Research report

Effects of alprazolam and cannabinoid-related compounds in an animal model of panic attack

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HIGHLIGHTS

• The pharmacological treatment of panic disorder is limited.
• One of the few animal models of panic is the response to KCN injection in rats.
• The KCN model is useful for screening new potential treatments for panic disorder.
• Cannabinoid compounds, which may alleviate anxiety, failed to inhibit KCN effects.
• Alprazolam, a benzodiazepine, abolished KCN behavioral and cardiovascular effects.

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ABSTRACT

Selective stimulation of carotid chemoreceptors by intravenous infusion of low doses of potassium cyanide (KCN) produces short-lasting escape responses that have been proposed as a model of panic attack. In turn, preclinical studies suggest that facilitation of the endocannabinoid system attenuates panic-like responses. Here, we compared the effects of cannabinoid-related compounds to those of alprazolam, a clinically effective panicolytic, on the duration of the escape reaction induced by intravenous infusion of KCN (80 μg) in rats. Alprazolam (1, 2, 4 mg/kg) decreased escape duration at doses that did not alter basal locomotor activity. URB597 (0.1, 0.3, 1 mg/kg; inhibitor of anandamide hydrolysis), WIN55,212-2 (0.1, 0.3, 1 mg/kg; synthetic cannabinoid), arachidonoyl-serotonin (1, 2.5, 5 mg/kg; dual TRP1 and anandamide hydrolysis inhibitor), and cannabidiol (5, 10, 20, 40 mg/kg; a phytocannabinoid) did not decrease escape duration. Alprazolam also prevented the increase in arterial pressure evoked by KCN, while bradycardia was unchanged. This study reinforces the validity of the KCN-evoked escape as a model of panic attack. However, it does not support a role for the endocannabinoid system in this behavioral response. These results might have implications for the screening of novel treatments for panic disorder.

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1. Introduction

Panic disorder is a subtype of anxiety disorder characterized by the occurrence of panic attacks, which comprises feelings of fear, discomfort and somatic symptoms of respiratory distress, hyperventilation, palpitations, and sweating [1]. These attacks can be classified as either ‘respiratory’ or ‘non-respiratory’, depending on its symptoms [2,3]. Some attempts to explain their neural substrates have been focused on brainstem structures such as the locus coeruleus, the nucleus raphe magnus, and the periaqueductal gray matter (PAG) [4–6]. Other theories emphasized prosencephalic structures such as the hypothalamus, the amygdala, and the prefrontal cortex [7–11].

Among the main theories regarding the neurobiology of panic attacks, Klein’s (1993) suffocation false alarm (SFA) theory states that clinical panic attacks are the misfiring of a suffocation alarm system. The false activation of the suffocation alarm would lead to dyspnea, fear, and hyperventilation [12,13]. The neural substrate of the ‘suffocation alarm system’, however, has remained elusive. In particular, the participation of the locus coeruleus in panic attacks was contradicted by the lack of aversiveness of its stimulation in humans [4]. The involvement of the amygdala was questioned by a study showing that patients with bilateral damage to the amygdala develop panic attacks both spontaneously and in response to the
inhalation of 35% CO2 and infusion of isoproterenol [14–16]. As to cognitive theories, it is proposed that the panic attack is the cortical ‘catastrophization’ of bodily symptoms [11]. Although all theories suggest that panic attacks are false alarms, the neural mechanisms that either predispose or trigger the response remain unknown.

In this context, recent data suggest that the PAG harbors a hypoxia-sensitive suffocation alarm system. Activation of this structure is hypothesized to trigger a respiratory-type panic attack and render the subject hypersensitive to CO2 [17,18]. Evidence for that were obtained from studies utilizing the escape reaction induced by potassium cyanide ( KCN ) as an animal model of panic attack. These studies showed that low doses of KCN induce an escape reaction that is blocked by clinically effective panicoletic drugs and electrolytic lesions of the DPAG [18,19]. Moreover, it was shown that the escape response was potentiated by hypercapnia [18]. Intravenous infusion of KCN leads to activation of peripheral chemoreceptors resulting in hypertension, bradycardia and tachypnea, which constitute the chemoreflex [20], along with a panic-like increase in locomotor activity.

Studies employing other animal models suggest a possible involvement of the endocannabinoid system in panic-like responses [21–25]. This system comprises the CB1 and CB2 receptors, the endogenous ligands anandamide and 2-arachidonoyl glycerol ( 2-AG ), and the enzymes mediating their synthesis and breakdown [26]. Activation of CB1 receptors in the PAG attenuates the escape reaction induced by stimulation of this structure [21–24,27,28]. CB1 agonists injected either systemically or in the PAG also attenuate the escape reaction induced by 20 kHz ultrasound and exposure to the elevated T maze [24,25,28]. Moreover, compounds that inhibit fatty acid amide hydrolase ( FAAH ), the main enzyme responsible for anandamide hydrolysis, also induce panicolytic-like effects [23,25]. Finally, these effects also occur after blockade of the transient receptor potential vanilloid type-1 ( TRPV1 ) channel, which is also a molecular target for anandamide [26]. Based on these models, CB1-mediated signaling might have a role in counteracting aversive reactions [29]. However, other models based on different theories of panic disorder could unveil a different role for the system in panic-like responses.

