SHORT COMMUNICATION

Cerebrospinal fluid concentrations of ghrelin in patients with multiple sclerosis

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Abstract

Ghrelin, an orexigenic peptide, exerts immunomodulatory and other effects in the CNS and has neuroprotective properties. Ghrelin is predominantly produced by X/A-like cells in the gastric mucosa. Hence, ghrelin's main source of production lies outside the CNS while important functions of ghrelin are operated by specific receptors in the CNS. We analyzed 82 samples of our repository (45 samples form patients with multiple sclerosis and 37 control samples) for cerebrospinal fluid (CSF) and corresponding serum concentrations of ghrelin. Desacyl ghrelin concentrations were measured with a commercially available enzyme immunometric assay. We validated the assay for CSF samples. The test-retest reproducibility for ghrelin in CSF samples was excellent. Ghrelin CSF concentrations were higher in patients with multiple sclerosis ($p<0.02$) compared with controls. CSF concentrations correlated with serum concentrations in patients with multiple sclerosis ($p<0.01$). No such correlation was found in controls. Our findings endorse existing hypotheses that ghrelin affects the central inflammatory process in MS. The correlation between serum and CSF concentrations in MS, but not in controls, suggests a differential regulation of blood-to-brain transport mechanisms for ghrelin in MS and indicates that central effects of ghrelin in MS might be amenable to pharmacological manipulation of the systemic ghrelin secretion.

INTRODUCTION

Ghrelin, the endogenous ligand for the growth hormone secretagogues receptor (GHSR) (Kojima *et al.* 1999), acts orexigenic (Asakawa *et al.* 2001), controls energy homeostasis and modulates gastrointestinal motility (Masuda *et al.* 2000; Edholm *et al.* 2004). In addition, ghrelin has immunomodulatory (Granado *et al.* 2005; Waseem *et al.* 2008) and neuroprotective (Jiang *et al.* 2008; Andrews *et al.* 2009) properties and influences CNS functions like cognition (Carlini *et al.* 2002), reward-associated behaviour (Naleid *et al.* 2005; Jerlhag *et al.* 2011) and sleep (Weikel *et al.* 2003; Kluge *et al.* 2010). Specific receptors for ghrelin (GHSR) have been described in various CNS regions (Zigman *et al.* 2006). The major source of ghrelin production is X/A-like cells in the gastric mucosa (Date *et al.* 2000).

Experimental data suggest that circulating ghrelin enters the brain by different (saturable and non-saturable) transporter systems as well as by

transmembrane diffusion. Banks and colleagues have shown in a mouse model that the extent to which circulating ghrelin enters the brain is determined by the physiological state and by the presence of blood-borne factors (Banks *et al.* 2008). Banks *et al.* conclude that blood-brain-barrier transport of ghrelin might be modified under pathophysiological conditions.

There are no data on the blood-brain-barrier permeability of ghrelin in humans. Only two studies investigated ghrelin cerebrospinal fluid (CSF) concentrations in healthy and diseased humans (Tritos *et al.* 2003; Popovic *et al.* 2004). Elevated ghrelin serum concentrations have been reported previously in patients with multiple sclerosis (Berilgen *et al.* 2005) and the authors hypothesized that elevated circulating ghrelin concentrations might counteract the proinflammatory process in CNS.

Considering that i) ghrelin is produced mainly outside the CNS, ii) the extent of ghrelin's transport across the blood-brain-barrier is complex and modifiable and iii) important biological functions are regulated by binding to specific receptors in the CNS, we investigated CSF and serum concentrations of ghrelin in controls and patients with multiple sclerosis.

METHODS

Subjects and samples

We analyzed CSF and corresponding serum samples of our biosample repository. The study was approved by the Ethical Committee of the Philipps-University Marburg, Germany. All subjects provided written informed consent that specimens may be used for scientific purposes. CSF and corresponding serum samples from 45 patients with multiple sclerosis (according to the revised McDonald Criteria 2005) (Polman *et al.* 2005) and 37 CSF and corresponding serum samples from controls were analyzed in this study. We did not differentiate between the 4 clinical subtypes of MS (i.e. relapsing-remitting MS, secondary-progressive MS, primary-progressive MS or progressive-relapsing MS). Control subjects were defined as having normal CSF findings for cell count, glucose, lactate and protein and as having no symptoms nor a history of inflammatory CNS disease or other neurological disorders. To prevent a potential bias caused by the high blood-CSF gradient for ghrelin, we excluded artificially ensanguined CSF samples.

Determination of ghrelin concentrations

Desacyl ghrelin concentrations were measured with a commercially available enzyme immunometric assay for desacyl ghrelin serum concentration (EIA, catalogue #A05119, SPI Bio, Montigny le Bretonneux, France) according to the manufacturer's instructions. We performed additional experiments to test the reliability of this assay for CSF samples. The test-retest reproducibility for desacyl ghrelin in CSF samples (10 samples measured in triplicate) was excellent (Pearson correlation coefficient: 0.809, $p<0.005$). Stability of desacyl ghrelin in frozen samples was assessed by freezing and thawing. Desacyl ghrelin concentrations remained stable over 4 cycles of freezing and thawing. Spike-recovery experiments with CSF samples showed a recovery >80%.

Statistical analyses

PASW statistics software version 19.0 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. For comparison of serum ghrelin concentrations we performed an analysis of covariance (ANCOVA) corrected for age, gender and serum albumin concentration. For comparison of CSF ghrelin concentrations we performed an ANCOVA corrected for age, gender, serum ghrelin concentration and albumin quotient (CSF/serum). We used partial correlation corrected for age, gender and albumin quotient (CSF/serum) for correlation analysis.

