Physiology & Behavior 189 (2018) 26–31

Contents lists available at ScienceDirect

Physiology & Behavior

journal homepage: www.elsevier.com/locate/physbeh

Glutamate-dependent regulation of food intake is altered with age through changes in NMDA receptor phenotypes on vagal afferent neurons

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ARTICLE INFO

Keywords:
NMDA receptor
Nodose ganglia
Food intake
Aging
Vagus nerve

ABSTRACT

Compared to younger individuals, older human subjects have significantly lower food intakes and an increased satiety response. *N*-methyl-D-aspartate (NMDA) receptors expressed by vagal afferent neurons originating from nodose ganglia (NG) are involved in modulating the satiety response. The present study investigated how NMDA receptor subunit phenotypes in NG neurons change with age and how these age-related alterations in food intake are modulated by presynaptic NMDA receptors in the NG of male Sprague Dawley rats (six week-old and sixty week-old). Food intake was measured at 30-, 60-, and 120-min following intraperitoneal administration of cholecystokinin (CCK) or the non-competitive NMDA receptor antagonist MK-801. Immunofluorescence was used to determine NMDA receptor subunit expression (NR1, NR2B, NR2C, and NR2D) in the NG. The results showed that, CCK reduced food intake at 30-, 60-, and 120-min post injection in both young and the middle-age animals, with no statistical difference between the groups at 30- and 60-min. In contrast, MK-801 produced an increase in food intake that was significantly higher in middle-age rats compared to young animals at all time points studied. NR1 subunit was expressed by almost all NG neurons in both age groups. In young rats, NR2B, NR2C, and NR2D subunits were expressed in 56.1%, 49.3%, and 13.9% of NG neurons, respectively. In contrast, only 30.3% of the neuronal population in middle-aged rats expressed NR2B subunit immunoreactivity, NR2C was present in 34.1%, and only 10.6% of total neurons expressed the NR2D subunit. In conclusion, glutamate-dependent regulation of food intake is altered with age and one of the potential mechanisms through which this age-related changes in intake occur is changes in NMDA receptor phenotypes on vagal afferent neurons located in NG.

1. Introduction

A decline in food intake and, consequently, body weight are typical characteristics of the aging process in humans [1–3] and rats [4]. Ordinarily, this age-related anorexia is a topic of interest because it can lead to nutritional deficiencies in a population that is already at a higher risk for a number of disease processes [5]. Although the direct cause for this decrease in food intake is not known; physiological studies have shown that as people age, they experience a decrease in taste and smell acuity, lower metabolic rate, and decreased physical activity [6,7].

Satiety signals are carried from the gastrointestinal (GI) tract via vagal afferents whose cell bodies lie in the nodose ganglia (NG) and synapse in the Nucleus of the Solitary Tract (NST) [8]. These vagal afferents release the neurotransmitter glutamate, which regulates food intake by signaling through *N*-methyl-D-aspartate (NMDA) receptors [9]. NMDA receptors are heteromeric ionotropic channels composed of multiple subunits. In general, each receptor consists of two NR1 and two NR2 or NR3 subunits [10]. Multiple receptor isoforms have been identified and their functional properties result from selective splicing of the NR1 transcript and expression of several different forms of the NR2 (NR2A, NR2B, NR2C, and NR2D) subunit [11].

Although NMDA receptors are commonly found postsynaptically, they have also been localized presynaptically in numerous higher brain regions, the cerebellum, and in peripheral afferent neurons (e.g. vagal) [12–15]. In the vagal system, both pre- and postsynaptic NMDA receptors participate in the control of food intake [9,16–18]. Evidence for the involvement of presynaptic NMDA receptors in the control of food

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https://doi.org/10.1016/j.physbeh.2018.02.042
Received 7 December 2017; Received in revised form 19 February 2018; Accepted 20 February 2018
Available online 21 February 2018
0031-9384/

intake came from a study that showed that administration of an NMDA receptor antagonist in the NST ipsilateral to unilateral NG removal did not elicit the typical increase in food intake observed by blocking NMDA receptors in the NST [16,17]. Thus, in the last decade, significant efforts have been made to establish the phenotype of NMDA receptors expressed in vagal afferents. A previous study examined NMDA receptor subunit immunoreactivity in vagal afferents of six week-old male rats and reported that in the NG nearly all neurons (92.3%) expressed the NR1 subunit. However, expression phenotype of the NR2 subunits, i.e. NR2-B, -C, and -D varied in different neuronal subsets [14].

Signaling through NMDA receptors in higher order brain regions, e.g. hippocampus and cortex, is primordial for long-term potentiation (LTP) and memory function. In rodents, previous research has shown that with age, there is a decline in NMDA receptor binding sensitivity [19,20]. Furthermore, Eckles-Smith et al. reported that compared to 3–6 month-old rats, 26 month-old rats had lower levels of expression of the NR1 NMDA receptor subunit [21]. Given these findings and the role of NMDA receptors in vagal afferent signaling, the current study aimed to investigate the phenotype of NMDA receptor expression in the NG of young and middle-age rats. It was hypothesized that the expression phenotype of the NR1, NR2B, NR2C, and NR2D NMDA receptor subunits in middle-age rats would differ from the phenotype observed in young rats, and that these differences would be reflected by differences in food intake in response to administration of MK-801, a non-competitive NMDA receptor antagonist. Immunofluorescence was used to determine NMDA receptor subunit expression (NR1, NR2B, NR2C, and NR2D) in the NG of six week-old [22] and sixty week-old (middle-age) male Sprague Dawley rats and measured changes in food intake in response to administration of the non-competitive NMDA receptor antagonist MK-801. Additionally, changes in food intake were measured following administration of cholecystokinin (CCK), which has been shown to exert a short-term inhibitory effect on food intake via vagal afferents through a non-NMDA dependent mechanism [23]. Thus, it was expected to observe a comparable decrease in food intake in young and middle-age rats following CCK administration. The results suggest that glutamate-dependent regulation of food intake is altered with age through changes in NMDA receptor phenotypes on vagal afferent neurons located in NG.

