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Feeding behaviour after injection of α-adrenergic receptor agonists into the median raphe nucleus of food-deprived rats

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ABSTRACT

This study investigated the participation of median raphe nucleus (MnR) α1-adrenergic receptors in the control of feeding behaviour. The α1-adrenergic agonist phenylephrine (PHE) and α2-adrenergic agonist clonidine (CLON) (at equimolar doses of 0, 6 and 20 nmol) were injected into the MnR of: a) rats submitted to overnight fasting (18 h); or b) rats maintained with 15 g of lab chow/day for 7 days. Immediately after the drug injections, the animals were placed in the feeding chamber and feeding and non-ingestive behaviours such as grooming, rearing, resting, sniffing and locomotion were recorded for 30 min. The results showed that both doses of PHE injected into the MnR of overnight fasted animals decreased food intake accompanied by an increase in the latency to start feeding. A reduction in feeding duration was observed only after treatment of the MnR with the 20 nmol dose of PHE. Both locomotion duration and sniffing frequency increased after injection with the highest dose PHE into the MnR. Feeding frequency and the other non-ingestive behaviours remained unchanged after PHE treatment in the MnR. Both doses of PHE injected into the MnR of food-restricted rats decreased food intake. This hypophagic response was accompanied by a decrease in feeding duration only after treatment of the MnR with the highest dose of PHE. The latency to start feeding and feeding frequency were not affected by injection of either dose of PHE into the MnR. While both doses of PHE increased sniffing duration, the highest dose of PHE increased resting duration and resting frequency. Treatment with CLON into the MnR did not affect feeding behaviour in either of the food deprivation conditions. The present results indicate the inhibitory functional role of α1-adrenergic receptors within the MnR on feeding behaviour.

1. Introduction

The raphe nuclei are constituted of serotonergic neurons grouped into eight nuclei located in the midline of the brainstem. These nuclei are considered to be the main source of prosencephalic serotonin (5-hydroxytryptamine) (5-HT) [1–4]. The dorsal raphe nucleus and the median raphe nucleus (MnR) also contain distinct subpopulations of non-serotonergic neurons that occur in equal or greater numbers compared to the serotonergic neurons [5–9]. In the MnR, GABAergic neurons are located in both midline and lateral regions across the rostro-caudal extent of the MnR. 5-HT neurons are found just lateral and adjacent to the population of GABAergic neurons at the midline with little overlap or co-localisation between the two populations [10]. Non-serotonergic neurotransmitters are co-localised with serotonergic neurotransmitters [10–14], and include gamma-amino butyric acid (GABA), glutamate and corticotropin-releasing factor (CRF) [15–17]. Thus, both 5-HT and non-5-HT neurotransmitters may be co-released within the raphe nuclei as well as in projection areas [18,19]. It is estimated that 20% of the 5-HT innervations originating in the MnR predominantly reach the dorsal hippocampus, medial septum and hypothalamus [5,20].

A great density of 5-HT_{1A} receptors is found in the MnR [21–24] and they function as autoreceptors that regulate the synthesis and release of 5-HT in their projection areas [21,25]. Agonists of 5-$HT1_A$ receptors, such as 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT), stimulate food intake when injected into the MnR [26–29]. Besides the high density of 5-HT receptors in the MnR, a great density of α1- and α2-adrenergic receptors in this nucleus has been reported [30–32]. MnR neurons receive noradrenergic innervation from the locus coeruleus/subcoeruleus, lateral tegmental area, and projections from the adrenaline (AD) (C1 + C2) medullary nuclei and A1/A2 cell groups [33–37]. The noradrenergic inputs to MnR exert tonic facilitatory control of 5-HT release through α1-adrenergic receptors and inhibitory control by α2-adrenergic receptors [21,30].

Previous studies in our laboratory revealed AD but not noradrenaline (NA) injected into the MnR decreased food intake and shortened

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meal duration in food-restricted rats [38]. On the other hand, injection of AD into the MnR of free-feeding rats increased food intake, feeding frequency and decreased the latency to start feeding [39]. Since the inhibitory action of 5-HT on feeding behaviour has been extensively reported [25,40–43] and due to the serotonergic innervation of the hypothalamic paraventricular nucleus, an important region where 5-HT has been implicated in feeding regulation arises from MnR serotonergic projections [41,44,45]. We have suggested that in food-restricted rats, AD-induced hypophagia may be due to the activation of α1-adrenergic receptors on 5-HT MnR neurons resulting in 5-HT release and an indirect inhibitory action of AD on feeding [38]. In free-feeding rats, the food intake effects evoked by AD injections may be attributed to the activation of α2-adrenergic receptors on 5-HT MnR neurons that could act to suppress the release of 5-HT and its inhibitory action on feeding behaviour [39].

This suggestion was reinforced by data showing that treatment with clonidine (CLON), an α2-adrenergic agonist, into the MnR of free-feeding rats resulted in a hyperphagic response with an intensity, duration as well as frequency similar to that induced by AD [46]. In this case, an inhibitory influence activated by adrenergic inputs was removed due to α2-adrenergic stimulation, which in turn decreased the concentration of catecholamines in the synapse. The feeding behaviour effects induced by CLON in the MnR were specifically mediated by α2-adrenergic activation since previous injection of an α2-adrenergic antagonist into the MnR blocked the hyperphagic response evoked by this drug [47]. In contrast, phenylephrine (PHE) treatment in the MnR failed to modify feeding behaviour in rats with free access to food. This lack of change in the feeding response after MnR α1-adrenergic receptor activation was attributed to the presence of elevated adrenergic inputs on MnR neurons which restrain food intake under free-feeding conditions [48]. This suggestion was reinforced by data showing that α1-adrenergic receptor blockade within the MnR increased food intake in free-feeding rats [47].

In order to strengthen the inhibitory functional role that α1-adrenergic receptors within the MnR exert on food intake, the present study was designed to evaluate the feeding behaviour effects evoked by injection of α1-adrenergic receptor agonists into the MnR of food-deprived animals since it has been reported that the inhibitory serotonergic tonus is lower in animals submitted to a food restriction regimen than in free-feeding animals [49–51]. In addition, the feeding effects caused by MnR α2-adrenergic receptor activation were also investigated.

