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Characterization of the relaxant action of urocortin, a new peptide related to corticotropin-releasing factor in the rat isolated basilar artery

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1 In addition to its well established neuroendocrine and neurotransmitter effects, corticotropin releasing factor (CRF) exerts a potent vasorelaxant action. Recently, a CRF-related peptide, urocortin, has been identified in the mammalian brain. In the present study, the cerebral vasomotor action of this peptide and the mechanism underlying its relaxant effect are characterized.

2 Ring segments obtained from the rat basilar artery were used for measurement of isometric force. The relaxant action of urocortin, CRF and sauvagine was studied in segments with a functionally intact endothelium.

3 In segments precontracted with prostaglandin $F_{2\alpha}$, urocortin, CRF and sauvagine induced concentration-related relaxation. The order of potency was as follows ($pD_2 \pm$ s.e.m. given in brackets): urocortin (9.32 ± 0.07) > sauvagine (9.08 ± 0.08) > CRF (7.50 ± 0.07). Complete relaxation was achieved with each agonist. Relaxation was not affected by removal of the endothelium but was markedly attenuated in segments precontracted with 50 mM K^+ Krebs solution. The relaxant effect of urocortin was inhibited by astressin in an apparently competitive manner. A pA_2 value of 7.52 was estimated for astressin. Inhibition of urocortin-induced relaxation was also observed in the presence of the adenylate cyclase inhibitor SQ22536 (pD_2 in the presence of 300 μM SQ22536, 9.36 ± 0.05) and the K^+ channel blockers tetraethylammonium (10 mM; pD_2, 8.65 ± 0.07), iberiotoxin (100 nM; pD_2, 8.88 ± 0.08) and apamin (10 nM; pD_2, 8.94 ± 0.07). However, the inhibitory actions of SQ22536 and apamin or iberiotoxin were not additive.

4 The results suggest that urocortin induces relaxation of cerebral arteries by activating CRF-R_2 receptors present in the vascular wall. Relaxation appears to be mediated by adenylate cyclase stimulation and activation of Ca^{2+}-dependent K^+ channels.

Keywords: CRF-related peptides; urocortin; adenylate cyclase; Ca^{2+}-dependent K^+ channels; cerebral artery

Introduction

Urocortin is a 40 amino acid peptide which was originally identified in rat brain tissue (Vaughan *et al*., 1995). Recently its human counterpart has been cloned successfully showing a 95% identity of its amino acid sequence with the rat peptide (Donaldson *et al*., 1996). Urocortin displays a high degree of structural homology to human corticotropin-releasing factor (CRF) as do the non-mammalian peptides urotensin I and sauvagine, originally identified in fish neurosecretory cells and frog skin, respectively (for review see Dieterich *et al*., 1997). These peptides may thus be grouped together and referred to as CRF-related peptides. In the mammalian brain the distribution of CRF differs from that found for urocortin, the latter being most prominent in the Edinger-Westphal nucleus and the lateral superior olive (Vaughan *et al*., 1995) although there may be some overlap as well (Wong *et al*., 1996).

In contrast to the distinct patterns of distribution of CRF and urocortin, the receptors mediating the actions of these compounds are widespread in the brain and in the periphery. So far two different types of receptors, CRF-R_1 and CRF-R_2 receptors, have been identified (Chang *et al*., 1993; Kishimoto *et al*., 1995; Lovenberg *et al*., 1995b; Perrin *et al*., 1995; Potter *et al*., 1994). In the brain the CRF-R_1 receptor is mainly expressed on pituitary cells and on neurons located in neocortical, cerebellar, and sensory relay structures while the CRF-R_2 receptor message is found predominantly in subcortical structures, in the cerebellum, in the choroid plexus, and in arteries and arterioles (Chalmers *et al*., 1995). Based on data obtained in rats the CRF-R_2 receptor may be further divided into two splice variants, CRF-$R_{2\alpha}$ and CRF-$R_{2\beta}$. While the CRF-$R_{2\alpha}$ receptor appears to be expressed in neurons and the choroid plexus (Dieterich *et al*., 1997), high densities of messenger RNA encoding for the CRF-$R_{2\beta}$ receptors have been found in non-neural tissues such as the heart and skeletal muscle and also in cerebral blood vessels (Lovenberg *et al*., 1995a).

In the central nervous system, CRF is involved in endocrine regulation and information processing (for review see Owens & Nemeroff, 1991). The presence of CRF receptors in peripheral tissues, however, indicates that CRF and related peptides may serve a variety of biological actions in addition to their well-known neuroendocrine and neuromodulator functions. Even in the first description of CRF its hypotensive effects was noted (Vale *et al*., 1981). Upon systemic application CRF and related peptides profoundly affect cardiovascular and respiratory functions as indicated by hyperventilation (Kübler *et al*., 1994) and hypotension in humans (Ehrenreich *et al*., 1997a,b; Kübler *et al*., 1994) and experimental animals (Lei *et al*., 1993; Lenz *et al*., 1985; MacCannell *et al*., 1982; Richter & Mulvany,

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1995; Vaughan *et al*., 1995). Hypotension was found to be accompanied by a facial flush in humans (Ehrenreich *et al*., 1997a,b; Kübler *et al*., 1994) and a dramatic increase of flow through the mesenteric artery in anaesthetized dogs (Lenz *et al*., 1985; MacCannell *et al*., 1982, 1984). Whether or not CRF receptor activation plays a role in regulating vascular tone under physiological or pathophysiological conditions is unclear as yet. However, in a previous study we found a markedly increased hypotensive reaction in early abstinent alcoholics following a bolus injection of human CRF (150 μg) (Ehrenreich *et al*., 1997a,b).

