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The hypocretin neurotransmission system in myotonic dystrophy type 1

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ABSTRACT

Background: Patients with myotonic dystrophy type 1 (DM1) frequently have symptoms of excessive daytime sleepiness (EDS). Some patients with DM1 show sleep-onset REM, similar to that observed in narcolepsy. Narcolepsy is characterized by impaired hypocretin (Hcrt) neurotransmission.

Objective: To test for dysregulation of Hcrt neurotransmission in a prospective cohort of patients with DM1.

Methods: Hcrt levels in CSF were measured by radioimmunoassay. Sleep physiology was assessed by overnight polysomnography (PSG) and a multiple sleep latency test (MSLT). Splicing of Hcrt receptor 1 and 2 (HcrtR1 and HcrtR2) mRNA was examined in postmortem samples of temporal cortex.

Results: Seventeen of 38 patients with DM1 reported symptoms of EDS. Among patients with DM1 with EDS who underwent PSG/MSLT, 7 of 13 showed reduced sleep latency, sleep-onset REM, or both. However, CSF Hcrt levels in DM1 (mean 277 pg/mL, n = 38) were not different from controls (mean 277 pg/mL, n = 33). Also, splicing of HcrtR1 and HcrtR2 mRNA in patients with DM1 was similar to controls.

Conclusions: Excessive daytime sleepiness and dysregulation of REM sleep occur frequently in patients with myotonic dystrophy type 1 (DM1). However, the pathophysiologic basis is distinct from narcolepsy, as patients with DM1 do not have a consistent defect of Hcrt release or receptor splicing. *Neurology*® 2008;70:226–230

GLOSSARY

AHI = apnea/hypopnea index; **DM1** = myotonic dystrophy type 1; **EDS** = excessive daytime sleepiness; **ESS** = Epworth Sleepiness Scale; **Hcrt** = hypocretin; **MIRS** = Muscular Impairment Rating Scale; **MSL** = mean sleep latency; **MSLT** = multiple sleep latency test; **PLM** = periodic limb movement; **PSG** = polysomnography; **SL** = sleep onset latency; **SOREMP** = sleep onset REM periods; **UM** = University of Milan, Italy; **URMC** = University of Rochester Medical Center.

Myotonic dystrophy type 1 (DM1) is caused by expansion of a CTG repeat in the 3′ untranslated region of the *DMPK* gene on chromosome 19. The mutant RNA forms ribonuclear inclusions,[1] sequesters splicing factors in the muscleblind family,[2] and interferes with regulated alternative splicing of pre-mRNA.[3] For example, myotonia in DM1 is associated with misregulated alternative splicing of the ClC-1 chloride channel.[4,5] A similar RNA-mediated disease process may underlie CNS symptoms of DM1.[6,7] Toxicity of expanded repeat RNA has also been implicated in other neurodegenerative disorders.[8,9]

Excessive daytime sleepiness (EDS) is a common and disabling feature of DM1.[10-12] However, the pathophysiology of the sleep disturbance is poorly understood. In some individuals with advanced disease, EDS may result from effects of DM1 on oropharyngeal and respiratory muscles, leading to obstructive sleep apnea and nocturnal hypoven-

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tilation.[13,14] Several lines of evidence suggest that DM1 may also have direct effects on sleep regulatory circuits in the CNS.[15-22]

Narcolepsy is associated with decreased levels of hypocretin 1 (Hcrt-1) in the CSF and loss of Hcrt-1-releasing neurons in the dorsolateral hypothalamus.[23] Hereditary narcolepsy in dogs is caused by mutations that affect splicing in the hypocretin receptor 2 (HcrtR2) gene.[24] Recently it was reported that Hcrt-1 was reduced in CSF in patients with DM1[25] and that sleep onset REM, similar to that observed in narcolepsy, was observed in patients with DM1.[15,16] To determine if DM1 is associated with abnormal Hcrt-1 action, we carried out a prospective study of CSF Hcrt-1 in patients with DM1 who did or did not have EDS.

METHODS

Study participants. Our cohort consisted of 38 patients with genetically confirmed DM1. Fifteen subjects were women and 23 were men (mean age 43 years, range 24 to 72 years). Twenty-five patients were enrolled at the University of Milan, Italy (UM), and 13 patients were enrolled at the University of Rochester Medical Center (URMC). The study was approved by respective Institutional Review Boards in both centers, and informed consent was obtained from all participants. Patients were excluded if they had contraindication for lumbar puncture, evidence for major respiratory involvement (vital capacity <60% of predicted), advanced muscle weakness (inability to walk 30 feet independently), congenital myotonic dystrophy, and use of medications known to affect wakefulness or sleep. Patients with a prior diagnosis of major depression, sleep apnea, or using nocturnal bilevel positive airway pressure system or CPAP were also excluded because of the EDS and altered sleep architecture that can occur in such patients. The duration of DM1 symptoms in our cohort ranged from 6 to 25 years. Muscle weakness ranged from mild to moderate (Muscular Impairment Rating Scale [MIRS] 2, 3, 4, and 5).[26]

Demographic characteristics, ESS, and MIRS did not differ between the UM and URMC patients. Patients were interview screened for cataplexy.