2. Materials and methods

2.1. Animals and surgery

All the experimental procedures described below were conducted in strict adherence to the recommendations found in the "Principles of animal care" and were approved by the local Committee for Ethics in Animal Research (CEUA-UFSC, protocol # PP00367). Male adult Wistar rats (n = 108) weighing 270–310 g at the time of surgery were used in this study and were group-housed in a room at 21 ± 2 °C under a 12 h lighting cycle (lights on at 07:00 h) with free access to food and water, except when food restriction was applied. The rats were anesthetised with ketamine hydrochloride (100 mg/kg, i.p.) and xylazine (20 mg/kg, i.p.) and were placed in a stereotaxic apparatus (Insight Instruments, Ribeirão Preto, SP, BRA) for unilateral stainless steel guide cannula (30 G) implantation according to the coordinates (AP ±7.8, L ±3.0 and DV ±9.0) described by Paxinos and Watson [52]. The guide-cannula was inserted at a lateral angle of 20° to avoid the sagittal sinus, and cerebral aqueduct obstruction and was aimed at 2 mm dorsal to the MnR. The cannula was anchored to the skull with jeweller screws and fixed with dental cement; the cannula was maintained patent between experiments by an inner removable stylet.

2.2. Drugs and injections

Phenylephrine hydrochloride (6 and 20 nmol, purchased from Sigma Chemical Co., St. Louis, MO, USA) and clonidine hydrochloride (6 and 20 nmol, purchased from Tocris Bioscience, Ellisville, MO, USA) were freshly dissolved in 0.9% saline, which was used as the vehicle in control experiments. The drug injections were made through an inner cannula (33 G) that extended 2 mm beyond the tip of the guide cannula connected by polyethylene tubing (PE10) to a Hamilton microsyringe (1 μl) fitted 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 cannula. The drug doses used in the experiments were derived from our previous studies [38,39,46,47].

2.3. Experimental procedures

For 1 week after surgery, the rats were housed individually with free access to food and water. After this period, the animals were separated into two experimental groups. 1) Food-restricted rats: in this group, the animals were submitted to a food restriction regimen whereby the daily food allotment was limited to 15 g of laboratory chow, delivered at 17:00 h for 7 days. The rats continued to have free accesses to water. After 4 days of food restriction, the body weight declined by approximately 10% and remained stable until the day of the experiment. The experimental session began on the eighth day of food restriction. The rats were habituated, on two occasions before the experiments, to the handling and injection procedures. During the habituation, the rats received mock injections to allow acclimation to the microinfusion procedures. No solutions were delivered on mock injection days by an inner cannula that exhibited the same guide cannula length. The rat chow not consumed at the end of the habituation sessions was offered to the animals at 17:00 h, in order to complete 15 g of chow pellets/day. 2) Overnight fast (18 h): in this group the animals were deprived of food overnight and the morning prior to all experiments (18 h), but continued to have free accesses to water. Each animal received only one drug treatment. All procedures were carried out between 13:00 h and 16:30 h. Immediately after the drug injection into the MnR, the rats were placed in the recording chamber constructed with transparent glass (49 × 34 × 32 cm), containing food and tap water (in a bottle placed outside the test cage with a spout projected through the wall of the cage). The session was recorded by a webcam perpendicularly located 60 cm above the cage floor for subsequent detailed behavioural analysis through Etholog 2.2 [53]. The back and lateral walls, as well as the floor cage, were coated with a black adhesive plastic paper. In order to facilitate behavioural recording, the front wall of the test cage had a mirror with the same dimensions arranged at a 45° angle in relation to the vertical plane. This mirror arrangement also prevented the animal from seeing its reflection in the mirror. At the end of the recording period, any food that occasionally spilled on the cage floor was recovered and weighed with the food that remained in the feeder. The difference between food or water weight at the beginning and at the end of the recording period was taken as the amount of food or water consumed. During the 30 min experimental session, the ingestive behaviours such as the latency to start feeding and drinking, the feeding and drinking duration, as well as the feeding and drinking frequency were evaluated. The duration and frequency of five non-ingestive behaviours (grooming, locomotion, rearing, resting and sniffing) were also assessed. The behaviour duration assessed in this study represents the sum of each episode occurred during the experimental session. All behaviour categories were defined in previous studies [54] and are described in Table 1.

An additional experimental group (experiment 3) was carried out in order to confirm whether the PHE effects on feeding behaviour were site-specific. In this group, the PHE dose that evoked feeding behaviour changes in experiments 1 and 2 was injected into areas in the vicinity of

Table 1
The behavioural categories used for behavioural analysis.
Based on Halford and Blundell [54].

Category	Description
Eating (food intake)	Biting, gnawing or swallowing food from wet mash dish directly or from front paws.
Drinking (water intake)	Licking the spout water bottles.
Grooming	Licking of the body, feet, and genitals. Scratching of coat or head with hind leg. Stroking whiskers with paws. Biting of the tail.
Locomotion	Walking around the cage or circling. Movements' involving all four paws.
Rearing	Front paws raised from the tank floor and either placed on the side of the tank or placed in front of the body.
Sniffing	Rapid wrinkling of nose (twitching of vibrissae) directed at some aspect of the environment. Head movement. Rear limbs immobile.
Resting	Animal inactive. Relaxed position with head curled to body or resting on the bottom of the cage, stretched out either on side or belly.

the MnR such as the pontine nuclei (Pn), pontine nuclei oral part (PnO), paramedia raphe nucleus (PMnR) reticulotegmental nucleus pons (RtTg), trigeminothalamic tract (tth), medial leminuscus (ml), and the feeding behaviour of both food-restricted and overnight fasted rats was evaluated.

