Substance P and Somatostatin Content and Transport in Vagus and Sciatic Nerves of the Streptozocin-Induced Diabetic Rat

DAVID B. MacLEAN

SUMMARY

Substance P (SP) and somatostatin (SS) are two widely distributed neuropeptides that within the vagus and sciatic nerves are localized predominantly in sensory fibers. The effect of diabetes mellitus on their content or transport in sensory nerves is unknown. With the nerve ligation technique, the peripheral orthograde 24-h transport of both peptides was quantified in the vagus nerve 3 days or 1 mo after induction of streptozocin (STZ) diabetes and in both the vagus and sciatic nerves after diabetes of 3 mo duration. In acute (3-day) diabetics, neuropeptide transport in the vagus was unaltered. After 1 mo, SP transport was significantly increased; content in unligated contralateral nerve was unaltered. Transport of SS was unchanged, and content in contralateral nerve was too low to reliably quantitate. After diabetes of 3-mo duration, transport of both peptides in the vagus nerve was increased in STZ-induced diabetic (STZ-D) rats versus both weight- and age-matched controls: SP 474 ± 17 (N = 10) vs. 358 ± 32 (N = 13) pg/24 h, STZ-D rats vs. controls, mean ± SE, $P < .03$; SS 29 ± 4 vs. 20 ± 3 pg/24 h, STZ-D rats vs. controls, $P < .02$. In the sciatic nerve, SP transport and content were unaltered. SS content was significantly reduced: 17 ± 3 vs. 30 ± 3 pg/3-mm nerve segment, STZ-D rats vs. controls, $P < .01$. SS transport in the sciatic nerve of diabetic rats was variably reduced ($P < .07$), and transport rates were increased (1.41 ± 0.13 vs. 0.96 ± 0.10 mm/h, STZ-D rats vs. controls, $P = .06$), suggesting increased SS turnover within diabetic nerves.

This study indicates that the diabetic state has little short-term adverse effect on neuropeptide synthesis and transport within sensory nerves. Decreased SS content in the sciatic nerve may be a useful marker of early diabetic neuropathy. *Diabetes* 36:390–95, 1987

From the Department of Medicine, Bowman Gray School of Medicine of Wake Forest University, Winston-Salem, North Carolina.

Address correspondence and reprint requests to Dr. David B. MacLean, Dept. of Medicine (Endocrinology), Bowman Gray School of Medicine, 300 South Hawthorne Rd., Winston-Salem, NC 27103.

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Diabetic polyneuropathy is a major contributor to the morbidity related to diabetes mellitus. Peripheral symmetrical polyneuropathy is manifested primarily by sensory symptoms, including pain, and may also contribute to ulceration and infection. Diabetic autonomic neuropathy has both motor and sensory components, although the relative contribution of each to the symptomatology is both clinically and pathophysiologically uncertain (1).

Recently several neuropeptides have been localized in primary sensory neurons, predominantly within small unmyelinated C-fibers of both the autonomic and peripheral nervous systems. Of these, the undecapeptide substance P (SP) (2) and the tetradecapeptide somatostatin (SS) (3) have been most categorized regarding their synthesis, transport, and possible functions within sensory nerves (4–7). The peptides are synthesized and largely processed within ganglion cell bodies and exported for axoplasmic transport by a microtubular-dependent rapid transport system. Most of the synthesized peptide is transported in a distal direction toward peripheral sites of innervation. One-fifth to one-third is transported centrally toward the central nervous system, i.e., from vagal sensory ganglia toward the medulla oblongata or from spinal ganglia toward the dorsal horn of the spinal cord (6,8,9).

The functions of SP and SS within sensory nerves remain to be fully determined. Central release of SP may mediate pain, chemical and temperature sensation, or (from vagal fibers) various visceral afferent reflexes (9–11). The peripheral release of SP may also modulate a variety of inflammatory, immune, and trophic functions of sensory nerves (12–15). SS may modulate SP release or actions or mediate specific sensory modalities (16); its roles within sensory neurons are otherwise unknown.

