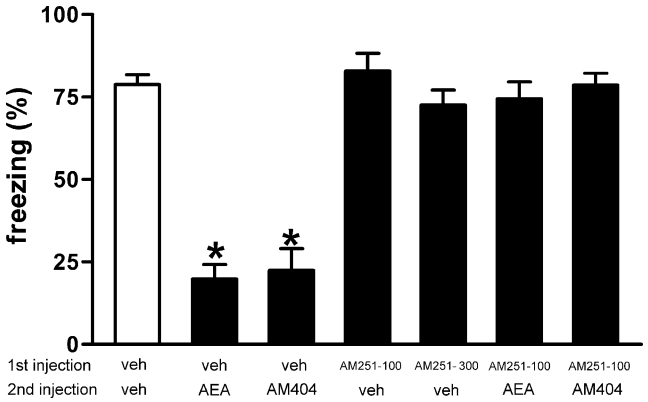

Fig. 2 Effects of unilateral microinjections of 200 nl of vehicle + vehicle (*veh*, $n=7$), vehicle + AEA (5 pmol, $n=6$), vehicle + AM404 (50 pmol, $n=6$), AM251 (100 pmol) + vehicle ($n=6$), AM251 (300 nmol) + vehicle ($n=8$), AM251 (100 pmol) + AEA ($n=6$), or AM251 (100 pmol) + AM404 ($n=6$) in the percentage of time spent in freezing behavior. *Columns* represent the means and *bars* the SEM, *asterisk* $p<0.05$ compared to vehicle + vehicle group

Discussion

In the present study, conditioned rats showed increased freezing and MAP and HR changes immediately after being placed in the test chamber, confirming that re-exposure to a context previously paired with an aversive stimulus (footshock) induces behavioral and autonomic responses typically observed in studies employing contextual fear-conditioning paradigms (Zhang et al. 2004, Resstel et al. 2006a, b). These responses were significantly reduced by intra-dlPAG injection of AEA, a cannabinoid receptor agonist, or AM404, an AEA transport inhibitor. Previous studies using animal models such as the EPM, Vogel conflict test, and chemical aversive stimulation have demonstrated that the dlPAG plays an important role in anxiety modulation in rats (Molchanov and Guimarães 2002; Moreira et al. 2007; Matheus et al. 1994). Our results, besides reinforcing the idea of an important role of dlPAG on conditioned fear responses (Amorapanth et al. 1999), also indicate that local endocannabinoid-mediated neurotransmission can modulate these responses.

The attenuation of fear responses by administration of AEA and AM404 into the dlPAG agrees with the anxiolytic effects observed after systemic administration of low doses of Δ9-tetrahydrocannabinol (the major psychoactive component of the *Cannabis sativa*), synthetic cannabinoids, or AM404 in rodents (Berrendero and Maldonado 2002; Patel and Hillard 2006; Bortolato et al. 2006). The anxiolytic effect of the latter compound is paralleled by an increase in AEA concentrations in the CNS (Bortolato et al. 2006; Kathuria et al. 2003), suggesting the involvement of this endocannabinoid. These results agree with the anti-aversive effects induced by intra-dorsal PAG injection of the CB1 receptor agonist HU210 in a model of chemically induced aversive response (Finn et al. 2003). Thus, our study reinforces the potential role of dlPAG as an important site for the anxiolytic action of cannabinoids.

AEA exerts most of its actions in the CNS via the CB1cannabinoid receptor (Matsuda et al. 1990; Rinaldi-Carmona et al. 1994). Thus, to verify if these receptors were involved in AEA and AM404 effects on fear-conditioned responses, AM251, a selective CB1 receptor antagonist, was used. The observation that the decrease in freezing and cardiovascular responses induced by dlPAG microinjection of AEA or AM404 was inhibited by pretreatment with AM251 reinforces the idea of the involvement of CB1 receptors on these effects (Chhatwal et al. 2005) and agrees with their proposed involvement in anxiety modulation (Bortolato et al. 2006; Kathuria et al. 2003; Patel and Hillard 2006).

