Diabetes Decreases Na^+-K^+ Pump Concentration in Skeletal Muscles, Heart Ventricular Muscle, and Peripheral Nerves of Rat

KELD KJELDSEN, HANS BRÆNDGAARD, PER SIDENIUS, JIM STENFATT LARSEN, AND AAGE NØRGAARD

SUMMARY

Na^+-K^+-ATPase or the Na^+-K^+ pump is essential for some specific properties of muscle and nerve tissue such as contractility and excitability. Previous studies have shown conflicting variations in Na^+-K^+-ATPase activity or Na^+-K^+ pump concentration of muscle cells in experimental diabetes. Our study demonstrates that early untreated diabetes in rats induced by injection of streptozocin is associated with decreases in [^{3}H]ouabain binding-site concentration of 24–48% in various skeletal muscles and 16% in peripheral nerves as well as a decrease in K^+-dependent 3-*O*-methylfluorescein phosphatase activity of 21% in the heart ventricle. These effects could be prevented by insulin treatment. They probably represent a decrease in the concentration of Na^+-K^+ pumps. There was no evidence for more than one population of Na^+-K^+ pumps in intact samples of skeletal muscle and nerves from normal, diabetic, and insulin-treated animals. The decrease in Na^+-K^+ pump concentration in nerve cells may be due to atrophy of the axons. In skeletal muscles, myocardium, and peripheral nerves, the observed decrease in Na^+-K^+ pump concentration may be important for the pathophysiology of diabetes. We emphasize that quantification of Na^+-K^+-ATPase or the Na^+-K^+ pump in muscle and nerve tissue from diabetic animals should preferably be performed with either intact samples or crude homogenates of whole tissue.

***Diabetes* 36:842–48, 1987**

Diabetes is a metabolic disorder that affects various organ systems, including skeletal muscles, the heart, and peripheral nerves (1–4). These changes are thought to involve both structural and metabolic defects, including enzymatic disorders of the plasma membrane.

Na^+-K^+-ATPase or the Na^+-K^+ pump generates the transmembranous Na^+-K^+ gradient and is essential for the specific properties of muscle and nerve tissue such as contractility and excitability (5). In a variety of tissues, including muscle and nerves, insulin has been demonstrated to enhance the transport of Na^+ out of the cell and of K^+ into the cell (6). The observation that these effects of insulin are inhibited by digitalis glycosides indicates that insulin exerts an acute regulation of existing Na^+-K^+ pumps by increasing their molecular activity (7). Furthermore, diabetes is associated with a number of metabolic disorders, including impaired protein synthesis and increased protein degradation, that might influence the Na^+-K^+ pump concentration or Na^+-K^+-ATPase activity.

Earlier studies have shown that in sciatic nerves of streptozocin (STZ)-treated rats the Na^+-K^+-ATPase activity is decreased by up to 40% (8,9). In the hearts of alloxan-treated dogs the [^{3}H]ouabain binding was unchanged compared with that of control dogs (10), whereas in the hearts of STZ-treated rats a 24% decrease in K^+-dependent *p*-nitrophenylphosphatase activity was observed (11). In skeletal muscles of STZ-treated rats a normal Na^+-K^+-ATPase activity was found compared with control rats (12).

Most studies characterizing Na^+-K^+-ATPase in muscle tissue are based on membrane fractions containing 0.2–18% of the total enzyme activity available in the starting material (13,14). Although this may not affect the interpretation of qualitative studies on the properties of the enzyme, it represents a serious disadvantage in quantitative estimates of changes in the total enzyme activity under various conditions. In contrast to such evaluations, measurements performed with intact tissue samples or crude homogenates thereof yield considerably higher values. In rat skeletal muscle we found good agreement between the concentration of Na^+-K^+ pumps as estimated either from [^{3}H]ouabain binding to intact muscle samples or from K^+-dependent 3-*O*-

From the Institute of Physiology, University of Aarhus (K.K., J.S.L., A.N.) and the Diabetes Research Laboratory, Second University Clinic of Internal Medicine, Aarhus Municipal Hospital (H.B., P.S.), Aarhus, Denmark.
Address correspondence and reprint requests to Keld Kjeldsen, Institute of Physiology, University of Aarhus, DK-8000 Aarhus C, Denmark.
Received for publication 28 August 1986 and accepted in revised form 3 February 1987.

methylfluorescein phosphatase (3-*O*-MFPase) activity, an enzyme closely associated with Na^+-K^+-ATPase, in crude homogenates (15).

On this basis it was relevant to assess the effect of diabetes on Na^+-K^+ pump concentration in skeletal muscles, heart ventricular muscle, and peripheral nerves of rats with high-accuracy methods. This was done by measuring the [^{3}H]ouabain binding-site concentration in intact muscle or nerve samples or the K^+-dependent 3-*O*-MFPase activity in crude homogenates, thus avoiding membrane purification.

