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MINIREVIEW ARTICLE

Nardilysin in human brain diseases: both friend and foe

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Abstract Nardilysin is a metalloprotease that cleaves peptides, such as dynorphin-A, α-neoendorphin, and glucagon, at the N-terminus of arginine and lysine residues in dibasic moieties. It has various functionally important molecular interaction partners (heparin-binding epidermal growth factor-like growth factor, tumour necrosis factor-α-converting enzyme, neuregulin 1, beta-secretase 1, malate dehydrogenase, $P42^{IP4}$/centaurin-α1, the histone H3 dimethyl Lys4, and others) and is involved in a plethora of normal brain functions. Less is known about possible implications of nardilysin for brain diseases. This review, which includes some of our own recent findings, attempts to summarize the current knowledge on possible roles of nardilysin in Alzheimer disease, Down syndrome, schizophrenia, mood disorders, alcohol abuse, heroin addiction, and cancer. We herein show that nardilysin is a Janus-faced enzyme with regard to brain pathology, being probably neuropathogenic in some diseases, but neuroprotective in others.

Keywords Nardilysin · Alzheimer disease · Schizophrenia · Alcoholism · Heroin addiction · Brain tumour

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Abbreviations

ACC	Anterior cingulate cortex
AD	Alzheimer disease
ADAM	A disintegrin and metalloprotease
APP	Amyloid precursor protein
BACE	Beta-site APP cleaving enzyme
CNS	Central nervous system
DLP	Dorso-lateral prefrontal cortex
DS	Down syndrome
EGF	Epidermal growth factor
erbB	Epidermal growth factor receptor (tyrosine kinase)
HB-EGF	Heparin-binding epidermal growth factor-like growth factor
HXXEH	His-Xaa-Xaa-Glu-His zinc–binding motif of the inverzincin/M16 family of metalloproteases
NRDc	Nardilysin
NRG	Neuregulin
PVN	Paraventicular nucleus
PNS	Peripheral nervous system
SON	Supraoptic nucleus
SH-SY5Y	Human neuroblastoma cell line
TACE	Tumour necrosis factor-α-converting enzyme
TNF-α	Tumour necrosis factor

Nardilysin: Introducing the enzyme

In this review, which includes some of our own recently published as well as unpublished findings, we will concentrate on the putative role(s) of nardilysin (N-arginine dibasic convertase; NRDc; EC 3.4.24.61) in human brain diseases, like Alzheimer disease (AD), Down syndrome (DS), schizophrenia, affective disorders, alcohol abuse,

heroin addiction, and cancer. For detailed information on enzymatic and biochemical properties of NRDc, the reader is referred to the excellent and comprehensive overviews published by Seidah and Prat (2002), Hospital and Prat (2004), and recently by Nishi (2013).

NRDc is a metalloprotease belonging to the inverzincin/M16 family of metalloendopeptidases (Rawlings and Barrett 1993; Hooper 1994). It was originally described as a somatostatin precursor processing enzyme that can be found in rat brain extracts (Gluschankof et al. 1984). Subsequently, it was revealed that besides somatostatin-28 NRDc can also cleave other peptides, such as dynorphin-A, α-neoendorphin, and glucagon, at the *N*-terminus of arginine and lysine residues in dibasic moieties (Chesneau et al. 1994; Pierotti et al. 1994; Bataille et al. 2006), although the generation of miniglucagon from glucagon apparently involves the proteolytic action of two different enzymes, NRDc and aminopeptidase B, associated in a structural complex (Fontés et al. 2005). Further analysis has then demonstrated that the NRDc is also capable of splitting at a single basic residue if the adjacent residue is hydrophobic (Chow et al. 2003). However, whether the established in vitro substrates of NRDc are also in vivo substrates of the enzyme is still an unanswered question. Optimal catalytic activity of NRDc is found at pH 8.9 (Chesneau et al. 1994). Its catalytic activity is inhibited by metal chelators, cations, and some polyamines (for details, see Nishi 2013), but up-regulated by retinoic acid (Draoui et al. 1997; Borrmann et al. 2011a). Like all members of the inverzincin/M16 family of metalloendopeptidases, NRDc has an approximately 200 amino acid conserved region containing the HXXEH-binding motif of catalytic Zn^{2+} (Seidah and Prat 2002; Hospital and Prat 2004). In addition, the NRDc molecule contains a unique acidic stretch of amino acids (acidic domain), located N-terminal from the HXXEH motif which serves as a polyamine binding site (Ma et al. 2001). The closest identified mammalian homologue within the M16 family of metalloendopeptidases is insulin-degrading enzyme, with 36 % overall identity (Seidah and Prat 2002; Hospital and Prat 2004).