Symptoms of sleepiness were assessed using the Epworth Sleepiness Scale (ESS), an 8-item self-reported questionnaire with a possible score ranging from 0 to 24.[27]

A *t* test was used for comparison between groups.

Hcrt-1 assays. Lumbar puncture was performed in the morning and CSF was immediately stored at −80 °C. Hcrt-1 levels were measured at the Stanford Center for Narcolepsy using a direct radioimmunoassay with I^{125}-labeled Hcrt-1 as previously described.[23] The CSF Hcrt-1 values in patients with DM1 were compared with 33 non-DM1 controls who did not have symptoms of a sleep disorder. The presence of HLA DQB1*0602 was determined using previously established PCR assays in the 13 URMC patients.[23]

Evaluation of daytime sleepiness and sleep-related breathing disorders. In addition to lumbar puncture, 13 URMC patients underwent overnight polysomnography (PSG) followed by a Multiple Sleep Latency Test (MSLT).

Sleep scoring. PSGs were scored in 30-second epochs according to Rechtschaffen and Kales criteria.[28] PSG scorers identified apnea/hypopnea and periodic limb movement (PLM) events based on standard criteria (American Academy of Sleep Medicine guidelines).[29] These events were tabulated to form an apnea/hypopnea index (AHI) and PLM index. A complete description of recording and scoring for the polysomnogram and MSLT is provided in the supplemental methods section on the *Neurology*® Web site at www.neurology.org.

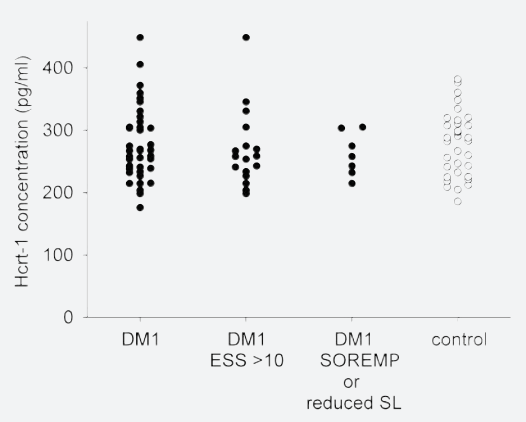
MSLT. This procedure was performed on the day following the PSG utilizing a four-nap protocol beginning no later than 10 AM and interspersed by 2-hour intervals. Standard procedures were used as specified by Carskadon et al.[30,31] A mean sleep latency (MSL) value from the four sessions was calculated for each patient. The number of sleep-onset REM periods during the study was determined.

Analysis of Hcrt receptor (HcrtR) splicing. We examined splicing of HcrtR1 and HcrtR2 transcripts in RNA isolated from temporal cortex of two patients with DM1, one control with Huntington disease, and one control with no neurologic disease. Neither patient with DM1 had participated in the CSF Hcrt-1 study, but both had reported symptoms of excessive sleepiness. *HcrtR1, HcrtR2,* and *DMPK* are all expressed in temporal cortex. The cortical tissue was dissected at the time of autopsy, flash frozen in liquid nitrogen, and then stored at −70 °C. We selected DM1 samples that previously had clearly shown misregulated alternative splicing, as determined by splicing of tau, amyloid precursor protein, and NMDA receptor 1.[6] We screened the entire coding sequence of each receptor in two overlapping RT-PCR products (707 or 785 bp length). These products were analyzed on laser-scanned agarose gels as described.[2] To test for splicing alterations having a small effect on the size of HcrtR cDNAs, we also analyzed each RT-PCR product after digestion with several different restriction enzymes (*Taq*I, *Alu*I, *Dpn*II, *Nla*III, *Bgl*I, *Alw*NI, or *Afl*II).

RESULTS

Hcrt-1 levels in CSF. CSF Hcrt-1 levels in 38 patients with DM1 (mean 277 pg/mL, range 176 to 448 pg/mL) did not differ from 33 controls (mean 277 pg/mL, range 186 to 382 pg/mL) (figure 1). The mean ESS score in 38 patients with DM1 was 10.4 (range 3 to 24). ESS scores greater than 10 are generally taken to reflect abnormal daytime sleepiness.[27] No significant difference in Hcrt-1 level was found between the patients with an ESS > 10 (n = 17; mean Hcrt-1 level 268 pg/mL; mean ESS 15.4) as compared to those having an ESS < 10 (n = 21; mean Hcrt-1 284; mean ESS 6.4). Three of the 13 URMC patients (23%) were HLA-DQB1*0602 positive, a frequency that was similar to the general population (12% to 35%). Of note, neither ESS scores ($p = 0.68$) nor Hcrt-1 levels ($p = 0.25$) correlated with the length of the CTG expansion in circulating blood cells (data not shown).

