Vol. 80, No. 2, 1978 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
January 30, 1978 Pages 398-404

Na^+,K^+-ATPase ENZYME UNITS IN SKELETAL MUSCLE FROM LEAN AND OBESE MICE

Ming Huey Lin*, Dale R. Romsos*, Tai Akera** and Gilbert A. Leveille*

Departments of Food Science & Human Nutrition*, and Pharmacology**
Michigan State University
East Lansing, Michigan 48824

Received December 8,1977

SUMMARY: [^{3}H]-Ouabain binding to muscle preparations was utilized to estimate the number of Na^+,K^+-ATPase enzyme units in hindlimbs from 8 week old lean and obese mice. Specific [^{3}H]-ouabain binding per mg particulate protein was 36% lower in obese mice; whereas, the affinity of the binding sites for ouabain was similar in obese and lean mice. Since obese mice had less muscle than lean mice, the number of Na^+,K^+-ATPase enzyme units in hindlimbs from obese mice was less than half the number observed in lean mice.

INTRODUCTION

The increased cellular thermogenesis in liver and skeletal muscle associated with thyroid hormone administration and with prolonged cold-exposure has been suggested to involve the Na^+ pump (1-5). Na^+,K^+-ATPase (EC 3.6.1.3) is the enzymatic equivalent of the Na^+ pump (6-8) and a change in thyroid status or cold exposure produces a concomitant change in the activity of this enzyme (1-5). The activity of Na^+,K^+-ATPase has also been suggested to control a major part of the basal metabolic rate of animals (9). Thus, under certain conditions at least, the energy demands of the Na^+/K^+ transport system may play an important role in the overall regulation of energy metabolism in an animal.

The balance between energy intake and cellular thermogenesis largely determines whether an adult animal will gain or lose body fat; however, there is a paucity of data on the possible involvement of Na^+,K^+-ATPase in the etiology of obesity. The genetically obese (ob/ob) mouse has a lower body temperature (10) and a lower heat production (unpublished observation from this laboratory) than lean littermates as well as a re-

0006-291X/78/0802-0398$01.00/0

duced ability to survive a cold stress (11). These observations suggest that the obesity in this model might be a consequence of reduced cellular thermogenesis which would in turn permit an increased portion of dietary energy to be retained as body fat.

The present study was undertaken as an initial step in evaluating the role of the Na^+ pump in obesity. The concentration of Na^+,K^+-ATPase enzyme units was estimated in skeletal muscle, a major thermogenic organ (12), of lean and obese mice. The number of enzyme units was assayed from the concentration of high affinity [^{3}H]-ouabain binding sites in the muscle preparation.

METHODS

Eight week old male obese (C57BL/6J-ob/ob) and lean (ob/+ or +/+) littermate mice were killed by cervical dislocation. The mice were immediately perfused via the heart with 10 ml of cold saline solution. The skin was removed from the carcass and both hindlimbs were severed perpendicular to the femur at the femur-pelvic junction. The muscle, stripped of all visible fat, was removed from the bone and homogenized in 9 volumes of an ice cold solution containing 0.25 M sucrose, 5 mM-histidine, 5 mM di-sodium EDTA, 0.1% sodium deoxycholate and 10 μM dithiothreitol (pH 7.0). Deoxycholate was included in the homogenization buffer to expose all possible ouabain binding sites (13,14). The homogenate was centrifuged at 100,000 x g for 60 minutes; the resulting pellet was resuspended in 10 mM Tris-HCl buffer (pH 7.4) and centrifuged again at 100,000 x g for 30 minutes. The final pellet was suspended in 10 mM Tris-HCl buffer (pH 7.4) and frozen at -20°C. All preparative procedures were performed at 0 to 4°C. The particulate fraction, rather than a purified sarcolemma membrane preparation, was utilized in the subsequent ouabain binding assay because it was not known to what extent obesity might alter the sedimentation characteristics of the subcellular particles and thereby influence the recovery of the enzyme.

