NOTE *Physiology*

Increased Locomotor Activity, Increased Food and Water Intake and Decreased PVN Neurons in H1 Calponin Gene-Deficient Mice

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ABSTRACT. Calponin (h1 or basic) is an actin-binding protein that is expressed abundantly in smooth muscle. Our previous study using h1 calponin-null mutant mice demonstrated that h1 calponin inhibits the shortening velocity of smooth muscle contraction without significantly affecting the amplitude of force production. Furthermore, early onset of osteogenesis and increased bone formation have been reported in mutated mice. In the present study, we examined the effect of h1 calponin depletion on the metabolism and behavior of mice and found that the mutated mice showed increased locomotor activity, as well as increased intake of food and water, associated with the decreased number of neurons in the paraventricular nucleus of the hypothalamus (PVN).

KEY WORDS: calponin knockout, metabolism, PVN.

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H1 calponin is an actin-binding protein that inhibits actin-activated myosin ATPase activity *in vitro* [6]. Although h1 calponin is widely used as a smooth muscle-specific marker, evidence has been accumulating that this protein is expressed in other types of cells, such as Ito cells, neuroblastomas and osteosarcomas [5, 7, 8]. For example, one study of non-smooth muscle cells revealed that h1 calponin suppressed bone formation [9]. However, the role of h1 calponin in other non-smooth muscle cells has been scarcely understood. During the course of our studies on smooth muscle contractility in h1 calponin-deficient mice [3, 10], we noticed that the mutated mice were more active than the wild-type mice. We report on this observation herein.

We first examined food and water intake in the wild-type and h1 calponin gene-deficient mice (C57BL/6-*CNh1*$^{tm/sst}$). Homozygous mutated mice were generated as reported previously (9). Twelve-week-old male mice were used. For 2 weeks, the amounts of food and water intake were measured every 4 days after the mice had become habituated in individual cages. All mice were housed under constantly controlled environmental conditions (room temperature: 23 ± 1°C, lights on from 07:00 to 19:00). As shown in Fig. 1, food and water intake were significantly increased in the mutated mice. During the course of the experiments, the increase in the body weight of the mutated mice did not differ from that of the control mice (data not shown).

We next examined the locomotor activity of these mice. The locomotor activity of each mouse was monitored with an infrared counter (Muromachi Kikai, Tokyo, Japan) and the data were collected as a series of total counts per 30 min-

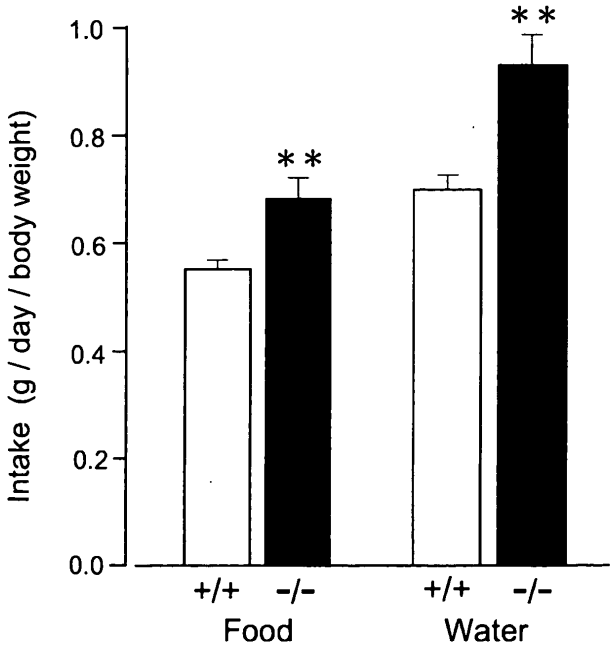

Fig. 1. Food and water intake of the wild-type mice (n=5, empty column) and h1 calponin-deficient mice (n=5, filled column). Each column and vertical bar represent the mean ± S.E.M. *; $p<0.05$ in Student's *t*-test.