Figure 1 CSF Hcrt-1 levels in patients with DM1 (n = 38), patients with DM1 with ESS > 10 (n = 17), patients with DM1 with multiple sleep latency test evidence of SOREMP or SL < 8 minutes (n = 7), and controls without a sleep disorder (n = 33)



Hcrt-1 = hypocretin 1; DM1 = myotonic dystrophy type 1; ESS = Epworth Sleepiness Scale; SOREMP = sleep onset REM periods; SL = sleep onset latency.

Table Hcrt-1 levels, percent of REM sleep on PSG, and MSLT data in 13 patients with myotonic dystrophy type 1

Patient ID	Hcrt-1, pg/mL	PSG, % REM	MSLT	
			Mean SL	SOREMP
1	274.4	33.2	3.0	2
2	240.6	11.3	20.0	0
3	233.3	36.3	19.0	0
4	231.7	12.6	7.6	1
5	257.7	30.6	18.4	1
6	198.2	33.4	10.3	0
7	266.8	27.6	9.9	0
8	242.5	34.8	6.8	2
9	226.3	38.5	8.0	0
10	267.3	29.0	8.3	0
11	304.6	37.9	1.5	4
12	214.4	31.9	4.0	0
13	303.0	39.7	1.8	0
Mean	250.8	30.5	9.1	0.8
SD	32.0	9.0	6.4	1.2

Hcrt-1 = hypocretin 1; PSG = polysomnography; MSLT = multiple sleep latency test; SL = sleep onset latency; SOREMP = sleep onset REM periods.

Overnight polysomnography. ***Sleep-related breathing disorders and periodic limb movements.*** Apnea, hypopnea, and periodic leg movements were not prominent in subjects with DM1 (table e-1 on the *Neurology*® Web site at www.neurology.org). DM1 patients exhibited a mean AHI of 5.9 (normal = 0 to 5; obstructive sleep apnea, mild = 5 to 20; moderate = 20 to 40; severe >40). Five subjects had AHIs between 5 and 20. The mean PLM index for the group was 7.2 with all subjects having a PLM index below 21.

Sleep continuity. When compared with normative values,[32] the mean value for sleep onset latency (SL) fell within the normal range in DM1 (mean DM1 = 18.7 minutes vs normal = 15 to 20 minutes) but this mean was driven by a very high SL in a single patient (no. 3; SL = 141 minutes). By removing this outlying datapoint, SL was reduced in subjects with DM1 (mean SL = 8.5 minutes) compared to normative data. Additionally, four patients had SL under 5 minutes (table e-1). Total sleep time (mean DM1 = 359.9 minutes vs normal = 375 to 425 minutes) was normal, and sleep efficiency (mean DM1 = 82.6 vs normal = 85 to 95%) was normal in all but three patients.

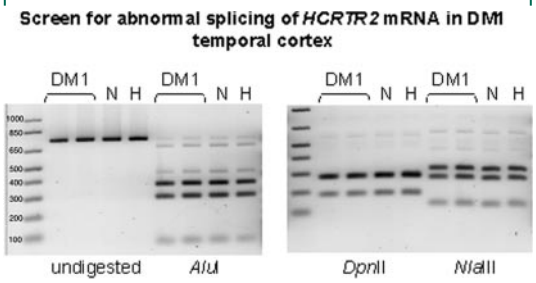
Sleep architecture. When compared with normative values,[32] the DM1 group exhibited a normal percentage of stage 1 sleep (DM1 = 6.1% vs normal = 2.5 to 7.5%) and stage 2 sleep (DM1 = 56.9% vs normal = 45 to 55%), whereas slow wave sleep was reduced (DM1 = 6.4% vs normal = 13 to 15%) (table e-2). The percentage of REM sleep was elevated in DM1 (DM1 = 30.5% vs normal = 20 to 25%), with 9 of 13 patients exhibiting a REM% above 30% (table). Finally, six patients had a REM latency less than 70 minutes, and in one patient the latency was less than 10 minutes (normal REM latency is 75 to 85 minutes).

Multiple sleep latency test. The mean MSLT sleep latency for patients with DM1 was 9.1, where a mean of ≤8 minutes is considered to represent pathologic sleepiness and means above 10 minutes are considered normal.[33,34] Seven subjects had mean sleep latencies ≤8 minutes, four of these were under 5 minutes, and three of the subjects with short latencies also had two or more sleep onset REM periods (SOREMPs) (table). Five subjects exhibited SOREMPs. There was no trend for reduced Hcrt-1 in patients whose MSLT testing showed reduced sleep latency or SOREMPs (figure 1).

