Lesions in the Lateral Part of the Dorsal Parabrachial Nucleus Caused Hyperphagia and Obesity

Katsuya Nagai,1 Hidetoshi Ino,1 Hideki Yamamoto,1 Hachiro Nakagawa,1,* Mariko Yamano,2 Masaya Tohyama,2 Sadao Shiosaka,2 Yahe Shiotani,2 Shinobu Inagaki,3 and Shozo Kitoh3

1Division of Protein Metabolism, Institute for Protein Research, Osaka University, Suita 565, Japan
2Department of Neuroanatomy, Institute of Higher Nervous Activity, Osaka University Medical School, Osaka 553, Japan
3The Third Department of Internal Medicine, Hiroshima University School of Medicine, Hiroshima 734, Japan

(Received February 20, 1987)

Summary For clarification of the role of CCK-8-like immunoreactive neurons in the lateral part of the nucleus parabrachialis dorsalis (PBD), which have fibers projecting to the ventromedial hypothalamus (VMH), the effects of unilateral and bilateral lesions in the lateral part of the PBD on increase in body weight, food and water intake, and metabolism were examined in female Sprague-Dawley rats. Unilateral and bilateral lesions caused significant increases in food and water intake, body weight gain, Lee’s index, and the weights of parametrial adipose tissue, liver, and kidney. These lesions resulted in increased serum concentrations of insulin and urea, while the liver phosphoenolpyruvate carboxykinase activity tended to be decreased. These effects were quite similar to, but slightly less than, the effects of bilateral lesions of the VMH. On the side of either unilateral or bilateral lesions, the VMH did not contain CCK-8-like immunoreactive fibers. These findings support the hypothesis that the CCK-8-like immunoreactive neurons in the lateral part of the PBD send signals to the VMH and by relaying signals there they participate in the regulation of food intake and metabolism, their firing suppressing food intake and insulin secretion. However, it is also possible that the lesions induced the above changes by destroying neurons descending and ascending to other brain sites or neural fibers passing through the lesion area.

*To whom correspondence should be addressed.
Recently Inagaki et al. [1] presented evidence suggesting that in rats a CCK-8 (C-terminal octapeptide of cholecystokinin)-like immunoreactive substance is present in neurons in the lateral part of the nucleus parabrahchialis dorsalis (PBD) and that fibers from these neurons extend to the ventromedial hypothalamus (VMH). On the other hand, cholecystokinin has been shown to decrease food intake [2], and recently we found that intrahypothalamic infusion of CCK-8 derivatives, such as glutaryl-CCK-8 and pyroglutamyl-CCK-8, reduced food intake in rats [3]. Furthermore results have indicated that the VMH is involved in not only regulation of food intake (as a satiety center) [4–6], but also regulation of energy metabolism [4–11]. These findings suggest the working hypothesis that CCK-8-like immunoreactive neurons in the PBD are involved in control of energy metabolism as well as feeding behavior through mediation of the function of the VMH. To examine this hypothesis, we studied the effects of bilateral or unilateral lesions in the lateral part of the PBD on food intake and metabolism.

As described in detail in this paper, we found that both unilateral and bilateral lesions in the PBD caused hyperphagia, obesity, and hyperinsulinemia.

MATERIALS AND METHODS

Seventy-one female Sprague-Dawley rats, initially weighing 130–180 g, were used. They were adapted to an animal room for 1 week and then divided into three groups. Using bipolar electrodes we made bilateral, electrolytic lesions in the lateral part of the PBD of rats in one group (n=37) under pentobarbital anesthesia. The coordinates of the lesions were 9.1 mm posterior to the bregma, 1.9 mm lateral to the midsagittal line, and 6.0 mm below the brain surface, and were determined according to the brain atlas of Paxinos and Watson [12]. The lesions were made with a current of 30 μA for 10 min. Since simultaneous lesions of the PBD on both sides results in death, a unilateral lesion was made first and the contralateral lesion was made 3 days later (bilateral lesions). Rats in another group (n=21) received a lesion in the left side of the PBD with a sham-operation (without current) on the right side 3 days later (unilateral lesion). Rats in the control group (n=13) received sham-operation on both sides in the same way (bilateral sham-operation).

The animals were housed individually in stainless steel cages, and given food (MF, Oriental Yeast, Tokyo) and water ad libitum. The animal room was maintained at 24 ± 1°C and 60 ± 10% relative humidity and illuminated for 12 h from 08:00 h every day. The rats were weighed once a week, and their mean daily water and food intake per week was measured from 3 weeks after the operation. Eighty-six days after the first operation, 26 of the rats with bilateral lesions, 19 with uni-
laterally one and 12 of the sham-operated animals were alive. Their naso-anal lengths were measured, the animals deprived of food, and they were then sacrificed 2 h later (about 16:00 h). At the time of sacrifice, trunk blood was collected, and the liver, kidneys and parametrial adipose tissues were removed and weighed. The liver and kidney were frozen until phosphoenolpyruvate carboxykinase activity was assayed.