Muscle preparations (1.0 to 1.3 mg protein) were incubated at 37°C in a total volume of 2 ml. The incubation mixture contained 1.0 mM inorganic phosphate (pH 7.4), 1.0 mM $MgCl_2$, 10 mM Tris-HCl buffer (pH 7.4). [^{3}H]-Ouabain (1.9 Ci/mmole) and nonlabelled ouabain were added at concentrations indicated in the results. At the end of the incubation the tubes were cooled in ice-cold water and then centrifuged at 100,000 x g for 30 minutes to separate bound ouabain from unbound ouabain. The amount of radioactivity and protein (15) in the pellet were then determined to estimate the amount of [^{3}H]-ouabain bound to the muscle preparations.

[^{3}H]-Ouabain binding to Na^+,K^+-ATPase follows classical Michaelis-Menten kinetics (16). The amount of saturable [^{3}H]-ouabain bound to Na^+,K^+-ATPase in the presence of a fixed concentration of labelled ligand, the affinity of the binding sites for the ligand and the number of ouabain binding sites in the muscle preparations were determined as previously described (16,17). Significant binding of [^{3}H]-ouabain to lower affinity nonspecific nonsaturable ouabain binding was determined in the presence

of 4 mM nonlabelled ouabain. Similar values for nonspecific [^{3}H]-ouabain binding were obtained when the incubation mixture contained 200 mM KCl and no phosphate ions, indicating that the saturable binding observed in the present study was related to the binding of ouabain to Na^+,K^+-ATPase (18).

Statistical comparisons were made with the paired-t-test (19).

RESULTS AND DISCUSSION

The body weight of the obese mice was greater than that of the lean mice; however, the weight of muscle from the hindlimbs was less in the obese mice than in the lean mice (table 1). These results are in agreement with previous reports which have shown that obese mice accumulate 4-5 times more fat, but less total protein, than lean mice from 3 to 8 weeks of age (20). During this period obese mice consumed only slightly more (9%) food than lean mice; thus the obese mice were much more efficient than lean mice in retaining ingested energy (20).

The time course of specific [^{3}H]-ouabain binding in vitro to muscle preparations from one pair of lean and obese mice is shown in figure 1. Binding of [^{3}H]-ouabain increased rapidly to reach a maximum at 3 to 5 minutes in both the lean and obese mouse preparations; however, the amount of [^{3}H]-ouabain bound in the obese mouse was less than in the lean mouse. Since the binding capacity decreased slightly between 5 and 50 minutes of incubation in both preparations, a 5 minute incubation was employed in the remaining assays.

The specific binding of [^{3}H]-ouabain to skeletal muscle preparations from obese mice was lower than observed in lean mice (table 1). Nonspecific, nonsaturable binding of ouabain to the preparations was also lower in obese mice than in lean mice; however, the differences were less pronounced than observed for specific [^{3}H]-ouabain binding.

The observed decrease in specific [^{3}H]-ouabain binding to muscle preparations from obese mice could result from either a decrease in binding site concentration or from a decrease in affinity of the binding sites for ouabain. Thus, K_d values (dissociation constant; reciprocal index of the affinity) for [^{3}H]-ouabain were estimated from the specific binding of [^{3}H]-

TABLE 1

BODY WEIGHT, HINDLIMB MUSCLE WEIGHT[1] AND [^{3}H]-OUABAIN BINDING TO MUSCLE PREPARATIONS FROM LEAN AND OBESE MICE.

