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

KATSUNI NODA, FUMIO UMEDA, HIROSHI ONO, AKITAKA HISATOMI, YOSHIHARU CHIJIIWA, HAJIME NAWATA, AND HIROSHI IBAYASHI

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 in NC-V 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. Diabetes 39:608-12, 1990

Vasoactive intestinal polypeptide (VIP), originally isolated from porcine 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 neurons (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.

RESEARCH DESIGN AND METHODS

Male Wistar rats aged 16 wk and weighing 270–340 g were divided into three groups. One (n = 11) was a control group that was given an intraperitoneal injection of citrate-saline buffer (pH 4.5) only. Another group (n = 3) was given an injection of 60 mg/kg i.p. STZ (lot 605114, Calbiochem-Behring, La Jolla, CA) that was dissolved in citrate-saline buffer just before injection. The STZ-D rats developed glycosuria, polyuria, hyperglycemia, and weight loss. The third group...
mogenizer. The homogenates were centrifuged at 10,000 rpm for 10 minutes in a refrigerated centrifuge. The supernatant was then collected. The collected supernatants were evaporated to dryness with a stream of nitrogen.

All samples were then resuspended in an appropriate volume of the solvents.

Electrophoresis:

The frozen tissues were boiled for 5 min in 10 mM Tris-HCl (pH 7.4) with 0.1% bovine serum albumin. The tissues were homogenized with a sonicator. The homogenates were centrifuged at 10,000 rpm for 10 minutes in a refrigerated centrifuge. The supernatants were collected and used for the determination of VIP concentration.

Electrode:

A standard electrode was used for the measurement of NCV. The electrode was filled with cold saline, and its needle was inserted 5 mm from the caudal end of the vertebral column. The electrode was connected to a non-polarized saline reference electrode.

NCV measurement:

NCV was measured with a modification of the method of Miyoshi and Goto (22) with Neuropack-1 MEB-51 (Nihon Kohden, Tokyo). Before and 8 wk after the onset of diabetes, all rats were anesthetized with an injection of pentobarbital sodium (50 mg/kg i.p.). The tail was clamped with a pair of pliers, and the bilateral sciatic nerves were surgically removed and placed in a glass tube. Electrical stimulation was applied to the sciatic nerve, and the NCV was calculated by dividing the distance between the two recording electrodes (exactly 5 cm) by the latency. To prevent tissue damage of the sciatic nerve before NCV assay, a constant subthreshold voltage was used for the calculation of NCV.

Statistical analysis:

All values are means ± SE. Student's t-test or Welch'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, the body weight was negatively correlated with plasma glucose levels (r = -0.082, P < 0.001, data not shown). The body weight was negatively correlated with plasma glucose levels (r = -0.082, P < 0.001, data not shown).

Radiomunnoassay of VIP:

As previously reported from our laboratory (24), VIP was measured by radiomunnoassay with specific antibody R-501, which was kindly provided by N. Yanaihara (Shizuoka College of Pharmacy, Shizuoka, Japan). The antibody was used at a final dilution of 1:180,000. Cross-reactivity of this serum with other polypeptides, e.g., secretin, gastrin, glucagon, motilin, substrate C peptide, somatostatin, cholecystokinin, and pancreatic polypeptide, was substantially negligible. Porcine VIP (2 μl) (Peptide Institute, Osaka, Japan) was labeled for 10 min with [125I]-labeled sodium (0 μCi, 5 μCi, 20 μCi) with lactoperoxidase. The tracer was purified on an SPC 25 Sephadex column 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 0.05% bovine serum albumin. 0.01% EDTA, 0.14 mM NaCl, and 250 KIU/ml aprotinin. After preincubation of the antibody and standard VIP or samples for 1 h, the samples were incubated for 48 h at 4°C. [125I]-labeled VIP was added. The assay tubes were further incubated for 48 h at 4°C. Bound and free VIP were separated with deoxtran-coated charcoal. All absorbances were measured at 490 nm. The assay was duplicated. The intra-assay coefficient of variation was 4.4%, and the interassay coefficient of variation was 15.1%. The detection limit of this assay was 1.6 pg/tube.

Changes in NCV:

NCV was measured twice before and 8 wk after STZ injection (Fig. 2). Before STZ injection, no differences were noted between the three groups (control, 27.2 ± 1.0 m/s; untreated STZ-D, 27.2 ± 1.0 m/s; insulin-treated STZ-D, 29.0 ± 0.7 m/s). After 8 wk, NCV was significantly increased in every group (control, 41.4 ± 0.8 m/s; P < 0.001; untreated STZ-D, 30.9 ± 0.6 m/s; P < 0.001; insulin-treated STZ-D, 36.3 ± 0.9 m/s; P < 0.001). A gradual decrease in NCV was observed in controls even with age was previously reported in rats (22,27). However, in the 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 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).

