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Urocortin stimulates tyrosine hydroxylase activity via the cAMP/protein kinase A pathway in rat Pheochromocytoma PC12 cells

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Abstract

Urocortin is a novel mammalian member of the corticotrophin releasing factor (CRF)-related peptides. We have investigated the expression, mechanism of action and second messenger for urocortin in rat pheochromocytoma PC12 cells. We initially confirmed the expression of urocortin and CRF-R2β, which is thought to be an endogenous receptor for urocortin, in PC12 cells. We also demonstrate that urocortin (≥1 nM) significantly elevates the level of cAMP in these cells. Moreover, α-helical CRF-(9-41), a more specific antagonist of CRF-R2 than CRF-R1 and the adenylate cyclase inhibitor SQ22536, inhibited the urocortin-induced increase in the level of cAMP. Thus, urocortin may exert its physiological role in chromaffin cells via CRF-R2β coupling to adenylate cyclase. Urocortin (≥1 nM) significantly increased the mRNA level and activity of tyrosine hydroxylase (TH), a rate-limiting enzyme in the biosynthesis of catecholamine. Furthermore, urocortin-induced changes in TH-mRNA and activity were inhibited by H89 (a PKA inhibitor) and SQ22536 as well as α-helical CRF-(9-41). However, urocortin did not affect DNA synthesis or catecholamine secretion in these cells. In conclusion, we have demonstrated that urocortin stimulates catecholamine biosynthesis via the cAMP/protein kinase A pathway in PC12 cells, where both urocortin and its receptor, CRF-R2, are expressed.

Keywords: Urocortin; Catecholamine; Tyrosine hydroxylase (TH); Protein kinase A; PC12 cells

Corticotrophin releasing factor (CRF) is a 41-amino acid peptide, initially found to stimulate corticotrophin secretion from the anterior pituitary [21]. Vaughan et al. [26] reported a novel CRF receptor-ligand, urocortin, which has significant homology to CRF. These CRF-related peptides are known to bind to several CRF receptors commonly coupled with adenylate cyclase. CRF receptor type 1 (CRF-R1) is highly expressed in the brain and the pituitary gland. Expression of CRF receptor type 2α(CRF-R2α) is restricted to the central nervous system, while CRF-R2β is expressed in the heart as well as other peripheral tissues, including the gastrointestinal tract, epididymis and brain [20].

Urocortin binds six times and 40 times more strongly than CRF to CRF-R1 and CRF-R2β, respectively. Indeed, urocortin is much more potent than CRF for the accumulation of cAMP in COS-M6 cells expressing CRF-R2β [21]. Thus, it would appear that urocortin is an endogenous ligand for CRF-R2β.

Although CRF-R2β is reported to be expressed in several peripheral tissues [16], it is unclear whether urocortin plays a direct role in these organs. Oki and coworkers reported that urocortin-like immunoreactivity was observed in adrenal cells [17,18] suggesting that urocortin plays a role in the regulation of catecholamine function in adrenomedullary chromaffin cells. However, it is not yet clear whether urocortin is involved in controlling catecholamine synthesis or secretion in chromaffin cells.

In adrenal medullary cells, tyrosine hydroxylase (TH) is a rate-limiting enzyme in the biosynthesis of catecholamine. TH-activity can be regulated by both short and long-term mechanisms. Short-term regulation of enzyme activity occurs at the post-transcriptional level, mainly activated via phosphorylation of the TH molecule [1,2,4,5,13,29]. Indeed, TH

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is phosphorylated and activated by different protein kinases, including cAMP-protein kinase A (PKA) [1,2,4,5,13,29]. Long-term regulation has been shown to be exerted at the level of TH-protein synthesis following TH-gene transcription [6,13]. Similarly, several protein kinases, including PKA, also induce elevated levels of TH-mRNA [6,13].

The major aim of this study was to determine the direct effect of urocortin on either catecholamine release or catecholamine synthesis in PC12 rat pheochromocytoma cells. We also examined the effect of urocortin on DNA synthesis in PC12 cells.

Unless stated otherwise, all reagents were purchased from Wako Seiyaku. Urocortin and α-helical CRF-(9-41) were obtained from the Peptide Institute (Osaka, Japan). SQ22536, which directly inhibits adenylate cyclase, was obtained from Biomol Research Laboratories (PA, USA). The experimental conditions for treatment of the cells with H89 or SQ22536 were identical to those described previously [3,7,14].

The PC12 cell line (RCB009) was obtained from the RIKEN Cell Bank (Ibaraki, Japan). Cells were maintained as described previously [23]. Total RNA was extracted as described previously [25]. First-strand cDNAs were amplified by PCR using 25 pmol of the appropriate synthetic oligonucleotide primers (urocortin, CRF-R1, CRF-R2α, CRF-R2β, respectively) according to the method of Nishikimi et al. [16].

