Microvascular Research 115 (2018) 44–51

Contents lists available at ScienceDirect

Microvascular Research

journal homepage: www.elsevier.com/locate/ymvre

Cutaneous and renal vasodilatory response to local pressure application: A comparative study in mice

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ARTICLE INFO

Keywords:
Microcirculation
Laser Doppler flux
Pressure-induced vasodilation
Endothelium dysfunction
Transient receptor potential vanilloid type 1

ABSTRACT

Background and aim: We have reported a novel relationship involving mechanical stimulation and vasodilation in rodent and human skin, referred to as pressure-induced vasodilation (PIV). It is unknown whether this mechanism exists in kidney and reflects the microcirculation in deep organs. Therefore, we compared the skin and kidney PIV to determine whether their changes were similar.

Methods: In anesthetized mice fed a normal salt-diet, laser Doppler flux (LDF) signals were measured when an increase in local pressure was applied to the surface of the head skin with the rate of 2.2 Pa/s (1 mm Hg/min) and to the left kidney with a rate of 4.4 Pa/s (2 mm Hg/min). The mechanism underlying renal PIV was also investigated. The skin and kidney PIV were also compared during salt load (4% NaCl diet).

Results: The kidney had higher baseline LDF and vascular conductance compared to those of the skin. Pressure application increased the LDF in the kidney as well as in the skin with a comparable maximal magnitude (about 25% from baseline value), despite different kinetics of PIV evolution. As we previously reported in the skin, the kidney PIV response was mediated by the activation of transient receptor potential vanilloid type 1 channels, the release of calcitonin gene-related peptide, and the participation of prostaglandins and nitric oxide. In the absence of hypertension, high salt intake abolished the cutaneous PIV response and markedly impaired the renal one.

Conclusion: PIV response in the mouse kidney results from a neuro-vascular interaction. Despite some differences between the skin and the kidney PIV, the similarities in their response and signaling mechanisms suggest that the cutaneous microcirculation could reflect, in part, the microcirculation of the renal cortex.

1. Introduction

The skin not only serves as a barrier to protect the body, but also contributes to the maintenance of the organismal homeostasis. Microvascular defects in the skin as well as those in the kidney and muscle have been shown to be linked to salt sensitivity, insulin resistance, and hypertension (Boegehold, 2002; de Jongh et al., 2007). The early detection of microvascular defects could be a predictor of cardiovascular and metabolic risk factors (Matsue et al., 2014). However, in daily clinical practice, the possibility of evaluating the microcirculation of deep territories with non-invasive tools is limited. In this regard, measurements of laser Doppler flux (LDF) on the skin surface presents the advantage of easy access, but it is unknown whether the skin microcirculation really reflects that in deep organs, particularly in the kidney. To our knowledge, only Coulon et al. (2012) reported that the skin LDF in post-occlusive reactive hyperaemia was correlated to the pulsed LDF measured in the renal interlobular arteries, but no mechanistic explanation was proposed.

We have reported a novel relationship in rodent and human skin (Fromy et al., 1998; Fromy et al., 2000) involving mechanical stimulation and vasodilation which is mediated by capsaicin-sensitive sensory nerves, referred to as pressure-induced vasodilation (PIV). Briefly, skin PIV is a response to an external non-noxious pressure application caused by the following steps, most likely in this order: first, pressure

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http://dx.doi.org/10.1016/j.mvr.2017.08.007
Received 19 April 2017; Received in revised form 23 July 2017; Accepted 28 August 2017
Available online 30 August 2017
0026-2862/

detection *via* acid-sensing ion channel 3 actives capsaicin-sensitive fibres leading to the release of neuropeptides such as calcitonin gene-related peptide (CGRP); then the release of prostaglandins and nitric oxide (NO) from the endothelium in response to the neuropeptides; lastly, vascular smooth muscle relaxation in response to the endothelial NO (Fromy et al., 2000; Fromy et al., 2012). Therefore, non-invasive PIV assessment by laser Doppler flowmeter evaluates the global neurovascular response to local pressure applied on the surface of tissue, and may be considered an index of local microcirculation. Using this technique, we previously reported PIV alterations during ageing (Gaubert et al., 2007; Fromy et al., 2010) and pathological states, such as diabetes and obesity (Demiot et al., 2006; Nguyen-Tu et al., 2013; Sigaudo-Roussel et al., 2004).

Capsaicin-sensitive sensory nerves have been identified in the kidney; their localization is not only limited to the renal pelvis (Knight et al., 1991; Mulder et al., 2013), but also extends from the renal arterial tree to the glomeruli as well as the renal tubules (Knight et al., 1991). The functional importance of these nerves has been highlighted by experiments showing that unilateral minor pressure increases in the renal pelvis lead to a contralateral natriuresis. This is the consequence of sensory nerve activation and its interaction with renal sympathetic nerves (Kopp et al., 1984). The transient receptor potential vanilloid type 1 (TRPV1) channels, also known as capsaicin receptors, are expressed almost exclusively in primary sensory nerves (Caterina et al., 1997; Caterina and Julius, 2001). Their activation leads to the release of CGRP and substance P, which are both potent vasodilators in most vascular beds (Withrington, 1992). The stimulation of capsaicin-sensitive sensory nerves increases renal blood flow, glomerular filtration rate and urinary sodium excretion in isolated kidney perfused at high pressure (Li and Wang, 2008). Interestingly, independently of renal hemodynamics, Li et al. recently reported a direct inhibitory effect of TRPV1 channels on sodium reabsorption in mouse cortical collecting ducts (Li et al., 2014). This may provide a possible explanation as to why the denervation of sensory nerve fibres impairs renal sodium excretion leading to salt-sensitive hypertension (Wang et al., 1998).

