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Ventromedial Hypothalamic Lesions Reduce the Number of Na^+,K^+-ATPase Enzyme Units in Skeletal Muscle of Weanling Rats[1] (41200)

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Abstract. Weanling rats with ventromedial hypothalamic (VMH) lesions retained 66% more body energy 1 to 4 weeks postsurgery than did nonlesioned control rats. The increased energy retention in VMH-lesioned rats resulted from diminished energy expenditure rather than from elevated energy intake. Numbers of Na^+,K^+-ATPase enzyme units in skeletal muscle and kidneys of these rats were estimated from saturable [^{3}H]ouabain binding to particulate fractions. Skeletal muscle of VMH-lesioned rats had 36% fewer Na^+,K^+-ATPase enzyme units than did skeletal muscle of control rats. VMH-lesions did not affect the number of Na^+,K^+-ATPase enzyme units in kidneys. These results are comparable to earlier reports on Na^+,K^+-ATPase enzyme units in muscle and kidneys of obese (ob/ob) mice and suggest that there is an association between development of obesity and reduced number of Na^+,K^+-ATPase enzyme units in skeletal muscle.

Weanling rats with lesions in the ventromedial hypothalamus (VMH) exhibit normal food intake and body weight gain, but they deposit more body fat than nonlesioned control rats (1). Increased fat deposition (and presumably increased total body energy retention) in the presence of normal food intake suggests that energy expenditure is reduced in VMH-lesioned, weanling rats. However, direct estimates of total body energy retention and energy expenditure in VMH-lesioned, weanling rats are not available.

ATP turnover associated with active transport of sodium is thought to contribute significantly to an animal's energy expenditure (2, 3), although there is disagreement on this issue (4–6). Genetically obese mice (ob/ob and db/db) have alterations in Na^+,K^+-ATPase (7–10), the enzyme associated with active sodium transport, and reduced energy expenditure (11, 12). To what extent the observed reductions in Na^+,K^+-ATPase in these obese animals are causally related to their development of obesity is unclear. Likewise, it is unclear how universal the association between altered Na^+,K^+-ATPase and development of obesity is in other animals. Bray *et al.* (9) found that Na^+,K^+-ATPase activities in livers of gold-thioglucose-lesioned mice, obese yellow mice, and Zucker obese rats (made hypothyroid or treated with exogenous triiodothyronine) were not different from respective lean control values. They did not examine Na^+,K^+-ATPase in other tissues of these obese animals. In obese humans the number of Na^+,K^+-ATPase enzyme units in erythrocytes is lower than in erythrocytes from nonobese controls (13), but again Na^+,K^+-ATPase in other tissues was not examined.

The purpose of this report was to obtain direct estimates of energy retention and metabolic rate in VMH-lesioned, weanling rats and to measure the number of Na^+,K^+-ATPase enzyme units in skeletal muscle and kidneys of these rats. These tissues were selected because we had previously shown that ob/ob mice exhibited a pronounced reduction in the number of Na^+,K^+-ATPase enzyme units in skeletal muscle (7, 10) whereas the number of enzyme units in kidneys of ob/ob mice was not altered (10).

Materials and Methods. Female Sprague–Dawley rats,[2] obtained at 21 days of age, were housed individually in stainless-steel cages and fed a high-carbohydrate diet (14) *ad libitum*. Lighting (on from 7 AM to 7 PM) and ambient temperature (23–25°) were

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[2] Spartan Research Animals, Inc., Haslett, Mich.

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TABLE I. ENERGY INTAKE, BODY WEIGHT GAIN, OXYGEN CONSUMPTION, AND ENERGY RETENTION OF SHAM-OPERATED AND VMH-LESIONED RATS[a]

	Sham operated	VMH lesioned
Metabolizable energy intake, kcal/3 weeks	1245 ± 30	1324 ± 141
Body weight gain, g/3 weeks	101 ± 2	107 ± 10
Oxygen consumption, ml/rat/hr[b]	315 ± 11	280 ± 9*
Energy retention, kcal/3 weeks[c]	263 ± 10	437 ± 71*

[a] Values represent means ± SEM for 11 sham-operated and 6 VMH-lesioned rats. At the start of the experiment, 1 week after surgery, sham-operated rats weighed 94 ± 1 g and VMH-lesioned rats weighed 93 ± 8 g. Asterisks indicate significant differences ($P < 0.05$) between VMH-lesioned and sham-operated rats as determined by Student's t test.