MATERIALS AND METHODS

Animals and induction of diabetes. Male Wistar rats (450–525 g), 23 wk old, were randomly allocated into one control and two experimental groups. Diabetes was induced by a single intravenous STZ injection (45 mg/kg body wt) under ether anesthesia. One experimental group was left untreated. Animals in the other group were given a daily subcutaneous injection of ultralente insulin for 4 wk according to blood glucose measurements made just before injection (16,17). Blood was obtained from a tail vein, and blood glucose was determined with a reflectance meter. All animals had free access to standard rat fodder and tap water. Room temperature was thermostatically controlled at 22°C and humidity was 50%. The light/dark cycle was 12 h.

Surgical procedures. Animals were killed by decapitation. The soleus, extensor digitorum longus, gastrocnemius, and diaphragm muscles were dissected out. The entire soleus muscle was immediately weighed. Samples of ~5 mg from the soleus, extensor digitorum longus, middle portion of the gastrocnemius, and peripheral part of the diaphragm muscles were prepared, frozen in liquid N_2, and stored at −20°C until determination of [^{3}H]ouabain binding. From the gastrocnemius muscle, samples of ~25 mg were prepared for determination of Na^+-K^+ content. The heart was dissected out. From the left ventricle, samples of ~25 mg were prepared for determination of Na^+-K^+ content, and the rest of the ventricle was minced with a scalpel for determination of 3-*O*-MFPase activity. Fifty millimeters of the sciatic nerves were dissected out of the midthigh, extraneural connective tissue was removed, and samples of ~5 mg were prepared for determination of [^{3}H]ouabain binding.

[^{3}H]ouabain binding. Measurement of [^{3}H]ouabain binding was performed as previously described in detail (18,19). To maintain the concentration of Na^+ and K^+ in the incubation medium at a level that would not interfere with the vanadate-facilitated binding of [^{3}H]ouabain, the samples were prewashed two times (10 min each in fresh unlabeled buffer) at 0°C in a Tris-sucrose buffer containing 10 mM Tris chloride, 250 mM sucrose, 3 mM $MgSO_4$, and 1 mM vanadate. Incubation took place in the same Tris-sucrose-vanadate buffer with the addition of 0.18–2.10 μCi/ml [^{3}H]ouabain and ouabain at a final concentration of $1–500 \times 10^{-8}$ M. All incubations were performed two to four times (60 min each in fresh buffer) with [^{3}H]ouabain and ouabain at 37°C. To correct for unspecific uptake of [^{3}H]ouabain, parallel experiments were performed in the presence of an excess of unlabeled ouabain (1×10^{-3} M). After the incubation all samples were washed four times (30 min each in fresh unlabeled buffer) at 0°C in the Tris-sucrose-vanadate buffer to reduce the fraction of [^{3}H]ouabain not bound to the receptor. The loss of specifically bound [^{3}H]ouabain during this procedure was assessed in separate washout experiments. Within the 1st min after blotting, the samples were weighed in minivials, and 0.5 ml 5% trichloroacetic acid was added. After overnight extraction and liquid scintillation counting, the total amount of [^{3}H]ouabain taken up by the samples was calculated based on the specific activity of the incubation medium and, after correction for unspecific uptake, expressed as picomoles per gram wet weight of muscle tissue. Additional corrections were made for radiopurity of the isotope (95%) and loss of specifically bound [^{3}H]ouabain during the washout (for details see ref. 14).

3-*O*-MFPase activity. The assay was performed as previously described in detail (20). After weighing, 1 g of the mince prepared from the heart ventricle was homogenized in 9 ml buffer containing 30 mM histidine, 2 mM EDTA, and 250 mM sucrose (pH 7.2) at 0°C. One hundred microliters of this preparation were suspended in 900 μl of buffer containing 20 mM imidazole, 2 mM EDTA, 250 mM sucrose (pH 7.0), and 0.08% (wt/vol) sodium deoxycholate at 24°C for 30 min to unmask latent ATPase activity. The assay medium contained 19.5 μM 3-*O*-MFP, 4 mM $MgCl_2$, 1 mM EDTA, 80 mM Tris (pH 7.6), and 10 μl of the detergent-treated homogenate corresponding to 100 μg wet wt of tissue in a total volume of 2600 μl. The K^+-dependent phosphatase activity was measured after addition of 13 μl of 2 M KCl to give a final K^+ concentration of 10 mM. Based on the slope of the lines recording the fluorescence, the phosphatase activities were calculated and expressed as micromoles per minute per gram wet weight of tissue. Correction for spontaneous hydrolysis of 3-*O*-MFP was made, and by subtracting the value obtained in the absence of K^+ from that obtained in the presence of K^+, the K^+-dependent 3-*O*-MFPase activity was calculated.

Na^+-K^+ contents. Total Na^+-K^+ content of the gastrocnemius muscle and left ventricle was determined by flame photometry with 3 mM LiCl as internal standard. Twenty-five milligrams of tissue were homogenized in 4 ml of 5% trichloroacetic acid and centrifuged; 1 ml of the clear supernatant was added to 1.5 ml of 5 mM LiCl, and measurements were performed with an FLM flame photometer (Radiometer, Copenhagen, Denmark).

