Journal of Neurochemistry
Lippincott Williams & Wilkins, Inc., Philadelphia

Localization of Na,K-ATPase α/β Isoforms in Rat Sciatic Nerves: Effect of Diabetes and Fish Oil Treatment

*†A. Gerbi, *S. Sennoune, *S. Pierre, §J. Sampol, †D. Raccah, †Ph. Vague, and *‡J.-M. Maixent

**Laboratoire de Recherche Cardiologique, Faculté de Médecine, †Laboratoire de Diabétologie, CHU Timone, ‡Laboratoire de Biochimie Fondamentale, Moléculaire, et Clinique, UFR de Pharmacie, and §Laboratoire d'Hématologie, Hôpital de la Conception, Marseille, France*

Abstract: The localization of the Na,K-ATPase isoenzymes in sciatic nerve remains controversial, as well as diabetes-induced changes in Na,K-ATPase isoforms. Some of these changes could be prevented by fish oil therapy. The aim of this study was to determine by confocal microscopy the distribution of Na,K-ATPase isoforms (α1, α2, α3, β1, and β2) in the sciatic nerve, the changes induced by diabetes, and the preventive effect of fish oil in diabetic neuropathy. This study was performed in three groups of rats. In the first two groups, diabetes was induced by streptozotocin and rats were supplemented daily with fish oil or olive oil at a dosage of 0.5 g/kg of body weight. The third one was a control group that was supplemented with olive oil. Five antibodies against specific epitopes of Na,K-ATPase isoenzymes were applied to stained dissociated nerve fibers with fluorescent secondary antibodies. The five isoenzymes were documented in nonspecific regions, Schwann cells (myelin), and the node of Ranvier. The localization of the α1, α2, and β1 isoenzymes was not affected by diabetes. In contrast, diabetes induced a decrease of the α2 subunit ($p < 0.05$) and an up-regulation of the β2 subunit ($p < 0.05$). These modifications were noted in both regions for α2 and were localized at the myelin domain only for the β2. Fish oil supplementation prevented the diabetes-induced changes in the α2 subunit with an additional up-regulation. The β2 subunit was not modified. A phenotypic change similar to nerve injury was induced by diabetes. Fish oil supplementation partially prevented some of these changes. **Key Words:** Na,K-ATPase—Isoforms—Immunocytochemistry—Confocal laser scanning microscopy—Image analysis—Diabetes—Dietary fish oil.
J. Neurochem. **73,** 719–726 (1999).

Neuropathy is a common and often disabling complication of diabetes (Greene et al., 1988). Diabetic peripheral nerve dysfunction reflects a complex interaction between disturbed metabolism and abnormal nerve structure. In diabetic rats and humans, Na,K-ATPase activity measured in the nerve is substantially reduced (Das et al., 1976; Greene and Lattimer, 1983, 1984; Scarpini et al., 1993). Some investigations attribute the acute reversible slowdown in nerve conduction to abnormalities in nerve Na^+ and Na^+-related metabolism mediated by a reversible Na,K-ATPase defect (Greene et al., 1984, 1987). This defect in peripheral conduction is reversible and resembles that seen earlier in human diabetes (Ward et al., 1971; Scarpini et al., 1993). Although the pathogenic mechanism underlying the defect in Na,K-ATPase activity remains obscure, Na,K-ATPase is a good marker of potential treatment efficiency. This defect in enzyme activity can be reversed in part by ganglioside treatment (Bianchi et al., 1988), by protein kinase C agonists (Greene and Lattimer, 1986), and by polyunsaturated fatty acid treatment, namely, γ-linolenic acid (Coste et al., 1997) and fish oil (Gerbi et al., 1998).

The functional Na,K-ATPase enzyme is a heterodimer composed of an α and β subunit in equimolar amounts (Cantley, 1981; Levenson, 1994). The α subunit contains the Na^+-dependent phosphorylation site and amino acids required for ouabain binding (Lingrel and Kuntzweiler, 1994). The β subunit is a glycoprotein required for enzyme activity, although its specific function is unknown.