2.4. Histological analysis

At the end of the experiments, the animals were deeply anaesthetised and transcardially perfused with 0.9% saline, followed by 10% formalin. The brains were removed and subsequently cut (on a vibratome) in the transverse plane (100 μm thick sections). Sections were stained with cresyl violet and the cannula loci were examined and documented through a camera lucida attached to a light microscope. Cannula placements were mapped onto the corresponding atlas drawings of Paxinos and Watson [52]. Data from the rats with misplaced cannulae in the MnR were not included in the analyses.

2.5. Statistical analysis

Separate one-way ANOVA tests were carried out to analyse the food-restricted data, for the different doses of PHE or CLON (0, 6 and 20 nmol). The same statistical procedures were employed for data analyses of the overnight fast group. The effects of treatment with saline (0) or PHE (20 nmol) in the MnR and non-median raphe nucleus (Pn, PnO, PMnR, RtTg, tth and ml) were analysed by two-way ANOVA (drug-dose × injected area). All these tests were followed, when appropriate, by Duncan's post-hoc test, and a $p<0.05$ was accepted as being statistically significant in these procedures. All statistical procedures were performed using the Statistica 9 software for Windows (StatSoft, Tulsa, OK, USA).

3. Results

Fig. 1 illustrates the injection sites within the MnR in coronal sections of the brain. Histological analysis indicated that 77 points of injection were within the MnR distributed between AP −7.44 mm and AP −8.40 mm. The misplaced injection sites included points in the Pn ($n=11$), Pno ($n=10$), RtTg ($n=4$), PMnR ($n=2$), tth ($n=2$) and ml ($n=2$).

3.1. Experiment 1: food-restricted rats (15 g chow/day for 7 days) — drug dose effects on ingestive and non-ingestive behaviours

The one-way ANOVA test revealed that food intake was affected by the drug PHE [$F(2,18)=10.36$; $p<0.001$]. Duncan's test revealed that both doses of PHE (6 and 20 nmol) injected into the MnR of food-restricted rats decreased food intake when compared with the control group. This hypophagic effect was accompanied by a decrease in feeding duration [$F(2,18)=3.65$; $p<0.046$] only after treatment of the MnR with the highest dose of PHE (Fig. 2). The latency to start feeding and the feeding frequency were not affected by injections of either dose of PHE into the MnR (Fig. 2). Drinking behaviour was also not modified by PHE treatment (Table 2). Except for resting and sniffing behaviours, the other non-ingestive behaviours did not change after PHE administration into the MnR. The highest dose of PHE (20 nmol) increased resting duration [$F(2,18)=5.27$; $p<0.015$] and resting frequency [$F(2,18)=3.69$; $p<0.045$] (Table 3). Both PHE doses increased sniffing duration [$F(2,18)=4.76$; $p<0.021$] (Table 3). Feeding (Fig. 3), drinking (Table 2) and non-ingestive behaviours remained unchanged after administration of both doses of CLON into the MnR (Table 3).

3.2. Experiment 2: overnight fast group — drug dose effects on ingestive and non-ingestive behaviours

The one-way ANOVA test demonstrated that both food intake [$F(2,16)=17.56$; $p<0.00009$] and the latency to start feeding [$F(2,16)=3.81$; $p<0.044$] were significantly affected by PHE treatment in the MnR. Feeding duration was marginally affected by PHE [$F(2,16)=3.30$; $p<0.062$]. Food intake decreased after injection of both doses of PHE into the MnR of overnight fasted rats (Fig. 4). This hypophagic response was evoked by both doses of PHE and was accompanied by an increase in the latency to start feeding (Fig. 4). A reduction in feeding duration was observed only after treatment of the MnR with the 20 nmol dose of PHE (Fig. 4). Drinking behaviour was not modified by PHE treatment (Table 2). Non-ingestive behaviours remained unchanged after the administration of both doses of PHE into the MnR (Table 4), except for locomotion duration, which was marginally affected by the drug [$F(2,16)=3.19$; $p<0.067$], and sniffing frequency which was significantly affected by PHE treatment [$F(2,16)=6.83$; $p<0.007$]. Both locomotion duration and sniffing frequency increased after injection of the highest dose PHE into the MnR (Table 4). Treatment of the MnR with both doses of CLON did not change feeding (Fig. 5), drinking (Table 2) and non-ingestive behaviours of overnight fasted rats (Table 4).

3.3. Experiment 3: PHE injections into the vicinity of the MnR

The highest dose of PHE (20 nmol) injected into the MnR of both food-restricted rats and overnight fasted rats, which had evoked a decrease in food intake, was also administered to other regions in the vicinity of the MnR (Pn, PnO, PMnR, RtTg, tth, ml). These injections sites were grouped as the non-median raphe region and food intake data after PHE treatment were used to confirm the specificity of the MnR as the site of PHE injections that evoke a hypophagic response. The two-way ANOVA test revealed that the food intake exhibited by food-restricted rats was affected by the drug [$F(1,26=10.40$; $p=0.003)$] and there was also a significant interaction between the drug and the injection site [$F(1,26=11.89$; $p=0.001)$]; in addition, the food intake response exhibited by overnight fasted rats was significantly affected by the drug [$F(1,26=7.24$; $p=0.012)$] and there was also a significant interaction between the drug and the injection site [$F(1,26=14.20$; $p=0.0008)$]. The food intake decrease evoked by the highest dose of PHE was circumscribed to the MnR in both food-restricted and overnight fasted rats (Fig. 6).

