The neurobiology of narcolepsy

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Narcolepsy is characterized by excessive sleepiness and abnormal manifestations of rapid eye movement (REM) sleep. Neurochemical studies of human and canine narcolepsy have demonstrated disturbed monoaminergic and cholinergic function and suggest that deficits of noradrenaline availability in specific brain regions may account for much of its disordered pathophysiology. Genetic susceptibility to narcolepsy is closely linked to a specific region of the major histocompatibility complex on chromosome 6 and an important direction for future research will be to unravel the relationship between this gene region and the neurochemical abnormalities of narcolepsy.

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Although narcolepsy was first described more than 100 years ago, its pathophysiologic link to REM sleep was not recognized until the 1960s and its frequent association with a specific region of chromosome 6 has been known for less than ten years. This review provides an overview of the clinical syndrome and a discussion of current concepts of the genetics and neurochemistry of narcolepsy.

Symptoms of narcolepsy usually become apparent between 15 and 35 years of age and continue throughout life. Although the onset may be attributed to stress, head trauma, or minor infections, these apparent precipitants may be just coincidental. Sleepiness, the most disabling symptom, is most evident during boring, sedentary situations and is partially alleviated by stimulation; in these respects, it is similar to what is experienced by normal people after sleep deprivation. However, narcoleptic sleepiness differs in that it is virtually continuous and cannot be fully relieved by any amount of nighttime or daytime sleep. Sleep 'attacks', or episodes of spontaneous sleep, are the culmination of a progressive increase in sleepiness to overwhelming proportions. Excessive sleepiness is more characteristic than increased sleep, and periods of daytime sleep are usually brief.

Other common symptoms include cataplexy, sleep paralysis, hypnagogic hallucinations, and disturbed nocturnal sleep. Cataplexy, a brief episode of muscular weakness unassociated with altered consciousness, is usually brought on by excitement or emotion. Severe attacks cause complete paralysis, while milder episodes lead to partial weakness involving facial, ocular, or limb muscles. Sleep paralysis refers to episodes of inability to move during sleep onset or upon awakening, and hypnagogic hallucinations, which may accompany sleep paralysis, are dream-like images that differ from dreams in that awareness of surroundings is partially preserved.

The frequent occurrence of rapid eye movement (REM) sleep at the onset of sleep is one of the most striking features of narcolepsy and the discovery of this abnormality provided the first clue to its pathogenesis. REM sleep is characterized by cortical activation with desynchronized EEG, increased brain metabolism, skeletal muscle atonia, rapid conjugate eye movements, and dreams. Substantial evidence suggests that the intrusion of REM sleep atonia into wakefulness is the cause of cataplexy and sleep paralysis and that hypnagogic hallucinations are due to the intrusion of dream imagery into the waking state. Thus, dissociated manifestations of REM sleep appear to be responsible for many of the symptoms of narcolepsy.

However, narcolepsy is not just a disorder of REM sleep. Frequent nocturnal awakenings that offset the increase in daytime sleep, daytime non-REM sleep episodes, and periods of ambiguous sleep that combine physiological features of REM and non-REM sleep are evidence of impaired sleep/wake regulation and reflect the indistinct boundaries between sleep states[1].

Neurochemical links between narcolepsy and REM sleep

The frequent occurrence of sleep onset REM periods and the link between narcoleptic symptoms and dissociated manifestations of REM sleep have led researchers to investigate brain structures and neurotransmitters involved in the control of REM sleep. Neuroanatomical studies using brainstem transections have demonstrated that the pons is the most critical site for the generation of REM sleep: REM sleep signs occur caudally but not rostrally with transections above the pons while the reverse is true with transections below the pons. Lesion studies suggest that regions of the pontine tegmentum involving the nucleus reticularis pontis oralis and the area just rostral to the locus coeruleus are essential components of the brain systems that permit REM sleep to occur. Single unit recording studies have identified neurons in these regions that fire selectively during REM sleep (Fig. 1), REM-on cells, while cells that are selectively silent during REM sleep, REM-off cells, are also located in these areas and in the closely adjacent locus coeruleus and dorsal raphé nucleus[2,3] (Fig. 2). The pontine regions responsible for REM sleep are modulated by diencephalic and forebrain structures and send projections to neurons of the spinal cord, oculomotor nuclei, thalamus, and cortex that mediate muscle atonia, rapid eye movements, and cortical activation.

