Emerging Therapies in Narcolepsy-Cataplexy

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Abstract: In the past, narcolepsy was primarily treated using amphetamine-like stimulants and tricyclic antidepressants. Newer and novel agents, such as the wake-promoting compound modafinil and more selective reuptake inhibitors targeting the adrenergic, dopaminergic, and/or serotoninergic reuptake sites (ie, venlafaxine, atomoxetine) are better-tolerated available alternatives. The development of these agents, together with sodium oxybate (a slow-wave sleep-enhancing agent that consolidates nocturnal sleep, reduces cataplexy, and improves sleepiness), has led to improved functioning and quality of life for many patients with the disorder. However, these treatments are all symptomatically based and do not target hypocretin, a major neurotransmitter involved in the pathophysiology of narcolepsy. In this review, we discuss emerging therapies in the area of narcolepsy. These include novel antidepressant or anticataplectic, wake-promoting, and hypnotic compounds. We also report on novel strategies designed to compensate for hypocretin deficiency and on the use of immunosupression at the time of narcolepsy onset.

Key Words: Treatment, narcolepsy, cataplexy

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INTRODUCTION

CURRENTLY AVAILABLE TREATMENTS FOR HUMAN NARCOLEPSY ACT SYMPTOMATICALLY AND DO NOT TARGET THE HYPOCRETIN (OREXIN) NEUROPEPTIDE SYSTEM, the primary neurotransmitter system involved in the cause of narcolepsy-cataplexy. In the last few years, however, much has been learned regarding the mode of action of currently available agents in the treatment of narcolepsy, thanks mostly to pharmacologic studies in a canine model of the disorder (see for review[1]). This well-characterized model, studied for more than 20 years,[1] has hypocretin receptor-2 (hcrtr-2) mutations[2] or sporadic hypocretin deficiency.[3] More recently, similar pharmacologic experiments are being conducted in murine models of narcolepsy genetically engineered to lack the hypocretin gene[4] or the hypocretin-producing cells.[5]

Amphetamine-like stimulants are primarily believed to improve sleepiness via presynaptic stimulation of dopaminergic transmission.[6-8] For amphetamine, these effects are mediated through the inhibition of the vesicular monoamine transporter (VMAT), an effect resulting in the emptying of vesicular dopamine (DA) stores in the cytoplasm, and reverse efflux of DA through the dopamine reuptake site (also called the DA transporter, DAT).[8-10] This effect produces a net increase in DA release and an associated reduction of presynaptic DA stores. Methylphenidate, another commonly used stimulant structurally distinct of catecholamines, has no effect on granular DA storage and primarily blocks DA reuptake.[8-11] Activation of DA transmission after methylphenidate is thus dependent of the underlying DA activity (ie, no increase in DA transmission in the absence of firing).[9,11] Other effects may be involved, for example stimulation of adrenergic transmission (Table 1). In canine narcolepsy, selective DA reuptake inhibitors such as GBR12909 (vanoxerine) have strong wake-promoting effects but no impact on cataplexy.[12] Modafinil, a recently developed wake-promoting compound with lower abuse potential and probably fewer cardiovascular effects[13,14] has similar effects in canine[6,15] and mice narcolepsy.[16] It has a debated mode of action but is also likely to target the DA reuptake system.[6-8,17,18]

The mode of action of anticataplectic antidepressants has also been studied in animal models of narcolepsy. These compounds reduce cataplexy in both the murine (evaluated as rapid eye movement-like transitions from wake[19]) and the canine model. [1,12,20,21] In canine, reduction is mediated by the inhibition of adrenergic and, to a lesser extent, serotonergic (5-HT) reuptake.[12,20] Differently from DA reuptake inhibitors, however, pure adrenergic and 5-HT reuptake inhibitors have only modest wake-promoting effects in animals.[1,6] Novel anticataplectic reuptake inhibitors available in humans include compounds with adrenergic (eg, atomoxetine) or dual adrenergic/serotoninergic (eg, venlafaxine) reuptake properties that do not have anticholinergic or alpha-adrenergic effects.[13,21]

Sodium oxybate (GHB), the most recent addition to our therapeutic arsenal in narcolepsy (see reference 22), is currently indicated for cataplexy. It also reduces daytime sleepiness and has an effect on disturbed nocturnal sleep.[22-25] The mode of action of GHB is debated and may involve stimulation of GABA-B receptors and possibly other GHB-specific receptors.[22,26] Interestingly, GHB has strong effects on DA transmission (probably mediated via GABA-B receptors on DA cells), acutely reducing cell firing, but with an uncoupling of DA synthesis, thereby resulting in increased DA store in animals.[27] Whether or not a reduction of DA transmission is important for sleep induction and the subsequent increased DA store is important for daytime vigilance has been speculated.[22] The compound has been shown to be efficacious in narcolepsy but, like amphetamine-like stimulants, is also abused

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Table 1—Currently Available Narcolepsy Treatments and Their Pharmacologic Properties

Compound	Pharmacologic Properties
Stimulants	
Amphetamine	Increases monoamine release (DA>NE>>5-HT). Primary effects due to reverse efflux of DA through the DAT. Inhibition of monoamine storage through the VMAT and other effects occur at higher doses. The D-isomer is more specific for DA transmission and is a better stimulant compound. Some effects on cataplexy (especially for the L-isomer) secondary to adrenergic effects occur at higher doses. Available as racemic mixture or pure D-isomer; various time-release formulations available.
Methamphetamine	Profile similar to amphetamine but more lipophilic with increased central penetration.
Methylphenidate	Blocks monoamine (DA>NE>>5-HT) uptake. No effect on reverse efflux or on VMAT. Short half-life. Available as racemic mixture or as pure D-isomer and in various time-release formulations.
Pemoline	DA uptake inhibition. Low potency. Rarely used due to occasional hepatotoxicity.
Selegiline (L-Deprenyl)	MAO B inhibitor with in vivo conversion into L-amphetamine and L-methamphetamine.
Modafinil*	Mode of action debated but probably involves relative selective DA reuptake inhibition. Fewer peripheral side effects. Low-potency compound. Available as a racemic mixture. Little if any addictive potential but less efficacious than amphetamine or methyphenidate. The R-isomer has a longer half-life and is in development.
Anticataplectic compounds	
Protryptiline	Tricyclic antidepressant. Monoaminergic uptake blocker (NE>5-HT>DA). Anticholinergic effects; all antidepressants have immediate effects on cataplexy, but abrupt cessation of treatment can induce very severe rebound in cataplexy
Imipramine	Tricyclic antidepressant. Monoaminergic uptake blocker (NE=5-HT>DA). Anticholinergic effects. Desipramine is an active metabolite.
Desipramine	Tricyclic antidepressant. Monoaminergic uptake blocker NE>>5-HT>DA). Anticholinergic effects.
Chlomipramine	Tricyclic antidepressant. Monoaminergic uptake blocker (5-HT>NE>>DA). Anticholinergic effects. Desmethylclomipramine (NE>>5-HT>DA) is an active metabolite. No specificity in vivo.
Venlafaxine*	Dual serotonin and adrenergic reuptake blocker (5-HT≥NE): very effective but some nausea. May have less sexual side effects than other antidepressants. Slightly stimulant, short half-life, extended-release formulation preferred.
Atomoxetine*	Specific adrenergic reuptake blocker (NE) normally indicated for attention-deficit/hyperactivity disorder. Slightly stimulant, short half-life, and reduces appetite.
Fluoxetine	Specific serotonin uptake blocker (5-HT>>NE=DA). Active metabolite norfluoxetine has more adrenergic effects. High therapeutic doses are often needed.
Other	
Sodium Oxybate* (GHB)	May act via GABA-B or specific GHB receptors. Reduces DA release. Need binightly dosing with immediate effects on disturbed nocturnal sleep; therapeutic effects on cataplexy and daytime sleepiness often delayed.

