Progress in Neuropsychopharmacology & Biological Psychiatry 79 (2017) 493–498

Contents lists available at ScienceDirect

Progress in Neuropsychopharmacology & Biological Psychiatry

journal homepage: www.elsevier.com/locate/pnp

B2-kinin receptors in the dorsal periaqueductal gray are implicated in the panicolytic-like effect of opiorphin

Caio César Sestile[a,*], Jhonatan Christian Maraschin[a], Marcel Pereira Rangel[a], Rosangela Getirana Santana[b], Hélio Zangrossi Jr[c,d], Frederico Guilherme Graeff[d], Elisabeth Aparecida Audi[a,*]

[a] Department of Pharmacology and Therapeutics, State University of Maringá (UEM), Maringá, PR, Brazil
[b] Department of Statistical, State University of Maringá (UEM), Maringá, PR, Brazil
[c] Department of Pharmacology, School of Medicine of Ribeirão Preto, University of São Paulo (USP), Ribeirão Preto, SP, Brazil
[d] Institute of Neurosciences and Behavior (INeC), Ribeirão Preto, Brazil

ARTICLE INFO

Keywords:
Opiorphin
Bradykinin
Panic model
5-HT$_{1A}$ receptor
Kinin B2 receptor

ABSTRACT

Reported results have shown that the pentapeptide opiorphin inhibits oligopeptidases that degrade brain neuropeptides, and has analgesic and antidepressant effects in experimental animals, without either tolerance or dependency after chronic administration. In a previous study we showed that opiorphin has a panicolytic-like effect in the dorsal periaqueductal gray (dPAG) electrical stimulation test (EST), mediated by the μ-opioid receptor (MOR). This study further analyzes the mechanism of opiorphin panicolytic action, using the EST and drug injection inside the dPAG. The obtained results showed that blockade of the 5-HT$_{1A}$ receptors with WAY-100635 did not change the escape-impairing effect of opiorphin, and combined injection of sub-effective doses of opiorphin and the 5-HT$_{1A}$-agonist 8-OH-DPAT did not have a significant anti-escape effect. In contrast, the anti-escape effect of opiorphin was antagonized by pretreatment with the kinin B2 receptor blocker HOE-140, and association of sub-effective doses of opiorphin and bradykinin caused a significant anti-escape effect. The anti-escape effect of bradykinin was not affected by previous administration of WAY-100635. Therefore, the anti-escape effect of opiorphin in the dPAG seems to be mediated by endogenous bradykinin, acting on kinin B2 receptors, which previous results have shown to interact synergistically with MOR in the dPAG to restrain escape in two animal models of panic.

Chemical compounds: Opiorphin (PubChem CID: 25195667); WAY100635 maleate salt (PubChem CID: 11957721); 8-OH-DPAT hydrobromide (PubChem CID: 6917794); Bradykinin (PubChem CID: 439201); HOE-140 (Icatibant) (PubChem CID: 6918173).

1. Introduction

Reported results obtained by our research group with escape responses performed by rats in two models of panic (Moreira et al., 2013) – the elevated T-maze (ETM) and electrical stimulation test (EST) of dorsal periaqueductal gray (dPAG) – indicate that endogenous opioids inhibit neurons that organize proximal defense in this brain region (Maraschin et al., 2016; Rangel et al., 2014; Roncon et al., 2013, 2015). Because panic attacks may be viewed as dysfunctional activation of proximal defense in the dPAG (Deakin and Graeff, 1991; Mobbs et al., 2007; Shuhama et al., 2016), these results implicate opioids in the vulnerability to panic attacks that is characteristic of panic disorder (Graeff, 2004, 2017). In this respect Preter and Klein (2008) have suggested that panic patients have a deficient opioid system that buffers panic attacks.