Although the neurochemistry of REM sleep has not been characterized fully, it is clear that acetylcholine plays an essential role. For example, systemic administration or local injection of cholinergic agonists into selected areas of the pontine tegmentum facilitates the appearance of REM sleep[4]. Numerous neurons with immunoreactivity for cholineacetyltransferase (ChAT) are present in the area of the locus coeruleus and peri-locus coeruleus and many pontine REM-on cells appear to be cholinergic or cholinoceptive or both[2,4].

Compelling evidence suggests that most of the

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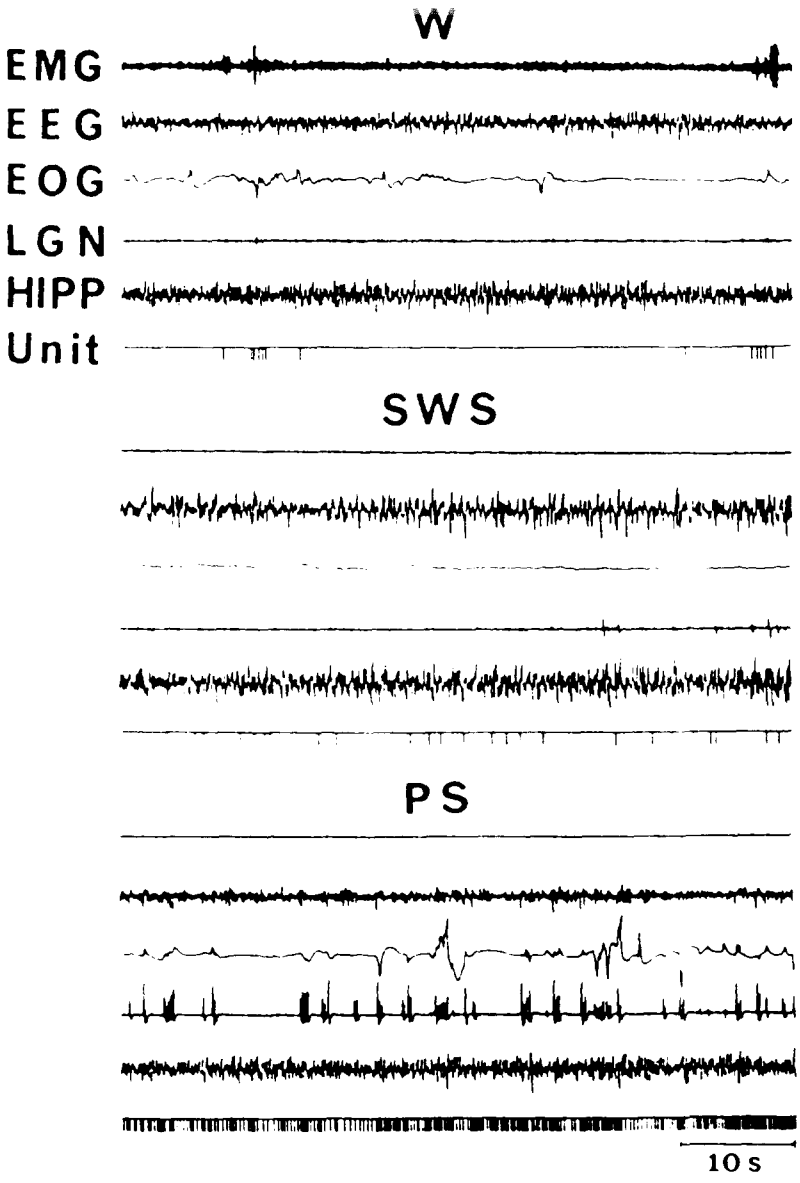

Fig. 1. *Spontaneous unit activity from a REM-on cell of the rostral pontine tegmentum. The discharge rate of the cell is low during quiet wakefulness (W), increases somewhat during non-REM slow wave sleep (SWS) and increases markedly during REM sleep [also known as paradoxical sleep (PS)]. Abbreviations: EEG, electroencephalogram; EMG, electromyogram; EOG, electro-oculogram; HIPP, hippocampus; LGN, lateral geniculate nucleus.* (Reproduced, with permission, from Ref. 40.)