In the nervous system, three different isoforms of the α subunit, termed α1, α2, and α3, and two β isoforms, termed β1 and β2, are expressed as the product of five distinct members of a multigene family (Shull et al., 1986; Martin-Vasallo et al., 1989). The α isoforms can be characterized by their pharmacological and functional properties (Sweadner, 1989; Berrebi-Bertrand et al., 1990; Gerbi et al., 1993; Jamme et al., 1997; Maixent et al., 1991, 1998; Pierre et al., 1999), suggesting that these

Received December 14, 1998; revised manuscript received March 22, 1999; accepted March 22, 1999.

Address correspondence and reprint requests to Dr. J.-M. Maixent at Laboratoire de Biochimie Fondamentale, UFR de Pharmacie, 27 bd J. Moulin, Marseille, Cedex 13005, France.

Abbreviations used: aa, amino acids; BSA, bovine serum albumin; FITC, fluorescein isothiocyanate; PB, phosphate buffer; STZ, streptozotocin.

TABLE 1. *Source and dilution of primary antibodies used for localization of Na,K-ATPase subunits in rat sciatic nerve*

	Subunit				
	α1	α2	α3	β1	β2
Monoclonal	+	+			
Polyclonal			+	+	+
Name	MCK1	MCB2	UBI	SpET β1	SpET β2
Dilution	1:50	1:100	1:50	1:1,000	1:1,000

MCK1 and MCB2 antibodies and anti-β1 and anti-β2 antibodies were generously provided by K. J. Sweadner and P. Martin-Vasallo, respectively. Anti-α3 antibody was purchased from UBI.

isoforms may have specific functions in the nervous system. All isoforms are expressed in peripheral nerves (Mata et al., 1991, 1993; Kawai et al., 1997). In their last study, Kawai et al. (1997) found that β2 acts as an adhesion molecule in peripheral nerve regeneration.

To determine whether in diabetic neuropathy the defect in nerve Na,K-ATPase activity reflects a reduction in the amount of polypeptide and whether such a defect is isoform specific, quantitative western blot analysis was used and led to divergent results on the distribution of the α subunits of Na,K-ATPase (Borghini et al., 1994; Fink et al., 1994; Gerbi et al., 1998). A major study on the distribution of the α subunit of Na,K-ATPase in rat brain by immunocytochemistry has been realized by McGrail et al. (1991). In view of these findings, we hypothesized that following diabetes and fish oil diet, a disturbing localization of Na,K-ATPase isoforms might be observed.

In the present study, we investigated by confocal laser scanning microscopy the distribution of Na,K-ATPase isoforms in the sciatic nerve and also evaluated the changes induced by diabetes and the preventive effect of fish oil.

MATERIALS AND METHODS

Animals and diets

Five-week-old male Sprague–Dawley rats weighing ~200 g were randomly divided into three groups of five. In two groups, diabetes was induced by intravenous injection of streptozotocin (STZ) at a dose of 60 mg/kg (Sigma, L'Isle d'Abeau, Chesne, France) diluted immediately before injection in citric acid buffer (0.01 mol/L, pH 5.5). In the control group, only citric acid buffer was injected. One group of diabetic animals was fed the standard rat chow diet supplemented with (n-3) fatty acid-enriched fish oil concentrate (MaxEPA; Pierre Fabre Santé, Castres, France) administered during 8 weeks at a daily dose of 0.5 g/kg of body weight by gavage. This supplement is rich in eicosapentaenoic acid [C20:5 (n-3)] and docosahexaenoic acid [C22:6 (n-3)] (Gerbi et al., 1999). The other group of diabetic animals was fed the standard rat chow diet supplemented with olive oil at the same dosage. Diabetic rats were not treated with insulin. The nondiabetic control group was also fed the standard rat chow diet supplemented with olive oil in a volume of 100 μl. Olive oil was chosen as the placebo because it does not contain (n-3) fatty acids (Gerbi et al., 1999). Rats had free access to water. All animal treatments adhered strictly to all institutional and national ethical guidelines.

Blood samples were collected regularly from the tip of the tail, and blood glucose was measured with a reagent strip (Reflolux; Boehringer Mannheim, Mannheim, Germany).