DA refers to dopamine; NE, norepinephrine; 5-HT, serotonin; DAT, dopamine transporter; MAO, monoamine oxidase; VMAT, vesicular monoamine transporter.
*Recent compounds that can be considered as first-intention treatments in narcolepsy-cataplexy, considering their benefit and side-effect profiles when compared to other older medications.

in the general population.[27]

At a recent meeting of the National Institute of Health entitled "Frontiers of Knowledge in Sleep & Sleep Disorders: Opportunities for Improving Health and Quality of Life," one key recommendation pertained to the education of physicians on the use of novel antidepressants and stimulants in the treatment of narcolepsy (http://www.nhlbi.nih.gov/ meetings/slp_front.htm). Indeed, old tricyclic antidepressants, such as clomipramine or protryptiline, together with amphetamines or methylphenidate are still too often used as first line treatments.[13] These therapies are effective and inexpensive, but recent alternatives such as sodium oxybate/GHB, modafinil, and novel reuptake inhibitors (Table 1) should be more frequently considered. Thanks to the recent progress and renewed interest in this area, novel therapies are also emerging, and will be discussed below.

NOVEL ADRENERGIC, SEROTONINERGIC, AND DOPAMINERGIC REUPTAKE BLOCKERS

The current success of modafinil, with its recently extended indications to shift work sleep disorder and residual sleepiness in

Table 2—Future Potential Narcolepsy Treatments and Their Pharmacologic Properties

Treatment Types	Advantages and Limitations
Non-hypocretin–based therapies	
Novel monoaminergic reuptake inhibitors	Inhibitors of DA reuptake likely to be mild stimulants; inhibitors of adrenergic reuptake likely to be anticataplectic agents. Possibly targeting multiple reuptake sites; may be developed in the context of depression, wake-promotion, attention-deficit/hyperactivity disorder treatments or as therapies for cocaine or stimulant abuse
Novel SWS enhancers	The efficacy of sodium oxybate (GHB) suggests that other hypnotics with SWS effect could have similar effects; possible agents in this class could include novel GABA-B agonists, GABA-A subtype specific compounds such as gaboxadol, longer-acting GBH analogues, and GABA reuptake inhibitors such as tiagabine or others.
Histaminergic H3 antagonists/inverse agonists	Autoreceptor of histaminergic neurons; will stimulate histaminergic transmission; effective on sleepiness and cataplexy in animals models; Effects in humans still uncertain, but multiple compounds available preclinically or in early human trials.
TRH analogues	Typically peptide analogues; effective in animal models but very high dose required; some compounds failed human trials on depression; limited activity for this area in the pharmaceutical industry
Hypocretin-Based Therapy	
Hypocretin-1 itself	Disappointing effects after intravenous, intracisternal, and intranasal administration to date, but extremely high doses could still be effective; would likely be effective if could be delivered intracerebroventricularly.
Hypocretin peptide agonists	Similarly to TRH analogues, could be effective at very high dose. Hypocretin is a larger peptide, and derivatives are unlikely to cross the blood-brain barrier sufficiently and will probably be unstable in vivo.
Nonpeptide agonists	Best possible hope, especially if targeting the hcrtr2 receptor; with central penetration; impossible to predict success to date; peptide receptor agonists are often difficult if not impossible to make.
Hypocretin cell transplantation	May one day provide a cure; results to date in other diseases are disappointing because of potential graft rejection, low survival rate of implant, and lack of supply for graft availability. This last problem could be solved on a long-term basis through stem cell technology, likely to be more than 10 years away.
Gene therapy	Promising in the future but need appropriate vector; potentially dangerous side effects; could be combined with cell-based therapies.
Immune-based therapies	
Steroids	Ineffective in 1 human and 1 canine case; unlikely to be useful.
IVIg	May be effective in decreasing symptoms but only if used before a year or so after onset; reported effects are still subjective and not confirmed through placebo-controlled trials; generally safe but occasionally life-threatening side effects.
Plasmapheresis	Similar to IV Ig but less available data; more invasive than IVIg.

DA refers to dopamine; SWS, slow-wave sleep; NE, norepinephrine, 5-HT, serotonin; H3, histamine receptor 3; TRH, thyrotropin-releasing hormone; IVIg, intravenous immunoglobulins.

sleep apnea,[13] together with the expanding use of stimulants for attention-deficit/hyperactivity disorders and the continued need for novel treatments for resistant depression, have fueled the growth of new products in this area. R-(-)-modafinil, the longer acting isomer of the currently available racemic modafinil mixture[28] is currently being evaluated in narcolepsy and sleep apnea. The half-life of R-(-)-modafinil is approximately 3 times longer than that of S-(+)-modafinil in humans. This variation will slightly increase the half-life of the product, facilitating a potential once-per-day administration. A number of companies have also developed improved delivery formulations and single isomer preparations for typical stimulants such as methylphenidate and amphetamines (see[8]).

A traditional problem with dopaminergic stimulants is their addiction potential. Cocaine, amphetamine, and methylphenidate have addiction potential, and all modulate DA release (amphetamine) and/or reuptake (methylphenidate, cocaine).[9,11] Interestingly however, not all DA reuptake inhibitors have a similar addiction potential.[11] Mazindol, a high-affinity DAT inhibitor, for example, is only moderately addictive. Current hypotheses for explaining these differences involve a combination of factors rather than a single property. These include pharmacokinetic differences (rapid brain penetration and onset of action, high potency, and solubility allowing the possibility of intravenous recreational use) and possibly combined effects on other monoamines (for example, 5-HT plus DA effects may change addiction po-

tential).[11,29] Differential effects on DA transmission (VMAT plus DAT inhibition for amphetamine; differential effects on basal versus stimulated DA release with some drugs) and distinct binding sites on the DAT protein itself may also be involved.[11,29,30] In this direction, federal agencies and companies have been engaged in the identification of DAT inhibitors that may not have strong (or any) addiction potential. These would be used to reduce exposure to more dangerous stimulants, a strategy akin to that taken by the methadone program for opiate abusers. In this direction, DA reuptake inhibitors with known stimulant effects, such as GBR12909 (vanoxerine), amineptine, or NS2359 (combined monoamine reuptake blocker), have been explored as a preventive treatment for cocaine abusers[31,32] or in the treatment of amphetamine withdrawal[33] and may become available for other indications (Table 2). A difficulty in this area remains the determination of what is abuse (eg, drug seeking and withdrawal symptoms) or misuse (eg, occasional use to counteract recreational sleep deprivation or to increase productivity). Other DA reuptake inhibitors such as amineptine (DAT inhibitor) and nomifensine (a dual DAT and adrenergic reuptake inhibitor) have been available in the past in Europe, only to be eventually withdrawn because of misuse or abuse.

A similar trend is also being seen in the area of antidepressant therapies where novel single, dual (duloxetine, milnacipram),[34] and even triple (DA, 5-HT, and adrenergic eg, DOV 216,303, NS 2359)[35] monoaminergic reuptake inhibitors are being studied. These compounds may be of interest as modulators of both cataplexy and sleepiness but are not novel in terms of mode of action (Table 2). Many may never be finally developed due to side effects, abuse potential, or other considerations. It is also likely that combination therapies with monoamine-selective inhibitors will often remain easier to titrate to control sleepiness and cataplexy separately.