Chemicals and isotopes. All chemicals were of analytic grade. Streptozocin was from Upjohn (Kalamazoo, MI), and ultralente insulin (bovine, heat treated, stored at pH 5.5) was from Novo (Copenhagen, Denmark). The glucose oxidase strip-operated reflectance meter was from Ames (Elkhart, IN). Ouabain was from Sigma (St. Louis, MO), vanadate was from Merck (Darmstadt, FRG), [^{3}H]ouabain (18.0 Ci/mmol) was from New England Nuclear (Boston, MA), and 3-*O*-MF and 3-*O*-MFP were from Sigma.

Statistics. Results are given as means ± SE. Statistical significance of any difference was ascertained with the two-tailed *t* test for nonpaired groups of observations. Linear regression analysis was performed with the method of least squares. $P < .05$ was used as the limit for statistical significance.

RESULTS

Body weight and blood glucose. All animals given STZ developed hyperglycemia on the following day (minimum

TABLE 1
Effect of diabetes with and without insulin treatment after 4 wk

	Control (n = 10)	Diabetes (n = 7)	P	Diabetes and insulin (n = 11)	P
Blood glucose (mM)	5.2 ± 0.1	28.7 ± 0.8	<.001	8.1 ± 1.1	<.02
Body weight (g)	496 ± 6.9	325 ± 11.2	<.001	497 ± 7.5	<.95
Soleus weight (mg)	184 ± 3.3	154 ± 4.2	<.001	185 ± 3.8	<.90
Na^+ content in gastrocnemius muscle (μmol/g wet wt)	19.3 ± 0.9	25.7 ± 2.0	<.02	19.7 ± 0.7	<.80
K^+ content in gastrocnemius muscle (μmol/g wet wt)	98.1 ± 1.4	99.2 ± 1.5	<.60	107.5 ± 1.6	<.001

Values are means ± SE. Diabetes was induced by streptozocin injection (45 mg/kg body wt i.v.); insulin treatment was daily subcutaneous injection of ultralente based on weight and plasma glucose.

19.4 mM). Untreated rats lost body and muscle weight during the 4 wk and had preserved hyperglycemia at death. These changes were prevented after treatment (Table 1).

[³H]ouabain binding to skeletal muscle. The basic characteristics of [³H]ouabain binding to samples of soleus muscles from control, diabetic, and insulin-treated animals were assessed in a series of experiments. The effect of the concentration of free [³H]ouabain was assessed by measurements in the concentration range 1–500 × 10^{-8} M. As shown in Fig. 1*A*, the specific binding of [³H]ouabain was saturable. In Fig. 1*B* the specific binding of [³H]ouabain is plotted as a function of specifically bound [³H]ouabain relative to the amount of free [³H]ouabain in the incubation medium. This Scatchard-type plot gives no evidence for more than one population of binding sites, with an apparent dissociation constant (K_d) in the range 3.0–3.6 × 10^{-8} M.