Although the cardiovascular responses induced by CRF or related peptides may be mediated, at least in part, by activation of centrally located receptors as part of an arousal reaction (Lehnert *et al*., 1993), a direct vascular action of these compounds is suggested by the presence of CRF receptor message in/around arteries (Chalmers *et al*., 1995; Lovenberg *et al*., 1995a). In fact, CRF and related peptides have been shown to induce relaxation of blood vessels studied *in vitro* (Lei *et al*., 1993; Rohde *et al*., 1996). Such experiments have mostly been performed in peripheral arteries with the exception of only one study that included cerebral arteries, but without addressing the underlying mechanisms (Lei *et al*., 1993). Recently, Yatsushiro *et al*. (1997c) using the perivascular microapplication technique developed by Wahl *et al*. (1970) found a dilating action of CRF and urocortin in cat pial arteries *in vivo*. The studies performed in cerebral blood vessels so far indicate that CRF and related peptides markedly affect cerebrovascular tone. The present study was, therefore, designed to characterize the cerebral vasomotor effects of urocortin and the underlying mechanisms using ring segments of the isolated rat basilar artery for measurement of isometric tension according to previously published studies (Feger *et al*., 1994; Ksoll *et al*., 1991; Schilling *et al*., 1995).

Methods

Experimental setup

Male Sprague-Dawley rats (270–450 g) were anaesthetized with ether and killed by bleeding from the carotid arteries. The brain was removed and immersed in ice-cold Krebs buffer (for composition, see below). The basilar artery was meticulously removed under an operation microscope and cut in four ring segments. These segments were mounted on two metal prongs of 70 μm diameter in a 5 ml organ bath for measurement of isometric force using Q11 transducers and MT233 amplifiers (Hottinger Baldwin, Frankfurt, Germany) coupled to a chart recorder (7005 Linseis, Selb, Germany) as previously described (Schilling *et al*., 1996). In some segments the endothelium was mechanically removed by repeated turning around the metal prongs or rubbing the intima with a human eyelash.

After mounting, all segments underwent a 60 min equilibration period during which the bath temperature was gradually increased to 37°C and resting tension adjusted to 2.5–3.5 mN. After equilibration, the bath solution was exchanged for a 124 mM K^+-Krebs solution (NaCl replaced by KCl) to check the contractile capacity of the segments (minimum tension required for inclusion, 2.5 mN). After a 45 min wash period endothelial function was tested by cumulative application of acetylcholine (10 nM–100 μM) following precontraction with 1 μM 5-hydroxytryptamine (5-HT). According to our previously used classification (Schilling *et al*., 1996), a functionally intact endothelium was assumed in segments displaying relaxation >30% of precontraction (E^+ segments) whereas a decrease of <15% of 5-HT-induced tone was taken to indicate successful removal of the endothelium by rubbing (E^- segments).

The relaxant actions of urocortin, sauvagine, and CRF were studied by cumulative application to segments precontracted with prostaglandin (PG) $F_{2\alpha}$ (3 μM) or by incubation in an iso-osmotic 50 mM K^+ Krebs solution (urocortin only). Since there was a significant decrease in the relaxant effect of urocortin upon repeated application, only one concentration-effect curve (CEC) was performed in each segment. The effects of the following antagonists and inhibitors on urocortin-induced relaxation were studied: astressin (10 nM–1 μM), a CRF receptor antagonist (Gulyas *et al*., 1995), SQ22536 (300 μM and 1 mM), an adenylate cyclase inhibitor (Harris *et al*., 1979), and the K^+ channel blockers glibenclamide (1 μM), tetraethylammonium (TEA, 100 μM or 10 mM), apamin (10 nM), and iberiotoxin (100 nM). In each case, the segments were preincubated with one of these inhibitors/antagonists for at least 30 min before precontraction with $PGF_{2\alpha}$ and application of urocortin.

Materials

Composition of the Krebs buffer was (mM): NaCl 119; KCl 3.0; NaH_2PO_4 1.2; $CaCl_2 \times 2H_2O$ 1.5; $MgCl_2 \times 6H_2O$ 1.2; $NaHCO_3$ 20; glucose 10. The bath solution was continuously bubbled with a humidified gas mixture (93% O_2/7% CO_2) to obtain a pH range around 7.35 at 37°C. Urocortin was purchased from Peninsula (Merseyside, U.K.); CRF, sauvagine, astressin, and iberiotoxin from Bachem (Heidelberg, Germany); SQ22536 from Calbiochem (Bad Soden, Germany); acetylcholine, glibenclamide and TEA from Sigma (Deisenhofen, Germany); apamin from RBI (Köln, Germany) and $PGF_{2\alpha}$ from Serva (Heidelberg, Germany). All other reagents were obtained from Merck (Darmstadt, Germany). Test solutions were prepared freshly on each day of the experiment and kept on ice throughout. Stock solutions were made up in distilled water, ethanol (glibenclamide), or degassed distilled water containing 1% acetic acid (urocortin, sauvagine and CRF).

