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NEW INSIGHTS ON AMYGDALA: BASOMEDIAL AMYGDALA REGULATES THE PHYSIOLOGICAL RESPONSE TO SOCIAL NOVELTY

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Abstract—The amygdala has been associated with a variety of functions linked to physiological, behavioral and endocrine responses during emotional situations. This brain region is comprised of multiple sub-nuclei. These sub-nuclei belong to the same structure, but may be involved in different functions, thereby making the study of each sub-nuclei important. Yet, the involvement of the basomedial amygdala (BMA) in the regulation of emotional states has yet to be defined. Therefore, the aim of our study was to investigate the regulatory role of the BMA on the responses evoked during a social novelty model and whether the regulatory role depended on an interaction with the dorsomedial hypothalamus (DMH). Our results showed that the chemical inhibition of the BMA by the microinjection of muscimol (γ-aminobutyric acid ($GABA_A$) agonist) promoted increases in mean arterial pressure (MAP) and heart rate (HR), whereas the chemical inhibition of regions near the BMA did not induce such cardiovascular changes. In contrast, the BMA chemical activation by the bilateral microinjection of *bicuculline methiodide* (BMI; $GABA_A$ antagonist), blocked the increases in MAP and HR observed when an intruder rat was suddenly introduced into the cage of a resident rat, and confined to the small cage for 15 min. Additionally, the increase in HR and MAP induced by BMA inhibition were eliminated by DMH chemical inhibition. Thus, our data reveal that the BMA is under continuous GABAergic influence, and that its hyperactivation can reduce the physiological response induced by a social novelty condition, possibly by inhibiting DMH neurons.

Key words: social novelty basomedial amygdala, dorsomedial hypothalamus, intruder rat.

INTRODUCTION

Specific brain nuclei are recruited during emotional and environmental stress, leading to several autonomic, endocrine and behavioral responses (Blanchard and Blanchard, 1989a,b; DiMicco et al., 2006; Szczepanska-Sadowska, 2008) that improve chances of survival. However, heightened stress promotes the continuous activation of neuronal pathways for compensatory adaptation, which compromises the organism's physiological integrity and the pattern of the aforementioned responses (Szczepanska-Sadowska, 2008; Chrousos, 2009). The neuronal circuitry involved in the stress response is not yet completely understood, specifically, the regions that can inhibit the stress response.

The amygdaloid complex and the dorsomedial hypothalamus are recognized today as important nuclei involved in the regulation of cardiovascular and behavioral responses evoked by emotional stress (Petrovich et al., 1996; Sah et al., 2003). However, the basomedial amygdala's (BMA) role in emotional regulation has been mostly neglected, even though there is evidence that this region could be important in influencing several emotional states. For instance, studies have shown that there is an increase in BMA neuronal activity in rats submitted to the inhibitory avoidance test (Silveira et al., 2001; de Andrade et al., 2012), suggesting that this region could be involved in controlling the animal's anxiety state. Moreover, matched lesions in the BMA and basolateral amygdala (BLA) reduced the conditioned fear responses, suggesting that the BMA could be involved in the neural pathway of fear control (Anglada-Figueroa and Quirk, 2005).

The BMA sends excitatory projections to the central amygdala (CeA) and the bed nucleus of stria terminalis (BNST) (Petrovich et al., 1996), both of which control the inhibitory tonus to the dorsomedial hypothalamus (DMH), an integratory region of the cardiovascular responses evoked by stressful situations (DiMicco et al., 1996; de Menezes et al., 2009; Fontes et al., 2011; Abreu et al., 2014). These observations support

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Abbreviations: BLA, basolateral amygdala; BMA, basomedial amygdala; BMI, *bicuculline methiodide*; BNST, bed nucleus of stria terminalis; CeA, central amygdala; DMH, dorsomedial hyphothalamus; $GABA_A$, γ-aminobutyric acid; HR, heart rate; MAP, mean arterial pressure; mPOA, medial preoptic area.

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a possible role of the BMA in the brain circuitry activated by stress.

To our knowledge, only one study has attempted to investigate the BMA's role in cardiovascular control. The study showed that while the injection of *bicuculline methiodide* (BMI; γ-aminobutyric acid ($GABA_A$) receptor antagonist) in the BMA of anesthetized rats increased arterial pressure and heart rate, the injection of muscimol ($GABA_A$ receptor agonist) did not alter those parameters (Yoshida et al., 2002). However, because anesthesia itself is known to affect cardiovascular parameters (Shimokawa et al., 1998), it remains unclear whether or not the BMA has a role in the brain related control of cardiovascular responsiveness in conscious animals.

