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Urocortin Induces Endothelium-Dependent Vasodilatation and Hyperpolarization of Rat Mesenteric Arteries by Activating Ca^{2+}-Activated K^+ Channels

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Seçilmiş, M.A., Özü, Ö.Y., Emre, M., Büyükafşar, K., Kiroğlu, O.E., Ertuğ, P., Karataş, Y., Önder, S. and Şingirik, E. *Urocortin Induces Endothelium-Dependent Vasodilatation and Hyperpolarization of Rat Mesenteric Arteries by Activating Ca^{2+}-Activated K^+ Channels*. Tohoku J. Exp. Med., 2007, **213** (1), 89-98 —— Urocortin, a member of corticotropin releasing factor (CRF) peptide family, has positive chronotrophic and inotropic effects on heart and also shows a vasodilatory effect. However, the mechanism underlying its vasodilatory effect has yet to be elucidated. Endothelium-dependent relaxation of resistance arteries is mainly achieved by activation of K^+ channels. Therefore, we investigated possible role of K^+ channels and hyperpolarization for the vasodilatory effect of urocortin using the isolated perfused rat mesenteric arteries. Urocortin (0.2 nM) produced a slow-onset decrease in the perfusion pressure of the mesenteric vascular bed, which was elevated by an α1-adrenoceptor agonist, phenylephrine (2-4 μM). Urocortin also hyperpolarized the main mesenteric artery. Removal of endothelium with saponin treatment considerably inhibited the relaxation and hyperpolarization induced by urocortin. In contrast, the hyperpolarization was not significantly changed by cyclooxygenase inhibitor, indomethacin (1 μM) and/or nitric oxide synthase inhibitor, N^{ω}-nitro-L-arginine (100 μM). Urocortin-induced relaxation was not affected by the combination of a guanylyl cyclase inhibitor, 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one (ODQ, 1 μM), indomethacin and N^{ω}-nitro-L-arginine. However, the relaxation and hyperpolarization were abolished by high extracelluler potassium concentration (40 mM) or by a large conductance Ca^{2+}-activated K^+ channel blocker, charybdotoxin (1 nM). Glibenclamide (1 μM), an ATP-dependent K^+ channel inhibitor, did not affect the relaxation and hyperpolarization. These results suggest that urocortin causes endothelium-dependent relaxation and hyperpolarization of rat mesenteric arteries, probably through the activation of charybdotoxin sensitive Ca^{2+}-activated K^+ channels. These findings also indicate an essential role of the endothelium for the urocortin-elicited vascular relaxation and hyperpolarization. ———— urocortin; Ca^{2+}-activated K^+ channels; charybdotoxin; mesenteric artery; hyperpolarization
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Urocortin, a member of corticotropin releasing factor (CRF) peptide family, is a 40-amino acid peptide, which was identified in rat (Vaughan et al. 1995) and in human being (Donaldson et al. 1996; Zhao et al. 1998). Both CRF and urocortin have effects on the central nervous system as neurotransmitters that mediate stress responses by acting corticotropin CRF receptors on the central neurons (Dunn and Berridge 1990; Herbert 1993; De Souza 1995). Intracerebral administration of CRF increases cardiac output and mean arterial pressure (Fisher et al. 1983; Overton et al. 1990), while it decreases mean arterial pressure when given intravenously (MacCannell et al. 1982; Overton and Fisher 1991). Actions of CRF-related peptide are mediated through their specific receptor subtypes, for instance, corticotropin CRF_1 receptor is highly expressed in the brain and pituitary (Potter et al. 1994). In the brain, the density of CRF_2 receptors is high in the hypothalamus, the amygdala, and the lateral septum. Corticotropin CRF_2 receptor mRNA is mainly expressed in peripheral tissue, particularly in heart, lung, skeletal muscle, ovary and gastrointestinal tract (Hillhouse et al. 2002; Rademaker 2004).