Statistical analysis

Relaxation was calculated in percentage of decrease in precontraction. For each individual CEC, the maximum relaxation (E_{max}) was determined and the pD_2 value calculated as the $-\log_{10}EC_{50}$ (i.e. that concentration at which the half maximal effect occurred). For statistical analysis one-way analysis of variance (ANOVA procedure) and posthoc LSD (least significant difference) tests for multiple comparisons of means were performed using the SPSS package (version 7.5.1). A P value of <0.05 was considered significant. All values given in the text and figures are means ± s.e.mean with n indicating the number of observations.

Results

In ring segments precontracted with $PGF_{2\alpha}$, cumulative application of urocortin, sauvagine and CRF induced concentration-related relaxation (Figure 1). The order of agonist potency was urocortin > sauvagine >> CRF (Table 1). Maximum relaxation was >100% of precontraction with each of these agonists (Table 1). Therefore, the effect of urocortin was also investigated in segments under resting

tension (i.e. without any pharmacological precontraction). In these experiments a concentration-related decrease of resting tension was again found (Figure 2a). The pD_2 value amounted to 9.47 ± 0.10 ($n=11$), very similar to that found in $PGF_{2\alpha}$-precontracted segments (level of precontraction: 3.1 ± 0.3 mN). Maximum decrease of resting tension induced by urocortin was 1.1 ± 0.2 mN.

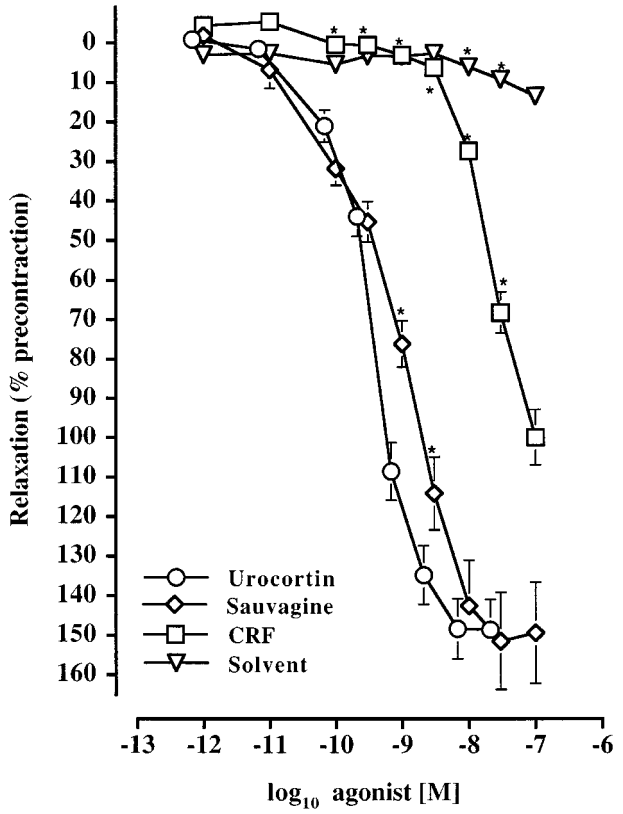


Figure 1 Concentration-related relaxation induced by urocortin (pooled control, $n=27$), sauvagine ($n=16$), and corticotropin-releasing factor (CRF, $n=18$) of rat basilar artery ring segments precontracted with prostaglandin (PG) $F_{2\alpha}$ (3 μM). Also included is the result obtained in time-matched solvent control experiments (see Methods section, $n=8$) Given are means $\pm$ s.e.mean. $^*P<0.05$ vs pooled controls.

Table 1 Comparison of the relaxant actions of urocortin, sauvagine and corticotropin-releasing factor (CRF) on isolated rat basilar artery ring segments

		pD_2	E_{max}
CRF ($n=18$)	E^+	7.50 ± 0.07*	121.5 ± 6.1
Sauvagine ($n=16$)	E^+	9.08 ± 0.08*	151.5 ± 12.3
Urocortin pooled control ($n=27$)	E^+	9.32 ± 0.07	149.7 ± 7.7
Urocortin ($n=10$)	E^-	9.28 ± 0.09	158.6 ± 15.2
Urocortin (50 mM K^+) ($n=9$)	E^+	8.04 ± 0.08*	35.5 ± 4.5*

Ring segments were either equipped with a functionally intact endothelium (E^+) or had the endothelium mechanically removed (E^-). Precontraction was achieved with $PGF_{2\alpha}$ (3 μM) or 50 mM K^+ Krebs solution. Given are the pD_2 and E_{max} values (means $\pm$ s.e.mean) with the number of observations indicated in brackets. $^*P<0.05$ vs pooled control for urocortin ($n=27$). Data were pooled from the control experiments performed in three subsets of experiments shown in Figure 3, Figure 4 and Figures 2 and 5, respectively.

