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Regional haemodynamic effects of urocortin in the anaesthetized rat

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Abstract

Urocortin is an endogenous vasodilator peptide that is related to corticotrophin-releasing factor. We examined the haemodynamic effects of urocortin in thiobutabarbital-anaesthetized rats, via the triple-isotope microspheres technique. Urocortin (3 nmol/kg, i.v. bolus) reduced mean arterial pressure (−25 mm Hg) through a decrease in total peripheral resistance (−43%). This was associated with an increase in cardiac output (+24%) and vasodilatation of the following tissues: heart and stomach (≈ 300% of baseline); liver, intestine, caecum/colon, skeletal muscle and skin (≈ 200%); and testes (≈ 150%). Arterial conductances of the kidneys, spleen and brain were unaffected by urocortin. Neither the vehicle (0.9% NaCl) nor a low dose of urocortin (0.3 nmol/kg) altered any measurements. Therefore, urocortin causes generalized vasodilatation as follows: heart and stomach>liver, intestine, caecum/colon, skeletal muscle and skin>testes.

Keywords: Urocortin; Arterial pressure; Peripheral resistance; Cardiac output; Blood flow; Arterial conductance

1. Introduction

Urocortin is a 40-amino acid peptide that belongs to the family of corticotrophin-releasing factor (Vaughan et al., 1995), which is also known as corticotrophin-releasing hormone. Urocortin has 45% sequence identity to rat and human corticotrophin-releasing factor and 63% sequence identity to fish urotensin (Vaughan et al., 1995). Urocortin mRNA has been detected in the brain (Takahashi et al., 1998) and periphery, which includes the heart (Okosi et al., 1998; Kimura et al., 2002), and the gastrointestinal tract, thymus and spleen (Kageyama et al., 1999). The actions of urocortin and related peptides are mediated through the activation of two distinct corticotrophin-releasing hormone receptors, CRF_1 and CRF_2 (Vaughan et al., 1995). Urocortin and corticotrophin-releasing factor have similar affinity for the CRF_1 receptor, but urocortin has a greater affinity than corticotrophin-releasing factor for the CRF_2 receptor (Vaughan et al., 1995). CRF_1 receptor mRNA is widely expressed in the brain and pituitary, and to a less extent, in peripheral tissues. CRF_2 receptors, on the other hand, exist in three functional splice variants, $CRF_{2A,2B,2C}$ (for a review, see Dautzenberg and Hauger, 2002). The mRNA for CRF_{2A} is found in the brain, whereas mRNA for CRF_{2B} is present in the brain as well as periphery, most notably in the heart, skeletal muscle and, to a less extent, in the lungs and intestine (Lovenberg et al., 1995). In addition, CRF_{2B} mRNA is found to locate predominantly on arterioles of the heart and brain (Lovenberg et al., 1995).

Intravenous injection of urocortin causes a long-lasting depressor response and tachycardia in conscious and anaesthetized rats (Parkes et al., 2001; Vaughan et al., 1995) and anaesthetized mice (Cohen et al., 2000), but a long-lasting pressor response in conscious sheep (Parkes et al., 1997). Urocortin induces the vasodilatation of in vitro preparations such as the isolated rat coronary (Huang et al., 2002), basilar (Schilling et al., 1998) and tail (Lubomirov et al., 2001) arteries, and isolated perfused human fetal placental artery (Leitch et al., 1998) and isolated rat heart (Terui et al., 2001).

The in vivo vasodilator profile of urocortin is not known. The aim of the present study was to examine the spectrum of the dilator action of urocortin in regional beds of the anaesthetized rat.

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2. Material and methods

2.1. Animal preparation

Male Sprague–Dawley rats (350–400 g) were anaesthetized with thiobutabarbital (100 mg/kg i.p.). Cannulae filled with heparinized normal saline (0.9% NaCl, 25 I.U./ml) were inserted into the left ventricle via the right carotid artery for the injection of radioactively labelled microspheres, and into the right femoral artery for blood withdrawal, as required for the measurement of flow and cardiac output (Pang, 1983). Cannulae were also inserted into the left femoral artery and vein for the recording of mean arterial pressure by a pressure transducer (PD23DB, Gould, Statham, CA, USA) and the injection of urocortin, respectively. Heart rate was derived electronically from the upstroke of the arterial pulse pressure by a Grass 7P4G tachograph.

