*Neuroscience* 152 (2008) 1015–1023

# GENDER-RELATED UROCORTIN 1 AND BRAIN-DERIVED NEUROTROPHIC FACTOR EXPRESSION IN THE ADULT HUMAN MIDBRAIN OF SUICIDE VICTIMS WITH MAJOR DEPRESSION

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**Abstract—In postmortem brains of patients with major depression, the expression of corticotrophin-releasing factor (CRF) is enhanced and that of brain-derived neurotrophic factor (BDNF) decreased. In mice over-expressing neuronal CRF (an animal model for depression) the expression of urocortin 1 (Ucn1) in the non-preganglionic Edinger-Westphal nucleus (npEW) is strongly down-regulated. Therefore, we hypothesized that an altered activity of Ucn1 neurons in the npEW would contribute to the pathogenesis of major depression. To test this hypothesis we measured Ucn1 mRNA and BDNF mRNA levels in the npEW of seven male and four female, drug-free suicide victims with major depression, and compared the data with those obtained from 10 male and seven female individuals without neurological and psychiatric disorders (controls). We show that compared with controls, the Ucn1-mRNA level in npEW neurons is about 9.12 times higher in male but unchanged in female suicide victims. Furthermore, BDNF mRNA expression in microdissections of npEW was 3.36 times lower in male suicide victims, but 5.27 times higher in female victims, compared with controls. Our data also show that male suicide victims had almost 11.47 times more Ucn1 and 4.26 times less BDNF mRNA in the npEW than female suicide victims. We discuss the significance of these data for npEW Ucn1 and BDNF, and propose that altered expressions of Ucn1 and BDNF in the npEW contribute to the pathogenesis of major depression and/or suicidality in a gender-specific manner.** 

**Key words: suicide, unipolar depression, non-preganglionic Edinger-Westphal nucleus, Q-RT-PCR, short postmortem delay.**

The lifetime prevalence of major depression is at least 10% (Weissman et al., 1996). It is a multifactorial disorder and one of the numerous risk factors is stressful life events (Reul and Holsboer, 2002; Goldberg, 2006). Upon stress corticotropin releasing factor (CRF) is released from the hypothalamus (Vale et al., 1981), and CRF is one of the principal mediators of the stress response (Chadwick et al., 1993). Not surprisingly, considerable evidence exists from cerebrospinal fluid studies, postmortem tissue receptor measurements, as well as CRF stimulation test studies to support the hypothesis that CRF is hypersecreted in depression (Nemeroff et al., 1984; Gold et al., 1986; Nemeroff, 1988, 1992; Murphy, 1991; Keck, 2006), and the cumulative evidence makes a strong case implicating dysregulation of CRF systems in the etiology and pathogenesis of major depression (Nemeroff, 1992; Arborelius et al., 1999; Reul and Holsboer, 2002; Hauger et al., 2006; Keck, 2006). However, since CRF receptors become rapidly desensitized in the presence of high agonist concentrations, enhanced CRF neurotransmission alone may not be sufficient to account for the neuropathology of major depression (Hauger et al., 2006). Thus, concomitant dysregulation of a mechanism controlling CRF receptor signaling may play a role as well. In search of such a mechanism, the discovery and characterization of novel members of the CRF neuropeptide family, such as urocortin 1 (Ucn1; Vaughan et al., 1995; Skelton et al., 2000; Latchman, 2002; Kozicz, 2007), urocortin 2 (or 'stresscopin-related peptide'; Reyes et al., 2001; Hsu and Hsueh, 2001) and urocortin 3 (or 'stresscopin'; Lewis et al., 2001; Hsu and Hsueh, 2001) have extended our insight into central peptidergic networks involved in stress adaptation and stress-induced brain diseases.