In precontracted arteries the relaxant action of urocortin was independent of the presence of a functionally intact endothelium. In segments in which the endothelial cells were mechanically removed, urocortin still induced concentration-related relaxation after $PGF_{2\alpha}$ precontraction (level of precontraction: 2.5 ± 0.2 mN) (Figure 2b, Table 1). However, in vessel segments precontracted by incubation in a 50 mM K^+ Krebs solution yielding 4.0 ± 0.5 mN tone, relaxation by urocortin was largely attenuated ($31.7 \pm 4.1\%$ relaxation with 30 nM urocortin) (Table 1, Figure 2b).

A significant decrease in the potency of urocortin was observed when it was applied twice on the same segment. The pD_2 values obtained in these segments were 9.43 ± 0.09 (first application) and 8.29 ± 0.05 (second application, $n=4$, $P<0.05$ vs first run), respectively. Therefore, only one CEC was constructed in each segment.

In the presence of astressin, a structurally constrained CRF analogue, the relaxant action of urocortin was inhibited in an apparently competitive manner. There was a rightwards shift of the CEC to urocortin with increasing concentrations of astressin (Figure 3). This shift calculated on the 75% relaxation level (which approximates half maximum relaxation under control conditions) was 3.5 fold with 100 nM astressin and 26.9 fold with 1 μM astressin, respectively. In additional experiments 100 nM astressin induced a 3.2 fold shift of the CEC for sauvagine (not shown). Based on the shift obtained with 100 nM astressin, a pA_2 value of 7.52 was estimated from the averaged CECs using the methodology of van Rossum (1963).

It has been shown that application of CRF and related peptides results in activation of adenylate cyclase and accumulation of cyclic AMP (cAMP) in several assay systems (Chen *et al*., 1986; Kishimoto *et al*., 1995; Perrin *et al*., 1995; Vaughan *et al*., 1995). We have, therefore, investigated the role of cyclic AMP in mediating urocortin-induced cerebroarterial relaxation. In segments in which the stimulation of adenylate cyclase was inhibited by preincubation with SQ22536 (300 μM or 1 mM) the relaxant action of urocortin was decreased in concentrations $\geqslant 1$ nM (Figure 4). The specificity of SQ22536 was proven in some segments by its ability to inhibit forskolin-induced relaxation (not shown).

The markedly diminished effect of urocortin in segments precontracted in a 50 mM K^+ Krebs solution raised the possibility that membrane hyperpolarization might be involved in the relaxant action of urocortin. Some segments were, therefore, studied after preincubation with different K^+ channel inhibitors. Relaxation induced by urocortin was neither affected by glibenclamide (1 μM, results not shown) nor by 100 μM TEA but was diminished in the presence of 10 mM TEA (Figure 5a) yielding a pD_2 value of 8.65 ± 0.07 ($P<0.05$ vs control). Statistically significant inhibition was also obtained in the presence of iberiotoxin (100 nM) and apamin (10 nM) (Figure 5b) yielding pD_2 values for urocortin of 8.88 ± 0.08 ($P<0.05$ vs control) and 8.94 ± 0.07 ($P<0.05$ vs control), respectively. In these experiments levels of $PGF_{2\alpha}$-induced precontraction were 3.8 ± 0.5 mN and 3.1 ± 0.6 mN, respectively. Apamin and iberiotoxin selectively inhibit Ca^{2+} dependent $K^+(K_{Ca}{}^{2+})$ channels with large and small conductance, but there was no additive effect seen in experiments in which both blockers were present in the bath (not shown). Furthermore, when segments were preincubated simultaneously with either apamin (10 nM) or iberiotoxin (100 nM) and SQ22536 (300 μM) there was no increase of inhibition compared with SQ22536 alone (Figure 6).