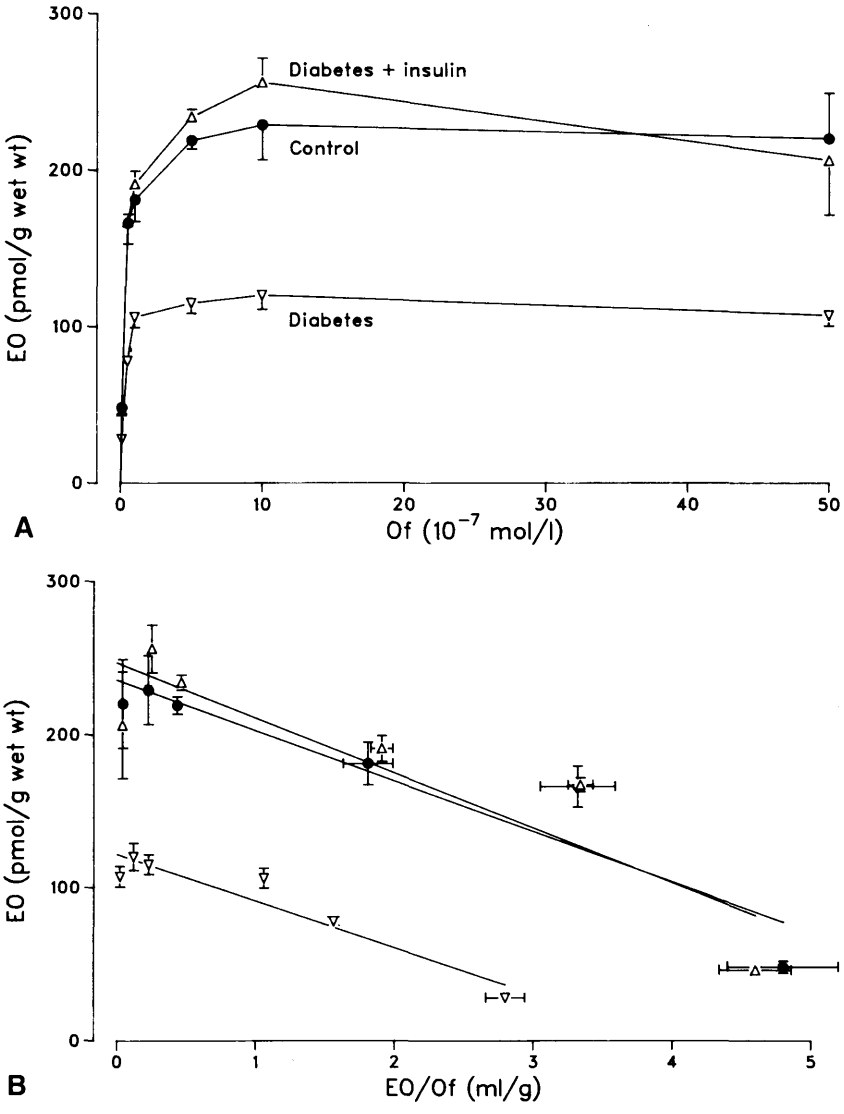

FIG. 1. [³H]ouabain binding (EO) plotted against ouabain concentration (Of) (*A*) and EO/Of (*B*) in biopsy specimens of rat soleus muscle. Treatment and number of animals as in Table 1. Incubation was 4 times (60 min each) at 37°C followed by 4 washouts (30 min each) at 0°C. Correction for unspecific retention of [³H]ouabain based on measurements performed by incubation with excess of unlabeled ouabain (1 × 10^{-3} M). Correction for loss of specifically bound [³H]ouabain during washout done by multiplication with 1.26. Each *point* represents mean of 5–10 observations; *bars* denote SE where it exceeds size of *symbols*. Regression lines of Scatchard-type plot constructed with method of least squares. Dissociation constants (K_d) were 3.3 × 10^{-8}, 3.0 × 10^{-8}, and 3.6 × 10^{-8} M; intercepts with ordinate in *B* (maximum binding capacity, EO_{max}) were 236, 122, and 247 pmol/g wet wt; and *r* values were .94, .95, and .91 for controls (●), diabetics (▽), and insulin-treated diabetics (△), respectively.

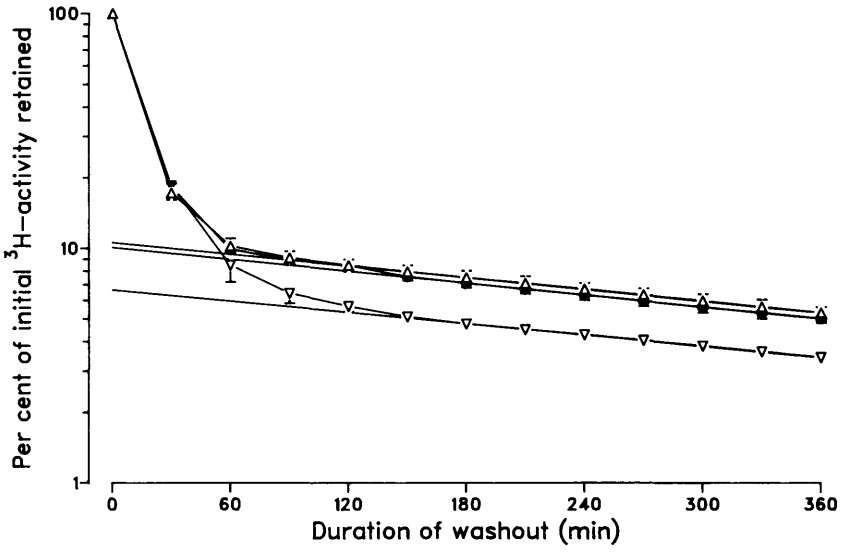

FIG. 2. Washout of [^{3}H]ouabain from biopsy specimens of rat soleus muscle. Treatment and number of animals as in Table 1. Experimental conditions as in Fig. 1 except that incubation occurred twice (60 min each) in buffer containing 20 μCi/ml [^{3}H]ouabain and unlabeled ouabain added to final concentration of 1×10^{-6} M. Each *point* represents mean of 4 observations; *bars* denote SE. *Straight lines* constructed by linear regression analysis with method of least squares and 8 values obtained during slow phase of washout. Decay constants were 5.7, 6.2, and 6.0 h and *r* values were all .999 for controls (●), diabetics (▽), and insulin-treated diabetics (△), respectively.

Whereas the K_d was almost the same for all three groups of animals, the maximum binding capacity showed a 48% decrease associated with untreated diabetes. This decrease was totally prevented by insulin treatment.

To evaluate the loss of specifically bound [^{3}H]ouabain during the standard washout in the cold after incubation with [^{3}H]ouabain, separate washout experiments were performed. Figure 2 shows the loss of [^{3}H]ouabain during the washout at 0°C. Whereas the initial, rapid phase of the curves probably represents the washout of [^{3}H]ouabain taken up but not specifically bound, the late, much slower phase probably represents release of specifically bound [^{3}H]ouabain. From the regression lines this release could be calculated to take place with a $t_{1/2}$ of ~6 h for all three groups of animals. Thus, the loss of specifically bound [^{3}H]ouabain during the standard washout (4 times, 30 min each) could be corrected by multiplying with a factor of 1.26. Note that the intercept values show a 34% reduction in samples obtained from diabetic rats.