Urocortin produces both positive chronotropic and inotropic effects on the sheep (Parkes et al. 1997) and rat heart (Nishikimi et al. 2000; Terui et al. 2001). It also causes a potent and long-lasting hypotension in the rats (Vaughan et al. 1995), making it therapeutically potential to be used in certain cardiovascular indications. Positive chronotropic and inotropic effects of urocortin are probably associated with an increase in cyclic AMP (cAMP) levels in the cardiac cell by the activation of CRF_2 receptors (Heldwein et al. 1996; Nishikimi et al. 2000). Hypotensive action of CRF-peptides may result from fall in total peripheral resistance (Vaughan et al. 1995), especially in the mesenteric bed (MacCannell et al. 1984, 1986; Gardiner et al. 2005).

Studies on the isolated preparations have shown that urocortin causes relaxation of rat arteries and veins (Schilling et al. 1998; Lubomirov et al. 2001; Terui et al. 2001), and human placenta (Leitch et al. 1998) and saphenous veins (Sanz et al. 2002). However, the underlying mechanisms of its vasodilatory actions are still unclear. On the other hand, some attempts have been made to elucidate possible mechanisms. It has been demonstrated that urocortin-induced relaxation is both endothelium-dependent and -independent in the coroner artery. Endothelium-dependent component of the relaxation is contributed by nitric oxide (NO) (Huang et al. 2002), while the other component is mediated by intracellular cAMP (Huang et al. 2003), which was also demonstrated in rat tail arteries (Lubomirov et al. 2001). On the other hand, in the isolated perfused rat heart, urocortin-induced coronary vasodilatation may partly depend on prostaglandins (Terui et al. 2001). These reports suggest that vasodilatory action mechanism of urocortin may show differences between species and preparations. The vasodilatory effects of CRF receptors were detected on both mesenteric circulation (MacCannell et al. 1984, 1986; Gardiner et al. 2005) and isolated mesenteric artery ring (Lei et al. 1993). Recently, it has been reported that hemodynamic effects of urocortin depend on activation of CRF_2 receptor and do not involve NO or prostanoids (Gardiner et al. 2005). However, the actions of urocortin and its possible vasodilatory mechanisms are not well studied in mesenteric vascular bed.

In light of in vivo and in vitro studies on the relaxant effects of CRF peptide family including urocortin, we aimed to investigate possible mechanisms underlying the vasodilatory response to urocortin in the isolated perfused rat mesenteric bed in the present study. Furthermore, the action of urocortin was also examined on the cell membrane potential of the isolated superior mesenteric artery.

Materials and Methods

Perfusion of the isolated rat mesenteric vascular bed

The protocol of this study was approved by the ethics committee of Medical Faculty at Çukurova University. Male Wistar rats (200-300 g) were killed by cervical dislocation, the abdominal cavity of rats was immediately opened. A polyethylene cannula (i.d. = 0.58, o.d. = 0.96) was inserted into the superior mesenteric

artery and the vascular bed flushed with heparinized Krebs-Henseleit solution. The mesenteric vascular bed was then dissected away from the connecting tissues and transferred to a warmed chamber where it was perfused with gassed (95% O_2 + 5% CO_2) Krebs-Henseleit solution (mM composition: NaCl 119, KCl 4.6, $CaCl_2$ 1.5, $MgCl_2$ 1.2, $NaHCO_3$ 15, $NaHPO_4$ 1.2 and glucose 11) maintained at 37°C with a constant flow rate of 6.5 ml/min using a peristaltic pump (Buchler Instruments, USA). The perfusion pressure of the mesenteric bed was continuously measured with a pressure transducer (Keller PRC 21K-10, Switzerland) and recorded on a polygraph (Gemini 7070, Italia). Under these conditions, any alterations in the perfusion pressure induced by a given drug were considered as changes in vascular tonus. After the equilibrium period of 60 min, the perfusion pressure was increased by the addition of phenylephrine (2-4 μM) to the perfusion fluid. Acetylcholine (0.01 μg/0.1 ml) was then applied close-arterially as bolus dose to each preparation in order to test the endothelial function.