Accordingly, in this study we compared the effects of alprazolam, a benzodiazepine with therapeutic use for the treatment of panic disorder, to those of cannabinoid-related compounds on the panic-like reaction induced by intravenous infusion of a low dose of KCN. We tested the anandamide hydrolase inhibitor, UR5897; the dual FAAH/TRPV1 blocker, arachidonoyl-serotonin; the non-selective cannabinoid agonist, WIN55,212-2; and cannabidiol ( CBD ), a phytocannabinoid that might act as a 5-HT1A receptor agonist [30] and presents anxiolytic ( humans and rodents ) and panicolytic ( rodents ) effects [31,32].

2. Materials and methods

2.1. Animals

Male Wistar rats ( 250–350 g ) from the animal facility of the Institute of Biological Sciences of UFMG were used. Animals were kept in a room with controlled temperature ( 24 °C ), 12-h light–dark cycle, and free access to food and water. They were housed in groups of five per cage. All procedures were approved by the Committee for Ethics in Animal Experimentation ( protocol 259/2013 ).

2.2. Drugs

All drugs were injected via ip route, except for KCN, which was injected via iv route. KCN ( Merck, Darmstadt, Germany ) was diluted in saline and the dose ( 80 μg/0.1 mL ) was chosen based on a dose–response curve performed in our laboratory ( data not shown ) and previous reports [19]. Alprazolam ( 1, 2, and 4 mg/kg; EMS Brazil ) was dissolved in a solution of saline and 2% Tween. WIN55,212-2, ( 0.1, 0.3, and 1 mg/kg; Cayman Chemicals ), UR5897 ( 0.1, 0.3, and 1 mg/kg; Cayman Chemicals ), and arachidonoyl-serotonin ( 1, 2.5, and 5 mg/kg; Cayman Chemicals ) were dissolved in a solution of ethanol, cremophor and saline in a proportion of 1: 1: 8. CBD ( 5, 10, 20, and 40 mg/kg ) was dissolved in a solution of Tween 5% and saline. Doses were chosen based on the following studies: [25,33,34].

2.3. Femoral artery and vein catheterization

One day before the experiments, under tribromoethanol ( 250 mg·kg−1·IP ) anesthesia, a small incision was made in theinguinal region to expose the femoral artery and vein. Polyethylene catheters ( PE-10 connected to PE-50; ClayAdams, Parsippany, NJ, USA ) filled with saline ( NaCl 0.9% ) were inserted into the abdominal aorta via the femoral artery and into the femoral vein. Both catheters were tunneled subcutaneously and exteriorized through the back of the neck. The catheter inserted in the femoral artery was used for recording of cardiovascular parameters and the catheter in the femoral vein was used for intravenous infusion. After surgeries, rats were placed in individual cages.

2.4. Chemoreflex stimulation

The peripheral chemoreflex was activated by intravenous infusion of 100 μL of KCN ( 80 μg/0.1 mL ) in accordance with the procedures described by [20] and previously validated for our experimental conditions [35,36].

2.5. Recording of cardiovascular parameters

The catheter in the femoral artery was flushed with heparinized saline ( 0.9% NaCl ) to prevent clotting and then connected to the pressure transducer ( model CDX III; Cobe Laboratories, Lakewood, CO, USA ). Pulsatile arterial pressure ( PAP ) was continuously recorded by an A/D data acquisition system ( MF100; Biopac Systems, Inc., Santa Barbara, CA, USA ). Mean arterial pressure ( MAP ) and heart rate ( HR ) was simultaneously derived from arterial pulse waves by software ( AcqKnowledge 5; Biopac Systems ).

2.6. Behavioral measurement

For measuring escape duration, animals were placed in a rectangular box ( 50 cm long, 30 cm wide, 20 cm high ) with opaque plastic walls. Animals were left in the box for 10 min for habituation. Afterwards, KCN was injected and the behavior response was recorded by a videocamera situated on top of the box. Escape duration was measured manually by an experimenter blind to the treatments.

2.7. Experimental protocols

For the behavioral experiments, animals received ip injections of cannabinoids and related drugs that act on the endocannabinoid system ( WIN55,212-2, UR5897, AA-5-HT, and CBD ) or alprazolam. Twenty minutes after the first injection they were placed in the box, left undisturbed during 10 min for habituation and received KCN infusion. The distance traveled was measured after the beginning of KCN infusion. In an independent experiment, for measurement of basal locomotor activity, the same protocol was followed, except that no KCN was administered. The distance traveled in the box was measured with the aid of the software Any-Maze, version 4.99 ( Stoelting ). For the cardiovascular recording, animals received one
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