Primary antibodies

Anti-Na,K-ATPase α- and β-isoform subunit antibodies were used for immunocytochemical localization (Table 1). Antibodies specific for each isoenzyme were used for the immunocytochemical detection of all isoenzymes expressed in sciatic nerve. The MCK1 (anti-α1) monoclonal anti-rat kidney antibody provided by K. J. Sweadner (Harvard University, Boston, MA, U.S.A.) interacts with amino acids (aa) 20–27 in the intracellular N-terminal domain of the H1 loop (Felsenfeld and Sweadner, 1988). The MCB2 (anti-α2) monoclonal anti-brain axolemma antibody of rat (Urayama et al., 1989) was a gift of K. J. Sweadner and the anti-α3 polyclonal antibody (purchased from Upstate Biotechnology UBI, Lake Placid, NY, U.S.A.) interacts with aa 320–514 of rat isoforms (Shyjan and Levenson, 1989). In contrast, the two β antibodies are polyclonal anti-human antibodies that interact with β1 at aa 70–303 of the human isoform, whereas anti-β2 interacts at aa 55–290 of the human isoform (Gonzales-Martinez et al., 1994). These two antibodies were provided by P. Martin-Vasallo (Universitat de la Laguna, Tenerife, Spain).

Immunofluorescence

After 8 weeks, rats of the three groups were killed, and sciatic nerves were dissected, desheathed, dissociated into individual fibers, and then treated with collagenase (240 U/ml type IV collagenase; Sigma) at 37°C for 1 h in survival solution (154 m*M* NaCl, 5 m*M* KCl, 2 m*M* $CaCl_2$, 1 m*M* $NaHCO_3$, 25 m*M* HEPES, pH 7.3) and 0.2 mg/ml trypsin inhibitor, which was renewed every 10 min.

Nerve preparations were then fixed with 4% paraformaldehyde in 0.1 *M* phosphate buffer (PB; pH 7.4) for 30 min. The nerves were rinsed successively with PB (3 × 5 min), incubated overnight with sucrose (68%) at 4°C, and transferred at −80°C until used.

When required, samples of sciatic nerve preparations were fixed de novo with 2% paraformaldehyde in 0.1 *M* PB for 10 min. The nerves were rinsed with PB (six times rapidly) and then rinsed successively with 50 m*M* NH_4Cl in PB (3 × 5 min), 1% bovine serum albumin (BSA) in PB (1 × 5 min), and 5% BSA and 0.2% Triton X-100 in PB (1 × 1 h). They were incubated overnight with primary antibodies (Table 1) in 1% BSA and PB and then rinsed with 1% BSA (six times rapidly

TABLE 2. *Body weights and plasma glucose levels for all groups of rats*

	n	Start weight (g)	Final weight (g)	Plasma glucose (mmol/L)
CO	5	213.7 ± 1.9	471.0 ± 11.9[a]	7.2 ± 0.5[a]
DO	5	207.9 ± 1.2	216.5 ± 11.5[b]	34.4 ± 4.4[b]
DM	5	210.7 ± 1.5	205.0 ± 11.2[b]	38.8 ± 3.9[b]

Data are group means ± SEM. CO, control + olive oil; DO, diabetic + olive oil; DM, diabetic + fish oil. Values in the same column not bearing the same superscript letters were significantly different at $p < 0.05$.

and then 3 × 5 min). The sample was then incubated in 5% BSA and PB (1 × 10 min at 4°C). The second antibody, which was Texas Red-conjugated $F(ab')_2$ fragment goat anti-rabbit IgG at the dilution of 1:200 (Immunotech SA, Marseille, France) or fluorescein isothiocyanate (FITC)-conjugated $F(ab')_2$ fragment goat anti-mouse IgG at the dilution of 1:100 (Immunotech SA) was then added in PB containing 1% BSA for 2 h at 4°C. After rinsing (six times rapidly and then 3 × 5 min) in PB, nerve fibers were teased apart under a dissecting microscope, and fibers were mounted in Mowiol (Rodriguez and Deinhard, 1960) and maintained at 4°C overnight.

The nerve fibers were observed under a Leica confocal laser scanning microscope with a ×63 objective.