[b] Oxygen consumption was measured in 13 sham-operated and 13 VMH-lesioned rats at 25° between 3 and 4 weeks after surgery.

[c] Body energy (kcal) measured at 4 weeks after surgery minus that estimated at 1 week after surgery (equations presented in Fig. 1).

controlled automatically. Water was available at all times.

After a period of 2 or 3 days to adapt to the laboratory and high-carbohydrate diet, rats were matched for body weight and divided into two groups. One group received bilateral electrolytic lesions in the VMH and the other group served as sham-operated controls. Rats were anesthetized with sodium pentobarbital (3.5 mg/100 g body wt, ip) and lesions were stereotaxically[3] placed within the VMH using coordinates suggested for weanling rats by Bernardis and Skelton (15). Lesions were produced by passing 1.5 mA of anodal current[4] through a stainless-steel needle which was insulated except for 0.5 mm at the tip. Current was allowed to flow for 10 sec between the needle tip and a second electrode attached to the incisor bar. Sham-operated rats were subjected to an identical procedure except no current was allowed to flow between electrodes. After surgery all rats were injected (im) with approximately 20,000 units of penicillin.[5] Locations of the lesions were examined histologically in 6 μ thick sections stained with cresyl violet.

Oxygen consumption was measured by placing each rat in a chamber maintained at 25°. A volume meter[6] delivered oxygen to the chamber and recorded the rate of oxygen consumption. Soda lime was used to remove CO_2 from the chamber. VMH-lesioned and sham-operated rats were killed 1 and 4 weeks after surgery to estimate body energy gain and efficiency of

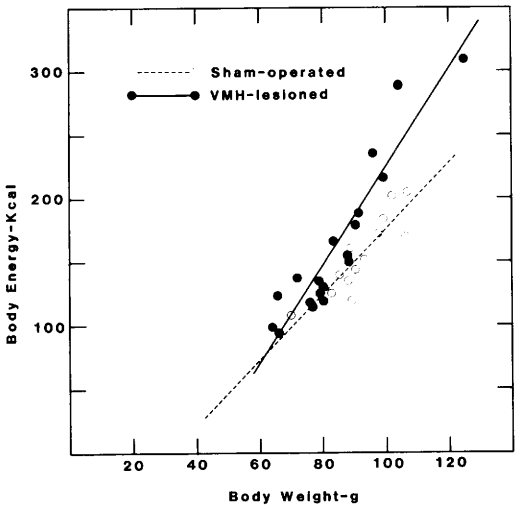

FIG. 1. Body energy of VMH-lesioned and sham-operated control rats as a function of body weight at 1 week after surgery. Linear regressions for VMH-lesioned and sham-operated rats were $y = 3.86x - 162.54$, standard error of the slope (SE_b) = 0.35, and $y = 2.57x - 81.79$, $SE_b = 0.41$, respectively. The slope of the linear regression calculated for VMH-lesioned rats was significantly greater ($P < 0.05$) than that calculated for sham-operated rats. These equations were used to estimate initial body energy of rats killed 4 weeks after surgery.

[3] David Kopf Instruments, Tujunga, Calif.

[4] Grass Instrument Co., Quincy, Mass.

[5] Combiotic, Chas. Pfizer and Co., Inc., New York, N.Y.

[6] Med Science Electronics, Inc., St. Louis, Mo.

energy retention. Carcass energy content was determined as previously described (14).