REM-off cells of the brainstem are monoaminergic, containing either noradrenaline or 5-HT. Lesions of noradrenergic neurons of the locus coeruleus pars alpha and of serotonergic neurons of the dorsal raphé lead to the appearance of many physiological features of REM sleep, suggesting that these structures and the REM-off cells that they contain inhibit REM-on cells and play a permissive role in REM sleep generation[5].

Most pharmacological treatments for narcolepsy facilitate monoaminergic activity, consistent with the concept that monoamine dependent inhibition of REM sleep is inadequate in narcolepsy. Amphetamines and other stimulants, the primary treatment for narcoleptic sleepiness, enhance synaptic availability of noradrenaline and suppress REM sleep; tricyclic antidepressants, the usual treatment for cataplexy, inhibit reuptake of monoamines. Agents that specifically inhibit either 5-HT reuptake or noradrenaline reuptake suppress cataplexy, suggesting that the cholinergic pathways presumed to be responsible for REM sleep atonia and cataplexy can be inhibited either by enhanced serotonergic activity of the dorsal raphé nucleus or by enhanced noradrenergic activity of the locus coeruleus. Prazosin, an α_1-adrenergic antagonist, causes a marked exacerbation of human cataplexy[6], consistent with an inhibitory role for α_1-adrenergic receptors in the control of cataplexy.

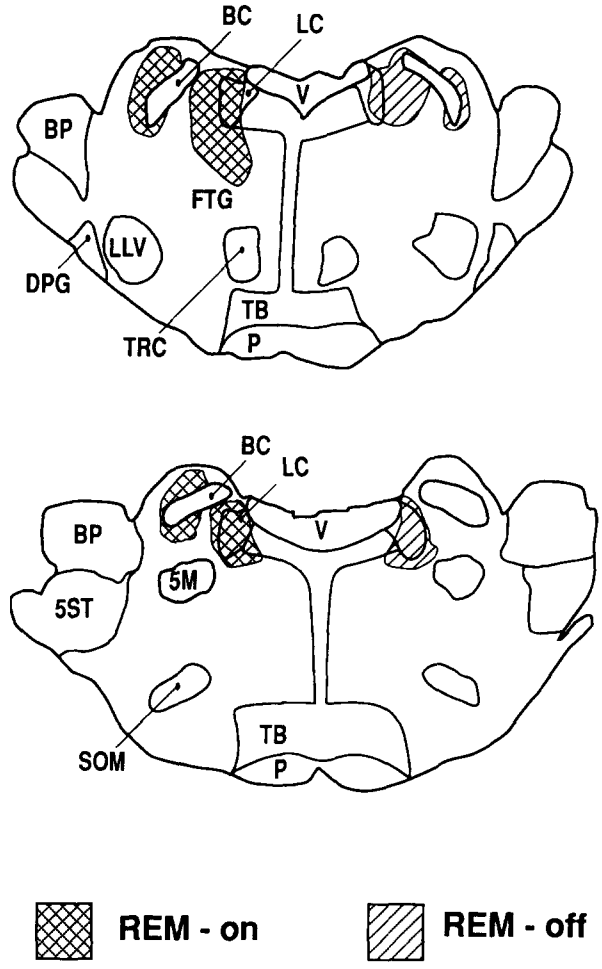

Fig. 2. *Pontine REM-on cells, shown on the left, and REM-off cells, shown on the right, in the cat. REM-on cells are located in the peribrachial region, adjacent to and within the locus coeruleus. REM-off cells are intermingled in this region and, when silent, may disinhibit the nearby REM-on neurons. Abbreviations: BC, brachium conjunctivum; BP, brachium pontis; DPG, dorsolateral pontine gray; FTG, gigantocellular tegmental field; LC, locus coeruleus; LLV, ventral nucleus of the lateral lemniscus; 5M, motor trigeminal nucleus; P, pyramidal tract; SOM, medial nucleus of the superior olive; 5ST, spinal trigeminal tract; TB, trapezoid body; TRC, tegmental reticular nucleus, central division; V, fourth ventricle. Sections shown are 3.1 mm (top) and 4 mm (bottom) caudal to stereotaxic zero.* (Redrawn from Refs 3 and 41.)

