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Mechanisms involved in the increased sensitivity of the rabbit basilar artery to atrial natriuretic peptide in diabetes

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ABSTRACT

Atrial natriuretic peptide (ANP) is a vasodilator with significant regional differences and controversial effects in the cerebral circulation, a vascular bed particularly prone to diabetes-induced complications. The present study has investigated how alloxan-induced diabetes modifies the mechanisms involved in the response of the rabbit basilar artery to ANP. ANP (10^{-12}–10^{-7} M) relaxed precontracted basilar arteries, with higher potency in diabetic than in control rabbits. In arteries from both groups of animals, endothelium removal reduced ANP-induced relaxations. Inhibition of NO-synthesis attenuated ANP-induced relaxation but this attenuation was lower in diabetic than in control rabbits. In control rabbits, indomethacin displaced to the left the concentration-response curve to ANP, without significantly modifying the E_{max} value. In diabetic rabbits, indomethacin significantly enhanced arterial relaxations to ANP. In KCl-depolarised arteries, relaxation to ANP was almost abolished both in control and in diabetic rabbits. Iberiotoxin inhibited relaxations to ANP in both groups of rabbits. Glibenclamide and 4-aminopyridine inhibited the ANP-induced relaxations more in diabetic than in control rabbits. Basilar arteries from diabetic rabbits showed decreased natriuretic peptide receptor C expression and no changes in natriuretic peptide receptor A, large conductance calcium-activated K^+ channels (BK_{Ca}), ATP-sensitive K^+ channels (K_{ATP}) and voltage-sensitive K^+ channels (K_V) expression. These results suggest that diabetes enhances the sensitivity of the rabbit basilar artery to ANP by mechanisms that at least include reduced expression of natriuretic peptide receptor C, and enhanced activity of K_{ATP} and K_V channels. Furthermore, diabetes reduces endothelial NO and prostacyclin which mediate arterial relaxation to ANP.

1. Introduction

Natriuretic peptides have emerged as important diagnostic and prognostic tools for cardiovascular disease. There are three distinct, but structurally related endogenous peptides: atrial (ANP), B-type (BNP) and C-type (CNP) natriuretic peptides (reviewed in Potter et al., 2006; Pandey, 2008; Schlueter et al., 2014). These peptides interact with three different types of natriuretic peptide receptors: A, B and C. Natriuretic peptide receptor A binds to both ANP and BNP, natriuretic peptide receptor B binds to CNP and natriuretic peptide receptor C binds with similar affinity to all three natriuretic peptides and is considered as a clearance receptor.

In addition to peripheral functions, these hormones act as neurotransmitters or neuromodulators in the brain (Hodes and Lichtstein, 2014) and could participate in the regulation of cerebral hemodynamics in physiological and pathophysiological conditions, such as acute brain injury including stroke (Guo et al., 2014, 2015). It has been suggested that therapeutic strategies targeting natriuretic peptides could have a potential neuroprotective usefulness after brain injury (Guo et al., 2014; Hodes and Lichtstein, 2014).

Diabetes induces structural and functional changes in the cerebrovascular bed, and vascular responses in the cerebral macro- and microvasculature are differentially affected. These local alterations may play an important role in the physiological regulation of brain perfusion and in the pathogenesis of cerebral complications of diabetes including stroke (Ergul et al., 2012).

It has been suggested the existence of a close link between natriuretic peptides, diabetes and insulin resistance. Plasma

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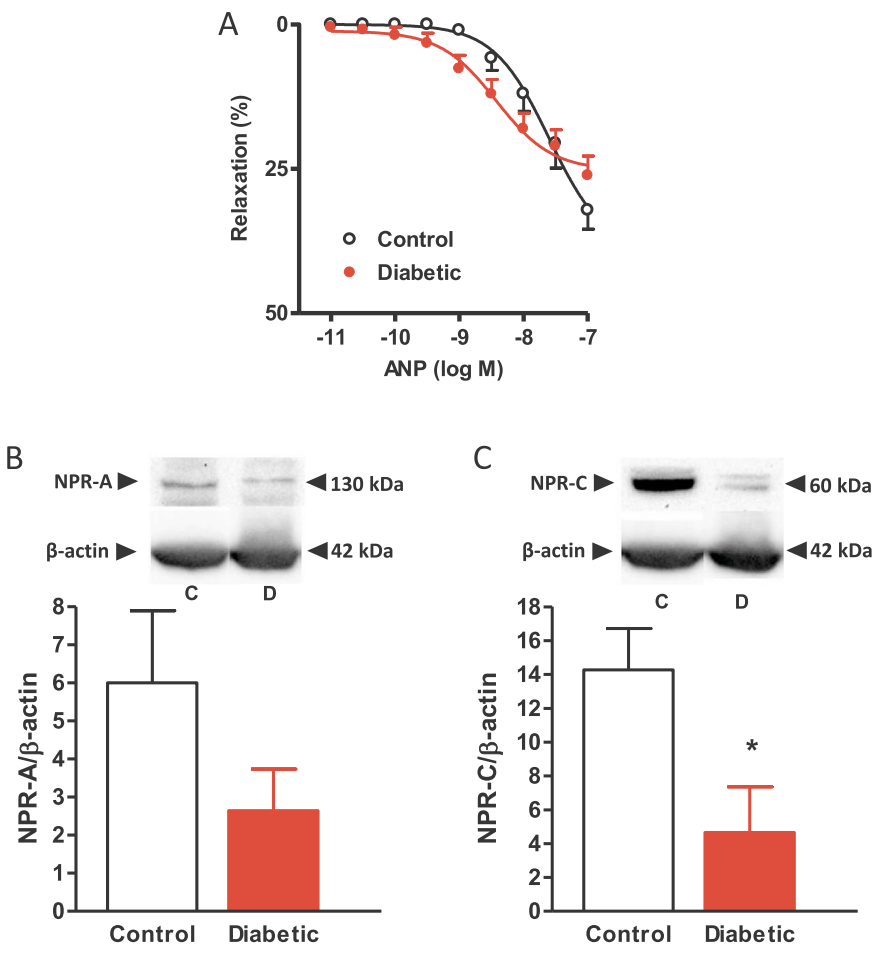