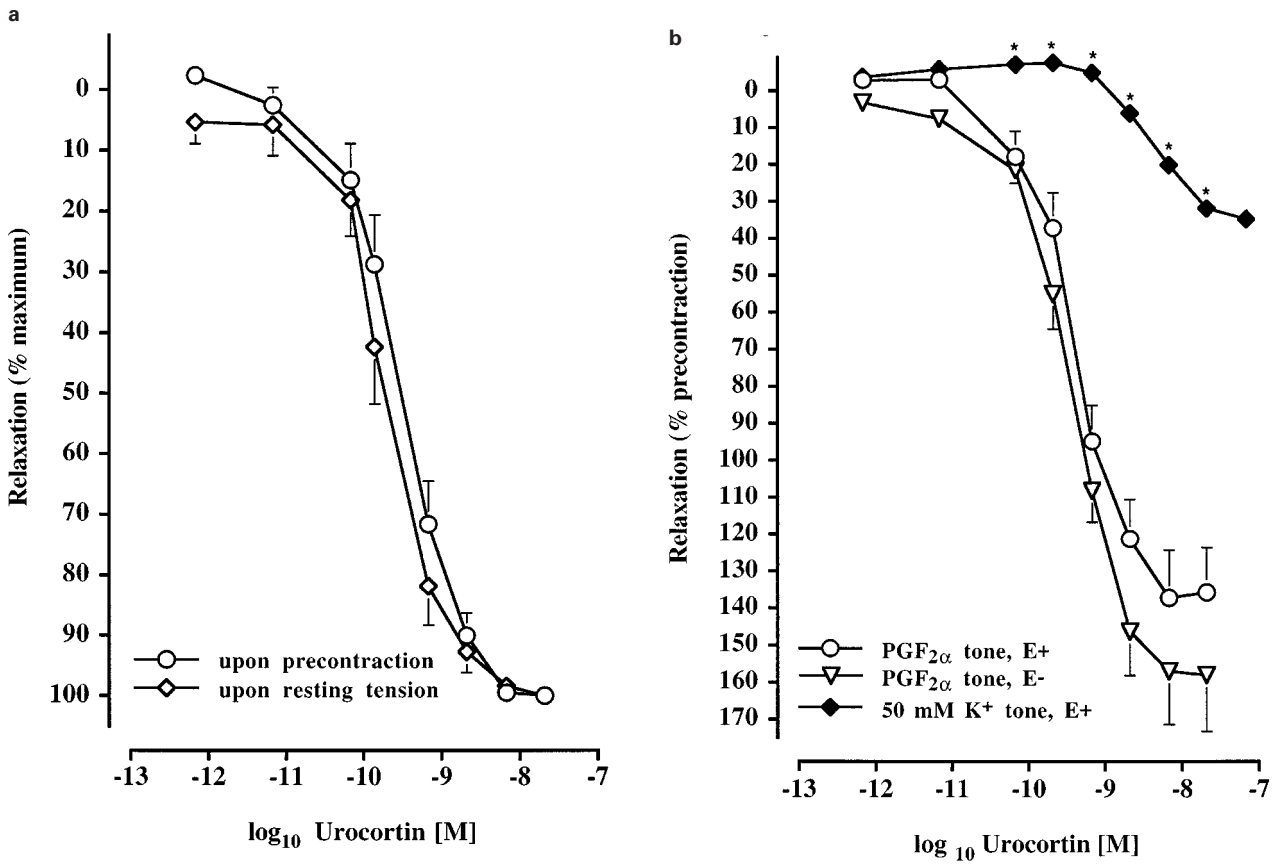


Figure 2 Comparison of concentration-related relaxation induced by urocortin in segments under resting tension or following precontraction by prostaglandin (PG) $F_{2\alpha}$ (3 μM) (a) and in segments precontracted by 50 mM K^+ Krebs or $PGF_{2\alpha}$ (3 μM) in segments with the endothelium intact (E^+) or mechanically removed (E^-) (b). Given are means $\pm$ s.e.mean of 9–10 observations. *$P<0.05$ vs relaxation induced by urocortin in that respective subset of experiments.

Discussion

The present study is the first one to characterize the vasomotor action of urocortin, a newly discovered member of the CRF-related peptide family in the cerebrovasculature. The main findings are as follows: (i) urocortin is significantly more potent than CRF in inducing cerebroarterial relaxation, (ii) the relaxant effect of urocortin is inhibited by astressin in an apparently competitive manner, and (iii) part of the relaxant action of urocortin may be induced by activation of K^+ channels, probably due to adenylate cyclase stimulation.

The order of agonist potency calculated on the EC_{50} level found in the present study was urocortin > sauvagine >> CRF. This result contrasts with findings of Yatsushiro *et al.* (1997c) who observed no differences in the potency of these agonists to induce dilatation of feline pial arteries *in vivo*. The reason for this contrasting result is not clear but may be related to the species, the position of the vessels in the cerebroarterial tree, or inherent to the methods used for constructing CECs (e.g. a truly cumulative application cannot be achieved by microapplication *in vivo*). The higher potency of sauvagine as compared to CRF in the rat basilar artery at least suggests activation of CRF-R_2 receptors (Chalmers *et al.*, 1996; Dieterich *et al.*, 1997; Rohde *et al.*, 1996) underlying relaxation induced by urocortin. This assumption is strengthened by several lines of evidence: firstly by the fact that urocortin, which is considered the naturally occurring CRF-R_2 receptor agonist (Vaughan *et al.*, 1995) was even more potent than sauvagine, and, secondly, by the presence of CRF-R_2 messenger RNA demonstrated in and around cerebral blood vessels (Chalmers *et al.*, 1995; Lovenberg *et al.*, 1995a; Yatsushiro *et al.*, 1997a). Further support is provided by the observation that the dilatation of feline pial arteries induced by topical application of CRF could not be blocked by a selective CRF-R_1 receptor antagonist (Yatsushiro *et al.*, 1997a).

In fact, molecular biology techniques revealed two splice variants of the CRF-R_2 receptor in rats, CRF-$R_{2\alpha}$ and CRF-$R_{2\beta}$, the latter obviously being expressed in non-neural tissues including cerebral arteries (Lovenberg *et al.*, 1995a). Whether or not activation of the CRF-$R_{2\beta}$ subtype is specifically indicated by the order of agonist potency found in the present study remains to be established. The results obtained with astressin, a conformationally restrained analogue of CRF do not provide any further information regarding the receptor subtype activated. Astressin is a non selective receptor antagonist (Gulyas *et al.*, 1995) thus resembling α-helical CRF_{9-41}, which has been extensively used in previous studies to inhibit the effects of CRF and related peptides. Astressin has been described to be the most potent CRF receptor antagonist available so far – at least in a mainly CRF-R_1 containing pituitary cell culture assay (Gulyas *et al.*, 1995). However, astressin also inhibits relaxation induced by urocortin and sauvagine to a similar degree suggesting the presence of one receptor subtype in the rat basilar artery wall, probably of the CRF-R_2 subtype as discussed earlier (see above). Inhibition occurred in a manner compatible with competitive antagonism as suggested by the apparently parallel rightwards shift of the CEC for urocortin in the presence of astressin (Figure 3).