Figure 3 shows the effect of untreated and treated diabetes on the concentration of [^{3}H]ouabain binding sites in various skeletal muscles determined at standard conditions, i.e., incubation two times (60 min each) in a buffer containing 1×10^{-6} M [^{3}H]ouabain. The decrease varied from 48% ($P < .001$) in the soleus muscle to 24% ($P < .02$) in the extensor digitorum longus muscle, whereas the reduction in the gastrocnemius and diaphragm muscles was between these values. This difference among muscles might be related to variation in fiber composition. In rats the soleus muscle contains predominantly type I fibers, the extensor digitorum longus muscle contains predominantly type II fibers, and the gastrocnemius and diaphragm muscles contain both type I and II fibers (21). In all muscles, insulin treatment prevented the decrease. By multiplying the total weight of the soleus muscle (Table 1) and the concentration of [^{3}H]ouabain binding sites (Fig. 3), the total number of [^{3}H]ouabain binding sites could be calculated and expressed as picomoles per muscle. In the rats with untreated

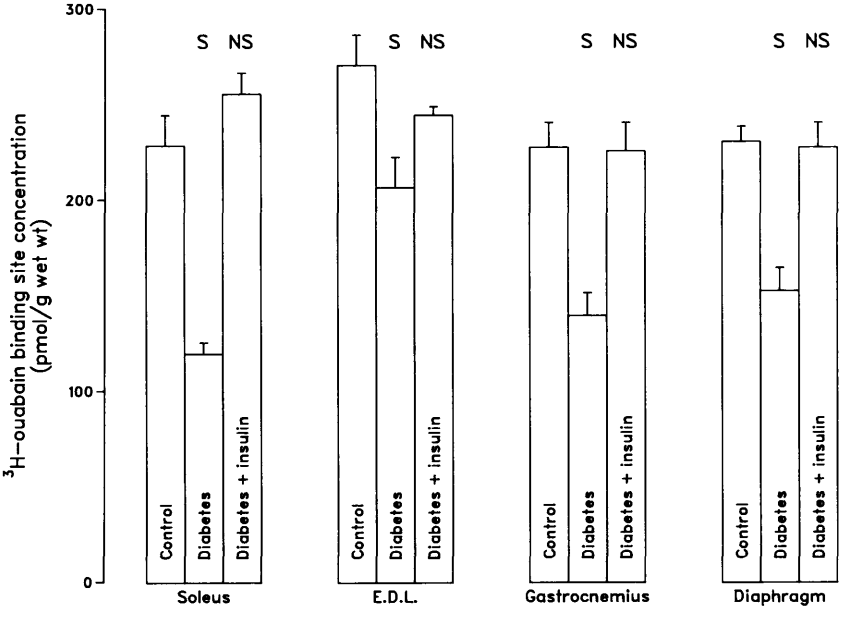

FIG. 3. Effect of diabetes and diabetes plus insulin treatment on concentration of [^{3}H]ouabain binding sites in biopsy specimens from rat soleus, extensor digitorum longus (E.D.L.), gastrocnemius, and diaphragm muscles. Treatment and number of animals as in Table 1. Experimental conditions as in Fig. 1 except that incubation occurred twice (60 min each) in buffer containing 1×10^{-6} M [^{3}H]ouabain. Each *column* represents mean of observations; *bars* denote SE.

TABLE 2
Effect of diabetes with and without insulin treatment on K⁺-dependent 3-*O*-MFPase in crude homogenates and Na⁺ and K⁺ content in left heart ventricle

	Control (n = 10)	Diabetes (n = 7)	P	Diabetes and insulin (n = 11)	P
3-*O*-MFPase activity (μmol · min⁻¹ · g⁻¹ wet wt)	1.26 ± 0.06	1.00 ± 0.08*	<.025	1.46 ± 0.08	<.10
Na⁺ content (μmol/g wet wt)	34.5 ± 1.8	40.1 ± 1.1	<.02	34.8 ± 1.1	<.90
K⁺ content (μmol/g wet wt)	75.7 ± 0.9	78.6 ± 2.5	<.30	80.1 ± 1.9	<.05

Values are means ± SE. 3-*O*-MFPase, 3-*O*-methylfluorescein phosphatase. Treatment as in Table 1.
*Values from only 6 samples.

diabetes the decrease in the soleus muscles was then even more pronounced (56% compared with control animals).

The decrease in the concentration of [³H]ouabain binding sites in the gastrocnemius muscle from diabetic animals was associated with a 33% increase in total Na⁺ content ($P < .02$), whereas the total K⁺ content was unchanged. After insulin treatment the total Na⁺ content was normalized, whereas the total K⁺ content was increased by 10% ($P < .001$) compared with control animals (Table 1).

3-*O*-MFPase activity in heart ventricular muscle. Due to the extremely low affinity of ouabain binding to rat heart, the concentration of Na⁺-K⁺ pumps could not be quantified by measurement of [³H]ouabain binding (20). Hence, the K⁺-dependent 3-*O*-MFPase activity was determined in crude homogenates of the left ventricle. As shown in Table 2, untreated diabetes was followed by a 21% decrease in K⁺-dependent phosphatase activity ($P < .025$). This was associated with a 16% increase in total Na⁺ content ($P < .02$). As in skeletal muscle, insulin treatment was associated with normal Na⁺ content and a 6% increase in K⁺ content ($P < .05$) compared with control animals.