At the end of the 4-week feeding period rats were killed by cervical dislocation. Gastrocnemius muscle and kidneys were rapidly removed after perfusing each rat with cold saline via the left ventricle of the heart. Numbers of Na^+,K^+-ATPase enzyme units (EC 3.6.1.3) in gastrocnemius muscle and kidneys were estimated by using the [^{3}H]ouabain binding method previously described (7, 10, 16). Protein content of the particulate fractions of muscle and kidneys was determined (17).

Results and Discussion. *Energy retention.* The VMH-lesioned, weanling rats did not consume more energy or gain more body weight than controls (Table I). Rates of oxygen consumption, however, were about 10% lower in VMH-lesioned rats than in control rats. Within 1 week after surgery VMH-lesioned rats already had slightly greater body energy content than control rats (Fig. 1). VMH-lesioned rats retained 66% more energy than control rats between 1 and 4 weeks postsurgery (Table I).

Efficiency of energy retention averaged 32 ± 2% (retained/intake) in VMH-lesioned rats and 21 ± 1% in control rats. Young obese (ob/ob) mice also retain a greater proportion of their energy intake than lean mice (18). In both VMH-lesioned, weanling rats and 1- to 3-week-old obese (ob/ob) mice (12, 18) the increased efficiency of energy retention results from diminished energy expenditure rather than from elevated energy intake.

[^{3}H]Ouabain binding in skeletal muscle. Gastrocnemius muscle weight 4 weeks postsurgery was 15% lower in VMH-lesioned rats than in control rats whereas particulate protein concentration in muscle was 20% higher in VMH-lesioned rats than in control rats (Table II). As a result particulate protein content per muscle was unchanged by the VMH-lesions. Nonspecific binding of [^{3}H]ouabain to the muscle preparations was estimated in the presence of excess nonlabeled ouabain. VMH lesions did not affect nonspecific binding of [^{3}H]ouabain to muscle (Table II). Specific binding of [^{3}H]ouabain to muscle preparations from VMH-lesioned rats was 35% lower than observed in preparations from control rats. Because the affinity of ouabain binding sites (K_d values are reciprocal indexes of affinity) for [^{3}H]ouabain was unaffected by VMH lesions, the lower specific binding of [^{3}H]ouabain to muscle from VMH-lesioned rats indicates that VMH lesions reduce the number of [^{3}H]ouabain binding sites in muscle. Binding site number was 36–37% lower in muscle of VMH-lesioned rats than in muscle of control rats (Table II).

TABLE II. GASTROCNEMIUS MUSCLE WEIGHT AND [^{3}H]OUABAIN BINDING TO MUSCLE PREPARATIONS FROM SHAM-OPERATED AND VMH-LESIONED RATS[a]

Gastrocnemius	Sham operated	VMH lesioned
Total weight, g	1.10 ± 0.03	0.94 ± 0.03*
Particulate protein, mg/g muscle	111 ± 7	131 ± 4*
Nonspecific [^{3}H]ouabain binding, pmole/mg protein[b]	1.09 ± 0.03	1.04 ± 0.06
Specific [^{3}H]ouabain binding, pmole/mg protein[c]	0.40 ± 0.03	0.26 ± 0.02*
K_d value, μM[d]	0.30 ± 0.03	0.32 ± 0.05
[^{3}H]Ouabain binding sites, pmole/mg protein[d]	0.73 ± 0.04	0.47 ± 0.04*
pmole/gastrocnemius muscle[d]	95 ± 11	60 ± 5*

[a] Values represent means ± SEM for nine pairs of sham-operated and VMH-lesioned rats, except only four or five pairs of rats were used to calculate K_d values. Asterisks indicate significant differences ($P < 0.05$) between VMH-lesioned and sham-operated rats.

[b] [^{3}H]Ouabain (0.4 μM) binding observed in the presence of excess nonlabeled ouabain (4 mM).

[c] [^{3}H]Ouabain (0.4 μM) binding minus [^{3}H]ouabain (0.4 μM) binding observed in the presence of excess nonlabeled ouabain (4 mM).

[d] Calculated as described by Akera and Cheng (16).