The level of cAMP in the cells was determined using a cAMP kit (Yamasa, Chousi, Japan) as described previously [7]. Tyrosine hydroxylase enzyme activity was measured using a method previously reported by Takekoshi et al. [23] and Kumai et al. [12]. TH mRNA level was determined by real-time PCR analysis as described previously by Kitaoka et al. [10]. DNA synthesis was measured as described previously [15]. Catecholamine content was determined as described previously [24,29].

Data were analyzed between groups by one-way analysis of variance (ANOVA) by means of the Statview computer software program (Abacus Concepts, Inc., Berkeley, CA). p values less than 0.05 were considered significant. All data are expressed as mean ± S.D.

As shown in Fig. 1, we initially demonstrated that urocortin and CRF-R2β mRNA (specific predicted length of transcripts 279 and 186 bases, respectively) were co-expressed in PC12 cells. This result suggests that urocortin exerts its action through its own receptor in a paracrine manner. In contrast, neither CRF-R1 nor CRF-R2α was expressed in PC12 cells, while these receptors were expressed in the brain. These observations are entirely consistent with previous reports, confirming that CRF-R2β is indeed a receptor associated with the peripheral site for urocortin. In contrast, CRF-R1 and CRF-R2α are receptors associated with the central nervous system [20].

As shown in Fig. 2, urocortin at 1, 10, 100 and 1000 nM significantly induced an increase in the level of cAMP by 24.7, 56.2, 52.3 and 51.5%, respectively ($p < 0.05$). Similar results have been reported for other systems such as cardiac myocytes [16]. We have also shown that urocortin-induced

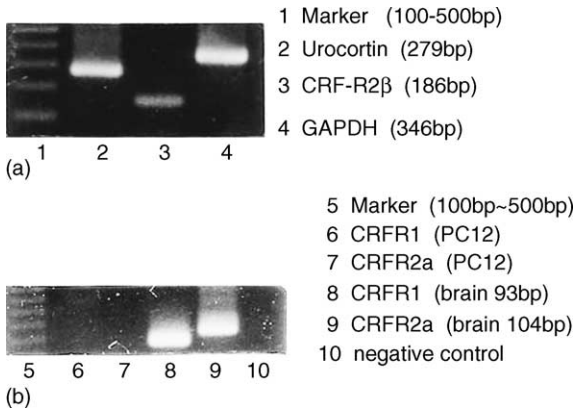

Fig. 1. Expression of urocortin and its receptor in PC12 cells. Total RNA (1 μg per lane) from PC12 cells were characterized by RT-PCR. Negative control indicates mRNA without prior RT (lane 10). The positive control indicates mRNA from brain following RT (lanes 8 and 9).

cAMP accumulation was inhibited by α-helical CRF-(9-41), as well as SQ22536, indicating that urocortin might act through CRF-R2β coupled with adenylate cyclase.

As shown in Fig. 3, urocortin at 1, 10, 100 and 1000 nM significantly increased TH enzyme activity by 16.3, 26.4, 20.3 and 18.7%, respectively. Furthermore, urocortin at 1, 10, 100 and 1000 nM significantly increased the level of TH mRNA by 18.9, 36.4, 36.8 and 31.1%, respectively (Fig. 4). These results show that urocortin simultaneously stimulates TH enzyme activity and up-regulation in the level of TH mRNA, suggesting that both mechanisms contribute to urocortin-induced catecholamine biosynthesis. Furthermore, the urocortin-induced increase in TH enzyme activity and mRNA level was inhibited by H89 (PKA inhibitor), as well as SQ22536, confirming that these effects are mediated through

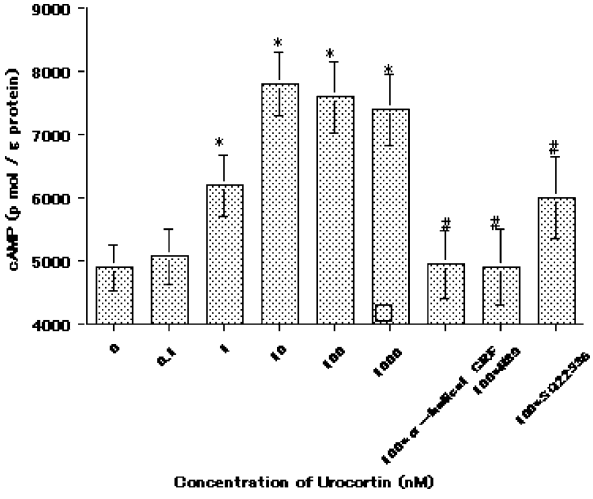

Fig. 2. Effects of urocortin on cAMP production in PC12 cells. Cells were incubated with various concentrations (0.1–1000 nM) of rat urocortin in the absence or presence of α-helical CRF (1 μM, 30 min pretreatment) or SQ22536 (0.5 mM, 15 min pretreatment). Intracellular cAMP was then subjected to EIA. The data shown are mean ± S.D. ($n = 4$). Significantly different ($^{*}p < 0.05$) from basal value and significantly different ($^{\#}p < 0.05$) from value induced by urocortin alone.