Two alternatively spliced variants of NRDc have been found for human and rat NRDc (NRDc1 and NRDc2). The two isoforms show similar biochemical and enzymatic properties but exhibit different species- and tissue-specific expression pattern (Hospital et al. 1997, 2002). In rodents, NRDc is highly expressed in endocrine organs, with the testis being a major expression site (Fumagalli et al. 1998). The expression of human NRDc mRNA in adult tissues has been shown to be remarkably high in the heart, skeletal muscle, and testis. Transcripts of NRDc were detected, although at lower levels, in most other tissues too, including developing and adult brain (Fumagalli et al. 1998; Bernstein et al. 2007). NRDc appears to have specific functions during the development. In foetal tissues, NRDc is abundantly expressed in placenta, heart, and brain. Remarkably, at early stages of mouse development, high levels of NRDc transcripts occur almost exclusively within neural tissues, pointing to specific roles of the enzyme neural development (Fumagalli et al. 1998). In addition to its peptidase activity, NRDc appears to be an important interaction partner of heparin-binding epidermal growth factor-like growth factor (HB-EGF). It binds to HB-EGF, which is known to be a potent stimulator of cell proliferation and migration (Nishi et al. 2001). Binding of NRDc to HB-EGF significantly modulates the HB-EGF-induced cellular response. Moreover, NRDc has been shown to enhance ectodomain shedding of HB-EGF through activation of the protease tumour necrosis factor-α-converting enzyme (TACE, also called ADAM 17), without involving the endogenous proteolytic activity of NRDc (Nishi et al. 2006). On the other side, the enzymatic activity of NRDc is known to be inhibited by HB-EGF. NRDc activity and HB-EGF binding are regulated by Ca^{2+} via the acidic domain (Hospital et al. 2002). Furthermore, there is some evidence that HB-EGF stimulates neurogenesis in proliferative zones of the adult brain by interacting with EGF receptor/ErbB1 and, possibly, NRDc (Jin et al. 2002). Of note, NRDc was subsequently shown to regulate further functionally important proteins, such as neuregulin 1 (NRG1), through its interaction with TACE and another protease, beta-secretase 1 (BACE1; Ohno et al. 2009). Other identified interaction partners of NRDc are the mitochondrial enzyme malate dehydrogenase (Chow et al. 2005) and the brain-specific $PtdInsP_3$ (phosphatidyl inositol 3,4,5 trisphosphate)/D-Ins(1,3,4,5)P_4 (inositol-1,3,4,5-tetrakisphophate)-binding protein $p42^{IP4}$/centaurin-α1 (Stricker et al. 2006).

Interestingly, tubulin was found to potentiate the interaction of NRDc with $p42^{IP4}$/centaurin-α1 (Borrmann et al. 2011b). Lastly, NRDc has recently been identified as an interaction partner of the histone H3 dimethyl Lys4, thus probably playing a significant role in tissue-specific gene regulation (Li et al. 2012).

Localization and some putative functions of nardilysin in non-diseased CNS

To better understand the putative implications of NRDc for neuropeptide metabolism, neuronal proliferation, differentiation, and migration, it is important to know its distribution patterns in normal developing and adult brain. Although NRDc was initially detected in, and partially purified from, rat brain cortex extracts (Gluschankof et al. 1984), the cellular sources of the enzyme within the brain were largely unknown until recently. Hence, we performed a detailed analysis to reveal the regional and cellular distribution of NRDc in human CNS (Bernstein et al. 2007).

In the prenatal human brain, we detected a nearly ubiquitous distribution of NRDc. This finding supported from a morphological viewpoint the notion that NRDc might play pivotal roles in brain development (Fumagalli et al. 1998; Nishi et al. 2001; Jin et al. 2002; Ohno et al. 2009). Beginning with the 18th gestational week, NRDc was immunohistochemically localizable in multiple neuroblasts and non-neuronal cells (most probably radial glia cells). In perinatal and adult brain, NRDc was confined to multiple neurons. In addition, a weak immunoreaction was detectable in some white matter oligodendroglial cells (Hiraoka et al. 2007) and a few astrocytes (Bernstein et al. 2007). Unexpectedly, double immunostaining experiments revealed that NRDc is not expressed in somatostatin-28 immunoreactive hypothalamic and cortical neurons. Interestingly, a considerable overlap was established between NRDc and $p42^{IP4}$ expression. With regard to the regional distribution, we detected that NRDc is widely, but unevenly distributed within human brain. NRDc immunopositive neurons were found in all brain areas studied. The highest density of NRDc expressing cells was found in discrete hypothalamic nuclei, followed by cerebellum, brain stem, and neocortex (Bernstein et al. 2007, 2009a).