4. Discussion

The results of the present study revealed that while the injection of PHE into the MnR decreased food intake in both food-deprived conditions, treatment with CLON in the MnR did not affect feeding

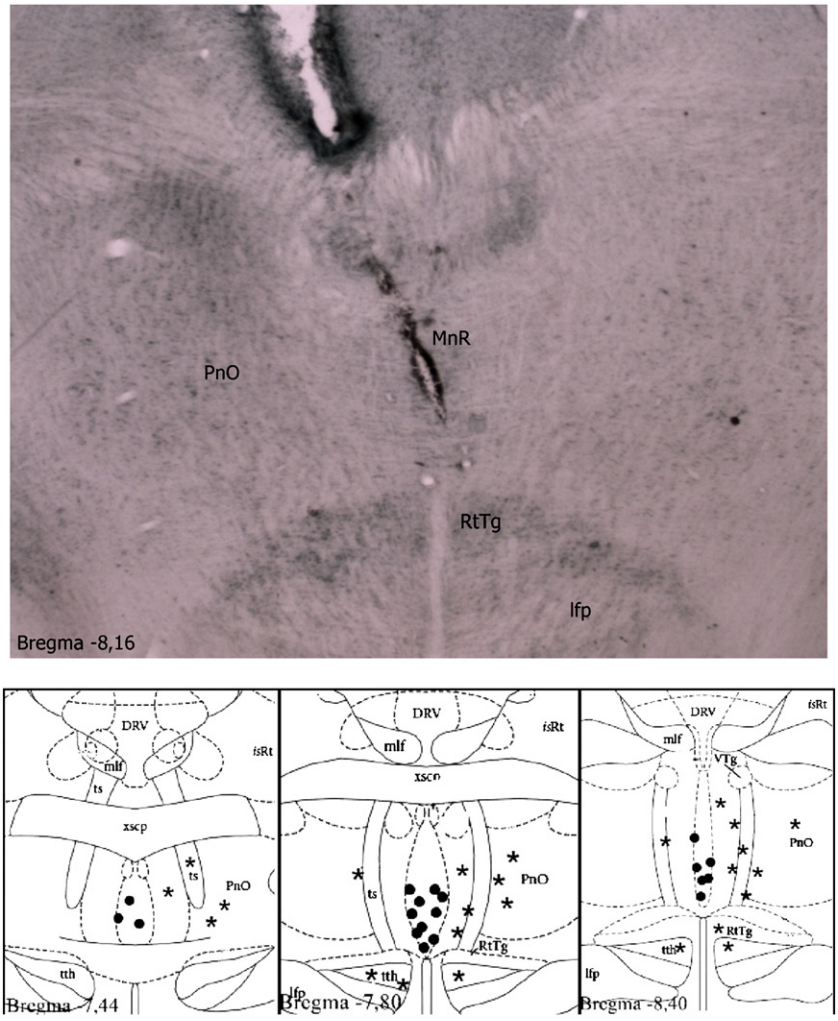

Fig. 1. Photomicrographs and schematic drawings of coronal sections through the median raphe nucleus (MnR) of the rats, illustrating the approximate sites (●) of phenylephrine (PHE)/clonidine (CLON) injections (at doses of 0, 6 and 20 nmol), and (★) phenylephrine (PHE) injections into the vicinity of the MnR. Arrows point to injection sites. Numbers at the top refer to anteroposterior stereotaxic coordinates from the rat brain atlas (Adapted from Paxinos and Watson, 2007). Scale bar = 100 μm. DRV = dorsal raphe nu, ventral part; isRt = isthmic reticular formation; lfp = longitudinal fasciculus pons; mlf = medial longitudinal fasciculus; MnR = median raphe nucleus; PnO = Pontine reticular nu, oral part; RtTg = reticulotegmental nu pons; ts = tectospinal tract; tth = trigeminothalamic tract; VTg = ventral tegmental nu; xscp = decussation sup cerebellar peduncle.

behaviour. This hypophagic effect evoked by PHE is specifically mediated by α1-adrenergic receptors within MnR, since treatment with the highest dose of PHE, which decreased food intake when administered into the MnR, did not change the feeding response when injected into the vicinity of the MnR. Opposite feeding responses were induced by the injection of α-adrenergic receptor agonists into the MnR of free-feeding rats. CLON injection into the MnR evoked hyperphagia while the PHE treatment did not change feeding behaviour [46,48].

Based on these results, we suggest that the hyperphagic effect evoked by CLON in free-feeding rats could be attributed to the inhibition of NA release from presynaptic stores, thus removing the α1-adrenergic stimulatory tone on serotonergic neurons within the MnR, since α2-adrenergic receptors seem to exert a tonic inhibitory influence on 5-HT release in the MnR [30]. The absence of feeding effects after treatment with PHE in the MnR of free-feeding rats could be due to elevated adrenergic inputs on serotonergic neurons [39,48]. It seems that the reduction of an adrenergic inhibitory influence in food-deprived rats allows for α1-adrenergic receptor activation by PHE, resulting in 5-HT release, since facilitatory control of 5-HT release is attributed to α1-adrenoceptors [21,30] situated on MnR serotonergic cell bodies [55]. In agreement with this suggestion, we have recently reported [47] that the blockade of α1-adrenergic receptors within the MnR of free-feeding rats resulted in hyperphagia accompanied by a reduction in the latency to start eating, an increase in feeding duration and an increase in feeding frequency. These data suggest the presence of tonic inhibitory inputs mediated by α1-adrenergic receptors on MnR neurons that restrain food intake under free-feeding conditions. The 5-HT released by MnR α1-adrenergic receptor activation could inhibit food intake through projections that terminate in the prosencephalic areas involved in the control of food intake such as the hypothalamus or amygdala [56–59].

It is estimated that 60% of MnR neurons are non-serotonergic [60]. It is possible that GABAergic or glutamatergic neurotransmission in the MnR [10,13,61] could be an alternative mechanisms involved in the hypophagic response evoked by PHE in food-deprived rats, since intra-MnR injection of muscimol [1,62,63] and baclofen [1] or the glutamatergic agonist (NMOLDA) [64] and glutamatergic antagonists (kynurenic acid and 2-amino-5-phosphonovaleric acid) increased food intake in free-feeding rats [65]. Raphe/neurokinin pathways could also mediate the feeding effects induced by PHE treatment in the MnR since the activation of neurokinin-3 (NK-3) receptors located on MnR serotonin cell bodies has been shown to decrease food intake [63].

