Digestive Diseases and Sciences, Vol. 40, No. 4 (April 1995), pp. 828–836

Effect of Streptozotocin-Induced Diabetes Mellitus on Release of Vasoactive Intestinal Polypeptide from Rodent Small Intestine

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Representative longitudinal muscle strips (6 × 10 mm) from proximal and distal small intestine were excised from control and streptozotocin-treated rats after one month of untreated and insulin-treated diabetes. Untreated diabetes significantly reduced tissue concentrations of vasoactive intestinal polypeptide (VIP) at both intestinal loci. Insulin treatment of the diabetic animals restored tissue VIP concentrations to control group levels, although the beneficial effect of insulin treatment was only significant in the duodenum. Spontaneous release of VIP was significantly attenuated by untreated diabetes at both intestinal sites. In the duodenum, insulin treatment of the diabetic animals restored VIP release to levels indistinguishable from control group values. In the ileum, insulin treatment produced levels of VIP release that were not significantly different from those of the control and untreated diabetic groups. Tetrodotoxin (5×10^{-6} M) significantly—but incompletely—inhibited VIP release from control group animals at both intestinal sites. These observations indicate that diabetes mellitus significantly diminishes VIP tissue concentrations and release from intestinal myenteric nerves. These abnormalities improve with insulin treatment. However, the mechanisms of VIP release from proximal and distal intestine appear to differ not only in their response to the diabetic state, but also in their response to insulin treatment.

KEY WORDS: diabetes mellitus, vasoactive intestinal polypeptide, gastrointestinal motility.

It is generally perceived that the main metabolic complications of diabetes mellitus consist of retinopathy, nephropathy, peripheral neuropathy, and vasculopathy. However, symptoms of gastrointestinal dysfunction are not uncommon among the diabetic population. In one study of diabetic outpatients, nearly 76% complained of nausea, vomiting, diarrhea, constipation, early satiety, or dysphagia (1). The etiology of these disturbances remains unclear, although a neuropathy of the myenteric plexus or of the extramural nerves that regulate gastrointestinal movements has been cited as a factor (2, 3).

Although there have been few exacting studies of the myenteric plexus from human diabetic subjects, a number of investigations have shown abnormalities of the innervation of the gastrointestinal tract in rodents with streptozotocin-induced diabetes. Immunohistologic studies disclose that after 12–18 months of strep-

Manuscript received December 10, 1993; revised manuscript received October 21, 1994; accepted November 16, 1994.

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This work was supported by a research grant from the Department of Veterans Affairs and by a grant (R29 DK38863-05) from the National Institutes of Health.

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tozotocin-induced diabetes the extrinsic paravascular mesenteric nerves and intramural nerves of the rodent small intestine contain numerous markedly swollen dystrophic axons, which stain intensely for tyrosine hydroxylase and dopamine-β-hydroxylase (4). After shorter durations of streptozotocin diabetes (eight weeks), the rodent ileum shows degenerative changes as well in the adrenergic and serotonin-like immunofluorescent nerves (5).

Vasoactive intestinal polypeptide (VIP) is a neurotransmitter that localizes to the neurons of the gastrointestinal tract and appears to have a pluripotential role in the regulation of gastrointestinal motility (6). Cholinergic mechanisms appear to be operant in both the release of VIP as well as the effect of VIP on intestinal smooth muscle contraction (7–9). There have been few critical studies examining whether enteric nerves containing VIP are affected by the diabetic state. One electron microscopic study showed signs of degeneration in the VIP-containing myenteric neurons (10), while another showed no evidence of dystrophic axonopathy in the peptidergic complement of neurons, including VIP (4). However, physiologic studies show evidence for abnormal cholinergic neuromuscular transmission in the myenteric plexus of streptozotocin-treated diabetic rats (11). The abnormality improves with insulin treatment.

While immunohistologic studies suggest that an increase in immunoreactive VIP is characteristic of the diabetic small intestine, the results of quantitative studies of radioimmunoassayable VIP are conflicting. Some studies of rodents diabetic for short durations (8–12 weeks) show an increase in the amount of radioimmunoassayable VIP, whether results are expressed per gram of tissue, per centimeter of intestinal length, or as the total amount of VIP per entire small intestine (12, 13). Interpretation of these observations is confounded by the fact that VIP content tends to vary along the length of the diabetic small intestine. VIP concentrations in the diabetic duodenum and jejunum appear not to be significantly different from those of nondiabetic animals (12). However, significantly more VIP is found in the diabetic ileum. Despite this, total small intestinal VIP content in diabetic animals is significantly higher than that found in nondiabetic control animals, a feature that likely reflects the increased mass of the diabetic intestine (14).

