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Cathepsin K generates enkephalin from β-endorphin: A new mechanism with possible relevance for schizophrenia

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ABSTRACT

Recent studies associate cathepsin K with schizophrenia. The endogenous substrates of this protease, however, remain to be identified. We show here that cathepsin K is capable of liberating met-enkephalin from β-endorphin (β-EP) in vitro. To verify if this process might possibly contribute in the pathogenesis of schizophrenia post mortem brains of patients suffering from this disease were analysed immunohistochemically for the presence and co-localization of cathepsin K and β-EP. In support of a functional role of the observed formation of met-enkephalin on the expense of β-EP increased numbers of cathepsin K immunoreactive cells, but diminished numbers of both β-EP-positive cells and double-positive (cathepsin K/β-EP) cells were found in left and right arcuate nucleus of schizophrenics. In addition a reduced density of β-EP-immunoreactive neuropil (fibres, nerve terminals) was estimated in the left and right paraventricular nucleus (PVN) of individuals with schizophrenia. Our results imply that cathepsin K, which becomes up-regulated in its cerebral expression by neuroleptic treatment, might significantly contribute to altered opioid levels in brains of schizophrenics, which have previously been reported by us and others, and might reinforce the interest in the putative roles of endorphin and enkephalins in neuropsychiatric disorders.

1. Introduction

There is preliminary evidence to suggest that the cysteine protease cathepsin K (EC 3.4.22.38) might play a role in schizophrenia (Bernstein et al., 2007). Its expression in the brain appears to be affected by the medication that patients suffering from schizophrenia receive. Cathepsin K was among the few genes the expression of which was found to be oppositely regulated by neuroleptics (haloperidol and clozapine) or amphetamine in the rat (Ko et al., 2006). Moreover, the human cathepsin K gene is located on chromosome 1q21.2, a region known to be linked to schizophrenia and bipolar disorders (for review see Craddock et al., 2005). We previously demonstrated a wide but uneven distribution of cathepsin K in adult rat and human brain (Bernstein et al., 2007). Cathepsin K expression was observed in multiple neurons as well as in astrocytes and white matter oligodendrocytes. Interestingly, individuals with chronic schizophrenia showed increased hypothalamic and striatal expression of cathepsin K when compared to matched controls (Bernstein et al., 2007). This increased expression may well be due to the long-term medication with haloperidol rather than resulting from the disease as such (Ko et al., 2006; Fehér et al., 2005).

Unfortunately, nothing is known yet about the precise functions of the enzyme in the brain. Cathepsin K is well-known for its collagenase activity. The crystal structure of cathepsin K in a complex with chondroitin sulfate has been resolved just recently (Li et al., 2008). It plays a prominent role in the turnover of bone tissue, which is of particular interest since patients suffering from schizophrenia have been reported to suffer from osteoporosis (Kishimoto et al., 2005). To what extent a dysregulated cathepsin K expression might be responsible for a decrease of bone density that is generally attributed to life habits of the patients (such as unbalanced diet, smoking and alcohol addiction, Kishimoto et al., 2005) remains to be resolved. Very recent data obtained in a mouse model of obesity demonstrate a direct participation of cathepsin K in mouse body weight gain and glucose metabolism (Yang et al., 2008). Cathepsin K via degrading fibronection, a matrix protein controlling adipogenesis, promotes lipid accumulation in human

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and mouse preadipocytes, a process that could be blocked by selective cathepsin K inhibition. In vivo, both cathepsin K deficiency and selective inhibition of cathepsin K in diet-induced and genetically created (ob/ob) obese mice reduced diet-induced body weight gain and serum glucose and insulin levels (Yang et al., 2008).

Remarkably, cathepsin K has been reported to be capable of cleaving neuroactive peptides (i.e. bradykinin and other kinins) (Godat et al., 2004). The apparent increase of cathepsin K immunoreactivity in brain areas rich in neuropeptides (and especially in endogenous opioids; Bernstein et al., 2007) of haloperidol-treated schizophrenics encouraged us to look for possible opioid-degrading activities of the enzyme. Special emphasis was given on β-endorphin as substrate, since the cellular expression and brain tissue concentrations of β-endorphin and some of its proteolytic fragments (Emrich, 1984; Wiegant et al., 1992; Bernstein et al., 2002; De Wied and Sigling, 2002), but not those of met-enkephalin (Zech and Bogerts, 1985; Mosnaim et al., 2008) were found to be altered in schizophrenia. In this study we demonstrate that cathepsin K proteolytically processes the neuropeptide β-endorphin *in vitro* leading to the formation of met-enkephalin. Furthermore, we show here by means of immunohistochemistry that cathepsin K appears partially co-expressed with β-endorphin in hypothalamic arcuate nucleus neurons, and that in haloperidol-treated schizophrenics, where the cellular expression of cathepsin K is up-regulated in these cells, the neuronal expression of β-endorphin immunoreactivity is reduced.

2. Materials and methods

2.1. Reagents

Pro-cathepsin K was obtained from InViTek (Berlin, Germany). Human β-endorphin was purchased from Bachem AG (Weil am Rhein, Germany).

2.2. Subjects

Case recruitment, acquisition of personal data, performance of autopsy, and handling of autoptic material were conducted in accordance with the Declaration of Helsinki, and have been approved by the responsible Ethical Committee of Magdeburg. Post mortem brains of seven patients with schizophrenia according to DSM-II-R (five males, two females, mean age = 50.9 years) and seven brains of controls without signs of neurological or psychiatric diseases (four males, three females, mean age = 49.5 years) were neuroanatomically studied. All brains were obtained from the New Magdeburg brain collection. Schizophrenic patients died between years 1988 and 1993, control individuals between years 1986 and 1999. A lifetime psychiatric diagnosis of schizophrenia was established according to DSM-III-R (American Psychiatric Association, 1987) by using the Diagnostic Instrument for brain studies (Dean et al., 2000), a structured instrument for the collection of clinical, pharmacological and other relevant information from case histories.