NOVEL HYPNOTICS WITH POTENTIAL ENHANCING EFFECTS ON SLOW-WAVE SLEEP

Another area of potential interest may be the use of novel sedative hypnotics in narcolepsy-cataplexy (table 2). Disturbed nocturnal sleep is a common and disabling symptom in narcolepsy. In the past, insomnia was treated using benzodiazepine-based hypnotics or other sedatives. These compounds are typically effective for insomnia but have little, if any, effects on daytime symptoms of narcolepsy. In contrast, GHB has proven to be remarkably efficacious in the treatment of multiple symptoms of narcolepsy: sleepiness, cataplexy, and disturbed nocturnal sleep.[22-25] As discussed above, the mode of action of GHB is debated but likely involves effects on GABA-B and possibly effects on less well-characterized GHB receptors.[22,26,27] A problem in its current formulation is the short half-life of the compound. The development of longer-acting GHB formulations or derivatives is ongoing. The short half-life is indeed an inconvenience but may improve safety and could be advantageous in avoiding residual sedation. It is also possible that the short half-life is important in preventing the development of tolerance and addiction. GHB addiction is not a problem in narcolepsy, and withdrawal symptoms are not observed upon abrupt cessation.[36] In contrast, GHB abusers experience withdrawal symptoms when stopping but are typically round-the-clock, high-dose GHB users, often also abusing multiple drugs.[37] Twenty-four–hour exposure to GHB may thus be critical to its addictive potential. Novel GABA-B agonists or modulators may also be of interest (development is limited by epileptogenic properties at high doses), but longer-acting GABA-B agonists, such as baclofen, are already available but have not been systematically evaluated in human narcolepsy.

At the pharmacologic level, GHB is unique as a strong hypnotic because of its ability to increase slow-wave sleep (SWS).[25] We have hypothesized that a core abnormality in hypocretin deficiency is the inability of patients to counteract even small amounts of sleep debt.[38] Whether the SWS-enhancing property of GHB, and the resulting decrease in homeostatic sleep debt, is needed for the beneficial effect of the compound on the various symptoms of narcolepsy is tantalizing.[22] This question will only be answered when other compounds with similar SWS-enhancing profiles, but distinct molecular modes of action, will be available. Currently studied or available GABAergic hypnotics with SWS-enhancing properties include gaboxadol, a GABAergic modulator with preferential effects on extrasynaptic GABAergic receptors containing the delta and alpha-4/5 subunits,[39] and tiagabine, a GABA reuptake inhibitor.[40] The existence of numerous other potential targets for hypnotics, such as 5-$HT_{2a/c}$ antagonists, histamine H_1 receptors antagonists, H_3 autoreceptor agonists, and ion channel blockers, together with the renewed interest of the pharmacologic sector in hypnotic therapies may also be beneficial to narcoleptic patients (see reference 41). Of note, ritanserin, a 5-HT_2 receptor antagonist, has been reported to have beneficial effects on disturbed nocturnal sleep in narcoleptic patients.[42]

HISTAMINE AGONISTS AND ANTAGONISTS

Like adrenergic and serotonergic cells, histaminergic cells significantly decrease activity during non-rapid eye movement and rapid eye movement sleep.[43] The sedative effects of H_1-receptor antagonists illustrate the importance of histamine in sleep regulation. The pioneering work of Lin and colleagues on the tuberomammillary nucleus also indicate a major role for this system.[44]

Hypocretin neurons have strong projections and excitatory effects on histamine transmission, an effect mediated by hcrtr2, the receptor mutated in canine narcolepsy.[2] The effects of hypocretin on alertness after intracerebroventricular (ICV) injections are diminished or abolished when histaminergic transmission is blocked,[45] suggesting the importance of the downstream effects of hypocretin on this neurotransmitter system in mediating wake promotion. We and others have also found that human narcolepsy, and possibly idiopathic hypersomnia, is associated with decreased histamine in cerebrospinal fluid (CSF).[46,47] The rationale for increasing histaminergic tone to treat narcolepsy and hypersomnia is thus strong from the pathophysiologic perspective.

The use of H_1-receptor agonists, though logically plausible, is made impossible by the lack of available centrally penetrating compounds and intolerable peripheral side effects. Current pharmaceutical industry interest is therefore mostly focused on the H_3 receptor (Table 2), a receptor known to be, among other actions, an autoreceptor located on brain histaminergic cell bodies.[48] Stimulation of this receptor is sedating, while antagonism promotes wakefulness (or reduces SWS) in rodents and dogs.[49,50] Experiments in narcoleptic canines have found anticataplectic and wake-promoting effects for some H_3 antagonists and inverse agonists.[49] Preliminary results in orexin/ataxin-3 narcoleptic mice, in which hypocretin-producing neurons are ablated,[5] indicate that

these mice are more sensitive to an H_3 antagonist in promoting wakefulness.[51] A significant number of pharmaceutical companies are currently developing or clinically exploring the use of H_3 antagonists or H_3 inverse agonists to promote wakefulness and cognition for various indications. Whether the promising results in animal models will also extend to humans, and whether these compounds will have enough efficacy, remains to be established.

THYROTROPIN-RELEASING HORMONE AGONISTS AND OTHER TARGETS

The use of thyrotropin-releasing hormone (TRH) direct or indirect agonists may also be potentially interesting (Table 2).[52] TRH is a small peptide of 3 amino acids, which penetrates the blood-brain barrier at very high doses. Small peptide derivatives with agonistic properties and increased blood-brain barrier penetration (eg, CG3703, CG3509, or TA0910) have been developed,[52,53] a success facilitated by the small nature of the parent TRH peptide. TRH (at the high dose of several mg/kg) and TRH agonists increase alertness and have been shown to be wake promoting and anticataplectic in the narcoleptic canine model.[52] TRH has excitatory effects on motoneurons[54] and enhances both DA and adrenergic neurotransmission,[55,56] properties that could contribute to its wake-promoting and anticataplectic effects. Interestingly, recent studies suggest that TRH may promote wakefulness by directly interacting with thalamocortical networks. Indeed, TRH itself and TRH receptor type 2 are abundant in the reticular thalamic nucleus,[57] and local thalamic application of TRH abolishes spindle wave activity.[58] In slices, TRH depolarizes thalamocortical and reticular/perigeniculate neurons by inhibiting a leak K+ conductance.[58] Unfortunately, however, human clinical studies at low doses in depression have shown limited efficacy and only moderate subjective alerting effects;[53,59,60] whether better compounds can or will be developed is unknown. Other possibly interesting development directions could involve inhibitors of the TRH-degrading enzyme, a relatively specific metallopeptidase.[61]

Other experimental targets for wake promotion could also involve novel neuropeptide systems and protein targets such as circadian clock proteins or kinases, novel ion channels, prokineticin,[62] or the recently described wake-promoting neuropeptide S.[63]

HYPOCRETIN PEPTIDE SUPPLEMENTATION: INTRAVENOUS, INTRANASAL, INTRACISTERNAL, AND ICV EFFECTS

The gold standard for narcolepsy treatment will one day likely be hypocretin replacement therapy. This could be achieved through the delivery of hypocretin peptides themselves, the use of prodrugs or agonists, or the use of genetic engineering or cell-replacement therapies (Table 2). Unlike the very small TRH molecule, hypocretin-1 and hypocretin-2 are peptides of medium size, 33 and 29 amino acids respectively.

Early experiments using radio-labeled hypocretin peptides have suggested that the peptide may cross the blood-brain barrier by passive diffusion.[64] Hypocretin-1 has been found to be more stable than hypocretin-2 in both the blood and the CSF,[64,65] a property that likely explains why hypocretin-1 is more active than hypocretin-2 after ICV injection. Subsequent pharmacologic experiments have therefore generally employed hypocretin-1.

