Decreased VIP Content in Peripheral Nerve From Streptozocin-Induced Diabetic Rats

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Decreased VIP Content in Peripheral Nerve From Streptozocin-Induced Diabetic Rats

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After induction of diabetes with streptozocin (STZ-D) in rats, we measured vasoactive intestinal polypeptide (VIP) content in sciatic nerve and spinal cord obtained from nondiabetic, untreated STZ-D, and insulin-treated STZ-D rats. Eight weeks after the onset of diabetes, caudal nerve conduction velocity (NCV) in the untreated STZ-D rats (n = 13) was slower than in the controls (n = 11; mean ± SE 30.9 ± 0.6 vs. 41.4 ± 1.8 m/s, P < 0.001). The decrease in NCV was less marked in the insulin-treated STZ-D rats (n = 11; 36.3 ± 0.9 m/s, P < 0.05 vs. control). VIP content in sciatic nerve decreased in the untreated STZ-D rats (1.33 ± 0.23 ng/g wet wt) compared with the other groups (control, 3.10 ± 0.44, P < 0.01; insulin-treated STZ-D, 2.44 ± 0.55, P < 0.05). However, in spinal cord, VIP content was not significantly different among the three groups. The VIP levels in sciatic nerve showed a positive correlation with NCV (r = 0.430, P < 0.01). In addition, an inverse correlation between VIP levels and blood glucose levels was observed (r = -0.5624, P < 0.001). NCV was also inversely correlated with blood glucose levels (r = -0.7662, P < 0.001). Together with a previous morphological study, these findings suggest a possible causal relationship between reduced VIP content and diabetic neuropathy.

V asoactive intestinal polypeptide (VIP), originally isolated from pork small intestine (1), has a wide range of biological activities, e.g., vasodilation and hypotension (1-4), stimulation of glycogenolysis and lipolysis (5,6), and stimulation of insulin and glucagon release (7,8). At first, VIP was considered to be a gut hormone (9), but immunohistochemical studies have demonstrated that VIP is found in neurons widely distributed throughout the body (10-14). VIP seems to act as a neurotransmitter, neuromodulator, or neurohormone in central and peripheral nervous systems, including autonomic and sensory nerves (15-19).

Immunohistochemical methods have demonstrated that VIP is also present in varicose autonomic nerve fibers in perivascular plexuses of vasa nervorum, i.e., blood vessels supplying blood to the peripheral nerves (20). Neurohumoral control of this small vessel is thought to be important in normal peripheral nerve function, and VIP might play a role in the pathogenesis of neuropathy associated with diabetes mellitus. In fact, Crowe et al. (14) and Gu et al. (21) reported that a reduction in VIP nerve innervation was observed in the penile erectile tissue of diabetic patients with impotence and streptozocin-induced diabetic (STZ-D) rats. However, these data neither proved nor disproved a cause-effect relationship between VIP and diabetic neuropathy.

The aim of our investigation was threefold. First, to compare the VIP content in the peripheral and central nervous systems in diabetes, we measured VIP content in two tissues from these nervous systems from STZ-D rats. Second, to investigate whether VIP is pathophysiologically related to diabetic neuropathy, nerve conduction velocity (NCV) was measured simultaneously with VIP as an indicator of peripheral nerve function. Third, diabetes-induced changes in these parameters were examined by linear regression analysis.
Mogenizer. The homogenates were centrifuged at 10,000 g for 15 min at 4°C. The supernatant was discarded, and the pellets were weighed and stored at -80°C until the extraction of VIP.

All recovery rates of VIP were 85 ± 5%.

Onset of diabetes, rats were killed by cervical dislocation. Characteristics of experimental rats after streptozocin injection. Values are means ± SE over 6 animals.

<table>
<thead>
<tr>
<th>Group</th>
<th>Body weight (g)</th>
<th>Blood glucose (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>before STZ</td>
<td>after STZ</td>
</tr>
<tr>
<td>Control</td>
<td>11</td>
<td>11.3 ± 1.31</td>
</tr>
<tr>
<td>Untreated diabetic</td>
<td>13</td>
<td>29.0 ± 4.4</td>
</tr>
<tr>
<td>Insulin-treated diabetic</td>
<td>13</td>
<td>30.6 ± 6.1</td>
</tr>
</tbody>
</table>

Values are means ± SE.