Hcrt receptor splicing. Splicing of HcrtR1 and HcrtR2 mRNA in patients with DM1 was not different from controls. Representative gels for analysis for HcrtR2 are shown in figure 2.

DISCUSSION Several lines of evidence suggest that DM1 may also have direct effects on sleep regulatory circuits in the CNS: 1) EDS may occur early in the disease process, when oropharyngeal and respiratory muscles are relatively pre-

Figure 2 Screen for abnormal splicing in HcrtR2 mRNA in myotonic dystrophy type 1 (DM1) temporal cortex



DM1 = two patients; N = normal control; H = patient with Huntington disease.

served[15,16]; 2) polysomnographic abnormalities in DM1 do not consistently correlate with evidence of sleep-disordered breathing[17]; 3) adequate treatment of sleep-disordered breathing does not always improve EDS[18,19]; 4) pulsatile secretion of cortisol and growth hormone is disrupted in DM1, suggesting a general disturbance of hypothalamic regulation and circadian rhythm[20,21]; and 5) central hypoventilation and hypersomnia were correlated in some patients with DM1 with autopsy findings of neuronal loss in the reticular formation and dorsal raphe.[22]

Based on previous observations of reduced Hcrt-1[25] and SOREMP in patients with DM1,[15,16] we postulated that EDS in DM1 may result from decreased Hcrt-1 release or misregulated Hcrt receptor splicing. Against our hypothesis, we found that none of 38 patients with DM1 in our cohort showed Hcrt-1 reductions in a range that is typically associated with narcolepsy (below 110 pg/mL in CSF).[23] Indeed, the mean level of Hcrt-1 in patients with DM1 was identical to controls, and Hcrt-1 levels in CSF showed no trend for reduction even among patients with DM1 who reported symptoms of EDS or displayed MSLT evidence for reduced sleep latency or SOREMPs. Our cohort did include individuals whose EDS was severe. For example, Patient 11 in the table was given a diagnosis of narcolepsy many years prior to the diagnosis of DM1, due to her severe hypersomnolence (ESS 16). Her MSLT met criteria for narcolepsy yet her Hcrt-1 level was 304.6 pg/mL. Furthermore, we found no abnormality of splicing for Hcrt receptors 1 or 2, even in DM1 samples that clearly showed spliceopathy for other transcripts.

Results of the present study differ from a previous study of six patients with DM1, in which one patient had Hcrt-1 levels below110 pg/mL in CSF, and three patients had Hcrt-1 levels between 110 and 200 pg/mL,[25] a range that is intermediate between levels observed in normal individuals and patients with narcolepsy.[23] By contrast, only 3 of 38 patients with DM1 in the current study, as compared to 2 of 33 controls, had levels in this intermediate range. As Hcrt-1 measurements for both studies were carried out by the same methods in the same laboratory, it is unlikely that technical factors related to assay performance can account for this difference. More likely, this disparity relates to differences in patient selection or methods of CSF collection, storage, or transport.

Results of our study confirm that DM1 is associated with SOREMPs. In particular, several of our patients had short mean sleep latencies across all four naps in the MSLT study, five had SOREMPs, and two patients had mean sleep latency <5 minutes and ≥ 2 SOREMPs. These results are similar to previous retrospective analyses.[15,16] SOREMPs are a characteristic feature of narcolepsy. Our patients with DM1 did not report symptoms of cataplexy; however, the physiologic and anatomic substrates of REM intrusion and cataplexy components are separable.[35] PSG data in patients with narcolepsy is typified by low sleep efficiency, frequent awakenings, REM sleep fragmentation, and increased percentage of stage 1 sleep. By contrast, a novel finding in our study was that patients with DM1 displayed increased REM sleep rather than stage 1 sleep. These results support the idea that hypersomnolence in DM1 may result from an intrinsic CNS defect that is distinct from narcolepsy.

Effects of DM1 on splicing regulation result in re-emergence of splice products that are normally expressed at an earlier stage of development. More specifically, in adult DM1 tissue there is inappropriate expression of alternative splice isoforms that are characteristic of late fetal or neonatal development.[2,3,6] In this regard, it is noteworthy that increased REM sleep is typically seen in neonates.[36] Based on results of the present study, it is possible that EDS and increased REM sleep in DM1 does not result from deficiency of Hcrt-1, but rather from reversion to neonatal patterns of alternative splicing in sleep regulatory circuits of the CNS, such as the GABAergic flip-flop switch in the mesopontine tegmentum,[35] resulting in partial recapitulation of neonatal sleep patterns.

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