Serum glucose was determined by the glucose oxidase method (Blood Sugar-GOD-Perid-Test Kit, Boehringer, Mannheim); serum insulin, by solid-phase radio-immunoassay (Phadebas Insulin Test, Daiichi Radioisotope Institute Co., Tokyo) with porcine insulin as a standard; and serum urea, by the method of Archibald [13]. Phosphoenolpyruvate carboxykinase (PEPCK), a key gluconeogenic enzyme in the liver and kidney, was assayed as described previously [14]. Protein concentration was determined by the micro-biuret method [15] with bovine serum albumin as a standard.

The brains of rats were fixed by perfusion with formalin, embedded in paraffin, sectioned, and stained with cresyl violet. Areas of lesions were examined microscopically. CCK-8-like immunoreactivity was examined in fibers in the area of the VMH by the indirect immunofluorescence technique with antibody against CCK-8 as described previously [1]. Data on animals (19 with bilateral lesions and 10 with unilateral one) in which the lesion(s) was incomplete or in which CCK-8-like immunoreactive fibers had not disappeared from the VMH on the side of the lesion were excluded from analyses.

Data were expressed as means ± standard errors of means. Statistical analyses were performed by Student's t-test and analysis of variance (ANOVA).

RESULTS

Histological examination of rat brain

Figure 1 shows a photomicrograph of the brain of a rat with bilateral lesions in the lateral part of the PBD. The lesion involved the lateral parts of the PBD bilaterally, parts of the ventral parabrachial nucleus, the superior cerebellar peduncle, the external and central nucleus of the inferior colliculus, the sagulum nucleus, the dorsal nucleus of the lateral lemniscus, the ventral spino-cerebellar tractus, and the area around the PBD. The fluorescence photomicrographs in Fig. 2 show the changes in CCK-8-like immunoreactive fibers in the VMH after a sham-operation (A) or after a unilateral (B) or bilateral (C) lesion is made in the lateral part of the PBD. Fluorescence due to CCK-8 was not detected on the side of the lesion(s) of the VMH.

Body weight and daily intake of food and water

As shown in Fig. 3, a unilateral lesion in the lateral part of the PBD resulted in a greater increase in body weight than seen in sham-operated rats, and bilateral lesions resulted in an even more pronounced increase. Figure 4 shows the mean
daily food and water intake from week 3 after the operation. Food intake by rats with bilateral lesions was much greater (mean intake from week 3 to week 12 after the operation: 21.9 g/day) than that by rats with unilateral lesions (20.7 g/day), while that by sham-operated rats was less (18.5 g/day) (Fig. 4A). Similar differences were observed in daily water intake: rats with bilateral lesions (mean intake from week 3 to week 12 after the operation: 30.9 ml/day) > those with unilateral lesion (29.5 ml/day) > sham-operated rats (27.4 ml/day) (Fig. 4B). These findings indicate that the lesions in the lateral part of the PBD result in an increase in body weight as well as in food and water intakes.

Lee's index and weights of parametrial adipose tissue, liver, and kidney

Figure 5 shows the change in naso-anal length 86 days after the operation. PBD lesions also resulted in increased naso-anal length, the order being as follows: bilateral lesion > unilateral lesion > sham-operation.

Lee's index, represented by $\sqrt{\frac{\text{body weight (g)}}{\text{naso-anal length (cm)}}}$, was also greater in rats with lesions, and the difference between the values for rats with bilateral lesions and sham-operated rats was statistically significant. In accordance with Lee's index, the weight of the parametrical adipose tissue was increased by the PBD lesion(s) in the following order: bilateral lesions > unilateral lesion > sham-operation. The weights of the liver and kidney were also increased.
PBD LESIONS CAUSED OBESITY

by the lesion(s). These findings show that lesion(s) induced in the lateral part of the PBD causes obesity.

Serum concentrations of glucose, insulin, and urea

As shown in Fig. 6, the serum glucose concentration after food deprivation for 2 h in rats with a PBD lesion(s) was similar to that in rats without the lesion(s). It should be noted, however, that the serum insulin level in rats with PBD lesions was higher than that in sham-operated rats, and that it was higher in rats with bilateral lesions than in those with a unilateral lesion. The serum urea concentration in rats with PBD lesions was higher than that in sham-operated rats, though the difference was small.

Phosphoenolpyruvate carboxykinase activity in liver and kidney

The changes in PEPCK activity after PBD lesions are shown in Fig. 7. The
Fig. 3. Change in body weight after lesions are made in the lateral part of the nucleus parabrachialis dorsalis. Bilateral and unilateral lesions, and sham-operation, are explained in the text. Numbers of animals used are indicated in parentheses. Values are mean ± SEM. ◦, Sham-operated (12); ▲, unilateral lesion (9); ●, bilateral lesions (7). The statistical significances of differences between groups by ANOVA are as follows: Sham-operated vs. unilateral lesion, p<0.0005; sham-operated vs. bilateral lesions, p<0.0005; unilateral lesion vs. bilateral lesions, p<0.0005.