Electrophysiological Studies

Rings of the superior mesenteric artery (8-10 mm) were opened longitudinally and pinned down on the rubber bottom of an organ chamber (10 ml) with the endothelial side upward. Tissue samples were superfused with Krebs-Henseleit solution, gassed with 95% O_2 and 5% CO_2 at 37°C at constant flow rate (4 ml/min). After 60 min of incubation, borosilicate capillary microelectrodes filled with 3 M KCl (tip resistances: 40-60 mΩ) were implanted into the smooth muscle cells from the intimal initial inner side. The microelectrode was coupled by an Ag/AgCl junction to a high impedance capacitance neutralizing amplifier (Nihon Kohden, Model MEZ-7200, Tokyo). An agar-bridge containing 3 M KCl was used as a reference electrode. The resting membrane potential was monitored continuously on a storage oscilloscope (Hitachi Model VC-6045, Tokyo). After a stable resting membrane potential for at least 3 min, acetylcholine was applied to test functional endothelium. We compared hyperpolarizing responses to urocortin before and after application of various test agents, which were added to the medium 10-30 min before the urocortin application.

Experimental protocol

Wistar albino rats were used to investigate the mechanical and electrical effect of urocortin on the isolated mesenteric arteries. Mechanical studies in the isolated perfused mesenteric vascular bed were as follows:

1) Vasorelaxant effect of urocortin on perfusion pressure in the mesenteric vascular bed,

2) The effect of cyclooxygenase inhibition by indomethacin on urocortin-induced relaxation,

3) The effect of NO-synthase inhibition by nitro-L-arginine in the presence of indomethacin,

4) The effect of guanylate cyclase by 1H-[1,2,4] oxadiazolo[4,3-a]quinoxalin-1-one (ODQ) in the presence of indomethacin and nitro-L-arginine,

5) Denudation of endothelium with a detergant substance, saponin,

6) The inhibition of K^+ channels with raised extracellular K^+ concentration,

7) The inhibiton of ATP-dependent K^+ channels with glibenclamide,

8) The inhibition of small conductance Ca^{2+}-activated K^+ channels with apamine, and

9) The inhibition of large conductance Ca^{2+}-activated K^+ channels with charybdotoxin.

Electrophysiological studies in mesenteric arterial strips were as also follows:

1) The effect of urocortin on the membrane potential,

2) The effect of cyclooxygenase inhibition with indomethacin on the urocortin-elicited changes in membrane potential,

3) The effect of NO-synthase inhibition with nitro-L-arginine in the presence of indomethacin,

4) The effect of denudation of endothelium with saponin,

5) The inhibiton of ATP-dependent K^+ channels with glibenclamide,

6) The inhibition of small conductance Ca^{2+}-activated K^+ channels with apamine, and

7) The inhibition of large conductance Ca^{2+}-activated K^+ channels with charybdotoxin.

Functional studies

After having a submaximal and stable vasoconstriction, which was induced by the addition of phenylephrine (2-4 μM) to perfusing fluid, urocortin was added to induce vasodilatation at a single concentration of 0.2 nM, which was chosen as an optimum concentration in preliminary experiments (data not shown). In the electrophysiological studies, both acetylcholine and urocortin were used at the same concentrations as those used in mechanical studies. In every individual perfusion experiment, a bolus dose of acetylcholine (0.01-0.1 μg) was

applied to the tissue to assess functional endothelium. The preparations not responding to phenylephrine and/or acetylcholine were discarded. For endothelium removal, mesenteric vascular bed was continuously perfused with saponin at the concentration of 50 mg/l for 10 min. Thereafter the vascular bed was immediately perfused with fresh Krebs solution without saponin for 1 hr before acetylcholine test.

Drugs and solutions

Following drugs were used: Acetylcholine chloride, urocortin (human), N^{ω}-nitro-L-arginine (nitro-L-arginine), ODQ, charybdotoxin, apamin, glibenclamide, indomethacin, phenylephrine hydrochloride (all purchased from Sigma Co, St. Lois, MO, USA) and saponin (from Fluka Chemie AG, Deisenhofen, Germany). Indomethacin, ODQ and glibenclamide were dissolved in dimethyl sulfoxide (DMSO). When added to 1 l perfusion reservoir (0.01% DMSO concentration), this concentration did not affect the phenylephrine-induced increase in perfusion pressure. Other chemicals were dissolved in distilled water except for nitro-L-arginine and saponin, which were dissolved in Krebs solution.