[³H]ouabain binding to peripheral nerves. The [³H]ouabain binding to samples of the sciatic nerves from control, diabetic, and insulin-treated animals displayed the same basic characteristics as the binding to skeletal muscle, although some minor differences were observed. The binding of [³H]ouabain was saturable and took place to one population of binding sites with an apparent K_d in the range 2.6–5.0 × 10^{-8} M (Table 3). Diabetes was associated with a 16% decrease in maximum binding capacity, whereas insulin treatment totally prevented this decrease. At 1 × 10^{-6} M [³H]ouabain, the concentration of [³H]ouabain binding sites decreased from 211 ± 5.4 to 182 ± 5.7 pmol/g wet wt (n = 17, $P < .005$). Correction for unspecific uptake and retention of [³H]ouabain corresponding to ~6% was done by subtraction. Correction for loss of specifically bound [³H]ouabain during the standard washout (4 times, 30 min each) at 0°C was deemed unnecessary because in nerve samples from control, diabetic, and insulin-treated rats the $t_{1/2}$ for this process exceeded 30 h.

TABLE 3
Effect of diabetes with and without insulin treatment on [³H]ouabain binding sites in biopsy specimens from rat sciatic nerve

	Control (n = 10)	Diabetes (n = 7)	Diabetes and insulin (n = 10)
K_d (M)	4.3 × 10^{-8}	2.6 × 10^{-8}	5.0 × 10^{-8}
EO_{max} (pmol/g wet wt)	213	179	218
r	.92	.98	.94

EO_{max}, maximum binding capacity. Treatment as in Table 1; conditions as in Fig. 1.

DISCUSSION

Our study demonstrates that early untreated diabetes is associated with a decrease in [³H]ouabain binding-site concentration in intact samples from skeletal muscle and peripheral nerves as well as with a decrease in K⁺-dependent 3-*O*-MFPase activity in crude homogenates from heart ventricle of rats. In rat skeletal and heart muscle, determinations of [³H]ouabain binding-site concentration and 3-*O*-MFPase activity, respectively, have proved adequate for the quantitative analysis of changes in the concentration of Na⁺-K⁺ pumps in response to hormones, drugs, or altered physiological conditions (13). Thus, the decrease in [³H]ouabain binding-site concentration and K⁺-dependent 3-*O*-MFPase activity observed in diabetes can be assumed to represent a decrease in the concentration of Na⁺-K⁺ pumps. This decrease could be totally prevented by insulin treatment. In several tissues, insulin stimulates Na⁺-K⁺-pump–mediated transport of Na⁺ and K⁺ across the cell membrane (6). In accordance with this, untreated diabetes was associated with an increase in total Na⁺ content of skeletal and heart muscle. This agrees well with an increase in intracellular Na⁺ content of ~33% in soleus muscles from rats 4 wk after STZ treatment (22). After insulin treatment, total Na⁺ content was normal, whereas total K⁺ content was increased.

In both STZ-treated rats (1) and newly diagnosed insulin-dependent diabetic patients (2), a reduction in skeletal muscle fiber size is seen. This is probably caused by impaired protein synthesis and increased protein degradation. Thus, a 70% reduction in protein synthesis has been found in the gastrocnemius muscle of STZ-treated rats (23). This may explain the reduction in weight of the soleus muscle observed in our study. It has been reported that the Na⁺-K⁺-ATPase activity in purified non–detergent-treated sarcolemma preparations from the extensor digitorum longus, anterior tibialis, and gastrocnemius muscles of rats was not changed 18 days after STZ treatment (12). We found a 24% decrease in [³H]ouabain binding-site concentration in the extensor digitorum longus muscle after 4 wk. This discrepancy can probably not entirely be explained by the difference

in observation time but could be due to incomplete recovery after intensive purification or lack of detergent treatment (13,14).

In deoxycholate-treated sarcolemma purified from heart ventricular muscle of rats, a 24% decrease in ouabain-sensitive K^+-dependent *p*-nitrophenylphosphatase activity was demonstrated 8 wk after STZ treatment (11). Like 3-*O*-MFPase, *p*-nitrophenylphosphatase is an enzyme closely associated with Na^+-K^+-ATPase. This agrees well with the 21% decrease in K^+-dependent 3-*O*-MFPase activity we observed after 4 wk. At variance with these observations, no change of the V_{max} of K^+-dependent *p*-nitrophenylphosphatase activity or maximum binding capacity of [^{3}H]ouabain was seen in isolated non–detergent-treated myocytes from the hearts of alloxan-treated dogs after 8 days (10).

In untreated insulin-dependent diabetic patients, a 28% decrease in free T_3 and T_4 has been demonstrated. After insulin treatment, free T_3 and T_4 were normalized (24). In skeletal muscle biopsy specimens from patients with hypothyroidism, a 50% decrease in the Na^+-K^+ pump concentration was seen (25). After hypothyroidism in rats, the decrease in Na^+-K^+ pump concentration was more pronounced in the soleus than in the extensor digitorum longus muscle (26). This is in good agreement with a more severe decrease in [^{3}H]ouabain binding-site concentration in the soleus than in the extensor digitorum longus muscle observed in our study. Thus, the decrease in Na^+-K^+ pump concentration in muscle cells in untreated diabetes may be associated with changes in thyroid status.