The deficits in monoamine activity may extend beyond those functions that are involved in the regulation of REM sleep. Human narcoleptic cerebrospinal fluid has low concentrations of free dopamine[7] and of homovanillic acid[8], suggesting lowered metabolism of dopamine. A reduction in dopamine activity in narcoleptics may be responsible for periodic leg movements, which are frequent in narcoleptics and often respond favorably to treatment with L-DOPA.

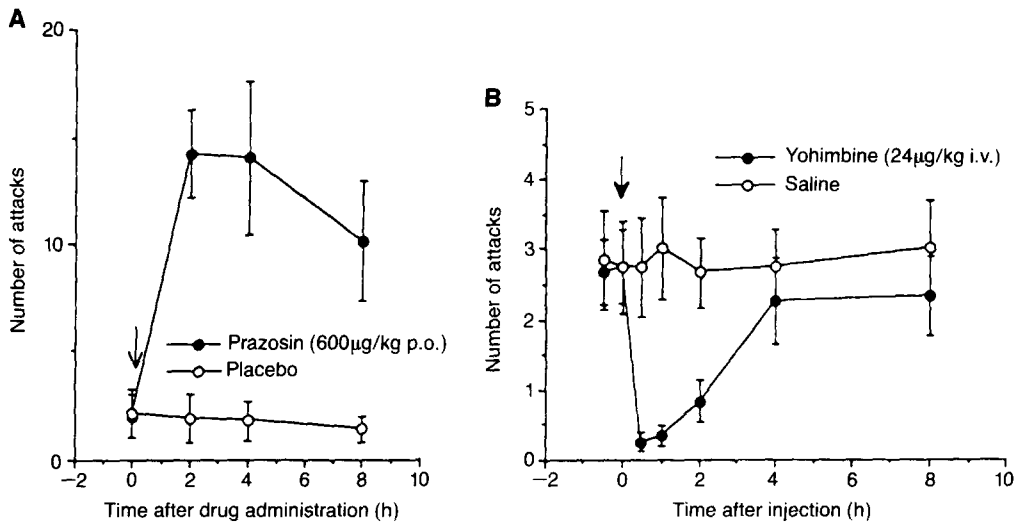

Fig. 3. *Effects of an α_1-adrenergic antagonist, prazosin* **(A)**, *and an α_2-adrenergic antagonist, yohimbine* **(B)**, *on canine cataplexy (n=6 in each group; mean ± SEM). Cataplexy was assessed using the food-elicited cataplexy test in which several pieces of food were placed at intervals along a runway; the time to complete the runway and the number of attacks during each trial were recorded. Prazosin produced a several-fold increase in the number of cataplectic attacks while yohimbine had an equally dramatic opposite effect.* (Reproduced, with permission, from Ref. 42.)

Canine narcolepsy provides a model of human narcolepsy

The discovery of a canine model of narcolepsy has provided an opportunity for more detailed neurochemical studies of narcoleptic brain function. Canine narcolepsy has the following similarities to human narcolepsy: (1) juvenile onset; (2) excessive sleepiness; (3) sleep onset REM periods; and (4) cataplectic attacks responsive to tricyclic antidepressants[9]. Canine narcolepsy differs from human narcolepsy in that it is often recessively inherited and it is not linked to the major histocompatibility complex.

Given the critical role of brainstem cholinoceptive neurons in REM sleep and cataplexy, it is not surprising that canine cataplexy is exacerbated by cholinergic agonists and inhibited by cholinergic antagonists. An increase in muscarinic M_2 receptors in the pontine reticular formation of narcoleptic dogs may indicate a defect in the availability of pontine acetylcholine with secondary up-regulation[10]. Although this finding suggests that cataplectic responses to muscarinic agonists may be due to supersensitivity to these agents, levels of ACh and of ChAT are not altered in the brainstem and muscarinic receptor abnormalities do not correlate with severity of cataplexy in the animals[11,12].