A suitable tool for learning more about functional implications of an enzyme for brain functions is to generate knock out mice. When doing so to the *Nrd1* gene, Ohno and colleagues registered that the majority of NRDc-deficient mice died within 48 h after birth and exhibited considerable growth retardation, which clearly points to an important pleiotropic role for NRDc (Ohno et al. 2009). It was observed that Nrd1 null mice had smaller brains and a thin cerebral cortex. Further, there were less myelinated nerve fibres with thinner myelin sheaths and smaller axon diameters in these animals. In addition to the changes in the CNS, hypomyelination was also present in the peripheral nervous system of Nrd1 null mice. Interestingly, neuron-specific over-expression of NRDc had the opposite effect, namely fibre hyper-myelination. Behaviourally, NRDc knock out mice had impaired motor activities and cognitive deficits. On the basis of these data, Ohno et al. (2009) proposed that NRDc is prominently involved in the regulation of axonal maturation and myelination in the CNS and PNS, in part, through the modulation of neuregulin-1 (NRG-1) shedding.

NRDc in the diseased human brain

NRDc in neurodegenerative disorders

Sporadic Alzheimer's disease (AD)

AD is a progressive degenerative encephalopathy, which is clinically characterized by profound behavioural disturbances, loss of memory, and reasoning, as well as personality changes. Neuropathological hallmarks of AD are accelerated atrophy and loss of neurons, reduction in synapses on surviving neurons, deposition of amyloid in neuritic plaques and within the walls of cerebral microvasculature, and appearance of neurofibrillary tangles (Braak and Del Tredici 2012). More than 95 % of the AD cases are sporadic in origin (discussed in Bernstein 2005).

To our knowledge, the first report linking NRDc to the pathophysiology of AD came from Maes et al. (2007), who found the expression of the NRDc encoding gene up-regulated in blood mononuclear cells of AD patients. In the same year, Hiraoka et al. (2007) showed in cell culture experiments that NRDc is able to greatly enhance the α-secretase activity of the ADAMs (a disintegrin and metalloproteases) 10 and 17. A prerequisite for the physiological significance of the α-secretase (ADAMs)-stimulating effect of NRDc in vivo is certainly the cellular co-expression of NRDc with ADAM10 and/or ADAM17. When analysing NRDc expression in cortical areas of AD and control brains, we estimated a significantly decreased numerical density of immunopositive neurons in AD (Bernstein et al. 2009a). Furthermore, by co-labelling experiments, we could show that in normal aged brains, depending on the cortex area and the cortical layer, 25–35 % of NRDc immunoreactive neurons co-express ADAM10, and 20–25 % of ADAM10-containing neurons are also immunopositive for NRDc. In AD brain, there was a significant reduction of co-expression in certain cortical layers. In addition, a majority of the diffuse and neuritic plaques were either immunoreactive for NRDc or ADAM10. In normal aged brains, the portion of NRDc immunoreactive cells co-expressing ADAM17 was in the same range as for ADAM10, whereas only about 15 % of ADAM17 immunoreactive neurons were also labelled for NRDc. In AD brains, a significant reduction in the density of double-labelled neurons was found in layers II and V/VII. Identified neuritic plaques never contained ADAM17. We concluded from our findings that (i) a molecular interaction of NRDc and ADAM10 and/or ADAM17 within the same cell in human brain neurons is quite possible and (ii) this interaction might be compromised in AD, which might contribute to the altered α-secretase expression/activity found in AD (Colciaghi et al. 2004; Hooper and Turner 2002; Bernstein et al. 2003, 2009a; Endres and Fahrenholz 2012; Lerner et al. 2012; Bekris et al. 2012; Lichtenthaler 2012; Vincent and Checler 2012). Since α-secretase activity represents an amyloid precursor protein (APP) processing pathway that prevents the formation of toxic Aβ peptides from APP and gives rise to the neurotrophic and neuroprotective cleavage product APPs-α (reviewed in Endres and Fahrenholz 2010, 2012), the ADAMs stimulating effect of NRDc can be regarded as