Quantitative analysis

Images were transferred to an IBM-compatible computer by real-time digitization of the video output of the confocal microscope with a PC Vision + card (Imaging Technology, Bedford, MA, U.S.A.), allowing 8-bit accuracy (i.e., 256 gray levels). The linearity of the acquisition was checked by digitizing artificial images constructed with the confocal microscope computer and spanning the whole range of intensities (Kaplanski et al., 1994). Images were then processed with a previously described analysis system developed by the same laboratory (André et al., 1990). The background fluorescence always comprised between level 20 and 30. The brightness of extracellular pixels was set equal to zero to allow direct visualization of cellular areas. The contour of labeled areas was then constructed with a standard boundary-follow algorithm, using a threshold higher than the background by 16 U. The staining was calculated as the ratio between total fluorescence of the area (total specific fluorescence) and the surface of this area (mean specific fluorescence). Area was expressed in pixels (a pixel represented a rectangular area of 0.25 × 0.25 mm^2). Mean values of six myelin and node of Ranvier domains for each rat of each group were then calculated.

Statistical analysis

All results are expressed as means ± SEM. Differences between control and diabetic groups were tested using Kruskal–Wallis ANOVA with Dunn's test (Sigmastat; Statistical Software). All statistical tests were considered significant at $p < 0.05$.

RESULTS

Changes in body weight and blood glucose

Rats given STZ exhibit a marked hyperglycemia and no gain of body weight at the end of the 8-week experiment (Table 2). Fish oil supplementation had no effect on blood glucose levels or body weight in diabetic rats.

Immunofluorescent staining of Na,K-ATPase isoenzymes in rat sciatic nerves

The immunocytochemical examination of dissociated nerve fibers, using the five specific antibodies against the Na,K-ATPase isoforms, showed isolated fibers in each preparation with the ultrastructural characteristic of nerve fibers (myelin Schwann cell, axon, and node of Ranvier). The number and integrity of fibers observed were dependent on the dissociation in Mowiol. Figure 1 shows the labeled fluorescence of some characteristic isolated fibers chosen on sciatic nerve fibers. Each image chosen was representative of the total area of the preparation and of the group studied. The control of each secondary antibody was realized in the absence of primary antibodies (Fig. 1). Both images showed a nonspecific staining of the secondary antibody at the laser intensity chosen. These controls were entirely devoid of staining and presented a nonquantifiable fluorescence under 1. This intensity of laser detection was chosen to be identical between the two fluorescent molecules. The threshold of immunodetection was fixed as the mean fluorescence quantified for the two controls (i.e., 1). The mean fluorescence detected in the different sections was specific for the isoform immunoreactivity. Moreover, the antibodies well described in the literature were very limited in their cross-reactivity. The fluorescent immunoreactivity described was specific for each isoform expressed in sciatic nerve fibers.

The α1 isoform immunoreactivity was localized in the node of Ranvier as well as in the membrane of the surrounding myelin (membrane of Schwann cells). A nonquantifiable stain was observed along some axons of isolated nerve fibers. Furthermore, detergent treatment was used to detect latent epitopes of Na,K-ATPase. The pattern of the α2 immunoreactivity was almost the same as that of the α1 immunoreactivity. These detections were realized with the anti-mouse FITC antibody, which conserves more integrity of the isolated nerve fibers than the Texas Red fluorescent anti-rabbit antibody. Immunodetection was nevertheless realized on each nerve fiber preparation.

The α3 immunoreactivity was detected in the node of Ranvier and in certain sections of the myelin sheath, and the staining of axons appeared more often than in nerve fibers labeled with α1 and α2 antibodies. β1 and β2 immunoreactivity patterns were approximately the same as that of α3 but with a more pronounced staining of the axons. The staining was always more pronounced in the node of Ranvier than in the myelin sheath. Nevertheless, this difference was not significant. The image quantification allowed the measurement of specific immunofluorescences for the five isoforms significantly detected in particular regions from nerve fibers (node of Ranvier and myelin sheath). So, the diabetes-induced alteration of Na,K-ATPase could be evaluated (Fig. 1). The amount and localization of the α1, α3, and β1 isoforms were not affected in diabetes. In contrast, the immunodetection of α2 and β2 isoforms was modified by diabetes in a specific manner (Fig. 1). The amount of the α2 isoform

