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European Journal of Pharmacology 493 (2004) 95–98

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Short communication

Urotensin-II, a neuropeptide ligand for GPR14, induces c-*fos* in the rat brain

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Received 7 April 2004; accepted 9 April 2004

Abstract

The vasoactive peptide urotensin-II and its receptor, GPR14 (now known as UT receptor), are localised in the mammalian central nervous system. Accordingly, various centrally mediated effects of urotensin-II on behaviour, neuroendocrine hormones and neurochemistry have been described. To investigate neuroanatomical substrates for the central actions of urotensin-II, expression of the immediate early gene c-*fos* was examined following intracerebroventricular administration to rats. Urotensin-II increased Fos expression in the cingulate cortex and periaqueductal grey, suggesting important central roles for urotensin-II and its receptor.

Keywords: Urotensin-II; UT receptor; GPR14; c-*fos*

1. Introduction

Urotensin-II is a peptide first isolated from the goby fish urophysis (Pearson et al., 1980), a structure homologous to the hypothalamic pituitary adrenal axis in mammals (Bern et al., 1985). A receptor for urotensin-II was recently identified when several groups showed urotensin-II functionally activates the G-protein-coupled receptor, GPR14 (now known as UT receptor) (Ames et al., 1999; Liu et al., 1999; Mori et al., 1999; Nothacker et al., 1999) which is identical to sensory epithelium neuropeptide-like receptor (SENR) (Tal et al., 1995).

Urotensin-II is best known for its vasoactive properties (reviewed by Maguire and Davenport, 2002) and is a more potent vasoconstrictor than endothelin-1 in non-human primates (Douglas et al., 2000). However, the localisation of both urotensin-II and UT receptor in areas distinct from the vasculature imply further roles for urotensin-II and its receptor.

Urotensin-II mRNA and protein are found in the central nervous system (CNS) with highest levels of mRNA found specifically in motor neurons of the spinal cord (Coulouarn et al., 1999; Ames et al., 1999). Accordingly, urotensin-II regulates intracellular Ca^{2+} in dissociated rat spinal cord neurons (Filipeanu et al., 2002) and stimulates spontaneous release from frog motor nerve terminals (Brailoiu et al., 2003). Highest levels of urotensin-II-like immunoreactivity are found in the ventral horn motor neurons of the spinal cord, the oromotor nuclei and in the acinar cells lining the thyroid follicles (Ames et al., 1999; Clark et al., 2001).

UT receptor is also well described in the mammalian CNS with highest mRNA expression in cortical areas, nucleus accumbens, substantia nigra, thalamus, cerebellum, choroid plexus and spinal cord motor nuclei (Ames et al., 1999; Gartlon et al., 2001; Liu et al., 1999; Tal et al., 1995). Interestingly, in situ hybridisation revealed that UT receptor mRNA co-localised specifically to cholinergic neurons in the mesopontine tegmental nuclei which are associated with motor function, arousal and sleep (Clark et al., 2001). High-density [^{125}I]urotensin-II binding sites are found in the lateral septal, medial habenula and interpenduncular nuclei of the rat (Clark et al., 2001) and in the cerebral cortex of humans (Maguire et al., 2000).

Given the localisation of both urotensin-II and its receptor in the mammalian CNS, the physiological

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doi:10.1016/j.ejphar.2004.04.009

actions of central urotensin-II have received little attention. We previously described the first evidence for behavioural and neuroendocrine effects following intracerebroventricular (i.c.v.) administration of urotensin-II to rats. Urotensin-II caused behavioural stimulation with increased grooming, rearing and motor activity. Neuroendocrine hormones were also affected with elevated levels of thyroid stimulating hormone and prolactin (Gartlon et al., 2001). Centrally mediated pressor and tachycardic effects following i.c.v. administration are also described (Lin et al., 2003).

Therefore, in the absence of localisation data for UT receptor protein, the aim of this study was to investigate neuroanatomical substrates for the central actions of urotensin-II. Expression of the immediate early gene c-*fos*, a marker of neuronal activation, was used to map the location of functionally active substrates for the response to urotensin-II following i.c.v. administration to rats. Expression of the gene product, Fos, was measured in various brain regions including cingulate cortex, paraventricular thalamus, dorsal amygdala, caudate, nucleus accumbens, hypothalamus, periaqueductal grey, habenula and lateral septum.