As with human narcolepsy, monoamine regulation of REM sleep appears to be deficient in canine narcolepsy. Drugs that block 5-HT or noradrenaline reuptake or that enhance noradrenaline release inhibit cataplexy in narcoleptic dogs[13], while α_1-adrenergic antagonists produce marked exacerbations (Fig. 3). The effects of α_1-adrenergic antagonists are prevented by atropine, consistent with the idea that the α_1-adrenergic receptors modulate the activity of brainstem cholinergic neurons that represent the outflow path for atonia. $Alpha_{1B}$ receptors with low affinity for prazosin are increased in the amygdala of narcoleptic dogs, perhaps a secondary up regulation as a result of deficient monoamine activity, and may play a role in the triggering of cataplexy by emotion[14–16].

The effects of α_2-adrenergic agonists and antagonists on canine cataplexy are markedly different from those of agents acting at α_1-receptors: α_2-adrenergic antagonists, such as yohimbine, inhibit cataplexy, while α_2-adrenergic agonists have relatively little effect[17]. Adrenergic receptors of the α_2 type, which are present on noradrenergic neurons of the locus coeruleus and appear to act as autoinhibitors of the actions of these neurons, are increased in the locus coeruleus of narcoleptic dogs[18], and may be partially responsible for the apparent deficit in noradrenaline activity.

There is also evidence of altered dopamine function in canine narcolepsy. Dopamine D_2 receptor density is elevated in the amygdala of dog narcoleptic brain, and relative concentrations of dopamine, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid are altered in the caudate nucleus and amygdala, consistent with a deficit in dopamine activity[19,20]. Cataplexy is suppressed by dopamine D_1 antagonists and D_2 antagonists while dopamine D_2 agonists aggravate cataplexy and D_1 agonists have little effect[21]. The effects of agents acting at dopamine D_2 receptors appear to be mediated by presynaptic dopamine autoreceptors that may interact with noradrenergic systems[21].

Do human neurochemical abnormalities parallel those of canine narcolepsy?

To date, only a limited number of postmortem studies have been performed on human narcoleptics. In histopathological studies, areas of gliosis noted in the ventrolateralcaudal pons, the periventricular anterior hypothalamus[22], and the pars reticularis of the substantia nigra[23], may be incidental to the disease. Neurochemical studies of human narcoleptic brains have demonstrated increased dopamine D_2 receptor density in the caudate and putamen, similar to the dopamine receptor changes