Untreated diabetes has been shown to influence the release of VIP from myenteric nerves (15). The electrically evoked—but not the basal—release of VIP and calcitonin gene-related peptide is attenuated in diabetic rodent ileum. However, the endogenous release of other substances, such as acetylcholine, serotonin, and substance P, appear to be unaffected by the diabetic state.

The purpose of the present investigation was to determine whether: (1) abnormalities in enteric tissue VIP concentration and release occur in the diabetic state, and (2) whether such putative abnormalities are improved by restoration of euglycemia with insulin treatment.

MATERIALS AND METHODS

Adult male rats (Holtzman Co., Madison, Wisconsin) weighing 400–500 g and ranging in age from 80 to 120 days were used in this study. The animals were divided into three age-matched groups. Group C was the control group (N = 12), group D was the diabetic group (N = 10), and group I was the insulin-treated diabetic group (N = 9). Experimental diabetes was induced by injecting 45 mg/kg body weight of streptozotocin in 5 ml of sterile saline into the tail vein of rats that had been fasted overnight. Nonfasting blood glucose concentrations >16.7 mmol/liter at 48 hr after administration of streptozotocin were required for the diagnosis of experimental diabetes. Animals not meeting this criterion received another injection of streptozotocin, 45 mg/kg body wt. Insulin-treated diabetic rats received 3 units of U-40 PZI insulin (Eli Lilly & Co., Indianapolis, Indiana) subcutaneously on day 2 and daily at 9:30 AM. Insulin doses were adjusted to maintain blood glucose concentrations near control group levels. Blood glucose concentrations up to 22.2 mmol/liter in the tail vein were determined at three- to four-day intervals at 9 AM in each nonfasted rat with a reflectance photometer (Miles Laboratories, Elkhart, Indiana). Blood glucose concentrations >22.2 mmol/liter were assigned a value of 22.2 mmol/liter.

On day 30, phenobarbital (50 mg/kg body wt) was injected intraperitoneally to anesthetize the animal. The abdomen of each rat was opened, and two segments of intestine, each approximately 2.0 cm long, were removed just distal to the pylorus and just proximal to the ileocecal valve. Using a dry cotton swab, the longitudinal muscle layer and the adherent myenteric plexus were dissected free from the circular muscle layer. This dissection yielded a longitudinal muscle–myenteric plexus tissue strip that was approximately 1.0 cm long and 0.6 cm wide with reference to the longitudinal axis of the intestine. Each strip was tied at each end with silk thread and placed in a 1.0-ml bath of Krebs' solution containing both albumin (0.1%) and protamine (.005 g/100 ml). The Krebs' solution was gassed constantly with 95% O_2–5% CO_2 and maintained at 36.5°C. The Krebs' solution had the following composition (mM): NaCl, 115.48; KCl, 4.63; NaH_2PO_4, 1.16; $NaHCO_3$, 21.91; $CaCl_2$, 2.47; $MgSO_4$, 1.16; and glucose, 11.5.

One end of the strips was fixed to the bottom of the organ bath while the other was attached to an adjustable rack-and-pinion assembly. Each strip was allowed to hang absolutely slack. The distance between the two ligatures on the muscle strip was carefully measured with a caliper. This was

called the initial length (L_i), and was defined as that length at which muscle tension is zero, but would increase with any further changes in muscle length. Further changes in muscle length were measured by the micrometer on the rack-and-pinion assembly. Each strip was progressively stretched in increments of 1.0 mm to 150% of the initial length. Preliminary studies showed that optimal release of neuropeptide occurred at the initial length or when the muscle strip was stretched to 150% of L_i (see below). In these studies the muscle strips were stretched to 150% of L_i, and this length was maintained as the baseline throughout the experiment.