Altered neuropeptide transport or content may be an early biochemical marker of sensory nerve dysfunction or disease in diabetes mellitus, e.g., altered regulation due to the metabolic derangements of diabetes (hyperglycemia and/or hy-

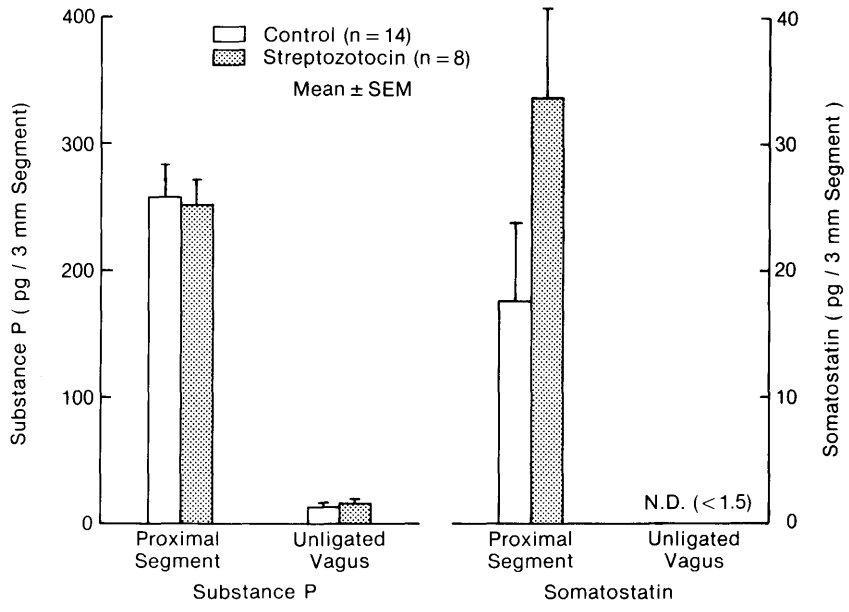

FIG. 1. Effect of acute (3-day) streptozocin-induced diabetes on neuropeptide transport in rat vagus nerve. Left cervical vagus nerve was ligated and resected 24 h later (see text). Proximal segment refers to net content (proximal minus vagus) in 3-mm nerve segment proximal (and adjacent) to ligature. Content in contralateral unligated nerve represents content in 3-mm unligated nerve (3 pooled segments ÷ 3). There was marked variability of transport of somatostatin (*right*) in streptozocin-treated animals; apparent increase is not significant ($P > .1$). Means ± SE. N.D., not detected.

poinsulinemia) or as an indirect result of neuropathy. In these studies, the nerve ligation model has been used to quantitate neuropeptide transport and, indirectly, synthesis. Placement of a ligature in the cervical vagus or sciatic nerve distal to their sensory ganglia results in a time-dependent accumulation of neuropeptide proximal to the site of ligature. Over a 24-h period, this amount is 6- to 10-fold greater than in an equal segment of unligated (contralateral) nerve. There is no net accumulation distal to the ligature, confirming the absence of significant retrograde transport (6–8). Kinetic studies in vitro employing both nodose (vagal) (17) and dorsal root (sciatic) (9) ganglia and attached nerve trunk have demonstrated that this accumulation adjacent to ligature results from ongoing neuropeptide synthesis within the ganglia and its subsequent axoplasmic transport. With the ligation model, the content and transport of SP and SS in the vagus nerve were measured in rats 3 days and 1 mo after the induction of streptozocin (STZ) diabetes and in both the vagus and sciatic nerves after diabetes of 3-mo duration.

MATERIALS AND METHODS

Sprague-Dawley rats (Charles River, Wilmington, MA) initially weighing 200–300 g were used for all the experiments. Animals were housed two per cage in the animal care facility of the Bowman Gray School of Medicine and fed a standard chow diet. All experimental procedures were approved by the school's Committee for Animal Care.

Chemicals and reagents. Synthetic SP and SS were purchased from Peninsula, Belmont, CA. STZ was purchased from Sigma (St. Louis, MO). Insulin (bovine Ultralente) was a gift from Lilly (Indianapolis, IN).

