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Blockade of median raphe nucleus α_1-adrenoceptor subtypes increases food intake in rats

Eduardo Simão da Silva *, Rafael Appel Flores, Elisa Carolina Cella, Brunno Rocha Levone, Ana Paula Taschetto, Larissa Kochenborger, Mariana Graciela Terenzi, Moacir Serralvo Faria, Marta Aparecida Paschoalini

Department of Physiological Sciences, Center of Biological Sciences — CCB, Federal University of Santa Catarina (UFSC), 88040-970, Florianópolis, SC, Brazil

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ABSTRACT

Previous studies have shown that the blockade of α_1-adrenoceptors in the median raphe nucleus (MnR) of free-feeding animals increases food intake. Since there is evidence for the presence of α_{1A}-, α_{1B}- and α_{1D}-adrenoceptors in the MnR of rats, this study investigated the involvement of MnR α_1-adrenoceptor subtypes in the control of feeding behavior, looking for possible differences on the role of each α_1-adrenoceptor in feeding. Male adult rats weighing 280–300 g with guide cannulae chronically implanted above the MnR were injected with antagonists of α_{1A}- (RS100329, 0, 2, 4 or 20 nmol), α_{1B}- (Rec 15/2615, 0, 2, 4 or 20 nmol) or α_{1D}-adrenoceptor (BMY 7378, 0, 2, 4 or 20 nmol). Subsequently, behavioral evaluation of ingestive and non-ingestive parameters was monitored for 1 h and the amount of food and water ingested was assessed for 4 h. The highest dose (20 nmol) of RS100329 and BMY 7378 increased food intake, feeding duration and frequency, and decreased the latency to start feeding. During the second hour 2 nmol dose of Rec 15/2615 increased food intake and all doses of BMY 7378 decreased water intake. No behavioral alterations were observed during the fourth hour. The results corroborate previous work from our lab in which we describe the involvement of α_1-adrenoceptors of MnR on food intake control. Moreover, we show evidence that α_{1A}- and α_{1D}-adrenoceptors mediate feeding responses to adrenaline injections and that the behavioral modifications are of considerable duration, persisting up to 2 h after injection of the antagonists.

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1. Introduction

The raphe nuclei are distributed rostro-caudally throughout the brainstem and present distinct neurochemical, morphological and projection characteristics (Wirtshafter, 2001; Adell et al., 2002; Hornung, 2003; Walther and Bader, 2003; Mokler et al., 2009; Takase and Nogueira, 2008). The median raphe nucleus (MnR) and the dorsal raphe nucleus send numerous serotonergic fibers to prosencephalic structures (Lucki, 1998; Hornung, 2003; Mokler et al., 2009). While the dorsal raphe nucleus innervates mainly the amygdala, ventral hippocampus and striatum (Azmitia and Segal, 1978), the MnR innervates preferentially the dorsal hippocampus, medial septum, nucleus accumbens, ventral tegmental area and several hypothalamic nuclei (Vertes et al., 1999; Lechin et al., 2006).

The MnR has a diversity of neurotransmitter systems involved in the control of food intake, it is rich in 5-HT_{1A} receptors (Sotelo et al., 1990; Kia et al., 1996; Cryan et al., 2002; Adell et al., 2002; Judge and Gartside, 2006) and the major part of these receptors is found in serotonergic neurons acting as autoreceptors (Hall et al., 1997; Adell et al., 2002). Injection of 8-OH-DPAT (a 5-HT_{1A} antagonist) into the MnR inhibits serotonergic neuronal firing, 5HT release at prosencephalic structures (Bonvento et al., 1992; Andrews et al., 1994; Avanzi and Brandão, 2001; Adell et al., 2002; Funk et al., 2005) and increases food intake (Currie et al., 1994). Along with 5HT receptors, GABA and glutamatergic receptors localized in the MnR also appear to participate in food intake regulation. Intra-MnR injection of kainic or quisqualic acid suppresses food intake in food-deprived rats, on the other hand antagonists of kainate/quisqualate receptors increase food intake in free-feeding rats (Wirtshafter and Krebs, 1990). Intra-MnR injection of Baclofen, a $GABA_B$ agonist, increases food and water intake and induces locomotor activity in non-deprived (free-feeding) rats (Wirtshafter et al., 1993).