In the mammalian brain including that of humans, Ucn1 is most abundantly expressed in the midbrain, namely in neurons in the non-preganglionic Edinger-Westphal nucleus (npEW; Vaughan et al., 1995; Kozicz et al., 1998; Bittencourt et al., 1999; Iino et al., 1999; Vasconcelos et al., 2003; Ryabinin et al., 2005; Gaszner et al., 2007). The activity of Ucn1-containing neurons in the npEW shows conspicuous changes in response to various acute and chronic challenges (Weninger et al., 2000; Kozicz et al., 2001; Gaszner et al., 2004; Korosi et al., 2005), and like CRF, Ucn1 acts on CRF1 and CRF2 receptors, stimulates the release of adrenocorticotropic hormone from the anterior pituitary as well as evokes anxiety (Skelton et al., 2000; Vetter et al., 2002; Latchman, 2002; Gysling et al., 2004). In addition, we have demonstrated that in mice over-expressing neuronal CRF (an animal model for anxiety and depression-like behavior), Ucn1 mRNA in the npEW is strongly down-regulated (Kozicz et al., 2004).

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*Abbreviations:* ANOVA, analysis of variance; BDNF, brain-derived neurotrophic factor; CRF, corticotropin releasing factor; $C_t$, cycle threshold; DPFC, dorsolateral prefrontal cortex; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; ir, immunoreactive; npEW, non-preganglionic Edinger-Westphal nucleus; RT-PCR, real-time polymerase chain reaction; Ucn1, urocortin 1.

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doi:10.1016/j.neuroscience.2007.12.050

The above finding suggests that an altered activity of the npEW-Ucn1 system may also contribute to the pathophysiology of major depression.

Newer hypotheses of depression neurobiology suggest closer study of involvement of neurotrophic factors, such as brain-derived neurotrophic factor (BDNF) in the regulation of mood disorders (for reviews see: Castren, 2005; Castren et al., 2006; Kato, 2007). BDNF expression is reduced in various brain areas upon stress, and essentially this reduction can be prevented essentially by all antidepressant drug treatment (for review see: Castren et al., 2006). In postmortem samples of the brain of depressed patients, the BDNF level is lower than in healthy persons, whereas antidepressant therapies restore this level to normal (Castren, 2004; Karege et al., 2005a,b). These studies have led to the formulation of the hypothesis, which emphasizes the role of BDNF as a tool of modifications in neuronal networks that regulate mood (for reviews see: Castren, 2004; Castren et al., 2006). Interestingly, in the rat midbrain, several BDNF expressing cells are present in the rostral periaqueductal gray (Hofer et al., 1990; Yan et al., 1997), and injecting BDNF into the midbrain has an antidepressant effect (Siuciak et al., 1997).

Taking the above data together, we hypothesize that Ucn1 and BDNF at the level of the npEW nucleus may play a substantial role in the pathogenesis of stress-induced brain diseases, such as major depression c.q. suicidality. As a first step in supporting this hypothesis, we have investigated (1) the possible co-existence of BDNF in Ucn1-containing neurons in the human microdissections of npEW with double fluorescence immunohistochemistry, and (2) using quantitative real-time polymerase chain reaction (RT-PCR) we have quantified Ucn1 and BDNF mRNA expressions in microdissections of npEW from controls and from an endophenotypically well-characterized group of patients with major depression viz. they all committed suicide. Gender differences in susceptibility to major depression are suggested by a large body of literature with the risk in women twice that in man (for reviews see: Desai and Jann, 2000; Kaminsky et al., 2006; Gorman, 2006; Goldstein and Gruenberg, 2007). In a recent study we have demonstrated that npEW-Ucn1 neurons express estrogen receptor beta (Derks et al., 2007), and estradiol decreases Ucn 1 promoter activity through estrogen receptor beta (Haeger et al., 2006). This has prompted us to extend our study to analyze Ucn1 and BNDF mRNA expression in microdissection of npEW samples with short postmortem delay both in men and women.

