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Changes in electrophysiological properties and noradrenaline response in vas deferens of diabetic rats

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Abstract

The purpose of this study was to study changes in the sympathetic nerves of the vas deferens in 10-week-old streptozotocin-induced diabetic rats. To assess the activity of autonomic neurons, we recorded the amplitude and frequency of spontaneous junction potentials in vasa deferentia from age-matched controls and streptozotocin-induced diabetic rats. No change in the resting membrane potential of the smooth muscle of the vas deferens was found in streptozotocin-induced diabetic rats. The frequency of spontaneous junction potentials was significantly increased in the streptozotocin-induced diabetic rats and their amplitude was also markedly increased. The dose–response curve for the contractile response of the vas deferens to noradrenaline was significantly shifted to the right and the apparent affinity (pD_2 value) was significantly decreased in streptozotocin-induced diabetic rats. These results suggest that degeneration of sympathetic neurons may occur in the vas deferens of 10-week streptozotocin-induced diabetic rats and that the greater amplitude of the spontaneous junction potentials may be related to an increase in Ca^{2+} mobilization, though the increase in Ca^{2+} mobilization does not lead to an enhanced contractile response.

Keywords: Vas deferens; Junction potential, spontaneous; Noradrenaline; Contraction

1. Introduction

The functional and biochemical deficits found in the autonomic nervous system of streptozotocin- or alloxan-induced diabetic rats have already been reviewed in detail (Tomlinson et al., 1992; Ozturk et al., 1996). It has been reported that diabetes causes degenerative changes in axons in sympathetic nerves (Monckton and Pehowich, 1980; Tomlinson and Yusof, 1983; Schmidt and Plurad, 1986; Kniel et al., 1986), but no evidence of autonomic neuropathy could be found in the mesenteric nerves in streptozotocin-induced diabetes (Schmidt and Scharp, 1982; Schmidt et al., 1983).

The responsiveness of the vas deferens to α-adrenoceptor agonists and to nerve stimulation has been reported to be increased in short-term diabetes but decreased in long-term diabetes (Longhurst, 1990, 1991; Ozturk et al., 1994). Furthermore, an increased Ca^{2+} influx and acceleration of phosphatidylinositol turnover has been found in the vas deferens of the streptozotocin-induced diabetic rat (Sakai and Honda, 1987; Sakai et al., 1989), although decreased calmodulin levels have been observed in the vas deferens of long-term streptozotocin-induced diabetic rats (Ozturk et al., 1994). The discrepancies among the above-mentioned results might be attributable to differences in the duration of the diabetes, or to the strain of rats or the experimental conditions. However, we felt that the disparate results in the literature might reflect different aspects of one and the same process, namely autonomic neuropathy in diabetes. One approach to this problem would be to examine any changes in neuromuscular transmission in the vas deferens that occur in diabetes. As a way of detecting and analyzing subtle changes in neuromuscular transmission, recordings were made of spontaneous junction potentials in the smooth muscle cells of vasa deferentia taken from age-matched controls and streptozotocin-induced diabetic rats. At present, no information is available concerning changes in spontaneous junction potentials in the vas deferens in streptozotocin-induced diabetes. We also examined the contractile response of the vas deferens to norepinephrine in controls and in streptozotocin-induced diabetic rats.

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2. Materials and methods

2.1. Animal model of diabetes

Male Wistar rats, 8-weeks-old and 220–250 g in weight, received a single injection via the tail vein of streptozotocin 60 mg/kg, dissolved in a citrate-buffer solution. Age-matched control rats were injected with buffer alone. Food and water were given ad libitum. The concentration of glucose in the plasma was determined by the *O*-toluidine method (Dubowski, 1962). This study was conducted in accordance with the Guide for the Care and Use of Laboratory Animals of Hoshi University which is accredited by the Ministry of Education, Sciences, Sports and Culture, Japan.

