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Nebivolol prevents ethanol-induced reactive oxygen species generation and lipoperoxidation in the rat kidney by regulating NADPH oxidase activation and expression

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ABSTRACT

We studied whether the β_1-adrenergic antagonist nebivolol would prevent ethanol-induced reactive oxygen species generation and lipoperoxidation in the rat renal cortex. Male Wistar rats were treated with ethanol (20% v/v) for 2 weeks. Nebivolol (10 mg/kg/day; p.o. gavage) prevented both the increase in superoxide anion (O_2^-) generation and thiobarbituric acid reactive substances (TBARS) concentration induced by ethanol in the renal cortex. Ethanol decreased nitrate/nitrite (NOx) concentration in the renal cortex, and nebivolol prevented this response. Nebivolol did not affect the reduction of hydrogen peroxide (H_2O_2) concentration induced by ethanol. Nebivolol prevented the ethanol-induced increase of catalase (CAT) activity. Both SOD activity and the levels of reduced glutathione (GSH) were not affected by treatment with nebivolol or ethanol. Neither ethanol nor nebivolol affected the expression of Nox1, Nox4, eNOS, nNOS, CAT, Nox organizer 1 (Noxo1), c-Src, $p47^{phox}$ or superoxide dismutase (SOD) isoforms in the renal cortex. On the other hand, treatment with ethanol increased Nox2 expression, and nebivolol prevented this response. Finally, nebivolol reduced the expression of protein kinase (PK) Cδ and Rac1. The major finding of our study is that nebivolol prevented ethanol-induced reactive oxygen species generation and lipoperoxidation in the kidney by a mechanism that involves reduction on the expression of Nox2, a catalytic subunit of NADPH oxidase. Additionally, we demonstrated that nebivolol reduces NADPH oxidase-derived reactive oxygen species by decreasing the expression of PKCδ and Rac1, which are important activators of NADPH oxidase.

1. Introduction

Chronic ethanol consumption can compromise kidney function and structure by inducing inflammation, tubular dysfunction and necrosis (De Marchi et al., 1994; Cecchin and De Marchi, 1996). Attempting to understand the clinical importance of the effects of ethanol on the kidney, several studies have been conducted to characterize the mechanisms underlying such effects. In this sense, increased generation of reactive oxygen species is described to play a central role in the pathogenesis of ethanol-induced nephropathy (Harris et al., 2015). Ethanol-induced increases in reactive oxygen species generation contribute to lipid peroxidation and oxidation of antioxidant proteins with further reduction of renal antioxidant capacity (Pourbakhsh et al., 2014; Harris et al., 2015). The enzyme nicotinamide adenine dinucleotide phosphate (NADPH) oxidase is an important source of reactive oxygen species in the kidney. NADPH oxidase catalyzes the transfer of electrons from NADPH to O_2, through the Nox catalytic subunit, to produce reactive oxygen species (Sedeek et al., 2013a). Seven NADPH oxidase isoforms have been identified (Nox1–5, Duox1–2), and Nox1, Nox2 and Nox4 have been identified in the kidney (Gill and Wilcox, 2006). Nox4 is the predominant isoform in the kidney, and this protein may be important in renal oxidative stress and kidney injury (Sedeek et al., 2013b).

Reactive oxygen species, such as superoxide anion (O_2^-), may react with nitric oxide (NO) to form peroxynitrite ($ONOO^-$), a highly oxidant compound that reacts with biological molecules, causing lipoperoxidation and oxidation of proteins and DNA. Additionally, the reaction between O_2^- and NO leads to a reduction in NO bioavailability (Pacher et al., 2007). In the kidney, NO is synthesized by the two constitutive isoforms of the NO synthase (NOS), termed endothelial NOS (eNOS)

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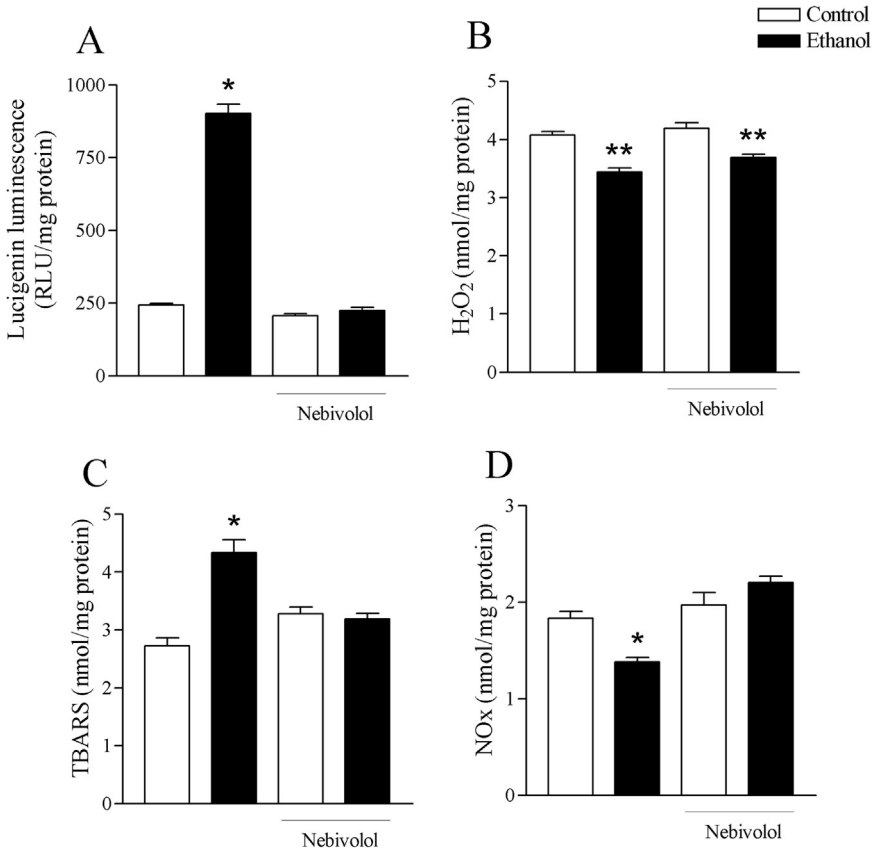

