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Research paper

Corticotrophin-releasing factor receptor 2 mediates the enhanced activation of satiety-related responses through oxytocin neurons in the paraventricular nucleus of the hypothalamus after adrenalectomy

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HIGHLIGHTS

- ADX increases OT neuronal activation in the PVN in response to feeding.
- $CRFr_2$ blockade reduces the high activation of PVN OT neurons after meal in ADX rats.
- PVN OT neurons might be downstream mediators of CRF on satiety responses after ADX.

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ABSTRACT

Adrenalectomy (ADX)-induced hypophagia is associated with increased activation of corticotrophin-releasing factor (CRF) and oxytocin (OT) neurons in the paraventricular nucleus of the hypothalamus (PVN) after refeeding. CRF_2- and OT-receptor antagonists abolish the hypophagia and the augmented activation of the nucleus of the solitary tract neurons induced by feeding after ADX. In addition, OT-receptor antagonist reversed CRF-induced anorexia. We evaluated the effect of intracerebroventricular pretreatment with CRF_2-receptor antagonist, antisauvagine-30 (AS30), on the activation of OT neurons of the PVN in response to refeeding of sham, adrenalectomized (ADX) and ADX rats replaced with corticosterone (ADX + B). In vehicle-pretreated animals, refeeding increased the number of Fos + OT double labeled neurons in the posterior parvocellular subdivision of the PVN (PaPo) of sham, ADX and ADX + B animals, with higher Fos expression and OT neuronal activation in the ADX group. AS30 reversed refeeding-induced increased activation of OT and non-OT neurons in the PaPo in the ADX group. In the medial parvocellular subdivision of the PVN (PaMP) of vehicle-pretreated animals, the number of Fos- and Fos + OT-immunoreactive neurons was increased after refeeding in ADX group. AS30 in the ADX group attenuated the enhanced Fos expression but not the number of Fos + OT double labeled neurons in the PaMP. In conclusion, CRF_2-receptor antagonist reverses the increased activation of OT neurons in the PaPo induced by feeding in ADX animals, suggesting that OT neurons might be downstream mediators of CRF effects on satiety-related responses after ADX.

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1. Introduction

Bilateral adrenalectomy (ADX) induces hypophagia [1–3] and is a well-established experimental model to investigate the mechanisms underlying the hypophagic effect observed in the primary adrenal insufficiency in humans [4]. Accordingly, glucocorticoid replacement to adrenalectomized (ADX) animals reverses the reduction of food intake [2–5]. ADX-induced hypophagia is associated with increased Fos expression in corticotrophin-releasing factor (CRF) and oxytocin (OT) neurons in the paraventricular nucleus of the hypothalamus (PVN), as well as enhanced neuronal

Abbreviations: ADX, adrenalectomy; B, corticosterona; CRF, corticotrophin-releasing factor; OT, oxytocin; PVN, paraventricular nucleus of the hypothalamus; NTS, nucleus of the solitary tract; TBE, 2,2,2-tribromoethanol; i.p., intraperitoneal; PaPo, posterior parvocellular subdivisions of the PVN; PaMP, medial parvocellular subdivisions of the PVN; PB, phosphate buffer; DAB, diaminobenzidine hydrochloride; AS30, D-Phe[11], His[12]-Sauvagine 11-40, Antisauvagine-30.

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activation in the nucleus of the solitary tract (NTS) induced by feeding, indicating that ADX stimulates satiety-related responses mediated by the hypothalamus and brainstem [1,2]. CRF and OT participate in the increased satiety-related responses after ADX, since the hypophagia and feeding-induced enhanced activation of NTS neurons following ADX were reversed by CRF_2- and OT-receptor antagonists [2,3,5]. Interestingly, the interaction between CRF e OT was demonstrated by Olson and co-workers [6], based on the abolition of the hypophagic effect of CRF by the pretreatment with OT-receptor antagonist, suggesting that OT might be a downstream mediator of CRF effects on food intake.

The present study was designed to evaluate the role of CRF on the activation of OT neurons in the PVN in response to food intake in ADX rats. Specifically, we investigated, in ADX rats, the effects of the central administration of a CRF_2-receptor antagonist on feeding-induced activation of OT neurons of the PVN.

2. Experimental procedures

2.1. Animals

Male Wistar rats, obtained from the Animal Facility of the Campus of Ribeirao Preto, University of Sao Paulo, Brazil, weighing 230–280 g, were housed in individual cages at a controlled temperature (23 ± 2 °C) with a fixed light-dark cycle (light from 6:00 AM to 6:00 PM). Animals had ad libitum access to pelleted rat chow and water, unless otherwise specified. To improve adaptation to the laboratory environment, the rats were daily handled during 7 days preceding the experiments. All experimental procedures were conducted between 7:00 AM and 12:00 PM and were approved by the Ethical Committee for Animal Use of the School of Medicine of Ribeirao Preto, University of Sao Paulo, Brazil (protocol number 092/2011).