2.2. Measurement of membrane potential with microelectrodes

After the rats had been anaesthetized with sodium pentobarbitone (50 mg/kg), they were exsanguinated. Vasa deferentia were removed and cleaned of adhering fat and connective tissue in Krebs–Henseleit solution (KHS) of the following composition (mM): NaCl 118.0, KCl 4.7, $NaHCO_3$ 25.0, $CaCl_2$ 1.8, NaH_2PO_4 1.2, $MgSO_4$ 1.2, dextrose 11.0. For experiments involving measurement of the membrane potentials of smooth muscle cells, 10- to 15-mm sections taken from the middle portion of the vas deferens were fixed to the bottom of an organ bath with a capacity of 1 ml. The KHS in the bath was bubbled with 95% O_2 and 5% CO_2 and periodically replaced with fresh solution pre-warmed to 37°C. Smooth muscle cells were impaled with glass microelectrodes that had resistances of 30 to 60 MΩ. The microelectrodes were made from glass capillaries with a fine glass core in their lumen and were filled with 3 M KCl a few hours before the experiment. The output of a microelectrode preamplifier (Nihon Kohden, model MEZ-7200, Tokyo, Japan) with high input impedance and capacity neutralization was fed into a dual-beam cathode ray oscilloscope (Nihon Kohden, model VC-11, Tokyo, Japan) to display the membrane potentials. Impalements were considered to be successful when spontaneous junction potentials were observed with a resting potential that was stable for more than 1 min.

2.3. Dose–response studies with in vitro vasa deferentia

For experiments involving measurement of contractions, the epididymal half of the vas deferens was mounted with a resting tension of 0.5 g in an organ bath containing 10 ml KHS and allowed to equilibrate for 60 min. The KHS in the bath was continuously bubbled with 95% O_2 and 5% CO_2 and its temperature was maintained at 37°C. Smooth muscle contractions were recorded isometrically using a force-displacement transducer and ink-writing recorder. The agonist was applied for 1 min and then washed out thoroughly, the tissue being washed three or four times with fresh medium in the 15 min before exposure to the next dose of agonist. For the dose–response curve, the peak tension developed after each dose was regarded as the response. Full dose–response curves were obtained by means of stepwise increases in the concentration of the agonist.

Because the animals used were not uniform in terms of body weight (control group, 500.6 ± 4.8 g; diabetic group, 221.5 ± 3.1 g), the tissue weights also varied. To minimize the variation due to the differences in tissue weight, the responses are expressed in a normalized fashion (mg/100 mg wet wt.).

2.4. Drugs

Streptozotocin and (−)noradrenaline hydrochloride were purchased from Sigma (St. Louis, MO, USA).

2.5. Statistical analysis

The data are expressed as the means ± S.E.M. When comparisons were made between groups, Dunnett's multiple comparison test was used. Differences were considered statistically significant at the 5% level.

3. Results

3.1. General

Ten weeks after treatment with streptozotocin, the concentration of glucose in the plasma was elevated significantly, from 158.2 ± 9.7 (mg/dl) in the age-matched controls to 607.8 ± 13.4 (mg/dl) in the diabetic rats.

3.2. Measurement of membrane potential with microelectrodes

There was no significant difference in mean resting membrane potential in the vas deferens, between age-matched controls (-57.6 ± 1.6 mV; 10 cells in six tissues; range -64.5 to -51.9 mV, $n = 10$) and streptozotocin-induced diabetic rats (-59.3 ± 0.8 mV; 10 cells in six tissues; range -61.0 to -56.0 mV, $n = 10$) (Fig. 1). The resting membrane potential was taken to be the highest value recorded in each cell. It should be noted, however, that the membrane potential was not completely steady in the cells from either group, but fluctuated slowly. This observation is consistent with previous findings (Burnstock and Holman, 1961).

