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Ingestive and locomotor behaviours induced by pharmacological manipulation of <Alpha>-adrenoceptors into the median raphe nucleus

Brunno Rocha Levone [a, *], Elisa Caroline Cella [a], Larissa Kochenborger [a], Eduardo Simão da Silva [a], Ana Paula Dambros Taschetto [a], Samira Schultz Mansur [b], Mariana Graciela Terenzi [a], Moacir Serralvo Faria [a], Marta Aparecida Paschoalini [a]

[a] Department of Physiological Sciences, Centre of Biological Sciences, Federal University of Santa Catarina (UFSC), 88040-900 Florianopolis, SC, Brazil
[b] Department of Morphological Sciences, Centre of Biological Sciences, Federal University of Santa Catarina (UFSC), Florianopolis, SC, Brazil

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ABSTRACT

The present study evaluated the involvement of α-adrenoceptors of the median raphe nucleus (MRN) in satiated rats, in food and water intake and motor behaviour. Control groups were treated with saline (SAL) or adrenaline (ADR), injected into the MRN seven minutes after injection of the vehicle used to solubilize the antagonists, propylene glycol (PLG) or SAL. Experimental groups were treated with an α-adrenoceptor antagonist, prazosin (α1, 20 or 40 nmol) or yohimbine (α2, 20 or 40 nmol) or phentolamine (non-selective α, 20 or 40 nmol), followed (later) by injection of ADR or SAL. Behaviour was recorded for 30 min. The injection of ADR and the blockade of α1 receptors resulted in hyperphagia whereas blocking α2 or α1 and α2 simultaneously did not change feeding behaviour. Pre-treatment with prazosin, followed by injection of ADR was not able to cause an increase in the amount of food ingested, while the higher dose of the α1 antagonist reduced the latency to start feeding. Pre-treatment with prazosin also caused hyperactivity. However, pre-treatment with phentolamine or yohimbine was able to block ADR-induced feeding. The present study supports the hypothesis that there is a tonic activation of α1-adrenoceptors in the MRN in satiated rats, which activates an inhibitory influence in areas that control food intake. Injection of ADR seems to activate α2 receptors, resulting in a decrease in the availability of endogenous catecholamines, which reduces the release of the signal that inhibits food intake, leading to hyperphagia.

1. Introduction

The raphe nuclei are a heterogeneous group of neurons located along the rostrocaudal axis of the brainstem (Hornung, 2003), with distinct morphologies, projections and neurochemical characteristics (Jacobs and Azmitia, 1992). The neurons of these nuclei are involved in multiple physiological functions such as the control of the sleep–wake cycle, hypothalamic neurohormones release, motivational behaviour, emotionality, responses to pain and energy balance (Berger et al., 2009; Jacobs and Azmitia, 1992; Jonnakuty and Gragnoli, 2008; Pytliak et al., 2011).

One of these raphe nuclei is the median raphe nucleus (MRN), which sends serotonergic and non-serotonergic (Vertes et al., 1999) projections to limbic structures, such as the hippocampus, and areas that influence food intake, such as hypothalamic nuclei (Descarries et al., 1982; Hopwood and Stamford, 2001; Vertes et al., 1999). This nucleus exerts control over anxiety- and depression-like behaviours, the reward circuitry, hippocampal plasticity, memory, and motor activity (Andrade et al., 2013; Inoue et al., 2014; Lopez Hill et al., 2013; Ohmura et al., 2010; Shim et al., 2014; Webb et al., 2012). Several studies have assessed the role of the MRN on feeding behaviour. Previous studies have shown that the injection of GABAergic agonists into the MRN causes an enormous increase in food and water intake (Fletcher, 1994; Klitenick and Wirtshafter, 1988; Wirtshafter et al., 1993). Furthermore, the injection of glutamatergic antagonists causes the same effect (Wirtshafter and

* Corresponding author. Tel.: +55 353 85 150 4160; fax: +55 48 3721 9672.
E-mail addresses: bulevone@gmail.com, brunno_rocha@hotmail.com (B.R. Levone), elisa_cella@hotmail.com (E.C. Cella), larissakochen@gmail.com (L. Kochenborger), edu_simao@yahoo.com.br (E.S. da Silva), anapaulataschetto@gmail.com (A.P.D. Taschetto), samirasmansur@hotmail.com (S.S. Mansur), mariana.terenzi@ufsc.br (M.G. Terenzi), m.serralvo@ufsc.br (M.S. Faria), marta@ccb.ufsc.br (M.A. Paschoalini).