The above-mentioned studies with rat models of panic have further shown that the anti-escape action of opioids in the dPAG was enacted through activation of the μ-opioid receptor (MOR), and that this receptor synergistically interacts with the serotonin 5-HT$_{1A}$ receptor (5-HT$_{1A}$R) (Rangel et al., 2014; Roncon et al., 2013). The latter has been shown to mediate the anti-escape effect of chronic, systemically administered fluoxetine (Roncon et al., 2012, 2015). Like with endogenous opioids, deficient 5-HT inhibition of proximal defense in the dPAG has also been supposed to underpin the vulnerability to panic attacks of panic patients (Del-Ben et al., 2001; Johnson et al., 2008). As a consequence, the cooperative interaction between 5-HT and endogenous opioids in the dPAG is thought to reconcile the opioid-deficiency and the 5-HT-deficiency hypotheses of panic disorder

* Corresponding authors at: Department of Pharmacology and Therapeutics, State University of Maringá, Av. Colombo 5790, Maringá, PR 87020-900, Brazil.
E-mail addresses: caiosestile86@gmail.com (C.C. Sestile), elisabethaudi8@gmail.com (E.A. Audi).

http://dx.doi.org/10.1016/j.pnpbp.2017.08.003
Received 16 June 2017; Received in revised form 3 August 2017; Accepted 5 August 2017
Available online 07 August 2017
0278-5846/

pathophysiology (Graeff, 2012, 2017).

Long-term administration of fluoxetine-like antidepressants that inhibit 5-HT membrane reuptake is the first-choice drug treatment of panic disorder. Although quite effective, these agents have drawbacks that prompt the search for new therapeutic medication, among which are the delay of several weeks for the therapeutic action to take place, the sizable portion of non-responders, and several untoward collateral effects (*e.g.*, Bandelow et al., 2013). In this regard, the suggested co-operative interaction between 5-HT_{1A}R and MOR in the dPAG for regulating panic attacks led to the proposal that additional opioid medication could enhance the panicolytic action of antidepressants, possibly accelerating the onset of action and making at least some drug-resistant patients responsive (Roncon et al., 2015). However, available agents that directly stimulate MOR, such as morphine and heroin, induce euphoria and are highly addictive (*e.g.*, Berrettini, 2016).

It follows from the above argument that one should look for chemicals that act on MOR indirectly. A promising option is opiorphin, a pentapeptide that was first isolated from human saliva. Opiorphin impairs the degradation of endogenous enkephalins by inhibiting a neutral endopeptidase and an aminopeptidase N (Wisner et al., 2006). As a result, it may enhance the effects of enkephalins, which are mediated by the activation of the δ-opioid receptor and/or MOR (Thanawala et al., 2008). Results obtained in experimental animals have shown that opiorphin has analgesic (Popik et al., 2010; Rougeot et al., 2010; Wisner et al., 2006) and antidepressant (Javelot et al., 2010; Yang et al., 2011) effects, and has low toxicity after acute administration (Bogeas et al., 2013). In addition, prolonged administration of opiorphin did not induced tolerance to the analgesic effect (Rougeot et al., 2010), as well as physiological (Popik et al., 2010) or behavioral (Popik et al., 2010; Rougeot et al., 2010) dependence. Because oral administration of opiorphin is ineffective, analogs that can be given *per os* are being developed (Poras et al., 2014).

These properties have prompt us to test opiorphin in animal models of panic. The obtained results showed that intravenous injection of opiorphin impaired escape performance in both the ETM and the EST. This panicolytic-like effect was also observed following the intra-dPAG administration of opiorphin, and local pretreatment with the selective MOR antagonist CTOP abolished the anti-escape effect of intra-dPAG opiorphin in both animal models, as well as antagonized the effect of intravenous opiorphin in the EST. Therefore opiorphin seems to exert a panicolytic-like action in the dPAG, mediated by MOR (Maraschin et al., 2016), possibly activated by endogenous enkephalins.

While enkephalin mediation is a plausible hypothesis, alternative or complementary mechanisms are possible, since neuropeptides other than enkephalins may be degraded by the oligopeptidases inhibited by opiorphin. In this regard, bradykinin (BK), a nonapeptide isolated from the precursor plasm protein kininogen, through the action of proteolytic enzymes (Beraldo and Andrade, 1997; Rocha e Silva et al., 1949), emerged as strong candidate. Not only BK-like immunoreactivity has been described in the dPAG (Perry and Snyder, 1984), but also BK injection in this brain region has been shown to increase the threshold intensity of the electrical current that elicits escape behavior when applied to the rat dPAG. Furthermore, the same panicolytic-like effect was determined by intra-dPAG injection of morphine, and the effect of either morphine or BK was antagonized by pretreatment with intraperitoneally injected naloxone, indicating MOR participation (Burdin et al., 1992).