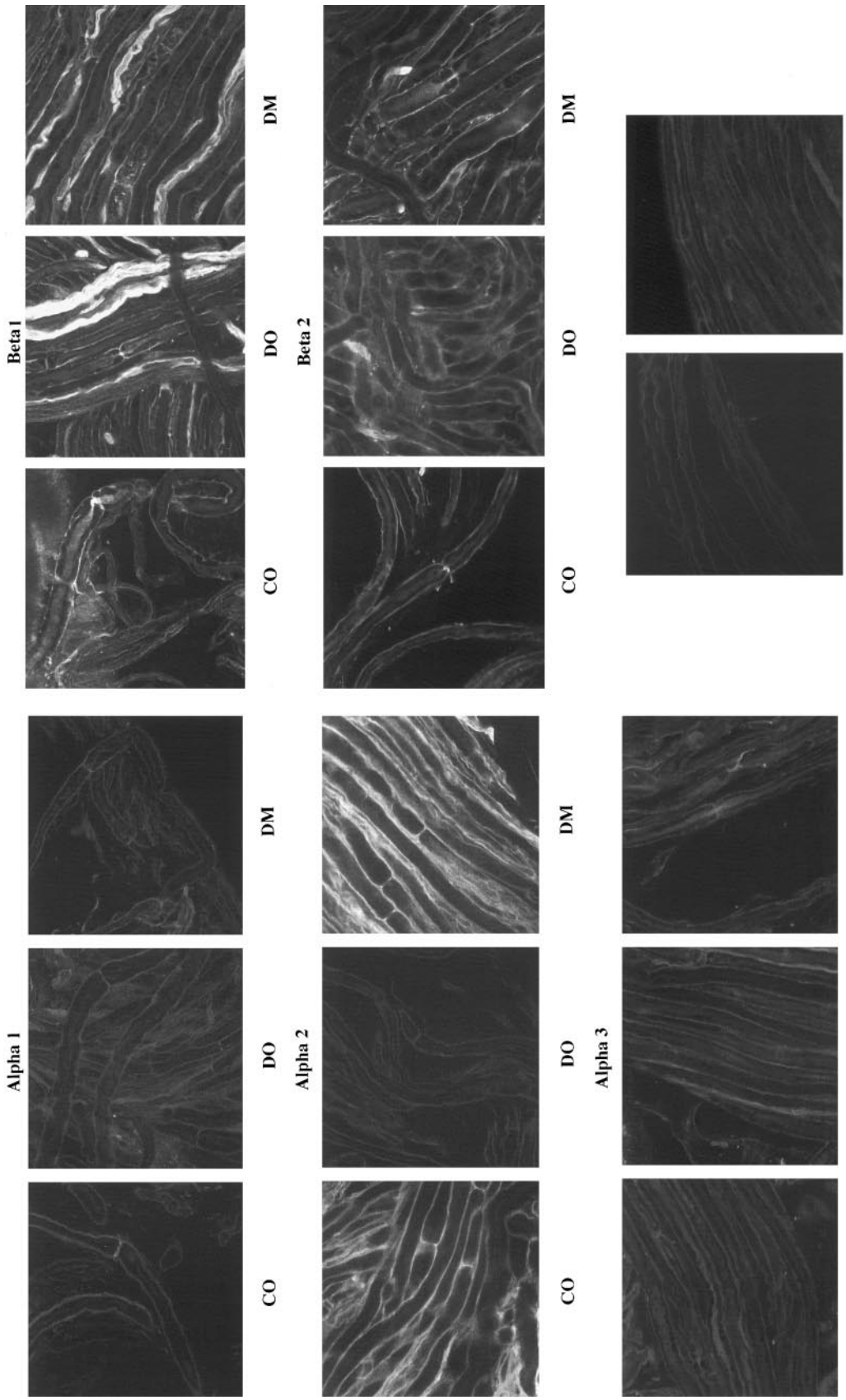

FIG. 1. Immunofluorescent staining of α (**left**) and β (**right**) subunits of Na,K-ATPase isoforms in rat nerve fibers. CO, control + olive oil; DO, diabetic + olive oil; DM, diabetic + fish oil. Two photographs representing the controls for staining of the different secondary antibodies (FITC and Texas Red) are shown at the bottom of the right panel. The mean fluorescence was not quantifiable under the threshold of detection of 1.