Statistical analysis

All data were expressed as means ± S.E.M. and evaluated in the Bonferroni-corrected test, which was used in the one-way analysis of variance (ANOVA). *P* values less than 0.05 were considered as significantly different from control. The increase in perfusion pressure induced by phenylephrine was expressed as mmHg. The vasodilatation in mesenteric vascular beds was measured as percent decrease in the perfusion pressure elevated by phenylephrine.

Results

Established tone and vasorelaxant responses to acetylcholine and urocortin in the rat isolated perfused mesenteric vascular bed

In all experiments, basal perfusion pressure was 20.3 ± 1.2 mmHg. After the phenylephrine (2-4 μM) was added to the perfusion fluid, the pressure increased by 52.2 ± 2.8 mmHg above the basal. As determined after preliminary experiments, this was the dose inducing submaximal increase in the perfusion pressure. Bolus injections of acetylcholine (0.01 μg) caused a sharp and transient decrease in the perfusion pressure. Urocortin perfusion (0.2 nM) also induced a slow developing vasodilatation at onset. Maximum vasodilatory responses to acetylcholine and urocortin were 53.9 ± 3.5% and 61.6 ± 2.7%, respectively (Figs. 1A and 2).

Effect of indomethacin, nitro-L-arginine and ODQ on the urocortin-induced vasodilatation

Neither indomethacin alone (1 μM) nor the combination of indomethacin with nitro-L-arginine (100 μM) affected relaxation induced by urocortin (0.2 nM). Furthermore, this relaxation was not changed by the addition of ODQ (1 μM) in the presence of indomethacin and nitro-L-arginine. The relaxations were 61.6 ± 2.7% in control, 54 ± 6.2% in indomethacin plus nitro-L-arginine, and 52.3 ± 6% in indomethacin plus nitro-L-arginine plus ODQ ($p < 0.05$, Figs. 1C and 2).

Effect of endothelium removal on acetylcholine- and urocortin-induced vasodilatation

Saponin perfusion (50 mg/l, for 10 min) successfully removed the endothelium of the mesenteric vascular bed because the endothelium-dependent vasodilatation induced by acetylcholine (0.01 μg) was almost abolished after saponin treatment. The vasodilatory response to urocortin was also significantly reduced to 8.75 ± 5.5% after saponin perfusion ($p < 0.05$, Figs. 1B and 2).

Effect of K^+ channel blockade on the urocortin-induced vasodilatation

Urocortin (0.2 nM)-induced vasodilatation was markedly decreased from 57.2 ± 2.9% (control) to 11.8 ± 2.5% in the presence of elevated (40 mM) extracellular KCl ($p < 0.05$). Likewise, charydotoxin (1 nM) reduced the relaxations to 6.0 ± 4.9% ($p < 0.05$). However, neither apamin (10 nM) nor glibenclamide (1 μM) affected the relaxation induced by urocortin. Urocortin-induced vasodilatations were 61.0 ± 6.0% and 65.8 ± 2.1 in the presence of apamin or glibenclamide respectively (Figs. 1D and 3).