The effect of diabetes on Na^+-K^+-ATPase in nerve tissues has been studied rather intensively. Thus, in only partially purified homogenates of sciatic nerves from rats, a decrease in Na^+-K^+-ATPase activity from 97 to 55 $\mu mol \cdot h^{-1} \cdot g^{-1}$ wet wt was observed 4 wk after STZ treatment (9). If a molecular activity of the Na^+-K^+-ATPase of 7500/min (27) is assumed, this corresponds to a decrease in Na^+-K^+ pump concentration from 215 to 122 pmol/g wet wt. This is in the same range as the decrease in [^{3}H]ouabain binding-site concentration from 213 to 179 pmol/g wet wt observed in our study. Also, in crude homogenates from the dorsal root ganglia of rats, a 46% decrease in Na^+-K^+- ATPase activity has been reported 8 wk after STZ treatment (28). In purified homogenates from sciatic nerves of normal rats, the binding of [^{3}H]ouabain took place to only one population of binding sites, with an apparent K_d of 2.2×10^{-8} M (29). This homogeneity is in accord with the results we obtained. However, the maximum binding capacity of [^{3}H]ouabain to the purified homogenates only corresponded to ~16 pmol/g wet wt.

The decrease in Na^+-K^+ pump concentration of sciatic nerves in our study after STZ treatment could be explained by the concomitant reduced fiber area and the enlargement of the endoneurial space (17,30). These changes could be prevented by insulin treatment. Furthermore, Na^+-K^+-ATPase activity has been found to be normalized when the osmolality of the incubation medium was increased to diabetic levels (31). Thus, there is no experimental evidence for a decrease in the total amount of Na^+-K^+ pumps or Na^+-K^+-ATPase in diabetic nerves.

During exercise, considerable amounts of K^+ are lost from the muscle cells to the interstitial fluid. Thus, in exercising mammalian skeletal muscles, interstitial K^+ concentrations as high as 9 mM have been recorded (32). Therefore, to avoid severe hyperkalemia with ensuing paralysis, the Na^+-K^+ pump must be able to rapidly reuptake this K^+. Hence, the decrease in Na^+-K^+ pump concentration might be associated with muscular symptoms such as fatigue in untreated diabetes.

Most studies characterizing Na^+-K^+-ATPase activity in various tissues are based on membrane fractions containing less than a few percent of the total enzyme activity available in the starting material. It is not known whether these fractions represent a true random sample of the plasma membrane or if they arise from localized or subspecialized regions (33). This represents a serious disadvantage in quantitative estimates of changes in the total Na^+-K^+-ATPase concentration under various conditions. In view of the uncertain quantification of Na^+-K^+-ATPase or the Na^+-K^+ pump as discussed above in muscle tissue from diabetic animals, it should be emphasized that such studies should preferably be performed with either intact samples or crude homogenates of whole tissue.

ACKNOWLEDGMENTS

We thank Torben Clausen for valuable discussion and Marianne Stürup-Johansen and Susanne Olesen for skilled technical assistance.

This study was supported in part by the Danish Medical Research Council, the Danish Heart Foundation, and the Nordic Insulin Foundation.

REFERENCES

1. Hegarty PVJ, Rosholt MN: Effects of streptozotocin-induced diabetes on the number and diameter of fibres in different skeletal muscles of the rat. *J Anat* 133:205–11, 1981
2. Jakobsen J, Reske-Nielsen E: Diffuse muscle fiber atrophy in newly diagnosed diabetes. *Clin Neuropathol* 5:73–77, 1986
3. Fein FS, Strobeck JE, Malhotra A, Scheuer J, Sonnenblick EH: Reversibility of diabetic cardiomyopathy with insulin in rats. *Circ Res* 49:1251–61, 1981
4. Thomas PK, Eliasson SG: Diabetic neuropathy. In *Peripheral Neuropathy*. Dyck PJ, Thomas PK, Lambert EH, Bunge R, Eds. Philadelphia, PA, Saunders, 1984, p. 1773–810
5. Skou JC: Enzymatic basis for active transport of Na^+ and K^+ across cell membrane. *Physiol Rev* 45:596–617, 1965
6. Moore RD: Effects of insulin upon ion transport. *Biochim Biophys Acta* 737:1–49, 1983
7. Clausen T: Regulation of active Na^+-K^+ transport in skeletal muscle. *Physiol Rev* 66:542–80, 1986
8. Das PK, Bray GM, Aguayo AJ, Raminsky M: Diminished ouabain-sensitive, sodium-potassium ATP-ase activity in sciatic nerves of rats with streptozotocin-induced diabetes. *Exp Neurol* 53:285–88, 1976
9. Greene DA, Lattimer SA: Impaired rat sciatic nerve sodium-potassium adenosine triphosphatase in acute streptozocin diabetes and its correction by dietary *myo*-inositol supplementation. *J Clin Invest* 72:1058–63, 1983
10. Onji T, Liu M: Effects of alloxan-diabetes on the sodium-potassium adenosine triphosphatase enzyme system in dog hearts. *Biochem Biophys Res Commun* 96:799–804, 1980
11. Pierce GN, Dhalla NS: Sarcolemmal Na^+-K^+-ATPase activity in diabetic rat heart. *Am J Physiol* 245:C241–47, 1983
12. Olson EN, Kelley DA, Smith PB: Characterization of rat skeletal muscle sarcolemma during the development of diabetes. *Exp Neurol* 73:154–72, 1981
13. Nørgaard A: Quantification of the Na,K-pumps in mammalian skeletal muscle. *Acta Pharmacol Toxicol* 58 (Suppl. 1):1–34, 1986
14. Kjeldsen K: Complete quantification of the total concentration of rat skeletal muscle Na^+ + K^+-dependent ATPase by measurements of ^{3}H-ouabain binding. *Biochem J* 240:725–30, 1986
15. Nørgaard A, Kjeldsen K, Hansen O: (Na^+ + K^+)-ATPase activity of crude homogenates of rat skeletal muscle as estimated from their K^+-dependent 3-*O*-methylfluorescein phosphatase activity. *Biochim Biophys Acta* 770:203–209, 1984
16. Rasch R: Control of blood glucose levels in the streptozotocin diabetic rat using a long-acting heat-treated insulin. *Diabetologia* 16:185–90, 1979