Previous results from our laboratory (Moreira et al. 2007) showed that administration of AEA into the dlPAG induced anxiolytic-like effects in the EPM, which were

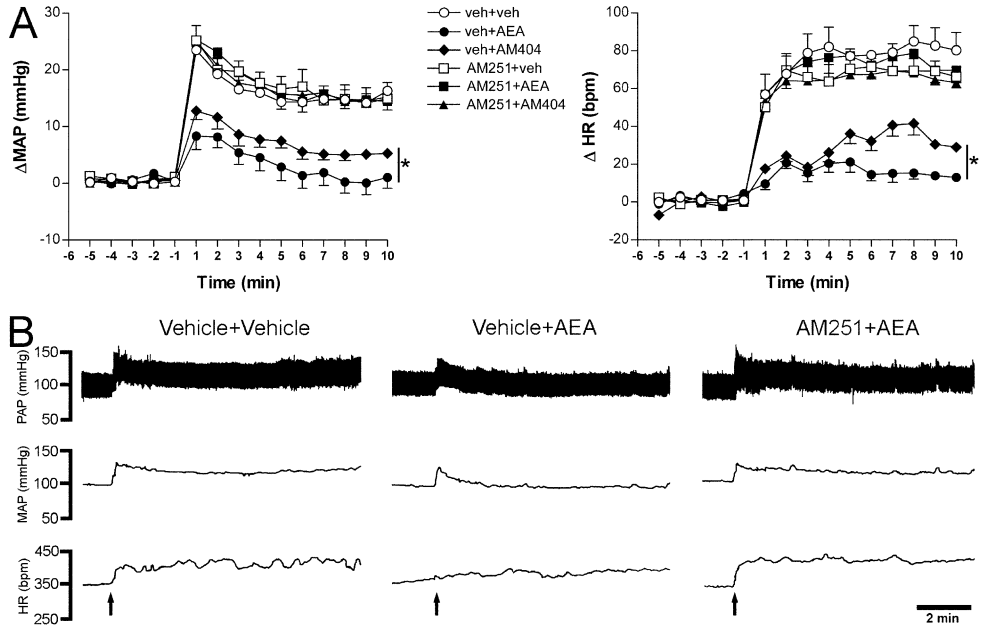

Fig. 3 **a** Time-course of the effects of unilateral microinjections of 200 nl vehicle + vehicle (veh, $n = 7$ per group), vehicle + AEA (5 pmol, $n = 6$), vehicle + AM404 (50 pmol, $n = 6$), AM251 + vehicle ($n = 6$), AM251 (100 pmol) + AEA ($n = 6$), and AM251 + AM404 ($n = 6$) in mean arterial pressure (ΔMAP) and heart rate (ΔHR). *Symbols* represent the means and *bars* the SEM. *Asterisk* indicates significant treatment difference ($p < 0.05$) over the whole footshock chamber exposure period compared to all other groups. **b** Representative individual recordings of pulsatile arterial pressure (*PAP*), mean arterial pressure (*MAP*), and heart rate (*HR*) showing the cardiovascular alterations evoked by re-exposure to context in conditioned rats treated with vehicle + vehicle, vehicle + AEA, or AM251 + AEA. *Arrows* indicate the start of the re-exposure

prevented by AM251. AM404, however, was ineffective by itself, whereas in the present study, this drug was able to attenuate fear-conditioned responses. Although at the moment it is only possible to speculate about the reasons for this difference, local PAG endocannabinoid system may be particularly engaged when animals are confronted with more intense aversive stimulus such as re-exposure to a fear-conditioned context, as compared to the EPM. Actually, whereas electrical footshocks have already been shown to increase AEA levels in the PAG (Hohmann et al. 2005),