Except for the latency to start feeding, the hypophagic response evoked by PHE injections into the MnR of overnight fasted rats

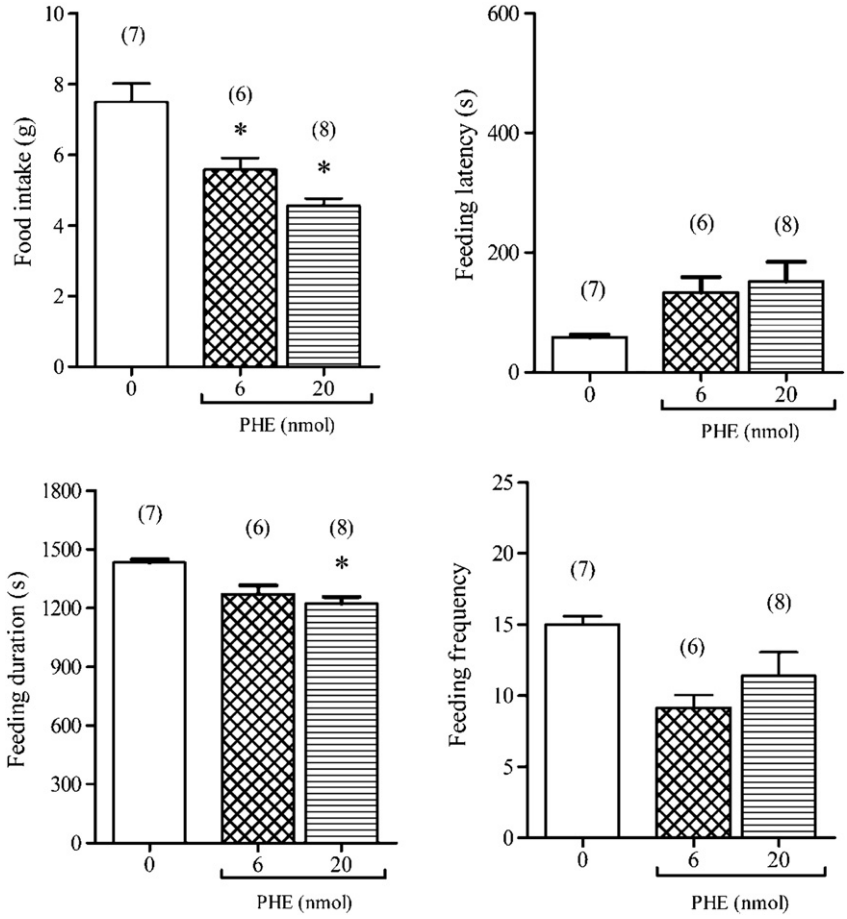

Fig. 2. Changes on food intake, feeding latency, feeding duration and feeding frequency after injection of vehicle (0) or phenylephrine (PHE, 6 nmol and 20 nmol) into the median raphe nucleus (MnR) of food restricted rats (15 g chow/day during 7 days). Values are mean ± S.E.M. *$p<0.05$ as compared to vehicle treatment (one-way ANOVA followed by Duncan's test for multiple comparisons). Numbers in parentheses indicate the number of animals per group.

was similar to that evoked by the same treatment in the MnR of food-restricted rats. The feeding duration decreased after the injection of the highest PHE dose into the MnR and feeding frequency was not affected by PHE treatment. In contrast, the latency to start feeding remained unchanged after treatment with PHE in the MnR of food-restricted rats, while both doses of PHE delayed the initiation of feeding in the overnight fasted rats. Feeding duration and the latency to start eating have been associated with different aspects of the control of feeding [66]. Changes in the latency to begin feeding may be associated with changes in satiety mechanisms or in the processing of those signals that inhibit eating between feeding. In this sense, the influence of MnR α1-adrenergic receptors on satiety mechanisms was presented only in the overnight fasted but not in the food-restricted rats. The reason for these discrepancies could be attributed to metabolic/hormone changes or patterns of orexigenic/anorexigenic neuropeptide gene expression associated with adaptations to a food restriction regimen [67–70]. On the other hand, changes in the feeding duration seemed to relate to satiation processes, or the mechanisms set in motion by nutrient-related signals that cause the end of a feeding bout. In this sense, we could

Table 2
Drinking behaviour after injection of phenylephrine (PHE) and clonidine (CLON) into the median raphe nucleus (MnR) of food restricted (15 g chow/day during 7 days) and overnight fast rats during 30 min. The number of animals used in each drug dose is illustrated in Figs. 2–4.

Behaviour	Food restricted rats			Overnigh fast		
		PHE	CLON		PHE	CLON
Water intake	0	0.0 ± 0.0	0.25 ± 0.1	0	0.1 ± 0.1	0.08 ± 0.08
	6	0.08 ± 0.08	0.50 ± 0.2	6	0.0 ± 0.0	0.3 ± 0.3
	20	0.12 ± 0.81	0.3 ± 0.2	20	0.1 ± 0.1	0.2 ± 0.2
Latency (s)	0	1800.0 ± 116.1	1577.7 ± 138.4	0	1681.3 ± 106.4	1627.4 ± 263.7
	6	1745.6 ± 125.5	1435.9 ± 138.4	6	1800.0 ± 114.9	1405.0 ± 244.2
	20	1547.0 ± 108.7	1639.4 ± 138.4	20	1632.0 ± 114.9	1296.8 ± 263.7
Duration (s)	0	0.0 ± 0.0	4.0 ± 2.7	0	2.2 ± 1.5	0.3 ± 0.3
	6	0.79 ± 0.79	5.2 ± 3.9	6	0.0 ± 0.0	1.4 ± 0.8
	20	10.6 ± 10.1	15.8 ± 14.7	20	3.0 ± 1.9	0.9 ± 0.6
Frequency (turn)	0	0.0 ± 0.0	0.8 ± 0.5	0	0.4 ± 0.3	0.2 ± 0.2
	6	0.3 ± 0.3	1.2 ± 0.8	6	0.0 ± 0.0	0.4 ± 0.2
	20	1.2 ± 1.1	1.2 ± 0.8	20	0.6 ± 0.6	0.3 ± 0.2

Values are mean ± S.E.M.