RESULTS

Demographic data and the mean desacyl ghrelin serum and CSF concentrations of the investigated subjects are summarized in Table 1. Ghrelin concentrations were approximately 50fold lower in CSF compared to the corresponding serum concentrations. There was no statistical difference between MS patients and controls in ghrelin serum concentrations (260.7±135.6 pg/mL versus 265.1±177.8 pg/mL; $p<0.75$). Ghrelin CSF concentrations were higher in MS patients compared with controls ($p<0.02$). The partial correlation analysis (corrected for age, gender and albumin quotient (CSF/serum)) revealed a statistically significant correlation between serum and CSF concentrations in the MS group (partial correlation coefficient 0.45, $p<0.01$). No such correlation was found in the control group (partial correlation coefficient 0.29). The correlation between ghrelin serum and corresponding ghrelin CSF concentrations is illustrated in Figure 1 as scatter blot of individual values.

DISCUSSION

We report increased ghrelin CSF concentrations as well as a correlation between serum and CSF concentrations in patients with multiple sclerosis.

We validated the desacyl ghrelin enzyme immunometric assay for CSF samples and our experiments show an excellent reproducibility for CSF samples. We controlled for a potential bias caused by the high blood-CSF gradient by excluding ensanguined CSF samples and by correcting for differences in blood-brain-barrier disintegrity in the statistical analysis. Our findings endorse the existing hypothesis that ghrelin affects the central inflammatory process in MS (Berilgen *et al.* 2005). The correlation between serum and CSF concentrations as well as the significantly higher CSF concentrations in MS suggests a differential regulation of blood-brain-

Tab. 1. Demographic data of the investigated subjects and group-specific mean values of serum desacyl ghrelin and CSF desacyl ghrelin.

	Controls	MS patients	*p*-value
Number of subjects	n=37	n=45	
Gender (male/female)	9/28	11/34	
Median Age in years [range]	43 [19-88]	30 [18-70]	
Mean desacyl ghrelin serum concentrations in pg/ml [± SD]	265.1±177.8	260.7±135.6	$p<0.75$
Mean CSF desacyl ghrelin concentrations in pg/ml [± SD]	3.8±2.0	4.6±2.5	$p<0.02$

barrier permeability and blood-to-brain transport systems for ghrelin. This concept of a differentially regulated blood-brain-barrier permeability for ghrelin has already been suggested by Banks *et al.* in a mouse model of starvation and obesity (Banks *et al.* 2008).

In contrast to a previously published study by Berilgen and colleagues (Berilgen *et al.* 2005) we did not find elevated serum ghrelin concentrations in MS. One reason for this divergent finding might be that we measured desacyl ghrelin concentrations, while Berilgen *et al.* measured total ghrelin concentrations. We did not investigate acyl ghrelin in addition to desacyl ghrelin concentrations because CSF concentrations of acyl ghrelin were below the threshold of detection. Ghrelin serum concentrations are subjected to a dynamic regulation in response to the individual's energy status. The investigated samples from our repository (that were collected at different times of day) are not suitable for comparing absolute serum concentrations. Having this in mind, the focus of this study was to compare CSF concentrations of two groups with very similar serum concentrations. We also controlled for blood-brain-barrier disintegrity in the statistical analyses. This implies that the finding of elevated CSF concentrations in MS is unlikely to be due to differences in serum concentrations or due to blood-brain-barrier disintegrity in MS patients.

In conclusion, our experiments show that desacyl ghrelin concentrations in human CSF can be measured reliably with a commercially available assay. Our data suggest a differential blood-to-brain transport for ghrelin in MS that occurs independently of MS-related blood-brain-barrier disintegrity. Another explanation for the elevated desacyl ghrelin CSF concentrations in patients with MS is an enhanced autochthon production of ghrelin in the CNS. The correlation between circulating and CSF concentrations of ghrelin in MS suggests that central effects of ghrelin are influenced by *circulating* ghrelin concentrations and might therefore be amenable to pharmacological manipulation of ghrelin secretion.

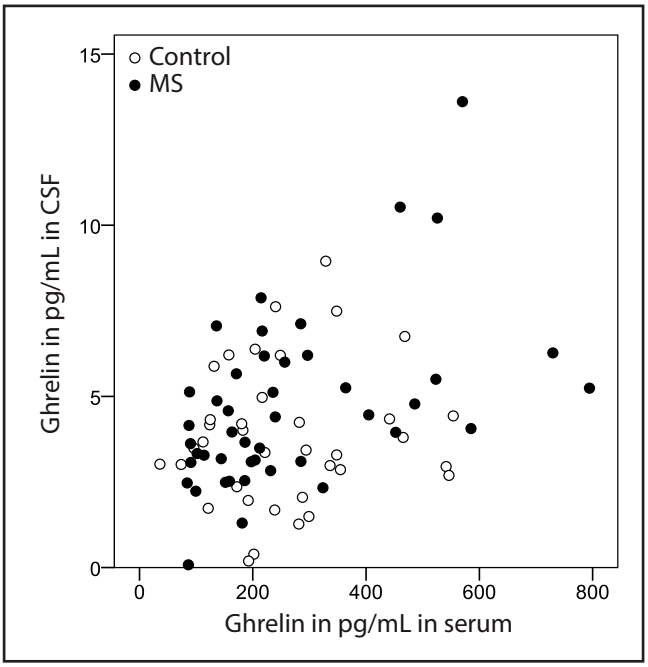

Fig. 1. Scatter plot of individual desacyl ghrelin serum concentrations and corresponding CSF concentrations. Black dots represent MS patients, circles represent controls.

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