2. Materials and methods

All experiments were carried out in accordance with the United Kingdom Animals (Scientific Procedures) Act, 1986. Male Sprague–Dawley rats (Charles River, 250–300 g) were implanted with an indwelling cannula directed towards the lateral ventricle (for detailed surgical methods, see Gartlon et al., 2001). Peptides or vehicle (0.9% saline solution) were administered by i.c.v. injection in a volume of 5 μl. Human urotensin-II (ETPDCFWKYCV) was obtained as a trifluoroacetate salt (GlaxoSmithKline, UK). Rats were exposed to a sham i.c.v. dosing procedure once daily for 5 days prior to the study to allow habituation of the immediate early gene response to dosing-induced stress (Melia et al., 1994). Urotensin-II [1 and 10 μg (doses taken from Gartlon et al., 2001), $n=6$] or vehicle (saline, 5 μl) were injected i.c.v. Two hours later, rats were deeply anaesthetised with sodium pentobarbitone (200 mg/kg i.p.) and intracardially perfused with heparinised saline solution, followed by 4% (w/v) paraformaldehyde in 0.1 M phosphate buffer. Fos immunohistochemistry was carried out according to the methods of Leslie et al. (1993) using sheep polyclonal primary c-Fos antiserum (Sigma-Genosys, UK), with an ABC (avidin–biotin complex)-peroxidase detection system using DAB (3,3′ -diaminobenzidine substrate) as the chromagen. Sections were examined by bright-field microscopy and cells which exhibited Fos-like immunoreactivity in pre-selected brain regions (cingulate cortex, paraventricular thalamus, dorsal amygdala, caudate, nucleus accumbens, hypothalamus, periaqueductal grey, habenula and lateral septum) were counted. Numbers of immunoreactive nuclei in a 200 × 200-μm square were determined by an observer blind to drug treatment and analysed (per region) by one-way analysis of variance followed by Duncan's New Multiple Range post hoc analysis.

3. Results

The 5-day sham dosing procedure resulted in low levels of Fos-like immunoreactivity in vehicle-treated animals (Fig. 2). Urotensin-II (1 and 10 μg) caused significant region-specific Fos induction. Fos-like immunoreactivity was increased dose-dependently in the cingulate cortex (Fig. 1) and periaqueductal grey ($P<0.05$; Fig. 2). No significant changes in Fos-like immunoreactivity were observed in the paraventricular thalamus, dorsal amygdala, caudate, nucleus accumbens, hypothalamus and habenula. There was a strong trend towards increased Fos-like immunoreactivity in the lateral septum, although this was not statistically significant.

4. Discussion

Urotensin-II caused highest c-*fos* expression in the cingulate cortex. The cingulate cortex is an important integrative centre of the brain, playing a central role in mediating

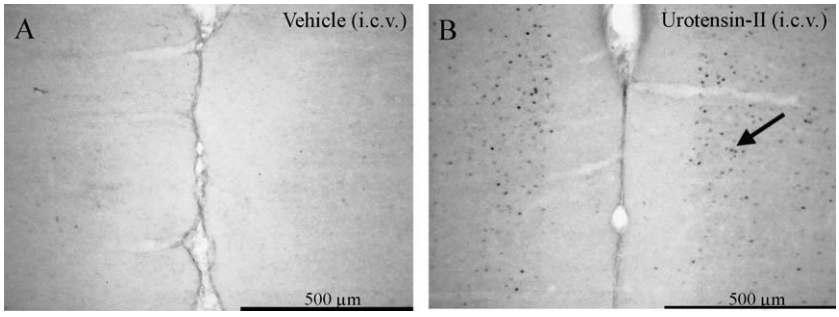

Fig. 1. Photomicrograph indicative of Fos-like immunoreactivity in the cingulate cortex (approximately 1.2 mm anterior to bregma) of a rat treated with (A) vehicle i.c.v., bar = 500 μm, (B) urotensin-II (10 μg), bar = 500 μm. Intense Fos-like immunoreactivity (arrow) was evident only in the brains of animals administered urotensin-II.

