Animal and Human Tissue Na,K-ATPase in Obesity and Diabetes: A New Proposed Enzyme Regulation

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ABSTRACT: *Background:* Na,K-ATPase is a membrane enzyme that energizes the Na-pump, hydrolyzing ATP and wasting energy as heat. It may play a role in thermogenesis, energy balance, and obesity development. Regulation of the enzyme by insulin is controversial. *Methods:* In animal and human obesity, tissue Na,K-ATPase was assayed by colorimetric measurement of released Pi. *Results:* Na,K-ATPase of hyperglycemic-hyperinsulinemic ob/ob mice (compared with lean control animals) was reduced in liver (−63%) and in kidney (−47%) ($P < 0.001$ in both instances). In contrast, in streptozotocin-treated hypoinsulinemic-diabetic Swiss mice, versus untreated animals, we found an increase of liver (+54%, $P < 0.01$) and kidney (+94%, $P < 0.001$) Na,K-ATPase. The enzyme was also increased (+99%, $P < 0.05$) in kidney from ob/ob mice made diabetic-hypoinsulinemic with streptozotocin (versus untreated obese animals). This is contrary to the occurrence of a genetic enzymatic defect and suggests regulation by hyperinsulinemia, present in ob/ob mice. A positive correlation between tissue enzyme activity and glycemia existed in both ob/ob and Swiss mice. In adipose tissue from obese patients (compared with lean subjects), Na,K-ATPase was reduced (−65%, $P < 0.001$) and negatively correlated with body mass index, oral glucose tolerance test--insulinemic area, and mean blood pressure. In vitro, in human liver tissue, 3 μg/mL glucagon exerted a statistically inhibitory effect on Na,K-ATPase (−44%). *Conclusion:* We hypothesize that animal and human obesity is associated with reduction of tissue Na,K-ATPase, linked to hyperinsulinemia, which may repress or inactivate the enzyme, influencing thermogenesis and energy balance. **KEY INDEXING TERMS:** Na,K-ATPase; Obesity; Diabetes; Insulin; NEFA; FFA. **[Am J Med Sci 2007;333(1):1–9.]**

The sodium (Na)- and potassium (K)-activated adenosine-triphosphatase (Na,K-ATPase; EC 3.6.1.37) is one of three ATPase enzymes, together with the H,K-ATPase and Ca2-ATPase. Na,K-ATPase is a complex membrane enzyme that converts chemical energy from the hydrolysis of adenosine triphosphate (ATP) into an active translocation of cations against an electrochemical gradient. Na,K-ATPase energizes the Na-pump responsible for maintaining high K and low Na intracellular concentrations, being an important thermogenetic mechanism that is endogenously regulated.[1,2] This pump, by maintaining the Na gradient between intracellular and extracellular compartment, influences cell volume, absorption processes in kidney or intestine, and excitability in nerve or muscle.[3] ATP is the energy source for this transport through the pump mechanism energized by Na,K-ATPase; the Na-pump, by hydrolyzing the ATP, produces energy that is released as heat.[2] This process can be regarded as a special *futile cycle* that consists of cyclic translocation of Na ions[4,5]; the active mechanism of pump (Figure 1), by wasting energy and occurring in all cells of the organism, may play a significant role in thermogenesis and energy balance regulation.

The rate of energy wasted by this mechanism has been estimated in different ways.[2,6,7] It was reported that 80% or more of the energy utilized by the kidneys is invested in this active transport.[7] It is therefore not surprising that the kidney is one of the tissues richest in Na,K-ATPase.[7] In recent decades, increasing information concerning amino acid sequence, structure, and specific isoforms of the Na,K-ATPase has been gained.[8] Some recent data demonstrate that in rat medullary thick ascending limb proinsulin C-peptide, via protein kinase-Cα (PKCα), stimulates Na,K-ATPase activity within a physiologic concentration range.[9]

Leptin (produced by the obesity ob-gene that controls energy intake and expenditure) was recently found to inhibit Na,K-pump activity, thus explain-

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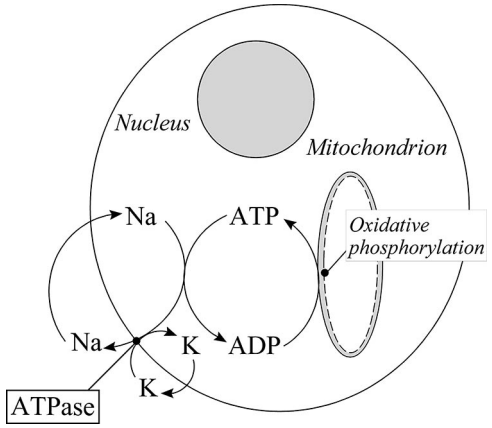

Figure 1. Mechanism of action of the Na,K-ATPase enzyme. Na,K-ATPase activity effects at cell surface the coupled countertransport of Na and K against their electrochemical gradients; this is associated with energy wasting (ATP degradation).

ing the physiologic natriuretic effect and cardiovascular or metabolic implications of this hormone.[10,11]