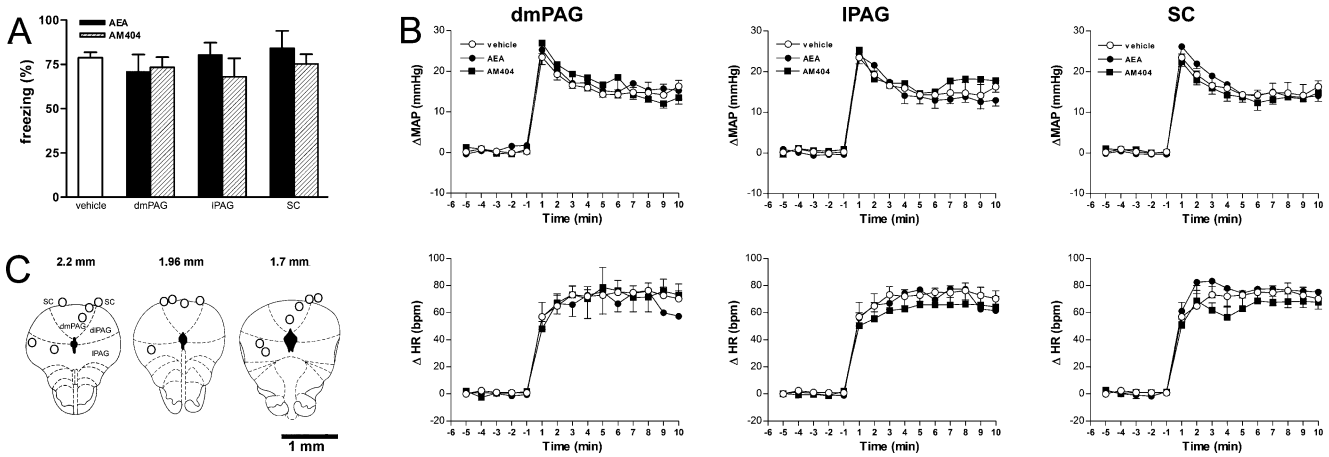

Fig. 4 **a** Lack of effect in the percentage of time spent in freezing behavior of unilateral microinjection of 200 nl AEA (5 pmol/200 nl) or AM404 (50 pmol/200nl) into the following dlPAG surrounding structures: dorsomedial PAG (*dmPAG*, $n=3$ per group), lateral PAG (*lPAG*, $n=3$ per group) and superior colliculus (*SC*, $n=3$–4 per group). Animals were compared to those injected with vehicle into the dlPAG ($n=7$). *Columns* represent the means and *bars* the SEM. **b** Time-course of the effects in the mean arterial pressure (*ΔMAP*) and heart rate (*ΔHR*) of unilateral microinjection of 200 nl AEA (5 pmol/200nl) or AM404 (50 pmol/200nl) into the following dlPAG surrounding structures: dorsomedial PAG (*dmPAG*, $n=3$ per group), lateral PAG (*lPAG*, $n=3$ per group) and superior colliculus (*SC*, $n=3$–4 per group). Animals were compared to those injected with vehicle into the dlPAG ($n=7$). *Symbols* represent the means and *bars* the SEM. **c** diagrammatic representation based on the rat brain atlas of Paxinos and Watson (1997) indicating injections sites of AEA or AM404 into the dmPAG, lPAG, and SC of conditioned animals

exposure to chronic mild stress failed to change these levels in several CNS regions, including the midbrain (Bortolato et al. 2007).

AM251, at the dose (100 pmol) that blocked the effects of exogenously administered AEA, failed to induce any change in the EPM (Moreira et al. 2007). This lack of AM251 effect was confirmed in the present study, even using with a higher dose (300 pmol). This result suggests that, under our experimental conditions, endogenous AEA does not play a significant tonic inhibitory role in the dlPAG.

Cannabinoid receptors are able to decrease neurotransmitter release (Gill et al. 1970). In the PAG, cannabinoid agonists inhibit glutamatergic synaptic transmission (Vaughan et al. 2000). Moreover, the inhibition of glutamatergic synaptic transmission in the PAG by the cannabinoid agonist WIN55,212-2 is reversed by the CB1-specific antagonist SR141716, indicating that it is mediated by activation of these receptors (Rinaldi-Carmona et al. 1994). Several studies have shown that antagonism of glutamate-mediated neurotransmission in the dlPAG produces anxiolytic responses in distinct animal models (Guimarães et al. 1991; Matheus et al. 1994; de Oliveira et al. 2001; Molchanov and Guimarães 2002). Direct administration of excitatory amino acids into this region, on the other hand, evokes intense defensive reactions (Bandler and Carrive 1988). Together, these pieces of evidence suggest that AEA and AM404 may be attenuating the expression of fear-conditioned responses by reducing glutamate release in the PAG due to CB1 receptor activation.

In conclusion, the present results indicate that facilitation of endocannabinoid-mediated neurotransmission in the dlPAG, through activation of local CB1 receptors, attenuates the expression of contextual fear responses.