The effects of intravenous (IV) and ICV administration of hypocretin-1 in hcrtr2-mutated canine narcolepsy has been examined by John et al[66] and Fujiki et al.[67] In 2002, John et al found that hypocretin-1, when injected at the low dose of 3 μg/kg IV, was wake-promoting and able to reverse cataplexy in hrctr-2 mutated Dobermans, while the IV administration of 4 μg/kg significantly worsened cataplexy.[66] This result was surprising from a pathophysiologic point of view, and, indeed, we found that, in our narcoleptic hcrtr2-mutated canines, a similar dose was ineffective in producing wakefulness, even when bolus ICV injections were employed.[67] Similar IV-injected doses per kilogram were also barely wake-promoting in normal dogs with functional receptors.[67] Significantly higher doses were later injected through both IV (for cataplexy and sleep) and ICV (for cataplexy) routes without significant effect (up to 24 μg/kg IV or 120 nmoles ICV) in hcrtr2-mutated dogs,[67] in disagreement with John's study.

A better model to assess the effects of hypocretin on narcolepsy may be to use hypocretin-deficient animals (rather than hcrtr2-mutated animals). In orexin/ataxin-3 narcoleptic mice, ICV hypocretin-1 (3 nmoles) can almost completely suppress episodes of behavioral arrests (cataplexy-like episodes) and reverse sleep fragmentation and sleep-onset rapid eye movement sleep periods in this model.[68] These experiments strongly suggest that if delivered to the right location, hypocretin-1 may prove to be a viable treatment in narcolepsy. In 2 hypocretin ligand-deficient canines (a close model of human narcolepsy), we administered high doses of IV hypocretin-1 (96-384 μg/kg) and found limited effects on cataplexy:[67,69] indeed, at best, we found that the 196- to 384-μg/kg doses decreased cataplexy, but for less than 15 minutes.[47] Whether this transient effect is a reflection of side effects rather than genuine therapeutic relief due to centrally penetrating hypocretin-1 is unknown. We also examined blood and CSF hypocretin-1 levels after IV administration and found extremely high concentrations in the blood (up to 10 million pg/mL in blood after 384 μg/kg IV) with minimal and variable increases in CSF hypocretin-1 levels (plus 400 pg/mL after 384 μg/kg IV; with exclusion of CSF samples containing blood).[67] These results indicate that the blood-brain barrier is, in fact, quite impermeable to hypocretin-1 (unless it is locally broken, for example with the insertion of a dialysis probe). Peripherally administered hypocretins are thus not likely to be effective in the treatment of narcolepsy, unless administered at much higher doses. The effects of even higher doses, similar to those found to be active for TRH (500 μg/kg to several mg/kg) in the canine model,[65] however, still need to be tested, as no significant peripheral side effects have been noted.

Another possible path towards delivering hypocretin-1 to the brain may be intranasal delivery.[70,71] Some investigators have reported CNS penetration for selected peptides and increased CSF levels in humans after intranasal administration, suggesting direct penetration from the nose to the brain.[71] In mice, we found that after intranasal ^{125}I-hypocretin-1 administration (5 nmol), high levels of putative labeled hypocretin-1 (10-1000 nmol) were found in multiple brain regions.[70] In addition to delivery to the brain, intranasal hypocretin-1 also resulted in delivery to the spinal cord, with a decreasing gradient from cervical (96 nmol) to lumbar (3 nmol) regions. However, a preliminary observation did not allow us to observe significant changes in locomotion in either control or narcoleptic mice after intranasal administration (J. Zeitzer, Ph.D., personal oral communication). Experiments in humans are needed to further test this hypothesis.

The finding that ICV administration was efficacious in rodents led us to implant a Medtronic pump in a 3 year-old hypocretin

deficient Weimaraner with a connection to the cisterna magna.[69] These pumps are commonly used to continuously administer analgesic (eg, opioids for pain) or spasmolytic (eg, baclofen for spasticity) compounds, and timing of the administration can be controlled remotely. It was our hope that hypocretin-1 would backflow through the foramina of Luschka into the ICV system, and that, if successful, a similar device could be implemented in humans through catheterization of the lumbar sac. Unfortunately, these experiments were not successful, even when up to 1200 nmol of hypocretin-1 (150 µg/kg) were injected, suggesting the impracticality of this approach. A possibility may be that hypocretin receptors are downregulated after long periods of hypocretin deficiency and therefore could not stimulated by the perfused hypocretin-1. This is however less likely, since neither hcrtr1 nor hcrtr2 mRNA have been found to be significantly decreased in hypocretin-deficient human brains (M. Honda, unpublished results using human cDNA arrays in postmortem human narcolepsy brains). We are planning to pursue this experimental approach with the direct lateral ventricle infusion of hypocretin-1 in hypocretin-deficient dogs.

HYPOCRETIN GENE THERAPY AND HYPOCRETIN CELL TRANSPLANTATION

Another possible experimental approach involves induction of endogenous hypocretins, which could involve gene therapy or hypocretin cell replacement therapy. Meieda et al, using mice nonspecifically overexpressing the hypocretin gene in the central nervous system (with a beta-actin/cytomegalovirus hybrid promoter), found that crossing these mice with orexin/ataxin-3 narcoleptic mice could rescue the phenotype of narcolepsy (both sleep abnormalities and behavioral arrests).[68] It is therefore theoretically possible that viral delivery of a transgene (or indirect delivery via cells carrying such a transgene but entering the central nervous system) resulting in the expression of hypocretin could be effective (despite the lack of proper anatomic distribution or physiologic regulation).

Closer on the horizon may be the use of cell transplantation. Transplanted cells are likely to keep their regulatory mechanisms intact. Transplantation of fetal hypothalamic tissue has, for example, been shown to rescue circadian abnormalities in suprachiasmatic nuclei-lesioned or clock-mutated animals.[72,73] In Parkinson disease, a large number of animal studies have indicated feasibility of cell transplantation, while clinical studies have shown variable effects (see reference 74). In a preliminary study, Arias-Carrión et al[75] recently found that the transplantation of neonatal rat hypothalami into the brainstem of adult rats might result in the development of stable grafts containing hypocretin neurons. Survival of the grafts was poor, however, and whether these grafts will restore function and project to their normal targets is unknown. Additional work is needed to further validate this approach in animal models.

In humans, it is estimated that about 70,000 hypocretin neurons exist in the brain and that narcolepsy is associated with a 85% to 100% loss.[76,77] Although the number of restored neurons needed to rescue the narcolepsy phenotype is unknown, narcolepsy typically presents when CSF hypocretin-1 levels are below 30% of control values.[78] It is thus likely that a minimum of 10% of the normal cell population may be required to have a clinical effect.

In models of Parkison disease, however, the survival rate of DA neurons of fetal mesencephalon grafts is only 5% to 10%.[79] This low survival rate suggests that it may be impossible to gather enough material from cadaver donors to achieve the required number of functioning cells. To solve the problem of supply, efficient cell-sorting or selection methods for hypocretin-containing cells may need to be developed. A similar problem has also been encountered in the field of type I diabetes, where intraportal islet-cell transplantation has been found to be effective, but donor material is scarce, and long-term benefits are still unclear.[80,81]

The possibility of immune reactions to the grafted hypocretin cells may be another concern, particularly if an autoimmune process indeed causes hypocretin deficiency in humans. A similar problem is emerging in the area of islet-cell transplantation.[80,81] The long-term solution for these problems may therefore be the genetic engineering of cells delivering hypocretins, either using stem-cell technology or genetically modified transplanted cells. In this area, like in others, narcolepsy is likely to benefit from parallel advances in other areas of medicine.