Induction of STZ diabetes. Fasted rats were anesthetized lightly with 30–40 mg i.p. pentobarbital sodium. The external jugular vein was exposed, and STZ, dissolved in sodium citrate, pH 5.5, was injected as a single bolus at a dose of 60–65 mg/kg i.v. (18). The animals were placed in metabolic cages for 3 days and glycosuria confirmed. In addition, glucose was measured in a single random tail vein sample by the glucose oxidase method. Animals with blood sugars of <350 mg/dl (< 5% of injected animals) were excluded from further study. Animals were subsequently housed two per cage and fed a standard chow diet. Control animals underwent the same surgical procedures, including the injection of citrate vehicle.

Determination of SP and SS transport in the vagus and sciatic nerves. Three days, 1 mo, and 3 mo after the induction of STZ diabetes, SP and SS transport were quantitated in the vagus nerve as previously described (7) and summarized briefly below. In addition, at 3 mo, the transport of both neuropeptides was also quantitated in the sciatic nerve. Under pentobarbital sodium anesthesia, the left cervical vagus nerve (3 days and 1 mo) or both the left cervical and sciatic nerves (3 mo) were surgically exposed and ligated with 4-0 suture. The wounds were closed and the animal replaced in its cage. Twenty-two hours later the animal was reanesthetized and the nerves resected. Ligated nerves were cut into 3-mm segments proximal and distal to the ligature and the contralateral unligated nerves cut into two (sciatic) or three (vagus) 3-mm segments for subsequent extraction and radioimmunoassay.

Neuropeptide content was measured with specific radioimmunoassays as previously described (7). SP antiserum 190 was raised in our laboratory and has been previously characterized (17). The SP assay is sensitive to 4 pg/tube with intra- and interassay coefficients of variation of 11 and 14%. SS antiserum was kindly provided by Dr. S. Reichlin (Boston, MA). The assay is sensitive to 2 pg/tube with intra- and interassay coefficients of variation of 9 and 15%. As previously determined, >95% of measured immunoreactivity in segments proximal to ligature and in unligated nerve coelutes with the synthetic peptides using both gel permeation and HPLC (7). Nerves from animals with diabetes of 3 days or 1 mo were included in a single assay. Animals with diabetes of 3-mo duration were studied later and neuropeptides measured in separate radioimmunoassays. For convenience, immunoreactive SP and immunoreactive SS are referred to simply as SP and SS.

Calculation of results. The net transport of neuropeptides is determined by subtracting the content of 3-mm segments of unligated nerve (content of pooled segments divided by

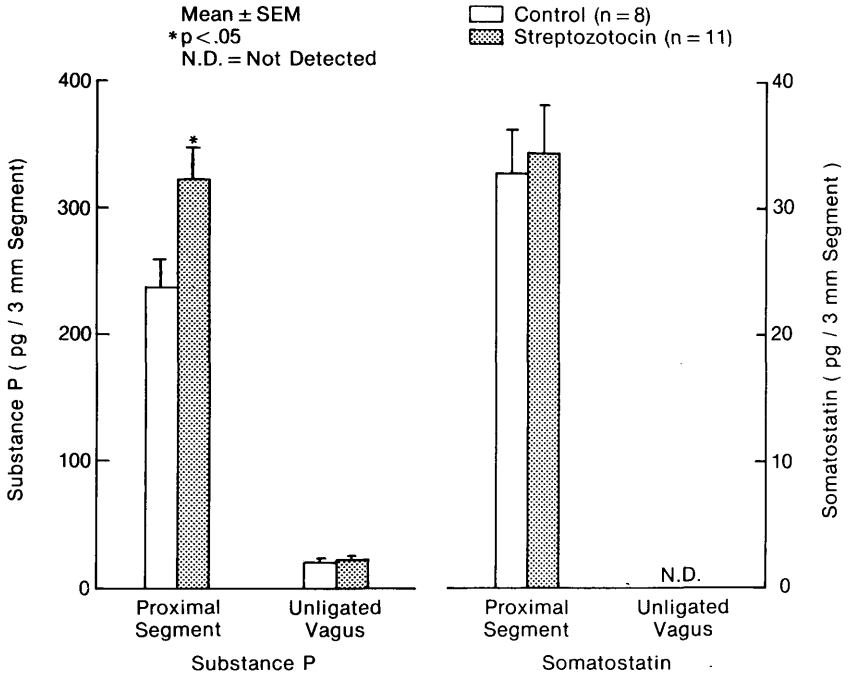

FIG. 2. Effect of subacute (1-mo) streptozocin-induced diabetes on neuropeptide transport in rat vagus nerve. Cervical vagus was ligated and subsequently resected. *Bars* indicate net content in segment proximal to ligature and in contralateral unligated nerve (means ± SE) as described in Fig. 1. Substance P transport, as indicated by increase in net proximal segment content, was increased in streptozocin-treated group; $P < .05$.

