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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