The tissues were allowed to stabilize for a 20-min baseline period. The organ baths were then drained and refilled with fresh Krebs' albumin solution. The baths were drained and refilled every 20 min for nine cycles, and the effluents were collected in test tubes containing Trasylol (0.05 ml/ml sample), frozen on ice, and saved for VIP assay. Some tissues were also incubated with 5×10^{-6} M tetrodotoxin to examine the effect of neural blockade on VIP release. At the end of the experiment, the ligatures were removed from the strips and the tissues were blotted dry and weighed.

In order to determine tissue VIP concentrations, longitudinal muscle–myenteric plexus tissue strips were dissected from proximal and distal small intestine. The strips were blotted to remove excess water and then weighed. The specimens were then placed in test tubes containing distilled water (0.05–0.10 g tissue/3 cc water) and boiled for 10 min. Glacial acetic acid was then added to the specimens to produce a 0.5 N solution. The specimens were homogenized and the slurry stirred for 2 hr at room temperature. After stirring, the specimens were centrifuged at 20,000 rpm at 4°C until the supernatant was free of particles. The volume of the supernatant was measured and then frozen on ice until ready for radioimmunoassay. The specifics of the VIP radioimmunoassay have been described in a previous communication (16). VIP concentration was expressed per milligram of tissue (blotted wet weight). Values were expressed as means ± standard error (SE) in a minimum of nine animals. Student's t test was used for statistical analysis. A probability value of ≤ 0.05 was used to indicate statistical significance.

Preliminary Studies. Preliminary studies were performed in order to determine the tissue preparation that would yield optimal release of VIP. As described above, adult male rats were anesthetized and a 6-cm-long segment of intestine was removed just distal to the pylorus. Two separate preparations were examined. In one preparation, segments consisting of both longitudinal and circular muscle were opened along their mesenteric insertions and stripped of their mucosae. In the other preparation, segments were further dissected and specimens consisting of only longitudinal muscle and adherent myenteric plexus were obtained. All specimens were then weighed, homogenized, and assayed for VIP content. Figure 1 shows that significantly higher concentrations of VIP are found in those tissues consisting of longitudinal muscle and adherent myenteric plexus than in those tissues consisting of both longitudinal and circular muscle with interposed myenteric plexus.

Muscle strips from both tissue preparations were also mounted in organ baths as previously described. The baths were drained at 20-min intervals and effluents assayed for VIP content. Figure 2 shows that the muscle strips consisting of longitudinal plus adherent myenteric plexus released significantly more VIP than those strips consisting of both circular and longitudinal muscle with interposed myenteric plexus.

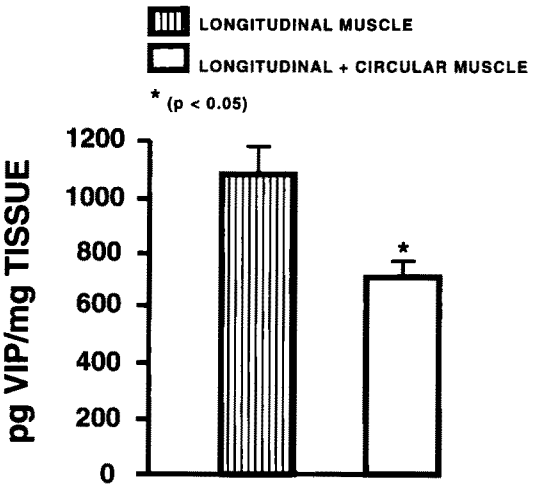

Fig 1. Tissue concentrations of VIP from the two preparations examined. Significantly more VIP is found in those tissues consisting of longitudinal muscle and adherent myenteric plexus (lined bar) than in those tissues consisting of both longitudinal and circular muscle with interposed myenteric plexus (open bar). Values are shown as the mean ± SE in a minimum of 10 animals.

In order to determine the optimum resting tension for VIP release, longitudinal muscle strips with adherent myenteric plexus were mounted in organ baths and either allowed to remain at zero resting tension (unstretched) or stretched to 150% of their initial length. Figure 3 shows that no significant difference in VIP release was seen between the two groups at each 20-min interval examined.

