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.

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 anorexigenic proopiomelanocortin and anorectic cocaine- and amphetamine-related transcript were significantly lower in midbrain-transected rats than in sham-operated rats. Our findings promise to elucidate the mechanisms of food intake and energy balance from the perspective of multifactorial regulatory systems that underlie functions such as neurohormonal integration.

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; AUC, area under the curve; GAPDH, glyceraldehyde 3-phosphate dehydrogenase

Keywords:

Food intake; Leptin signaling; Energy homeostasis

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, that 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 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 with 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 to 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 to 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 [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 (age, 16–18 weeks; 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).

2.3 Enzyme-linked immunosorbent assay

Blood samples were obtained from the rats 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, Yokohama, Japan).

2.4 Quantitative real-time PCR

The hypothalami of rats from each group (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 relative to the level of glyceraldehyde 3-phosphate dehydrogenase (GAPDH) mRNA.

2.5 Statistical analysis

Data were analyzed by using the Statistical Package in the Graph Pad Prism software version 6.0. All data were expressed as means ± standard error of the mean. 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 gained weight more rapidly than the sham-operated rats during weeks 8 through 20 after operation (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 response 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 at 90 min after insulin injection than those in the sham-operated rats (Fig. 2B, left), but not at other timepoints. However, 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).

3.3. Plasma leptin levels and mRNA expression levels in hypothalamus

Plasma levels of leptin were significantly higher in midbrain-transected rats than in sham-operated rats (Fig. 3A); however the mRNA levels of ObRb in the hypothalamus were significantly lower in midbrain-transected rats than in sham-operated rats (Fig. 3B). To examine the involvement of anorectic and orexigenic substances produced in the hypothalamus, we evaluated the mRNA levels of AGRP, NPY, POMC, and CART in the midbrain-transected and sham-operated rats. The hypothalamic mRNA levels of AGRP and NPY did not differ between sham-operated rats and midbrain-transected rats (Fig. 4A, B), whereas those of POMC and CART were significantly lower in midbrain-transected rats than in sham-operated rats (Fig. 4C, D).

4. Discussion

In the present study, we characterized the energy balance of midbrain-transected rats, in which the neural pathway from the hindbrain to the hypothalamus had been severed. In general, the hypothalamus is thought to be important for integrating information about energy metabolism provided from the periphery via the bloodstream, neural pathways, or both [11]. In addition, the hindbrain, including the NTS, relays peripheral information about feeding to the hypothalamus [11, 12]. Indeed, signals from some meal-related metabolites, monoamines, and peptides, as well as mechanical and chemical stimuli, are transmitted to the NTS [13]. Thus, the neural pathway from the hindbrain to the hypothalamus plays a crucial role in maintaining energy homeostasis. Several gastrointestinal hormones, such as CCK, PYY, and GLP-1, function as satiety signals that are transmitted to the NTS via the vagal afferents [14]. Furthermore, we recently showed that peripheral coinjection of leptin with CCK or GLP-1 synergistically and significantly decreases food intake, and this effect is abolished in midbrain-transected rats [8, 9]. Thus, the chronic disruption of satiety signal transmission from the periphery to the central nervous system in the midbrain-transected rats may be expected to result in obesity with overeating. Consistent with this, our data demonstrated that both food intake and body weight were higher in the midbrain-transected rats than in the sham-operated rats.

Our midbrain-transected rats also showed glucose intolerance and insulin resistance. Considering that obesity is a component of metabolic syndrome and a risk factor for diabetes, we speculated that the obesity of the midbrain-transected rats induced the abnormal glucose metabolism. To investigate whether the disruption of the neural pathway from the hindbrain to the hypothalamus directly causes glucose intolerance and insulin resistance, the midbrain-transected rats will need to be examined before they become obese.

Leptin, a circulating hormone derived from white adipose tissue and the stomach, plays a key role in regulating food intake by conveying anorectic information to the brain to regulate the energy balance [15, 16]. The genetic absence of leptin results in marked hyperphagia and severe obesity in both rodents and humans [17, 18], whereas plasma leptin levels in animals and humans with diet-induced obesity are raised significantly in proportion to the increase in fat mass [19-21]. Leptin directly binds to the ObRb receptor located in several hypothalamic nuclei, including the arcuate nucleus, ventromedial hypothalamic nucleus, dorsomedial hypothalamic nucleus, lateral hypothalamus, and hypothalamic paraventricular nucleus; these nuclei strongly contribute to feeding regulation and maintenance of energy homeostasis [22]. In this study, we showed that plasma leptin levels of midbrain-transected rats were higher than those of sham-operated rats. This result correlates with the obesity of the midbrain-transected rats. However, we also showed that midbrain-transected rats had lower levels of hypothalamic ObRb mRNA than sham-operated rats. Obese animals and humans show leptin resistance as well as hyperleptinemia. Although the molecular mechanism of leptin resistance has yet to be completely elucidated, leptin resistance is thought to arise from a defect in signal transduction through ObRb [23, 24]. In this study, we failed to clarify how the disruption of the neural pathway from the hindbrain to the hypothalamus is associated with the downregulation of ObRb; however, our data at least show that leptin signaling is not fully functional in the midbrain-transected rats.