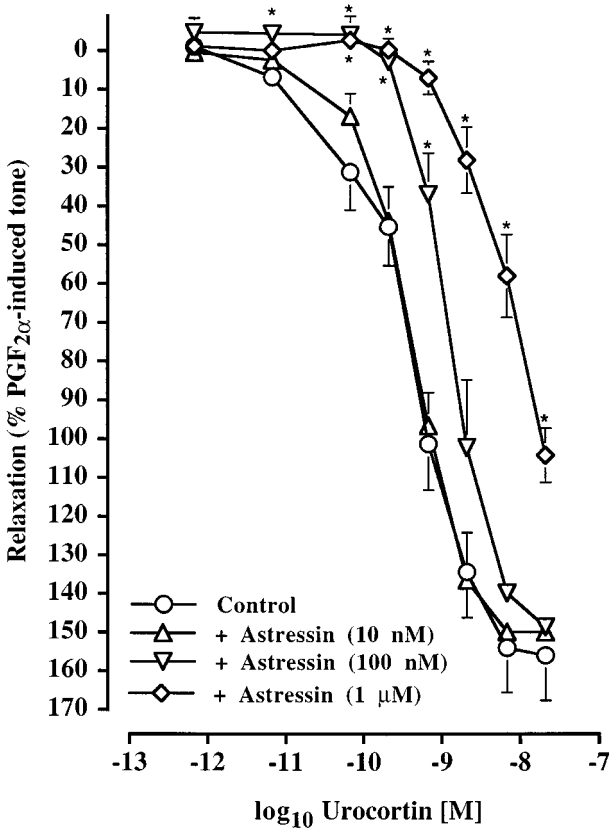


Figure 3 Inhibition by astressin of urocortin-induced relaxation of the isolated rat basilar artery precontracted with prostaglandin (PG) $F_{2\alpha}$ (3 μM). There is an apparently parallel rightwards shift of the concentration-effect curve for urocortin related to the concentration of astressin. Given are means ± s.e.mean of 6–14 observations. *$P<0.05$ vs relaxation induced by urocortin in that respective subset of experiments.

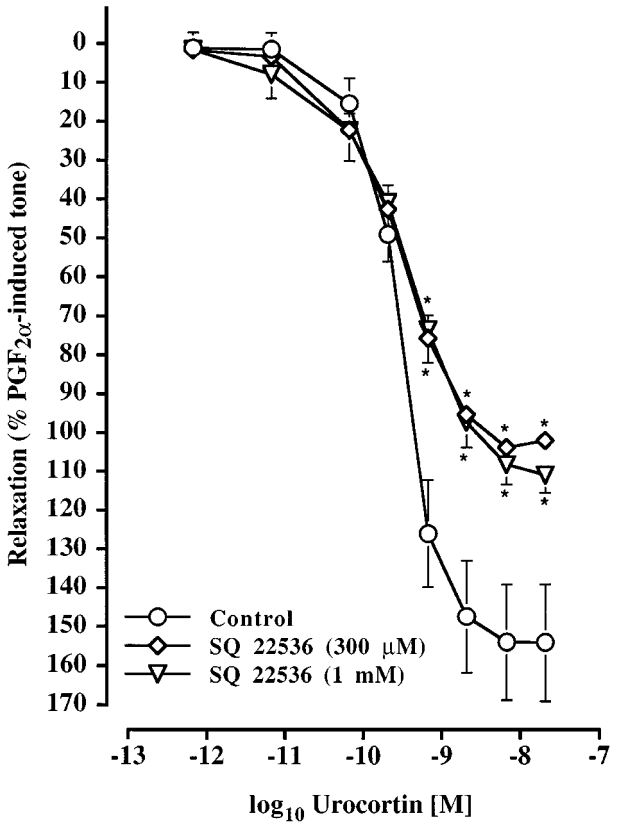
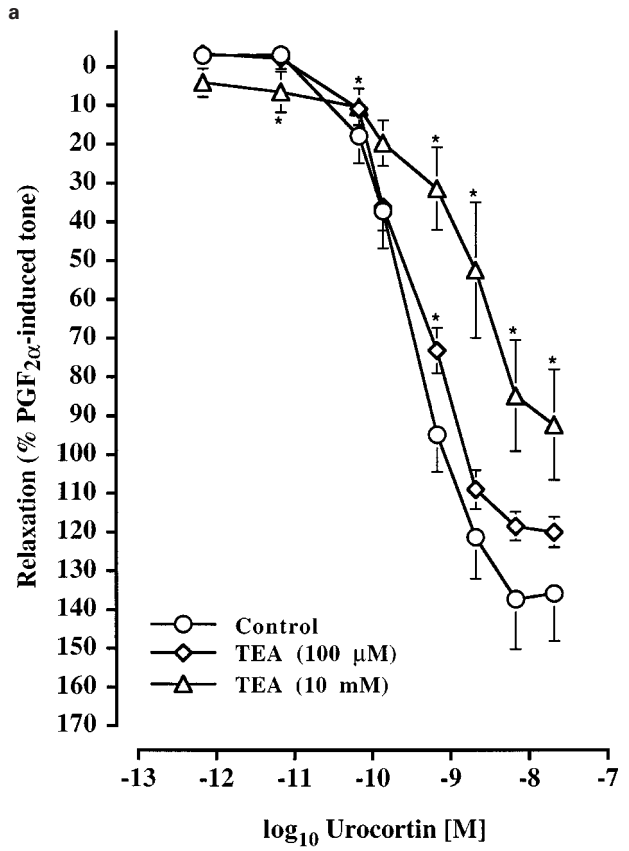