Parameter	Group	
	Lean	Obese
Body weight-g	25 ± 1[a]	39 ± 1[b]
Hindlimb muscle:		
Total weight-g	2.1 ± 0.1[a]	1.3 ± 0.1[b]
Particulate protein-mg/g muscle	74 ± 2[a]	73 ± 2[a]
Specific [^{3}H]-ouabain binding[2]- pmoles/mg protein	1.59 ± 0.05[a]	1.04 ± 0.07[b]
Nonspecific [^{3}H]-ouabain binding[3]- pmoles/mg protein	1.71 ± 0.03[a]	1.43 ± 0.03[b]
K_D value[4]-nM	36 ± 4[a]	30 ± 2[a]
[^{3}H]-ouabain binding site concentration[5]- pmoles/mg protein	1.74 ± 0.06[a]	1.11 ± 0.07[b]
pmoles/total hindlimb muscle	269 ± 14[a]	111 ± 12[b]

[1] Means ± SEM for 10 pairs of lean and obese mice. Values followed by different superscript letters are significantly ($P<0.001$) different.

[2] [^{3}H]-ouabain (0.4 uM) binding minus [^{3}H]-ouabain (0.4 uM) binding observed in the presence of excess nonlabelled ouabain (4 mM).

[3] [^{3}H]-ouabain (0.4 uM) binding observed in the presence of excess nonlabelled ouabain (4 mM).

[4] See figure 2.

[5] Calculated as described elsewhere (16).

ouabain determined in the presence of various concentrations of nonlabelled ouabain. In this type of assay the use of a [^{3}H]-ouabain concentration (20 nM) lower than the K_d value is essential (16); this low concentration of [^{3}H]-ouabain kept the magnitude of the nonspecific binding relatively low. A plot of [^{3}H]-ouabain binding versus increasing concentration of nonlabelled ouabain in muscle preparations from 3 pairs of lean and obese mice is presented in figure 2. Values calculated from individual mice are pre-

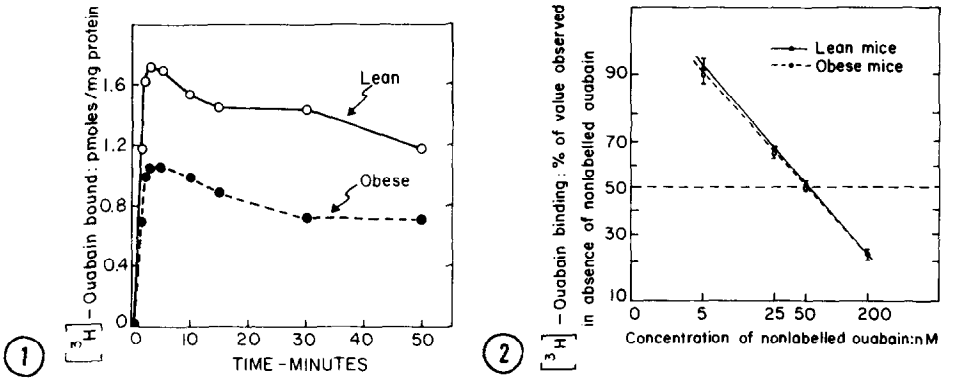

Figure 1: Time course of specific [^{3}H]-ouabain binding to skeletal muscle preparations from one pair of lean and obese mice. Tissue preparations (1 to 1.3 mg protein) were incubated in 1 mM $MgCl_2$, 1 mM inorganic phosphate, 10 mM Tris-HCl buffer (pH 7.4) and 0.4 µM [^{3}H]-ouabain (1.9 Ci/mmole) at 37°C for the indicated times. Specific [^{3}H]-ouabain binding was obtained by subtracting nonspecific non-saturable ouabain binding observed in the presence of 4 mM nonlabelled ouabain from values observed in the absence of the nonlabelled ligand.

Figure 2: Binding of 20 nM [^{3}H]-ouabain to skeletal muscle particulate fractions in the presence of various concentrations of non-labelled ouabain. The ordinate is in probit scale and the abscissa is in logarithmic scale. Each point represents the mean ± SEM for 3 lean or 3 obese mice.

sented in table 1. The K_d values obtained in obese mice were similar to those observed in lean mice suggesting that the number of ouabain binding sites was lower in muscle preparations from obese mice, whereas the affinity of each binding site for ouabain remained unchanged. Likewise, the affinity of skeletal muscle Na^+,K^+-ATPase for ouabain also remained unchanged, but the number of binding sites changed, when rats were made hyperthyroid (17).

