

Claudio L. Bassetti
Martin Hersberger
Christian R. Baumann

CSF prostaglandin D synthase is reduced in excessive daytime sleepiness

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Prof. Dr. med. C. L. Bassetti (✉) ·
Ch. R. Baumann, MD
Department of Neurology
Universitätsspital Zürich
Frauenklinikstrasse 26
8091 Zürich, Switzerland
Tel.: +41-1/255-55-03
Fax: +41-1/255-43-80
E-Mail: claudio.bassetti@usz.ch

M. Hersberger
Institute of Clinical Chemistry
Universitätsspital
Zürich, Switzerland

■ **Abstract** Lipocalin-type prostaglandin D synthase (L-PGDS) is a brain enzyme, which produces prostaglandin D₂, a substance with endogenous somnogenic effects. Using a standardized protocol for immunonephelometric determination of cerebrospinal fluid (CSF) L-PGDS levels, we show that CSF L-PGDS levels are significantly lower in 34 patients with excessive daytime sleepiness when compared with levels in 22 healthy controls. Thus, L-PGDS may represent the first neurochemical measure of excessive daytime sleepiness.

■ **Key words** hypersomnia · excessive daytime sleepiness · prostaglandin · betatrace · sleep · narcolepsy

Introduction

Excessive daytime sleepiness (EDS) is a common symptom, which affects up to 5% of the population. The differential diagnosis of EDS includes sleep deprivation, sleep-associated breathing disorders, and such neurological disorders as narcolepsy, idiopathic hypersomnia, and posttraumatic hypersomnia.

The pathophysiology of EDS is unknown in most of these conditions. No neurochemical measure of EDS is known. In narcolepsy, an abnormal hypocretin transmission with low cerebrospinal fluid (CSF) levels of hypocretin-1 (orexin A) was recently discovered [1]. Since hypocretin deficiency (1) is usually detected only when cataplexy is present and (2) is rare in non-narcoleptic patients with EDS, other neurochemical pathways are probably implicated in EDS [2, 3].

The enzyme lipocalin-type prostaglandin D synthase (L-PGDS; also known as β -trace) is involved in sleep-wake regulation in rodents, primates and humans [4]. L-PGDS is synthesized in the brain and secreted into the CSF, where it is the sole source of prostaglandin D₂ (PGD₂), which has potent somnogenic effects. In normal conditions, a 32:1 CSF-serum concentration gradient has been found for L-PGDS [4]. In humans, serum L-PGDS concentrations show a circadian pattern and are reduced during sleep deprivation; furthermore, CSF PGD₂ is increased in African sleeping sickness [5, 6].

We hypothesized that changes in CSF L-PGDS levels may be found in patients with EDS secondary to narcolepsy, other neurological disorders, and chronic sleep deprivation.

Subjects and methods

Subjects

We enrolled 14 patients with narcolepsy with cataplexy (EDS-NC; seven women, seven men, mean age 36 years, range 19–63, SD 14), 16 patients with EDS secondary to other (mainly neurological disorders (EDS-N; five women, 11 men, mean age 37 years, range 20–67, SD 13), four patients with EDS due to chronic sleep deprivation (CSD; four men, mean age 40 years, range 30–66, SD 17), and 22 controls without sleep-wake disturbances or other neurological disorders (11 women, 11 men, mean age 34 years, range 17–60, SD 13) in this study, which was performed according to local ethical committee standards. All subjects gave informed consent for the study.

Sleep disorders were diagnosed clinically and using sleep studies, according to international criteria [7]. Narcolepsy with cataplexy was diagnosed by sleep questionnaires and additional investigations (including hypocretin-1 determination in CSF, HLA phenotyping in serum, polysomnography, multiple sleep latency tests). Diagnoses in EDS-N included idiopathic hypersomnia (n=4), periodic hypersomnia (n=1), narcolepsy without cataplexy (n=2), posttraumatic hypersomnia (n=4), postischemic/postinfectious hypersomnia (n=1/1), familial hypersomnia of unknown origin (n=2), and EDS with Parkinson's disease (n=1). In CSD patients, no sleep-wake or neurological disorders were identified. None of our patients or controls had normal pressure hydrocephalus, spinal canal stenosis, posttraumatic or postinfectious leptomeningeal dysfunctions, or any significant cardiovascular, neurological or renal disorder known to influence L-PGDS levels.