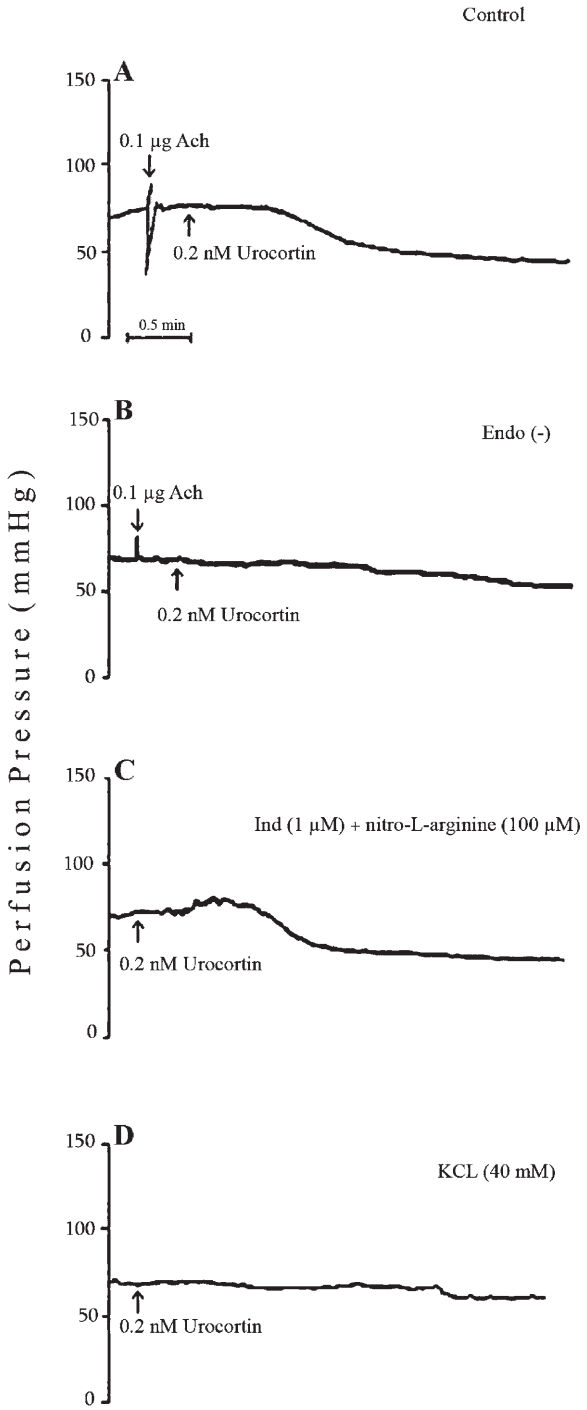


Fig. 1. Tracings showing the relaxant effects of 0.2 nM urocortin in the isolated perfused rat mesenteric vascular bed: control (A), in the absence of endothelium (B), in the presence of 1 μM indomethacin + 100 μM nitro-L-arginine (C), and in the presence of 40 mM KCl (D).

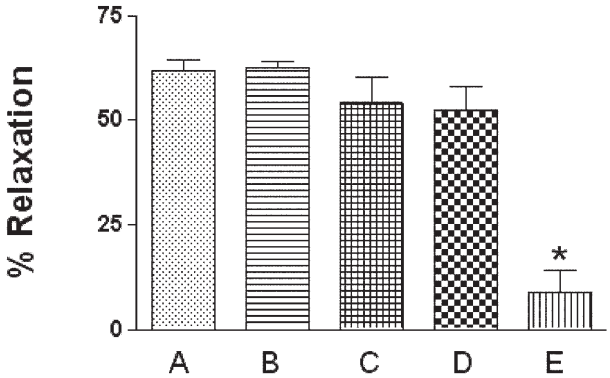


Fig. 2. Urocortin-induced vasodilatation in the isolated perfused rat mesenteric bed; the pressure was elevated by 2-4 μM phenylephrine: control (A), in the presence of 1 μM indomethacin (B), 1 μM indomethacin + 100 μM N^{ω}-nitro-L-arginine (nitro-L-arginine) (C), 1 μM indomethacin+100 μM nitro-L-arginine + 1 μM 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one (ODQ) (D), and in the absence of endothelium (E). Relaxation is expressed as percentage of phenylephrine-induced contraction (mean ± S.E.M.) *$p < 0.05$, significantly different from control (n = 4-12).