Kinetic analyses revealed that the concentration of [^{3}H]-ouabain binding sites per mg particulate protein was 36% lower in obese mice than in lean mice (table 1). Since the weight of muscle from hindlimbs of the obese mice was also lower, total binding site number in the hind-limbs of obese mice was less than half the number observed in hindlimbs of lean mice. The magnitude of difference observed in ouabain binding

sites in muscle of lean and obese mice was comparable to the change in Na^+,K^+-ATPase activity (21) or in the number of ouabain binding sites (17) in muscle of rats subjected to large doses of thyroxine. Na^+-dependent respiration in muscle was also stimulated to a similar extent when rats were cold-acclimated (5).

The results of this study demonstrate that skeletal muscle from obese mice contains a markedly lower concentration of Na^+,K^+-ATPase enzyme units and suggest a possible involvement of the Na^+/K^+ transport system in the etiology of the obesity. The lower body temperature, the reduced heat production, and lower maintenance energy requirement of the obese mouse are consistent with the hypothesis that reduced skeletal muscle Na^+,K^+-ATPase plays an important role in the altered energy metabolism observed in the obese mouse.

ACKNOWLEDGMENTS

Supported in part by NIH AM 15847. DRR is the recipient of Career Development Award NIH K04 AM 00112. Michigan Agricultural Experiment Station Journal Article No. 8352.

REFERENCES

1. Ismail-Beigi, F., and Edelman, I.S. (1970) Proc. Natl. Acad. Sci. U.S.A. 67, 1071-1078.
2. Ismail-Beigi, F., and Edelman, I.S. (1971) J. Gen. Physiol. 57, 710-722.
3. Ismail-Beigi, F., and Edelman, I.S. (1973) Am. J. Physiol. 225, 1172-1177.
4. Ismail-Beigi, F., and Edelman, I.S. (1974) Proc. Soc. Exp. Biol. Med. 146, 983-988.
5. Guernsey, D.L., and Stevens, E.D. (1977) Science 196, 908-910.
6. Skou, J.C. (1965) Physiol. Rev. 45, 596-617.
7. Nechay, B.R., and Nelson, J.A. (1970) J. Pharmacol. Exp. Ther. 175, 717-726.
8. Hoffman, J.F. (1969) J. Gen. Physiol. 54, 3434-3505.
9. Himms-Hagen, J. (1976) Ann. Rev. Physiol. 38, 315-351.
10. Joosten, H.F.P., and Vander, P.H.W. (1974) Metabolism 23, 425-436.
11. Mayer, J., and Barnett, R.J. (1963) Yale J. Biol. Med. 26, 38-45.
12. Jansky, L. (1972) Biol. Rev. 48, 85-132.
13. Jorgensen, P.L., and Skou, J.C. (1971) Biochim. Biophys. Acta 233, 366-380.
14. Besch, H.R., Jr., Jones, L.R., and Watanabe, A.M. (1976) Circ. Res. 39, 586-595.
15. Gornall, A.G., Bardawill, C.J., and David, M.M. (1949) J. Biol. Chem. 177, 751-756.
16. Akera, T., and Cheng, V.K. (1977) Biochim. Biophys. Acta (in press).

17. Lin, M.H., and Akera, T. (1978) J. Biol. Chem. (in press).
18. Tobin, T., and Sen, A.K. (1970) Biochim. Biophys. Acta 198, 120-131.
19. Steel, R.G.D., and Terrie, J.H. (1960) Principles and Procedures of Statistics, McGraw-Hill Book Co., Inc., New York.
20. Lin, P.Y., Romsos, D.R., and Leveille, G.A. (1977) J. Nutr. 107, 1715-1723.
21. Asano, Y., Liberman, V.A., and Edelman, I.S. (1976) J. Clin. Invest. 57, 368-379.