Methods

All patients were medication-free; a drug washout of 14 days had been performed prior to the examinations. We assessed the severity of EDS subjectively by the Epworth sleepiness scale, and objectively by the multiple sleep latency test (MSLT). L-PGDS levels were determined in the CSF with albumin as reference protein by an immunonephelometric assay (Dade-Behring GmbH, Marburg, Germany) on an automated analyzer [8]. Lumbar CSF was collected only between 10 am and 1 pm to minimize the influence of circadian variation of L-PGDS levels. Thereafter, CSF was immediately frozen at -80°C for storage, and crude CSF was not defrosted until determination of L-PGDS. Measurements of all samples were performed in the same assay to prevent influence of inter-assay variability, and have been repeated in a second and third assay. CSF hypocretin-1 determinations were performed by a commercially available radioimmuno assay (Phoenix Pharmaceuticals, Belmont, CA, USA; determination procedure: as described before) [9]. Furthermore, we determined CSF/serum albumin ratio to assess the integrity of the blood-brain barrier [10].

Statistical analyses were performed by Student's t-tests, ANOVA, and Pearson correlation tests. Significance was declared at $p < 0.05$ level.

Results

The results are summarized in figure 1 and table 1. Mean Epworth Sleepiness Scale scores were 15.9 (SD 3.6) in EDS-NC, 13.8 (SD 5.5) in EDS-N, and 14.0 (SD 2.0) in CSD. Mean sleep latencies on MSLT were 2.3 minutes (SD 1.7) in EDS-NC, 4.3 minutes (SD 2.6) in EDS-N, and 3.8 minutes (SD 0.8) in CSD. CSF

hypocretin-1 levels were undetectable/low in 11 of 14 EDS-NC patients, and normal in all other subjects.

L-PGDS levels in EDS-NC (mean 14.7 mg/L, range 7.0–19.0, SD 3.8, $p=0.002$), EDS-N (mean 16.6 mg/L, range 11.0–24.8, SD 3.8, $p=0.011$), and CSD (mean 13.8 mg/L, range 12.9–15.9, SD 1.4, $p=0.042$) were significantly lower than in controls (mean 21.3 mg/L, range 11.7–36.8, SD 6.8). These results have been confirmed by repeated determinations with a second and third assay. Mean levels did not differ significantly between EDS-NC, EDS-N and CSD patients. In the control group, CSF L-PGDS levels were higher in men (mean 22.7 mg/L) than in women (mean 19.8 mg/L), but this finding was not significant ($p=0.33$). Within the narrow time interval of CSF withdrawal, there was no association between L-PGDS levels and the time of lumbar puncture.

There were no correlations between CSF L-PGDS levels and age, Epworth Sleepiness Scale score, or mean sleep latency on MSLT. However, mean sleep latencies and L-PGDS levels were lower in EDS with undetectable CSF hypocretin-1 levels (n=9; mean sleep latency: 1.7 minutes, SD 1.0; mean L-PGDS 13.1 mg/L, SD 3.3) than in patients with detectable levels (n=5; mean sleep latency: 4.2 minutes, SD 2.5; mean L-PGDS 16.9 mg/L, SD 3.6). CSF hypocretin-1 levels correlated positively with L-PGDS levels (n=56, $r=0.43$, $p=0.001$). Mean CSF/serum albumin ratio in EDS patients (EDS-NC and EDS-N) was 4.6 (SD=1.3, range 2.1–6.8), compared with the mean of 4.3 (SD=1.1, range 2.2–5.9) in the controls ($p=0.62$). Mean CSF/serum albumin ratios did not correlate with CSF L-PGDS levels.

