Hypocretin (orexin) deficiency in human narcolepsy

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Alterations in the hypocretin receptor 2 and preprohypocretin genes produce narcolepsy in animal models. Hypocretin was undetectable in seven out of nine people with narcolepsy, indicating abnormal hypocretin transmission.

The cause of human narcolepsy is unknown.[1,2] The disorder affects 0·03–0·1% of the general population[2] and is usually treated with monoaminergic amphetamine-like stimulants and antidepressants.[1] Narcolepsy is caused by an interplay of genetic and environmental factors, and is associated with HLA-DR2 and DQB1*0602.[2] Positional cloning has identified hypocretin receptor-2 gene mutations as the cause of narcolepsy in a canine model.[3] Additionally, preprohypocretin knockout mice have symptoms reminiscent of narcolepsy.[4]

We hypothesised that a disruption in hypocretin neurotransmission causes human narcolepsy. Immunoreactive hypocretin was measured in the cerebrospinal fluid (CSF) of nine people with narcolepsy (aged 48·6 [SD 14·4] years; 4 women) and 8 controls (40·3 [13·3] years; 5 women). All subjects gave their informed consent for the study. Volunteers were recruited through advertising in the newsletter of the Dutch Narcolepsy Patient Association and the Leiden University Medical Center and had a lumbar puncture between 9.30 am and 3.45 pm. The diagnosis of narcolepsy and the presence of cataplexy were confirmed by a physician experienced with narcolepsy (GJL). All patients were HLA-DR2/DQB1*0602 positive. Samples were immediately frozen, coded and shipped blindly to Stanford University. Hypocretin-1 was extracted from 1 mL of CSF (second fraction of 1·5 mL) with a reversed phase SEP-PAK C18 column. Iodine-125 hypocretin-1 radioimmunoassay (Phoenix Pharmaceuticals, Mountain View, CA, USA) was used to measure levels in reconstituted aliquots (duplicate analyses were done on all of the samples).

Hypocretin-1 was detectable in all controls, with little inter-individual variation (table) and no relation to the time of the lumbar puncture (data not shown). In seven of nine patients, however, hypocretin concentrations were below the detection limit of the assay (<40 pg/mL) (p=0·007, Mann-Whitney U test between groups). Undetectable levels were observed in patients regardless of duration of illness, medication, age, or gender. Two subjects with an unquestionable diagnosis of narcolepsy-cataplexy had similar (255 pg/mL) and elevated (638 pg/mL) levels compared with controls.

Our results show that hypocretin neurotransmission is deficient in some people with narcolepsy. These results, together with the observation that hypocretin receptor and peptide gene alterations induce narcolepsy in animal models,[3,4] suggest that hypocretin deficiency contributes to the development of the sleep disorder. In contrast to the canine and mouse models, however, human narcolepsy is rarely familial and typically involves environmental factors on an HLA susceptibility background.[2] Decreased hypocretin neurotransmission in these patients is thus not likely to be due to highly penetrant hypocretin mutations. Rather, an HLA-associated autoimmune-mediated destruction of hypocretin-containing neurons in the lateral hypothalamus might produce narcolepsy in these patients.

The 2 patients with detectable hypocretin-1 were both HLA-DQB1*0602 positive and clinically indistinguishable from the other narcoleptic patients. A possible explanation might involve receptor/effector mediated deficiency (as opposed to a defect in hypocretin production). Indeed, CSF hypocretin-1 levels are consistently detectable in hypocretin receptor-2 mutated narcoleptic dogs (dobermans) at levels

Subjects	Age (yrs)	Sex	MSLT		Cataplexy	Duration of illness (yrs)	Current pharmacological treatment (daily dose)	Hypocretin-1 (pg/mL)
			SL (min)	SOREMP				
Patients								
1	27	M	1·0*	3*	+	9	GHB 5·6 g/methylphenidate 5–10 mg	<40
2	34	M	0·9	5	+	4	untreated for 2·5 months	<40
3	39	F	2·0*	2*	+	1	Clomipramine 10 mg	<40
4	45	F	3·0	2	+	14	Methylphenidate 30 mg	255
5	50	M	6·3*	3*	+	19	Clomipramine 30 mg/GHB 3·0 g	638
6	50	M	1·2	3	+	32	GHB 5·4 mg/modafinil 400 mg	<40
7	53	F	1·2	1	+	19	GHB 4·0 g	<40
8	69	F	2·8	2	+	38	Clomipramine 10 mg/modafinil 200 mg	<40
9	70	M	2·1	2	+	53	untreated for 20 years	<40
Controls								
1	22	M	na	na	–	na	–	285
2	23	F	na	na	–	na	–	285
3	33	M	na	na	–	na	–	250
4	45	M	na	na	–	na	–	280
5	45	F	na	na	–	na	–	280
6	46	F	na	na	–	na	–	285
7	48	F	na	na	–	na	–	280
8	61	F	na	na	–	na	–	285

na=not applicable; MSLT=Multiple Sleep Latency Test; SL and SOREMP=Mean Sleep Latency and number of Sleep Onset REM Periods in 5 or 4 (marked by *) naps. All CSF cell counts, protein and glucose were within normal range. Recovery rate for the extraction of hypocretin-1 was 60·2 (3·8)%, and intra-assay variability for the measurement (extraction and RIA) was 3·8%. All samples were measured twice with comparable results.

Table: **CSF hypocretin-1 levels and clinical features of narcoleptic and control subjects**

similar to those found in control dobermans (narcoleptic, n=33 mean 273·5 [SD 33·3] pg/mL, control, n=9, 258·0 [19·8] pg/mL, unpublished data). The high hypocretin levels observed in patient no 5 may also indicate an upregulation of hypocretin-1 production.