their number) from the content in the 3-mm segment proximal to ligature. Protein content in the vagus is very low and variable, and results are more uniformly expressed as neuropeptide per nerve segment; all values in this article are reported in that form.

The apparent transport rate is calculated from the equation $r = a/ct$, where r equals the rate in mm/h, a equals net accumulation in the proximal segment, c equals content in 1-mm unligated nerve, and t equals the duration of ligation in hours. The absolute transport velocity does not change under ordinary experimental circumstances (see ref. 19 for review). Only a portion of neuropeptide transported within either vagus or sciatic nerve may actually be at any given moment within a rapid transport compartment (6–8). The remainder is in an apparent stationary phase. The net transport rate, therefore, more accurately represents the partitioning of neuropeptide into transport and apparent stationary compartments.

Statistics. Data are expressed as means ± SE. The significance of differences between groups was calculated by Student's t test for unpaired data.

RESULTS

Streptozocin diabetes. All of the STZ-induced diabetic (STZ-D) rats included in the study had glycosuria and blood sugars >350 mg/dl. Animals with diabetes of 3-days duration lost 2.5 ± 0.7% of their initial body weight, whereas controls gained 8.1 ± 0.3%. Animals with diabetes of 1 mo gained no weight (−0.59 ± 1.1%), whereas controls gained 27.2 ± 1.8%. The animals with diabetes of 3-mo duration all appeared ill with marked muscle atrophy, increased mesenteric fat, hyperdefecation, and with an overall weight loss of 12.6 ± 0.8% (starting weight 329 ± 19 and weight at time of ligation 294 ± 11 g). A subgroup of rats was treated with daily Ultralente insulin, 6 U/kg, during the 3rd mo. In this group there was excess mortality, with only 5 survivors out of an initial group of 14. Among the survivors, weight loss stabilized, and weight at the time of ligation was 409 ± 11 g ($P < .01$ vs. untreated diabetic rats). Because of the large size of the vehicle-injected animals in the 3-mo experiment (575 ± 15 g), a second control group, weight matched to the diabetic rats, was also studied. Their weight when killed was 337 ± 2 g. There were no differences between these two control groups regarding neuropeptide transport, and the results were pooled.

The effects of STZ-induced diabetes of 3-days, 1-mo, or 3-mo duration on neuropeptide transport. Three days after the induction of streptozocin diabetes, there were no significant changes in SP or SS transport or content in the vagus nerve (Fig. 1). There was, however, a large variation in SS transport (net proximal segment content). After diabetes of 1-mo duration, there was a significant ($P < .05$) increase in net SP content in the segment proximal to ligature, indicating increased transport (Fig. 2). There was a small but insignificant increase in content in an equal segment of unligated nerve. There was no change in SS content in the segment proximal to ligature (Fig. 2, *right*), whereas content in unligated nerves was below the limits of reliable detection (4 pg/3 mm). In animals with chronic diabetes of 3-mo duration, changes in SP transport were more pronounced (Fig. 3). Net SP content in the proximal segment of ligated vagus nerve was increased nearly one-third in STZ-D rats compared with controls ($P < .01$). Content of SP in the contralateral unligated nerve was also significantly increased in diabetic animals: STZ-D rats ($N = 12$) 48 ± 4 vs. controls ($N = 13$) 35 ± 3 pg/3-mm segment, $P < .01$. Although the number of survivors in the insulin-treated group was small, the changes observed in the diabetic rats appeared to be partially reversed. SP transport and content were both reduced relative to untreated animals and were not significantly different from controls (Fig. 3). At this 3-mo time point, SS proximal segment content was also significantly increased