## EXPERIMENTAL PROCEDURES

### Subjects

Brains from suicide victims with major depression ($n=10$; six males and four females) and from controls ($n=17$; 10 males and seven females) were used in this study. The average age of male controls and suicide victims was $53.36\pm1.94$ and $44.86\pm6.33$ respectively. The average age of female controls and suicide victims was $72.25\pm3.88$ and $42.7\pm4.67$, respectively. Two-way analysis of variance (ANOVA) revealed significant effect of victim ($F_{(3,26)}=20.07$; $P=0.0001$) and gender by victim interaction ($F_{(3,26)}=6.19$; $P=0.02$). Using Fisher post hoc analysis, no statistical difference was measured between the average age of male controls and suicide victims ($P=0.12$) as well as male and female suicide victims ($P=0.68$). All subjects died without neurological or affective disorder. Brains were obtained at autopsy at the Department of Forensic Medicine of Semmelweis University, Budapest, micro-dissected, and stored in the Human Brain Tissue Bank, Budapest. The medical, psychiatric and drug history of all suicide victims was obtained through chart review coupled with interviews with the attending physician(s)/psychiatrist(s) and family members. For every suicide victim, a psychiatric diagnosis of affective disorder was retrospectively made and confirmed by experienced psychiatrists on the basis of *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition* criteria. The diagnosis was major depressive disorder with recurrent episodes. Suicide victims with a history of schizophrenia, epilepsy, alcohol or other drug abuse were not included in the studies. Victims had died by hanging ($n=9$; six males and three females) or drug overdose ($n=1$; one female). Some of the suicide subjects of this study had been previously treated for major depression, however details on the exact dose of medications as well as on level of compliance with therapy could not reliably obtained in all cases. Nevertheless, based on psychological autopsy and medical records, persons included in the study had not used antidepressant medication for at least 2 months before death. In addition, toxicological tests on blood samples did not reveal the presence of drugs or alcohol in cases of death by hanging. The causes of death in control subjects were acute cardiac-respiratory failure, myocardial infarction. Their medical records confirmed the absence of a history of psychiatric illness, antipsychotic medications, and alcohol or drug abuse during the last 10 years. In addition, the neuropathological analysis (histopathology) did not show any neurodegenerative disorders in these cases. Both controls and suicide victims were Caucasian from Hungary (Budapest region). Harvesting of tissues was approved by the local ethics committee of Semmelweis University, and informed consent had been obtained from next of kin.

Brains were obtained between 1 and 6 h after death with the exception of two brain samples of female suicide victims, where the postmortem delay was 11 h ($3.69\pm0.61$ and $2.55\pm0.37$ h for male victims and controls, respectively, and $7.00\pm2.31$ and $4.13\pm0.41$ h for female victims and controls, respectively). Two-way ANOVA revealed significant effect of gender ($F_{(3,26)}=11.31$; $P=0.002$) and victim ($F_{(3,26)}=7.73$; $P=0.009$). Using Fisher post hoc analysis, no statistical difference was measured between the average postmortem delay of male/female controls and suicide victims ($P=0.15$ for males and $P=0.056$ for females) as well as between male and female controls ($P=0.059$). Postmortem times of female suicide victims were different from that that of male controls ($P=0.003$) and male suicide victims ($P=0.01$). At the time of the dissection, the midbrains were sliced into 1–1.5 mm thick coronal sections at a temperature between 0 and $-10$ °C. Under a stereomicroscope, samples from the rostral ventral portion of the midbrain, including the ventral central gray, oculomotor nuclei and the Cajal nucleus, but dominated by the Edinger-Westphal nucleus, were microdissection of with a 1.5 mm micropunch needle. This approach allows minimal variations in the size and weight of microdissected samples. Tissue samples from three consecutive sections were collected in Eppendorf tubes and stored at $-70$ °C until use.

### Double labeling immunocytochemistry

Human rostral midbrains including the npEW of two male controls and two male suicide victims were microdissected, immersion fixed in 4% paraformaldehyde for 48 h at 4 °C. Coronal slices (30 $\mu$m) at the level of the superior colliculus were cut on a freezing microtome (Microm GmBH, Walldorf, Germany). Sections were rinsed $4\times15$ min in 0.1 M PBS, followed by 0.5% Triton X-100 (Sigma-Aldrich, Zwijndrecht, The Netherlands) in 0.1 M