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Review

Total renal denervation reduces sympathoexcitation to different target organs in a model of chronic kidney disease

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ABSTRACT

It is known that increased sympathetic nerve activity in chronic kidney disease (CKD) progressively worsens kidney function and hypertension. We tested the hypothesis that total renal denervation contributes to reduce sympathetic activation to different beds and improves renal function in 5/6 nephrectomy model of CKD in male Wistar rats. After eight weeks of 5/6 nephrectomy surgery there was an increase in mean arterial pressure (CKD 179 ± 22 mm Hg, n = 6 vs. control animals 108 ± 9; $p < 0.05$, n = 6) with no changes in heart rate (HR). Sympathetic nerve activity was increased at different levels to the remaining kidney, splanchnic and lumbar beds compared to control (CTL) group (CKD rSNA: 150 ± 50, n = 9 vs. CTL 96 ± 15, n = 9; CKD sSNA: 129 ± 51, n = 5 vs. CTL 34 ± 14, n = 6; CKD lSNA: 203 ± 35, n = 8 vs. CTL 146 ± 21, spikes/s, n = 7, $p < 0.05$). Three weeks after total renal denervation (DNX) MAP was normalized in the CKD rats (124 ± 19 mm Hg, n = 5, $p < 0.05$), with no change in HR. The lSNA was normalized (151 ± 40, n = 5, vs. CKD 203 ± 35 spikes/s, n = 8) and sSNA was decreased in 49% (64 ± 34, n = 5 vs. CKD 129 ± 51 spikes/s, n = 5, $p < 0.05$). Renal function, assessed by creatinine plasma levels was improved after renal denervation (CKD 1.50 ± 0.64, n = 8; vs. CKD + DNX 0.82 ± 0.22 mg/mL, n = 8, $p < 0.05$). These findings demonstrate that renal nerves contribute to the maintenance of hypertension in CKD by increasing sympathoexcitation to other beds.

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1. Introduction

Chronic kidney disease (CKD) is a progressive and multifactorial disorder with high risk of cardiovascular morbidity and mortality that affects about 5–7% of the world's population (Greenberg et al., 2014; Levey and Coresh, 2012; Tonelli et al., 2006; Whaley-Connell et al., 2013).

Clinical and experimental studies have shown that increased sympathetic vasomotor activation is involved in the pathophysiology of all stages of CKD (Campese, 1997; Campese et al., 2011; Converse et al., 1992; Masuo et al., 2010; Paterno et al., 2012). Increased sympathetic nerve activity (SNA) to the kidneys has been shown in 5/6 nephrectomy rats, an experimental model of CKD (Paterno et al., 2012). Additionally, baroreflex control of sympathetic drive to the kidney and other targets was impaired in a genetic model of CKD, which was mediated in part by angiotensin II (Yao et al., 2015).

Renal nerves influence the renal control of blood pressure (BP) by modulating renin secretion, which activates the renin-angiotensin system, renal blood flow and sodium balance. In fact, renal SNA (rSNA) inappropriately augmented plays a crucial role in the onset and maintenance of clinical and experimental arterial hypertension (DiBona and Kopp, 1997; Esler et al., 2012). Thus, the renal nerves have been a potential therapeutic target in patients with neurogenic hypertension (Esler et al., 2012). Interestingly, renal denervation restored early-stage of CKD by decreasing blood pressure and improving renal function in patients with resistant hypertension (Kiuchi et al., 2015). However, the influence of renal nerves on sympathetic activation to other beds has not been fully elucidated in CDK.

Thus, the present study tested the hypothesis that renal denervation improves renal function and influences the sympathoexcitation to other targets that are crucial in the maintenance of hypertension in an experimental model of CKD.

2. Methods

All experiments protocols were approved by the Research Ethics Committee of the Escola Paulista de Medicina - Universidade Federal de São Paulo (UNIFESP) – (process no. 0270/12). Male Wistar rats (240–350 g) were obtained from central animal house of UNIFESP. The animals were housed in groups, had free access to rat chow and water, and were maintained in a temperature-controlled room (22 °C) on a 12 h light/12 h dark cycle.

