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.
Mogenizer. The homogenates were centrifuged at 10,000 g for 10 min with 75% acetone. The collected solvents were evaporated with radioimmunoassay buffer as stated below. Mean sample was resuspended in an appropriate volume of water.

Radioimmunoassay of VIP. As previously reported from our laboratory (24), VIP was measured by radioimmunoassay with specific antibody R-501, which was kindly provided by N. Yanahara (Shizuoka College of Pharmacy, Shizuoka, Japan). The antigen was used at a final dilution of 1:180,000. Cross-reactivity of this serum with other polypeptides, i.e., secretin, gastrin, glucagon, motilin, substance P, C-peptide, somatostatin, cholecystokinin, and paracrine polypeptide, was substantially negligible. PIP VIP (2 μg; Peptide Institute, Osaka, Japan) was labeled for 10 min with 125I-labeled sodium (0.5 μCi) with lactoperoxidase. The tracer was mixed with an SP-2 Sephadex column (1 × 10 m) of 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 added. The assay tubes were further incubated for 48 h at 4°C. Bound and free VIP were separated with diethylamide-coated charcoal. All samples were measured in duplicates. 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.

Statistical analysis. All values are means ± SE. Student’s t-test or Wilcoxon rank sum test was applied if an equal distribution was not assumed. The significance level was 0.05.

RESULTS

Development of diabetes. Before STZ injection, mean ± SE body weights of the three groups were as follows: control (418 ± 18 g), untreated STZ-D rats (303 ± 16 g), and insulin-treated STZ-D rats (2.1 ± 14 g). However, 3 days after 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 = 0.966, P < 0.001).

Radioimmunoassay of VIP. The standard curve of VIP as shown in Figure 1. Two parallel longitudinal incisions were made 5 mm from the caudal end of the vertebral column. A total of 12500 (Nihon Kohden, Tokyo). In brief, before and after STZ injection, no significant difference in NCV was observed among the three groups (control, untreated STZ-D, and insulin-treated STZ-D). However, 3 days after STZ injection, plasma glucose in the untreated STZ-D and insulin-treated STZ-D rats increased compared with controls. This study demonstrates that VIP content in sciatic nerve is decreased in diabetic neuropathy. Linear regression analysis using linear regression analysis between NCV and VIP content in sciatic nerve restored the decreased VIP content in this nerve to a level similar to controls. These results suggest that VIP is associated with a decrease in NCV in the diabetic nerve.

Discussion

This study demonstrates that VIP in sciatic nerve is significantly reduced in STZ-D rats. VIP levels in sciatic nerve were negatively correlated with blood glucose levels. In this nerve to a level similar to controls. These results suggest that VIP is associated with a decrease in NCV in the diabetic nerve.
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 NCV in rats aged 10 wk over 8 wk of STZ-D. Indeed, Miyoshi and Goto (22) found an increase in the lumbosacral segment around the ligature on sciatic nerve disappeared. This reduction, as suggested by Jakobsen (29), could be explained by some alteration in nerve fibers related to diabetic rats rather than an inhibition of nerve growth. Our investigation proves a significant correlation between VIP and NCV in nondiabetic and STZ-D rats. Obviously, this evidence does not necessarily prove causal relationship between VIP and neuropathy. However, several lines of circumstantial evidence that suggest an etiologic role for VIP in the development of diabetic neuropathy are present, as discussed below. Interestingly, this study shows, in diabetic rats, VIP content in sciatic nerve but not spinal cord is decreased VIP content in spinal cord of STZ-D rats was not different from controls. Previous reports concerning the distribution of VIP immunoreactivity in spinal cord stated that affective fibers are the main source of VIP in spinal cord, and in particular, the lumbosacral segment receives large numbers of VIP-containing visceral afferents (30-33). The existence of VIP-containing nerve fibers other than visceral afferents in the spinal cord has been confirmed in two independent investigations (34,35). VIP-containing neurons in the spinal cord probably include affective fibers, supraspinal nerve fibers, and intrinsic spinal neurons, although according to several studies, spinal nerve presumably includes only two groups of VIP-containing nerve fibers, i.e., sympathetic postganglionic fibers and somatic afferents (36-38). Because of these findings, the decrease of VIP in visceral or somatic afferents cannot explain our observation in STZ-D rats of a loss of VIP immunoreactivity in sciatic nerve but not in spinal cord. Therefore, our observation strongly suggests that the decrease of VIP associated with STZ-D is due to the change of VIP content in the sympathetic postganglionic fibers. Lundberg et al. (29) also found in experimentally diabetic rats of a loss of VIP immunoreactivity in sciatic nerve fibers around the ligature on sciatic nerve disappeared. This reduction of VIP in sciatic nerve postganglionic 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 particular peripheral nerves in the rat has been demonstrated by the immunohistochemical study of Appenzeller et al. (30). VIP is a major vasodilatory 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 effenter fibers might cause a decrease of VIP in the microvasculature in peripheral nerves, and as a consequence, ischemic damage of the nerve might occur. In fact, Tuck et al. (39) found that disturbance in endoneurial blood flow with constant insufficient O2 supply is an important etiologic factor of diabetic peripheral neuropathy. Although there is little doubt that multiple biochemical abnormalities, including hyperglycemia, polyuria metabolism, or other derangements, in diabetics 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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FIG. 3. Nervous intestinal polypeptide (VIP) content in spinal cord (left) and sciatic nerve (right) from control (A), untreated streptozotocin induced diabetic (STZ-D) (B), insulin-treated STZ-D (C) rats. Values are means ± SE. *P < 0.05 vs. control. †P < 0.05 vs. insulin-treated STZ-D rats.

FIG. 4. Relationship between nerve conduction velocity (NCV) and vasoactive intestinal polypeptide (VIP) content in sciatic nerve of experimental rats. • Control rats, • untreated streptozotocin-induced diabetic (STZ-D) rats, ■ insulin-treated STZ-D rats. Values are means ± SE.