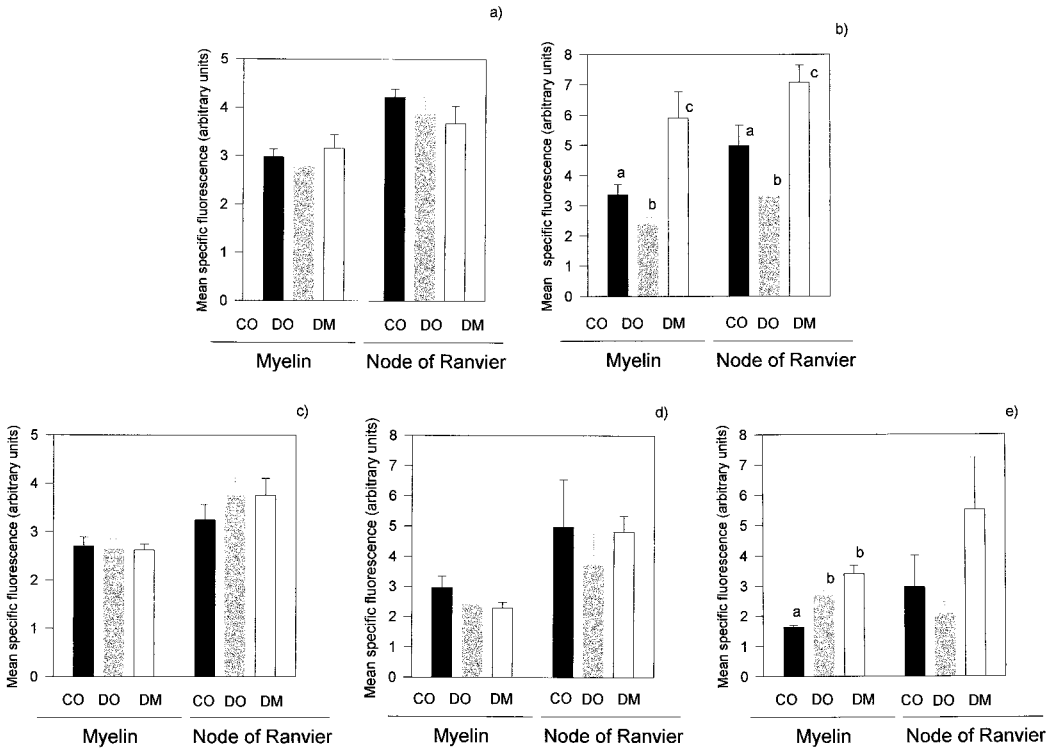

FIG. 2. Quantitative evaluation of the immunofluorescent staining of Na,K-ATPase isoforms in Schwann cells and nodes of Ranvier of sciatic nerve fibers: $\alpha 1$ (**a**), $\alpha 2$ (**b**), $\alpha 3$ (**c**), $\beta 1$ (**d**), $\beta 2$ (**e**). The staining was calculated as the ratio between the total fluorescence of the area and the surface of this area. Mean values of six myelin and node of Ranvier domains for each rat of each group were then calculated. $p < 0.05$, statistical difference between groups a, b, and c. CO, control + olive oil; DO, diabetic + olive oil; DM, diabetic + fish oil.

(Fig. 2) was decreased in the myelin and in the node of Ranvier (control + olive oil vs. diabetic + olive oil, $p < 0.05$), whereas the amount of $\beta 2$ was specifically increased in the myelin sheath (control + olive oil vs. diabetic + olive oil, $p < 0.05$) and not affected in the node of Ranvier.

The fish oil supplementation induced an up-regulation of the $\alpha 2$ isoform in the two regions detected (control + olive oil vs. diabetic + fish oil and diabetic + olive oil vs. diabetic + fish oil, both different at $p < 0.05$), and no supplementary effect was identified for the $\beta 2$ isoform (Fig. 2). The other isoforms ($\alpha 1$, $\alpha 3$, and $\beta 1$) were not affected by the fish oil treatment.