Fig. 4. Changes in mean daily food (A) and water (B) intake after formation of PBD lesions. Food and water intake was measured from week 3 after the operation. Numbers of animals used and other details are the same as for Fig. 3. ◦, Sham-operated, ▲, unilateral lesion; ●, bilateral lesions. Statistical significances of differences between groups by ANOVA are as follows: Food intake, sham-operated vs. unilateral lesion, p<0.0005; sham-operated vs. bilateral lesions, p<0.0005; unilateral lesion vs. bilateral lesions, p<0.05. Water intake: sham-operated vs. unilateral lesion, p<0.05; sham-operated vs. bilateral lesions, p<0.001; unilateral lesion vs. bilateral lesions, p<0.05.

enzyme activity in the liver was significantly lower in rats with bilateral lesions in the PBD than in rats with unilateral one, and tended to be lower in rats with bilateral lesions in the PBD than in sham-operated rats. PEPCK activity in the kidney was not affected by PBD lesions.
DISCUSSION

In this work we found that lesions in the lateral part of the PBD caused 1) increases in food and water intake, 2) an increase in body weight with increased weights of the parametrial adipose tissue, liver, and kidney, 3) increases in naso-anal length and Lee's index, 4) increases in serum concentrations of insulin and urea, and 5) a tendency of a decrease in liver PEPCK activity in addition to disappearance of CCK immunoreactive fibers in the VMH. These changes are quite similar to those seen after bilateral lesions of the VMH [4, 7-10, 16, 17]. Furthermore, concerning the central action of CCK in rats, McCaleb and Myers [18] and we [3] reported that intrahypothalamic infusions of CCK and CCK-8 derivatives elicited suppression of food intake due to noradrenaline and circadian rhythm, respectively. From these facts the present findings support the working hypothesis that the CCK-8-like immunoreactive neurons in the lateral part of the PBD send axons to the VMH and by mediation of the latter suppress food intake and insulin secretion. However, it is also possible that the destruction of neurons descending and ascending to other brain sites or of neural fibers passing through the lesioned area by the PBD lesions might induce the above changes.

We have found that in female Sprague-Dawley rats, the increase in body weight by 12 weeks after formation of bilateral lesions in the VMH and sham-operations were about 300 g and 80 g, respectively (unpublished observations). In the present study we found that increases in body weight at 12 weeks after bilateral lesions were made in the PBD and after sham-operations were about 210 g and 130 g, respectively (Fig. 3). The ages of the rats used in the two experiments were slightly different, but bilateral lesions in the VMH apparently caused a greater increase in body weight than bilateral lesions in the PBD, judging from the ratios of body weight increase in rats with lesions to that in sham-operated rats (VMH lesions vs. PBD lesions: 2.3 vs. 1). Thus we speculate that regulation of food intake by the VMH is not controlled only by the CCK-8 neurons in the lateral part of the PBD.

Bilateral lesions in the VMH of rats is reported to result in enhanced insulin
secretion and hyperinsulinemia [4, 7, 10] and also an increase in the blood urea concentration [9, 16, 17]. Consistent with these reports, we found in this work that the serum insulin concentration was significantly higher and the serum urea concentration slightly higher in rats with unilateral and bilateral PBD lesions than in sham-operated rats (Fig. 6). These findings raise the questions of whether increases in insulin and urea concentrations are brought about indirectly by hyperphagia and obesity or are induced directly by modification of the neuronal activity in the VMH by the PBD lesions. Our finding that hyperinsulinemia and increased serum urea concentration due to PBD lesions occurred even after deprivation of food for 2 h (Fig. 6) supports the latter possibility.

We reported previously that after bilateral lesions of the VMH, PEPCK activity decreased in the liver, and increased in the kidney [8]. In the present study PBD lesions elicited a tendency toward a decrease in liver PEPCK activity (Fig. 7), although they did not affect the kidney enzyme activity. The change in liver PEPCK activity induced by PBD lesions is similar to that induced by VMH lesions. Thus it seems quite likely that CCK-8-like immunoreactive neurons in the lateral part of the PBD are involved in suppression of food intake and insulin secretion by modification of the neuronal activity in the VMH. This possibility requires further study for its verification. We also found that water intake increased after PBD lesions. It has been shown that in rats, at least 70% of the water intake depends on food intake (prandial drinking) [19]. The question of whether the increase in water intake due to PBD lesions is simply caused by hyperphagia or whether it is caused by some other mechanism must also be studied.

Recently, gustatory and visceroreceptive afferent neural connections were found to project to the nucleus parabrachialis through the nucleus tractus solitarius [20–22], and these afferent neural connections were suggested to influence feeding behavior and metabolism [23, 24]. Thus, the CCK-8-like immunoreactive neurons in the PBD, projecting to the VMH, may well be involved in these mechanisms.

REFERENCES


