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Beneficial effect of an ACTH-(4-9) analog on peripheral neuropathy and blood pressure response to tyramine in streptozocin diabetic rats

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Patients with long-standing diabetes mellitus often display symptoms and signs of peripheral as well as autonomic neuropathy. In rats rendered diabetic by a single injection of streptozocin (STZ) the peripheral neuropathy has been well documented by a slowing of motor and sensory nerve conduction velocities and aberrations in the fiber size distribution of the afflicted nerves (Greene et al., 1987). We demonstrated recently that chronic treatment with the neurotrophic synthetic ACTH-(4-9) analog, ORG 2766 (50 μg/kg s.c.; 3 times/week), prevents the slowing of the conduction velocities and the alteration in fiber size distribution in the sciatic nerve of STZ diabetic rats (Van der Zee et al., 1989). The autonomic neuropathy in STZ diabetic rats is associated with damage to the sympathetic nervous system and microvascular pathology, affecting control of blood pressure and flow (Ramos, 1988). It had been shown that pressor responses to tyramine and noradrenaline can be used as a test for functional sympathetic impairment (Provoost et al., 1973). We now report for the first time that chronic treatment of STZ diabetic rats with the peptide, ORG 2766, prevents the diminished responsiveness to tyramine-induced changes in blood pressure.

Male rats of an inbred Wistar strain (CpB, TNO, Zeist, The Netherlands), weighing 200 g, were used. Streptozocin (Zanosar, Upjohn, Kalamazoo, MI) was injected once (50 mg/kg i.v.), inducing blood glucose levels > 16.7 mM throughout the experiment, with the first measurement 7 days after the injection. Age-matched non-diabetic rats served as controls. Met(O_2)-Glu-His-Phe-D-Lys-Phe (ORG 2766, Organon Int. B.V., Oss, The Netherlands) was dissolved in saline and administered in a dose of 50 μg/kg s.c., 3 times/week (Mo, We, Fri) for 9 weeks, beginning immediately after the STZ injection. Dose and treatment schedule were based on our previous study in which 50 but not 5 μg/kg protected against the diabetic peripheral neuropathy (Van der Zee et al., 1989). The H-related sensory nerve conduction velocity (SNCV) was measured as described in detail before (Van der Zee et al., 1989). The blood pressure response 3 days later, 48 h after the last peptide administration, was measured in anaesthetized rats via indwelling carotid arterial and jugular venous cannulae as described by Provoost et al. (1973). The increase in systolic and diastolic blood pressure following administration of the postsynaptic adrenoceptor agonist, phenylephrine (L-phenylephrine hydrochloride, Sigma, St. Louis, MO (USA); 0.75, 1.5, 3, 4.5 μg/kg), and the indirect sympathomimetic para-tyramine (para-tyramine hydrochloride, Regis

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Chemical co., Chicago, III (USA); 40, 100, 250 μg/kg), was recorded.

The STZ diabetic rats had a significantly lower body weight following 9 weeks of diabetes, as compared to age-related non-diabetic controls (250 ± 15 g and 363 ± 11 g, respectively). Treatment with the peptide, ORG 2766, did not influence either body weight or the increase in blood glucose level (data not shown). The saline-treated diabetic rats showed a significantly decreased SNCV (mean ± S.E.M.: 47 ± 3 m/s; n = 11) compared to ORG 2766-treated diabetic rats (64 ± 5 m/s; n = 12) and age-matched non-diabetic control rats (72 ± 3 m/s; n = 7; one-way analysis of variance followed by a supplemental t-test, $P < 0.05$). These data confirm and extend our recent observations on the protective effect of ORG 2766 in peripheral neuropathy, carried out at 5-7 weeks following STZ injection (Van der Zee et al., 1989).

The basal mean blood pressures of both the saline- and ORG 2766-treated diabetic rats (means ± S.E.M.: 79.4 ± 6.4 (n = 7) and 81.4 ± 8.3 mmHg (n = 5), respectively) were not significantly different from that of age-related controls (89.8 ± 3.3 mmHg; n = 5). In non-diabetic control rats there was, as expected, a dose-dependent increase in systolic and diastolic blood pressure following injection with phenylephrine and tyramine (fig. 1a,b; only the systolic data are shown). Saline-treated diabetic rats showed a significantly lower responsiveness to both tyramine and phenylephrine. The tyramine dose-response curve in peptide-treated diabetic rats was significantly different from that of the saline-treated diabetic rats but not of the age-matched controls (fig. 1b). The impaired responsiveness to phenylephrine was not influenced by ORG 2766 (fig. 1a). In a pure sympathetic neuropathy one would expect diminished tyramine responsiveness and enhanced phenylephrine responsiveness, the first as a result of presynaptic malfunction and the second due to postsynaptic receptor supersensitivity. Such is indeed the case in 6-OHDA sympathectomy (Provoost et al., 1973). However, there are apparently in the diabetic animal, in addition to presynaptic noradrenergic deficits, defects at the postsynaptic vascular site as was also shown by Jackson and Carrier (1983). Irrespective of the mechanism of

