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Research report

Alpha$_1$ receptor antagonist in the median raphe nucleus evoked hyperphagia in free-feeding rats☆

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ABSTRACT

Serotonergic neurons in the median raphe nucleus (MnR) are stimulated by α_1-adrenergic agonists and inhibited by α_2-agonists. This study investigated the effect of the blockade of the MnR α_1-adrenergic receptors of free feeding rats as an attempt to elucidate the functional role of these receptors in the control of feeding behavior. In addition, an α_2-receptor antagonist was also administered in the MnR in order to strengthen the previous suggestion that α_2-adrenergic receptors participate in the control of feeding behavior, probably decreasing the facilitatory influence on MnR serotonergic neurons. The α_1-adrenergic antagonist prazosin (PRA, 40 nmol) or vehicle was injected into the MnR 15 min before treatment with phenylephrine (PHE, 0.2 nmol). The α_2-adrenergic antagonist yohimbine (YOH, 40 nmol) was administered 15 min before clonidine (CLO, 20 nmol) or vehicle in free-feeding rats. After the injections, the animals were placed in the feeding chamber for 30 min to evaluate the ingestive and non-ingestive behaviors. At the end of the experiment the quantity of food and water consumed were measured. While treatment with PRA in the MnR followed by PHE did not change the feeding behavior, PRA injection alone into the MnR caused hyperphagia accompanied by a reduction in the latency to start eating, an increase in feeding frequency and an increase in the feeding duration. Pretreatment with YOH in the MnR blocked the hyperphagic effect induced by CLO. The present data reinforce our previous suggestion that the MnR α_2-adrenergic receptors participate in the control of feeding behavior, probably decreasing the facilitatory influence on MnR serotonergic neurons of free-feeding animals. Furthermore, these results indicate that this influence is tonically mediated by α_1-adrenergic receptors upon MnR neurons, which inhibit food intake.

Introduction

The raphe nuclei are a collection of neurons with varied morphologies, projections and neurochemical characteristics. They flank the midline along the rostrocaudal extension of the brainstem and constitute the main source of serotonin (5-hydroxytryptamine, 5-HT) in the central nervous system (Adell, Celada, Teresa Abellán, & Artigas, 2002; Mokler, Dugal, Hoffman, & Morgane, 2009; Takase & Nogueira, 2008; Walther & Bader, 2003). It is estimated that 20% of the 5-HT innervation of the forebrain originates in the median raphe nucleus (MnR) (Descarries, Watkins, Garcia, & Beaudet, 1982), which is located from the caudal limit of the decussation of the superior cerebellar peduncle to the level of the trigeminal motor nucleus (Hensler, 2006). The MnR projections ascend within the medial forebrain bundle and reach structures of the forebrain (Bonvento, Scatton, Claustre, & Rouquier, 1992; Bortolozzi & Artigas, 2003) such as the hypothalamus (Hensler, 2006) implicated in the regulation of behavior such as food intake (Prat, Blackstone, Connolly, & Skelly, 2009; Wirtshafter, 2001).

The MnR is one of the brain areas with the highest density in 5-HT$_{1A}$ receptors (Adell et al., 2002; Cryan, Page, & Lucki, 2002; Judge, Young, & Gartside, 2006; Kelley, Baldo, Pratt, & Will, 2005; Kia et al., 1996; Sotelo, Cholley, Mestikawy, Goslan, & Hamom, 1990). The great majority of these receptors is found on serotonergic neurons and functions as autoreceptors that regulate the synthesis and release of 5-HT in their projection areas (Adell et al., 2002; Currie & Coscina, 1993). Injections of the 5-HT$_{1A}$ receptor agonist 8-hidroxy-2-(di-*n*-propylamino) tetralin (8-OH-DPAT) into the MnR inhibit 5-HT cell firing and reduce its release in both the cell body and terminals (Adell et al., 2002; Andrews, Hogg, Gonzalez, & File, 1994; Avanzi & Brandão, 2001; Bortolozzi & Artigas, 2003;

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Funk, Li, Fletcher, & Le, 2005; Shim, Javaid, & Wirtshafter, 1997). Injections of 8-OH-DPAT into the MnR have been reported to increase food intake (Currie, Fletcher, & Coscina, 1994).

In addition to the high density of 5-HT receptors, a great density of α_1 and α_2-adrenergic receptors is also found in the MnR (Adell & Artigas, 1999). It has been reported that endogenous noradrenaline in the MnR exerts facilitatory control of 5-HT release via α_1-adrenergic receptors and inhibitory control via the α_2 subtype (Adell & Artigas, 1999). MnR neurons receive noradrenergic innervation from the locus coeruleus/subcoeruleus, lateral tegmental area and A1 and A2 cell groups (Adell et al., 2002; Cryan et al., 2002; Hopwood & Stamford, 2001). The α_2-adrenergic agonist clonidine (CLO) injected into the MnR decreases the dialysate level of 5-HT to 30% of basal values into this nucleus, while α_2-adrenergic antagonists into the MnR enhance its release (Adell & Artigas, 1999). In addition, injections of α_1-adrenergic agonists into the MnR either decrease 5-HT release (Hopwood & Stamford, 2001) or do not change 5-HT basal levels (Adell & Artigas, 1999). It has also been shown that injections of α_1-adrenergic receptor antagonists into the MnR decrease extracellular levels of 5-HT in this nucleus as well as in forebrain structures such as the hippocampus and the striatum (Adell & Artigas, 1999; Bortolozzi & Artigas, 2003).