How could hypocretin deficiency induce narcolepsy? Hypocretin neurons are discretely localised in the lateral hypothalamus, but have diffuse projections.[5] Of special interest are the dense projections to monoaminergic cell groups and the excitatory nature of this neuropeptide.[3,5] Hypocretin deficiency may decrease monoaminergic tone, an abnormality previously suggested to underlie the narcolepsy symptomatology, and could explain the beneficial effect of currently prescribed narcolepsy treatments. These findings may lead to treatments increasing hypocretin transmission.

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1 Nishino S, Mignot E. Pharmacological aspects of human and canine narcolepsy. *Prog Neurobiol* 1997; **52:** 27–78.
2 Mignot E. Genetic and familial aspects of narcolepsy. *Neurology* 1998; **50** (suppl 1)**:** S16–S22.
3 Lin L, Faraco J, Li R, et al. The sleep disorder canine narcolepsy is caused by a mutation in the hypocretin (orexin) receptor 2 gene. *Cell* 1999; **98:** 365–76.
4 Chemelli RM, Willie JT, Sinton CM, et al. Narcolepsy in orexin knockout mice: molecular genetics of sleep regulation. *Cell* 1999; **98:** 437–51.
5 Peyron C, Tighe DK, van den Pol AN, et al. Neurons containing hypocretin (orexin) project to multiple neuronal systems. *J Neurosci* 1998; **18:** 9996–10015.

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Malarial anaemia in African children associated with high oxygen-radical production

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Reactive oxygen intermediates are thought to be involved in both the illness and parasite destruction during malaria. We measured innately increased reactive oxygen intermediate production in Gabonese children with severe malaria and anaemia.

The underlying mechanisms of anaemia in malaria are still unknown. Phagocytosis, lysis of parasitised and uninfected erythrocytes, and impaired erythropoiesis occur.[1] During their immune response against malaria parasites, monocytes and granulocytes use reactive oxygen intermediates (ROI) which contribute to both parasite destruction and to pathological processes, including anaemia, as has been shown in mice models.[2]

To study the role of ROI production in human malaria we monitored ROI production in 100 Gabonese children attending the paediatric ward of the Albert-Schweitzer-Hospital in Lambaréné with severe *Plasmodium falciparum* malaria. The children, with mainly severe anaemia and hyperparasitaemia, were compared with 100 children matched for age and sex, with mild malaria. Inclusion criteria according to WHO definition and clinical parameters of both groups on admission are described elsewhere.[3] Venous blood samples were taken on admission and 6 months later, when children were healthy and aparasitaemic. As a measure of ROI production, luminol-enhanced chemiluminescence was assessed, and expressed in kilo relative light units (kRLU). We measured basal chemiluminescence in whole blood preparations and chemiluminescence after stimulation with phorbol-12-myristate-13-acetate (PMA), in whole blood and granulocyte preparations. Granulocytes were additionally stimulated with the chemotactic peptide N-formyl-methionyl-leucyl-phenylalanine (FMLP) and tumour necrosis factor (TNF).

	Median (median absolute deviation) chemiluminescence (kRLU×10³)		p*
	Mild malaria	Severe anaemia and hyperparasitaemia	
Admission			
Basal†	163 (122)	192 (147)	NS
PMA†	3458 (1214)	3183 (1549)	NS
FMLP†	749 (466)	539 (339)	NS
Basal‡	337 (256)	563 (413)	0·014
TNF‡	525 (405)	778 (585)	NS
Month 6			
Basal†	80 (48)	112 (83)	NS
PMA†	2263 (822)	2810 (1123)	0·009
FMLP†	441 (203)	781 (430)	0·004
Basal‡	160 (85)	232 (168)	NS
TNFF‡	275 (126)	511 (296)	0·009

NS=not significant. *Mann-Whitney *U* test. †Integral of 30 min interval. ‡Integral of 70 min interval.

Chemiluminescence in isolated granulocytes after stimulation

We found substantially increased basal and stimulated chemiluminescence in whole blood on admission, compared with 6 months later. Comparison of both groups during the acute attack revealed significantly higher chemiluminescence in the whole blood of children with severe disease (basal 3·2 *vs* 2·6 kRLU, p<0·005; after stimulation with PMA 51·8 *vs* 33·3 kRLU, p<0·01) whereas after 6 months, differences were not significant. In pure granulocytes, we measured a much higher basal chemiluminescence in children with severe anaemia and hyperparasitaemia on admission, and a higher stimulated ROI release when they were healthy 6 months later compared with children with mild malaria (table).

On admission, within the group of children with severe malaria, packed cell volume and basal chemiluminescence in

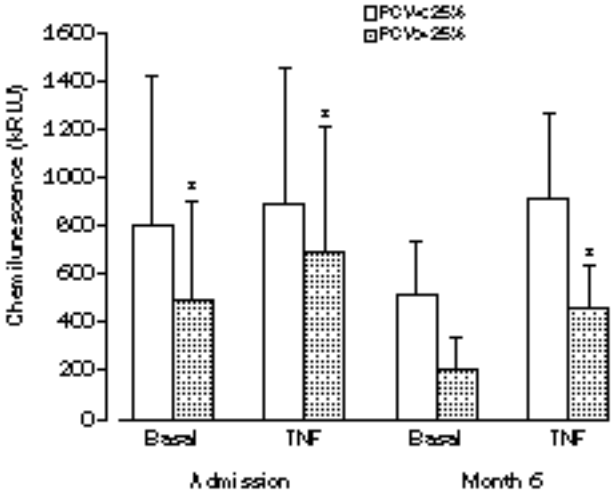

Figure 1: **Chemiluminescence after stimulation with TNF in severe malaria**
Isolated granulocytes of children with different packed cell volume on admission. Values are median and median absolute deviation. *p<0·05.