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ScienceDirect
European Journal of Pharmacology 578 (2008) 242–248
www.elsevier.com/locate/ejphar

The effect of urocortin II administration on the coronary circulation and cardiac function in the anaesthetized pig is nitric-oxide-dependent

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Received 5 June 2007; received in revised form 19 September 2007; accepted 23 September 2007
Available online 2 October 2007

Abstract

We planned to determine the primary effects and mechanisms of urocortin II, a member of the corticotrophin-releasing factor (CRF) family highly expressed in the cardiovascular system, on coronary blood flow and myocardial function *in vivo*. Urocortin II was infused into the left anterior descending coronary artery in 25 anaesthetized pigs whilst measuring haemodynamic variables, coronary blood flow, ventricular dP/dt_{max} cardiac output and percentage of segmental shortening. This infusion was repeated after blockade of the autonomic nervous system, nitric-oxide synthase (NOS) or subtype 2 of the CRF receptors. In all experiments changes in heart rate and aortic blood pressure were prevented. Intracoronary urocortin II increased, within 60 s, coronary blood flow (15±3.2%, $P<0.05$), dP/dt_{max} (12.7±2.6%, $P<0.05$), cardiac output (16±2.3%, $P<0.05$) and percentage of segmental shortening (19.8±3.8%, $P<0.05$). Blockade of NOS abolished only the coronary effects whereas blockade of subtype 2 of the CRF receptors abolished all cardiac and coronary effects. It was shown for the first time that urocortin II administration primarily increases coronary blood flow and myocardial function through the release of nitric oxide and activation of subtype 2 of the CRF receptors in the anaesthetized pig. This provides a mechanism through which a local increase of urocortin II levels can help improve a compromised cardiovascular function.

Keywords: Coronary blood flow; Corticotrophin releasing factor; Myocardial contractility; Nitric oxide; Urocortin II

1. Introduction

The peptide urocortin II, a member of the corticotrophin-releasing factor (CRF) family, binds selectively to the subtype 2 of CRF receptor, which is highly expressed in the myocardium and the blood vessels of humans (Kimura et al., 2002; Wiley and Davenport, 2004) and animals (Oki and Sasano, 2004), with no appreciable activity at the subtype 1 (Reyes et al., 2001; Lewis et al., 2001). Recent evidence has highlighted a potential clinical importance for urocortins in that it was shown to have cardioprotective effects against ischemic reperfusion injury in animals (Garcia-Villalon et al., 2004, 2005; Brar et al., 2004), and that its systemic administration has beneficial cardiovascular effects in heart failure in animals (Rademaker et al., 2005; Bale et al., 2004) as well as in humans (Davis et al., 2007). Despite this, the primary effect of urocortin on the coronary circulation *in vivo* has remained unknown. Moreover the information regarding the role of nitric oxide in the mechanisms of action is scarce and discordant.

For instance, intravenous administration of urocortin II to normal human volunteers or animals has been reported to result in variable changes of arterial pressure indices, an increase in heart rate, cardiac output and left ventricular inotropic state (Rademaker et al., 2005; Bale et al., 2004; Davis et al., 2005; Mackay et al., 2003; Gardiner et al., 2005). These changes can confound results about coronary blood flow, when considering *in vivo* experiments. Thus, urocortin administration to conscious sheep was found to increase left ventricular contractility and heart rate, and to result in coronary vasodilation (Parkes et al., 1997). In the rat isolated perfused heart urocortin resulted in positive inotropic effects and coronary vasodilation, which was not related to nitric-oxide release (Terui et al., 2001). Regional variation is also known to occur. In anaesthetized rats urocortin was found to increase the

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doi:10.1016/j.ejphar.2007.09.018

cardiac and gut vascular conductance without affecting the spleen, brain or the kidney (Abdelrahman and Pang, 2003). Also, urocortin was found to relax isolated coronary arteries of the rat through both endothelium-dependent and independent mechanisms (Huang et al., 2002, 2003; Yao et al., 2002), though this effect was smaller than that on basilar and tail artery segments (Sanz et al., 2003). Finally, the administration of urocortin II in conscious rats resulted in mesenteric and hindquarter nitric-oxide-independent vasodilation, though without affecting renal vascular conductance (Gardiner et al., 2005).

The present work was therefore planned, in controlled experiments in anaesthetized pigs, to investigate the primary *in vivo* effect of the administration of urocortin II on coronary blood flow and related myocardial function and to determine the mechanisms involved. This was achieved by infusing the peptide locally into the coronary circulation whilst preventing changes in heart rate and aortic blood pressure to avoid the secondary interference by reflex and local metabolic and physical effects.