RESULTS

Table 1 shows some characteristics of the three groups of animals. All three groups had a similar mean body weight at the onset of the study. The control group animals gained body weight between the time of entry into the study and the time of sacrifice, while the untreated diabetic animals failed to show any increase in body mass. The insulin-treated diabetic animals gained nearly 14% in body weight, an increase similar to that of the nondiabetic controls. The fasting, nondiabetic control animals showed a mean blood glucose concentration of 5.5 ± 0.3 mmol/liter at the onset of the study. Both the untreated diabetic group and the insulin-treated diabetic group showed similar levels of hyperglycemia after induction of diabetes. Two animals from the insulin-treated diabetic group required another injec-

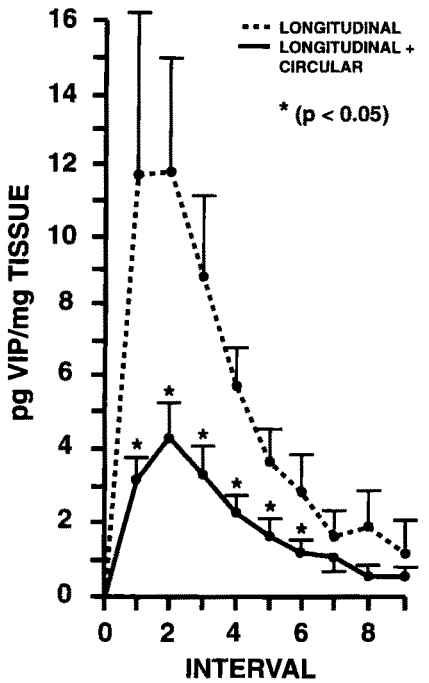

Fig 2. Spontaneous VIP release from isolated muscle strips from the small intestine. Muscle strips were placed in organ baths and allowed to remain slack at zero resting tension. Longitudinal strips consist of only longitudinal muscle and adherent myenteric plexus. Strips labeled longitudinal and circular consist of longitudinal and circular muscle with interposed myenteric plexus. Significantly more VIP is released from the "longitudinal" strips than from the other group containing both muscle layers. Values are shown as the mean ± SE in a minimum of 10 animals.

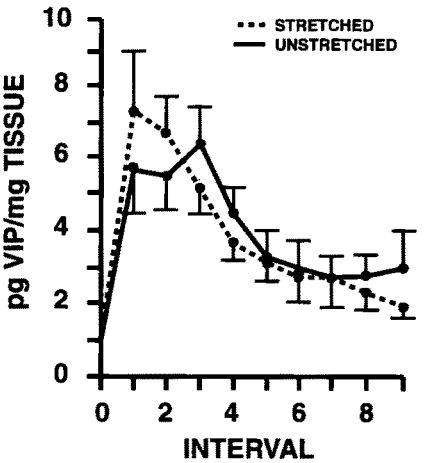

Fig 3. Effect of resting tension on spontaneous VIP release from isolated strips of small intestine. In this study, strips of longitudinal muscle with adherent myenteric plexus were placed in organ baths and either allowed to remain slack at zero resting tension (unstretched) or stretched to 150% of their initial length. No significant differences in VIP release are seen between the two groups at each interval examined. Values are shown as the mean ± SE in a minimum of 10 animals.

tion of streptozotocin. The untreated diabetic animals continued to maintain the same level of hyperglycemia throughout the duration of the study, while the insulin-treated diabetic group manifested blood glucose concentrations that approached those of the control group.

Longitudinal muscle with adherent myenteric plexus from the duodenum of control animals contained nearly 1380 pg VIP/mg tissue, while similar tissue from the ileum contained nearly 1200 pg VIP/mg tissue ($P < 0.05$; Figure 4). Experimental diabetes produced an approximate 40% decrease in tissue VIP levels in the duodenum and ileum ($P < 0.05$, respectively). Insulin treatment of the diabetic animals restored tissue VIP concentrations at both loci in the intestine to levels that were indistinguishable from control group values. However, the beneficial effect of insulin treatment on tissue VIP levels was significant only in the duodenum and not in the ileum.

Figure 5 shows that control tissues from the duodenum and ileum released a maximum of 11.6 and 7.5 pg VIP/mg tissue/20 min, respectively ($P < 0.05$). Untreated diabetic animals released significantly less VIP per milligram tissue from both sites in the intestine. In the duodenum, insulin treatment of the diabetic animals restored VIP release to levels that were indistinguishable from control values at all intervals examined. In contrast, in the ileum, insulin treatment of the diabetic animals produced levels of VIP release that were intermediate to those of the control and the untreated diabetic group.