2.1. Experimental protocols

Rats were distributed into three independent experimental groups: control rats (CTL), chronic kidney disease (CKD) and CKD submitted to renal denervation (CKD + DNX).

CKD was induced by nephrectomy 5/6 method and experiments were performed after 8 weeks. We previously reported that at this period, there is already a renal sympathoexcitation, arterial hypertension and reduction of kidney function (Bergamaschi et al., 1997; Paterno et al., 2012). In the CKD + DNX group, rats were submitted to total renal denervation five weeks after CKD induction and experiments were performed 3 weeks after renal denervation, before any complete reinnervation could have occurred (Mulder et al., 2013). All cardiovascular, neural, biochemical and histological analyses were performed 8 weeks after induction of CKD.

Euthanasia was performed with KCl intravenous administration in deep anesthetized rats.

2.2. Induction of chronic kidney disease (CKD)

To induce 5/6 nephrectomy, the rats were anesthetized with ketamine (60 mg/kg ip - Vetbrands, Brazil) and xylazine (20 mg/kg ip - Vetbrands, Brazil). Briefly, after a ventral laparotomy, removal of the right kidney and ligation of two of the three branches of the left renal artery were performed, resulting in the infarction of two-thirds of the left kidney (Paterno et al., 2012). We have not performed sham nephrectomy in the control rats, since we (Romero et al., 2016) and others (Pawlak et al., 2016; Shibata et al., 2016; Singh et al., 2016) have showed that sham nephrectomy maintains renal physiological and histological parameters intact. Thus, we avoided unnecessary surgery for ethical reasons.

2.3. Cardiovascular parameter analysis in conscious rats

Eight weeks after CKD surgery the rats were instrumented for intravenous injection of drugs and direct arterial pressure recording. The animals were anesthetized with 2.0–2.5% halothane-O_2 (Cristália, SP, Brazil) and fitted with femoral venous and arterial catheters. In our previous experience, 24 h later halothane anesthesia all cardiovascular parameters and SNA were returned to normal values (Biancardi et al., 2007; Perry et al., 2014). After surgical recovery (~24 h) and signal stabilization, the pulsatile blood pressure, mean arterial pressure (MAP) and heart rate (HR) were recorded and averaged for at least 20 min in conscious rats through an analog-to-digital converter PowerLab (ADInstruments, Australia), with a minimum frequency of acquisition of 2 kHz.

2.4. Renal, splanchnic and lumbar sympathetic nerve activity analysis in anesthetized rats

Rats were slowly anesthetized with thiopental (50 mg/kg - for the induction dose and followed by 5 mg/kg/h for continuous infusion via infusion pump-iv). A tracheotomy was performed to reduce airway resistance and inspired air was enriched with 100% oxygen.

For the renal (rSNA), splanchnic (sSNA) and lumbar (lSNA) sympathetic nerve activity recordings, the left renal, splanchnic and lumbar nerves were retroperitoneally exposed, placed on bipolar silver electrodes and once the conditions for nerve recording was established, the nerve and electrode were covered with vaseline oil. The signal from the nerves were displayed on an oscilloscope (TDS 220; Tektronix, Portland, OR), and the nerves activity was amplified (gain 20 K, Neurolog; Digitimer, Welwyn Garden City, UK), and filtered by a band-pass filter (100–1.000 Hz). At the end of the experiments, the background noise level of SNA was determined following hexamethonium bromide administration (30 mg/kg, iv) (Sigma-Aldrich). However, for the lSNA considering that some of the fibers are preganglionic and does not respond to hexamethonium the electrical signal between SNA bursts was used to estimate the background noise, as previously reported (Stocker and Muntzel, 2013). The total nerve activity was expressed in spikes per second (spikes/s), as we were interested on cardiovascular fibers (Malpas and Ninomiya, 1992). It was previously shown that during baroreceptor stimulation the major action was on the occurrence of frequency of rSNA and not on the amplitude of burst (Malpas and Ninomiya, 1992).