AGRP, NPY, POMC, and CART are hypothalamic neuropeptides involved in feeding and energy metabolism [25]. Defective leptin signaling is associated with increased levels of orexigenic AGRP and NPY and reduced levels of anorexigenic POMC and CART mRNA [26-29]. Recently, we

reported that coinjection of subthreshold doses of GLP-1 and leptin significantly increased POMC mRNA expression, dramatically reducing feeding [7]. In addition, we showed that coinjection of subthreshold doses of CCK and leptin significantly increased CART mRNA expression, resulting in a decrease in food intake [8]. In contrast, coinjection of GLP-1 or CCK with leptin did not affect the mRNAs of AGRP and NPY [7, 8]. GLP-1 and CCK are anorectic signaling molecules produced in the small intestine, and which are thought to be transported to the hindbrain at least in part via the vagal afferents [30, 31]. The NTS in the hindbrain transmits the anorectic or orexigenic information produced in the periphery to the hypothalamus [32]. In the current study, AGRP and NPY mRNA levels did not differ between sham-operated rats and midbrain-transected rats, whereas POMC and CART mRNA levels were significantly decreased in the midbrain-transected rats. Taken together, our findings suggest that anorectic signals such as those generated by GLP-1, CCK, and leptin are abolished in the midbrain-transected rats, which may lead to decreases in POMC and CART mRNA. Our data demonstrate that chronic interruption of anorectic signals disturbs the hypothalamic circuits that control energy balance and may induce obesity in midbrain-transected rats. To elucidate how the neural pathway from the periphery to the central nervous system controls energy balance, further investigations, including identification of neurotransmitters produced in the hindbrain, will be required.

Here we have described the metabolic characteristics of rats whose neural pathways from the hindbrain to the hypothalamus were severed. Our findings should help to understand the mechanisms of food intake and energy balance from the perspective of multifactorial regulatory systems that underlie functions such as neurohormonal integration.

Conflict of interest

No conflicts of interest, financial or otherwise, are declared by the authors.

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Figure legends

Fig. 1. Food intake and body weight. Food intake (A) and change in body weight (B) of sham-operated rats and midbrain-transected rats fed *ad libitum* after the operation. Data represent means ± SEM (n = 13–14 each). *, $P < 0.05$ *vs.* sham-operated rats, **, $P < 0.01$ *vs.* sham-operated rats, ***, $P < 0.001$ *vs.* sham-operated rats (two-way ANOVA followed by Holm-Sidak test).

Fig. 2. Glucose and insulin tolerance tests. Blood glucose levels in sham-operated rats and midbrain-transected rats were assessed with glucose tolerance tests (A) and insulin tolerance tests (B). Data represent means ± SEM (n = 4–5 each). *, $P < 0.05$ *vs.* sham-operated rats, **, $P < 0.01$ *vs.* sham-operated rats, ***, $P < 0.001$ *vs.* sham-operated rats (two-way ANOVA followed by Holm-Sidak test or Student's t-test). AUC, area under the curve.

Fig. 3. Expression of leptin and its long-form receptor. Plasma leptin levels (A) and hypothalamic ObRb mRNA levels (B) were measured in sham-operated rats and midbrain-transected rats. The expression levels of ObRb were normalized to the amount of GAPDH. Data represent means ± SEM (n = 6–7 each). **, $P < 0.01$ *vs.* sham-operated rats, ***, $P < 0.001$ *vs.* sham-operated rats (Student's t-test).

Fig. 4. Feeding-associated molecules in hypothalamus. Quantitative PCR analysis of agouti-related protein (AGRP; A), neuropeptide Y (NPY; B), proopiomelanocortin (POMC; C), and anorectic cocaine- and amphetamine-regulated transcript (CART; D) in sham-operated and midbrain-transected rats. Data were normalized to the amount of GAPDH. Data represent means ± SEM (n = 6–7 each). *, $P < 0.05$ *vs.* sham-operated rats (Student's t-test).