2.2. Measurement of cardiac output and regional blood flow

A well-stirred suspension (100–200 μl) containing 20,000–40,000 microspheres (15 μm diameter) labelled with either ^{57}Co, ^{113}Sn or ^{103}Ru (Perkin-Elmer Life Sciences, Boston, MA, USA) was injected and flushed over 10 s into the left ventricle. Beginning at 10 s before the injection of each set of microspheres, blood was withdrawn (Harvard infusion/withdrawal pump) from the right femoral arterial cannula into a heparinized saline-filled syringe at 0.35 ml/min for 45 s. The order of the administration of the microspheres for each group was as follows: Co–Sn–Ru ($n=2$), Sn–Ru–Co ($n=2$) and Ru–Co–Sn ($n=2$). At the end of the experiments, blood samples, whole organs, as well as 30 g each of skeletal muscle (from areas of the chest, abdomen, back and forelimb) and skin (from areas of the chest, abdomen and back) were removed for the counting of radioactivity (in counts per min or cpm) using a 1185 Searle Automatic Gamma Counter (Nuclear-Chicago, IL, USA) with a 3-in. NaI crystal. Corrections were made for the spillover of radioactivity from ^{113}Sn into the ^{57}Co channel (24%), and for spillover of ^{103}Ru into the ^{57}Co and ^{113}Sn channels (30% and 8%, respectively).

2.3. Experimental protocol

Rats were divided into two groups ($n=6$) and given 30 min to stabilize. A first set of microspheres was injected into the two groups to determine baseline flow followed by injection of the other two sets at intervals of 20 min. From the results of our preliminary studies, two doses of urocortin were chosen: a low dose (0.3 nmol/kg) that caused a small (-5 mm Hg, ED_{10-20}) decrease in mean arterial pressure, and a higher dose (3 nmol/kg, ED_{85-95}) that induced prolonged hypotension. These doses were determined from additional preliminary experiments. Both doses of urocortin or the vehicle (equivalent volumes of 0.9% NaCl) were injected at the plateau phase of response to urocortin, at $\cong 3-5$ min before the injection of each set of microspheres.

2.4. Calculations

$$\mathrm{CO(ml/min)} = \frac{\text{Blood withdrawal rate(ml/min)} \times \text{total injected cpm}}{\text{cpm in withdrawn blood}}$$

$$\text{Total peripheral resistance(TPR, mm Hg min/ml)} = \frac{\mathrm{MAP(mm\ Hg)}}{\mathrm{CO(ml/min)}}$$

$$\text{Blood flow(ml/min)} = \frac{\text{Blood withdrawal rate(ml/min)} \times \text{tissue cpm}}{\text{cpm in withdrawn blood}}$$

$$\text{Arterial conductance(ml/mm Hg min)} = \frac{\mathrm{Blood\ flow(ml/min)}}{\mathrm{MAP(mm\ Hg)}}$$

2.5. Drugs

Urocortin (Sigma, MO, USA) was dissolved in distilled water and kept in aliquots at $-20\,^{\circ}$C until use. Thiobutabarbital (Inactin) was from Sigma.

2.6. Statistical analysis

All data are shown as mean ± standard error of the mean (S.E.M.). To render the homogeneity of variance, the data of flow and conductance were log-transformed prior to statistical analysis by repeated-measures analysis of variance, followed by Tukey's test, with $P<0.05$ selected as the criterion for statistical significance.

3. Results

Intravenous injection of saline did not significantly affect mean arterial pressure, heart rate, cardiac output and total peripheral resistance (Fig. 1) or blood flow to any organs or tissue (Table 1). Since the vehicle did not alter mean arterial pressure or regional flow, it also did not affect arterial conductances (ratio of blood flow to mean arterial pressure) of any organs or tissue (results not shown).