Table 3
Duration of non-ingestive behaviours 30 min after injection of vehicle (0) phenylephrine (PHE) or clonidine (CLON) into the median raphe nucleus of food restricted rats (15 g chow/day during 7 days). The number of animals used in each drug dose is illustrated in Figs. 2 and 3.

Behaviour	Phenylephrine (PHE)			Clonidine (CLON)		
	Dose (nmol/0.2 μl)	Duration (s)	Frequency (turn number/30 min)	Dose (nmol/0.2 μl)	Duration (s)	Frequency (turn number/30 min)
Locomotion	0	124.8 ± 19.4	33.4 ± 4.1	0	231.2 ± 48.7	60.5 ± 8.7
	6	158.2 ± 30.2	42.3 ± 9.3	6	180.7 ± 42.5	48.5 ± 12.5
	20	125.4 ± 29.9	30.5 ± 8.3	20	170.6 ± 19.1	42.8 ± 5.4
Resting	0	8.1 ± 4.3	2.7 ± 1.6	0	10.4 ± 5.5	2.8 ± 1.3
	6	10.0 ± 6.4	1.8 ± 0.7	6	22.8 ± 15.1	3.5 ± 1.6
	20	115.6 ± 40.9*	8.5 ± 2.4*	20	61.9 ± 36.7	3.3 ± 2.6
Rearing	0	57.8 ± 11.7	18.8 ± 2.7	0	86.8 ± 15.8	36.6 ± 7.9
	6	101.8 ± 29.7	26.6 ± 7.8	6	105.6 ± 24.7	29.7 ± 5.9
	20	39.5 ± 11.8	15.5 ± 4.8	20	84.5 ± 15.9	25.2 ± 4.0
Sniffing	0	70.6 ± 13.6	20.2 ± 3.8	0	99.9 ± 13.5	32.0 ± 3.8
	6	151.2 ± 30.0*	29.6 ± 4.1	6	155.9 ± 43.6	35.5 ± 9.0
	20	159.8 ± 23.1*	25.5 ± 4.7	20	132.5 ± 25.5	29.5 ± 3.6
Grooming	0	16.4 ± 4.2	2.2 ± 0.4	0	35.7 ± 10.3	4.5 ± 1.4
	6	31.9 ± 4.5	4.0 ± 0.4	6	61.0 ± 13.9	4.5 ± 1.1
	20	50.0 ± 15.9	4.7 ± 1.6	20	74.0 ± 24.0	5.2 ± 1.6

Values are mean ± S.E.M. *$p<0.05$ as compared to the vehicle treatment (one-way ANOVA followed by Duncan's post-hoc test).

suggest that α1-adrenergic receptors in the MnR can be associated with neural circuits involved in satiation induced by signals arising from the digestive tract [71–74], characterised in this study by a reduction in the meal size and shortening feeding duration in both food-restricted and overnight fasted rats.

The hypophagia evoked by PHE injection into the MnR of 7-day food-restricted rats could be attributed to an increase in the duration of sniffing behaviour, an indication of ambient exploration. This increase in exploratory behaviour induced by both doses of PHE could have interfered with the feeding response such that the animal stopped eating not because he was satiated, but because the drug could have stimulated a different behaviour such as exploration. However, the noticeable increase in resting (or sleep-like) behaviour after PHE injection into the MnR may be interpreted as an integral part

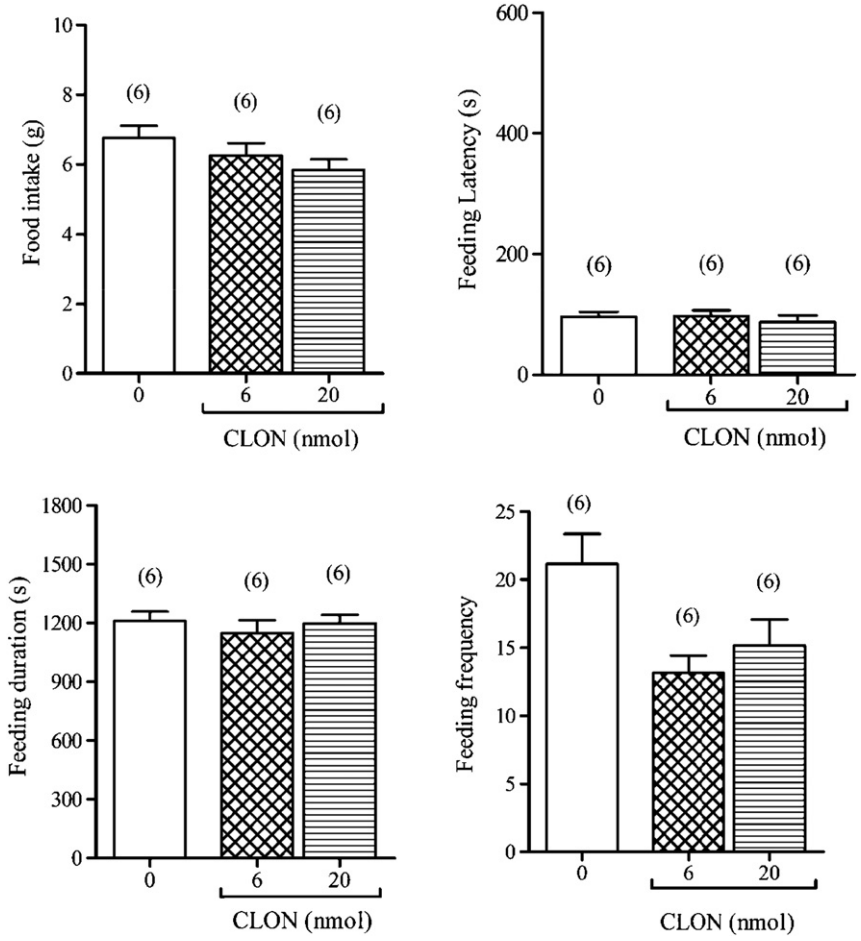

Fig. 3. Changes on food intake, feeding latency, feeding duration and feeding frequency after injection of vehicle (0) or clonidine (CLON, 6 nmol and 20 nmol) into the median raphe nucleus (MnR) of food restricted rats (15 g chow/day during 7 days). Values are mean ± S.E.M. Numbers in parentheses indicate the number of animals per group.

