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Treatment with the xanthine oxidase inhibitor, allopurinol, improves nerve and vascular function in diabetic rats

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Abstract

Several putative sources of reactive oxygen species could potentially contribute to diabetic neuropathy and vasculopathy. The aim was to assess the involvement of elevated xanthine oxidase activity. After 6 weeks of streptozotocin-diabetes, groups of rats were given 2 weeks of high-dose allopurinol treatment (50 and 250 mg/kg) to gauge the effect of maximal blockade of xanthine oxidase. In the final experiments, rats were subjected to sensory testing and, under butabarbital anaesthesia, measurements were made on nerve conduction velocities and neural tissue blood flow estimated by hydrogen clearance microelectrode polarography. Further groups were used to study detailed responses of the isolated mesenteric vascular bed after 4 weeks of diabetes and allopurinol (150 mg/kg) treatment. Diabetes caused 20% and 14% reduction in motor and sensory conduction velocity, which were 78% and 81% corrected by allopurinol treatment respectively, both doses giving similar results. Diabetic rats showed tactile allodynia and thermal hyperalgesia, which were completely corrected by allopurinol, whereas mechanical hyperalgesia was only 45% ameliorated. Sciatic nerve and superior cervical ganglion blood flow was halved by diabetes and allopurinol corrected this by approximately 63%. Mesenteric endothelium-dependent vascular responses to acetylcholine, which depend upon nitric oxide and endothelium derived hyperpolarizing factor, were attenuated by diabetes. Allopurinol treatment gave approximately 50% protection for both components. Thus, xanthine oxidase is an important source of reactive oxygen species that contributes to neurovascular dysfunction in experimental diabetes. Inhibition of xanthine oxidase could be a potential therapeutic approach to diabetic neuropathy and vasculopathy.

Keywords: Diabetic neuropathy; Xanthine oxidase; Allopurinol; Blood flow; Vascular endothelium; Nitric oxide; EDHF

1. Introduction

Peripheral nerve perfusion is reduced by diabetes and this makes an important contribution to neuropathy in patients and animal models (Tuck et al., 1984; Cameron et al., 2001a); elevated oxidative stress has been implicated in this neurovascular dysfunction (Keegan et al., 1995; Pieper and Siebeneich, 1997; Low et al., 1997; Cameron and Cotter, 1999). Treatment with scavengers of reactive oxygen species improves nerve blood flow, oxygenation and function in experimental diabetes (Cameron et al., 1994a; Nagamatsu et al., 1995; Love et al., 1997). The nitric oxide and endothelium-derived hyperpolarizing factor (EDHF) systems of the vascular endothelium are vulnerable to diabetes and oxidative stress (Pieper and Siebeneich, 1997; Cameron and Cotter, 1999; Cameron et al., 2001a). Together, deficits in these vasodilators could substantially account for impaired nerve perfusion (Kihara and Low, 1995; Maxfield et al., 1997; Terata et al., 1999). To effectively prevent these neurovascular complications, experiments in animal models show that the dose needed to ensure that sufficient molecules of a scavenger such as vitamin E are present wherever reactive oxygen species are formed is prohibitively high for use in man (Keegan et al., 1995; Cotter et al., 1995). An attractive alternative strategy to scavenging reactive oxygen species after they have formed is to inhibit their formation by targeting the processes responsible.

There are numerous potential sources of reactive oxygen species in diabetes, including autoxidation of glucose and

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metabolites, advanced glycation, mitochondrial respiratory chain leakage, nitric oxide synthase and NAD(P)H oxidase, and elevated xanthine oxidase activity (Low et al., 1997; Cameron and Cotter, 1999; Baynes and Thorpe, 1999; Nishikawa et al., 2000; Cotter and Cameron, 2003). The latter mechanism is the subject of this investigation. Circulating xanthine oxidase levels are increased in experimental diabetes and this contributes to superoxide production (Desco et al., 2002; Matsumoto et al., 2003; Aliciguzel et al., 2003). In diabetic patients, treatment with the xanthine oxidase inhibitor, allopurinol, improved endothelium-dependent vasodilation and lowered circulating levels of the oxidative stress marker, malondiadehyde (Butler et al., 2000). One aim was to assess the involvement of xanthine oxidase in nerve dysfunction in diabetic rats, using high-dose allopurinol treatment to ensure that maximal effects were observed. A further aim was to examine the details of allopurinol's vascular action using the in vitro mesenteric vascular bed preparation (Cameron et al., 2001b), focussing on resistance vessel endothelium-dependent vasodilation mediated by nitric oxide and EDHF.