Tetrodotoxin significantly inhibited VIP release from the control group animals in both the duodenum

TABLE 1. CHARACTERISTICS OF THE THREE GROUPS OF RATS*

	Control (N = 12)	*Diabetic* (N = 10)	*Insulin-treated diabetic* (N = 9)
Mean weight (g)			
Initial	484 ± 25	507 ± 20	475 ± 27
Final	577 ± 21	504 ± 31	540 ± 21
Blood glucose concentration (mmol/liter)			
After induction of diabetes		18.9 ± 0.6	20.3 ± 0.6
Mean values during experiment	6.8 ± 0.4	19.9 ± 0.8	6.8 ± 0.6

*Values are expressed as mean ± SE.

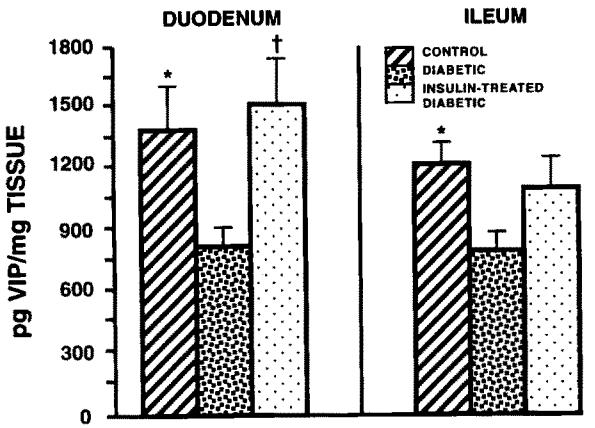

Fig 4. Tissue concentrations of VIP from the proximal and distal small intestine of control, diabetic, and insulin-treated diabetic rats. Untreated diabetes produces a significant diminution in tissue VIP concentrations. In the duodenum, insulin treatment restores tissue VIP concentrations to a level that is similar to control values and significantly different from that of the untreated diabetic state. In the ileum, the insulin-treated diabetic animals show tissue VIP concentrations that are intermediate to the control and untreated diabetic animals and not significantly different from either. In this and in subsequent figures values are given as the mean ± SE in 12 control, 10 diabetic, and 9 insulin-treated diabetic animals. $^{*}P < 0.05$, control vs diabetic; $^{\dagger}P < 0.05$, insulin-treated diabetic vs untreated diabetic.

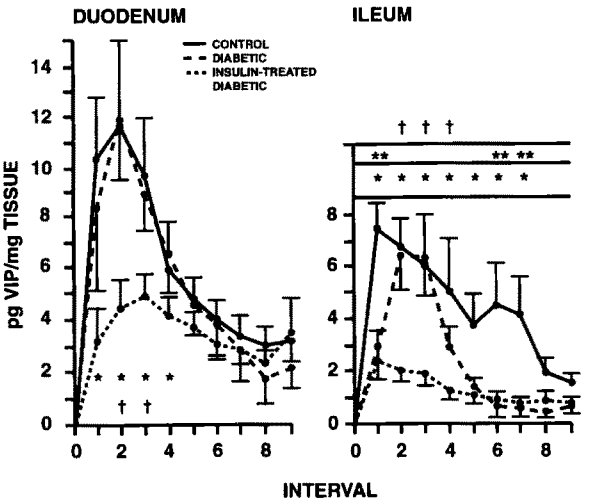

Fig 5. Spontaneous VIP release from duodenal and ileal muscle strips from control, diabetic, and insulin-treated diabetic animals. Tissues are incubated in the presence of Krebs' solution only. Control tissues from both the duodenum and ileum release significantly more VIP than the untreated diabetic tissues at most of the intervals examined. In the duodenum insulin treatment of the diabetic animals restores VIP release to levels that are indistinguishable from control values. In contrast, ileal tissues from the insulin-treated diabetic animals release quantities from VIP that are intermediate to those of the control and untreated diabetic groups and are significantly different at most of the intervals examined. This suggests that the duodenum and ileum differ with respect to the effect of insulin on the mechanisms of VIP release. $^{*}P < 0.05$, control vs diabetic; $^{\dagger}P < 0.05$, untreated diabetic vs insulin-treated diabetic; $^{**}P < 0.05$ control vs insulin-treated diabetic.