neuroprotective. Recently, Ohno (2011) could demonstrate that in mice with forebrain-specific NRD over-expression, which were inter-crossed with AD model mice (over-expressing the APP mutant APP695swe and a mutant presenillin 1), there was a reduced number of plaques compared to the number found in AD model animals. This result shows convincingly that NRDc is capable of preventing amyloid plaque formation by enhancing α-secretase activity in vivo (Ohno 2011). Thus, finding a way to simultaneously increase α-secretase (ADAMs) and NRDc activities would be a powerful tool to enhance the non-amyloidogenic APP pathway. Such a "killing-two-birds-with-one-stone" strategy is probably the use of retinoic acid in AD therapy (for clinical trials, see Ono and Yamada 2012). It is well known that retinoids may influence APP processing by stimulating the expression of the putative α-secretase ADAM10 (Endres and Fahrenholz 2012; Lerner et al. 2012), what makes retinoic acid administration a treatment option for AD. Interestingly, however, retinoids also stimulate NRDc expression (Borrmann et al. 2011a), which in addition to the direct stimulation of ADAM10 activity might help further to facilitate the non-amyloidogenic APP pathway. Whether NRDc, via its interaction with the putative β-secretase BACE1 (Ohno et al. 2009), also promotes the formation of amyloid remains to be established. Besides its influence on APP processing, NRDc might contribute to AD pathophysiology because of its neuropeptide-splitting activity. Dysregulation of dynorphins and elevated levels of the somatostatin-28, which appear in parallel to a general somatostatin deficit, have repeatedly been reported in AD (for review, see Bernstein et al. 2009a). Although there is only little, if any, overlap of NRDc and somatostatin-28 immunoreactive neurons in human brain (Bernstein et al. 2007), a reduced somatostatin-28 metabolizing function of NRDc (Csuhai et al. 1998) cannot be ruled out in AD. Lastly, NRDc has been demonstrated to interact with $p42^{IP4}$/centaurin-α1 (Stricker et al. 2006). Centaurin-α1 was found to be up-regulated in AD (Reiser and Bernstein 2002, 2004). Recent studies show that it is required for Aβ-induced loss of dendritic spines (Szatmari et al. 2013), thus playing a prominent role in AD pathophysiology. Thus, interaction between NRDc and centaurin-1α might contribute to brain structural damage observed in AD.

Down syndrome

Down syndrome (DS, trisomy 21) is a disease which is caused by the presence of a third copy of chromosome 21, where the gene coding for APP is located. DS is associated with a decrease in cognitive ability (mental retardation) and retarded physical growth. Neuropathologically, DS comprises AD-like lesions such as the massive appearance of neuritic plaques (reviewed in Cheon et al. 2008). Similar to the situation in sporadic AD, we found strongly decreased neuronal NRDc expression in DS. Compared to unaffected children, the density of NRDc immunoreactive neurons in the cortex of one and 6-year-old DS children was already reduced. In the brains of adult DS patients, the density of NRDc immunoreactive neurons was well below that of controls. Single NRDc immunoreactive neuritic plaques were observed in adult DS cases. Moreover, cell counts revealed a significant reduction (up to 65 % of controls) of double-labelled neurons in all layers of the superior temporal cortex. Some neuritic plaques in adult DS cases contained ADAM10 only, others were immunoreactive for both NRDc and ADAM10 (Bernstein et al. 2009a).

Hence, NRDc seems to have a protective effect in both AD (as highlighted in Gough et al. 2011) and DS due to its interaction with the ADAM10.

No other neurodegenerative disorders in humans have yet been identified as implying abnormal NRDc activity and/or cellular distribution.

