Changes in Sensitivity of the Rat Stomach Fundus to Various Drugs in Streptozotocin-Induced Diabetic Rats

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Abstract—The changes in sensitivity of the rat stomach fundus to acetylcholine (ACh), norepinephrine (NE), isoproterenol (ISO) and to vasoactive intestinal peptide (VIP) were examined in control rats and in streptozotocin (STZ)-induced diabetic rats. The dose-response curves for drugs were constructed 8 weeks after treatment with STZ. The dose-response curve for ACh in diabetic rats was shifted to the left as compared to the control curve, whereas the dose-response curves for NE, ISO and VIP were shifted to the right. These results suggest that functional changes in the autonomic nervous systems of the rat stomach fundus may occur in STZ-induced diabetic rats.

An accumulating body of evidence suggests that autonomic neuropathy is a common complication in diabetic patients. In fact, it has been reported that diarrhea is common in patients with diabetes mellitus (1), suggesting that diabetes is associated with dysfunction of the autonomic nervous system of the gut. Lincoln et al. (2) reported degeneration of adrenergic nerves, together with changes in the cholinergic and serotonergic innervation of the myenteric plexus of the ileum, in STZ-induced diabetic rats. Thus, it might be expected that the sensitivity of cholinergic and adrenergic postsynaptic receptors in the rat stomach would be changed. Peptidergic innervation of the gut has recently been reported, and one of the active peptides is the vasoactive intestinal peptide (VIP) (3). Recent electron microscopic studies have revealed degenerative changes in the myenteric nerve fibers of diabetic rats, and many of the fibers were shown to contain VIP (4). The purpose of the present study was to determine whether denervation-like postsynaptic supersensitivity can be induced in STZ-induced diabetic rats.

Diabetes was induced in male Wistar rats which weighed from 200–250 g by a single injection (60 mg/kg, i.v.) of STZ (Sigma, St. Louis, U.S.A.) in 0.02 M citrate saline. Successful induction of diabetes was assessed in blood samples by the o-toludine method (5). Levels of glucose in the blood of rats with STZ-induced diabetes (after 8 weeks) were significantly elevated (447.1±17.9 mg/dl, n=20) when compared with those of the age-matched control rats (121.4±7.0 mg/dl, n=20). Rats were sacrificed by a blow on the head 8 weeks after injection of STZ or saline. The stomach was excised, and fundus strip preparations of approximately 20 mm in length and 2 mm in width were made essentially by the method of Vane (6). The strip was cut out along the longitudinal muscle and the mucous layer was removed. The fundus strips were suspended in a 10 ml organ bath which contained modified Krebs-Ringer bicarbonate buffer solution at 37°C, aerated with 95% O₂ and 5% CO₂. The tension loaded on each strip was 1.0 g. Contractions and relaxations were recorded isotonically with an isotonic transducer (Nihon Kohden) connected to an ink-writing recorder (Nihon Kohden). The composition of the modified Krebs-Ringer solution was as follows (mM): NaCl, 120; KCl, 4.7; CaCl₂, 2.2; MgCl₂, 1.2; NaHCO₃, 25; KH₂PO₄, 1.2; and glucose, 14. All data are expressed as a mean±S.E.M. A minimum of 8 animals was used in each experimental and control group. Statistical
analysis of data was performed using Student's t-test, and a probability level of <0.05 was regarded as significant. pD2 (-log M of EC50) and IC50 were determined by graphical estimation.

When ACh was added cumulatively to the bath after an equilibration period of 30 min, the longitudinal strips of the fundus contracted in a dose-dependent manner. (Fig. 1). The difference in the sensitivity to ACh between the control and the diabetic stomach was determined by a comparison of the geometric pD2 values obtained from dose-response curves. pD2 values for ACh were 6.59±0.17 and 7.58±0.13 in the control and diabetic rats, respectively (n=8–15, P<0.01). NE, ISO and VIP produced dose-dependent relaxations in the fundus strips which were caused to precontract by the presence of 10^{-6} M serotonin. When contractions were recorded isotonically, no differences in the contractile height of the fundus induced by 10^{-6} M serotonin were observed between control and diabetic rats. In contrast to the response to ACh, the sensitivities to NE, ISO and VIP were significantly reduced in diabetic rats (Fig. 2), whereas maximal relaxation of the fundus induced by relaxants was not changed. IC50 values for NE, ISO and VIP in control and diabetic rats were as follows: 5.20±0.86x10^{-8} M and 8.85±1.23x10^{-8} M (n=13–15, P<0.01); 3.28±0.42x10^{-9} M and 9.16±1.36x10^{-9} M (n=8–15, P<0.01); 2.42±0.04x10^{-9} M and 3.94±0.50x10^{-9} M (n=10–11, P<0.05).

In the present study, we found that an increased response of the rat stomach fundus to ACh and a decreased response to NE, ISO and VIP was induced in the rats with STZ-induced diabetes. The phenomenon of changes in sensitivity in smooth muscle has been reviewed (7) and such a condition can be elicited in many ways, for example, by denervation or by inhibition of axonal transport (8–10). It has been reported that neuropathy of the autonomic nervous system and markedly decreased axonal transport occur in rats with experimentally induced diabetes (11). It is likely, therefore, that diabetes may produce changes in postsynaptic receptors for ACh by cholinergic degeneration or by an interruption of the influence of some neurotrophic factor(s), e.g., trophic factor, via an inhibitory effect of the fast axonal transport system. However, the relaxations of the stomach fundus induced by NE, ISO and VIP were significantly decreased in diabetic rats, indicating that there was a decrease in the

![Fig. 1. Dose-response curves for contractions of the rat stomach fundus induced by ACh in control (●) and diabetic (○) rats. The evoked contractions (mean±S.E.M.) are expressed as a percentage of the maximal contraction caused by ACh (10^{-3} M) in each individual experiment. n=8–13. *P<0.05, **P<0.01 (Student's t-test).](image)
number of postsynaptic $\beta$-receptors and receptors for VIP. Although it is unclear, at present, why diabetes produces an increased response to ACh and a decreased response to NE, ISO and VIP, functional changes in the autonomic nervous system may occur in rats with STZ-induced diabetes.

References