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Krebs, 1990). Thus, treatments which reduce neural activity in the MRN lead to an increase in food intake (Wirtshafter et al., 2011), which leads to the conclusion that the MRN exerts an inhibitory influence on food intake. Similarly, neurons within the MRN influence locomotor activity (Wirtshafter and McWilliams, 1987). These cells seem to be part of a system that supresses locomotion which works simultaneously with lateral activating systems (Wirtshafter and McWilliams, 1987).

Noradrenaline (NA), its synthesizing enzymes and its transporters are found in the MRN (Adell et al., 2002; Saavedra et al., 1976). There is a high density of α1-and α2-adrenoceptors in this nucleus (Adell and Artigas, 1999), however, no β-adrenoceptors have ever been detected, although these receptors are seen in the pontine nuclei, adjacent to the MRN (Wanaka et al., 1989). The main noradrenergic innervation to these nuclei originates from the locus coeruleus/subcoeruleus, the lateral tegmental area and the A1/A2 cell groups, whereas the adrenergic innervation arises basically from C1/C2 medullary nuclei (Adell et al., 2002; Cryan et al., 2002; Hopwood and Stamford, 2001; Vertes et al., 1999). It is suggested that endogenous catecholamine exerts a stimulatory control on the release of serotonin (5-HT), through activation of α1-adrenoceptors, and an inhibitory influence through activation of α2-adrenoceptors (Adell and Artigas, 1999). The MRN also has a high density of the somatodendritic autoreceptor 5-HT_{1A}, that may regulate the release of 5-HT in its projections (Adell et al., 2002). The injection of 8-OH-DPAT, a 5-HT_{1A} receptor agonist, into the MRN may reduce the release of 5-HT in its projection areas (Currie et al., 1994; Hopwood and Stamford, 2001). Several others neurotransmitters can modulate the activity of neurons in the MRN, such as dopamine, glutamate, gamma-aminobutyric acid (GABA), glycine, opioids and others (Adell et al., 2002; Pineyro and Blier, 1999; Shim et al., 2014).

Studies in our laboratory have shown that the injection of adrenaline (ADR) into the MRN causes hypophagia in rats deprived of food (Maidel et al., 2007). On the other hand, the injection of ADR in satiated rats caused hyperphagia (dos Santos et al., 2009). The injection of phenylephrine, a selective α1-adrenoceptor agonist, in satiated rats, was not able to modify the ingestive behaviour, but clonidine, a selective α2-adrenoceptor agonist, caused a hyperphagia similar to that caused by ADR (Mansur et al., 2010). Conversely, in rats deprived of food, phenylephrine caused hypophagia and clonidine was not able to alter feeding behaviour (Ribas et al., 2012). Thus, it is suggested that, in rats deprived of food, ADR acts on α1-adrenoceptors, which allows the release of a signal that inhibits feeding, possibly 5-HT (Maidel et al., 2007). In satiated rats, it is suggested that ADR acts on α2-adrenoceptors (dos Santos et al., 2009), which decreases the availability of endogenous catecholamines (Adell and Artigas, 1999), inhibiting the release of the inhibitory signal and thus increasing food intake (dos Santos et al., 2009). The present study aims to evaluate changes in feeding behaviour after treatment of the MRN with selective or non-selective antagonists of α-adrenoceptors, followed or not followed by ADR.

Table 1
Ingestive and non-ingestive variables. Based on Halford et al. (1998).