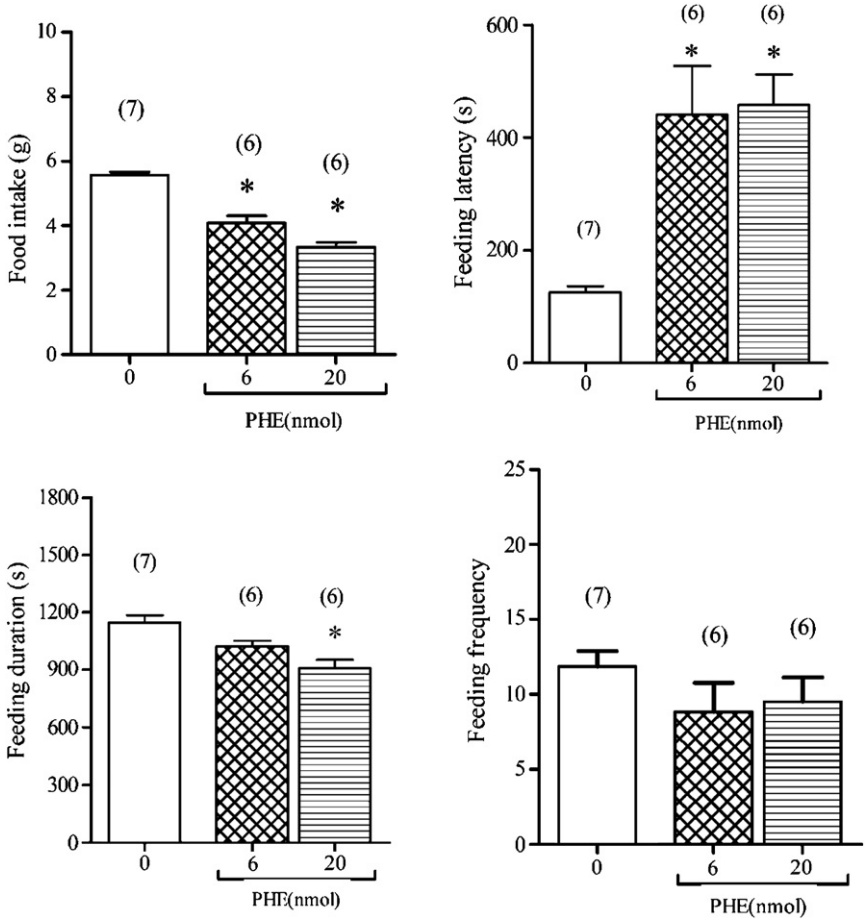

Fig. 4. Changes on food intake, feeding latency, feeding duration and feeding frequency after injection of vehicle (0) or phenylephrine (PHE, 6 nmol and 20 nmol) into the median raphe nucleus (MnR) of overnight fast. Values are mean ± S.E.M. *$p<0.05$ as compared to vehicle treatment (one-way ANOVA followed by Duncan's test for multiple comparisons). Numbers in parentheses indicate the number of animals per group.

of the normal behavioural satiety sequence (feeding–maintenance–resting) [54,75,76]. Furthermore, the highest dose of PHE injected into the MnR of overnight fasted rats evoked an increase in both the sniffing frequency and duration of locomotion, but the lowest PHE dose evoked a hypophagic response with no changes in non-ingestive behaviours. Taken together, these data suggest that α1-adrenergic receptors within the MnR seem to specifically participate in the control of food intake.

We have previously shown that injection of AD into the MnR of free-feeding rats decreased the latency to start drinking [39]. These data suggest the involvement of MnR adrenergic receptors in circuits

Table 4
Duration of non-ingestive behaviours 30 min after injection of vehicle (0) phenylephrine (PHE) or clonidine (CLON) into the median raphe nucleus of overnight fast . The number of animals used in each drug dose is illustrated in Figs. 4 and 5.

Behaviour	Phenylephrine (PHE)			Clonidine (CLON)		
	Dose (nmol/0.2 μl)	Duration (s)	Frequency (turn number/30 min)	Dose (nmol/0.2 μl)	Duration (s)	Frequency (turn number/30 min)
Locomotion	0	181.8 ± 14.6	50.8 ± 5.5	0	145.1 ± 15.6	41.6 ± 4.2
	6	230.1 ± 12.3	52.6 ± 4.4	6	181.5 ± 14.7	51.4 ± 3.2
	20	252.7 ± 31.4*	65.0 ± 9.2	20	183.3 ± 26.7	46.2 ± 6.5
Resting	0	6.3 ± 3.1	2.0 ± 1.1	0	26.1 ± 11.5	7.3 ± 2.7
	6	69.0 ± 45.0	6.5 ± 3.0	6	12.9 ± 3.7	4.7 ± 1.4
	20	55.9 ± 28.8	8.3 ± 2.0	20	12.7 ± 4.3	4.3 ± 1.2
Rearing	0	124.9 ± 19.5	35.8 ± 3.7	0	77.2 ± 8.6	28.2 ± 2.3
	6	126.3 ± 40.0	32.3 ± 5.6	6	95.6 ± 14.1	33.0 ± 3.6
	20	93.4 ± 11.8	32.3 ± 3.6	20	91.6 ± 13.9	28.2 ± 4.3
Sniffing	0	197.1 ± 48.4	36.0 ± 4.0	0	104.3 ± 11.5	31.0 ± 3.4
	6	169.5 ± 18.1	37.1 ± 5.0	6	131.8 ± 15.2	34.9 ± 4.1
	20	305.1 ± 34.0	60.3 ± 6.5*	20	142.3 ± 38.2	33.2 ± 7.5
Grooming	0	62.1 ± 13.9	6.6 ± 1.4	0	43.1 ± 15.1	4.0 ± 1.5
	6	75.6 ± 11.3	4.5 ± 0.5	6	87.2 ± 18.2	4.7 ± 0.5
	20	112.8 ± 23.0	9.3 ± 1.7	20	54.3 ± 12.0	3.3 ± 0.5

Values are mean ± S.E.M.
* $p<0.05$ as compared to the vehicle treatment (one-way ANOVA followed by Duncan's post-hoc test).