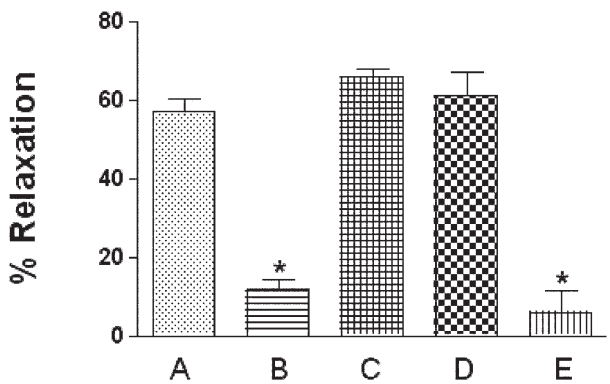


Fig. 3. Urocortin-induced vasodilatation in the isolated perfused rat mesenteric bed, the perfusion pressure of which was elevated by 2-4 μM phenylephrine: control (A), in the presence of 40 mM KCl (B), 1 μM glibenclamide (C), 10 nM apamin (D) or 1 nM charybdotoxin (E). Relaxation is expressed as percentage of phenylephrine-induced contraction (mean ± S.E.M.) *$p < 0.05$, significantly different from control (n = 4-8).

Effect of urocortin on the resting membrane potential in the mesenteric artery

The resting membrane potential of the smooth muscle cells of superior mesenteric artery was –53.6 ± 0.5 mV. The same amount of urocortin concentration (0.2 nM) used to induce relaxation in the isolated perfused mesenteric bed in this study produced hyperpolarization in the membrane potential by –11.4 ± 0.9 mV (Fig. 4).

Effects of nitro-L-arginine and indomethacin on the urocortin-induced hyperpolarization

The treatment of the mesenteric artery by nitro-L-arginine (100 μM) alone or by the combination with indomethacin (1 μM) depolarized smooth muscle cells to –49.4 ± 0.4 and –48.8 ± 0.4 mV, respectively. In the presence of nitro-L-arginine alone or nitro-L-arginine plus indomethacin, urocortin induced hyperpolarizations by 11.8 ± 0.6 mV and 11.4 ± 0.9 mV, respectively, which were not statistically different from control.

Effects of removal of endothelium on the urocortin-induced hyperpolarization

Urocortin (0.2 nM)-induced hyperpolarization was abolished by the removal of endothelium, while resting membran potential was not affected (Table 1). Effectiveness of saponin was checked by the substantial inhibition of acetylcholine-evoked hyperpolarization. Resting membran

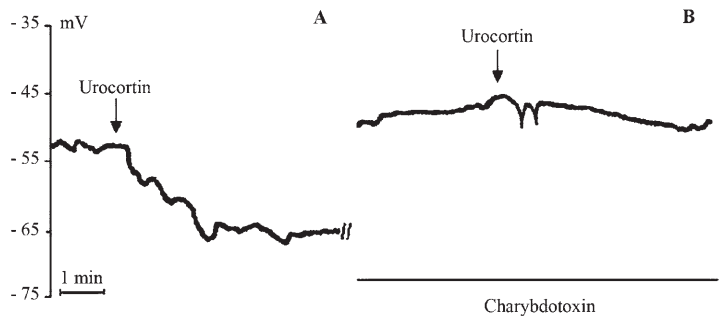


Fig. 4. A tracing showing the effects of 0.2 nM urocortin on the membrane potential of the main mesenteric artery in the absence and presence of 1 nM charybdotoxin, a large conductance Ca^{2+}-activated K^+ channels blocker.

TABLE 1. Effects of putative inhibitors and endothelium removal on urocortin-induced hyperpolarization in smooth muscle of mesenteric arteries.

	In the absence of blocker (mV)		In the presence of blocker (mV)	
	RMP	Uro-induced hyperpolarization	RMP	Uro-induced hyperpolarization
Nitro-L-arginine (100 μM)	–53.6 ± 0.5 (n = 6)	11.4 ± 0.9 (n = 6)	–49.4 ± 0.4* (n = 6)	11.8 ± 0.6 (n = 6)
Nitro-L-arginine (100 μM) + indomethasine (1 μM)	–52.2 ± 0.5 (n = 8)	12.6 ± 1.3 (n = 8)	–48.8 ± 0.4* (n = 7)	11.4 ± 0.9 (n = 7)
Glibenclamide (1 μM)	–52.8 ± 0.6 (n = 6)	11.8 ± 0.8 (n = 6)	–49.6 ± 0.5* (n = 6)	9.8 ± 0.8 (n = 6)
Endothelium Removal	–54.7 ± 1.8 (n = 8)	11.4 ± 1.3 (n = 5)	–51.8 ± 1.1 (n = 8)	0.2 ± 1.0+ (n = 5)
Charybdotoxin (1 nM)	–52.8 ± 0.4 (n = 5)	12.2 ± 0.4 (n = 5)	–45.0 ± 0.7* (n = 5)	2.8 ± 1.4+ (n = 5)
Apamin (10 nM)	–52.4 ± 0.8 (n = 5)	13.0 ± 1.0 (n = 5)	–50.8 ± 0.4 (n = 5)	7.0 ± 0.3+ (n = 5)