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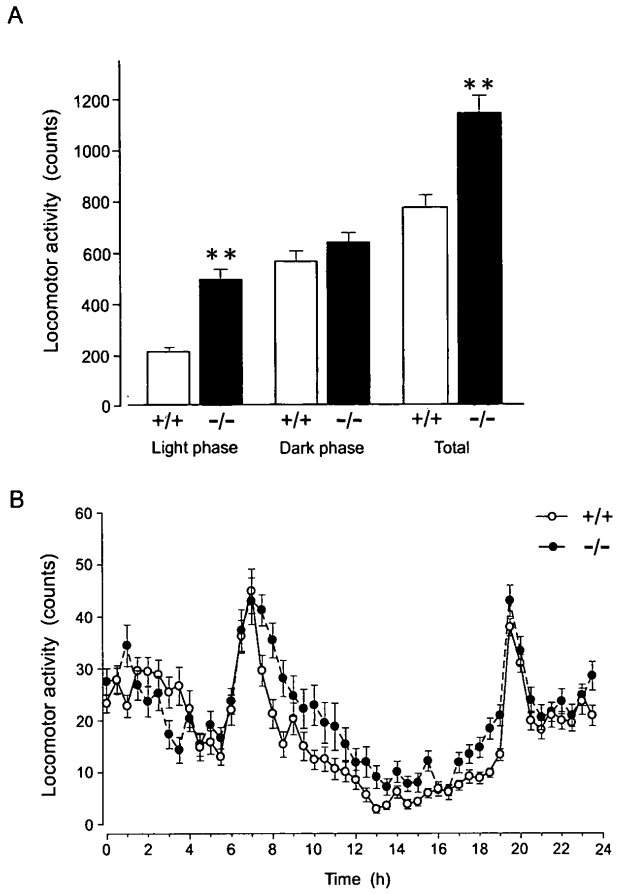

Fig. 2. A: Locomotor activity of wild-type mice (n=9, empty column) and mutated mice (n=8, filled column). Values are the sums of light and dark phase counts. Each column and vertical bar represent the mean ± S.E.M. *; $p<0.05$ in Student's *t*-test. B: Averaged (± S.E.M) daily pattern of locomotor activity of the same mice (open circle, wild type; closed circle, mutated mice).

utes in a microcomputer (9801 VM, NEC, Tokyo, Japan). The results showed that light-phase locomotor activity was significantly increased in the mutated mice, while dark-phase activity in the mutated mice was the same as that in the wild-type mice (Fig. 2A). The peak phase of activity was observed at the beginning and end of the dark phase in either mice (Fig. 2B).

Because the hypothalamus is the region that regulates feeding and locomotor activity, we performed histological analyses of the hypothalamus of the wild-type and mutated mice. The mice were anesthetized with sodium pentobarbital (50 mg/kg body weight) and perfused intracardially with 0.85% physiological salt saline followed by a fixative consisting of 4% paraformaldehyde in 0.1 M phosphate-buffered saline (pH 7.4). The brain was dissected out, immersed in the same fixative for 2 hr at 4°C, and stained with 0.1% cresyl violet acetate (Sigma, St. Louis MO, U.S.A.). The slides were observed and photos were taken under light microscopy. Cresyl-violet-positive cells in the paraventricular nucleus of the hypothalamus (PVN) and in the suprachiasmatic nucleus of the hypothalamus (SCN) were counted on the photos. The results showed that the number of cresyl-violet-positive cells in SCN was comparable between the wild-type and the mutated mice. In contrast, the number of the cells in PVN was significantly lower in the mutated mice (Fig. 3).

Hypothalamus is a central region that regulates appetite and energy expenditure and PVN- or VMH-lesioned rats are known to be hyperphagia and obese (1, 11). The locomotor activity of PVN-lesioned rats retains a circadian rhythm, meanwhile that of VMH-lesioned rats is disrupted (11). In the present study, h1 calponin gene-deficient mice displayed hyperphagia, associated with the decreased number of cresyl-violet-positive cells in PVN. The mutated mice, how-