2. Methods

2.1. Animals

Male Sprague-Dawley rats (6 week-old, n = 19 and 60 week-old, n = 19; Simonsen Labs, Gilroy, CA) were individually housed in a temperature-controlled vivarium and allowed to acclimate to the animal facility for 3–5 days prior to starting experiments. Rats were housed individually in regular shoe-box cages with wire floors and were given free access to standard pellets of rat chow (Harlan Teklad F6 Rodent diet, Madison, WI), unless otherwise specified, and water. Vivarium lights maintained a 12:12-h light: dark cycle, with lights on 0700–1900-h. All animal procedures were approved by the Washington State University Institutional Animal Care and Use Committee and conform to National Institutes of Health guidelines for the use of vertebrate animals (publication No. 86–23, revised 1985).

2.2. Drugs and treatments

Dizocilpine (MK-801, Sigma), an NMDA receptor antagonist, and CCK octapeptide sulfate (CCK-8; American Peptide, Sunnyvale, CA) were dissolved in a vehicle solution of sterile 0.15 M NaCl saline for injection to a final concentration of 40 μg/kg and 3 μg/kg, respectively. MK-801, CCK, or the vehicle alone was administered intraperitoneally immediately prior to the presentation of chow pellets. Although due to the age difference between the tested groups body weights were significantly different and thus were the doses administered to each group, it is universally customary to use body weight as the main criterion for dosage calculation since it follows that substances administered to act after absorption into the body become diluted in proportion to blood volume and are transported by the blood stream in a relative concentration.

2.3. Experimental protocol: food intake

Feeding tests were conducted every other day at 0900-h in their home cage and the animals were allowed to free feed between testing days. Prior to each test, rats were fasted overnight (16-h). On test day, they received an intraperitoneal injection of either vehicle, CCK, or MK-801 immediately prior to food presentation. Food intake was measured during the following 2 h by weighing the amount of food left, including spillage, at 30-, 60-, and 120-min and subtracting it from total food added to the cage. A total of three feeding tests were conducted following a vehicle injection to establish an intake baseline, as well as to mitigate the stress produced by the injection itself and handling. On the fourth test day, the animals received a CCK injection. Following a three-day rest period, the same protocol was followed to measure food intake after MK-801 injection.

2.4. Immunofluorescence and counter-staining

Seven days after completion of the feeding tests, animals were prepared for tissue collection and processing following the protocol previously described by Czaja, et al. [14]. Briefly, rats (n = 6 per group) were deeply anesthetized with an intramuscular injection of acepromazine, ketamine, and xylazine (0.1 ml/100 g), exsanguinated, and transcardially perfused with a sequence of phosphate-buffered saline (PBS) and 4% paraformaldehyde solution (pH 7.4). Immediately after the perfusion, NG were collected, post-fixed (4% paraformaldehyde, 30 min), and immersed in a cryoprotectant solution of 18% sucrose in PBS and NaN_3 overnight. The ganglia were cut longitudinally into 20 μm serial sections and mounted in four series on glass slides (Superfrost Plus), with every fourth section on the same slide. This procedure yielded 28–32 20 μm-thick sections per ganglion, with each slide containing seven or eight 20 μm sections, with an interval of 60 μm between adjacent sections. After drying on the slides, the sections were rehydrated and processed for detection of selected NMDA receptor subunits according to conventional immunofluorescence protocols. The slide-mounted sections were immersed for 15 min in a 0.1% sodium borohydride1 solution to reduce autofluorescence. Subsequently, the sections were incubated overnight in a blocking solution consisting of 10% normal horse serum in Tris sodium phosphate buffer (TPBS; pH 7.4). The blocking solution was washed from the tissues, and each section was incubated in affinity-purified polyclonal primary antisera (Table 1) raised in goat (Santa Cruz Biotechnology, Santa Cruz, CA) against the NR1, NR2B, NR2C, or NR2D NMDA receptor subunit for 48 h at room temperature. After three washes in TPBS, sections were incubated with a secondary antibody raised in donkey (CY3; 1:200; 705–165-003; Jackson Immunoresearch, West Grove, PA) overnight at room temperature. Following overnight incubation, the sections were washed with Tris-buffered PBS, counterstained with DAPI (Molecular Probes) to reveal neuronal and glial nuclei, and mounted in ProLong (Molecular Probes) to reduce photo bleaching.

2.5. Counting of NG neurons

Images of the NG were viewed and captured with a Zeiss Axioplan 2 imaging photomicroscope equipped with a digital camera (Axio Cam MRc) and appropriate filters for DAPI, CY3, and Alexafluor 488. With the aid of a computer, the captured images were evaluated with the Axio Vision 4.0 Imaging System (Carl Zeiss Vision, Oberkochen, Germany). For preparation of microscopic illustrations, Corel Graphic