and ileum (Figure 6). In the duodenum, significantly less VIP was released in the presence of tetrodotoxin from the diabetic tissues compared to controls at most of the intervals examined. Insulin treatment of the diabetic animals produced levels of tetrodotoxin-resistant VIP release that, in the duodenum, were intermediate to those of the control and the untreated diabetic animals and not significantly different from either group at any of the intervals examined. In contrast, in the ileum, few differences in the levels of tetrodotoxin-resistant VIP release were noted among the control, diabetic, and insulin-treated animals. This suggests that the diabetic state—treated or untreated—has little influence on the tetrodotoxin-resistant mechanisms of VIP release from the distal intestine.

Figure 7 shows the total mean VIP released from the three groups of animals in the presence of Krebs' solution only (upper panels) as well as tetrodotoxin (middle panels). As suggested by the interval studies, untreated diabetes significantly inhibited total VIP release in both duodenal and ileal tissues (upper panels). Insulin treatment restored total duodenal VIP release to levels similar to the control group. In contrast, while insulin treatment improved total ileal VIP release, the values were still significantly less than those of the nondiabetic controls.

The middle panels of Figure 7 show the effect of tetrodotoxin on total mean VIP release. In the duodenum, the untreated diabetic animals showed significantly attenuated VIP release, while insulin treatment restored total VIP release to control group levels. In contrast, no significant differences in tetrodotoxin-resistant mean VIP release were noted from ileal tissues from control, diabetic, and insulin-treated diabetic animals.

The bottom panels of Figure 7 show the effect of tetrodotoxin on VIP release from the three groups of animals, expressed as a percentage of peptide released when the tissues are incubated with Krebs' solution only. In the duodenum, tetrodotoxin inhibited VIP release to a similar extent in the three groups, ie, between 40 and 50%. Thus, the diabetic state appears to have no specific effect on the relative release of duodenal VIP from tetrodotoxin-sensitive nerves. Likewise, insulin treatment of the diabetic animals also seems to have no influence on VIP release from the duodenal tissues.

As in the duodenum, nearly 50% of the VIP re-

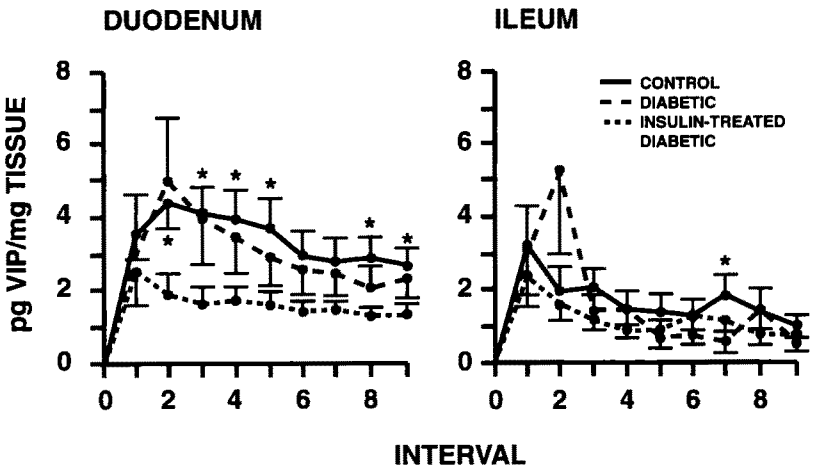

Fig 6. Effect of tetrodotoxin (5×10^{-6} M) on spontaneous VIP release from duodenal and ileal muscle strips in the duodenum. The diabetic tissues release significantly less VIP than control tissues at most of the intervals examined. The insulin-treated diabetic animals show levels of VIP release that are intermediate to the control and untreated diabetic groups at most of the intervals studied. In the ileum no significant differences in VIP release are noted among the three groups of animals with one exception. This suggests that the diabetic duodenum and ileum differ with respect to tetrodotoxin-resistant mechanisms of VIP release. *$P < 0.05$, control vs diabetic.

leased from the ileal tissues was through a tetrodotoxin-sensitive mechanism. However, and in contrast to the duodenum, the diabetic state significantly inhibited the amount of VIP released from tetrodotoxin-sensitive nerves. Furthermore, insulin treatment of the diabetic animals significantly increased the percentage of VIP released from tetrodotoxin-sensitive nerves to a level indistinguishable from that of the control group. These observations suggest that proximal and distal intestine react differently to the diabetic state with respect to the mechanisms of VIP release.