Fig. 1. Effects of nebivolol in ethanol-induced oxidative stress in the rat renal cortex. Bar graphs represent O_2^- levels evaluated by lucigenin-derived chemiluminescence assay (A). The concentrations of H_2O_2 were determined fluorometrically (B). The levels of TBARS (C) and NOx (D) were determined colorimetrically. Results are show as the means ± S.E.M. of 7–10 experiments. *Compared to control, control+nebivolol and ethanol+nebivolol; **Compared to control and control+nebivolol ($P < 0.05$, two-way ANOVA followed by Bonferroni's comparison test).

and neuronal NOS (nNOS) (Mount and Power, 2006). The physiological actions of NO in the kidney include the regulation of renal and glomerular haemodynamics, inhibition of tubular sodium reabsorption, maintenance of medullary perfusion, and modulation of renal sympathetic nerve activity (Mattson et al., 1992; Majid and Navar, 1994; Eppel et al., 2003). Since NO has numerous important functions in the kidney, reduced synthesis or availability of NO is associated with renal dysfunction (Roczniak et al., 2000; Mount et al., 2005).

Nebivolol is a third-generation β-adrenergic receptor antagonist with high selectivity for β_1-adrenergic receptors. Additional effects of nebivolol include NO production via activation of β_3-adrenergic receptors, inhibition of NADPH oxidase and direct O_2^- scavenging (Oelze et al., 2006; Maffei and Lembo, 2009; Serg et al., 2012). Reactive oxygen species play an important role in both acute and chronic kidney diseases and due to its antioxidant actions nebivolol has been shown to be effective in the treatment of various states of renal diseases (Shamekhi Amiri, 2016). Additionally, nebivolol elicits renal vasodilation and increases renal blood flow and glomerular filtration rate (Greven and Gabriëls, 2000).

Drugs with antioxidant properties offer protection against the oxidative damage caused by ethanol in the kidney, further improving kidney function and histoarchitecture (Mailankot et al., 2009; Shanmugam et al., 2010). In the kidney, reactive oxygen species-mediated toxicity is considered the primary mechanism by which ethanol induces renal injury (Scott et al., 2000; Rodrigo and Rivera, 2002). Considering that nebivolol displays renoprotective action in different models of renal injury and that this effect is related to its antioxidant properties, we tested the hypothesis that nebivolol would display a protective action against ethanol-induced oxidative stress, which is the primary mechanism triggered by ethanol to induce renal dysfunction. In the present study, we investigated the effects of nebivolol in an early phase of ethanol-induced renal changes, which is characterized by oxidative stress.

2. Materials and methods

2.1. Ethanol administration

Experimental protocols were approved by the Animal Ethics Committee of the University of São Paulo – *Campus* of Ribeirão Preto (#14.1.357.53.2). Male Wistar rats weighting between 250–280g (60–70 days old), were randomly divided into four groups: control, ethanol, control+nebivolol (10 mg/kg/day, p.o. gavage) (Ceron et al., 2013), and ethanol + nebivolol. Control rats received water ad libitum, whereas rats from the ethanol group received 20% (v/v) ethanol in their drinking water. Rats from the ethanol groups were submitted to a period of adaptation in which they received increasing doses of ethanol (5%, 10% and 20% during the first, second and third week, respectively). At the end of the third week, the experimental stage was initiated and lasted for two weeks (Passaglia et al., 2015; Marchi et al., 2016). At the end of the treatment period, rats were anaesthetized with urethane (1.25*g*/kg, intraperitoneally [i.p.], Sigma–Aldrich, St. Louis, MO, USA). The kidneys were removed and the renal cortex frozen in liquid nitrogen and stored at −80 °C. Blood samples were collected and centrifuged at 6500×*g* for 15 min at 4 °C, and serum was separated and stored at −80 °C.

2.2. Determination of creatinine, sodium and potassium serum levels

Creatinine serum levels were determined colorimetrically (at 540 nm) following the instructions of a commercially available kit