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Research report

Amygdalar excitatory/inhibitory circuits interacting with orexinergic neurons influence differentially feeding behaviors in hamsters

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HIGHLIGHTS

- Increased food consumption following infusion of hamster BlA with ORX-A.
- Treatment of CeA with ORX-B prevailed on water consumption.
- ORX-A/-B + NMDA/zolpidem increased or reduced eating/drinking behaviors, respectively.
- The same treatments accounted for up and down ORX-2R expression levels.
- ORX-A/B + combined treatment support new therapeutic bearings on psychiatric disorders.

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ABSTRACT

Recently, environmental stimuli on different neurobiological events, via participation of distinct amygdalar (AMY) ORXergic fibers have aroused wide interests in view of their ability to modify neuronal linked stressful and physiological homeostatic conditions. Results of the present study indicate that ORXergic (ORX-A/B) circuits of the facultative hibernating golden hamster (*Mesocricetus auratus*) central AMY (CeA) and basolateral AMY (BlA) nuclei constitute major sites of feeding behaviors. Indeed, hamsters after treatment of BlA with ORX-A frequently ingested greater quantities of food as compared to controls, while ORX-B in CeA induced a very ($p < 0.001$) great consumption of water. The same nuclei treated separately with either ORX-A or ORX-B ± the selective α_1 $GABA_A$ benzodiazepine receptor agonist (zolpidem) dedicated less time to eating and drinking sessions. Conversely, hamsters that received the same neuropeptides but this time with the glutamatergic agonist NMDA displayed greater hyperphagic effects above all for ORX-A. When behavioral changes were compared to the expression of the specific ORXergic receptor (ORX-2R), an up-/down-regulating pattern was detected in some limbic areas (AMY, hippocampus and hypothalamus) following treatment with ORX-A or ORX-B plus NMDA. Overall, indications deriving from this study strongly point to hamster BlA-enriched ORX-A fibers in combination with either inhibitory or excitatory signals as main targets of hyperphagic responses while CeA ORX-B activities in presence of these same neuronal signals predominantly induced drinking motivational behaviors. The distinct behavioral activities of these two neuropeptides may have useful clinical bearings toward psychiatric and sleeping disorders such as bulimia and narcolepsy.

1. Introduction

The two excitatory neuropeptides hypocretins or orexins (ORX-A, B) that are produced from a single mRNA transcript plus pre-pro-hormone of the lateral hypothalamic area (LHA) seem to be capable of interacting with two receptor subtypes, i.e. ORX-1R and ORX-2R. In particular ORX-1R binds more selectively with ORX-A while ORX-2R has an equal affinity for both ORX-A and ORX-B [43]. Hypothalamic ORXergic circuits have demonstrated to be actively involved with feeding habits plus altering anxiety states, motor behaviors and sleep-wakening states [5]. Deletion of the pre-pro-orexin gene produced a narcoleptic-like phenotype in mice, which is similar to that of humans [10]. At the same time, intracerebroventricular (i.c.v.) administration of ORX-A accounted for a large number of LHA ORXergic neuronal fields promoting an appetite-stimulating type of activity in non-fasted rats [43]. In addition, ORXergic fibers of this hypothalamic area by cross-talking with the cerebral cortex (COR), hippocampus (HIP), septum, and amygdala (AMY) appear to control the different motivational and motor performances [28].

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At the brain level, it has already been shown that some AMY nuclei are not only actively involved with the modulation of negative affective states such as fear [25] but above all with the promotion of appetitive processes, including feeding behaviors linked to reward tasks [21]. From a morpho-functional point of view, AMY exhibits distinct and segregated functional domains [44] that include a posterodorsal "cortical-like" basolateral amygdala nucleus (BlA) and a central amygdala nucleus (CeA), both of which are connected to hypothalamic and brainstem autonomic systems [49]. Recent works have reported that both AMY sites serve as major feeding input regions receiving and sending neuronal signals to LHA and nucleus accumbens shell (nAc) [39]. In the case of CeA, it serves as an output relay for intra-amygdaloid connections originating from BlA and so tends to emphasize the participation of these afferent fibers, which are innervated by gustatory signals [32] on feeding responses [55].

It is important to note that feeding behaviors depend, aside ORXergic signals, on the interaction of other neuroreceptor systems such as dopamine, opioids, glutamate and γ-amino-butyric acid (GABA) as shown by their role on food seeking and consumption plus feeding reward processes [22]. Works have shown that certain subpopulations of $GABA_A$ receptors ($GABA_A$Rs), characterized by specific subunit compositions exert critical effects on feeding and motor activities [6]. This supramolecular pentameric structure, composed of at least 20 different classes of subunits: α (1–6), β(1–4), γ (1–3), δ, ε, θ, π, ρ (1–3), forms a complex $GABA_A$R ionophore molecule [36]. Due to the involvement of the α subunit with the structural assembly of this neuroreceptor system plus the expression of pharmacological functions as shown by $\alpha_{1,2,3,5}$ exhibiting varying degrees of sensitivity to benzodiazepines (BZD) underlies the importance of such a subunit on the execution of socio-sexual and motor behaviors [7]. Working along these lines of interest, the differing α-containing $GABA_A$R neuronal circuits represent a major facet of appetitive processes, including food reward behaviors [22]. In particular telencephalic GABAergic activities deriving from the nAc shell have been shown to influence hyperphagic effects [41].