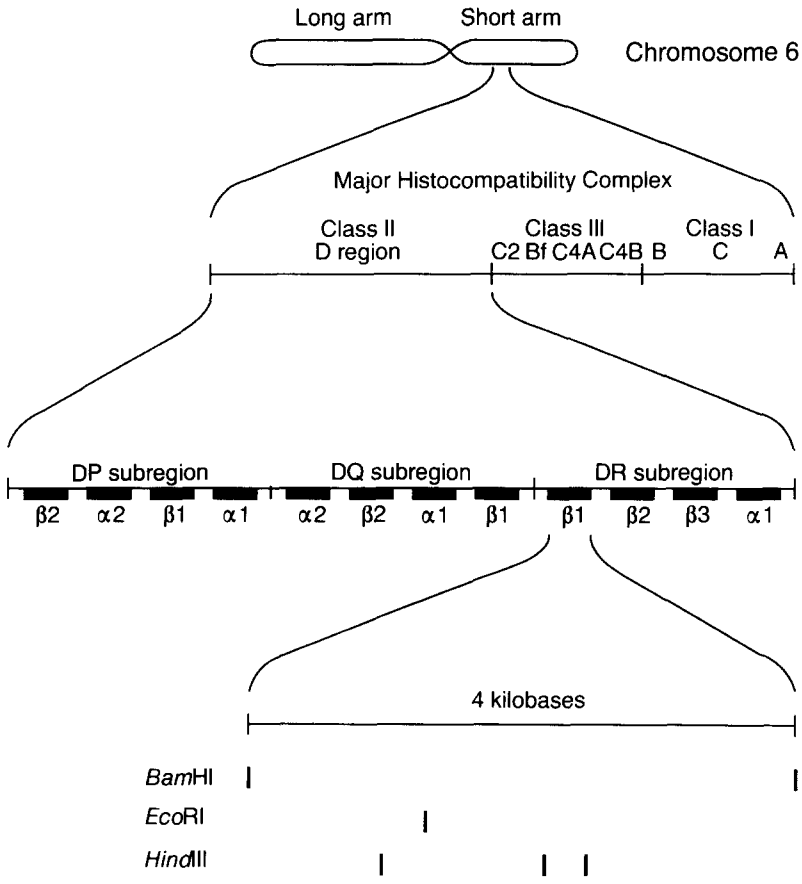

Fig. 4. *The genes of the major histocompatibility complex (MHC) regulate immune function by controlling the expression of cell surface glycoproteins that bind to antigens. Class I antigens, which are designated as HLA-A, -B, and -C, are expressed on all nucleated cells and platelets. Class III genes code for serum proteins involved in the complement system as well as for other unrelated proteins. Class II antigens, which are expressed only on lymphocytes, monocytes, macrophages, and dendritic cells, are composed of four subunits of α and β chains. The subunits include two outer extracellular domains, which are the main sites of polymorphism, and two inner subunits, each of which contains an inner extracellular domain, a transmembrane component, and a small intracellular portion. Polymorphisms of DR-β1, the main site of variability of DR antigens, have been investigated with a number of restriction endonucleases that recognize segments of DNA from this region, including* Bam*HI,* Eco*RI, and* Hind*III.*

that occur with canine narcolepsy, and increased dopamine D_1 receptor density in the caudate and the medial globus pallidus[24,25]. Mamelak has proposed that a deficit in neurotransmission at noradrenergic synapses that have α_1-adrenergic receptors leads to an increase in turnover of noradrenaline and 5-HT, leading in turn to reduction of activity of pedunculopontine neurons, secondary up regulation of pontine muscarinic receptors, and up regulation, via the substantia nigra compacta, of dopamine receptors (M. Mamelak, pers. commun.). An alternative explanation is that a presynaptic defect leads to reduced synaptic release of monoamines and acetylcholine, leading to receptor up regulation in specific areas.

Human narcolepsy is a genetic disease

While the occasional occurrence of narcolepsy in families has been known for many years, the genetic basis was unknown until the recent discovery of an extraordinarily strong linkage of narcolepsy to certain Class II human leukocyte antigens (HLA), indicating that a specific genetic substrate, presumably a 'narcolepsy susceptibility' gene closely linked to the major histocompatibility complex on chromosome 6, is almost always present (Fig. 4). After initial reports of this association from Japanese investigators[26], subsequent studies worldwide demonstrated an incidence exceeding 90% of the HLA-DR2 and HLA-DQw1 antigens or both compared to a 20–35% incidence in the normal population (see citations in Ref. 27).

Subtypes of HLA-DR2 and HLA-DQw1 are now recognized and preliminary studies have shown a 100% association of the DRw15 subtype of HLA-DR2 and the DQw6 subtype of HLA-DQw1 with narcolepsy[28,29]. Of the HLA-D antigens, which are identified using mixed lymphocyte culture techniques rather than the serologic testing used to identify DP, DQ, and DR antigens, narcolepsy has a strong association with Dw2. The high correlations of DR2 (DRw15), DQw1 (DQw6), and Dw2 do not occur with other disorders causing excessive sleepiness and are the highest known for diseases with HLA associations. The incidence of HLA-DR2, while elevated worldwide, varies in different populations. In Japanese narcoleptics with cataplexy the incidence exceeds 99%, while in Caucasians the incidence is 91–98%. North American black narcoleptics have an incidence of DQw1 exceeding 90% but the incidence of DR2 is only 65 to 70% (Ref. 30).