Radionurommunity of VIP. As previously reported from our laboratory (24), VIP was measured by radionurommunity with specific antibody R-5011), which was kindly provided by N. Yanaihara (Shizuoka College of Pharmacy, Shizuoka, Japan). The antibody was used at a final dilution of 1:100,000. Cross-reactivity of this serum with other peptides, e.g., secretin, glucagon, motilin, substance P, PII, somatostatin, cholecystokinin, and pancreatic polypeptide, was substantially negligible. Pork VIP (2 µg, Peptide Institute, Osaka, Japan) was labeled for 10 min with 125I-labeled sodium (5, mCi) with lactoperoxidase. The tracer was purified on an SPC 25 Sephadex column (1 × 100 cm) in 0.1 M ammonium acetate (pH 7.4) and eluted with 1.2 M ammonium acetate (pH 7.4) with 0.1% bovine serum albumin. The assay buffer was 0.01 M phosphate buffer (pH 7.4) with 0.5% bovine serum albumin. 0.01% EDTA, 0.14 M NaCl, and 250 KIU/ml aprotinin. After precipitation of the antibody and standard VIP or samples for 24 h at 4°C, 125I-labeled VIP was adsorbed. The assay tubes were further incubated for 48 h at 4°C. Bound and free VIP were separated with deproteinized-organic solvent. All samples were measured in triplicate. The intra-assay coefficient of variation was 4.8%, and the inter assay coefficient of variation was 3.4%. The detection limit of this assay was 1.6 pg/tube.

Statistical analysis. All values are means ± SE. Student's t test or Wilcoxon's method after inspection of variance was used for the nonpaired or paired comparison with 5% significance level. Linear regression analysis was also used.

RESULTS

Development of diabetes. Before STZ injection, mean ± SE body weights of the three groups were not different (control, 313 ± 2.0 g; untreated STZ-D, 309 ± 6.4 g; insulin-treated STZ-D, 315 ± 4.4 g). After 3 days of STZ injection, plasma glucose in the untreated STZ-D and insulin-treated STZ-D rats increased compared with controls. Long-term insulin injection effectively prevented extreme hyperglycemia and progressive weight loss in STZ-D rats (Table 1). Body weight was negatively correlated with plasma glucose levels in the experimental rats (r = 0.956, P < 0.001).

Radioimmununmetry of VIP. The standard curve of VIP was shown in Fig. 1. Dilution curves of the samples from sciatic nerve and spinal cord were parallel to the standard curve of VIP.

Changes in VIP. NCV was measured twice before and 8 wk after STZ injection (Fig. 2). Before STZ injection, non-NCV in untreated STZ-D rats was observed between the three groups (control, 27.1 ± 0.5 m/s; untreated STZ-D, 27.1 ± 0.5 m/s; insulin-treated STZ-D, 29.0 ± 0.7 m/s). After 8 wk, NCV was significantly increased in each group (control, 41.8 ± 1.8 m/s; untreated STZ-D, 30.6 ± 0.6 m/s; insulin-treated STZ-D, 36.3 ± 0.9 m/s). A gradual decrease in NCV with age was previously reported in rats (22,27). However, in untreated STZ-D rats, this increase was less marked than the controls. NCV value of the insulin-treated STZ-D rats was between the NCV levels of the other two groups, indicating that insulin treatment partially improved impaired peripheral nerve function. Furthermore, there was a negative correlation between plasma glucose levels and NCV in the experimental rats (r = -0.762, P < 0.001; data not shown).

VIP content in tissues. Eight weeks after STZ injection, the weight of sciatic nerve and spinal cord in the untreated STZ-D rats decreased compared with controls. However, this reduction was completely prevented by the insulin treatment (Table 2).