Recent evidence suggests that the skin acts cooperatively with the kidney to regulate electrolyte homeostasis and salt sensitivity (Wiig et al., 2013). We thought it was of interest to study the regulation of the microcirculation by the neuro-vascular interaction in these two organs and practically, to determine whether the reactivity of skin microcirculation correlates with that of the kidney. For this purpose, we have chosen to assess the PIV response under different experimental conditions. First, the skin and kidney PIV responses were evaluated in mice fed a normal salt diet and the mechanism underlying the renal PIV was also investigated. Secondly, we challenged the skin and kidney PIV responses by using a salt load, a condition previously reported to alter the structure of the vascular endothelium even in the absence of increased arterial pressure (Kusche-Vihrog et al., 2015).

2. Methods

2.1. Animals

Male C57Bl/6 mice (10–14 week old) from Janvier Laboratory (Le Genest Saint Isle, France) were housed in controlled conditions (temperature 21 ± 1 °C and 12-hour light/dark cycle). Mice had free access to tap water and standard mice diet containing 0.25% sodium (Elevage A04, SAFE, Augy, France). All experiments were performed under animal care procedures and in accordance with protocols that were approved by the Ethics Committee of Animal Experimentation of the University Claude Bernard Lyon 1 (Protocol BH2012-79).

2.2. PIV assessment on the skin and the kidney

PIV was assessed in mice anesthetized with thiopental (40 mg/kg, *i.p.*, Nesdonal, Mérial, Lyon, France). Mice were placed in an incubator (Mediprema, Chambray-les-Tours, France) to maintain the skin temperature at 35 ± 0.5 °C. The skin temperature was monitored by a patch probe placed at the back of the neck, close as possible the pressure application site, while the kidney temperature was recorded using a needle probe in contact with the superior pole of the left kidney. Systolic blood pressure (SBP) was monitored using a non-invasive tail cuff system (XBP1000, Kent Scientific, Torrington, Connecticut, USA).

Two days prior to the skin PIV experiment, mice were slightly anesthetized under isoflurane (2% in oxygen) to remove the hair from the head and the back with a depilatory lotion. An implant (5 mm in diameter) was inserted under the skin of the head to isolate this part of the skin from the underlying tissues. PIV was assessed using a weighbridge adapted to hold a laser Doppler probe (PF408, Perimed, Sweden), as previously described in rodents (Fromy et al., 2000). A laser Doppler contact probe was connected to a flowmeter (PF4001 Master, Perimed, Sweden) and allowed for simultaneous local pressure application and LDF signal measurements on the surface of the local pressure application site. For the kidney PIV assessment, through a left flank incision, the left kidney with intact capsule was carefully placed in a plastic spoon to avoid the movements induced by breathing; the renal PIV was measured with the same system described above for skin PIV. Using a computerized acquisition system (Biopac, Santa Barbara, California, USA), the skin and kidney LDF signals were digitized with a 20 Hz sampling frequency and averaged every 30 s to reduce the instantaneous variability of the signals.

2.3. Transcutaneous iontophoresis

The endothelium-dependent vasodilatation was assessed on the hairless back of anesthetized mice (thiopental, 40 mg/kg, *i.p.*) using iontophoretic delivery of 2% acetylcholine (Ach) with an anodal current application of 100 μA for 10 s. The response to iontophoretic delivery was expressed as the maximal increase from baseline levels. This technique was chosen to evaluate *in vivo* the cutaneous microcirculation without any systemic effect.

2.4. Drugs

Indomethacin, an inhibitor of cyclooxygenase; N^{ω}-Nitro-L-arginine (LNNA), an inhibitor of NO synthase; CGRP8-37, a selective competitive antagonist at CGRP receptors, and capsazepine, a competitive antagonist of TRPV1 channels as well as Ach were purchased from Sigma-Aldrich (Saint-Quentin-Fallavier, France). Indomethacin, LNNA and CGRP8-37 were diluted in saline. Capsazepine was prepared in dimethyl sulfoxide (DMSO) as a solution stock (20 mg/ml) and diluted with a mixture of 3% DMSO, 10% Tween 80 and saline to improve solubilization.

2.5. Protocol 1: renal PIV compared to cutaneous PIV in mice fed a standard diet

Cutaneous and renal PIV were assessed in two separated groups of anesthetized adult mice. Basal LDF signals were measured during a 1-min control period. The local pressure was then progressively increased at a rate of 2.2 Pa/s (1 mm Hg/min) on the surface of the head skin and at a rate of 4.4 Pa/s (2 mm Hg/min) on the surface of the left kidney for a whole period of 20 min. According to the results of our preliminary study, the application of 4.4 Pa/s on the kidney was chosen to elicit a maximal magnitude of PIV response, which was comparable to that observed on the skin (about 25% from the baseline values).

2.6. Protocol 2: mechanisms involved in renal PIV in mice fed a standard diet

Adult mice were divided into four groups to investigate the role of NO synthase, cyclooxygenase, CGRP, and TRPV1 channels in the renal