All subjects with schizophrenia had histories of inpatient hospitalization. Only patients with well preserved and extensive clinical records were selected for the present study. Demographic and histological data are summarized in Table 1. The mean duration of illness was 19.9 years with a range from 8 and 28 years. Since cathepsin K is regarded a marker of adiposity (Chiellini et al., 2003; Funicello et al., 2007) we selected only controls and cases with schizophrenia who had a normal body mass index (well below the recently calculated cut-off value of 28.7 kg/m^2 for the metabolic syndrome; Tirupati and Chua, 2007; Bernstein et al., 2008) in order to exclude an influence of a disturbed fat metabolism on the cerebral expression of the enzyme. Knowing body weight and height of all cases with schizophrenia controls we were able to determine individual body mass indices (Table 1). Importantly, the average body mass index (BMI) of lean controls (23.7 ± 2.1 kg/m^2) did not significantly differ from that of cases with schizophrenia (24.0 ± 2.2 kg/m^2).

All patients received antipsychotic medication during the course of their disease (haloperidol). Control brains of patients without a history of neuropsychiatric disorders were obtained from the same pathological institutes or medical examiner's office. Brains with lifetime reports of abuse of alcohol, drugs, dementia, neurological illness, trauma, chronic terminal disease known to affect the brain were excluded. Additionally, quantitative neuropathological changes due to neurogenerative disorders were ruled out by an experienced neuropathologist as earlier described (Danos et al., 2003). Brain volumes were calculated by the weight method using fresh whole brain weight and brain density (Yamada et al., 1999). There were no significant differences (Student's t test, χ^2 analysis) between patients with schizophrenia and controls with regard to age, gender, and storage delay.

2.3. Influence of haloperidol on cathepsin K expression in tissue culture

The human SH-SY5Y neuroblastoma cell line was purchased from DSMZ (Braunschweig, Germany; ACC 209) and grown to 80% confluency in Dulbecco's modified Eagle's medium (DMEM; PAA Laboratories, Linz, Austria), supplemented with 15% heat-inactivated fetal calf serum (PAA). To study the effect of haloperidol, cells were seeded into 6-well plates at a density of 2×10^6 cells/3 ml and cultured in the absence or presence of haloperidol as indicated in the figure for 24 h. Cells were then washed twice in PBS and RNA preparation was started by directly adding the lysis buffer of the RNeasy Mini Kit (Qiagen, Hilden, Germany). RNA was prepared according to the manufacturer's instructions. A 1 μg quantity of total RNA was transcribed into cDNA. A 25 μl reaction mixture consisted of 1× SensiMix (Quantace, UK), 0.5 μl of SYBR-Green I (Quantace), 1 μl cDNA, and 0.3 μmol/l of the specific primers for cathepsin K (Hs_CTSK_1_SG QuantiTect Primer Assay; Qiagen, Hilden, Germany), pro-opiomelanocortin (forward: CTACTCCATggAgCACTTCC; reverse: CgTCTggCTCTTCTCggAgg), pro-enkephalin A (forward: ggAgCTCCTgCAgCTgTC; reverse: gTCggAgggCAgAgCCTC), GAPDH (forward: TCCAAAATCAAgTggggCgATgCT; reverse: ACCACCTggTgCTCAgTgTgACCC, BioTeZ, Berlin, Germany). PCR was performed in an iCycler (BioRad, Munich, Germany). Initial denaturation and polymerase activation at 95 °C for 10 min was followed by 40 cycles with denaturation at 95 °C for 30 s, annealing at 60 °C for 30 s, and elongation at 72 °C for 60 s.

2.4. Tissue processing and immunohistochemistry

The brains for histological and immunohistochemical analysis were processed in a standard manner, including immersion-fixation in 8% phosphate-buffered formaldehyde for 2 months, embedded in Paraplast and cut with a microtome (whole-brain coronal sections, 20 μm thickness; Bernstein et al., 1998). Volume shrinkage was determined for each brain before and after dehydration and embedding of tissue. Volume shrinkage factors were calculated using the formula: $VF = (A_1/A_2)^{3/2}$ (VF = volume shrinkage factor; A_1 = cross-sectional area before processing of tissue; A_2 = cross-sectional area after processing of tissue). Sections at

Table 1
Demographical data of the patients.

Case/sex (m/f) age (years)	Post mortem interval (h)	Storage time (months)	Cause of death	BMI (kg/m^2)
Control cases				
1/f/49	28	7.0	Myocardial infarction	22.7
2/m/56	30	7.3	Pulmonary embolism	20.2
3/m/45	33	4.5	Heart failure	25.4
4/f/61	48	5.0	Pulmonary embolism	25.0
5/f/38	24	7.9	Pulmonary embolism	24.0
6/m/46	37	9.6	Heart failure	23.9
7/m/50	22	7.0	Heart failure	24.0
Patients with schizophrenia				
1/m/49	24	12.1	Heart failure	24.0
2/f/60	48	4.8	Acute heart failure	25.3
3/f/53	31	3.6	Myocardial infarction	27.2
4/m/46	48	6.9	Pulmonary embolism	22.2
5/m/51	38	9.6	Ileus	20.9
6/m/49	28	6.5	Myocardial infarction	24.2
7/m/48	40	6.8	Suicide (hanging)	25.3