Fig. 1. Relaxant effects of ANP and natriuretic peptide receptor expression in the rabbit basilar artery. Concentration-response curves for ANP in UTP precontracted basilar arteries isolated from control (n = 10) and diabetic (n = 15) rabbits (**A**). Values are expressed as percentages of the active tone and represent means ± S.E.M. of "n" arterial segments from at least 4 animals. Representative Western blots and relative abundance of natriuretic peptide receptor A (**B**) and natriuretic peptide receptor C (**C**) in basilar arteries from control (n = 4) and diabetic (n = 4) rabbits. Levels of β- actin are shown as a loading control. Relative densities of immunoreactive bands were normalised to the density of corresponding bands for β-actin and values represent means ± S.E.M. of "n" pool of 3 arteries isolated from 3 animals. *$P < 0.05$ significantly different from control rabbits.

concentrations of ANP are increased in patients with diabetes mellitus (Nannipieri et al., 2002; McKenna et al., 2005). In addition, increased plasma concentrations of ANP are related to a poor glycaemic control (McKenna et al., 2005). Given their effects on lipid and glucose metabolism (Welsh and McMurray, 2012; Heinisch et al., 2012), and on arterial blood pressure, natriuretic peptides provide a particularly promising target to simultaneously address common cardiovascular and metabolic disorders (Schlueter et al., 2014). In general, ANP is well known to elicit direct vasorelaxation but information on the direct effect of ANP in the cerebral circulation is controversial (Guo et al., 2014, 2015), and information about cerebral vasoactive effects of this peptide in the diabetic state is lacking. We have previously reported that diabetes induces hyporeactivity of the rabbit carotid arteries to ANP by a mechanism that at least includes changes in vascular regulatory prostanoids and a reduced participation of K^+-channels (Marrachelli et al., 2011). The aim of the present study was to investigate the influence of experimental alloxan-induced diabetes on the mechanisms that participate in the response of the rabbit basilar artery to ANP, including the possible contribution of natriuretic peptide receptors, nitric oxide (NO), K^+ channels, and prostanoids on the vascular activity of ANP.

2. Materials and methods

One hundred male New Zealand White rabbits (Granja San Bernardo, Tulebras, Spain) were used in the present study. Animals were randomly divided into two experimental groups: fifty in the control group and fifty destined for induction of diabetes. Thirty-four/group were used for isometric tension recording, twelve/group for Western blotting, and four/group for enzyme immunoassay. Housing conditions and experimental procedures were in compliance with the European Union (Directive 2010/63/EU) and Spanish (RD 53/2013) regulations on the use of animals for scientific purposes and approved by the Ethics Committee for Animal Experimentation and Welfare from the University of Valencia (ref. A11295344586921).

The induction of experimental diabetes with alloxan, euthanization, extraction of basilar arteries and the preparation procedures for isometric tension recording in arterial segments under optimal resting tension of 4.9 mN were achieved following previously described protocols (Alabadí et al., 2004; Centeno et al., 2013). Hyperglycemia higher than 12 mM occurred within 24–48 h after alloxan injection and remained increased until euthanization (18.5 ± 1.1 mM). Diabetic rabbits also presented polyuria, polydipsia, and a delay in body-weight increase. In comparison with control rabbits, diabetic rabbits presented significantly decreased plasma values of albumin, total proteins, sodium, and chloride and increased values of glucose, alanine amino transferase, amylase and potassium, without significant differences in the rest of standard plasma parameters analysed.

2.1. Concentration-response curves

Concentration-response curves to ANP (10^{-12}–10^{-7} M) were obtained cumulatively in basilar arteries previously contracted with UTP (10^{-4} M). The active tone induced by UTP in basilar arteries from control rabbits (9.1 ± 0.4 mN) was similar to that obtained in basilar arteries from diabetic rabbits (8.4 ± 0.5 mN). To assess the influence of the endothelium, concentration-response curves to ANP were obtained in endothelium-denuded arteries (rubbed arteries); endothelium removal was achieved by rubbing the intimal surface with a scored stainless steel rod. To explore the participation of NO in the effects of ANP, the response to ANP was obtained in arterial segments incubated