Neuropsychiatric disorders

Schizophrenia

Schizophrenia is a debilitating mental illness caused by a combination of genetic factors and environmental insults. Human NRDc gene is localized on chromosome 1, at location 1p32.2p32.1. Already, in 2003, two patients with a deletion of p32.1p32.3 on the short arm of chromosome 1 were described, who showed intracranially a diffusely thinned corpus callosum, increased T2 signal in periventricular white matter and an enlarged 3rd ventricle, as well as a delay in neurodevelopment and serious learning difficulties (Zinner and Batanian 2003). Cognitive deficits, hypo-myelination, and enlarged ventricles (as seen in Nrd1 null mice and the aforementioned patients) are key hallmarks of schizophrenia (reviewed in Bernstein et al. 2009b). Moreover, genome-wide linkage analysis has shown that NRDc is associated with an increased risk of schizophrenia (Fallin et al. 2003). Putting these findings together, and keeping in mind the interaction between NRDc and neuregulin-1 (NRG-1, Ohno et al. 2009), a well-replicated schizophrenia susceptibility gene (for a recent comprehensive and balanced review on this topic, see Banerjee et al. 2010), it is conceivable that NRDc plays a role in the pathophysiology of schizophrenia. We therefore studied post-mortem brains of medicated individuals with schizophrenia ($N = 8$) and matched controls ($N = 11$). We determined the number and density of NRDc- and NRG-1-expressing neurons, and the co-expression of NRDc with (pan) NRG-1, or the specific isoforms NRG-1α, and NRG-1β, in the dorso-lateral prefrontal and anterior cingulate

cortex of medicated schizophrenics and controls (Bernstein et al. 2011, see Fig. 1a–e). Compared to controls, we found a nearly 50 % increase in the density of NRDc immunoreactive neurons in both prefrontal areas in schizophrenia. This increase was most pronounced in cortical layers 3, 4, and 5. In control brains, double immunolabelling revealed that NRDc is co-localized with (pan) NRG-1 (overlap between both antigens 85 ± 3 %) and with NRG-1 isoform 1β (overlap 88 ± 7 %). NRG-1α, which was found to be restricted to a limited number of human brain grey and white matter interstitial neurons (Bernstein et al. 2006; Connor et al. 2009), was co-localized with NRDc in about 65 % of the neurons. As shown in Table 1, in schizophrenia, the number of NRG-1β expressing neurons was significantly higher than in controls, which is in full accordance with a previous report (Law et al. 2004), whereas the number of NRG-1α is lower (Bertram et al. 2007). However, the percentage of nerve cells which co-expressed NRDc and NRG-1α or NRG-1β did not differ between cases with schizophrenia and controls. Taking into account the "schizophrenia-like" behavioural and anatomical properties of NRDc-deficient mice mentioned above, we had expected to find a reduction in NRDc expression in patients with schizophrenia. Instead, an increased density of NRDc immunoreactive neurons in schizophrenia was observed. However, this is perfectly in line with increased mRNA levels for NRDc that have been measured in different brain areas in schizophrenia (Akil et al. 2006). Ohno et al. (2009) in their experiments found that over-expression of NRDc in rodents leads to hyper-myelination, which definitely does not occur in schizophrenia. However, our schizophrenia patients had received long-term treatment with neuroleptics. Therefore, an effect of medication on cerebral NRDc expression is possible. Neuroleptics are known to increase the expression of the NRG isoform 1β, but not 1α, in rat brain (Wang et al. 2008). Antipsychotic treatment may also in part be responsible for the observed increase of NRG-1β in schizophrenia. Further, up-regulation of NRDc in neurons from patients with schizophrenia could be a compensatory mechanism counteracting oligodendrocyte cell loss and myelination impairment, which are frequently observed in schizophrenia (Bernstein et al. 2009b). Thus, the high degree of cellular overlap of (pan) NRG-1/NRG-1β and NRDc expression, as we observed in human brain neurons, gives strong neuromorphological support the notion proposed by Ohno and colleagues that NRDc is an important modulator of the shedding of NRG-1. In summary, there is some evidence for a role of NRDc in the pathophysiology of schizophrenia, possibly by mediating the shedding of neuregulin-1. However, further studies are clearly needed to substantiate these preliminary results.

Mood disorders

There are two groups of mood disorders (also called affective disorders): unipolar (major depression) and bipolar disorder (BD). The division is based on whether a manic or hypomanic episode has ever been observed. During depressive episodes, individuals may experience a plethora of symptoms, including persistent feelings of sadness, hopelessness or helplessness, serious cognitive impairment, and suicidal thoughts or attempts (Lin et al. 2013). Very little is yet known about the possible implication of NRDc for affective disorders. One recent report, however, shows that NRDc is down-regulated (−1.21-fold) in brains of individuals with BD (Konradi 2012).