Figure 4 Inhibition by SQ22536 of urocortin-induced relaxation of the isolated rat basilar artery precontracted by prostaglandin (PG) $F_{2\alpha}$ (3 μM). There is no difference in the inhibitory effect of 300 μM and 1 mM SQ22536. Given are means ± s.e.mean of 6–11 observations. *$P<0.05$ vs relaxation induced by urocortin in that respective subset of experiments.

When applied twice to the same segment the potency of urocortin was markedly reduced. This observation suggests partial desensitization of the CRF-R_2 receptor by urocortin – an effect which has previously been reported for CRF-R_1 receptors following stimulation with CRF (Reisine & Hoffman, 1983). Despite this partial desensitization a pA_2 value for astressin was estimated from the averaged CECs for urocortin under control conditions and in the presence of 100 nM astressin using the methodology of van Rossum (1963). The obtained value of 7.52 suggests a relatively high affinity of astressin for the CRF-R_2 receptor.

In cultured cells stably expressing CRF-R_1 or CRF-R_2 receptors, in pituitary cells, and in brain homogenates CRF receptor activation results in an increased cyclic AMP content indicating that both types of CRF receptors are coupled to stimulation of adenylate cyclase activity (Chen *et al*., 1986; Reisine & Hoffman, 1983). The same mechanism may also be involved in mediating urocortin-induced relaxation of cerebral arteries as indicated by the inhibitory effect of SQ22536. This compound has been described to antagonize PGE_1-stimulated cyclic AMP accumulation in human blood platelets in the concentration range of 30 μM–1 mM (Harris *et al*., 1979). SQ22536 (300 μM) also inhibits forskolin-induced relaxation of isolated rat basilar artery (unpublished observation) indicating its efficacy in this vascular preparation. However, inhibition of urocortin-induced relaxation by SQ22536 became obvious only at 1 nM urocortin and higher, i.e. above the EC_{50} level. This may indicate that activation of adenylate cyclase by urocortin occurs only in the high concentration range, but it may also reflect differences in the efficacy of SQ22536 to inhibit receptor-independent stimulation of adenylate cyclase by forskolin and receptor-dependent stimulation by agonists such as urocortin.

Cyclic AMP is an important second messenger mediating vascular relaxation by activation of several effector pathways (Murray, 1990). Membrane hyperpolarization by activating K^+ channels appears to be a major pathway as suggested by recent findings (Paternò *et al*., 1996; Taguchi *et al*., 1995). In the present study first evidence in favour of membrane hyperpolarization involved in the vasomotor action of urocortin came from the observation that relaxation was greatly reduced in K^+-precontracted segments. In the following experiments with different K^+ channel blockers glibenclamide did not affect urocortin-induced relaxation. Since glibenclamide specifically inhibits ATP-sensitive K^+ channels in cerebral arteries (Parsons *et al*., 1991) activation of this channel type can be excluded. However, the relaxant action of urocortin was inhibited in the presence of 10 mM TEA, but not with 100 μM TEA. In contrast, TEA did not affect relaxation induced by CRF in rat mesenteric arteries (Lei *et al*., 1993) suggesting that agonist-transduction coupling may differ with the origin of the vessels. From the results obtained with TEA an activation of K_{Ca}^{2+} channels can be inferred. In accord, iberiotoxin (100 nM) and apamin (10 nM) also inhibited urocortin-induced relaxation. However, these compounds which are considered selective inhibitors of high and