Spontaneous junction potentials were observed in all impaled cells, both from age-matched controls and from streptozotocin-induced diabetic rats (Fig. 1). For each group, spontaneous junction potentials were recorded over a 5-min period in each of six cells. As shown in Fig. 1,

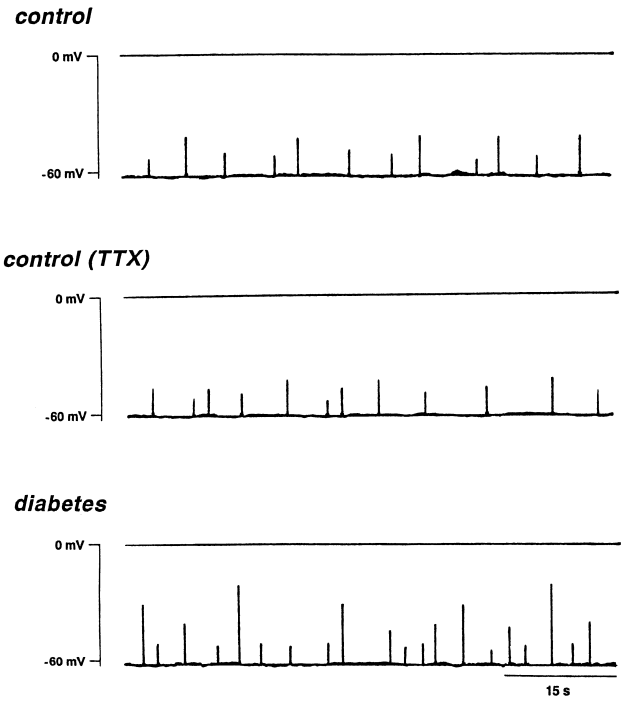

Fig. 1. Continuous intracellular recording showing spontaneous junction potentials in a smooth muscle cell of the age-matched controls and streptozotocin-induced diabetic rats. To examine the effect of 10^{-6} M tetrodotoxin (TTX), the vas deferens was exposed to these agents for 30 min.

spontaneous junction potentials could be recorded even in solutions which contained tetrodotoxin (10^{-6} M). In diabetic rats, the frequency and amplitude of the spontaneous junction potentials were markedly increased (Fig. 1). While we observed spontaneous junction potentials, the postsynaptic membrane did not spontaneously depolarize.

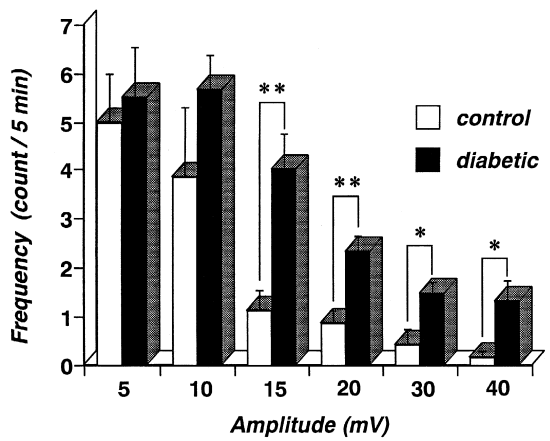

Fig. 2. Relationship between frequency and amplitude of spontaneous junction potential in vasa deferentia from age-matched controls and streptozotocin-induced diabetic rats. Frequency data broken down according to the amplitude of the spontaneous junction potentials. Each value is the mean of six determinations and vertical bars represent the S.E. $*\ P < 0.05$, $**\ P < 0.01$.

A distribution of amplitude vs. frequency of spontaneous junction potentials from these 5-min periods of recording for each group was obtained (Fig. 2). The amplitude distribution was asymmetrical in both groups. The spontaneous junction potentials occurred with greater frequency and amplitude in the steptozotocin-induced diabetic rats than in the controls (Fig. 2). The total number of spontaneous junction potentials was also greater in the streptozotocin-induced diabetic rats than in the controls (Fig. 1).

3.3. Dose–response studies with in vitro vasa deferentia

The dose–response curve for the contractile response of vasa deferentia to noradrenaline was shifted to the right in streptozotocin-induced diabetic rats (Fig. 3).