It has been established that thyroid hormone stimulates oxygen consumption and active Na and K transport in many mammalian tissues, regulates the Na,K-ATPase rate in some tissues, and exerts most of its calorigenic effects by stimulating Na,K-ATPase in liver, kidney, heart, and skeletal muscle (but not in brain).[12–14] On these grounds, some authors studied the Na,K-ATPase activity in various tissues of genetically obese rodents: a remarkable reduction of activity was demonstrated in liver, kidney, and muscle (but not in brain) of the genetic ob/ob mouse,[15] as well as in liver of the genetically db/db mouse,[16] but not in liver of fatty rats.[16] Bray et al[15] hypothesized that genetic obesity in mice might be due to the deficiency of Na,K-ATPase and to the consequent failure of thyroid hormone to stimulate the enzyme and therefore to elevate thermogenesis. This genetic enzyme deficiency (by affecting the thermogenic capacity of the animals) would reduce wasting of energy, favoring a positive caloric balance and, therefore, the occurrence of obesity.[15] The enzyme regulation by insulin is controversial: in a recent comprehensive review, we analyzed animal and human tissue Na,K-ATPase, its level, regulation, and behavior in some hyperinsulinemic and insulin-resistant states, and we attempted to organize the body of literature on this complicated topic.[17]

In the present paper, we study the Na,K-ATPase activity (and its regulation) in some tissues from mice and human subjects with obesity or diabetes, the possible correlation of enzyme activity with hyperinsulinemia and insulin resistance, and its probable link to hypertension and nonesterified fatty acids (NEFA).

Materials and Methods

Studies in Mouse Tissues

In our animal studies, we followed the guidelines indicated by the Committee on Care and Use of Laboratory Animals concerning the Guide for the Care and use of Laboratory Animals (DHHS, NIH, publ no 85–23, 1985, Institute of Laboratory Animal Resources, NRC, Washington).

Hyperglycemic-Hyperinsulinemic ob/ob Mice. For this experiment, we used male obese-hyperglycemic mice (C57B1/6J-ob/ob strain) (n = 20) and matched lean littermate animals (n = 15), of 12-week age, obtained from the Jackson Laboratories (Bar Harbor, ME). Animals were housed in standard cages and were fed ad libitum with laboratory chow (also during the night). In the morning, the animals were weighed (mean ± SEM body weight was 47.79 ± 0.71 g in ob/ob mice versus 23.17 ± 0.75 g in normal mice), and then were killed by decapitation. Their glycemia was measured by commercial reagent strips that were read by a glucometer; mean ± SEM morning glycemia was 6.88 ± 0.43 mmol/L for the normal and 11.38 ± 1.88 mmol/L for the ob/ob mice (+65%, $P < 0.05$). A fragment of liver and kidney was quickly removed, washed in cold saline (carefully deprived of blood), weighed, cut into small segments and immersed in cold homogenization medium at pH 7.0 (in a tissue-to-medium ratio of 1:9). The medium was composed as follows: 10 mmol/L TRIS with 0.25 mol/L sucrose and 1.25 mmol/L EDTA, according to Ismail-Beigi and Edelman.[12] The tissue slices were then homogenized at 2 to 3° C with six strokes in a Teflon-glass Elvehjem-Potter homogenizer. The homogenates were diluted with the homogenization medium (1:3 for liver and 1:5 for kidney) and centrifuged at 3000 *g* for 10 minutes in a refrigerated centrifuge. Finally, the clear supernatants were used for the enzyme assay, which was performed soon after the homogenate preparation. The Na,K-ATPase of homogenates was assayed according to the method of Ismail-Beigi and Edelman[12] and was given as the difference between the inorganic phosphate (Pi) concentration in the reaction mixture expressing the total Mg,Na,K-ATPase activity and that in the mixture containing ouabain (to assay ouabain-insensitive Mg-ATPase activity). The homogenates were warmed at 37° C for 5 minutes prior to the assay, and the reaction was started by the addition of 0.1 mL of homogenate in 0.9 mL of a medium at pH 7.4 composed as follows (in mmol/L): 25 TRIS, 137.5 Cl, 5 ATP (disodium salt), 5 Mg, 115 Na, 12.5 K, and 5 Na-azide (to prevent ATP regeneration by the mitochondria present in the homogenate). The reaction was terminated after 15 minutes by the addition of 0.40 mL of 20% cold trichloroacetic acid. For each assay, three tubes were prepared: one for the blank without homogenate (which was added after the addition of trichloroacetic acid), one for total activity, and one into which ouabain (0.01 mol/L) was added (all volumes were adjusted with buffer). A 10-minute centrifugation at 3000 *g* was performed, and the colorimetric measurement of Pi released in the supernatant was made according to the method of Fiske and SubbaRow.[18] Protein content of homogenates was determined by the method of Lowry and coworkers,[19] and the Na,K-ATPase activity was expressed as nmoles Pi/min/mg protein. All data are given as mean ± SEM. Results were statistically evaluated by means of (paired and unpaired) Student *t* test. Substrates used in the enzyme assay were obtained from Boehringer (Mannheim, Germany). All other chemicals used were of the highest commercially available grade.

Diabetic-Hypoinsulinemic Swiss Mice. To study the behavior of Na,K-ATPase in a condition of hypoinsulinemia, 12 male normal Swiss mice (body weight 27.95 ± 1.06 g), aged 24 weeks, obtained from Stefano Morini (S. Polo d'Enza, RE, Italy), were made diabetic by a single intraperitoneal injection of streptozotocin (STZ) (200 mg/kg body weight), dissolved in citrate buffer at pH 4.5 immediately before the treatment. Control animals (n = 24) were injected intraperitoneally with buffer only. During the next 24 hours, the STZ-injected mice were allowed to drink a 10% glucose solution to reduce the risk of mortality due to the hypoglycemic episodes caused by the insulin release from pancreatic

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