Data are expressed as mean ± S.E.M.

*$p < 0.05$ vs resting mebrane potential (RMP).

+$p < 0.05$ vs urocortin-induced hyperpolarization in before.

potential was −54.7 ± 1.8 mV (n = 8). Acetylcholine significantly reduced the membran potential to −63.4 ± 0.8 mV ($p < 0.05$, n = 8) in intact vessels, whereas it was −51.8 ± 1.1 mV (n = 8) after saponin treatment.

Effects of selective K^+ channel blockade on the hyperpolarization induced by urocortin.

Glibenclamide (1 μM), an ATP-dependent K^+ channel inhibitor, did not significantly alter the resting membrane potential and the hyperpolarization induced by urocortin (0.2 nM) (Table 1). Charybdotoxin (1 nM), a large conductance Ca^{2+}-activated K^+ channel blocker, depolarized the cell from −52.8 ± 0.4 mV to −45 ± 0.7 mV ($p < 0.05$). It also decreased the hyperpolarization induced by urocortin from 12.2 ± 0.4 mV to 2.8 ± 1.4 mV ($p < 0.05$) (Table 1, Fig. 4). Apamin (10 nM), a small conductance Ca^{2+}-activated K^+ channel blocker, also decreased the hyperpolarization by urocortin from 13.0 ± 1.0 mV to 7 ± 0.3 mV, while it had no effect on the resting membrane potential (−50.8 ± 0.4 mV, Table 1).

Discussion

Urocortin is an endogenous ligand for corticotropin CRF receptors, binding to its subtype corticotropin CRF_2 receptor with high affinity (Heldwein et al. 1996; Nishikimi et al. 2000), and leads to an increases in intracellular Ca^{2+} levels (Kiang 1997). Activation of these receptors by urocortin results in the relaxation of vascular tissues by endothelium-dependent and -independent pathways. Indeed, the role of endothelium in vasodilatory response to urocortin in the rat uterine artery (Jain et al. 1999), and coronary arteries was established (Huang et al. 2003). Consistently, in the present study, removal of endothelium caused a marked attenuation in the urocortin induced-relaxation in the isolated perfused rat mesenteric beds. Hyperpolarization observed in the mesenteric strips was also abolished. This shows that the endothelium may play a significant role in urocortin-elicited hyperpolarization and vascular relaxation.

It was reported that urocortin-induced coronary vasodilatation was reduced by cyclooxygenase (COX) inhibition by indomethacin, suggesting that prostacyclin may be involved in the vasodilatory effect of urocortin (Terui et al. 2001). On the contrary, Sanz et al. (2002) reported that urocortin-induced relaxation was potentiated by the presence of COX inhibition with meclofenamate and suggested that this effect may result from inhibition of vasoconstrictor prostanoids in the human saphenous veins. In this study, vasodilatory responses induced by urocortin were not affected by the presence of COX inhibition with indomethacin, implying vasodilatory prostacycline is not involved in urocortin-induced relaxation in the mesenteric arteries.