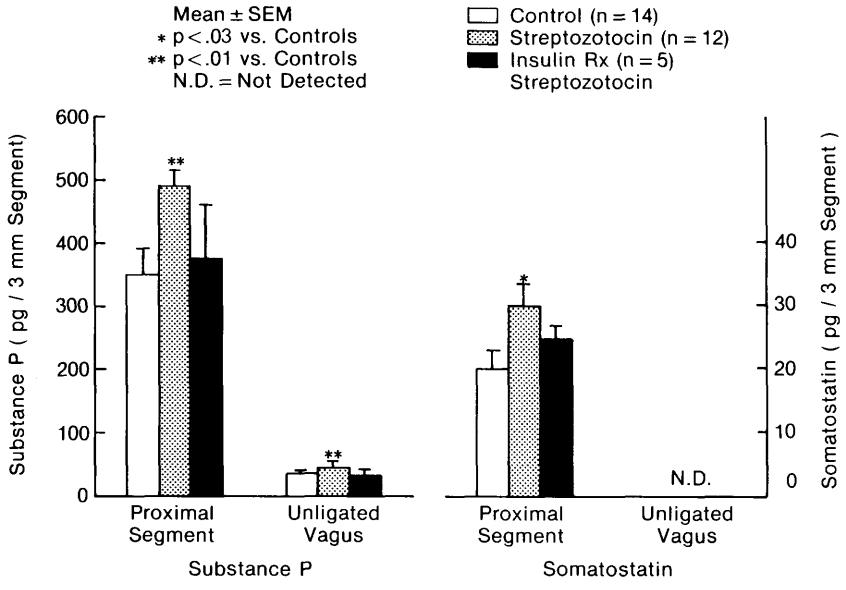

FIG. 3. Substance P and somatostatin transport in vagus nerve of chronic (3-mo) streptozocin-induced diabetic rat. *Bars* indicate net neuropeptide content in segment proximal to ligation and in unligated segments as described in Fig. 1. In this experiment, a small group was insulin treated (*solid bars*). Both peptides were significantly increased in streptozocin-induced diabetic rats compared with controls in segment proximal to ligature, indicating increased transport. At this time point, substance P was also increased in contralateral unligated nerve. Differences between insulin-treated animals and other groups were not significant.

in the vagus nerve and by a similar percentage to that of SP. Insulin administration partially reversed this increase. The low content of SS in unligated vagal segments allowed no comparison between the treated and control groups.

Because of the somewhat surprising changes noted at 1 mo, neuropeptide transport and content in the sciatic nerves of the same animals were also measured in this 3-mo experiment (Fig. 4). There was no change in SP transport or content. In the insulin-treated group, both transport and control segment content were modestly reduced. The findings regarding SS were more striking (Fig. 4, *right*). Net proximal segment content, or transport, was variably reduced ($P < .07$). However, the content in unligated nerves of diabetic animals was significantly reduced by 44% compared with controls: STZ-D rats 17 ± 3 vs. controls 30 ± 3 pg/3 mm, $P < .01$. Observed differences in the insulin-treated group were not significant compared with controls, in part because of the larger variation and smaller sample size.

The apparent transport rates were calculated for SP in the vagus nerve and for both peptides in the sciatic nerve with the equation $r = a/ct$. There were no significant differences in calculated transport rates for SP in the vagus nerve of the 3-day or 1-mo diabetic rats. Also, because of the proportional increases in net proximal and unligated nerve segment contents, calculated apparent transport rates for both groups were nearly identical at 3 mo (Table 1). Because of the low content of SS in the unligated vagus nerve, its transport rates could not be calculated. In the sciatic of chronic STZ-D animals, the apparent transport rate of SP was unchanged, whereas the apparent transport rate of SS was variably increased >40% compared with controls (Table 1, *right*; $P = .06$). This increase in apparent rate is an arithmetic reflection of the decreased content in unligated nerves. As detailed previously, this is not an indication of increased absolute transport velocity but more likely indicates an increased proportion of SS within a rapid transport compartment, i.e., probable increased turnover in nerves despite less transport overall.