Substance abuse

Alcoholism

Alcoholism (aka alcohol abuse or alcohol dependence) is a complex psychiatric disease, which is behaviourally characterized by compulsive and uncontrolled consumption of alcoholic beverages. Usually, alcohol abuse has tremendous consequences for the drinker's health, personal relationships, and social standing. As to the brain, excessive alcohol intake can cause structural and functional abnormalities, including grey and white matter shrinkage, as well as neuronal alterations and degeneration (reviewed in Crews and Nixon 2009). There are at least four good reasons to assume that NRDc might play a role in the pathomechanisms of alcohol dependence: (i) a recent meta-analysis has provided strong evidence in favour of a genetic link between NRDc1 and alcohol dependence (Wang et al. 2011), (ii) some of the enzyme's substrates and interaction partners are differently expressed in neural and non-neural tissues under the influence of ethanol intake (dynorphin, Przewłocka et al. 1992; Chang et al. 2007, 2010), α-neoendorphin (Przewłocka et al. 1992), somatostatin-28 (Ferriero et al. 1992), TNF-α (Chen et al. 2006), and malate dehydrogenase (Crow et al. 1982), (iii) HB-EGF has been shown to be protective against ethanol-induced cell pathology (Nash et al. 2009), and (iv) NRDc mRNA is slightly increased in the Nuc. accumbens (but not in the amygdala) of alcohol-preferring rats (Rodd et al. 2008). Recently, we could show a significantly decreased expression of NRDc in human neuroblastoma cells under the influence of ethanol stress and a reduction in neuronal densities of the NRDc protein in discrete brain regions in severe alcoholics. NRDc expression was significantly reduced after 96 h of SH-SY5Y cells exposure to 200 mM ethanol. In heavy drinkers, there was a significantly reduced density of NRDc immunoreactive neurons in Nuc. basalis of Meynert, hypothalamic paraventricular (PVN),