Ingestive variables	Food: Eating food, jaw movements (chewing) Water: Drinking/licking water from the bottle-feeder
Quantity	Weight difference between the beginning and the end of the experiment
Duration	Total time spent on the behaviour
Frequency	Number of times the behaviour was initiated
Latency	Time until the onset of the behaviour
Non-ingestive variables	Frequency and duration of the following behaviours
Food exploration	Any exploration of the food, except eating
Rearing	Exploration of the test chamber with both front paws elevated from the floor; an increase may indicate hyperactivity
Grooming	Self-cleaning, licking or gentle biting the body or tail or stroking whiskers with the paws
Locomotion	Exploration of the test chamber, walking around using all four paws; an increase may indicate hyperactivity
Non-locomotor exploration	Horizontal movements of the front paws or the head (including sniffing), the hind paws stay in place, not moving
Immobility	Absence of any movement of the head or paws, resting state; an increase may indicate sedation

2. Materials and methods

2.1. Animals and cannula implantation

Male Wistar rats (from the animal breeding facility of the Federal University of Santa Catarina) were housed in groups of five in a temperature controlled (21 ± 2 °C) room. Food and water were provided *ad libitum* on a 12/12 h light/dark cycle (lights on at 7:00 am). All procedures were conducted in accordance with the recommendations of Brazilian College of Animal Experimentation and approved by the Animal Experimentation Ethics Commission of the Federal University of Santa Catarina.

After a 7-day period of adaptation to the testing room, animals weighing between 280 and 300 g were intraperitoneally anesthetised with a ketamine (87 mg kg^{-1}) and xylazine (13 mg kg^{-1}) mixture. Using stereotaxic techniques, a unilateral stainless steel guide cannula (30G) was implanted 2 mm above the MRN and fixed with a screw and dental acrylic. The guide cannula was positioned at a 20° of inclination, to avoid damage to the mesencephalic aqueduct and sagittal venous sinus. The coordinates, based on the Paxinos Atlas for the rat brain (Paxinos and Watson, 2007), were: antero-posterior: 7.8 mm, lateral: 3 mm, dorso-ventral: 7 mm.

2.2. Experimental procedures and drugs

After the surgery, the rats were housed in individual cages, with food and water *ad libitum*. After five days, the rats were habituated to the experimentation environment for two consecutive days (30 min each day). On the seventh day, injections were made using a needle 2 mm longer than the guide cannula, which was connected to a Hamilton micro syringe by a polyethylene tube. All drugs were injected in a volume of 0.2 μl, for a period of 60 s and another 60 s were allowed for the solution to diffuse through the brain tissue. Every rat received two injections and after the second, they were immediately placed in the testing chamber and the behaviours recorded for 30 min. All experimental procedures were carried out from 10:00 am to 2:00 pm (light cycle).

Drugs used included adrenaline (ADR, adrenergic agonist, 20 nmol, Sigma–Aldrich), prazosin (PRZ, α1-adrenoceptor antagonist, 20 and 40 nmol, Sigma–Aldrich), yohimbine (YBN, α2-adrenoceptor antagonist, 20 and 40 nmol, Sigma–Aldrich) and phentolamine (PNT, nonspecific α-adrenoceptor antagonist, 20 and 40 nmol, Sigma–Aldrich). ADR and PNT were dissolved in saline (SAL), and PRZ and YBN in propylene glycol (PLG). The dose of ADR chosen was the smallest effective dose able to promote hyperphagia in satiated rats, as stated in a previous study from our laboratory (dos Santos et al., 2009). Control groups were treated (7 min before) with the vehicle of the antagonist (PLG or SAL), followed by an injection of SAL or ADR. Experimental groups were treated with the antagonists PRZ, YBN or PNT (7 min before), followed by an injection of SAL or ADR.

2.3. Feeding and locomotor behaviours

Video-recorded data were analysed using the software Etholog 2.2.5 (Ottoni, 2000). A biobehavioural assay was performed to verify the drug action on appetite, using the protocol by Halford et al. (1998), listed in Table 1 (Halford et al., 1998). This protocol is still in use and provides an assessment of drug effects on a range of behaviours and is able to indicate whether the ingestive effects are merely secondary to sedation, ataxia or psychomotor stimulation (Rodgers et al., 2010).

2.4. Histological analysis

Immediately after the experiments, rats were deeply anaesthetised with carbon dioxide and then transcardially perfused with 0.9% saline, followed by 10% formalin. Brains were sectioned in a cryostat, in the coronal plane, 50 μm thick. The sections were stained with cresyl violet and the position of the injection was assessed on a light microscope. The Paxinos Atlas was used to evaluate the position of the injection sites.

2.5. Statistical analysis

Separate one-way ANOVA tests were performed to compare all antagonist groups with the corresponding control (PLG or SAL) and the group pre-treated with