DISCUSSION

Immunohistochemical studies suggest that there is a qualitative increase in the intensity and density of VIP-containing axons in the diabetic rodent small intestine (10, 12, 13, 17). The apparent increase in immunoreactive VIP has been localized to the mucosa, the myenteric ganglia, and muscle layers of the small intestine (4, 10, 13, 18). These changes in immunoreactive VIP occur after diabetes of short (eight weeks) or long (12–18 months) duration (4, 17, 18).

Several mechanisms can explain the diminished release of VIP. The simplest is that the reduced tissue content of VIP noted in the present investigation translates into diminished release of the peptide. An alternative explanation is that diminished release of VIP might be due to an impaired mechanism of release at the nerve terminal or the defective axonal transport of the peptide. Such a mechanism is supported by observations that defects in axonal transport have been demonstrated in rodents with autonomic neuropathy (19–21). A third consideration is that attenuated release of the peptide may be due to a derangement of the intrinsic neural control regulating its release. Which of these factors is actually responsible for the attenuated VIP release cannot be stated with certainty, although reduced tissue content of the peptide appears to be the most plausible explanation.

The significance of the diminished tissue VIP concentration as well as its role in gastrointestinal motility disturbances and its attenuated release in the diabetic intestine likewise remains unclear. The effect of VIP on motility of the gastrointestinal tract tends to vary with the species and tissue in question (22–25). On a cellular level, VIP has been shown to induce release of acetylcholine from myenteric synaptosomes of the guinea pig small intestine (26). In intact tissues, VIP has been shown to contract the guinea pig small intestine not only via release of acetylcholine from postganglionic neurons but also by stimulation of noncholinergic excitatory neurons and through the direct stimulation of smooth muscle itself (9). Consequently, loss of tissue VIP may be a factor in the defective intestinal cholinergic neuromuscular trans-