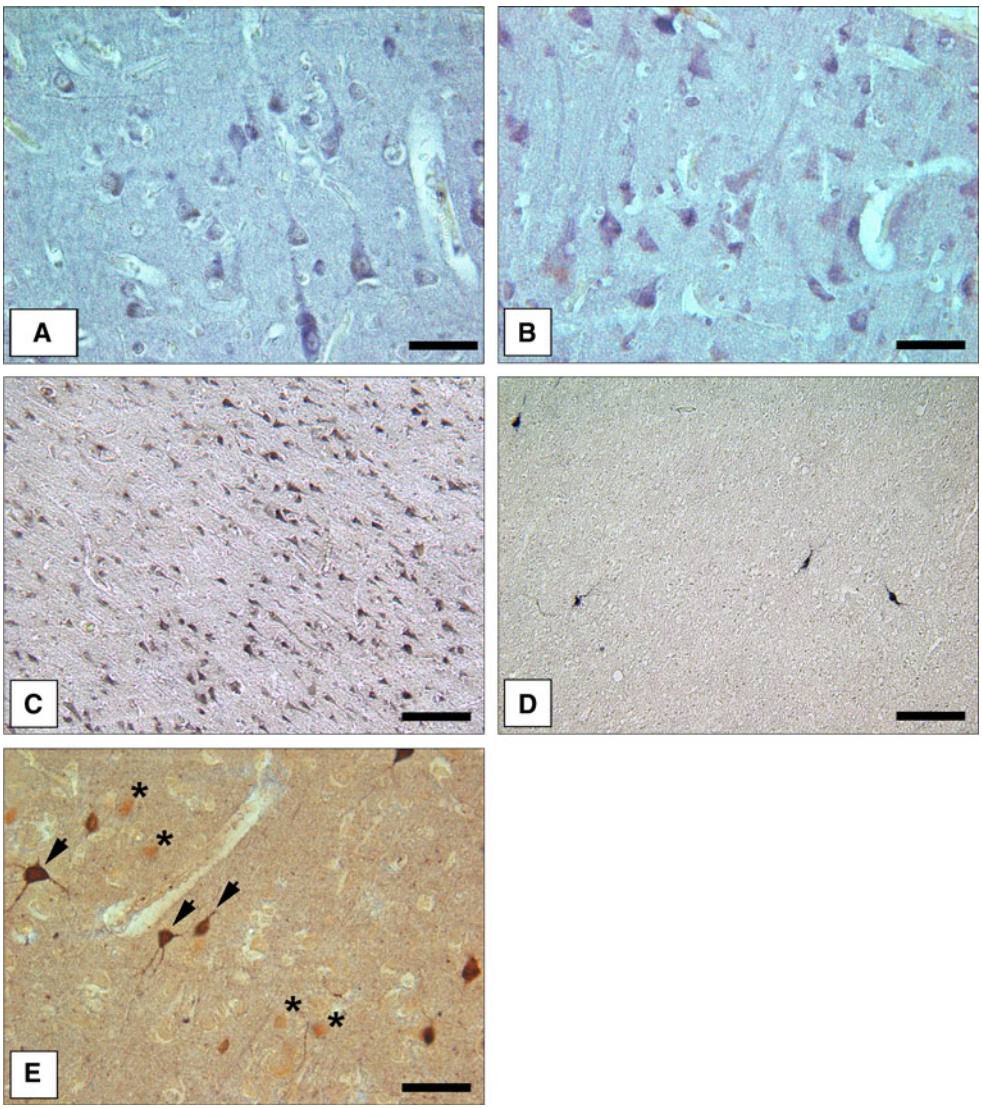

Fig. 1 NRDc and NRG immunoreactive prefrontal neurons in brains of schizophrenics and control cases. **a** NRDc expressing neurons in the DLP of a control case. *Bar* 40 μm. **b** Multiple NRG-1β expressing neurons in the DLP of an individual with schizophrenia. *Bar* 40 μm. **c** White matter neurons immunoreactive for NRG-1α. *Bar* 120 μm. **d** Low-power microphotograph showing multiple NRG-1β immunoreactive neurons in the DLP in schizophrenia. *Bar* 120 μm. **e** Double immunolabelling for NRDc and NRG-1β. *Black–brown* neurons express both antigens (*arrows*); *golden brown* neurons contain NRG-1β, but not NRDc (*asterisks*). *Bar* 60 μm

and supraoptic (SON) nuclei, but not in cortical areas (Bernstein et al. 2013). These alcohol-dependent reductions of NRDc in cell culture and nervous tissue point to an implication of the enzyme in the pathophysiology of alcoholism. However, the precise pathomechanisms underlying this decrease have to be established in forthcoming studies.

Opiate addiction

Opiate drugs exert their effects by binding to three different opioid receptor types (μ, δ, and κ) and mimicking the actions of endogenous opioid peptides (endorphins, endomorphins, enkephalins, and dynorphins). The μ-opioid receptor subtype is critical for the rewarding effects of heroin and morphine. Blockade of μ-opioid receptor but not other opioid receptors attenuates opiate self-administration (De Vries and Shippenberg 2002). Replicated findings showing a prominent involvement of the dynorphin-neoendorphin system in opioid and cocaine abuse and withdrawal (recently reviewed in Clarke et al. 2012) prompted us to look for possible alterations in the neuronal NRDc expression in post-mortem brains of heroin victims. We investigated eight persons (five males, three females; aged 24 ± 7 years) that died after a "golden shot" (heroin overdose). All had a long history of heroin abuse and,

Table 1 Density (Mean ± SD cells/mm^3) of NRDc, NRG-1β, and NRG-1α immunoreactive neurons in the left and right hemispherical dorso-lateral prefrontal (DLP) and anterior cingulate cortex (ACC) in schizophrenic (S; $N = 8$) and control (C; $N = 11$) groups

	NRDc	NRG-1β	NRG-1α
DLP left			
C	720 ± 120	680 ± 90	9.95 ± 2.3
S	1,410 ± 210***	940 ± 150**	4.6 ± 2.2***
DLP right			
C	840 ± 110	750 ± 85	10.0 ± 2.4
S	1,210 ± 160***	990 ± 110**	4.3 ± 2.1***
ACC left			
C	642 ± 90	635 ± 112	9.20 ± 1.9
S	850 ± 105***	890 ± 140*	5.5 ± 2.0***
ACC right			
C	630 ± 140	740 ± 160	10.3 ± 2.1
S	950 ± 160***	1,050 ± 130**	6.2 ± 2.0*

Significance: * $p < 0.05$, ** $p < 0.02$, *** $p < 0.01$

Table 2 Density (Mean ± SD cells/mm^3) of NRDc immunoreactive neurons in the left and right hemispherical paraventricular (PVN) and supraoptic (SON) nuclei in individuals addicted to heroin (AH; $N = 8$) and controls cases (C; $N = 8$)

Hypothalamic nucleus	NRDc expressing neurons
PVN left	
C	7,410 ± 410
AH	6,160 ± 380**
PVN right	
C	7,840 ± 355
AH	6,580 ± 420***
SON left	
C	6,330 ± 290
AH	5,850 ± 405 n.s.
SON right	
C	6,570 ± 310
AH	5,525 ± 280***

n.s. not significant

Significance: ** $p < 0.02$, *** $p < 0.01$

possibly, other illegal drugs. Eight individuals without a history of drug abuse (four males, four females; aged 32 ± 4 years) served as a control group. All control persons died of natural causes. Qualitatively, NRDc expression in hypothalamic neurons of heroin victims was much weaker than in control cases (Fig. 2a–d). When counting NRDc immunopositive neurons in hypothalamic PVN and SON nuclei, we found a statistically significant reduction in the numerical density in the left and right PVN and the right SON (Table 2). This is the first evidence for a