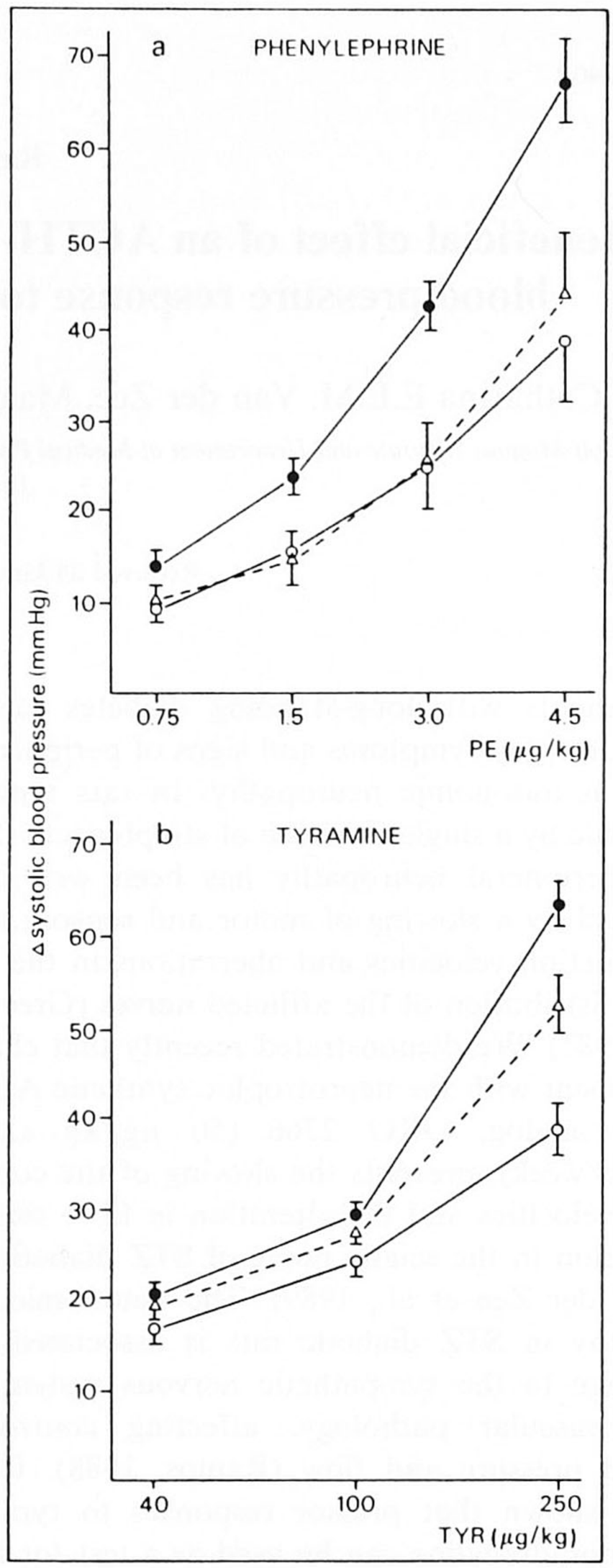

Fig. 1. Increase in systolic blood pressure in streptozocin-diabetic and age-matched control rats. (a) The increase in blood pressure following phenylephrine (PE) was significantly reduced in both saline (○ n = 7)- and ORG 2766 (△ n = 5)-treated diabetic rats, compared to control rats (● n = 5); $F(2,14) = 6.5$, $P < 0.01$. (b) The increase in blood pressure following tyramine (TYR) was significantly reduced in saline-treated diabetic rats (○ n = 22), compared to control rats (● n = 20) and to ORG 2766-treated diabetic rats (△ n = 22); $F(2,61) = 6.95$, $P < 0.002$. The blood pressure values for ORG 2766-treated diabetic rats were not significantly different from those for control rats. The data were analyzed by analysis of variance with repeated measures (MANOVA).

the sympathetic vascular pathology in the STZ diabetic rat, the data obtained with the ORG 2766 treatment suggest amelioration of the presynaptic deficit. This is consistent with the known neurotrophic activity of ORG 2766 (Van der Zee et al., 1989). These and our previous data (Van der Zee et al., 1989) point to a protective effect of chronic treatment with ORG 2766 on peripheral and autonomic neuropathy in diabetic rats. Further clinical studies on the efficacy of this non-toxic neuropeptide in the treatment of diabetes mellitus associated neuropathies are warranted.

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References

Green, D.A., S.A. Lattimer and A.A.F. Sima, 1987, Sorbitol, phosphoinositides, and sodium-potassium-ATPase in the pathogenesis of diabetic complications, N. Engl. J. Med. 316, 599.

Jackson, C.V. and G.O. Carrier, 1983, Influence of short-term experimental diabetes on blood pressure and heart rate in response to norepinephrine and angiotensine II in the conscious rat, J. Cardiovasc. Pharmacol. 5, 260.

Provoost, A.P., J.A. De Kemp and W. De Jong, 1973, Effect of neonatal 6-hydroxydopamine treatment on the blood pressure response to noradrenaline and tyramine in rats, European J. Pharmacol. 23, 297.

Ramos, O.L., 1988, Diabetes mellitus and hypertension, Hypertension 11 (Suppl I), I-14.

Van der Zee, C.E.E.M., R. Gerritsen van der Hoop and W.H. Gispen, 1989, Beneficial effect of ORG 2766 in treatment of peripheral neuropathy in streptozocin-induced diabetic rats, Diabetes 38, 225.