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Research paper

Role of the neural pathway from hindbrain to hypothalamus in the regulation of energy homeostasis in rats

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HIGHLIGHTS

- Food intake and body weight increased in rats with a midbrain transection.
- The midbrain-transected rats showed insulin resistance and hyperleptinemia.
- Hypothalamic POMC and CART decreased in the midbrain-transected rats.

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ABSTRACT

Recent evidence suggests that neural pathways from the hindbrain to the hypothalamus are important for informing the hypothalamus of the body's condition with regard to energy metabolism. Here we examined energy metabolism in rats with transections of the midbrain that severed the neural pathway from the hindbrain to the hypothalamus, and then investigated the levels of various molecules associated with control of energy metabolism in these rats. Food intake and body weight were higher in the midbrain-transected rats than in sham-operated rats. In addition, the midbrain-transected rats showed insulin resistance and hyperleptinemia. Furthermore, the hypothalamic mRNA levels of anorectic proopiomelanocortin and cocaine- and amphetamine-related transcript were significantly lower in midbrain-transected rats than in sham-operated rats. Our findings elucidate the mechanisms of food intake and energy balance from the perspective of multifactorial regulatory systems that underlie functions such as neurohormonal integration.

1. Introduction

Adult humans and animals usually maintain a balance between their energy intake and energy expenditure levels, as demonstrated by the constancy of body weight and body composition. Food intake is finely regulated by a complicated interaction of many orexigenic and anorectic signals produced in the brain and peripheral tissues. Several peripheral hormones, such as leptin, peptide YY (PYY), glucagon-like peptide-1 (GLP-1), cholecystokinin (CCK), and ghrelin, are associated with feeding and energy metabolism [1,2]. These hormones regulate orexigenic neurons, anorectic neurons, or both, which are located in the hypothalamus, resulting in the maintenance of energy homeostasis [3,4]. Our research group has been investigating the linkage between feeding-related hormones and neurons; we have found that ghrelin, CCK, and PYY are not only transported to the brain via the circulation, but also modulate vagal afferent pathways and neural pathways from the hindbrain to the hypothalamus [5,6]. Furthermore, we recently showed that intraperitoneal (ip) coinjection of subthreshold levels of GLP-1 and leptin, or CCK and leptin, which individually have no effect on feeding, dramatically reduces food intake [7,8]. In addition, we demonstrated that these synergistic actions on feeding are

Abbreviations: PYY, peptide YY; GLP-1, glucagon-like peptide-1; CCK, cholecystokinin; ip, intraperitoneal; NTS, nucleus of solitary tract; GTT, glucose tolerance testing; ITT, insulin tolerance testing; ObRb, leptin receptor; AGRP, agouti-related protein; NPY, neuropeptide Y; POMC, proopiomelanocortin; CART, anorectic cocaine- and amphetamine-regulated transcript; ELISA, enzyme-linked immunosorbent assay; HOMA-IR, homeostasis model assessment for insulin resistance; AUC, area under the curve; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; SEM, standard error of the mean.

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abolished in rats with bilateral transections of the neural pathways from the region of the hindbrain containing the nucleus of the solitary tract (NTS), which receives vagal afferents, to the hypothalamus [8,9]. These data suggest that the neural pathways from the hindbrain to the hypothalamus inform the hypothalamus of the state of energy metabolism.

In this study, we examined energy metabolism in midbrain-transected rats and investigated the effect of the transection on the expression of molecules involved in energy metabolism. We first observed the midbrain-transected rats and sham-operated rats for 20 weeks and compared their food intake and body weight. Next, we assessed the glucose metabolism of midbrain-transected rats by glucose tolerance testing (GTT) and insulin tolerance testing (ITT). Finally, we investigated plasma leptin levels and feeding-associated molecules in the hypothalamus, including the long-form leptin receptor (ObRb), agouti-related protein (AGRP), neuropeptide Y (NPY), proopiomelanocortin (POMC), and anorectic cocaine- and amphetamine-regulated transcript (CART).

2. Materials and methods

2.1. Experimental animals

Male Wistar rats (8–10 weeks old; Charles River Japan, Shiga, Japan) weighing 300–350 g were used for all experiments. Rats were given standard laboratory chow and water *ad libitum*. They were housed individually in plastic cages at constant room temperature on a 12:12-h light:dark cycle (lights on, 0800–2000 h). Food intake and body weight were monitored once a week throughout the experiment. All procedures were performed in accordance with the Japanese Physiological Society's guidelines for animal care. Our experimental protocol was approved by the Ethics Review Committee for Animal Experimentation of the Faculty of Medicine, University of Miyazaki, Japan.