2. Materials and methods

Experiments were performed in accordance with the United Kingdom 'Animal Procedures Act, 1986' and the National Institutes of Health 'Principles of Laboratory Animal Care, 1985 revised version'.

2.1. Experimental groups and diabetes induction

Male Sprague–Dawley rats (Aberdeen University colony), 19 weeks old at the start of the study were used. Diabetes, induced by intraperitoneal streptozotocin (Astra-Zeneca Pharmaceuticals, Macclesfield, Cheshire, UK) at 42.5 mg/kg freshly dissolved in sterile 0.9% saline solution, was verified 24 h later by estimating hyperglycaemia and glycosuria (Visidex II and Diastix; Ames, Slough, UK). Blood samples were taken from the tail vein or by cardiac puncture (mesenteric bed study) for plasma glucose determination (GOD-Perid method; Boehringer Mannheim, Mannheim, Germany) just before final experiments.

Three investigations were undertaken, in which diabetes duration was 4–8 weeks. The first used a reversal paradigm to examine the effects of allopurinol on sciatic endoneurial blood flow and large nerve fibre-mediated motor and sensory conduction velocity: treatment was given daily 2 weeks following 6 weeks of untreated diabetes. Two high doses of allopurinol were employed approximately 50 mg/kg, which inhibits xanthine oxidase activity by more than 90% in rats (Klein et al., 1996), and approximately 250 mg/kg to ensure that the maximal effects of the drug were assessed. At the higher dose, the effect of cotreatment with the nitric oxide synthase inhibitor N^G-nitro-L-arginine (10 mg/kg) was also examined. Using the same paradigm, further treated diabetic groups were added using lower doses (1.8–50 mg/kg) of allopurinol to determine the ED_{50} values for correction of nerve conduction velocity deficits. In a second set of experiments, also using the reversal paradigm, rats were subjected to sensory testing before and after high dose (150 mg/kg) allopurinol treatment to assess effects on nociceptive system function. These rats were then also used for measurements of superior cervical ganglion blood flow. A third set of experiments investigated the vascular effects of allopurinol in the mesenteric vascular bed preparation. This used a preventive paradigm over 4 weeks, treatment (150 mg/kg) being given from diabetes induction. Groups comprised non-diabetic control, untreated diabetic and treated diabetic rats. Allopurinol was given as a dietary supplement, doses being based on rat body weight and prior observation that diabetic rats consume approximately 50 g of food per day. N^G-nitro-L-arginine was given at an appropriate concentration dissolved in the drinking water, diabetic animals consuming approximately 120 ml per day.

2.2. Nerve function tests

The sciatic nerve was exposed between sciatic notch and knee and motor conduction velocity was measured in the nerve branch to tibialis anterior muscle as previously described (Cameron et al., 1989). Saphenous nerve sensory conduction velocity was measured between groin and calf. Rectal and near nerve temperatures were monitored and regulated between 36.5 and 37.5 °C with radiant heat.

Tactile allodynia and mechanical stimulation thresholds of the foot were measured by an electronic von Frey hair apparatus and the Randall–Sellito test (Randall and Sellito, 1957), respectively. Latencies for foot withdrawal reflexes to noxious thermal stimulation were estimated by the Hargreaves plantar test (Hargreaves et al., 1988). All tests were carried out using commercially available equipment (Ugo-Basile, Comerio, Italy). Measurements were made in a constant room temperature at the same time each day, and rats were given a 3-day period for familiarisation with handling, the environment, equipment, and experimental procedure.

Tactile allodynia was estimated on a single day. Stimulus intensity started at 2 g and if a withdrawal response to stimulation did not occur within 8 s, the stimulus was increased by a 0.2 log unit increment and reapplied. When a positive withdrawal response was obtained, stimulus intensity was lowered by 0.2 log units. Thus, stimulus intensity tracked the threshold, and a run of 6 near-threshold responses was used to estimate the 50% threshold value using lookup tables (Chaplan et al., 1994). Measurements were made for both feet, the average being taken to represent the 50% threshold for an individual rat. After 12 days of treatment, allodynia thresholds were reassessed.

Mechanical pressure withdrawal thresholds were estimated twice per day for each foot over a 3-day period before allopurinol treatment commenced. After 13 days of treatment, thresholds were again determined over 3 days. Data from the 3 pre-treatment and 3 end-of-treatment days were averaged to give pressure threshold values. Following mechanical testing, rats were placed in the thermal testing apparatus. After 30 min acclimatisation, a constant power infrared stimulus was focussed onto the sole of the foot and the latency for reflex foot withdrawal recorded via a photoelectric monitor. For each