Data from our lab have shown that adrenaline (AD) injected into the MnR increases food intake in free-feeding rats (Maidel et al., 2007), but reduces food intake in food-deprived rats (Dos Santos et al., 2009). These opposite effects of AD were attributed to differential activation of pre-synaptic and post-synaptic α-adrenoceptors that is dependent on the nutritional state of the animal. Later data showed that MnR injections of prazosin (Mansur et al., 2011), a α_1-adrenoceptor antagonist, and clonidine, a α_2-adrenoceptor agonist, increased food intake in

* Corresponding author. Tel.: +55 48 37219352; fax: +55 48 37219672.
E-mail address: edu_simao@yahoo.com.br (E.S. da Silva).

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free-feeding rats (Mansur et al., 2010), supporting the hypothesis of a differential activation by AD. On the other hand, MnR injections of phenylephrine, a α_1-adrenoceptor agonist, decreased food intake in food-deprived rats while clonidine had no effect (Ribas et al., 2012). Collectively, these results allowed us to conclude that in free-feeding animals, the activation of MnR α_1-adrenoceptor receptors stimulates an inhibitory pathway on food intake. The activity of this inhibitory pathway appears to be reduced in food-deprived conditions.

MnR neurons receive afferents from the locus coeruleus, lateral tegmental area, A1/A2 cell group and AD C1/C2 medullary nuclei (Hopwood and Stamford, 2001; Cryan et al., 2002; Adell et al., 2002; Lechin et al., 2006) and express mRNA of α_1-adrenoceptor subtypes α_{1A}, α_{1B} and α_{1D} (Day et al., 1997). There is evidence that a noradrenergic input to the MnR exerts tonic facilitatory control of 5-HT release through α_1-adrenoceptors (Adell and Artigas, 1999). Given the presence of α_1-adrenoceptor subtypes in the MnR, the aims of our work are to study the effect of various α_1-adrenoceptor antagonists injected into the MnR on feeding behavior and to determine the duration of such effects by prolonging the period of observation of food and water intake used in previous studies.

2. Materials and methods

2.1. Animals

Male Wistar rats (weighing 270–300 g at the time of surgery) were group-housed at 22–24 °C, 12:12 light–dark cycle (lights on at 6:00 AM) with standard rodent chow and water available *ad libitum*. The animals were housed in groups of five per cage until the day of the experiments. After surgery, rats were housed in individual cages. The experimental procedures were conducted in compliance with the recommendations of the Ethics Committee for the use of Experimental Animals (CEUA) of the Federal University of Santa Catarina, SC, Brazil (CEUA protocol: #PP0075-2010). All efforts were made to minimize the number of animals used and their suffering.

2.2. Stereotaxic surgery

The rats were anesthetized (ip) with a ketamine hydrochloride (87 mg kg^{-1}) and xylazine (13 mg kg^{-1}) mixture and stereotaxically implanted with a unilateral stainless steel guide cannula (30G, 18 mm length). The target for this cannula was 2 mm above the MnR based on the atlas of The Rat Brain (Paxinos and Watson, 2007). The following coordinates from bregma were used: AP = −7.8 mm; L = 3.0 mm; and DV = 7.0 mm from the surface of the skull, at an angle of 20° from the vertical plane to avoid the sagittal sinus and the cerebral aqueduct. The cannula was anchored to the skull with dental cement and the whole implant stabilized with jeweler screws and more dental cement. A removable stylet was introduced to keep the cannula free from blockages until the day of the experiment.