HYPOCRETIN PEPTIDE ANALOGUES

An obvious solution considering the lack of central nervous system penetration of exogenous hypocretin peptides may be to develop centrally acting hypocretin agonists. The molecular size and innate water solubility of compounds are some of the important issues to consider when attempting to deliver peptides effectively into the brain parenchyma.[82] The fact that most of the narcolepsy phenotype is recapitulated by hcrtr2-deficient animals[2,19] suggests that hcrtr2- rather than hcrtr1-targeted agonists may be most appropriate. Hypocretin-2, a peptide with higher hcrtr2 versus hcrtr1 affinity, may be a useful starting point, but it has a very short biologic half-live;[64,65] more-stable molecular entities will be needed for pharmacologic delivery.

The modification of the native peptide or the design of precursor molecules (ie, prodrug) may potentially overcome these hurdles.[83] Substitution scans, truncated peptide analysis, and cross-species comparisons indicate that the C-terminal amide portion of both hypocretin peptides, most notably the last 8 amino acids (a region of high homology between hypocretin-1 and 2) is most critical.[84,85] Selected identified peptide substitutions have also been found to have increased selectivity toward hcrtr1 and hcrtr2.[84,85] Unfortunately, none of the provided structures is small enough to be a likely viable drug. However, further study of these modified peptides at very high doses, together with further structural improvement to increase stability and central penetration, may still prove promising.

HYPOCRETIN-RECEPTOR AGONISTS

Direct agonists with adequate pharmacokinetic properties would be ideal therapies. For most G-protein coupled receptor (GPCR) systems, it is however typically more difficult to identify agonists than antagonists. Indeed, agonists must not only bind the receptor, but must also interact tightly at the molecular level to stimulate secondary messenger systems. This may be even more dificult for peptide-receptor systems, considering the size of the ligand and the potential complexity of the associated molecular interactions. In spite of these difficulties, a dozen nonpeptide agonists for GPCR peptide receptors, including the urotensin-II receptor (GPR14),[86] opioid receptor-like (ORL1) receptor,[87] and galanin receptor[88] are currently under development.

Several companies have succeeded in identifying small molecular hypocretin-receptor antagonists,[89-92] with both hcrtr1 and hcrtr2 selectivity. These molecules are active in *vitro* and in *vivo*, but it is still too early to predict whether some of these compounds have the appropriate characteristics to become viable drugs. It is also unclear if these drugs will have acceptable side-effect profiles and whether a proper indication will be found within or outside the sleep disorder market. Whether nonpeptide hypocretin-receptor agonists can or will be identified and successfully developed based on the above, is unknown.

IMMUNOMODULATION AS A PREVENTIVE TREATMENT FOR NARCOLEPSY

The combined observation of hypocretin cell loss[76,77] and HLA association[93] suggests an autoimmune basis for narcolepsy. If this is the case, it is likely that the process is only reversible prior to near complete ablation of cells. Studies in young children near the abrupt onset of symptoms and presumed disease onset (typically 3 months to 1 year) indicate that, in most cases, CSF hypocretin-1 levels are undetectable or very low at the time of presentation, even when the subject does not have cataplexy.[78,94,95] This unfortunately suggests that symptoms only appear after the majority of the cell population is destroyed. Indeed, rat studies indicate that a 70% cell loss only results in a 50% decrease in CSF hypocretin-1, suggesting compensation in cases of partial cell loss.[96] However, it is also possible that the loss of CSF hypocretin-1 is a reflection of decreased cell function without actual cell death and that the destruction can still be partially reversed at this early stage with immunosuppression (Table 2).

To address this issue, we first attempted to reverse narcolepsy using prednisone in an 8-year-old child with an abrupt onset 3 months prior to diagnosis.[94] The child had already undetectable CSF hypocretin-1 (normal CSF protein, CSF cell count and fluid-attenuated inversion recovery magnetic resonance imaging), no cataplexy, and a positive Multiple Sleep Latency Test. Prednisone was selected as a broad cell-mediated and antibody-mediated immunosuppressant. Repeat Multiple Sleep Latency Test and CSF evaluation were performed after 3 weeks, but no clinical improvement was noted. This child now has developed cataplexy that is controlled by venlafaxine and modafinil. A similar prednisone trial was also performed in a hypocretin-deficient narcoleptic dog (Weimaraner) diagnosed 2 weeks after an abrupt onset, with similar negative results.[69]

In a patient with recent-onset cataplexy, combining IV immunoglobulins (IVIg) and prednisone reduced cataplexy and sleepiness subjectively, but the patient was unable to continue treatment.[97] Dauvilliers et al[98] and Zuberi et al[99] studied 4 additional patients each with IVIg alone; 6 with recent onset, 2 with more-distant disease onset. Four monthly treatments using 2 g/kg over 2 days were typically performed. Subjective effects on sleepiness and/or cataplexy were observed in recent-onset cases but not in established narcolepsy cases (defined as onset of more than a few years). Side effects were mild and included short-lasting fever in some cases. Most notably, in the Dauvilliers study,[98] cataplexy was significantly reduced and anticataplectic drugs were no longer needed 9 months after ending the IVIg treatment, suggesting long-term preventive effects.

Problematically, however, all the reported effects were subjective. Repeat CSF hypocretin-1 measurements in the Dauvilliers study[98] indicated persistence of low CSF hypocretin-1 levels, although in 1 case a possible small increase (from undetectable to 79 pg/mL) was noted. Whether a small improvement in hypocretin deficiency, without increasing CSF hypocretin-1 levels above the limit of detection was present, will have to be addressed by improving sensitivity of the reported measurements. Similarly, reports by Zuberi[99] indicated more improvement in subjective sleepiness than in cataplexy. Finally, while Multiple Sleep Latency Test and Maintenance Wakefulness Test evaluations in the Dauvillier's study indicated a nonstatistically significant improvement in sleep latency in 2 cases, the phenotype persisted.[98] Indeed, sleep-onset rapid eye movement periods were still observed in all situations. To confirm these results, proper double-blind, placebo-controlled trials will be needed. This will require close coordination of research and clinical resources in our field, as reports of narcolepsy within a few months of disease onset are, at present, extremely rare. It may also be interesting to explore the effect of other immunomodulatory treatments, for example those acting on various lymphokines.

The mode of action of IVIg on these symptoms will also need to be studied further. IVIg therapy is believed to act by clearing autoantibodies, yet most attempts at detecting such pathogenic antibodies in human narcolepsy have failed.[100,101] A notable exception may be the recent report of Smith et al,[102] who found that passive transfer of immunoglobulin from a patient with narcolepsy into mice may result in secondary M3 cholinergic hypersensitivity (central cholinergic hypersensitivity is one of most well-documented characteristics of canine narcolepsy)[103,104] in bladder strips.

However, the IVIg mixture is complex, and modulation of other arms of the immune system is possible. It remains possible that the mode of action of immune-modulatory drugs is symptomatic, as experiments at the University of California Los Angeles, in hcrtr2-mutated canines (presumably without autoimmune abnormalities) also suggest a paradoxical preventive effects on the development of cataplexy.[105] To address this issue, studying the effect of plasmapheresis, another treatment for antibody-mediated diseases, may provide further answers. We recently reported a case in which plasmapheresis had some temporary efficacy in an adult with hypocretin deficiency, unusually severe cataplexy, and late onset at age 60.[106] Whether immune modulation close to the onset and associated preventive measures, such as the monitoring of children at risk (eg, family members with the disease HLA haplotype), as performed in with type I diabetes[107] in Scandinavia, will one day prove feasible, remains to be seen.