DISCUSSION

Our study presents data on the localization of the five isoenzymes of Na,K-ATPase previously described in rat sciatic nerves (Kawai et al., 1997; Gerbi et al., 1998). Indeed, as revealed by specific polyclonal and monoclonal antibodies against the five isoforms of the Na,K-ATPase ($\alpha 1$, $\alpha 2$, $\alpha 3$, $\beta 1$, and $\beta 2$), a nonspecific localization was observed in the present study. All isoforms were expressed in the myelin sheath and in the node of Ranvier. In both of these regions, the mean fluorescence could be quantified and compared under two different conditions: diabetes and fish oil-treated diabetic rats. The main results were that diabetes induced a decrease of the amount of the $\alpha 2$ subunit ($p < 0.05$) and an up-regulation of the amount of the $\beta 2$ subunit ($p < 0.05$). These modifications were noted in both regions for the $\alpha 2$ subunit and localized at the myelin domain only for the $\beta 2$ subunit. Fish oil supplementation prevented these changes in the $\alpha 2$ subunit with an additional up-regulation. In contrast, the $\beta 2$ subunit was not modified. A phenotypic change similar to nerve injury (Kawai et al., 1997) with a specific effect on $\alpha 2$ and $\beta 2$ in Schwann cells was induced by diabetes. Fish oil supplementation partially prevented some of these changes.

These results can be compared with our previous work in rats on biochemical, physiological, and histological parameters (Gerbi et al., 1998, 1999). Concerning diabetes-induced alterations, we observed, in the biochemical study, a decrease in the enzymatic activity of Na,K-ATPase that correlated with a slowdown in nerve conduction velocity (Gerbi et al., 1999). The membrane Na,K-ATPase activity defect was also associated with a decrease in protein amounts of $\alpha 1$ and $\alpha 3$ subunits. The decrease in nerve conduction velocity could also result from histological damage (Gerbi et al., 1999). Fish oil treatment always showed a preventive effect on these alterations (Gerbi et al., 1998, 1999). Additionally, we

confirm in the present study a beneficial effect of fish oil treatment.

The effect of diabetes on the abundance of Na,K-ATPase can be questioned. Indeed, we found discrepant results in studying this effect between the present study in situ and the previous one using isolated membrane fractions and western blot analysis. Borghini et al. (1994) did not find any alteration in protein amounts of α1 and α2 subunits of Na,K-ATPase. In contrast, Fink et al. (1994) found a specific decrease in the quantity of the α1 isoform of Na,K-ATPase. We can explain some of the divergences in western blot analysis by the membrane purification procedure and by the antibodies (monoclonal/polyclonal) used to reveal the quantity of Na,K-ATPase isoforms, which differed between these studies.

In this work, we used another assay to assess the diabetes-induced alteration of Na,K-ATPase isoforms, which has the advantage of being in situ with intact nerve structures. For the preventive treatment of diabetic neuropathy, such approaches are new and complete our previous studies on this topic. With these tools, we have been able to detect all the isoforms in sciatic nerves. All isoforms were detected in Schwann cells as well as at the node of Ranvier. Prior studies on the localization of Na,K-ATPase in the central nervous system with polyclonal antisera have localized the Na,K-ATPase in the nodal axolemma of central (Wood et al., 1977; Schwartz et al., 1981) and peripheral (Ariyasu et al., 1985) myelinated nerve fibers. Ariyasu and Ellisman (1987) concluded from their data that Na,K-ATPase is uniformly distributed along unsheathed portions of axons without evidence of detectable focal concentrations of the enzyme and that the catalytic subunit of Na,K-ATPase along unsheathed axons is distinct from the α isoform found in Schwann cells and other organs. Immunocytochemical studies of the sciatic nerves have suggested a more widespread distribution of Na,K-ATPase activity in these tissues with more reaction in the nodes of Ranvier than in the internodal areas of the axolemma (Vorbrodt et al., 1982; Inomata et al., 1983; Nasu, 1983). Furthermore, in situ hybridization studies with riboprobes specific for the three α isoforms showed expression of α1 mRNA both in axons and in Schwann cells, whereas that of α3 was observed exclusively in axons and was undetectable in Schwann cells (Mata et al., 1991). Moreover, the α2 and α3 isoforms were clustered mainly at the axolemma in the node of Ranvier (Ariyasu et al., 1985). More recently, Kawai et al. (1997) suggested that α3 and β1 were axonal and that α2 and β2 were found exclusively in Schwann cells.