Nitric oxide (NO) is synthesized in the endothelium from L-arginine by NO synthase (NOS), and NO activates soluble guanylate cyclase of vascular smooth muscle and increases intracellular cyclic GMP levels, resulting in the relaxation of vascular smooth muscle (Moncada et al. 1991). It is generally accepted that NO plays little role in response to endothelium-dependent vasodilators in resistance arteries (Hwa et al. 1994; Tomioka et al. 1999). Indeed, in this study urocortin-induced hyperpolarization and relaxation were not affected by the presence of N^G-nitro-L-arginine (nitro-L-arginine), which inhibits NOS activity. Similar findings were also observed by the others, who reported that urocortin-induced relaxation was not affected by the inhibition of NOS in the coronary arteries (Terui et al. 2001), human saphenous veins (Sanz et al. 2002), in male rat renal arteries (Sanz et al. 2003), and in the rat pulmonary arteries (Chan et al. 2004). Furthermore, in this study vasodilatory response to urocortin was not affected in the presence of ODQ, which is an inhibitor of soluble guanylate cyclase (Tseng et al. 2000). These findings suggest that hyperpolarization and relaxation produced by urocortin was not mediated by NO. In contrast, inhibition of NOS partly reduced the relaxation induced by urocortin in the pregnant rat uterine artery (Jain et al. 1999), in the rat coronary artery (Huang et al. 2003) and in the female rat renal artery (Sanz et al. 2003). Similarly, Chen et al. (2005) reported that urocortin-induced relaxation in the human internal mammary artery primarily involves NO, which stimu-

lates Ca^{2} -activated K^{+} channels via cyclic GMP (Chen et al. 2005). These findings suggest that the role of endothelium, in response to urocortin, may vary depending on differences in vascular beds, gender, species and other factors, such as concentrations used. From the present study, it is clear that urocortin could hyperpolarize the smooth muscle of mesenteric arteries endothelium-dependently. The urocortin-induced relaxation may be mediated by the activation of K^{+} channels, since it was abolished by raised extracellular K^{+}. It is known that when the extracellular K^{+} was raised to 25-40 mM (Chen et al. 1988; Adeagbo and Triggle 1993) or blocking of specific K^{+} channels, hyperpolarization of smooth muscle was abolished. In vascular smooth muscles, hyperpolarization can be induced by cromakalim and inhibited by glibenclamide (Quast and Cook 1988), which is mediated by ATP-dependent K^{+} channels. As the activation of glibenclamide-sensitive K^{+} channels also mediates smooth muscle hyperpolarization to NO (Garland and McPherson 1992) and other endothelium-derived factors, there may be a possibility that hyperpolarization and relaxation induced by urocortin is involved in this type of K^{+} channel. However, glibenclamide did not affect hyperpolarization or vasodilatation induced by urocortin in mesenteries, suggesting that ATP-dependent K^{+} channels are not involved. Similar findings were also observed in cerebral (Schilling et al. 1998) and uterine vessels (Jain et al. 1999). The fact that glibenclamide had no effects on the urocortin-induced hyperpolarization and vasodilatation points out that this response may be mediated by other potassium channels, for instance Ca^{2+} activated K^{+} channels. Indeed, urocortin-induced hyperpolarization and relaxation were markedly attenuated by the presence of charybdotoxin, a selective blocker of large conductance Ca^{2+}-activated K^{+} channels. Our findings in mesenteric arteries support previous studies carried out in rat basilar arteries (Schilling et al. 1998), human saphenous veins (Sanz et al. 2002) and rat renal arteries (Sanz et al. 2003), in which vasorelaxations induced by urocortin were affected by blockade of Ca^{2+}-activated K^{+} channels. Interestingly, apamin, a selective blocker of small conductance Ca^{2+}-activated K^{+} channels, significantly attenuated the hyperpolarization induced by urocortin, while it did not affect the vasodilatation. The discrepancy may have resulted from different experimental protocols in the electrophysiological and perfusion experiments in this study. Simultaneous detection of membrane potential and concomitant vascular tone may be needed to clarify this paradox.

In conclusion, the results of this study support the finding that urocortin, an endogenous ligand for corticotropin CRF receptors, elicits a slow-developing vasodilatation in vasculatures. It is also concluded that urocortin may cause endothelium-dependent hyperpolarization and relaxation through the activation of charybdotoxin-sensitive Ca^{2+}-activated K^{+} channels in rat mesenteric arteries.

Acknowledgments

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