Relationship between VIP content and NCV:

To evaluate the significance of reduced NCV in the pathogenesis of diabetic peripheral neuropathy, linear regression analysis was performed between NCV and VIP content in sciatic nerve (Fig. 3). However, no significant correlation between NCV and VIP content was found (correlation coefficient = -0.04, P = 0.76). Nevertheless, VIP content in sciatic nerve increased significantly in the insulin-treated STZ-D rats compared with untreated STZ-D rats (1.2 ± 0.2 ng/g wet wt). Therefore, these results indicate that insulin treatment reversed the decreased VIP content in diabetic sciatic nerve. To examine whether insulin treatment affected VIP content in diabetic sciatic nerve, the levels of VIP were determined in diabetic and insulin-treated diabetic rats. As previously reported from our laboratory (24), VIP was measured by radiomunnoassay with specific antibody R-501, which was kindly provided by N. Yanaihara (Shizuoka College of Pharmacy, Shizuoka, Japan). The antibody was used at a final dilution of 1:180,000. Cross-reactivity of this serum with other polypeptides, e.g., secretin, gastrin, glucagon, motilin, substrate C peptide, somatostatin, cholecystokinin, and pancreatic polypeptide, was substantially negligible. Porcine VIP (2 μl) (Peptide Institute, Osaka, Japan) was labeled for 10 min with [125I]-labeled sodium (0 μCi, 5 μCi, 20 μCi) with lactoperoxidase. The tracer was purified on an SPC 25 Sephadex column 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 0.05% bovine serum albumin. 0.01% EDTA, 0.14 mM NaCl, and 250 KIU/ml aprotinin. After preincubation of the antibody and standard VIP or samples for 1 h, the samples were incubated for 48 h at 4°C. [125I]-labeled VIP was added. The assay tubes were further incubated for 48 h at 4°C. Bound and free VIP were separated with deoxtran-coated charcoal. All absorbances were measured at 490 nm. The assay was duplicated. The intra-assay coefficient of variation was 4.4%, and the interassay coefficient of variation was 15.1%. The detection limit of this assay was 1.6 pg/tube.

TABLE 1

Characteristics of experimental rats after sham-nociceptor injection

<table>
<thead>
<tr>
<th>Group</th>
<th>Body weight (g)</th>
<th>Blood glucose (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>341 ± 6</td>
<td>8.0 ± 0.6</td>
</tr>
<tr>
<td>Untreated diabetic</td>
<td>296 ± 4.8</td>
<td>12.2 ± 2.6</td>
</tr>
<tr>
<td>Insulin-treated diabetic</td>
<td>306 ± 6.5</td>
<td>5.1 ± 0.5</td>
</tr>
</tbody>
</table>

Values are means ± SE.

Relationship between VIP content and NCV:

To evaluate the significance of reduced NCV in the pathogenesis of diabetic peripheral neuropathy, linear regression analysis was performed between NCV and VIP content in sciatic nerve (Fig. 3). However, no significant correlation between NCV and VIP content was found (correlation coefficient = -0.04, P = 0.76). Nevertheless, VIP content in sciatic nerve increased significantly in the insulin-treated STZ-D rats compared with untreated STZ-D rats (1.2 ± 0.2 ng/g wet wt). Therefore, these results indicate that insulin treatment reversed the decreased VIP content in diabetic sciatic nerve. To examine whether insulin treatment affected VIP content in diabetic sciatic nerve, the levels of VIP were determined in diabetic and insulin-treated diabetic rats. As previously reported from our laboratory (24), VIP was measured by radiomunnoassay with specific antibody R-501, which was kindly provided by N. Yanaihara (Shizuoka College of Pharmacy, Shizuoka, Japan). The antibody was used at a final dilution of 1:180,000. Cross-reactivity of this serum with other polypeptides, e.g., secretin, gastrin, glucagon, motilin, substrate C peptide, somatostatin, cholecystokinin, and pancreatic polypeptide, was substantially negligible. Porcine VIP (2 μl) (Peptide Institute, Osaka, Japan) was labeled for 10 min with [125I]-labeled sodium (0 μCi, 5 μCi, 20 μCi) with lactoperoxidase. The tracer was purified on an SPC 25 Sephadex column 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 0.05% bovine serum albumin. 0.01% EDTA, 0.14 mM NaCl, and 250 KIU/ml aprotinin. After preincubation of the antibody and standard VIP or samples for 1 h, the samples were incubated for 48 h at 4°C. [125I]-labeled VIP was added. The assay tubes were further incubated for 48 h at 4°C. Bound and free VIP were separated with deoxtran-coated charcoal. All absorbances were measured at 490 nm. The assay was duplicated. The intra-assay coefficient of variation was 4.4%, and the interassay coefficient of variation was 15.1%. The detection limit of this assay was 1.6 pg/tube.

TABLE 2

Wt. weights of sciatic nerve and spinal cord in control and sham-nociceptor-injected rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Wt. weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>110 ± 3.4</td>
</tr>
<tr>
<td>Untreated diabetic</td>
<td>105 ± 4.7</td>
</tr>
<tr>
<td>Insulin-treated diabetic</td>
<td>125 ± 4.7</td>
</tr>
</tbody>
</table>

Values are means ± SE.
abiotic 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 diabetic NCV in rats aged 10 wk or diabetic sciatic nerve of diabetic rats. 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. Gillon et al. (28) also found an increase in diabetes rather than an inhibition of nerve growth. Values are means ± SE. *P < 0.05 vs. control. TP < 0.05 vs. insulin-treated STZ-D rats.

FIG. 3. Nociceptive intestinal polypeptide (VIP) content in sciatic nerve (left) and sciatic nerve (right) from control (A), untreated streptozocin-induced diabetic (STZ-D) (B), and insulin-treated STZ-D (C) rats. Values are means ± SE. *P < 0.05 vs. control. TP < 0.05 vs. insulin-treated STZ-D rats.