To further explore the seemingly panicolytic action of BK in the dPAG, we have carried out a study with the EST, and the obtained results showed that intra-dPAG injection of BK increased escape threshold in a dose-dependent way. Both the selective kinin B2 receptor (B2R) antagonist HOE-140 and the selective MOR antagonist CTOP blocked this panicolytic-like effect. Reciprocally, the same effect of the selective MOR agonist DAMGO was antagonized by pre-treatment with either CTOP or HOE-140, indicating cross-antagonism between MOR and B2R. Importantly, intra-dPAG injection of captopril, a drug that inhibits the enzymatic degradation of BK (Taddei and Bortoloto, 2016), impaired escape in a dose-dependent way, and this effect was blocked by pretreatment with HOE-140, suggesting mediation by endogenous BK (Sestile et al., 2017). Therefore, BK may physiologically restrain panic attacks in the dPAG through MOR/B2R stimulation.

The present study aimed to further investigate the mechanism of action of opiorphin using intra-dPAG injection and the EST. If the panicolytic-like effect of opiorphin were mediated by enkephalins through activation of MOR, it should be affected by drugs that act on the 5-HT_{1A}R, as it happens with other MOR agonists and antagonists (Rangel et al., 2014; Roncon et al., 2013). To test this prediction, the first experiment probed whether the selective 5-HT_{1A}R-blocker WAY-100635 would antagonize opiorphin, and the second, whether co-administration of sub-effective doses of opiorphin and the selective 5-HT_{1A}R-agonist 8-OH-DPAT would result in a significant panicolytic-like effect. Since both gave negative results, the following experiments focused on BK. Thus, in the third experiment, we measured the influence of pretreatment with the selective B2R antagonist HOE-140 on anti-escape action of opiorphin, and in the fourth, we assessed the panicolytic-like effect of combined administration of sub-effective doses do BK and opiorphin. Finally we checked whether BK itself could interact with the 5-HR_{1A}R using WAY-100635 as a tool.

2. Methods

2.1. Animals

Male Wistar rats (State University of Maringá) weighing 220–250 g were housed in group of five per cage under a 12/12 h light/dark cycle (lights on at 40 lx intensity from 07:00 to 19:00 h) at 22 °C ± 1 °C with free access to food and water. The experimental procedures were approved by the State University of Maringá Committee of Ethical Conduct in the Use of Animals in Experiments (1121010415/CEUA) and are in accordance with the International Guiding Principles for Biomedical Research Involving Animals (WHO, 1985). All efforts were made to minimize the number of animals used and their suffering.

2.2. Drugs

Opiorphin (Bachem, USA); Bradykinin (H-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg-OH acetate salt) (Bachem, USA) and HOE-140 (Arg-Arg-Pro-Hyp-Gly-Thi-Ser-Tic-Oic-Arg) (Sigma, USA) were dissolved in a distilled water. (±)-8-hydroxy-2-(di-*n*-propylamino) tetralin hydrobromide (8-OHDPAT, Sigma, USA) and WAY-100635 (Sigma, USA) was dissolved in 0.9% sterile saline. All drugs were administered into the dPAG. For injections a needle (0.3 mm outer diameter) was introduced through the guide cannula until its tip was 1.0 mm below the cannula end, and a volume of 0.2 µl was infused over 120 s using a 10 µl microsyringe (Hamilton 701-RN) that was attached to a microinfusion pump (Insight, Brazil).

2.3. Apparatus

Escape behavior induced by electrical stimulation of the dPAG was evaluated in a 40 cm diameter circular arena that was surrounded by 40 cm high walls made of transparent Plexiglas. The stimulation current (peak to peak) was generated by a sine-wave stimulator and monitored with an oscilloscope (Minipa, Brazil). The brain electrode was connected to the stimulator by means of an electromechanical swivel and flexible cable, allowing ample movement of the animal inside the experimental cage.

2.4. Surgery

The rats were anesthetized with an intramuscular injection of ketamine (75 mg/kg; União Química, Brazil) and xylazine (10 mg/kg;