Previous studies have shown that the activation of α_2-adrenergic receptors by clonidine injection into the MnR of free-feeding rats evoked hyperphagia (Mansur, Terenzi, Marino-Neto, Faria, & Paschoalini, 2010), while the injection of α_1-adrenergic agonist phenylephrine into this nucleus did not change feeding behaviors (Mansur, Terenzi, Marino-Neto, Faria, & Paschoalini, 2011). In the present study, the MnR α_1-adrenergic receptors of free feeding rats were blocked as an attempt to determine the functional role of these receptors in the control of feeding behavior. In addition, the MnR α_2-receptors were also antagonized in order to verify the previous suggestion that α_2-adrenergic receptors participate in the control of feeding behavior, probably decreasing the facilitatory influence on MnR serotonergic neurons.

Methods

Animals

Male Wistar rats (weighing 260–290 g at the time of surgery) were housed in a room at 22–24 °C, 12:12 h light–dark cycle (lights on at 6:00 AM, provided by a 100-W bulb 2.75 m above the center of the room, approximately 100 lx at central floor level), with standard rodent chow and water available *ad libitum*. The animals were housed in groups of five per cage until the start of the experiments. After surgery, all rats were housed individually. The experimental procedures were conducted in compliance with the recommendations of the Ethics Committee for the use of Experimental Animals of the Federal University of Santa Catarina, SC, Brazil. All efforts were made to minimize the number of animals used and their suffering.

Stereotaxic surgery

The rats were anesthetized (ip) with a mixture of ketamine hydrochloride (87 mg kg^{-1}) and xylazine (13 mg kg^{-1}) and stereotaxically implanted with a unilateral stainless steel guide cannula (30 G, 18 mm length). The target 2 mm above the MnR was based on coordinates taken from the atlas of The Rat Brain (Paxino & Watson, 2007). The following coordinates from bregma were used: AP = −7.8 mm; L = 3.0 mm; DV = 7.0 mm from the skull surface, 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 jeweller screws and more dental cement. A removable stylet was introduced to keep the cannula free from blockages until the day of the experiment.

Drug injections

After 7 days, recovery period, injections were made by inserting a needle (33 G, 20 mm length) protruding 2 mm beyond the ventral tip of the guide cannula and connected by polyethylene tubing to a Hamilton microsyringe (1 μl) fitted to an injection pump. Prazosin (PRA), an α_1 adrenergic antagonist, or yohimbine (YOH), an α_2 adrenergic antagonist, at the dose of 40 nmol in 0.2 μl, were injected into the MnR 15 min before treatment with phenylephrine (PHE, 0.2 nmol) or clonidine (CLO, 20 nmol) into the MnR of free-feeding rats. The doses of the antagonists (Yada et al., 1997) and the agonists were chosen based on the results of previous studies (Mansur et al., 2010, 2011). The antagonists were dissolved in propylene glycol and the agonists were dissolved in 0.9% sterile saline (SAL). These solvents (saline and propylene glycol, SPG or SAL) were used as control solutions. Each rat received only one treatment.

Experiments

The α_1-receptor antagonist was used in order to remove the adrenergic facilitatory influence on serotonergic neurons found in the MnR that we assume could be elevated in free-feeding rats. The α_2-receptor antagonist was used to blocked the hyperphagic effect evoked by clonidine (α_2-receptor agonist) in order to reinforce that this effect is specifically mediated through α_2-receptors. During the experimental session, naïve free-feeding rats were treated in the MnR with SPG-SAL (n = 7), SPG-PHE (n = 7), PRAZ-SAL (n = 7), PRAZ-PHE (n = 6), YOH-SAL (n = 6), SPG-CLO (n = 7) or YOH-CLO (n = 6). Immediately after the second injection, the animals were placed in the feeding recording chamber to evaluate the ingestive and non-ingestive-behaviors.

Food intake behavior

Each animal was placed for 30 min in a feeding recording chamber containing a known weight of standard rodent chow and a previously measured volume of tap water. At the end of the session, the 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 by a webcam for subsequent behavior analysis with the Etholog 2.25 (Ottoni, 2000). During the session, the latencies to start feeding and drinking, durations and frequencies of feeding and drinking as well as the frequencies of risk assessment and the durations of locomotion, rearing, grooming and immobility were evaluated. See Lopes et al. (2007) for details about these procedures.

Histological analysis

At the end of the experiments, the animals were deeply anaesthetized and transcardially perfused with SAL (0.9%) and formalin (10%). The brains were removed, maintained in formalin and sliced (vibratome) in the coronal plane (100 μm). Sections were mounted on gelatinized slides and stained with cresyl violet. The cannulae placements were identified under a microscope by comparison of the sections with the photographs and diagrams of the atlas of The Rat Brain (Paxino & Watson, 2007). Only data from rats with cannulae correctly placed into the MnR were included in the study.

Statistical analysis

The previous data inspection revealed a normal distribution and a homogenous variance (Levene and Brown & Forsythe tests) for all