2. Material and methods

The experiments were carried out in 25 domestic pigs, weighing 63 to 70 kg, supplied by an accredited dealer (Azienda Agricola Invernizzi, Olengo, Novara, Italy). The animals, fasted overnight, were anaesthetized with intramuscular ketamine (20 mg/kg; Parke-Davis, Milan, Italy) followed after about 15 min by intravenous sodium pentobarbitone (15 mg/kg; Siegfried, Zofingen, Switzerland), and artificially ventilated with oxygen-enriched air using a respiratory pump (Harvard 613, Harvard Apparatus, South Natick, Mass., USA). Anaesthesia was maintained throughout the experiments by continuous intravenous infusion of sodium pentobarbitone (7 mg/kg/h) and assessed as previously reported (Linden and Mary, 1983), using responses of the animals to somatic stimuli. The experiments were carried out in accordance with European Union Guidelines (no. 86/609/CEE) and with the Guide for the Care and Use of Animals as adopted and promulgated by the United States National Institutes of Health.

Blood pressures in the ascending aorta and in the right atrium were recorded via catheters connected to pressure transducers (Statham P23 XL; Gould, Valley View, Ohio, USA) inserted into the right femoral artery and the right external jugular vein, respectively. The chest was opened in the left fourth intercostal space and, after applying a positive end-expiratory pressure, the pericardium was cut and an ultrasound flowmeter probe was positioned around the proximal part of the left anterior descending coronary artery (Transonic Systems Inc. model 420, NY, USA) to obtain the coronary blood flow.

Left ventricular pressure was measured by means of a catheter inserted through the left atrium. The frequency response of the catheter-manometer system was found to be flat (±5%) up to 40 Hz.

In order to measure regional contractile function, pairs of 2 mm ultrasonic segment length microtransducer crystals (Sonometrics, London, Ont., Canada) were implanted in the left anterior ventricular wall in the distribution area of the left anterior descending coronary artery. Each pair of crystals was implanted in the midmyocardial layer approximately 10 mm apart and parallel to the fibers direction. A further crystal was implanted in the left ventricular posterior wall in order to calculate the ventricular volume. To pace the heart, electrodes were sewn on the left atrial appendage and connected to a stimulator (model S8800, Grass Instruments, Quincy, Mass., USA) delivering pulses of 3–5 V with 2-ms duration at the required frequency. To prevent changes in aortic blood pressure during the experiments, a cannula was introduced into the left internal mammary artery and connected to a reservoir containing Ringer's solution kept at 38 °C. The reservoir was pressurized using compressed air, which was controlled with a Starling resistance, and pressure within the reservoir was measured by a mercury manometer. This method has been shown in anaesthetized pigs to allow the aortic blood pressure to be maintained at steady levels without significant changes in filling pressures of the heart or the haematocrit (e.g. Vacca et al., 1999). The acid–base status of the animals was monitored with a gas analyzer and kept within normal limits as previously reported (Linden and Mary, 1983). Coagulation of the blood was avoided by the injection of heparin (Parke-Davis; initial doses of 500 i.u./kg, and subsequent doses of 50 i.u./kg every 30 min). The rectal temperature of the pigs was monitored and kept between 38 and 40 °C using an electric pad.

All cardiovascular parameters were recorded via a micro1401 A/D converter (Cambridge Electronic Design, Cambridge, UK) and processed by using Spike2 Software (CED). Sonomicrometer crystals data were digitally processed by specific hardware and software (Sonometrics, London, Ont., Canada).

To calculate coronary vascular resistance, the difference between mean aortic blood pressure and mean left ventricular pressure during diastole was considered as the coronary pressure gradient. Coronary vascular resistance was calculated as the ratio between this pressure gradient and mean diastolic coronary blood flow during the steady-state. At the end of the experiment, each animal was killed by an intravenous injection of 90 mg/kg sodium pentobarbitone.

2.1. Experimental protocol

The experiments were begun after at least 30 min of steady-state conditions with respect to measured haemodynamic variables. In all the experiments, in order to avoid the interference of any possible changes in heart rate and aortic blood pressure during the experiments, the heart was paced to a frequency 20 beats/min higher than that observed during the steady state and the arterial system connected to the pressurized reservoir. In the 25 pigs the experiments were carried out by infusing either urocortin II (Sigma) or the vehicle (saline) only into the left anterior descending coronary artery, through a butterfly needle inserted into the proximal part of the vessel. The infusions were completed within a period of 5 min (1 ml/min) by using an infusion pump (model 22, Harvard Apparatus).

In each pig, a mean dose of urocortin II of 0.069±0.008 (0.06–0.09) μg/min for each ml/min of measured coronary blood flow, was infused into the coronary artery; that corresponded to the optimal dose of 6 μg/kg found in a preliminary dose–response study. The amount of urocortin II was similar to the one given in rats