TABLE III. KIDNEY WEIGHT AND [^{3}H]OUABAIN BINDING TO KIDNEY PREPARATIONS FROM SHAM-OPERATED AND VMH-LESIONED RATS[a]

Kidney	Sham operated	VMH lesioned
Weight of both kidneys, g	0.76 ± 0.02	0.71 ± 0.07
Particulate protein, mg/g kidney	111 ± 2	107 ± 4
Nonspecific [^{3}H]ouabain binding pmole/mg protein[b]	0.94 ± 0.04	0.91 ± 0.05
Specific [^{3}H]ouabain binding, pmole/mg protein[c]	0.87 ± 0.05	0.81 ± 0.04
K_d value, μM[d]	8.32 ± 0.29	8.11 ± 0.24
[^{3}H]Ouabain binding sites,		
pmole/mg protein[d]	18.92 ± 1.01	17.26 ± 0.90
pmole/both kidneys[d]	1539 ± 97	1279 ± 142

[a] Values represent means ± SEM. Nine pairs of sham-operated and VMH-lesioned rats, except only four or five pairs of rats were used to calculate K_d values. No significant differences were observed.
[b,c,d] See Table II.

The [^{3}H]ouabain binding data in muscle of VMH-lesioned rats are consistent with our earlier observations in hindlimb muscles of obese (ob/ob) mice (7, 10). In neither VMH-lesioned rats nor obese mice was nonspecific [^{3}H]ouabain binding or affinity of binding sites for [^{3}H]ouabain altered. However, in muscles of both VMH-lesioned rats and obese mice the number of Na^+,K^+-ATPase enzyme units, as indicated by the number of ouabain binding sites, is diminished. Further, the extent of reduction in enzyme units in muscle of VMH-lesioned rats (36–37%) is comparable to that observed in muscle of 4- to 8-week-old obese mice (34–59%) (10).

[^{3}H]Ouabain binding in kidneys. Table III presents data on kidney weights and [^{3}H]ouabain binding parameters in VMH-lesioned and control rats. None of the parameters measured were affected by VMH lesions. The number of ouabain binding sites is not diminished in kidneys of obese mice either (10).

General Discussion. A primary role for reduced energy expenditure, rather than increased energy intake, in the initial development of obesity in weanling VMH-lesioned rats and in young obese (ob/ob) mice (10, 18) and young obese (fa/fa) rats (19, 20) is now evident. Diminished nonshivering thermogenesis has been identified as the major contributor to reduced energy expenditure in obese mice maintained at standard laboratory room temperature (21), but less is currently known about the source of reduced energy expenditure in VMH-lesioned rats or obese (fa/fa) rats. Diminished nonshivering thermogenesis may not be a major contributor to reduced energy expenditure in weanling VMH-lesioned rats because weanling VMH-lesioned rats consume less oxygen (225 ± 7 ml/rat/hr) even at 30° than control rats (260 ± 5 ml/rat/hr) (unpublished observation).

Numbers of Na^+,K^+-ATPase enzyme units are reduced in skeletal muscle, but not in kidneys, of both VMH-lesioned and obese (ob/ob) mice (10). This suggests the involvement of a common mechanism influencing Na^+,K^+-ATPase in these two obese animals. Because Na^+,K^+-ATPase in several tissues is dependent on the thyroid hormone status of the animal (3), it is possible that the subtle impairments that have been noted in thyroid hormone status of VMH-lesioned rats (22) and ob/ob mice (22, 23) contribute to the diminished Na^+,K^+-ATPase in skeletal muscle of these animals. Another possibility is that the hyperinsulinemia and associated insulin resistance in skeletal muscle observed in these obese animals influences Na^+,K^+-ATPase (24, 25).

Gold-thioglucose administration produces lesions in the hypothalamus. As indicated earlier, Na^+,K^+-ATPase activity in livers of mice with these lesions was unaltered (9). Since we did not examine Na^+,K^+-ATPase in livers of VMH-lesioned rats, we are unable to indicate if Na^+,K^+-

ATPase responds similarly in these two obese animals.

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