Bilateral midbrain transection was performed under isoflurane anesthesia (DS Pharma Animal Health, Osaka, Japan), as previously described in Ref. [8,10]. The head was fixed in a stereotaxic instrument in a 2.4-mm nose-down position. A steel knife blade 1.5 mm wide was used to penetrate the brain in the coronal plane at two points: at 0.5 mm on either side of the midline, 1 mm in front of the lambdoid suture. For each incision, the blade penetrated to a depth 7.7 mm below the dura. In the sham operation, the skull was exposed but the brain was left intact. We removed the brains after all experiments and histologically verified the exact locations of the lesions.

2.2. GTT and ITT

GTT and ITT were performed on the sham-operated rats and midbrain-transected rats at 16–18 weeks after the operation ($n = 4$ or 5 per group). Rats were fasted overnight for the GTT and then given an ip injection of 2 g/kg body weight glucose at 09:00. Blood glucose levels in blood drawn from the tail vein were measured immediately prior to injection (time point 0) and then at 30, 60, 90, and 120 min after injection by using the glucose oxidase method (Ascensia Breeze 2; Bayer Medical, Leverkusen, Germany). For the ITT, rats were fasted for 4 h and then given an ip injection of 1 U/kg body weight insulin (Novo Nordisk, Mainz, Germany) at 13:00. Blood glucose levels were measured immediately prior to injection (time point 0) and then at 30, 60, 90, and 120 min after injection. The area under the curve (AUC) was calculated by using Graph Pad Prism software version 6.0 (San Diego, CA). Plasma insulin levels were measured in midbrain-transected and sham-operated rats after overnight fasting by using enzyme-linked immunosorbent assay (ELISA) kits (Morinaga Institute of Biological Science, Yokohama, Japan). The homeostasis model assessment for insulin resistance (HOMA-IR) was used to assess insulin resistance, which was calculated from the fasting insulin and glucose concentrations by using the formula [26 × fasting insulin (ng/mL) × fasting glucose (mg/dL)/405] [11].

2.3. Plasma leptin levels

Blood samples were obtained from the rats 20 weeks after the operation ($n = 6$ or 7 per group) after they had been fasted overnight. The plasma was stored at −30 °C until analyzed. Plasma leptin levels were measured with an ultrasensitive rat leptin ELISA kit (Morinaga Institute of Biological Science).

2.4. Quantitative real-time PCR

The hypothalami of rats from each group 20 weeks after the operation ($n = 6$ or 7 per group) were removed after overnight fasting, and total RNA was rapidly extracted with TRI reagent (Molecular Research Center, Cincinnati, OH). First-strand cDNA was synthesized from 500 ng total RNA by using PrimeScript RT Master Mix (Takara Bio, Shiga, Japan), and the resulting samples were subjected to quantitative PCR. Quantitative real-time PCR was conducted on a LightCycler system (Roche Diagnostics, Mannheim, Germany) by using the SYBR Premix Ex Taq mix system (Takara Bio). The primer set for ObRb consisted of the forward primer 5′-TGTCAGAAATTCTATGTGGTTTTGT-3′ and reverse primer 5′-TTGGATAGGCCAGGTTAAGTG-3′. The primer sequences used for the other genes have been described elsewhere [8]. The abundance of all reaction products was normalized to the level of glyceraldehyde 3-phosphate dehydrogenase (GAPDH) mRNA.

2.5. Statistical analysis

Data were analyzed by using the Statistical Package in Graph Pad Prism software version 6.0. All data were expressed as means ± standard error of the mean (SEM). Statistical significance was evaluated by using Student's *t*-test (two-tailed tests) or a two-way ANOVA with a post hoc Holm-Sidak test. *P* values less than 0.05 were considered significant.

3. Results

3.1. Food intake and body weight gain

The food intake of all midbrain-transected rats significantly increased after the operation compared with the food intake of the sham-operated rats (Fig. 1A). The midbrain-transected rats also gained weight more rapidly than the sham-operated rats (Fig. 1B).

3.2. GTT and ITT

In the GTT, blood glucose levels in the midbrain-transected rats were significantly higher at 30, 60, 90, and 120 min after glucose injection than those in the sham-operated rats (Fig. 2A, left). The AUC for the glucose was significantly higher in the midbrain-transected rats than in the sham-operated rats (Fig. 2A, right). In the ITT, blood glucose levels in the midbrain-transected rats were significantly higher than those in the sham-operated rats at 90 min after insulin injection (Fig. 2B, left), but not at other time-points. The AUC for the glucose response in the ITT was significantly higher in the midbrain-transected rats than in the sham-operated rats (Fig. 2B, right). The fasting insulin level and HOMA-IR were significantly higher in the midbrain-transected rats than in the sham-operated rats (Fig. 2C and D).