For bilateral adrenalectomy and sham surgeries, animals were deeply anesthetized with intraperitoneal (i.p.) injection of 2.5% 2,2,2-tribromoethanol (TBE, 1 mL/100 g of body weight). Surgeries were performed via a dorsal midline approach with a single incision in the skin and small bilateral sections through the muscle layer at the angle between the last rib and vertebral column. Sham-operated animals underwent similar surgical procedures but without removal of the adrenal glands and were given tap water with 0.5% ethanol to drink. Adrenalectomized animals without hormone replacement (ADX) were given 0.9% saline with 0.5% ethanol as drinking fluid, and adrenalectomized rats with hormone replacement were given 0.9% saline with corticosterone diluted in 0.5% ethanol at a concentration of 25 mg/L to drink (ADX + B) [3].

2.2. Intracerebroventricular (icv) cannulation

Wistar rats deeply anesthetized with i.p. injection of 2.5% TBE (1 mL/100 g of body weight) were placed in a stereotaxic instrument (Kopf, model 900) with bregma and lambda in a horizontal plane. A stainless steel guide cannula (10.0 mm long, 0.6 mm o.d., 0.4 mm i.d.) was implanted into the right lateral ventricle using coordinates from the atlas of Paxinos and Watson [7]: 0.6 mm caudal to bregma, 1.5 mm lateral to the mid-line and 3.5 mm below the dura mater. The cannula was fixed to the cranium using dental acrylic resin and two jeweller's screws. A 30-gauge metal obturator filled the cannula except during the injections. After surgery, the rats received a prophylactic injection of penicillin (50,000 U, intramuscular) and were allowed to recover for 7 days, during which they were daily handled to minimize stress during the experimental procedure. Cannula placement was verified by sectioning the brains of all rats with the cryostat at the end the experiment.

2.3. Fos and OT immunohistochemistry

Rats were anesthetized with i.p. injection of 2.5% TBE (1 mL/100 g of body weight) and transcardially perfused with 200 mL of cold isotonic saline containing heparin (50 UI/L), followed by 500 mL of cold 4% paraformaldehyde solution in 0.1 M phosphate buffer (PB), pH 7.2. The brains were removed, post-fixed for 1 h in 4% formaldehyde solution and stored at 4 °C in PB containing 30% sucrose. Coronal sections of 30 μm were obtained with a cryostat (Microm) and preserved at −20 °C in cryoprotectant solution until further processing.

Sections were processed for Fos immunoreactivity (ir) by incubating overnight at room temperature with rabbit anti-Fos antibody (Ab-5, Oncogene Science, Manhasset, NY, USA) and diluted 1:10,000 in 0.1 M phosphate buffer (PB) containing 2% normal goat serum and 0.3% Triton X-100. Free-floating sections were washed with PB and incubated first with goat biotin-labeled anti-rabbit immunoglobulin (Vector Laboratories Inc., Burlingame, CA, USA, 1:200 dilution in 1.5% normal goat serum-PB) and then with avidin–biotin–peroxidase complex (Vector Laboratories Inc., Burlingame, CA, USA, 1:200 in PB) for 1 h at room temperature. Immunoperoxidase labeling was detected using diaminobenzidine hydrochloride (DAB, Sigma Chemical Co., St. Louis, MO, USA) intensified with 1% cobalt chloride and 1% nickel ammonium sulphate, which generates a blue–black reaction product. Next, the sections were incubated for 48 h at 4 °C with rabbit anti-OT (Peninsula Lab. Inc., USA, 1:10,000). Then, sections were rinsed and subjected to the protocol described above, except that non-intensified DAB, which generates an insoluble brown reaction product, was used. The sections were mounted on gelatinized slides, air-dried overnight, dehydrated, cleared in xylene and placed under a cover slip with Entellan (New Jersey, USA).

PVN subdivisions were identified with the aid of the atlas of Paxinos and Watson. Medial (PaMP) and posterior (PaPo) parvocellular subdivisions of the PVN were considered at −1.80 mm and −2.12 mm from bregma, respectively. Numbers of immunoreactive (ir) neurons were counted with the aid of a Leica microscope equipped with a DC 200 Leica digital camera attached to a contrast enhancement device. Fos-ir neurons exhibited a conspicuous blue-black immunoreaction product in the cell nucleus, while OT labeling exhibited a brown cytoplasmic reaction. Sections were counted bilaterally in 3–4 sections of 4–9 animals from each experimental group by participants blind to the experimental protocols.

2.4. Experimental protocol

2.4.1. Effects of pretreatment with CRF_2-receptor antagonist on neuronal activation in the PVN in the fasting-refeeding regimen

After icv surgery, animals were subjected to sham and ADX surgeries and divided into sham, ADX and ADX + B groups. Six days later, the animals were fasted for 16 h, and on day 7 they were icv injected with D-Phe^{11}, His^{12}-Sauvagine 11-40 (Antisauvagine-30, AS30, Peninsula/Bachem, 5 μg/5 μL) or vehicle (0.9% NaCl/5 μL). Fifteen minutes following the injections, half of the animals were given access to food and, 2 h later, all rats were anesthetized and perfused. Brain tissue was collected and processed for immunohistochemistry.

2.5. Statistical analysis

The data were expressed as means ± SEM, which were tested using three-way ANOVA, followed by the Newmann–Keuls post hoc test. Differences were considered significant at $P < 0.05$.

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