The negative logarithm of the EC_{50} (pD_2) for norepinephrine was 5.1 ± 0.1 M ($n = 8$) in age-matched control and 4.9 ± 0.1 M ($n = 7$) in streptozotocin-induced

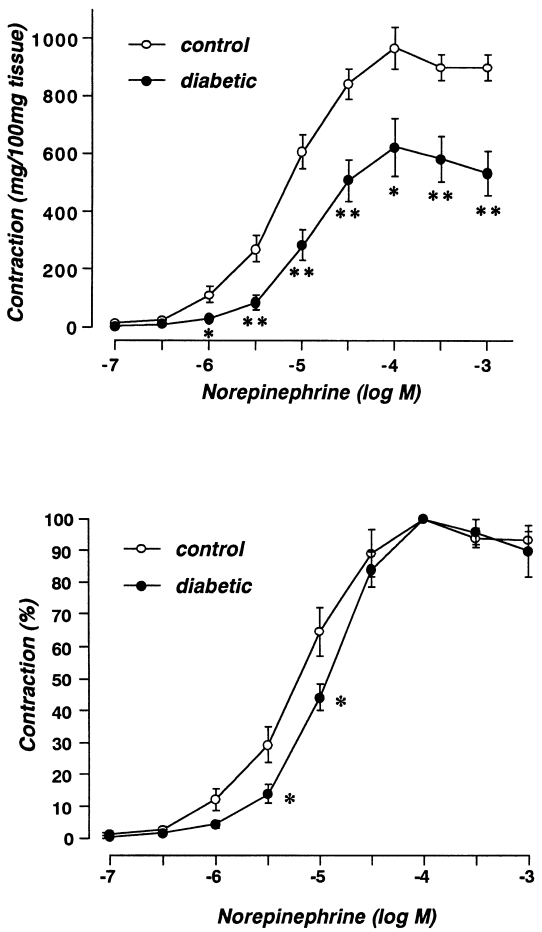

Fig. 3. Dose–response curve for noradrenaline-induced contractile response in vasa deferentia from age-matched controls and streptozotocin-induced diabetic rats. Upper panel: dose–response curves for noradrenaline-induced contractile response (controls, $n = 8$; diabetes, $n = 7$). Lower panel: the dose–response curves were normalized. Each data point is the mean of 7–8 determinations and vertical bars represent the S.E. $*\ P < 0.05$, $**\ P < 0.01$.

diabetic rats. The maximum tension developed by the controls on exposure to noradrenaline was 986.86 ± 73.8 mg/100 mg ($n = 8$) but was only 623.4 ± 99.7 mg/100 mg ($n = 7$) in the vasa deferentia from streptozotocin-induced diabetic rats.

4. Discussion

In the present study, we found that spontaneous junction potentials occurred with greater frequency and amplitude in vasa deferentia of streptozotocin-induced diabetic rats than in those from the age-matched controls, and that the dose-dependent contraction of the vas deferens to noradrenaline was significantly reduced in streptozotocin-induced diabetic rats.

It has been reported that, in the vas deferens, spontaneous junction potentials are due to the spontaneous release of transmitter (noradrenaline) from axon varicosities (Holman, 1970; Goto et al., 1977). To investigate changes in this spontaneous release of noradrenaline, we now used isolated vas deferens. In such isolated tissue, the frequency of spontaneous junction potentials is not directly related to sympathetic activity, because the cell bodies of the sympathetic neurons are not present. Changes in the frequency of spontaneous junction potentials in the isolated vas deferens may, instead, reflect the stability of sympathetic nerve endings (Goto et al., 1977; Goto, 1983). In the present study, we found that the frequency of spontaneous junction potentials was significantly greater in streptozotocin-induced diabetic rats, suggesting that the spontaneous release of noradrenaline from sympathetic nerve endings might be increased. While we observed spontaneous junction potentials, the postsynaptic membrane did not spontaneously depolarize, suggesting that an increase in spontaneous junction potentials is not due to spontaneous depolarization of the postsynaptic membrane. As mentioned in Section 1, diabetes causes degenerative changes in the axons in sympathetic nerves (Monckton and Pehowich, 1980; Tomlinson and Yusof, 1983; Schmidt and Plurad, 1986; Kniel et al., 1986), though no evidence of autonomic neuropathy has been found in the mesenteric nerves in streptozotocin-induced diabetes (Schmidt and Scharp, 1982; Schmidt et al., 1983). The present results are consistent with the notion that sympathetic nerve endings may be unstable because of neuropathy, thereby leading to an increased frequency of spontaneous junction potentials, at least in the vas deferens of the streptozotocin-induced diabetic rat.