2.3. Drugs and injections

Injections were made using a needle (33G, 20 mm length) extending 2 mm beyond the ventral tip of the guide cannula and connected by polyethylene tubing (PE10) to a Hamilton microsyringe (1 μl) attached to an injection pump. The injected volumes (0.2 μl) were administered over a period of 60 s and a further 60 s was allowed for the solution to diffuse from the needle. RS100329 a α_{1A}-adrenoceptor antagonist, Rec15/2615 a α_{1B}-adrenoceptor antagonist, and BMY7378 a α_{1D}-adrenoceptor antagonist were given at doses of 2, 4 and 20 nmol respectively, purchased from TOCRIS Biosciences (Ellisville, MO, USA) and dissolved in 0.9% sterile saline with 5% DMSO. Each animal received only one injection: a dose of one drug or the corresponding vehicle.

2.4. Experimental procedures

Animals were acclimatized to the feeding recording chamber for 1 h for two consecutive days immediately before the experimental session. The experiment was designed to evaluate the effects of RS100329 (n = 10 for each dose), Rec 15/2615 (n = 10 for each dose) or BMY7378 (n = 9 for each dose) injection (0, 2, 4 or 20 nmol) into the MnR on ingestive and non-ingestive behaviors during the first hour. The ingestive behavior was also evaluated 2 and 4 h after drug injection.

2.4.1. Behavioral assessment

After they have been injected, the animals were placed for 60 min in a cage containing a known weight of standard rodent chow and a known volume of faucet water. At the end of the session, the measured difference between food and water at the beginning and at the end was taken as the amount of food or water consumed. The experiment was recorded with a webcam for subsequent behavioral analysis with the Etholog 2.25 (Ottoni, 2000). During the session the duration (time spent in a given behavior during experimental time), frequency (number of episodes of a given behavior) and latencies (time in seconds to initiate a given behavior) for eating, drinking, locomotion, sniffing, immobility, rearing and grooming were evaluated. See Ribas et al. (2012) for details about these procedures and description of behaviors. In addition, food and water intake recordings were also taken 2 and 4 h post-injection.

2.5. Histological analysis

At the end of the experiments, the animals were deeply anesthetized and transcardially perfused with saline (0.9%) and formalin (10%). The brains were removed, kept in formalin and sliced in the coronal plane (50 μm). Sections were mounted on gelatinized slides and stained with cresyl violet. The cannula placements were identified under a microscope by comparison of the sections with photographs and diagrams of The Rat Brain atlas (Paxinos and Watson, 2007). Only data from rats with cannula correctly placed in the MnR were included in the study (approximately 80% of the total of implanted animals).

2.6. Statistical analysis

Behavioral data were analyzed by one-way ANOVA followed by Duncan's post hoc. The amount of food and water consumed during the experiment was analyzed by repeated measures ANOVA. Results are expressed as mean ± standard error of the mean (SEM). When appropriate, the ANOVA was followed by Duncan's post hoc test for multiple comparisons. Only probability values of less than 5% were considered significant.

3. Results

3.1. α_{1A}-Adrenoceptor antagonist

All animals included in the statistical analysis had their injection site verified by histological assessment. The ANOVA of repeated measures revealed that a 20 nmol dose of RS100329 (Fig. 1) significantly increased food intake in the first hour ($F(6, 68) = 3.52$; $p = 0.03$). This treatment increased both duration ($F(3, 34) = 6.5429$; $p = 0.001$) and frequency of feeding ($F(3, 34) = 7.49$; $p = 0.0005$); in addition, a decreased latency ($F(3, 34) = 4.1198$; $p = 0.01$) to start eating was observed (Table 1). The 20 nmol dose of RS100329 did not modify drinking or non-ingestive behaviors. Both lower doses of RS100329 produced no effect on any behavior registered in this study (Table 2). Water intake was not significantly altered at any interval studied.

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