DISCUSSION

This study quantitates neuropeptide transport and content in nerves of the diabetic rat for the first time. There are two

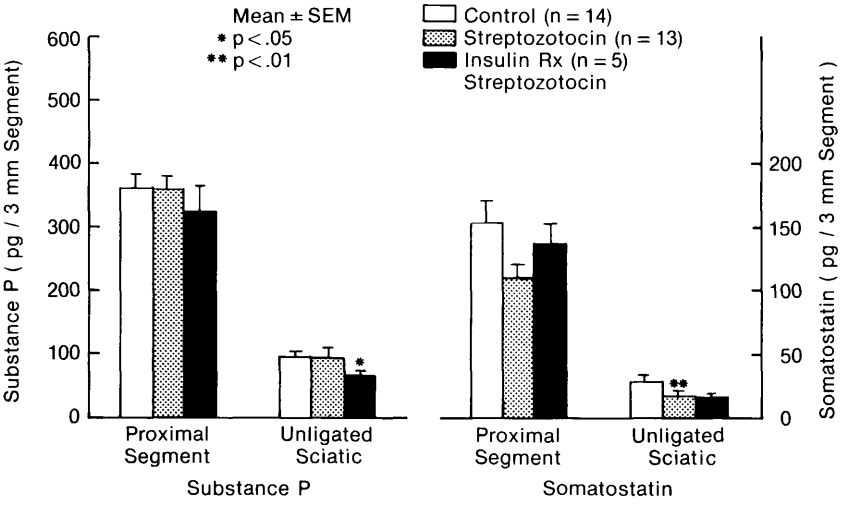

FIG. 4. Neuropeptide transport in sciatic nerve of the chronic (3-mo) streptozocin-induced diabetic rat. Substance P transport was not altered, whereas somatostatin transport (net proximal segment content) ($P < .07$), and particularly content ($P < .01$), were reduced in diabetic group. Insulin administration to diabetic animals during 3rd mo had minor effects on sciatic neuropeptide transport or content.

TABLE 1
Net transport rates of neuropeptides in the vagus and sciatic nerves of the chronic streptozocin-induced diabetic (STZ-D) rat

	Vagus nerve		Sciatic nerve	
	Substance P (mm/h)	Somatostatin (mm/h)	Substance P (mm/h)	Somatostatin (mm/h)
Controls	1.14 ± 0.15	*	0.54 ± 0.04	0.96 ± 0.10
STZ-D	1.14 ± 0.13	*	0.62 ± 0.06	1.41 ± 0.13†

Net transport rate is calculated from equation $r = a/ct$, where a equals net accumulation proximal to ligation, c equals peptide content in 1 mm unligated nerve, and t equals time of ligation in hours. See text for details.
*Somatostatin transport rate was not calculated because of small quantity of peptide in unligated nerve.
†Net transport rate of somatostatin in sciatic nerve of diabetic rats was increased ($P = .06$), indicating increased turnover within nerve.

principal findings. First, the absence of change in SP and SS transport in the vagus nerve in acute (3-day) diabetes suggests that hypoinsulinemia or hyperglycemia per se are unlikely to acutely alter neuropeptide synthesis and transport. The absence of decreases after 1 mo further demonstrates that the diabetic state and its metabolic sequelae do not directly inhibit neuropeptide synthesis/transport. Second, the decline in SS content in the sciatic nerve at 3 mo may be a marker of early sensory neuropathy.

Axonal transport in experimental diabetes has been studied predominantly in the sciatic nerve; quantitative studies in the vagus have not been previously reported. Modest changes have been demonstrated in acetylcholinesterase and choline acetyltransferase transport (20); in one study, no change in the anterograde transport of acetylcholinesterase or dopamine β-hydroxylase was noted (21). Two groups have reported abnormalities of retrograde transport of radiolabeled nerve growth factor (NGF) (22,23). For example, Sidenius and Jakobsen (23) reported a modest 15% reduction in retrograde transport of NGF during 24 h in rats with diabetes of 3- to 5-wk duration.