If Schwann cells play an important role in maintaining the integrity and function of the peripheral nerve, the role of Na,K-ATPase in Schwann cells is not really understood. We believe that functional complexes produce and maintain the Na^+ gradient across the axolemma at the node of Ranvier for the nerve action potential. The present study shows in Schwann cells and at the node of Ranvier a nonspecific localization of isoforms in the sciatic nerves of rat. However, the effect of diabetes on the abundance of Na,K-ATPase can be questioned. Two studies carried out by our group apparently gave different results when the immunological techniques of western blotting and in situ analysis were used. Western blot analysis needs membrane fractionation and purification of enriched Na,K-ATPase, where the characterization of cell origin is lost. Using confocal microscopy, we were able to visualize the ultrastructural distribution of individual isoforms in compartments such as the Schwann cell and node of Ranvier. However, the axon, which represents an important part of the sciatic nerve, has not been unambiguously defined in accordance with a previous study done by Mata et al. (1991). One reason is the lack of accessibility of antibodies to their respective axon binding sites. Membrane fractionation has the advantage of giving access to the axon and its isoform pattern. Thus, the two techniques are complementary, and the divergent effect of diabetes on isoform abundance in appearance lies in the different abundance and subcellular distribution of isoforms within each cellular type. In another study (Gerbi et al., 1999), we evidenced axonal degeneration as the main histological damage resulting from diabetic neuropathy. These alterations were prevented by a fish oil diet. Such effect could be observed in membrane fractions (Gerbi et al., 1998) but not by immunocytochemical confocal microscopy in the present study. As for Borghini et al. (1994), they explained the diabetes-induced decrease in Na,K-ATPase activity by a different phosphorylation state of the catalytic α subunit and did not find any decrease in the quantity of the α subunit. In contrast, Fink et al. (1994) found a specific decrease in the quantity of the α1 isoform. We think that at least part of the divergences in these results could be due to the membrane fractionation and the western blot analysis.

It is well known that diabetes alters Na,K-ATPase activity (Greene and Lattimer, 1983, 1984). Indeed, a decreased excitability of the node of Ranvier attributable to decreased nodal Na^+ permeability in the axon is responsible for the slowdown in nerve conduction velocity (Brismar and Sima, 1981). As the extrusion of intra-axonal Na^+ is dependent on normal Na,K-ATPase activity, this correlates with the reduced Na,K-ATPase activity in diabetic neuropathy (Greene et al., 1984), which is reversible.

This in situ evaluation suggests a specific implication of the α2 isoenzymes in the reduction of Na,K-ATPase activity and reduced nerve conduction velocity. The increase in the β2 isoform, known to be an adhesion molecule in Schwann cells, could be a marker of nerve injury, as suggested by Kawai et al. (1997).

In this study, we have also evaluated the preventive treatment of fish oil supplementation. We previously observed a beneficial effect of fish oil supplementation on functional, biochemical, and anatomical changes of peripheral nerve fibers induced by diabetes (Gerbi et al., 1998, 1999). We have observed a specific preventive effect on the decrease of the α2 isoenzyme. Moreover, a significant up-regulation of this immunoreactivity has

been observed in the myelin sheath and at the node of Ranvier. In contrast, a supplementary effect was not observed for the localization of other isoenzymes.

Our results show a nonspecific distribution of all Na,K-ATPase isoforms in Schwann cells and nodes of Ranvier of sciatic nerves; a specific effect on the $\alpha 2$ and $\beta 2$ isoforms of Na,K-ATPase in diabetes in these cells; and a specific restoration by fish oil supplementation of the $\alpha 2$ isoform of Na,K-ATPase. This could explain the beneficial effect of fish oil on the altered nerve cell conduction during diabetic neuropathy (Gerbi et al., 1998, 1999).

Acknowledgment: This work was supported in part by grants from Pierre Fabre Santé. We thank R. Pistoresi and E. Cosa for their help with confocal microscopy.

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