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References

Adams IB, Compton DR, Martin BR (1998) Assessment of anandamide interaction with the cannabinoid brain receptor: SR 141716A antagonism studies in mice and autoradiographic analysis of receptor binding in rat brain. J Pharmacol Exp Ther 284:1209–1217

Amorapanth P, Nader K, LeDoux JE (1999) Lesions of periaqueductal gray dissociate-conditioned freezing from conditioned suppression behavior in rats. Learn Mem 6:491–499

Bandler R, Carrive P (1988) Integrated defence reaction elicited by excitatory amino acid microinjection in the midbrain periaqueductal grey region of the unrestrained cat. Brain Res 439:95–106

Bandler R, Keay KA, Floyd N, Price J (2000) Central circuits mediating patterned autonomic activity during active vs. passive emotional coping. Brain Res Bull 53:95–104

Berrendero F, Maldonado R (2002) Involvement of the opioid system in the anxiolytic-like effects induced by Delta(9)-tetrahydrocannabinol. Psychopharmacology (Berl) 163:111–117

Blanchard RJ, Blanchard DC (1969) Passive and active reactions to fear-eliciting stimuli. J Comp Physiol Psychol 68:129–135

Bortolato M, Campolongo P, Mangieri RA, Scattoni ML, Frau R, Trezza V, La Rana G, Russo R, Calignano A, Gessa GL, Cuomo V, Piomelli D (2006) Anxiolytic-like properties of the anandamide transport inhibitor AM404. Neuropsychopharmacology 31:2652–2659

Bortolato M, Mangieri RA, Fu J, Kim JH, Arguello O, Duranti A, Tontini A, Mor M, Tarzia G, Piomelli D (2007) Antidepressant-like activity of the fatty acid amide hydrolase inhibitor URB597 in a rat model of chronic mild stress. Biol Psychiatry 62:1103–1110

Carrive P, Leung P, Harris J, Paxinos G (1997) Conditioned fear to context is associated with increased Fos expression in the caudal ventrolateral region of the midbrain periaqueductal gray. Neuroscience 78:165–177

Carrive P, Lee J, Su A (2000) Lidocaine blockade of amygdala output in fear-conditioned rats reduces Fos expression in the ventrolateral periaqueductal gray. Neuroscience 95:1071–1080

Chhatwal JP, Davis M, Maguschak KA, Ressler KJ (2005) Enhancing cannabinoid neurotransmission augments the extinction of conditioned fear. Neuropsychopharmacology 30:516–524

de Oliveira RM, Del Bel EA, Guimarães FS (2001) Effects of excitatory amino acids and nitric oxide on flight behavior elicited from the dorsal periaqueductal gray. Neurosci Biobeh Rev 25:679–685

Devane WA, Dysarz FA 3rd, Johnson MR, Melvin LS, Howlett AC (1988) Determination and characterization of a cannabinoid receptor in rat brain. Mol Pharmacol 34:605–613

Devane WA, Hanus L, Breuer A, Pertwee RG, Stevenson LA, Griffin G, Gibson D, Mandelbaum A, Etinger A, Mechoulam R (1992) Isolation and structure of a brain constituent that binds to the cannabinoid receptor. Science 258:1946–1949

Egertova M, Cravatt BF, Elphick MR (2003) Comparative analysis of fatty acid amide hydrolase and cb(1) cannabinoid receptor expression in the mouse brain: evidence of a widespread role for fatty acid amide hydrolase in regulation of endocannabinoid signaling. Neuroscience 119:481–496

Fanselow MS (1980) Conditioned and unconditional components of post-shock freezing. Pavlov J Biol Sci 15:177–182

Finn DP, Jhaveri MD, Beckett SR, Roe CH, Kendall DA, Marsden CA, Chapman V (2003) Effects of direct periaqueductal grey administration of a cannabinoid receptor agonist on nociceptive and aversive responses in rats. Neuropharmacology 45:594–604

Gill EW, Paton WD, Pertwee RG (1970) Preliminary experiments on the chemistry and pharmacology of cannabis. Nature 228:134–136

Guimarães FS, Carobrez AP, De Aguiar JC, Graeff FG (1991) Anxiolytic effect in the elevated plus-maze of the NMDA receptor antagonist AP7 microinjected into the dorsal periaqueductal grey. Psychopharmacology (Berl) 103:91–94

Herkenham M, Lynn AB, Johnson MR, Melvin LS, de Costa BR, Rice KC (1991) Characterization and localization of cannabinoid receptors in rat brain: a quantitative in vitro autoradiographic study. J Neurosci 11:563–583