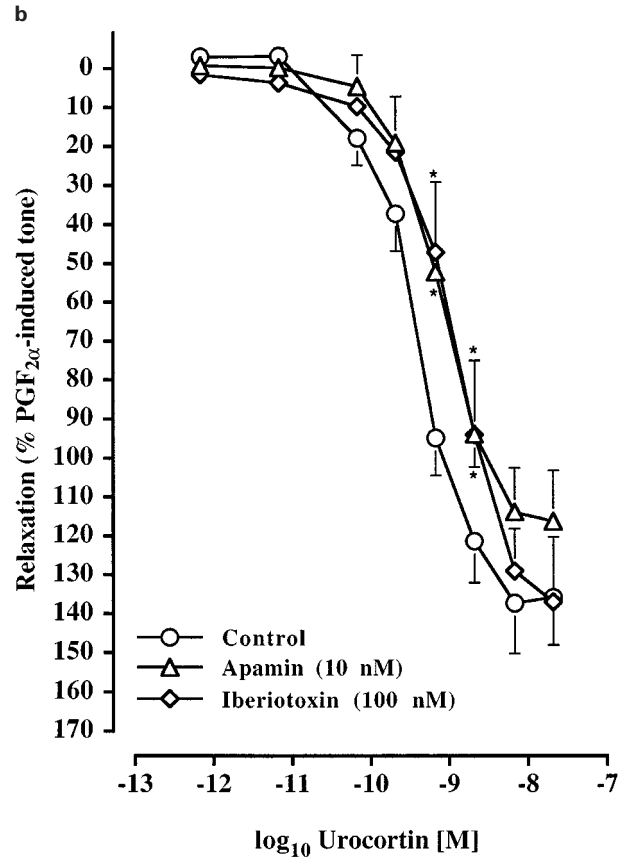


Figure 5 Inhibition of urocortin-induced relaxation of the isolated rat basilar artery by tetraethylammonium (TEA) (a) and by iberiotoxin or apamin (b). Ring segments were precontracted by prostaglandin (PG) $F_{2\alpha}$ (3 μM). Inhibition with TEA became apparent only with 10 mM but not with 100 μM. There is a comparable degree of inhibition induced by 100 nM iberiotoxin and 10 nM apamin. Given are means ± s.e.mean of 9–12 (a) and 7–9 (b) observations. *$P<0.05$ vs relaxation induced by urocortin in that respective subset of experiments.

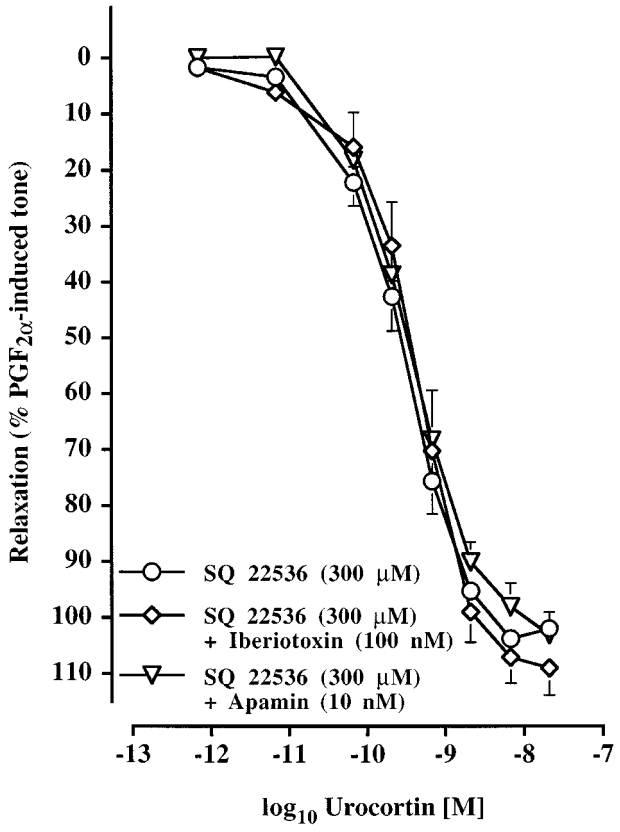


Figure 6 Inhibition of urocortin-induced relaxation of the isolated rat basilar artery by iberiotoxin or apamin in the presence of the adenylate cyclase inhibitor SQ22536. Ring segments were precontracted by prostaglandin (PG) $F_{2\alpha}$ (3 μM). There is no addition in the inhibitory action of SQ22536 by either iberiotoxin or apamin. Given are means ± s.e.mean of 7–11 observations.

low conductance K_{Ca}^{2+} channels were equally effective. Therefore, in the rat basilar artery some overlap in the range of K^+ channels affected by these toxins may be inferred. This assumption is further supported by the fact that there was no additive effect when both blockers were applied together. Although inhibition of urocortin-induced relaxation by either of the three K^+ channel blockers tested was small it reached statistical significance at 1 nM urocortin and above. Interestingly, the inhibitory action of SQ22536 became apparent at the same concentration level suggesting that K^+ channel activation induced by urocortin is mediated by an increase in cyclic AMP content due to adenylate cyclase stimulation. In accordance, there is no additive effect in the inhibitory actions of SQ22536 and either apamine or iberiotoxin.

In the present study, urocortin proved to be an extremely potent relaxant agent in the rat basilar artery. Based on this finding, urocortin may be considered a promising therapeutic agent in pathophysiological situations in which a severe decrease of (regional) cerebral blood flow may develop. However, results obtained in experimental studies using CRF and antagonists were inconsistent. On one hand, CRF receptor antagonists proved to be neuroprotective in models of ischemia- and glutamate-induced neuronal damage (Maecker

et al., 1997; Strijbos *et al*., 1994; Yatsushiro *et al*., 1997b), but it is unclear whether these effects are due to inhibition of receptors located in the vasculature or on neurons. On the other hand, application of a receptor agonist, i.e. CRF itself has been observed to result in a decrease of tracer extravasation in the early phase after experimental brain injury (Wei & Gao, 1991) and a better posthypoxic recovery of neuronal function in the hippocampal slice preparation (Fox *et al*., 1993). The results of the former study suggest implication of CRF receptors located in the vasculature while those of the latter study indicate pathophysiological importance of CRF receptors located on neurons. Thus, the apparent discrepancy in the effects of CRF receptor agonists and antagonists may reflect differences in the expression pattern and in the functional consequences following activation of CRF receptors in cerebral blood vessels and neurons, rendering successful design of new CRF-related therapeutic strategies a complex task.

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