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Role of the non-opioid dynorphin peptide des-Tyr-dynorphin (DYN-A_{2-17}) in food intake and physical activity, and its interaction with orexin-A.

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ABSTRACT

Food intake and physical activity are regulated by multiple neuropeptides, including orexin and dynorphin (DYN). Orexin-A (OXA) is one of two orexin peptides with robust roles in regulation of food intake and spontaneous physical activity (SPA). DYN collectively refers to several peptides, some of which act through opioid receptors (opioid DYN) and some whose biological effects are not mediated by opioid receptors (non-opioid DYN). While opioid DYN is known to increase food intake, the effects of non-opioid DYN peptides on food intake and SPA are unknown. Neurons that co-express and release OXA and DYN are located within the lateral hypothalamus. Limited evidence suggests that OXA and opioid DYN peptides can interact to modulate some aspects of behaviors classically related to orexin peptide function. The paraventricular hypothalamic nucleus (PVN) is a brain area where OXA and DYN peptides might interact to modulate food intake and SPA. We demonstrate that injection of des-Tyr-dynorphin (DYN-A_{2-17}, a non opioid DYN peptide) into the PVN increases food intake and SPA in adult mice. Co-injection of DYN-A_{2-17} and OXA in the PVN further increases food intake compared to DYN-A_{2-17} or OXA alone. This is the first report describing the effects of non-opioid DYN-A_{2-17} on food intake and SPA, and suggests that DYN-A_{2-17} interacts with OXA in the PVN to modulate food intake. Our data suggest a novel function for non-opioid DYN-A_{2-17} on food intake, supporting the concept that some behavioral effects of the orexin neurons result from combined actions of the orexin and DYN peptides.

1. Introduction

Food intake is a complex behavior regulated by multiple neuromodulators [1,2], including the orexin (also called hypocretin) and dynorphin (DYN) neuropeptides [3,4]. The DYN peptides are derived from a single precursor, prepro-dynorphin [5]. These peptides are widely expressed throughout the brain [6], including hypothalamic orexin neurons [7] and can be classified as opioid or non-opioid, depending on whether they bind to opioid receptors [8]. It is well established that the opioid peptide DYN-A_{1-13} promotes food intake by activating kappa opioid receptors across multiple brain regions [9], but our understanding of the physiological role of non-opioid DYN peptides is limited. A classic example of a non-opioid DYN peptide is des-Tyr-dynorphin (DYN-A_{2-17}) [10]. *In vitro*, DYN-A_{2-17} has excitatory post-synaptic effects [11–15], yet its receptor and cellular mechanisms of action are undefined. DYN-A_{2-17} has been linked to pain, drinking and thermal regulation [16–20]. However, its the role on food intake and other behaviors related to energy balance are unclear.

The orexins are two neuropeptides (orexin-A and orexin-B) [21,22] produced by neurons located in the lateral, perifornical and dorsomedial hypothalamus that project widely throughout the brain [23,24]. The biological effects of the orexin peptides are mediated through two G-protein coupled receptors (orexin receptor 1 and 2) [21,22]. Pharmacological data indicate orexin-A (OXA) has more salient behavioral effects as compared to orexin-B, and thus best exemplifies the biological role of the orexin peptides [1]. OXA increases food intake and non-structured physical activity of low intensity known as spontaneous physical activity (SPA) [25]. In humans, SPA includes time spent fidgeting, standing and ambu-

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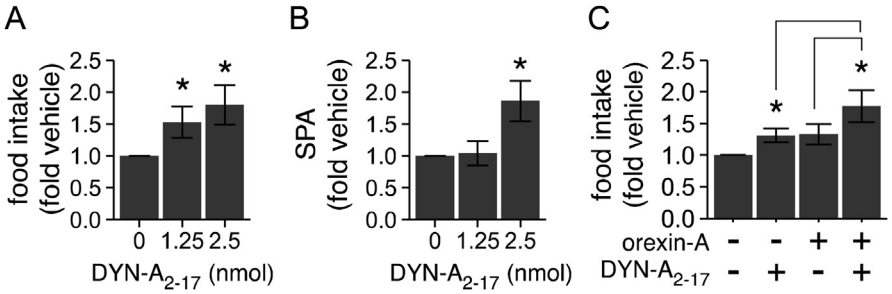

Fig. 1. Effects of non-opioid DYN-A_{2-17} on food intake and SPA and its interaction with orexin-A. DYN-A_{2-17} injected into PVN of Balb/c mice ($n = 11$) increases (A) food intake and (B) SPA 2 h post-injection. (C) Co-injection of DYN-A_{2-17} (0.625 nmol) with orexin-A (0.150 nmol) increased their individual effects on food intake ($n = 21$) Data presented as averaged fold change within mice. *, P-value < 0.05 as compared to vehicle. Brackets above bars, $P < 0.05$ for comparison between treatments. All pairwise comparisons corrected for multiple comparisons with Holm's correction. Y-axis, mean ± SEM.

lating [26]. In rodents, SPA is measured as locomotor activity in an open field or home cage after an acclimation period to differentiate SPA from the novelty-induced locomotion [27]. Although OXA promotes feeding, activation of the orexin neurons drives a negative energy balance, largely due to increased SPA [1,28].