VIP content in spinal cord was unchanged in value among the three groups (Fig. 3A). In contrast, VIP in sciatic nerve (Fig. 3B) was reduced in the untreated STZ-D rats (1.3 ± 0.23 ng/g wet wt) and restored by insulin to a level similar to that of the controls (1.6 ± 0.4 ng/g wet wt; P < 0.01) in the insulin-treated STZ-D rats (2.4 ± 0.55 ng/g wet wt; P < 0.06). VIP content in control and insulin-treated STZ-D rats was not significantly different. These results indicate that insulin treatment reversed the decreased VIP content in diabetic sciatic nerve. To examine whether loss of fat tissue in diabetes masked a decrease in VIP content in the spinal cord, total VIP content was compared. Total VIP content in spinal cord of untreated STZ-D rats (1.61 ± 0.12 ng/g total wet wt) showed a tendency to be lower than for the other groups (control, 2.09 ± 0.29 ng/g wet wt; insulin-treated STZ-D, 2.12 ± 0.23 ng/g wet wt), but these differences were not significant. In addition, VIP levels in the sciatic nerve negatively correlated with plasma glucose levels (r = -0.5624, P < 0.001; data not shown).

Relationship between VIP content and NCV. To evaluate the significance of reduced VIP in the pathogenesis of diabetic peripheral neuropathy, linear regression analysis between VIP content and NCV in sciatic nerve was examined. Separate analysis for each group did not result in significant correlation (r = 0.082, P = 0.264, and 0.348 in control, untreated STZ-D, and insulin-treated STZ-D rats, respectively). However, linear regression analysis for all three groups revealed a positive correlation between VIP content and NCV in sciatic nerve (Fig. 4). However, these results did not necessarily prove the cause-effect relationship between VIP and diabetic neuropathy.

DISCUSSION

This study demonstrates that VIP content in sciatic nerve is significantly reduced in STZ-D rats. VIP levels in sciatic nerve were negatively correlated with blood glucose levels. Insulin treatment restored the decreased VIP content in this nerve to a level similar to controls. These results suggest that hyperglycemia and/or insulin deficiency leading to other metabolic disturbances is involved in the decreased VIP content.
abnormal derangement may cause the decrease in VIP content in diabetic peripheral nerve. However, the precise mechanism of this reduction of VIP content in the diabetic sciatic nerve was not clarified in this study.

We observed the increase in NCV in rats aged 10 wk over 8 wk of age (STZ-D). Indeed, Miyoshi and Goto (22) found that NCV in Wistar rats aged 10 wk increased during growth up to 23 wk of age. The increase in NCV during this period was 17 m/s. Gilton et al. (28) also found an increase in NCV of the sciatic nerve during growth. This increase in NCV is presumably due to growth of nerve fibers. Also, in their study, NCV in diabetic rats was slower than in controls. Peripherally, the decrease of VIP associated with STZ-D is due to the change of VIP content in the sympathetic postganglionic fibers. Lundberg et al. (36) reported that after crush on spinal cord neurones, large nerve fibers around the ligature on sciatic nerve disappeared. This finding suggests that the major population of VIP-containing nerve fibers in the sciatic nerve is sympathetic postganglionic nerve fibers. In general, these sympathetic nerves innervate and regulate the microvascular circulation, including the vasa nervorum of the peripheral nerve. The presence of VIP in nerves in peripheral nerves has been demonstrated by the immunohistochemical study of Appenzeller et al. (20). VIP is a major vasodilator factor in the regulation of blood flow in the gastrointestinal tract and genital (18) tracts. Therefore, it is reasonable to speculate that a decrease of VIP content in the sympathetic efferent fibers might cause changes of the microvascular circulation in peripheral nerves, and as a consequence, ischemic damage of the nerve might occur. In fact, Tuck et al. (39) found that disturbance in endothelial blood flow with constant coronary insufficient O2 supply was an important etiological factor of diabetic peripheral neuropathy.

Although there is little doubt that multiple biochemical abnormalities, including hyperglycemia, polyol metabolism, or other derangements, in diabetes play an etiologic role in the development of diabetic neuropathy (40,41), we speculate that reduced VIP in the autonomic nerve might be another important pathogenetic factor for diabetic peripheral polyneuropathy.

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