CONCLUSION

In conclusion, the treatment of human narcolepsy is rapidly evolving. Much progress has recently been made through the improvement of currently available symptomatic therapies that enhance monoaminergic signaling. Novel stimulants and hypnotics are being developed and may further benefit narcoleptic patients. More exciting, however, may be the hypocretin-based therapies that are being designed. The most promising avenues include hypocretin agonists and hypocretin cell transplantation therapies, but these modalities are most likely decades away. Recent results using IVIg, although preliminary, also suggest the possibility of early intervention to limit disease progression, if associated with early diagnosis close to disease onset.

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REFERENCES

1. Nishino S, Mignot E. Pharmacological aspects of human and canine narcolepsy. Prog Neurobiol 1997;52:27-78.
2. Lin L, Faraco J, Li R, Kadotani H, et al. The sleep disorder canine narcolepsy is caused by a mutation in the hypocretin (orexin) receptor 2 gene. Cell 1999;98:365-76.
3. Ripley B, Fujiki N, Okura M, Mignot E, Nishino S. Hypocretin levels in sporadic and familial cases of canine narcolepsy. Neurobiol Dis 2001;8:525-34.
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. Hara J, Beuckmann CT, Nambu T, et al. Genetic ablation of orexin neurons in mice results in narcolepsy, hypophagia, and obesity. Neuron 2001;30:345-54.
6. Nishino S, Mao J, Sampathkumaran R, Shelton J, Mignot E. Increased Dopaminergic transmission mediates the wake promoting effects of CNS stimulants. Sleep Res On line; 1:49-61.
7. Wisor JP, Nishino S, Sora I, Uhl GH, Mignot E, Edgar DM. Dopaminergic role in stimulant-induced wakefulness. J Neurosci 2001;21:1787-94.
8. Nishino S, Mignot, E. CNS stimulants in sleep medicine: basic mechanisms and pharmacology. In: Kryger MH, Roth T, Dement WC eds. Principles and Practice of Sleep Medicine. Philadelphia: WB Saunders; 2005:468-98.
9. Russell V, de Villiers A, Sagvolden T, Lamm M, Taljaard J. Differences between electrically-, ritalin- and D-amphetamine-stimulated release of [3H]dopamine from brain slices suggest impaired vesicular storage of dopamine in an animal model of attention-deficit hyperactivity disorder. Behav Brain Res 1998;94:163-71.
10. Fukui R, Svenningsson P, Matsuishi T, Higashi H, Nairn AC, Greengard P, Nishi A. Effect of methylphenidate on dopamine/DARPP signaling in adult, but not young, mice. J Neurochem 2003;87:1391-401.
11. Volkow ND, Fowler JS, Wang GJ, Ding YS, Gatley SJ. Role of dopamine in the therapeutic and reinforcing effects of methylphenidate in humans: results from imaging studies. Eur Neuropsychopharmacol 2002;12:557-66.
12. Mignot E, Renaud A, Nishino S, Arrigoni J, Guilleminault C, Dement WC. Canine cataplexy is preferentially controlled by adrenergic mechanisms: evidence using monoamine selective uptake inhibitors and release enhancers. Psychopharmacology 1993;113:76-82.
13. Mignot E. An update on the pharmacotherapy of excessive daytime sleepiness and cataplexy. Sleep Med Rev. 2004;8:333-8.
14. US Modafinil. Randomized trial of modafinil as a treatment for the excessive daytime somnolence of narcolepsy: US Modafinil in Narcolepsy Multicenter Study Group. Neurology 2000;54:1166-75.
15. Shelton J, Nishino S, Vaught J, Dement WC, Mignot E. Comparative effects of modafinil and amphetamine on daytime sleepiness and cataplexy of narcoleptic dogs. Sleep 1995;18:817-26.
16. Willie JT, Renthal W, Chemelli RM, et al. Modafinil more effectively induces wakefulness in orexin-null mice than in wild-type littermates. Neuroscience 2005;130:983-95.
17. Mignot E. Mignot E, Nishino S, Guilleminault C, Dement WC. Modafinil binds to the dopamine uptake carrier site with low affinity. Sleep 1994;17:436-7.
18. Saper CB, Scammell TE. Modafinil: A drug in search of a mechanism of action. Sleep 2004;27:11-2.
19. Willie JT, Chemelli RM, Sinton CM, et al. Distinct narcolepsy syndromes in Orexin receptor-2 and Orexin null mice: molecular genetic dissection of Non-REM and REM sleep regulatory processes. Neuron 2003;38:715-30.
20. Nishino S, Arrigoni J, Shelton J, Dement WC, Mignot E. Desmethyl metabolites of serotonergic uptake inhibitors are more potent for suppressing canine cataplexy than their parent compounds. Sleep 1993;16:706-12.
21. Nishino S. Anticataplectic medications. In: Bassetti C, Billiard M, Mignot E eds. Narcolepsy and Hypersomnia. New York: Marcel Dekker; 2005: In Press.
22. Arnulf I, Mignot E. Sodium oxybate for excessive daytime sleepiness in narcolepsy-cataplexy. Sleep 2004;27:1242-3.
23. Broughton R, Mamelak M. The treatment of narcolepsy-cataplexy with nocturnal gamma-hydroxybutyrate. Can J Neurol Sci 1979;6:1.
24. Scrima L, Hartman PG, Johnson FH, Hiller FC. Efficacy of gamma-hydroxybutyrate versus placebo in treating narcolepsy-cataplexy: double-blind subjective measures. Biol Psychiatry 1989;26:331-43.
25. Mamelak M, Black J, Montplaisir J, Ristanovic R. A pilot study on the effects of sodium oxybate on sleep architecture and daytime alertness in narcolepsy. Sleep 2004;27:1327-34.
26. Maitre M. The gamma-hydroxybutyrate signalling system in brain: organization and functional implications. Prog Neurobiol 1997;51:337-61.
27. Itzhak Y, Ali SF. Repeated administration of gamma-hydroxybutyric acid (GHB) to mice: assessment of the sedative and rewarding effects of GHB. Ann N Y Acad Sci. 2002;965:451-60.
28. Donovan JL, Malcolm RJ, Markowitz JS, DeVane CL. Chiral analysis of d- and l-modafinil in human serum: application to human pharmacokinetic studies. Ther Drug Monit 2003;25:197-202.
29. Lile JA, Wang Z, Woolverton WL, France JE, Gregg TC, Davies HM, Nader MA. The reinforcing efficacy of psychostimulants in rhesus monkeys: the role of pharmacokinetics and pharmacodynamics. J Pharmacol Exp Ther 2003;307:356-66.
30. Johnson RA, Eshleman AJ, Meyers T, Neve KA, Janowsky A. [3H]substrate- and cell-specific effects of uptake inhibitors on human dopamine and serotonin transporter-mediated efflux. Synapse 1998;30:97-106.
31. Preti A. Vanoxerine National Institute on Drug Abuse. Curr Opin Invest Drugs 2000;1:241-51.
32. Gorelick DA, Gardner EL, Xi ZX. Agents in development for the management of cocaine abuse. Drugs 2004;64:1547-73.
33. Srisurapanont M, Jarusuraisin N, Jittiwutikan J. Amphetamine withdrawal: II. A placebo-controlled, randomised, double-blind study of amineptine treatment. Aust N Z J Psychiatry 1999;33:94-8.
34. Kent JM. SNaRIs, NaSSAs, and NaRIs: new agents for the treatment of depression. Lancet 2000;355:911-8.
35. Beer B, Stark J, Krieter P, Czobor P, Beer G, Lippa A, Skolnick P. DOV 216,303, a "triple" reuptake inhibitor: safety, tolerability, and pharmacokinetic profile. J Clin Pharmacol 2004;44:1360-7.
36. The abrupt cessation of therapeutically administered sodium oxybate (GHB) does not cause withdrawal symptoms. J Toxicol Clin Toxicol 2003;41:131-5.
37. Tarabar AF, Nelson LS. The gamma-hydroxybutyrate withdrawal syndrome. Toxicol Rev 2004;23:45-9
38. Zeitzer JM, E. M. In: Bassetti C, Billiard M, Mignot E eds. Narcolepsy and Hypersomnia. New York: Marcel Dekker; 2005: In Press.
39. Krogsgaard-Larsen P, Frolund B, Liljefors T, Ebert B. GABA(A) agonists and partial agonists: THIP (Gaboxadol) as a non-opioid analgesic and a novel type of hypnotic. Biochem Pharmacol 2004;68:1573-80.
40. Mathias S, Wetter TC, Steiger A, Lancel M. The GABA uptake inhibitor tiagabine promotes slow wave sleep in normal elderly subjects. Neurobiol Aging 2001;22:247-53.