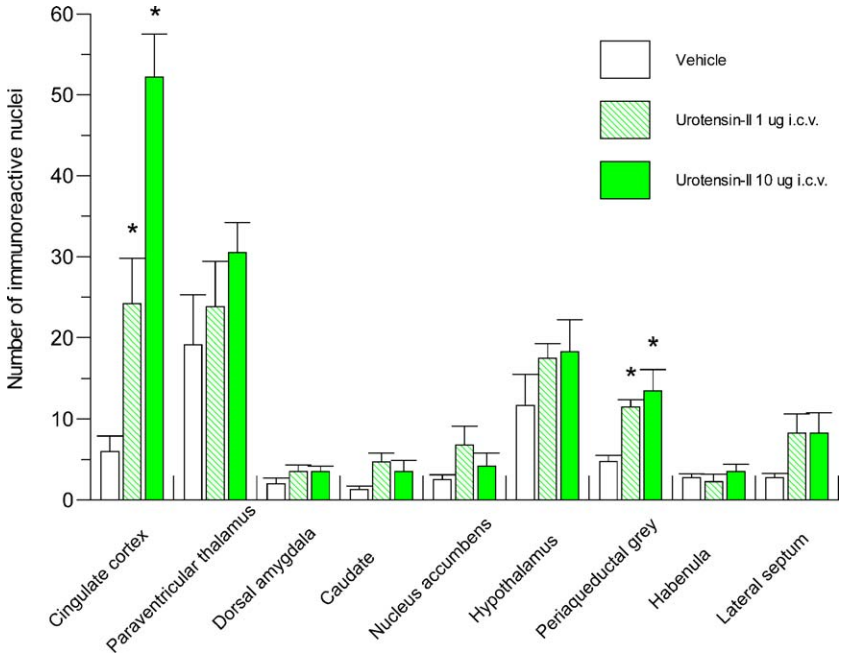

Fig. 2. Number of Fos-like immunoreactive nuclei were determined in brains perfused within 2 h after i.c.v. administration of vehicle or urotensin-II (1 or 10 μg). Fos-like immunoreactivity was counted in a 200 × 200 μm grid in the cingulate cortex, paraventricular thalamus, dorsal amygdala, caudate, nucleus accumbens, hypothalamus, periaqueductal grey, habenula and lateral septum. Data shown as means (+S.E.M.). Significant differences from vehicle are shown by $*P < 0.05$.

cognitive and emotional responses and controlling motor, endocrine and autonomic functions. Dose-dependent increases in Fos-like immunoreactivity were also observed in the periaqueductal grey, which is a brain area involved in mediating pain perception as well as many somatic and visceral stereotypical behaviours such as the "panic" response. Expression in these areas is consistent with the localisation of UT receptor mRNA (Gartlon et al., 2001) and further suggest important central roles for urotensin-II. Interestingly, Fos-like immunoreactivity was not significantly increased in the lateral septum and habenula, sites previously shown to exhibit high density [^{125}I]urotensin-II binding (Clark et al., 2001).

Overall, there is a discrepancy between the expression of urotensin-II stimulated Fos and the distribution of UT receptor which may be explained in several ways. Firstly, reports of UT receptor expression are also inconsistent and vary according to the type and sensitivity of the detection system used [distributions by reverse transcriptase-polymerase chain reaction, dot-blot, in situ hybridisation and receptor autoradiography are reviewed by Maguire and Davenport (2002). For example, [^{125}I]urotensin-II binding in lateral septal, medial habenula and interpenduncular nuclei is found in the absence of UT receptor mRNA expression (Clark et al., 2001). Secondly, it is suggested that urotensin-II binding sites found in the absence of UT receptor expression represent pre-synaptic autoreceptors (Clark et al., 2001; Liu et al., 1999). Finally, Fos is expressed in response to many extracellular stimuli and, therefore, it is conceivable that urotensin-II stimulated Fos expression could be independent of UT receptor activation.

Despite evidence for a role for urotensin-II in fish and mammalian neuroendocrine regulation (Conlon et al., 1997; Gartlon et al., 2001), there was no Fos induction in the hypothalamus, which is the major controlling centre of pituitary output. However, previous findings suggest that a more detailed analysis of the hypothalamus, and also other brain regions, may reveal urotensin-II induced Fos in discrete areas or specific nuclei. For example, the neuropeptide neuromedin-U causes widespread centrally mediated effects (Gartlon et al., submitted for publication) but induces Fos only in discrete areas; including specific nuclei of the hypothalamus (Ozaki et al., 2002).

In summary, the induction of c-*fos* in the cingulate cortex, periaqueductal grey and lateral septum further suggests important central roles for urotensin-II and its receptor. In addition, this work demonstrates the utility of immediate early gene (c-*fos*) expression to help determine neuronal substrates of centrally mediated responses in the absence of detailed protein distribution data. Further studies should assess in more detail the CNS distribution of Fos protein, and other immediate early genes, after i.c.v. urotensin-II administration to rats.

Acknowledgements

The authors are very grateful for the surgical and technical assistance provided by Karen Davis, Susan

Barber, Jackie Colledge, Jonathon Barford and Louise Chamberlain.

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