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Research report

Calorie restriction alters physical performance but not cognition in two models of altered neuroendocrine signaling

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Abstract

A major neuroendocrinological effect of calorie restriction (CR) is induction of neuropeptide Y (NPY) in the arcuate nucleus (ARC). Aside from its appetite-stimulating effects, NPY is thought to be involved in the modulation of behavioral processes including anxiety and learning and memory. In the present study physical fitness, anxiety, and learning/memory-related tasks were assessed in mice lacking NPY or a functional ARC after dietary manipulation by CR. Physical fitness was improved by CR when measured by inclined screen and rotarod, and this diet effect was not affected by NPY or ARC status. As has been observed previously, the NPY knockout mice displayed heightened anxiety in an open field. This phenotype was not fully recapitulated in the ARC-lesioned model. CR affected neither total locomotor activity in the open field nor thigmotaxic behavior in these models. Neither NPY nor CR had a significant effect on Morris water maze performance; however, ARC-damaged mice were unable to learn the task, and this deficit was not corrected by CR. We conclude that despite established effects of CR on ARC signaling, our results suggest a mechanistic separation between the two where behavior is concerned.
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Keywords: Behavior; Calorie restriction; Cognition; Hypothalamus; Learning; Monosodium glutamate (MSG); Neuroendocrine system; Neuropeptide Y (NPY)

1. Introduction

Lifespan extension aside, calorie restriction (CR) consistently confers numerous beneficial functional adaptations upon laboratory animals [24,51]. Of these, the behavioral changes that have been associated with calorie restriction include enhanced physical and cognitive performance [25,26,35,53]. Enhanced physical performance may be attributed in large part to improved body composition in CR animals compared to fully fed controls, but the mechanisms responsible for the cognitive effects are not fully understood. Hypotheses linking CR to altered cognitive capability include reduction of neural oxidative stress, promotion of synaptic plasticity and induction of various stress proteins and neurotrophic/neuroprotective factors [33,45]. How CR might trigger such neuromodulation has not been fully characterized and is thus the subject of this study.

The arcuate nucleus (ARC) in the hypothalamus of rodents is the first-order processing unit for nutrient status in the brain [18,60]. The sum of peripheral signals relating information regarding nutrient status (e.g. leptin from adipose tissue and ghrelin from the gut) converge on the ARC where they are converted to a neurochemical report (e.g. Neuropeptide Y (NPY), pro-opiomelanocortin (POMC)) that is transmitted to brain regions involved in the activation of food-related behavior. As would be expected, ARC output is altered by CR such that expression of orexigenic NPY is upregulated [4,5,13,30,38,55].

While CR-induced NPY upregulation was established more than 15 years ago [5], causative roles for the ARC and NPY in mediating the effects of CR remain putative. Since, mice and rats treated neonatally with monosodium glutamate (MSG) exhibit neuronal death specifically in the ARC [7,40] owing to blood-brain barrier permeability at the median eminence [44], this

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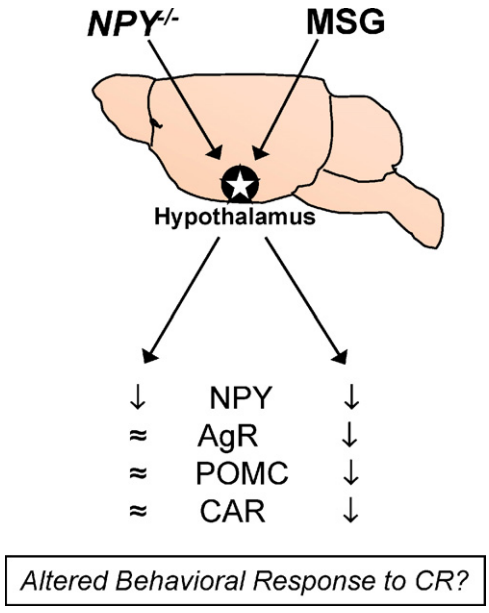

Fig. 1. Two models of altered hunger signaling were used in this study to assess the involvement of the NPY and the ARC in the behavioral response to CR. In $NPY^{-/-}$ mice NPY expression is selectively downregulated, whereas global ARC lesioning by MSG treatment leads to a broad-range reduction in key appetitive ARC neuropeptides. Whether either of these neurochemical adaptations leads to an altered behavioral response to CR is the central question of this work.

technique can be employed to evaluate the role of the ARC in CR-related adaptations. Neonatal MSG-induced neurotoxicity in mice results in a pronounced reduction in ARC neuropeptide expression [6,36] including NPY [1,6,29,31,36] and is a well-characterized model for evaluating the functional importance ARC neurons [9,23,49].