17. Jakobsen J: Early and preventable changes in peripheral nerve structure and function in insulin-deficient diabetic rats. *J Neurol Neurosurg Psychiatry* 42:509–18, 1979
18. Nørgaard A, Kjeldsen K, Hansen O, Clausen T: A simple and rapid method for the determination of the number of ^{3}H-ouabain binding sites in biopsies of skeletal muscle. *Biochem Biophys Res Commun* 111:319–25, 1983
19. Nørgaard A, Kjeldsen K, Clausen T: A method for the determination of the total number of ^{3}H-ouabain binding sites in biopsies of human skeletal muscle. *Scand J Clin Lab Invest* 44:509–18, 1984
20. Nørgaard A, Kjeldsen K, Hansen O: K^+-dependent 3-*O*-methylfluorescein phosphatase activity in crude homogenate of rodent heart ventricle: effect of K^+-depletion and changes in thyroid status. *Eur J Pharmacol* 113:373–82, 1985
21. Close RI: Dynamic properties of mammalian skeletal muscles. *Physiol Rev* 52:129–97, 1972
22. Moore RD, Munford JW, Pillsworth TJ Jr: Effects of streptozotocin diabetes and fasting on intracellular sodium and adenosine triphosphate in rat soleus muscle. *J Physiol* (Lond) 333:277–94, 1983
23. Pain VM, Garlick PJ: Effect of streptozotocin diabetes and insulin treatment on the rate of protein synthesis in tissues of the rat in vivo. *J Biol Chem* 249:4510–14, 1974
24. Schmitz O, Alberti KGMM, Hreidarsson AB, Laurberg P, Weeke J, Ørskov H: Suppression of the night increase in serum TSH during development of ketosis in diabetic patients. *J Endocrinol Invest* 4:403–407, 1981
25. Kjeldsen K, Nørgaard A, Gøtzsche CO, Thomassen A, Clausen T: The effect of thyroid status on the number of Na-K pumps in human skeletal muscle. *Lancet* 2:8–10, 1984
26. Kjeldsen K, Everts ME, Clausen T: The effects of thyroid hormones on ^{3}H-ouabain binding site concentration, Na,K-contents and ^{86}Rb-efflux in rat skeletal muscle. *Pfluegers Arch* 406:529–35, 1986
27. Jensen J, Nørby JG, Ottolenghi P: Binding of sodium and potassium to the sodium pump of pig kidney evaluated from nucleotide-binding behaviour. *J Physiol* (Lond) 346:219–41, 1984
28. Green RJ, King RHM, Thomas PK, Baron DN: Sodium-potassium-ATPase activity in the dorsal root ganglia of rats with streptozotocin-induced diabetes. *Diabetologia* 28:104–107, 1985
29. Lombet A, Laduron P, Mourre C, Jacomet Y, Lazdunski M: Axonal transport of Na^+,K^+-ATPase identified as a ouabain binding site in rat sciatic nerve. *Neurosci Lett* 64:177–83, 1986
30. Jakobsen J: Axonal dwindling in early experimental diabetes. 1. A study of cross-sectioned nerves. *Diabetologia* 12:539–46, 1976
31. Greene DA, Lattimer S: Impaired energy utilization and Na,K-ATPase in diabetic peripheral nerve. *Am J Physiol* 246:E311–18, 1984
32. Hnik P, Holas M, Krekule I, Kriz N, Mejsnar J, Smiesko V, Ujec E, Vyskocil F: Work-induced potassium changes in skeletal muscle and effluent venous blood assessed by liquid ion-exchanger microelectrodes. *Pfluegers Arch* 362:85–94, 1976
33. Jones LR, Besh HR: Isolation of canine cardiac sarcolemmal vesicles. In *Methods in Pharmacology*. Vol. 5. Schwartz A, Ed. New York, Plenum, 1984, p. 1–12