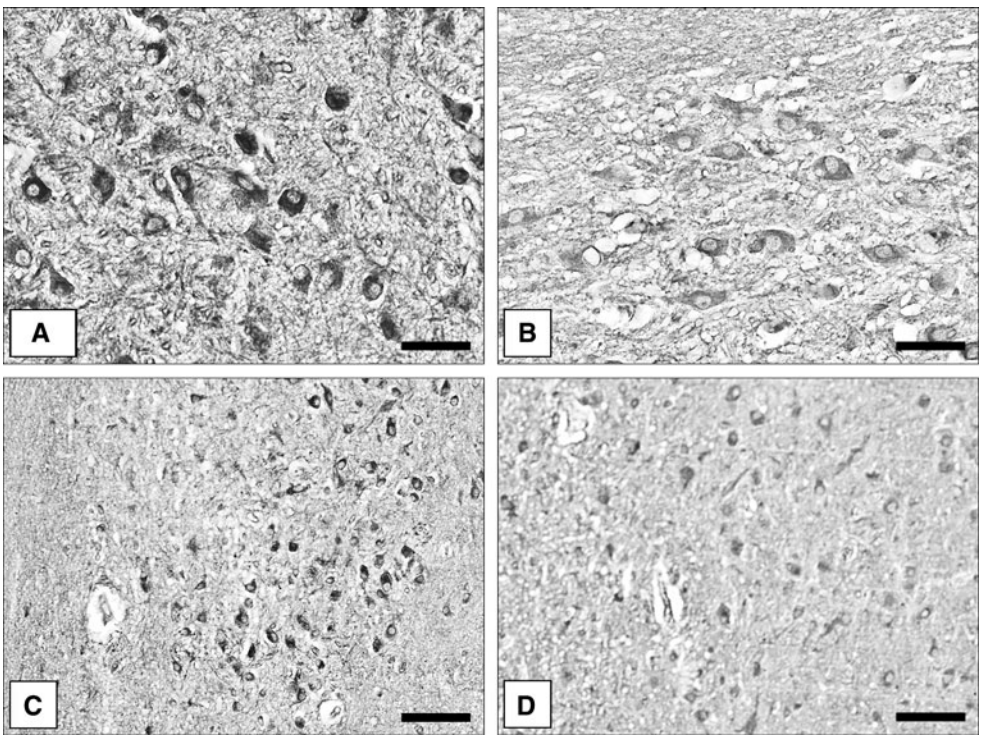

Fig. 2 NRDc expressing hypothalamic neurons in individuals addicted to heroin and control cases. **a** Moderate NRDc immunoreactivity in SON neurons of a control case. *Bar* 30 μm. **b** Weak NRDc immunoreactivity in SON neurons of an individual addicted to heroin. *Bar* 30 μm **c** Moderate NRDc immunoreactivity in PVN neurons of a control case. *Bar* 80 μm. **d** Weak NRDc immunoreactivity in PVN neurons of an individual addicted to heroin. *Bar* 80 μm

possible contribution of NRDc to brain alterations in heroin addiction.

Brain tumours

All intracranial solid neoplasms are referred as brain tumours. NRDc has been shown to play prominent roles in some extracranial tumours, such as breast cancer (Nishi et al. 2001) and gastric cancer (Kanda et al. 2012). In particular, its interaction with p53 and its mutants influences the invasiveness of the tumour cells (Coffill et al. 2012). There is yet no direct clinical or experimental evidence in favour of an involvement of NRDc in brain tumour development and/or progression. However, there is data showing that deletions of, and mutations in, the chromosome region where the NRDc gene is located are frequently found in meningioma (Sulman et al. 1998) as well as in some astrocytoma and oligodendroglioma (summarized in Ichimura et al. 2009). This and the replicated high expression of NRDc in neuroblastoma cells (Draoui et al. 1997; Hiraoka et al. 2007; Borrmann et al. 2011a, b; Bernstein et al. 2013) make NRDc a—yet possibly underestimated—candidate for brain tumour pathology.

Conflict of interest None.

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