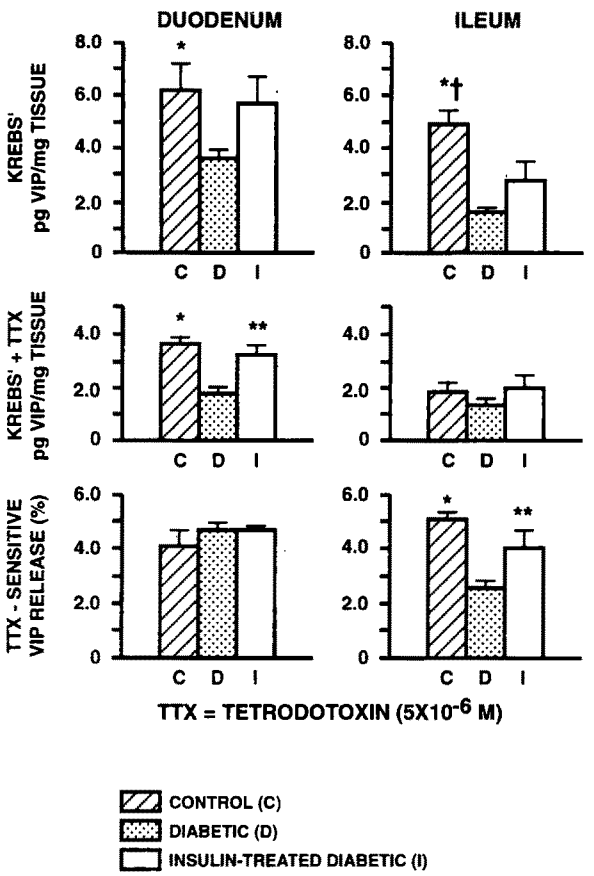

Fig 7. Total mean VIP release from the three groups of animals during incubation with Krebs' solution alone (top panels) or Krebs' solution with tetrodotoxin (middle panels). The lower panels show the total mean amount of VIP released (expressed in percent) that is inhibited by tetrodotoxin. The upper panels show that insulin treatment restores duodenal VIP release to near control-group levels, while the insulin-treated ileal tissues show levels of VIP release that are still significantly lower than controls. The middle panels show that tetrodotoxin-resistant VIP release is significantly diminished in the duodenum in the diabetic animals and restored to near control-group levels by insulin treatment. In contrast, no significant differences in tetrodotoxin-resistant VIP release are noted among these three groups in the ileum. The bottom panels show that there is no significant difference among the three groups in tetrodotoxin-sensitive VIP release in the duodenum. In the ileum, however, the diabetic tissues show significantly less VIP release, an abnormality that is improved by insulin treatment. $^{*}P < 0.05$, control vs diabetic; $^{\dagger}P < 0.05$, control vs insulin-treated diabetic; $^{**}P < 0.05$, insulin-treated diabetic vs untreated diabetic.

mission previously described in the streptozotocin-induced diabetic rodent (11).

Differences in experimental methodology likely explain the discrepancy between the present study and earlier investigations, which show an increase in intestinal VIP content in streptozotocin-treated diabetic rats (12, 13). In some investigations the intestinal mucosa was not removed (12). In another, increases in tissue VIP were noted when the results were expressed per centimeter of intestine, but not when expressed per gram of intestinal weight (13). The inconsistency is likely due to the fact that the diabetic intestine undergoes significant longitudinal and circumferential growth in the untreated diabetic state as well as dramatic mucosal proliferation (14).

The duration of the untreated diabetic state also appears to affect tissue neuropeptide concentration. One study showed that after eight weeks of streptozotocin-induced diabetes, the level of VIP in the myenteric plexus and muscle layers of the proximal rodent colon was significantly increased compared to age-matched nondiabetic control rats (27). However, 16 and 25 weeks after induction of diabetes, VIP levels were lower than those noted at eight weeks and had returned to levels similar to those of the nondiabetic animals. Similar adaptive changes occur in the contractile responses of the diabetic distal intestine to electrical field stimulation (28). After one month of diabetes, a significantly attenuated response is noted. After two months, some recovery occurs, while after three months the response is indistinguishable from that of nondiabetic control animals.

The present study shows that a basal release of VIP continues despite incubation with the neurotoxin tetrodotoxin. While tetrodotoxin-resistant mechanisms might be inferred as an explanation for this phenomenon, it is a least equally likely that a substantial proportion of VIP is passively released from neurons traumatized and torn in the dissection process. Of note is the observation that tetrodotoxin inhibited VIP release to a similar extent (40–50%) in duodenal tissues from the control, diabetic, and insulin-treated diabetic animals. In contrast, tetrodotoxin had significantly less influence in inhibiting VIP release from diabetic ileal tissues (approximately 23% inhibition). These observations suggest that in the diabetic state proximal and distal intestine may differ with respect to the mechanisms for VIP release. These findings are consistent with the observation that the effect of streptozotocin diabetes on regulatory peptide concentration is not uniform throughout the small intestine (12).

In the present study insulin treatment restored tissue VIP concentrations to normal or near normal levels. This beneficial effect of insulin treatment in experimental diabetes has been previously described. In one study defective enteric neuromuscular transmission in streptozotocin-treated diabetic rats was ameliorated after exogenous insulin administration

(11). In another, degenerating enteric axons characteristic of untreated diabetes were not found in those rodents that had undergone pancreatic islet cell transplantation several weeks after induction of streptozotocin diabetes (29).

In conclusion, the present investigation shows that streptozotocin-diabetes of 30 days' duration is associated with a significant decrease in VIP tissue concentration in the seromuscular layers of the proximal and distal rodent small intestine. This reduction is associated as well with diminished VIP release from the myenteric nerves. Insulin treatment of the animals shortly after induction of diabetes returns tissue VIP concentrations and spontaneous VIP release to near control-group levels. These findings argue that the abnormalities in VIP metabolism are secondary to the diabetic state and not to a toxic effect of streptozotocin. Statements regarding the physiologic significance of altered tissue VIP concentration and release must remain conjectural as the precise nature of the motility disturbance in the diabetic intestine likewise remains unclear.

ACKNOWLEDGMENTS

The authors wish to thank Ms. Brenda Edwards for her secretarial expertise in the preparation of this manuscript.

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