Approximately 96% of orexin producing neurons express DYN peptides [7,29] and data show co-release of orexin and DYN peptides in some brain areas [29–31]. Thus, some of the cellular and behavioral effects mediated by orexin neurons could be due in part to the combined actions of these peptides. For example, OXA and DYN-A_{1-13} have opposite behavioral effects in ventral tegmental area [29] but both peptides increase activity of post-synaptic neurons in tuberomammillary nucleus (TMN) [30]. In TMN, DYN-A_{1-13} inhibits GABAergic tone while OXA has direct post-synaptic excitatory effects [30]. Finally, although both the loss of orexin neurons and orexin peptide deficiency cause fragmentation of wake behavior, only mice lacking orexin neurons have increased susceptibility to obesity [32–34]. Collectively, these data support the hypothesis that physiological effects of the orexin neurons depend on the combined effect of orexin and DYN peptides with brain-site specific mechanisms and behavioral outcomes. However, whether orexin and non-opioid DYN peptides interact to modulate behavior remains to be determined.

The role of the paraventricular hypothalamic nucleus (PVN) in the regulation of food intake and SPA is well characterized [2,35]. Injection of DYN-A_{1-13} into the PVN increase food intake [9] and OXA injections in PVN increase both food intake and SPA [36,37]. Thus, we hypothesized that non-opioid DYN-A_{2-17} in the PVN would also increase food intake and SPA. Furthermore, we hypothesized that OXA and DYN-A_{2-17} could interact to modulate food intake.

2. Materials and methods

2.1. Animals

Adult male Balb/c mice ($n = 40$, Instituto Salud Publica, Santiago, Chile) were used in these experiments. Mice (20–25 g and 8–12 weeks old upon arrival) were housed individually in clear solid bottom cages with corn-cobb bedding and environmental enrichment materials. Mice were maintained on a 12-h light/12-h dark cycle (lights on at 07:00 AM) in a temperature-controlled environment (21–24 °C). Food (ProLab RMH-3000, Lab Diets, MO, USA) and water were available *ad libitum*. All procedures were approved by the Institutional Bioethics Committee at Universidad Andres Bello.

2.2. Surgeries

Animals were anaesthetized with isofluorane gas (Baxter, TX, USA, 5% induction and 1% maintenance) and implanted with a stainless steel cannula (28 gauge, Plastics One, VA, USA) aimed at the PVN (stereotaxic coordinates from the mouse brain atlas of Paxinos and Watson relative to bregma: −0.9 mm rostral, −0.2 mm lateral, 3.6 mm below skull surface [38]) based on standard stereotaxic procedures. The coordinates were chosen such that a 33 gauge cannula injector extended 1 mm beyond the end of the cannula. Animals recovered for one week after surgery before experiments began.

2.3. Peptides

DYN-A_{1-13}, OXA and DYN-A_{2-17} (all peptides from Bachem, Torrence, CA, USA) were dissolved in artificial cerebrospinal fluid (aCSF; NaCl 150 mM, KCl 3 mM, $CaCl_2{\cdot}2H_20$ 1.4 mM, $MgCl_2{\cdot}6H_20$ 1.7 mM, $Na_2HPO_4{\cdot}7H_20$ 1.5 mM, $NaH_2PO_4{\cdot}7H_20$ 0.22 mM, all chemicals from Winkler, Santiago, Chile) at the following concentrations: DYN-A_{1-13} 0.725–3 nmol/0.25 μL, OXA 0.150 nmol/0.25 μL, DYN-A_{2-17} 0.625–2.5 nmol/0.25 μL. All peptides were aliquoted into single use vials and stored at −80 °C before use.

2.4. Injections

All experiments were performed using a repeated measures design, with doses of each peptide randomized over days in a counterbalanced design with at least 48 h between injections. Injections were performed between 09:00 and 11:00 AM. Before injecting the peptides, all mice were injected with aCSF once per day on three consecutive days to acclimate them to the injection procedure. All peptides were injected in a volume of 0.25 μl over 30 s and injector was kept in place for an additional 30 s. For the co-injection studies, mice were injected into PVN with four treatments: (1) aCSF/aCSF, (2) DYN-A_{2-17} (0.625 nmol)/aCSF, (3) OXA (0.150 nmol)/aCSF and (4) the combination of both peptides DYN-A_{2-17} (0.625 nmol)/OXA (0.150 nmol) in a single aliquot of 0.25 μl.

On each day of injections, bedding and enrichment material were removed from the home cage at least 2 h pre-injection. Food intake and spillage were measured 2 h post-injection. Physical activity was recorded continuously throughout for 2 h post-injection with a video camera at floor level positioned perpendicular to the longitudinal axis of the cage. Mice were allowed free access to food and water throughout the procedure. Bedding and enrichment materials were returned to the home cage after completion of the experiment.

For all animals used in experiments shown in Fig. 1A–C, cannula placement was verified first by the ability of DYN-A_{1-13} (3 nmol) or orexin-A (0.4 nmol) to increase short-term food intake (2 h) after intra-PVN injection compared to aCSF injection. Animals were excluded from the study if DYN-A_{1-13} or OXA at the specified doses failed to increase food intake. These doses were selected based on previous studies [39,40] (Table 1). At the end of the experiment,