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European Journal of Pharmacology 484 (2004) 361–362
www.elsevier.com/locate/ejphar

Rapid communication

Chronic vasopeptidase inhibition normalizes diabetic endothelial dysfunction

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Received 28 October 2003; accepted 4 November 2003

Abstract

Type 2 diabetes mellitus is a major cause of vascular morbidity but animal models for this disease have not been adequately characterized. We demonstrate that endothelial dysfunction is present in the Zucker diabetic fatty (ZDF) rat. Vasopeptidase inhibition with AVE7688 (7-[[(2*S*)-2-(acetylthio)-1-oxo-3-methylpropyl]amino]-1,2,3,4,6,7,8,12*b*-octahydro-6-oxo-(4*S*,7*S*,12*bR*)-pyrido[2,1-*a*][2]benzazepine-4-carboxic acid), 45 mg/kg/day in chow for 6 weeks, normalized acetylcholine mediated relaxation of mesenteric artery rings. Thus, chronic vasopeptidase inhibition may prevent vascular complications related to type 2 diabetes mellitus.

Keywords: Zucker diabetic fatty rat; Diabetic vasculopathy; Vasopeptidase inhibition

Cardiovascular complications are the major cause of morbidity and mortality in diabetic patients. In order to evaluate potential novel treatments for diabetic vasculopathy preclinically, suitable animal models are urgently needed. However, the available type 2 diabetic rat strains have not adequately been characterized with regard to vascular complications. The Zucker diabetic fatty (ZDF) rat is a prototype type 2 diabetes model, characterized by obesity, insulin resistance and diabetes mellitus (Unger and Orci, 2001).

The novel vasopeptidase inhibitors, which simultaneously inhibit both angiotensin-converting enzyme and neutral endopeptidase, have been developed for potentially superior effects over pure angiotensin-converting enzyme inhibitors but their therapeutic value in diabetic end organ damage has not yet been fully explored.

Therefore, we characterized endothelial function and tested the effect of the vasopeptidase inhibitor, AVE7688, in the ZDF rat.

The animal experiments were performed in accordance with the European Community guidelines for the use of experimental animals, with the German law for the protection of animals, and with current Aventis guidelines. Male homozygous ZDF rats (Gmi-fa/fa) were treated with either placebo ($n=10$) or the vasopeptidase inhibitor AVE7688 (7-[[(2*S*)-2-(acetylthio)-1-oxo-3-methylpropyl]-amino]-1,2,3,4,6,7,8,12*b*-octahydro-6-oxo-(4*S*,7*S*,12*bR*)-pyrido[2,1-*a*][2]benzazepine-4-carboxic acid; $n=9$). The dose of AVE7688 was 45 mg/kg/day in chow, based on previous experience where this dose prevented diabetic nephropathy in the ZDF rat (Schäfer et al., 2003). Five heterozygous, lean rats received placebo and served as nondiabetic controls. At age 35 weeks, when glycosylated haemoglobin (HbA1c) exceeded 11% in the homozygous rats indicating fully established diabetes, treatment was started and continued for 6 weeks. Then, the animals were sacrificed and three rings from the mesenteric artery of each animal were mounted on force transducers in organ baths gassed with carbogen at 37 °C and pH 7.4, as described previously (Löhn et al., 2002). After equilibration for 1 h, contraction was induced by KCl (60 mM) and, after washout, by phenylephrine (1 μM). Thereafter, stepwise endothelium-dependent relaxation was induced by acetylcholine (1 nM to 1 μM) in the continuous presence of phenylephrine. Data are expressed as mean ± S.E.M. Differences between groups were tested for significance using unpaired *t*-tests.

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doi:10.1016/j.ejphar.2003.11.008

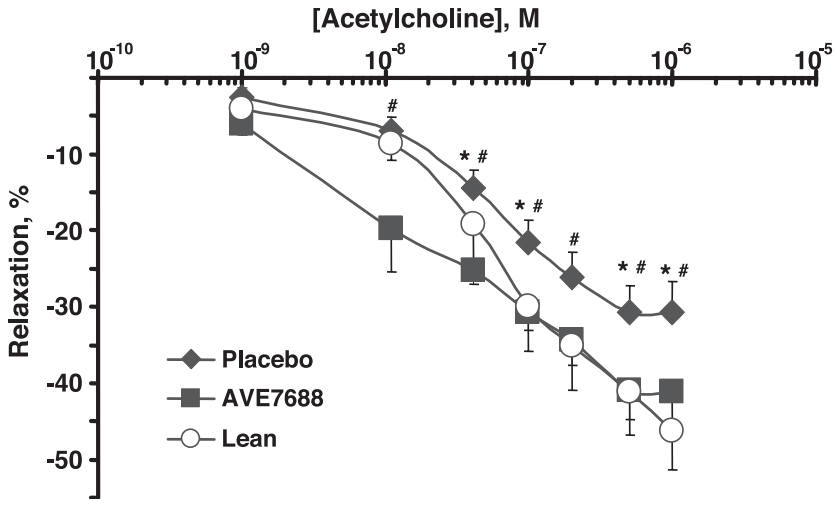

Fig. 1. Acetylcholine mediated relaxation of phenylephrine-precontracted rings from mesenteric arteries. Each group consists of three rings each from 5 to 10 animals. Note that maximal relaxation is reduced in homozygous, diabetic animals compared to heterozygous, lean controls. AVE7688 normalizes endothelial function in mesenteric rings from homozygous animals. Data are mean ± S.E.M. $^{*}P<0.05$ vs. lean; $^{\#}P<0.05$ vs. AVE7688.

The contractile responses to either KCl or phenylephrine of the mesenteric arteries were not different between the groups. In contrast, chronic treatment with the vasopeptidase inhibitor AVE7688 normalized endothelium-dependent relaxation over the entire range of concentrations of acetylcholine tested (Fig. 1). Maximal endothelium-dependent relaxation was significantly reduced to 30.7 ± 3.5% in precontracted mesenteric arteries from diabetic animals compared to lean (46.2 ± 5.2%, $P<0.05$ vs. ZDF placebo).

The present study is the first to characterize endothelial dysfunction in a type 2 diabetic rat model. Using this model, we demonstrate that diabetic endothelial dysfunction can be normalized by vasopeptidase inhibition.

Agents blocking the renin–angiotensin system have been proven to reduce cardiovascular complications in diabetic patients (HOPE Investigators, 2000; Lindholm et al., 2003). In experimental studies, dual inhibition of angiotensin-converting enzyme and neutral endopeptidase (i.e., vasopeptidase inhibition) ameliorates hypertensive endothelial dysfunction and prevents diabetic nephropathy even more effectively than angiotensin-converting inhibition alone (d'Uscio et al., 2001; Davis et al., 2003; Schäfer et al., 2003). The diabetic endothelium is characterized by an excess oxidative stress and lack of available nitric oxide, resulting in endothelial dysfunction and, ultimately, diabetic target organ damage (Bayraktutan, 2002). Neutral endopeptidase catalyzes the breakdown of a number of organoprotective peptides, including bradykinin, substance P, and the natriuretic factors. Blockade of the neutral endopeptidase can therefore be assumed to increase the tissue concentrations of these peptides, augment receptor-mediated generation of nitric oxide, and improve vascular function. In line with this hypothesis, the present study shows that AVE7688 can normalize diabetes-induced endothelial dysfunction and hence may be useful in prevention and treatment of diabetic cardiovascular complications.

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