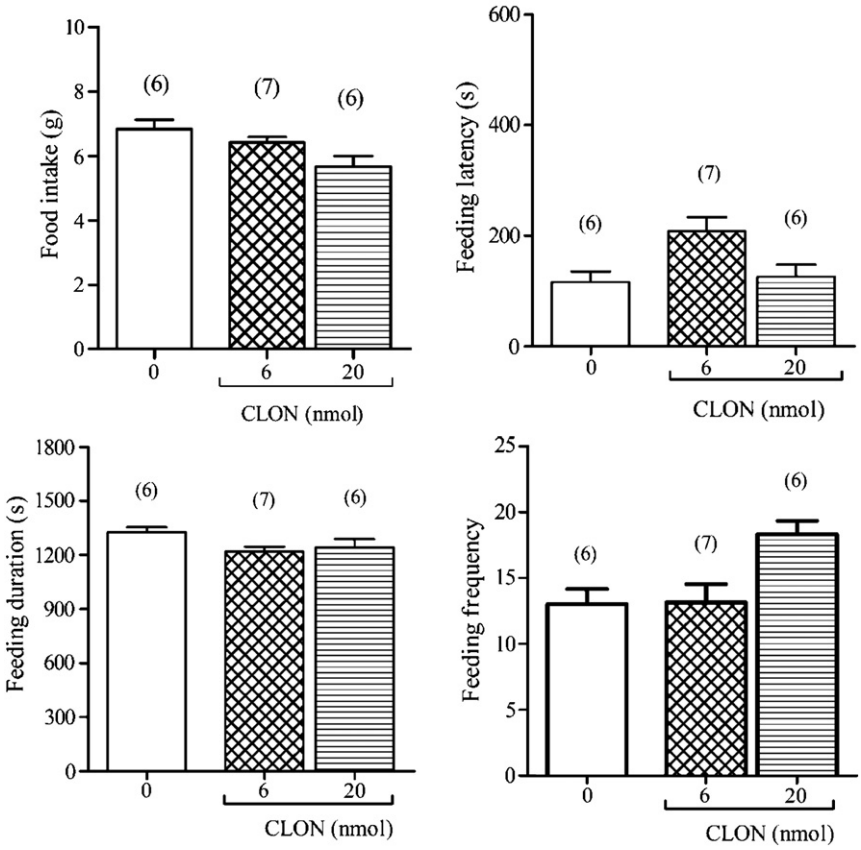

Fig. 5. Changes on food intake, feeding latency, feeding duration and feeding frequency after injection of vehicle (0) or clonidine (CLON, 6 nmol and 20 nmol) into the median raphe nucleus (MnR) of overnight fast. Values are mean ± S.E.M. Numbers in parentheses indicate the number of animals per group.

controlling water intake. The present results exclude the participation of α-adrenergic receptors within the MnR in the control of water intake since treatment with either α1- or α2-adrenergic receptor agonists in the MnR failed to affect drinking behaviour. Furthermore, the adrenergic control of drinking and feeding behaviours seems to include separate circuits in the MnR since α1-adrenergic receptor activation in this nucleus increased food intake but was not associated with changes in drinking behaviour.

In conclusion, the present results reinforce the inhibitory functional role of α1-adrenergic receptors within the MnR on feeding behaviour. This inhibitory influence tonically restrains food intake under free-feeding conditions since MnR α1-adrenergic receptor blockade increased food intake in rats with free access to lab chow [47]; the intensity of this inhibitory mechanism seemed to decline as food availability decreased.

Financial disclosure

Anderson Savaris Ribas, Rafael Appel Flores, Aparecida Marcelino de Nazareth, Moacir Serralvo Faria, Mariana Graciela Terenzi, José Marino-

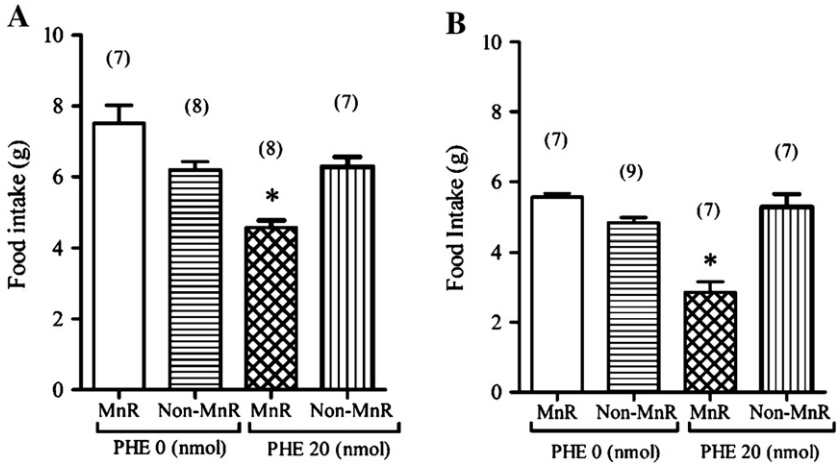

Fig. 6. Changes on food intake after injection of vehicle (0) or phenylephrine (PHE, 20 nmol) into the median raphe nucleus (MnR) and surrounding area (non-MnR) of food restricted rats (15 g chow/day during 7 days) (A) and overnight fast rats (B). Values are mean ± S.E.M. *$p<0.05$ as compared to vehicle treatment in both areas and the PHE treatment in the non-MnR (two-way ANOVA followed by Duncan's test for multiple comparisons). Numbers in parentheses indicate the number of animals per group.

Neto, Marta Aparecida Paschoalini report no biomedical financial interests or other potential conflicts of interest.

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