The greater amplitude of the spontaneous junction potentials in our diabetic rats may have been due to a higher concentration of noradrenaline being released spontaneously in the vas deferens. Since the spontaneous junction potentials are driven by an influx of Ca^{2+}, this result may imply that the mobilization of Ca^{2+} is increased in streptozotocin-induced diabetic rats. Indeed, Sakai and Honda (1987) reported that Ca^{2+} influx in the vas deferens was greater in diabetic rats than in controls. It is likely, therefore, that the greater amplitude of the spontaneous junction potentials in our diabetic rats was due to an increased mobilization of Ca^{2+} in the smooth muscle of the vas deferens.

The smooth muscle contractile responses induced by various agonists are known to be associated with an increased intracellular Ca^{2+} concentration. If, as mentioned above, Ca^{2+} mobilization is increased in streptozotocin-induced diabetic rats, we should expect such rats to show a shift to the left of the dose–response curve for the contractile responses induced by noradrenaline. However, we found both a shift and a significant decrease in the EC_{50} (pD_2) value for noradrenaline. Moreover, the maximum contractile response to noradrenaline was also decreased in our streptozotocin-induced diabetic rats. Of particular interest, when we try to explain this apparent discrepancy, is the observation of Ozturk et al. (1994) that rats suffering from streptozotocin-induced diabetes for 8 weeks exhibit a significant decrease in tissue calmodulin level in the vas deferens. Calmodulin plays an essential role in smooth muscle contraction by activating myosin light chain kinase, which in turn modulates the interaction between actin and the myosin cross-bridges in response to changes in the intracellular Ca^{2+} level (Adelstein and Eisenberg, 1980; Adelstein et al., 1980). An explanation that is consistent with all the above results is that increased Ca^{2+} mobilization in the diabetic vas deferens does underlie the increased amplitude of the spontaneous junction potentials, but that such an increase in intracellular Ca^{2+} in response to noradrenaline does not lead to an increased contractile response because there is a decreased level of calmodulin in the diabetic state. In addition, our finding that the EC_{50} (pD_2) value in streptozotocin-induced diabetic rats was significantly decreased suggests that α-adrenoceptor density also is decreased in the diabetic vas deferens. This would be expected to reduce the response to noradrenaline.

It has been reported that glycosylation of actin, myosin and calmodulin is significantly increased in the platelets, brain and lens from diabetic rats (Cohen et al., 1989; Muruganandam et al., 1993; Pekiner et al., 1993; Evcimen and Nebioglu, 1996). It is unclear at present, however, whether the glycation of these proteins is responsible for the decreased contractile response of the vas deferens to noradrenaline in the diabetic rats; this requires further investigation.

5. Conclusion

We found that spontaneous junction potentials occurred with greater frequency and were of greater amplitude in streptozotocin-induced diabetic rats than in age-matched controls. This result suggests that degeneration of sympathetic neurons in the vas deferens may occur in 10-week streptozotocin-induced diabetic rats. The present study also

showed that, while the greater amplitude of the spontaneous junction potentials may be related to an increase in Ca^{2+} mobilization, such an increase in Ca^{2+} mobilization does not lead to an enhanced contractile response to noradrenaline. These results strongly suggest that diabetes mellitus causes changes in Ca^{2+} mobilization in the smooth muscle as well as in sympathetic nerve endings. As suggested by Tomlinson et al. (1982), these findings indicate that rats with chronic streptozotocin-induced diabetes exhibit pathological changes in the noradrenergic nerves supplying the vas deferens.

Acknowledgements

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