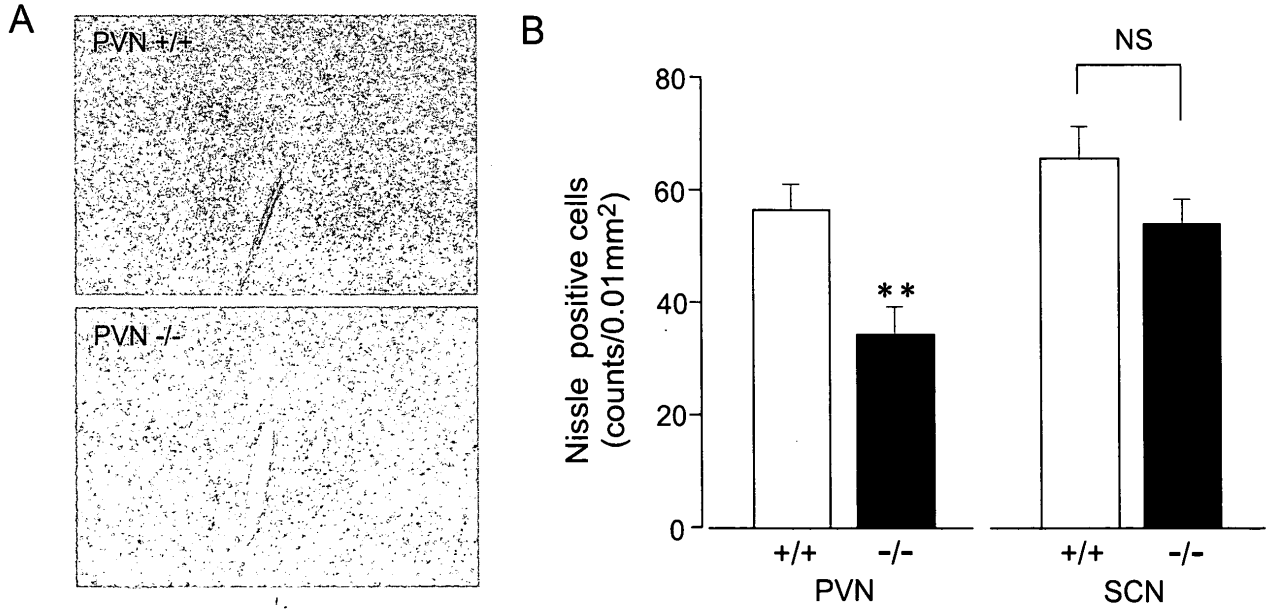

Fig. 3. A: Microscopic images of cresyl-violet-positive cells in PVN (upper: wild-type mice; lower: mutated mice). B: The number of cresyl-violet-positive cells in 0.01 mm^2 in PVN and SCN of wild-type (empty column, n=6) and mutated mice (filled column, n=7). Each column and vertical bar represent the mean ± S.E.M. *; $p<0.05$ in Student's *t*-test.

ever, were not obese. The locomotor activity of the mutated mice was higher than that of the wild type mice, which might have resulted in higher energy expenditure and escaped obesity. The result that the mutated mice were still more active in the dark phase than in the light phase may suggest that the hyperphagia is mediated by the impairment of PVN, but not VMH. On the other hand, it has been reported that the feeding is also controlled by gastrointestinal contractility through vagal afferent fibers [2], and our previous study showed that smooth muscle from calponin gene-deficient mice indicated a higher cross-bridge cycling rate than that from the wild-type mice [3]. Therefore, it is possible that the change in smooth muscle contractility of the gastrointestinal tract influenced the food intake in the mutated mice. Further studies are needed to identify the precise role of h1 calponin in mammalian feeding behavior.

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REFERENCES

1. Williams, G., Bing, C., Cai, X. J., Harrold, J. A., King, P. J. and Liu, X. H. 2001. *Physiol. Behav.* **74**: 683–701.
2. Marthis, C., Moran, T. H. and Schwartz, G. J. 1998. *Am. J. Physiol.* **274**: R280–286.
3. Takahashi, K., Yoshimoto, R., Fuchibe, K., Fujishige, A., Mitsui-Saito, M., Hori, M., Ozaki, H., Yamamura, H., Awata, N., Taniguchi, S. I., Katsuki, M., Tsuchiya, T. and Karaki, H. 2000. *Biochem. Biophys. Res. Commun.* **279**: 150–157.
4. Turek FW. 1985. *Annu. Rev. Physiol.* **47**: 49–64.
5. Ueki, N., Ohkawa, T., Yamamura, H., Takahashi, K., Tsutsui, T., Kawai, Y., Yokoyama, Y., Amuro, Y., Hada, T. and Higashino, K. 1998. *Biochim. Biophys. Acta.* **1403**: 28–36.
6. Winder, S. J. and Walsh, M. P. 1990. *J. Biol. Chem.* **265**: 10148–10155.
7. Yagi, M., Sugimoto, T., Mine, H., Horii, Y., Matsumura, T., Sawada, T., Abe, T., Sano, K., Goshi, J. and Takahashi, K. 1998. *Hum. Cell.* **11**: 231–238.
8. Yamamura, H., Yoshikawa, H., Tatsuta, M., Akedo, H. and Takahashi, K. 1998. *Int. J. Cancer.* **79**: 245–5020.
9. Yoshikawa, H., Taniguchi, S. I., Yamamura, H., Mori, S., Sugimoto, M., Miyado, K., Nakamura, K., Nakao, K., Katsuki, M., Shibata, N. and Takahashi, K. 1998. *Genes Cells.* **3**: 685–695.
10. Yoshimoto, R., Hori, M., Takahashi, K., Taniguchi, S. I., Katsuki, M., Ozaki, H. and Karaki, H. 2000. *Jpn. J. Pharmcacol.* **84**: 474–476.
11. Tokunaga, K., Matsuzawa, Y., Fujioka, S., Kobatake, T., Keno, Y., Odaka, H., Matsuuo, T. and Tarui, S. 1991. *Brain Res. Bull.* **26**: 393–396.