41. Mignot E, Taheri S, Nishino S. Sleeping with the hypothalamus: emerging therapeutic targets for sleep disorders. Nat Neurosci 2002;5:1-6.
42. Mayer G. Ritanserin improves sleep quality in narcolepsy. Pharmacopsychiatry 2003;36:150-5.
43. Steininger TL, Alam MN, Gong H, Szymusiak R, McGinty D. Sleep-waking discharge of neurons in the posterior lateral hypothalamus of the albino rat. Brain Res 1999;840:138-47.
44. Lin JS. Brain structures and mechanisms involved in the control of cortical activation and wakefulness, with emphasis on the posterior hypothalamus and histaminergic neurons. Sleep Med Rev 2000;4:471-503.
45. Huang ZL, Qu WM, Li WD, Mochizuki T, Eguchi N, Watanabe T, Urade Y, Hayaishi O. Arousal effect of orexin A depends on activation of the histaminergic system. Proc Natl Acad Sci U S A 2001;98:9965-70.
46. Kanbayashi T, Kodama T, Hondo H, et al. CSF histamine and noradrenaline contents in narcolepsy and other sleep disorders. Sleep 2004;27:A236.
47. Nishino S, Sakurai E, Nevisimalova S, et al CSF histamine content is decreased in hypocretin-deficient human narcolepsy. Sleep 2002;25):A476.
48. Chen J, Liu C, Lovenberg TW. Molecular and pharmacological characterization of the mouse histamine H3 receptor. Eur J Pharmacol 2003;467:57-65.
49. Tedford CE, Edgar DM, Seidel WF, Mignot E, Nishino S, Pawlowski GP, Yates SL. Effects of a novel, selective, and potent histamine H3 receptor antagonist, GT-2332, on rat sleep/wakefulness and canine cataplexy. Neuroscience 1999;25:abstract 1134.
50. Barbier AJ, Berridge C, Dugovic C, et al. Acute wake-promoting actions of JNJ-5207852, a novel, diamine-based H3 antagonist. Br J Pharmacol 2004;143:649-61.
51. Shiba T, Fujiki N, Wisor J, Edgar D, Sakurai T, Nishino S. Wake promoting effects of thioperamide, a histamine H3 antagonist in orexin/ataxin-3 narcoleptic mice. Sleep 2004;:A241-2.
52. Nishino S, Arrigoni J, Shelton J, et al. Effects of thyrotropin-releasing hormone and its analogs on daytime sleepiness and cataplexy in canine narcolepsy. J Neurosci 1997;17:6401-8.
53. Griffiths EC, Bennett GW. Clinical applications of thyrotropin-releasing hormone. Clin Sci 1987;73:449-57.
54. Nicoll RA. Excitatory action of TRH on spinal motoneurons. Nature 1977;265:242-3.
55. Sharp T, Bennett GW, Marsden CA. Thyrotropin-releasing hormone analogues increase dopamine release from slices of rat brain. J Neurochem 1982; 39:1763–6.
56. Keller HH, Bartholini G, Pletscher A. Enhancement of cerebral noradrenaline turnover by thyrotropin-releasing hormone. Nature. 1974: 248:528–9.
57. Heuer H, Schafer MK, O'Donnell D, Walker P, Bauer K. Expression of thyrotropin-releasing hormone receptor 2 (TRH-R2) in the central nervous system of rats. J Comp Neurol 2000;428:319-36.
58 Broberger C. Neurotransmitters switching the thalamus between sleep and arousal: functional effects and cellular mechanism. Presented at Showa University International Symposium for Life Science. 1st Annual Meeting New Frontiers in Neuroscience Research. Showa University Kamijo Hall (Tokyo), August 31, 2004.
59. Vogel HP, Benkert O, Illig R, Muller-Oerlinghausen B, Poppenberg A. Psychoendocrinological and therapeutic effects of TRH in depression. Acta Psychiatr Scand 1977;56:223-32.
60. Bunevicius R, Matulevicius V. Short-lasting behavioural effects of thyrotropin-releasing hormone in depressed women: results of placebo-controlled study. Psychoneuroendocrinology 1993;18:445-9.
61. Schomburg L, Turwitt S, Prescher G, Lohmann D, Horsthemke B, Bauer K. Human TRH-degrading ectoenzyme cDNA cloning, functional expression, genomic structure and chromosomal assignment. Eur J Biochem 1999;265:415-22.
62. Cheng MY, Bullock CM, Li C, Lee AG, Bermak JC, Belluzzi J, Weaver DR, Leslie FM, Zhou QY. Prokineticin 2 transmits the behavioural circadian rhythm of the suprachiasmatic nucleus. Nature 2002;417:405-10.
63. Xu YL, Reinscheid RK, Huitron-Resendiz S, et al. Neuropeptide S: a neuropeptide promoting arousal and anxiolytic-like effects. Neuron 2004;43:487-97.
64. Kastin AJ, Akerstrom V. Orexin A but not orexin B rapidly enters brain from blood by simple diffusion. JPET 1999;289:219-23.
65. Yoshida Y, Fujiki N, Maki RA, Schwarz D, Nishino S. Differential kinetics of hypocretins in the cerebrospinal fluid after intracerebroventricular administration in rats. Neurosci Lett 2003;346:182-6.
66. John J, Wu MF, Siegel JM. Systemic administration of hypocretin-1 reduces cataplexy and normalizes sleep and waking durations in narcoleptic dogs. Sleep Res Online 2000;3:23-8.
67. Fujiki N, Ripley B, Yoshida Y, Mignot E, Nishino S. Effects of IV and ICV hypocretin-1 (orexin A) in hypocretin receptor-2 gene mutated narcoleptic dogs and IV hypocretin-1 replacement therapy in a hypocretin ligand deficient narcoleptic dog. Sleep 2003;6:953-9.
68. Mieda M, Willie JT, Hara J, Sinton CM, Sakurai T, Yanagisawa M. Orexin peptides prevent cataplexy and improve wakefulness in an orexin neuron-ablated model of narcolepsy in mice. Proc Natl Acad Sci U S A 2004;101:4649-54
69. Schatzberg SJ, Barrett J, Cutter Kl, Ling L, Mignot E. Case study: Effect of hypocretin replacement therapy in a 3-year-old Weimaraner with narcolepsy. J Vet Internal Med 2004;18:586-8.
70. Hanson LR, Martinez PM, Taheri S, Kamsheh L, Mignot E, Frey II WH. Intranasal administration of hypocretin 1 (Orexin A) bypasses the blood-brain barrier & targets the brain: a new strategy for the treatment of narcolepsy. Drug Delivery Technol 2004;4:66-71.
71. Hallschmid M, Benedict C, Born J, Fehm HL, Kern W. Manipulating central nervous mechanisms of food intake and body weight regulation by intranasal administration of neuropeptides in man. Physiol Behav 2004;83:55-64.
72. DeCoursey PJ, Buggy J. Circadian rhythmicity after neural transplant to hamster third ventricle: specificity of suprachiasmatic nuclei. Brain Res 1989;500:263-75.
73. Vogelbaum MA, Galef J, Menaker M. Factors determining the restoration of circadian behavior by hypothalamic transplants. J Neural Transplant Plast 1993;4:239-56.
74. Roitberg B, Urbaniak K, Emborg M. Cell transplantation for Parkinson's disease. Neurol Res 2004;26:355-62.
75. Arias-Carrion O, Murillo-Rodriguez E, Xu M, Blanco-Centurion C, Drucker-Colin R, Shiromani PJ. Transplant of hypocretin neurons into the pontine reticular formation: preliminary results. Sleep 2004:27:1465-70.
76. Peyron C, Faraco J, Rogers W, et al. A mutation in a case of early onset narcolepsy and a generalized absence of hypocretin peptides in human narcoleptic brains. Nat Med. 2000;6:991-7.
77. Thannickal TC, Moore RY, Nienhuis R, , et al. Reduced number of hypocretin neurons in human narcolepsy. Neuron 2000;27:469-74.
78. Mignot E, Lammers GJ, Ripley B, et al. The role of cerebrospinal fluid hypocretin measurement in the diagnosis of narcolepsy and other hypersomnias. Arch Neurol 2002;59:1553-62.
79. Bjorklund A. Neurobiology. Better cells for brain repair. Nature 1993;362:414-5.
80. Couzin J. Diabetes. Islet transplants face test of time. Science 2004;306:34-7.
81. Naftanel MA, Harlan DM. Pancreatic Islet Transplantation. PLoS Med 2004;1:e58.
82. Pardridge WM. Transport of small molecules through the blood-brain barrier: biology and methodology. Adv Drug Deliv Rev 1995;15:5-36.
83. Prokai-Tatrai K, Prokai L. Modifying peptide properties by prodrug design for enhanced transport into the CNS. Prog Drug Res