On the basis of the above features, it was the intention of the present study to establish the type of feeding responses induced by separate i.c.v. infusions of BlA and CeA with the two ORX neuropeptides (ORX-A; ORX-B) as well as together with either the BZD agonist zolpidem, specific for α_1 $GABA_A$R subunit, or the glutamate agonist N-methyl-D-aspartate (NMDA) in the facultative Syrian hibernating hamster (*Mesocricetus auratus*). The selection of this rodent was largely based on its permissive facultative hibernating features, which allow us to correlate motor performances to the various feeding requirements during certain stages of this physiological state. Hibernation is a unique physiological state that permits animals to survive under extraordinary climatic and stressful conditions. This condition has been largely studied on *M. auratus* that displays profound decreases in oxidative metabolism and body temperature during bouts of prolonged torpor interrupted every 5–14 days by brief periodic arousals. The different physiological states feature a decrease in body temperature, with short periods of inter-bout euthermy when body temperature rises to ∼37 °C and is maintained for 12–48 h before reentry into torpor [12]. Indeed, the decrease in body temperature to values <5 °C contributes to the reduction of metabolic and enzymatic activities reaching 2–4% of normal rates, which are restored rapidly to near-normal levels so to avoid complications prior to arousal state [47,48].

In the case of AMY sites, BlA and CeA have shown to be tightly linked with the onset of explorative and feeding type of behaviors [1]. For this reason the behavioral effects were compared to the expression differences of the main ORX receptor subtype (ORX-2R) in some limbic areas and precisely BlA, CeA, hippocampal dentate gyrus (DG), lateral amygdalar nucleus (Lat), LHA the periventricular hypothalamic nucleus (Pe), the supraoptic nucleus (SON) that have been recognized as key ORXergic sites controlling different motor performances [33]. In addition, ORX-2R was preferred for this study due to its comparable affinity for both neuropeptides with respect to the greater affinity of ORX-1R for mostly ORX-A [42] plus to its importance on behavioral plasticity events [52]. The prevailing and distinct effects exerted by these neuropeptides may supply further insights regarding AMY-related feeding activities especially during some psychiatric disorders such as anorexia and bulimia, which are characterized by a disrupted motivational state [38].

2. Materials and methods

2.1. Animals and surgery

All experimental procedures described below were approved by the local Committee for Ethics in Animal Research (CEUA-UFSC, protocols #PP00091/CEUA and 23080.010535/2007-26) plus in accordance with suggestions and indications provided by the "Principles of animal care" (NIH, 1985). For the present study adult Syrian facultative hibernating hamsters (150–180 g body weight; Charles River, Como Italy) that were initially housed not more than 3 per cage were maintained at 22–24 °C, under a 14:10 light–dark cycle (lights on 6 a.m.) with free access to food and water. After 1 week of habituation period, hamsters were anesthetized with ketamine hydrochloride (100 mg/kg, i.p.) and xylazine (20 mg/kg, i.p.) and some animals were stereotaxically implanted with a unilateral stainless steel guide cannula (30 G) aimed 2 mm dorsal to the CeA (n = 47) while other animals were implanted for treatment of BlA (n = 47), according to previously described studies [5]. The cannula was positioned onto the skull with jeweler screws, fixed with dental cement and assured during the entire experiment by an inner removable stylet (Fig. 1).

2.2. Drugs and injection

ORX-A (20 nM), ORX-B (60 nM), zolpidem (100 nM) and NMDA (0.1 mM) all purchased from Sigma Chemical Co. (St. Louis, MO, USA) were freshly dissolved in saline 0.9%. Injections were made through an inner cannula (33 G) that extended 2 mm beyond the tip of the guide cannula, which was connected to a Hamilton microsyringe (1 μl) by polyethylene tubing. Injection volumes (1 μl) were given over a period of 60 s plus a further 60 s time-interval was used to consent the solution to diffuse from the cannula.

2.3. Experimental procedures

Seven days after surgery, some free-feeding hamsters bearing CeA guide cannula received daily either doses of ORX-A (n = 5), ORX-B (n = 5), zolpidem (n = 5) and NMDA (n = 5) alone or in combination (ORX-A/B ± zolpidem, n = 10; ORX-A/B ± NMDA, n = 10) for one week with respect to controls (C, n = 5) that received injections of 1 μl vehicle (0.9% saline). Other hamsters, this time bearing BlA guide cannula received the same above treatments. Immediately after injections, hamsters were initially placed for 30 min in the recording chamber to familiarize with its new environment. Subsequently, 30 min after the habituation period, three daily behavioral observations (10 a.m. to 6 p.m.) were conducted for the above treated hamsters during the entire observational session. Each day latency, duration and frequency of six previously defined behavioral categories and namely eating, drinking, grooming plus some typical motor performances such as moving about in the cage, following other hamsters, darting, upright position and resting [16], were continuously recorded by a webcam positioned perpendicularly at 60 cm above the cage floor, after which detailed behavioral analyses were conducted by using a Etholog 2.2 program [37]. At the end of the recording period, food that occasionally spilled onto the floor was recovered and weighed with food that remained on the feeder. The differences between food and water at beginning and end of recording period were calculated as total amount of food or water consumed. Also body weight differences of hamsters belonging to same treatment groups were compared to those of C at the end of the observation interval. In order to avoid interferences during behavioral recordings, back and lateral walls along with the floor cage were coated with a black adhesive plastic paper, while the front wall of the testing cage had a mirror of equal dimensions held at a 45° angle to the vertical plane so that it prevented animals from seeing its reflection.

2.4. Histological analysis

At the end of behavioral procedures, hamsters were anesthetized and 1 μl Evans blue dye (1%) was injected to verify the correct sites of the cannula within either CeA (n = 2) or BlA (n = 2; Fig. 1). Brain sections (30 μm) obtained at a vibratome were stained with cresyl violet and cannula loci were established by using a camera lucida attached to a light microscope and then mapped onto corresponding schemes of the hamster atlas [30].