Evidence suggests the ARC and NPY have functional roles in cognition. For example, ARC-lesioned mice display impaired memory [43]. Central administration of NPY has been shown to enhance memory retention in mice [16], although a regulatory role for NPY in learning and cognition is still putative [47]. Whether the ARC or NPY have been involved in behavioral adaptations following CR is presently unknown, although neuroendocrine signaling has been proposed to play an important role in mediating the response to CR [37,52,56]. We hypothesize that the response by the ARC (and fluctuations in ARC NPY in particular) act to help mediate downstream effects of CR. To test this hypothesis we employed two mouse models (Fig. 1), an MSG-induced ARC-lesioned mouse (MSG) and an NPY knockout mouse ($NPY^{-/-}$) [14] and compared their performance with control animals on a battery of behavioral tests after CR or ad libitum (AL) feeding.

2. Materials and methods

2.1. Mice and diets

Male and female $NPY^{-/-}$ mice (129S-Npy^{tm1Rpa}/J) were obtained from the Jackson Laboratory (Bar Harbor, ME) and bred in a vivarium maintained by the Intramural Research Program at the National Institute on Aging (Baltimore, MD). After two generations of breeding, offspring were genotyped and all animals were confirmed to be homozygous knockout for *NPY*. Male wild-type ($NPY^{+/+}$) controls (129S1/SvImJ) were purchased from the Jackson Laboratory directly. The C57BL/6J (B6) mice were bred at the Jackson Laboratory and injected subcutaneously with either 4 mg/g monosodium glutamate (MSG) or physiological saline (SAL) on postnatal day 5. The 129S1 mice were 3–5 months of age, and the B6 mice were 2 months of age when they were divided randomly into diet groups. Eighty mice in total (10 for each condition and diet) were used for this study, all of which were males. The mice were housed individually in ventilated caging on a 12 h light/dark cycle at 22 °C and 35% humidity with ad libitum access to water. All testing was performed during the light phase using procedures outlined and approved by the Animal Care and Use Committee at the National Institute on Aging.

The diet, AIN93-G, was obtained from Bio-Serv (Frenchtown, NJ) and was used for both AL and CR diets. AL mice were fed unlimited quantities of the diet, and the CR animals were fed daily weighed amounts equivalent to 70% of AL intake. Animals were maintained on the study diets for 6 weeks before behavioral testing commenced and for the 2 weeks during the behavioral testing.

2.2. Body composition analysis

Measurements of lean and fat mass were acquired simultaneously using the Minispec LF90 (Bruker Optics, Billerica, MA), an NMR analyzer for whole body composition assay of live, unanesthetized mice.

2.3. Rotarod

A five-station Rota-Rod Treadmill for mice (Med Associates, St. Albans, VT) consisting of a mechanized rotating bar (3 cm diameter) suspended 16 cm above a platform was used for this experiment. On the first day the mice were habituated to the rotarod for 120 s at a constant speed of 4 rpm. The following day testing was completed in three trials of increasing speed from 4 to 40 rpm. Mice were tested for a maximum of 300 s in each trial. The latency to fall was recorded for each trial and averaged for each group. Results shown are group means of the averaged three trials per mouse.

2.4. Inclined screen

Mice were placed in a tilted, open field (55 cm^2 surface area constructed from a 0.6 cm^2 wire mesh grid with black sides extending 15 cm above the grid) and movement was recorded for 300 s using Field 2020 tracking software from HVS Image (Buckingham, UK). Results were averaged for total distance traveled for each mouse.

2.5. Open field locomotor activity

Mice were placed in a level, open field (same as for inclined screen) and movement was recorded for 300 s using Field 2020 tracking software from HVS Image. Results were averaged for both total distance traveled and time spent in the center of the field (comprised of the interior 40 cm^2) or around the periphery (the area extending 7 cm inside around the sides of the field).

2.6. Morris water maze

The apparatus consisted of a white circular plastic pool (100 cm diameter and 70 cm high) which was filled with water (24 ± 1 °C) rendered opaque by addition of white Dry Temp® paint powder (Palmer Paint Products Inc., Troy, MI, USA). Spatial navigation cues were affixed to a clear, plastic cylinder concentric with the pool and extending approximately 30 cm above the top of the pool. A circular (10 cm diameter) escape platform (the target) was submerged just below the water surface. Each acquisition trial (four trials per day for 6 days) was started by placing a mouse in the water facing the wall of the tank. The location of entry of the mouse changed every trial such that mice entered the maze from each direction once each day, and the order of start position was set randomly for each mouse. A trial lasted until the mouse found the platform or until 60 s had elapsed. If a mouse did not find the platform within 60 s, it was placed on the platform for 30 s in order to familiarize it with the location of the platform. After completion of the fourth trial on each day, the mouse was returned to its home