The low dose of urocortin (0.3 nmol/kg) did not significantly affect mean arterial pressure, total peripheral resistance, heart rate, cardiac output, or regional arterial flows and

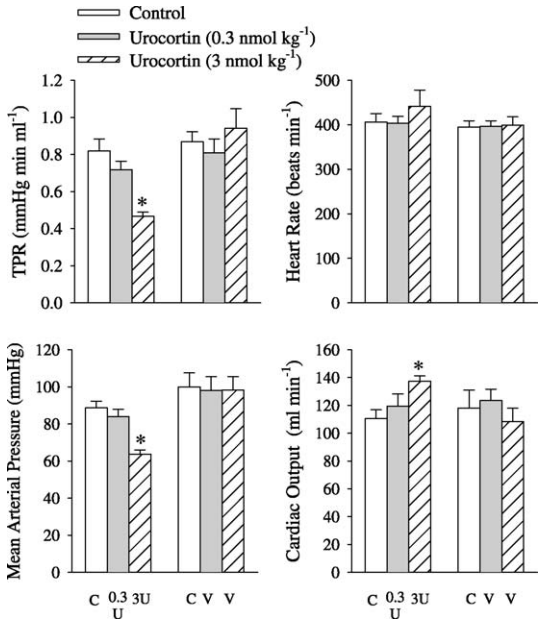

Fig. 1. Effects (means ± S.E.M.) of urocortin (0.3 and 3 nmol/kg) and vehicle (0.9% NaCl) on mean arterial pressure, heart rate, cardiac output and total peripheral resistance (TPR) in two groups of thiobutabarbital-anaesthetized rats ($n=6$). C = pretreatment baseline; V = vehicle; 0.3U = urocortin (0.3 nmol/kg), and 3U = urocortin (3 nmol/kg). *Significantly different from baseline ($P<0.05$).

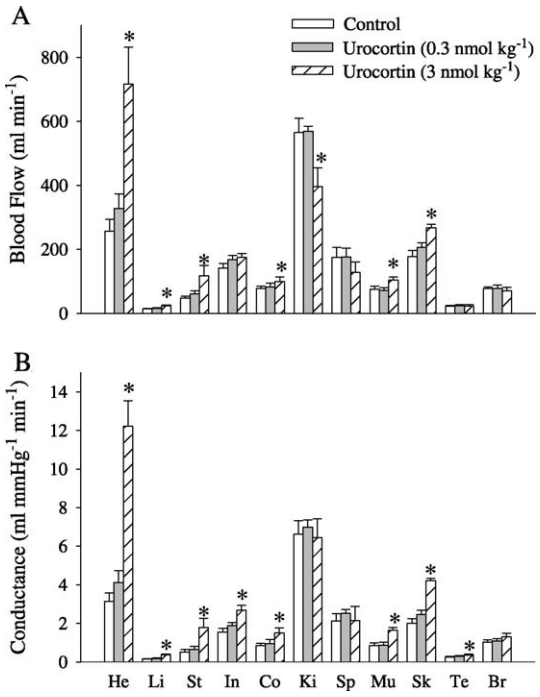

Fig. 2. Effects (means ± S.E.M.) of urocortin (0.3 and 3 nmol/kg) on blood flow (A) and arterial conductance (B) in thiobutabarbital-anaesthetized rats ($n=6$). Values of flow and conductance are expressed as per 100 g of tissues from the heart (He), liver (Li), stomach (St), intestine (In), colon plus caecum (Co), kidneys (Ki), spleen (Sp), testes (Te) and brain (Br), and as per 1000 g of tissues from the skeletal muscle (Mu) and skin (Sk). *Significantly different from baseline ($P<0.05$).

conductance (Figs. 1 and 2). The high dose (3 nmol/kg) decreased mean arterial pressure (−25 mm Hg) and total peripheral resistance (−43%) and increased cardiac output (+24%), but it did not significantly affect heart rate. The high dose also increased flows to the heart, liver, stomach, caecum and colon, skeletal muscle and skin, but it reduced flow to the kidneys (Fig. 2). When flow was normalised by arterial pressure to reflect intrinsic vascular tone, the high dose was found to increase arterial conductance of all tissues, except the kidneys, spleen and brain (Fig. 2). Its dilator influence was more prominent in the heart and stomach (≈ 300% of baseline) than the liver, intestine, caecum/colon, skeletal muscle and skin (≈ 200%), and the testes (≈ 150%).