In this study, we investigated the regulatory role of the BMA in the cardiovascular response evoked by social novelty and whether that regulatory role was influenced by an interaction with the DMH. To that end, we examined the influence of acute inhibition of BMA neurons by injecting muscimol on basal cardiovascular parameters. We have also evaluated the effect of BMA disinhibition by injecting BMI bilaterally on the cardiovascular parameters of an intruder Wistar rat, when it was suddenly introduced into the home cage of a resident rat. Furthermore, we assessed the functional connection between the BMA and the DMH, by examining the influence of acute inhibition of the DMH on responses evoked by BMA acute inhibition.

EXPERIMENTAL PROCEDURES

Ethical approval

All procedures were previously approved by the ethics committee for animal research of the Federal University of Ouro Preto (CEUA-UFOP; #2012/51) and were performed according to the regulations set forth by the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals (8th edition; 2011) in accordance with the rules and regulations of articles focused on animal experimentation. All investigators understand the ethical principles under which Neuroscience operates, and that our work complies with this journal's animal ethics checklist. Care was taken to minimize the number of animals used and to avoid their unnecessary suffering.

Animals

The experiments were carried out on 25 male Wistar rats (220-240 g) from the University Centre of Animal Science. The animals were housed collectively in cages with dimensions of 41 × 34 × 17 cm (three animals per cage) that were maintained at an average temperature of 23 ± 1 °C in a light/dark cycle of 12 h, and were allowed free access to water and food (commercial feed Nuvilab®, Brazil). After the completion of the first surgical procedure (described below), the animals were housed individually in cages with dimensions of 30 × 19 × 13 cm.

Surgical procedures

Surgical procedures started when the rats reached a weight of 300 ± 20 g. The animals were anesthetized (80 mg kg^{-1} ketamine and 11.5 mg kg^{-1} xylazine, ip, supplemented when necessary) to perform the implant guide cannula (23G) for drug injection in the BMA unilaterally, the BMA bilaterally, the DMH bilaterally, and in a region near the BMA, as previously described (de Menezes et al., 2006, 2008, 2009). For this procedure, the rats were placed in a stereotaxic apparatus with the tooth bar fixed at −3.3 mm below the interaural line. After dissecting and cleaning the skull region, we made a 1-mm incision for stainless steel screws and guide cannula implants. Guide cannulas were positioned according to the coordinates of the Paxinos and Watson atlas (Paxinos and Watson, 2007) using the bregma as a reference point. Coordinates for the BMA: 2.3 mm posterior, 4.1 mm lateral, 7.3 mm ventral; and for the DMH: 3.2 mm posterior, 0.6 mm lateral, 7.5 mm ventral. Screws and dental acrylic secured the guide cannulas. After 7 days of recovery, the animals were placed under the anesthetic isoflurane (2.5% isoflurane in 3 L/min O_2; Cristalia, Brazil), and a polyethylene catheter was inserted into the femoral artery for later measurement of cardiovascular parameters. Briefly, an incision was made in the inguinal region of the rat, and the catheter was inserted into the femoral until it reached the aorta (about 4 cm). The catheter was tunneled subcutaneously and exteriorized on the back of the neck. Analgesic (ketoflex 4 mg/kg, 0.1 ml/300 g s.c., Mundo Animal, Brazil) and antibiotics (0.2 ml/100 g, s.c., Fort Dodge Animal Health, Brazil) were administered after both surgical procedures. We initiated the experimental procedures 48 h after the last surgery, during which the catheter was connected to a data acquisition system for obtaining cardiovascular parameters.

Cardiovascular measurements

The catheter, connected to the data acquisition system (PowerLab/400, ADInstruments, NSW, Sydney, Australia), allowed for the measurement of the pulsatile blood pressure of the animals. The pressure oscillations that were captured were amplified and converted into signals that were sent to a data acquisition board by an analogical-to-digital converter. The software Chart 7.0 for Windows (ADI Instruments, NSW, Sydney, Australia) held a continuous collection of pulsatile blood pressure, and then calculated mean arterial pressure (MAP) and heart rate (HR).

Experimental design

On the day of the experiment, the animals were brought to the experimental room in their home cages. The experiment began after the stabilization of physiological parameters (MAP and HR) for at least 30 min. For injecting the drugs, injection cannulas (30 gauge, 1 mm longer than the guide cannula) were connected to a Hamilton syringe (5 μL), filled with the compound to be microinjected, using a Teflon tubing (ID 0:12 mm; OD