Hohmann AG, Suplita RL, Bolton NM, Neely MH, Fegley D, Mangieri R, Krey JF, Walker JM, Holmes PV, Crystal JD, Duranti A, Tontini A, Mor M, Tarzia G, Piomelli D (2005) An endocannabinoid mechanism for stress-induced analgesia. Nature 435:1108–1112

Kathuria S, Gaetani S, Fegley D, Valino F, Duranti A, Tontini A, Mor M, Tarzia G, La Rana G, Calignano A, Giustino A, Tattoli M, Palmery M, Cuomo V, Piomelli D (2003) Modulation of anxiety through blockade of anandamide hydrolysis. Nat Med 9:76–81

Krieger JE, Graeff FG (1985) Defensive behavior and hypertension induced by glutamate in the midbrain central gray of the rat. Braz J Med Biol Res 18:61–67

LeDoux JE, Iwata J, Cicchetti P, Reis DJ (1988) Different projections of the central amygdaloid nucleus mediate autonomic and behavioral correlates of conditioned fear. J Neurosci 8: 2517–2529

Matheus MG, Nogueira R, Carobrez AP, Graeff FG, Guimarães FS (1994) Anxiolytic effect of glycine antagonists microinjected into the dorsal periaqueductal grey. Psychopharmacology 13:565–569

Matsuda LA, Lolait SJ, Brownstein MJ, Young AC, Bonner TI (1990) Structure of a cannabinoid receptor and functional expression of the cloned cDNA. Nature 346:561–564

Mechoulam R, Ben-Shabat S, Hanus L, Ligumsky M, Kaminski NE, Schatz AR, Gopher A, Almog S, Martin BR, Compton DR et al (1995) Identification of an endogenous 2-monoglyceride, present in canine gut, that binds to cannabinoid receptors. Biochem Pharmacol 50:83–90

Molchanov ML, Guimarães FS (2002) Anxiolytic-like effects of AP7 injected into the dorsolateral or ventrolateral columns of the periaqueductal gray of rats. Psychopharmacology (Berl) 160:30–38

Moreira FA, Aguiar DC, Guimarães FS (2007) Anxiolytic-like effect of cannabinoids injected into the rat dorsolateral periaqueductal gray. Neuropharmacology 52:958–965

Patel S, Hillard CJ (2006) Pharmacological evaluation of cannabinoid receptor ligands in a mouse model of anxiety: further evidence for an anxiolytic role for endogenous cannabinoid signaling. J Pharmacol Exp Ther 318:304–311

Paxinos G, Watson C (1997) The rat brain in stereotaxic coordinates. 2.ed. Academic, Sydney

Resstel LB, Joca SR, Guimarães FG, Correa FM (2006a) Involvement of medial prefrontal cortex neurons in behavioral and cardiovascular responses to contextual fear conditioning. Neuroscience 143:377–385

Resstel LB, Joca SR, Moreira FA, Correa FM, Guimarães FS (2006b) Effects of cannabidiol and diazepam on behavioral and cardiovascular responses induced by contextual conditioned fear in rats. Behav Brain Res 172:294–298

Rinaldi-Carmona M, Barth F, Heaulme M, Shire D, Calandra B, Congy C, Martinez S, Maruani J, Neliat G, Caput D et al (1994) SR141716A, a potent and selective antagonist of the brain cannabinoid receptor. FEBS Lett 350:240–244

Smith PB, Compton DR, Welch SP, Razdan RK, Mechoulam R, Martin BR (1994) The pharmacological activity of anandamide, a putative endogenous cannabinoid, in mice. J Pharmacol Exp Ther 270:219–227

Sugiura T, Kondo S, Sukagawa A, Nakane S, Shinoda A, Itoh K, Yamashita A, Waku K (1995) 2-Arachidonoylglycerol: a possible endogenous cannabinoid receptor ligand in brain. Biochem Biophys Res Commun 215:89–97

Vaughan CW, Connor M, Bagley EE, Christie MJ (2000) Actions of cannabinoids on membrane properties and synaptic transmission in rat periaqueductal gray neurons in vitro. Mol Pharmacol 57:288–295

Zhang WN, Murphy CA, Feldon J (2004) Behavioural and cardiovascular responses during latent inhibition of conditioned fear: measurement by telemetry and conditioned freezing. Behav Brain Res 154:199–209