Table 1
Effects (means ± S.E.M.) of vehicle (0.9% NaCl) on blood flow (ml/min) in thiobutabarbital-anaesthetized rats ($n=6$ each)

Organ	Baseline	Vehicle	Vehicle
Heart	321 ± 32	362 ± 45	296 ± 37
Liver	15 ± 3	16 ± 3	13 ± 2
Stomach	65 ± 11	60 ± 7	67 ± 9
Intestine	127 ± 17	141 ± 16	130 ± 13
Colon + caecum	110 ± 16	107 ± 13	87 ± 10
Kidneys	516 ± 43	554 ± 62	484 ± 66
Spleen	199 ± 36	140 ± 47	202 ± 42
Muscle	87 ± 10	85 ± 11	66 ± 7
Skin	167 ± 32	182 ± 21	166 ± 17
Testes	25 ± 2	30 ± 4	32 ± 4
Brain	66 ± 9	80 ± 17	80 ± 13

Flows (per 100 g tissue), except for muscle and skin (per 1000 g tissue).

4. Discussion

The low dose of urocortin caused insignificant changes in arterial pressure and haemodynamics, which were generally along the same direction as those produced by the high dose. The high dose of urocortin decreased arterial pressure via vasodilatation as revealed by the marked decrease in total peripheral resistance. Urocortin slightly increased cardiac output, and this was likely due to decreased flow resistance, since heart rate was unchanged. Depressor response (Vaughan et al., 1995; Parkes et al., 2001) and tachycardia (Parkes et al., 2001) to intravenous injection of urocortin have been reported. The lack of tachycardic response to urocortin in the present study was likely secondary to the effect of the anaesthetic thiobutabarbital which possibly depressed baroreflex activity. Our results also show that the most prominent dilator action of urocortin is in the heart and stomach, followed by the liver, skin, skeletal muscle, colon/caecum, intestine and, lastly,

the testes. There was, however, no increase in flow conductance in the kidneys, spleen and brain, suggesting that these tissues are the least sensitive to the dilator effect of urocortin. Urocortin has been shown to increase coronary arterial flow in the isolated perfused rat heart (Terui et al., 2001) and to cause relaxation of isolated rat coronary artery (Huang et al., 2002).

The vasodilator action of urocortin in the gastrointestinal (stomach, intestine, colon/caecum) and skeletal muscle beds in the present study has not been reported previously. It is of interest that the related peptide corticotrophin-releasing factor has been shown to increase mesenteric and hindquarter flows of conscious rats (Gardiner et al., 1988). Corticotrophin-releasing factor has also been shown to increase flows in the mesenteric bed of conscious dogs (Lenz et al., 1985), and both mesenteric and common iliac arteries in anaesthetized cynomologus monkeys (Udelsman et al., 1986). In contrast, MacCannell et al. (1984) have reported that corticotrophin-releasing factor causes selective dilatation of the mesenteric but not coeliac artery. The dilator actions of urocortin in vascular beds of the liver (hepatic arterial) and testes have not been reported previously.

There are indications that urocortin may have a physiological and/or pathophysiological role. Immunoreactivity and mRNA for urocortin have been detected in the brain as well as the periphery, such as the heart, gastrointestinal tract and arterioles (see review by Parkes et al., 2001). Furthermore, urocortin has been shown to bind strongly to CRF_{2B} receptors, and CRF_{2B} mRNA is present on resistance arteries and the heart (Lovenberg et al., 1995). As well, depressor effect of urocortin is absent in mice with deficient CRF_2 receptors (Bale et al., 2000; Coste et al., 2000), and mice with deficient CRF_2 receptors have elevated blood pressure (Coste et al., 2000). Lastly, the results from the present show clearly that exogenously administered urocortin causes a depressor response through peripheral vasodilatation. Taken together, these results suggest a physiological vasodilator role for urocortin.

To summarise, a depressor dose of urocortin causes prominent vasodilatation of the heart and stomach, and to a less extent, dilatation of the liver, intestine, caecum/colon, skeletal muscle, skin and testes.

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