The mechanism of increased SP transport in the vagus of animals with diabetes of 1- or 3-mo duration is unclear. First, it may have been a response to long-term alterations in metabolism, e.g., hyperglycemia, an effect not apparent after diabetes of 3-days duration. Second, the increase in transport may have been in response to chronic functional activation of vagal sensory fibers. For example, the chronic hyperphagia of the diabetic rats may result in both increased gastric distention and small bowel peristalsis. SP may be a mediator of reflexes triggered by gastric distention (12); in addition, vagal SP release has been measured in response to peristalsis of the isolated small intestine (24). At this time, it is not known whether chronic recurrent activation of vagal sensory fibers in vivo results in up- rather than downregulation of neuropeptide synthesis/transport. Acute 24-h changes in membrane polarization in vitro have either no effect or decrease SP synthesis or transport (25). Third, the increase may be a response to early pathological or metabolic alterations within the sensory fibers and cells, e.g., altered polyphosphoinositide synthesis/turnover, and a related decrease in Na^+-K^+-ATPase activity (26,27). Whether the activities of these membrane lipid- and ion-transport systems regulate sensory neuropeptide biosynthesis or transport is not known. It is also unknown whether these noted increases are markers of, or even participants in, altered sensory autonomic function.

Within the sciatic nerve, SP and SS are localized within separate populations of dorsal root ganglia neurons and their small-diameter unmyelinated fibers. It can again be inferred that hypoinsulinemia or chronic hyperglycemia does not decrease the synthesis/transport of SP in the sciatic nerve. Also, because SP synthesis and transport in the sciatic nerve of adult animals probably remain dependent on NGF (26), this study further suggests that alterations in synthesis or retrograde transport of NGF are probably not important in the early pathogenesis of diabetic neuropathy. In humans, a decrease in SP content has been observed in peripheral nerves of patients with chronic diabetic neuropathy (29). The unchanged levels in rats suggest that this decrease in humans is due to late anatomic degeneration of SP-containing fibers rather than a functional decline in SP synthesis/transport.

Somatostatin content was very decreased in the sciatic nerves of STZ-D animals ($P < .01$). Although SS transport was not quite significantly decreased ($P < .07$) nor were transport rates significantly increased ($P = .06$), the findings effectively rule out that the decreased content is due to increased turnover alone. Rather, the overall pattern of findings suggests that disease or degeneration of a subpopulation of sensory fibers is occurring after 3 months of STZ-induced diabetes. There is other evidence that SS and SP are differentially regulated within dorsal root neurons. SS but not SP content is also decreased in the sciatic nerve of the aging Fisher rat (30). Mudge (31) has demonstrated in cultures of dorsal root neurons that SS synthesis/content depends on support or Schwann cells, whereas SP in the same cultures does not. Their findings, the differential sensitivity of the two peptides to the diabetic state, and the pathologic observations of myelin degeneration in experimental diabetes (32) together suggest that loss of Schwann cell integrity may play a role in early diabetic neuropathy.

The number of surviving insulin-treated animals was unfortunately small; surviving animals did regain some weight, suggesting that the treatment was partially effective. Although there was a consistent trend toward reversal of neuropeptide changes in STZ-D animals, a larger study is necessary to confirm that trend.

In summary, sensory neuropeptide content and transport have been quantitated in the STZ-D rat. Increased neuropeptide transport in diabetes of 1- and 3-mo duration may be a marker of altered sensory vagal function in that disorder. Finally, the decreased SS content and evidence of increased turnover in the sciatic nerve after 3 mo of diabetes may indicate early disease of sensory fibers. These alterations in neuropeptide transport/content could serve as useful markers in the study of the early pathophysiology of experimental diabetes.

ADDENDUM

Since this article was revised, I obtained samples of sciatic nerve from rats with STZ-induced diabetes of 6-wk duration (D. B. M., Z. Shihabi, and W. Lorentz, unpublished observations). Because of the experimental design, transport

could not be measured. A subgroup of diabetic rats (N = 12) was treated with the aldose reductase inhibitor sorbinil, 20 mg/kg daily. No changes in sciatic content of SP were found among the three groups. Decreased content of SS was again found in the sciatic nerves of diabetic rats: controls (N = 12) 25.6 ± 1.6 pg/3-mm segment vs. STZ-D rats (N = 12) 17.6 ± 1.0 pg/3 mm, means ± SE, $P < .01$. SS content in sorbinil-treated animals (24.0 ± 1.6 pg/3 mm) was similar to controls and significantly increased compared with untreated diabetic rats ($P < .01$).

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