2003;61:155-88.
84. Asahi S, Egashira S, Matsuda M, et al. Development of an orexin-2 receptor selective agonist, [Ala(11), D-Leu(15)]orexin-B. Bioorg Med Chem Lett 2003;13:111-3.
85. Darker JG, Porter RA, Eggleston DS, et al. Structure-activity analysis of truncated orexin-A analogues at the orexin-1 receptor. Bioorg Med Chem Lett 2001;11:737-40.
86. Croston GE, Olsson R, Currier EA, et al. Discovery of the first nonpeptide agonist of the GPR14/urotensin-II receptor: 3-(4-chlorophenyl)-3-(2- (dimethylamino)ethyl)isochroman-1-one (AC-7954). J Med Chem 2002;45:4950-3.
87. Zaveri N. Peptide and nonpeptide ligands for the nociceptin/orphanin FQ receptor ORL1: research tools and potential therapeutic agents. Life Sci 2003;73:663-78.
88. Saar K, Mazarati AM, Mahlapuu R, et al. Anticonvulsant activity of a nonpeptide galanin receptor agonist. Proc Natl Acad Sci U S A 2002;99:7136-41.
89. Smart D, Sabido-David C, Brough SJ, et al. SB-334867-A: the first selective orexin-1 receptor antagonist. Br J Pharmacol 2001;132:1179-82.
90. Porter RA, Chan WN, Coulton S, et al. 1,3-Biarylureas as selective non-peptide antagonists of the orexin-1 receptor. Bioorg Med Chem Lett 2001;11:1907-10.
91. Langmead CJ, Jerman JC, Brough SJ, Scott C, Porter RA, Herdon HJ. Characterisation of the binding of [3H]-SB-674042, a novel nonpeptide antagonist, to the human orexin-1 receptor. Br J Pharmacol 2004;141:340-6.
92. Hirose M, Egashira S, Goto Y, et al. N-acyl 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline: the first orexin-2 receptor selective non-peptidic antagonist. Bioorg Med Chem Lett 2003;13:4497-9.
93. Mignot E, Lin L, Rogers W, et al. Complex HLA-DR and -DQ interactions confer risk of narcolepsy-cataplexy in three ethnic groups. Am J Hum Genet. 2001;68:686-99.
94. Hecht M, Lin L, Kushida CA, et al. Report of a case of immunosuppression with prednisone in an 8-year-old boy with an acute onset of hypocretin-deficiency narcolepsy. Sleep 2003;26:809-10.
95. Arii J, Kanbayashi T, Tanabe Y, et al. SF hypocretin-1 (orexin-A) levels in childhood narcolepsy and neurologic disorders. Neurology 2004; 63:2440-2.
96. Gerashchenko D, Murillo-Rodriguez E, Lin L, et al. Relationship between CSF hypocretin levels and hypocretin neuronal loss. Exp Neurol 2003;184:1010-6.
97. Lecendreux M, Maret S, Bassetti C, Mouren MC, MT. Clinical efficacy of high-dose intravenous immunoglobulins near the onset of narcolepsy in a 10-year-old boy. J Sleep Res 2003;12:347-8.
98. Dauvilliers Y, Carlander B, River F, Touchon J, Tafti M. IVIG treatment in narcolepsy: Report on two new cases. J Sleep Res 2004;13(Suppl1):167.
99. Zuberi SM, Mignot E, Ling L, MacArthur I. Variable response to intravenous immunoglobulin therapy in childhood narcolepsy. J Sleep Res 2004;13(Suppl1):828.
100 Black JL 3rd, Krahn LE, Pankratz VS, Silber M. Search for neuron-specific and nonneuron-specific antibodies in narcoleptic patients with and without HLA DQB1*0602. Sleep 2002;25:719-23.
101. Taheri S, Krempetz M, Jackson M, Paterno J, Mignot E. Investigation of the autoimmune basis of narcolepsy using Western blot analysis of lateral hypothalamus protein extract with serum and cerebrospinal fluid (CSF) Sleep 2003;26:A285.
102. Smith AJ, Jackson MW, Neufing P, McEvoy RD, Gordon TP. A functional autoantibody in narcolepsy. Lancet 2004;364:2122-4.
103. Reid MS, Tafti M, Geary J, et al. Cholinergic mechanisms in canine narcolepsy: I. Modulation of cataplexy via local drug administration into pontine reticular formation. Neuroscience 1994;59:511-22.
104. Nishino S, Shelton J, Reid MS, Siegel JM, Dement WC, Mignot E. A cholinoceptive site in the basal forebrain is involved in canine narcolepsy. Soc Neurosci 1992:880.
105. Boehmer LN, Wu MF, John J, Siegel JM. Treatment with immunosuppressive and anti-inflammatory agents delays onset of canine genetic narcolepsy and reduces symptom severity. Exp Neurol 2004;188:292-9.
106. Chen W, Black J, Einen M, Mignot E. Therapeutic effects of plasmapheresis: a case report. Sleep 2005;(suppl):In Press.
107. Sadeharju K, Hamalainen AM, Knip M, et al. Enterovirus infections as a risk factor for type I diabetes: virus analyses in a dietary intervention trial. Clin Exp Immunol 2003;132:271-7.