Although initial reports of a 100% incidence of HLA-DR2 with narcolepsy suggested that a gene closely linked or identical to the gene expressing the HLA-DR2 antigen might be uniquely responsible for genetically determined 'narcoleptic susceptibility', family studies and well-documented isolated cases of narcolepsy with cataplexy that are negative for HLA-DR2 and HLA-DQw1 indicate that this hypothesis is probably incorrect[31,32], although the possibility that rare crossover events account for HLA-DR2 negative cases cannot be excluded. In some families with multiple affected members, all narcoleptics are negative for HLA-DR2 and in at least one family, affected members probably had no haplotype linkage, even though they were all positive for HLA-DQw1, because the responsible genes came from one parent in some family members and from the other parent in others[33]. The existence of familial HLA-DR2-negative narcolepsy suggests that there may be a second narcolepsy susceptibility gene, perhaps the human homolog of the gene responsible for canine narcolepsy. It is also possible that the gene linked to HLA-DR2 is necessary in most but not all cases for the expression of the actual 'narcolepsy gene' or genes, which could be located anywhere in the human genome.

The relative frequencies of homozygotes and heterozygotes for DR2 (DRw15), DQw1 (DQw6) and Dw2 support a dominant mode of inheritance[34], but the penetrance is incomplete, since the incidence of narcolepsy in relatives of narcoleptics is less than 5% and in some families with two or more

narcoleptics, the same HLA haplotypes occur in affected and unaffected individuals[33,35]. The additional environmental or genetic factors that lead to the expression of narcolepsy in persons who carry the HLA-linked susceptibility gene are unknown. Certain haplotypes associated with HLA-DR2 increase the likelihood of narcolepsy but the associated haplotypes are not the same in Japanese and Caucasian narcoleptics. While genetic susceptibility is an important determinant, the discordance for narcolepsy in several pairs of monozygotic twins provides strong evidence that environmental influences are also highly significant. It is uncertain whether the HLA-linked gene may be expressed as a sleep disturbance other than narcolepsy.

Studies using restriction fragment length polymorphisms have found no polymorphisms of DNA fragments close to the HLA-DQ (α) and HLA-DQ (β) loci, and studies using techniques to clone and sequence the genes responsible for the α and β DQw1 chains and the Dw2 molecule have not shown significant differences between narcoleptics and controls[29,36,37]. The DR-β gene sequence, the region of greatest variability, appears identical in narcoleptics and controls with the DR2 (DRw15)/DQw6/Dw2 haplotypes when studied with polymerase chain reactions and oligonucleotide probes specific for DR2/Dw2 (Ref. 38). These findings suggest that the HLA-linked susceptibility gene for narcolepsy is not identical to the genes coding for DR and DQ antigens on the short arm of chromosome 6.

The association of HLA-DR2 and DQw1 with some autoimmune diseases, the presence of HLA-DR antigens in normal human brain, and the reduced binding of muramyl peptides to B lymphocytes of individuals with the DR2/DQw1 phenotype[39] suggest the possibility of an immunologic basis for narcolepsy. Although there is no clear evidence yet of an immunologic defect, it is possible that as with juvenile-onset diabetes, an immunologic event precipitates narcolepsy causing, for example, immune-mediated destruction of a neuronal cell surface protein with subsequent permanent down regulation of activity at noradrenergic α_1 synapses.

Concluding remarks

The data presented here indicate that while the neurochemical basis responsible for narcolepsy is becoming more apparent, much work remains to be done. Abnormalities involving α_1- and α_2-adrenergic receptors in canine narcolepsy need to be confirmed in human narcolepsy, but it remains possible that disturbed noradrenaline metabolism is secondary to some other primary neurobiologic defect. Of crucial importance will be the identification of the specific DNA fragment in the HLA-D region that is responsible for narcoleptic susceptibility and the determination of the relationship between this gene region and neurotransmitter function. The contribution of environmental influences and other genetic factors to the disease also remains to be elucidated. A better understanding of the neurochemical and genetic basis of narcolepsy should lead ultimately to more effective and specific treatments for this chronic disabling disease.

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