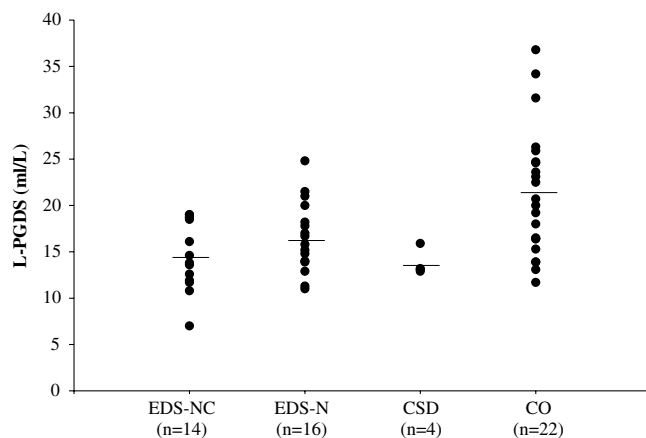


Fig. 1 L-PGDS levels in 34 EDS patients and 22 controls. L-PGDS levels (β -trace, in mg/L) in 14 patients with narcolepsy-cataplexy (EDS-NC), 16 patients with excessive daytime sleepiness (EDS) due to other neurological disorders (EDS-N), 4 patients with EDS due to chronic sleep deprivation (CSD), and 22 controls (CO). Horizontal lines indicate mean levels

Table 1 Patients' characteristics EDS-NC: patients with narcolepsy-cataplexy, EDS-N patients with excessive daytime sleepiness due to other neurological disorders, CSD: patients with chronic sleep deprivation, CO: controls. Hcrt: hypocretin-1 levels in pg/ml. ESS=Epworth sleepiness scale. n.a.=not available

Diagnosis	n	Sex m:f	Age (years)			ESS		MSL (min)		L-PGDS (mg/L)			Hcrt (pg/mL)	
			mean	range	SD	mean	SD	mean	SD	mean	range	SD	mean	SD
EDS-NC	14	7:7	36	19–63	14	15.9	3.6	2.3	1.7	14.7	7.0–19.0	3.8	125	230
EDS-N	16	11:5	37	20–67	13	13.8	5.5	4.3	2.6	16.6	11.0–24.8	3.8	470	139
CSD	4	4:0	40	30–66	17	14.0	2.0	3.8	0.8	13.8	12.9–15.9	1.4	495	113
CO	22	11:11	34	17–60	13	n.a.	n.a.	n.a.	n.a.	21.3	11.7–36.8	6.8	526	102

Discussion

Our data demonstrate that L-PGDS activity – as reflected by lumbar CSF levels – is decreased in EDS secondary to narcolepsy, other neurological disorders, and chronic sleep deprivation. Considering the recent finding of decreased serum L-PGDS levels during total sleep deprivation, [5] these observations suggest a reduction (downregulation?) of the prostaglandin D system activity in the brain in the presence of EDS and increased sleep propensity. The discrepancy between our finding of low CSF L-PGDS levels (which has been replicated in repeated measurements) and the recent report of increased serum levels in narcoleptic patients cannot be explained yet [11]. The finding of discrepant serum and CSF L-PGDS concentrations was recently found to be useful to detect CSF leaks [12]. Similarly, earlier reports found that CSF L-PGDS levels can be used in conjunction with CSF/serum albumin ratio to distinguish between different neurological pathologies associated with CSF protein increase and blood-brain barrier dysfunctions [13]. Due to our finding of similar CSF/serum albumin ratios in EDS patients and in controls, however,

we assume that alterations in the blood-brain barrier function may not be the primary cause for our finding. Thus, further studies to determine simultaneously L-PGDS in serum and CSF are required.

The large overlap in CSF L-PGDS levels between EDS patients and controls indicates that this test may not serve as a diagnostic tool for EDS in clinical practice.

Conclusion

This study does not allow to elucidate the role of L-PGDS in the pathophysiology of EDS. Considering the neurochemical complexity of sleep-wake regulation and the heterogeneity of disorders included in our study, it is likely that the link between decreased L-PGDS levels and EDS is complex. Rather we postulate a decrease of L-PGDS activity reflecting the interaction, recently shown experimentally, between the prostaglandin D system, arousal-promoting monoaminergic neurons in the brainstem, and sleep-active neurons in the preoptic area [14,15].

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