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Neuropharmacology of Sleep and Wakefulness

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Synopsis

The development of sedative/hypnotic molecules has been empiric rather than rational. The empiric approach has produced clinically useful drugs but for no drug is the mechanism of action completely understood. All available sedative/hypnotic medications have unwanted side effects and none of these medications creates a sleep architecture that is identical to the architecture of naturally occurring sleep. This chapter reviews recent advances in research aiming to elucidate the neurochemical mechanisms regulating sleep and wakefulness. One promise of rational drug design is that understanding the mechanisms of sedative/hypnotic action will significantly enhance drug safety and efficacy.

Keywords

Neurotransmitters; Receptors; Translational Research; Drug Development

Sleep states are comprised of a constellation of physiological and behavioral traits, and the mechanisms by which sedative/hypnotic medications alter these traits remain unclear. Drugs that enhance states of sleep also alter autonomic physiology, behavior, cognition, and affect. The complexities of brain neurochemistry and the extensive neural circuits regulating levels of behavioral arousal contribute to the present inability to understand exactly how sedative/hypnotics promote sleep. An additional complexity is that many sedative/hypnotic drugs have behavioral state-specific actions. For example, some sedative/hypnotic drugs promote the non-rapid eye movement (NREM) phase of sleep at the expense of decreasing the rapid eye movement (REM) phase of sleep. In spite of the foregoing limitations, there has been progress in developing sleep medications that maximize desired actions such as rapid sleep onset, minimal next day effect, low or no abuse potential, and creation of a drug-induced state that is indistinguishable from physiological sleep. To date, however, no sedative/hypnotic produces all of these desired effects.

Rational drug design is an approach that has been successful in the development of antibiotic medications. Rational drug development of sedative/hypnotic medications is an approach based on understanding the receptor-binding properties of a molecule and how a molecule alters ligand binding, neurotransmitter synthesis, release, reuptake, and

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degradation. All of the foregoing cellular mechanisms can then be interpreted in the context of the overall drug effect. For sedative/hypnotic medications the desired action is, of course, promoting a safe and restorative sleep-like state. This chapter and Figure 1 provide an overview of neurotransmitters and brain regions currently known to modulate states of sleep and wakefulness. This overview of sleep neuropharmacology can be read as a précis of a recent chapter¹ and interested readers are referred elsewhere for detailed reviews on sleep.^{2–8}

γ-AMINOBUTYRIC ACID (GABA)

GABA is the major inhibitory neurotransmitter in the brain, and activation of GABA_A receptors causes neuronal inhibition by increasing chloride ion conductance. Because of their powerful inhibitory effects, GABA_A receptors are the targets of most sedative/hypnotic and general anesthetic drugs. GABA_A receptors exist as multiple subtypes (reviewed in⁹) and these subtypes are differentially located throughout the brain (reviewed in¹⁰). The differences in clinical effects caused by various benzodiazepine (e.g., diazepam) and non-benzodiazepine (e.g., eszopiclone) sedative/hypnotics are attributed to the relative selectivity of these drugs for different GABA_A receptor subtypes.¹⁰ The complexity imparted by the numerous GABA_A receptor subtypes is humbling. Although there is detailed knowledge about the many subunit isoforms that comprise GABA_A receptor subtypes,⁹ information is lacking about which of the many possible subtypes actually are expressed in specific brain regions,^{11–13} and which subtypes are localized synaptically versus extrasynaptically.¹⁴ Extrasynaptically localized GABA_A receptors possess a delta subunit and have particular relevance for sleep medicine.^{15, 16}

A better understanding of the *in vivo* characteristics and anatomical localization of GABA_A receptor subtypes will contribute to rationale drug development. The preclinical studies described in this section illustrate the complexity of the problem and provide examples of how the effects of GABAergic drugs on behavior vary as a function of brain region. For example, although systemic administration of GABA-mimetic drugs promotes sleep, sedation, or general anesthesia, enhancing GABAergic transmission within the pontine reticular formation actually increases wakefulness and decreases sleep. The pontine reticular formation is part of the ascending reticular activating system and contributes to the generation of REM sleep. Direct administration into the pontine reticular formation of drugs that increase GABAergic transmission increases wakefulness and inhibits sleep.^{17–20} Similarly, pharmacologically increasing the concentration of endogenous GABA within the pontine reticular formation increases the time required for isoflurane to induce general anesthesia.²¹ Consistent with this finding are data showing that endogenous GABA levels in the pontine reticular formation are greater during wakefulness than during REM sleep²² or during the loss of wakefulness caused by isoflurane.²¹ Inhibiting GABAergic signaling at GABA_A receptors within the pontine reticular formation causes an increase in REM sleep and a decrease in wakefulness^{18, 19, 23, 24}. Likewise, decreasing extracellular GABA levels in the pontine reticular formation of rat decreases wakefulness and increases sleep,²⁰ and shortens the time required for isoflurane to induce loss of consciousness.²¹ Furthermore, blocking GABA_A receptors in the pontine reticular formation increases time needed to regain wakefulness after isoflurane anesthesia.¹⁸ Considered together, these data demonstrate a wakefulness-promoting role for GABA in the pontine reticular formation.

In brain regions containing neurons that promote wakefulness, GABAergic inhibition has been shown to cause an increase in sleep. These brain regions include the dorsal raphe nucleus (Fig. 1; DRN), tuberomammillary nucleus of the posterior hypothalamus (Fig. 1; TMN), medial preoptic area (Fig. 1; MPO), and ventrolateral periaqueductal gray²⁵ (for reviews see^{7, 26, 27}).

ACETYLCHOLINE

Acetylcholine is distinguished as being the first identified neurotransmitter. Although the first neurochemical theory of sleep²⁸ correctly posited that acetylcholine plays a primary role in generating the brain-activated states of wakefulness and REM sleep, cholinergic drugs are not part of the standard pharmacological armamentarium of sleep disorders medicine. Nonetheless, understanding the mechanisms by which cholinergic neurotransmission generates and maintains REM sleep is crucial, because acetylcholine interacts with other transmitter systems that are targets of sleep pharmacotherapy (e.g., GABAergic and monoaminergic). Much of the research on the regulation of sleep by acetylcholine has focused on transmission mediated by muscarinic cholinergic receptors. Five subtypes (M₁–M₅) of the muscarinic receptor have been identified,²⁹ and the M₂ subtype plays a key role in the generation of REM sleep.³⁰

Cholinergic signaling originating from the laterodorsal tegmental and pedunculopontine tegmental nuclei (LDT/PPT) and the basal forebrain (see Fig. 1) promotes the cortically activated states of wakefulness and REM sleep (reviewed in³¹). LDT/PPT neurons can be divided into two populations based on discharge pattern. One population discharges maximally during wakefulness and REM sleep (referred to as Wake-On/REM-On) and another population fires only during wakefulness (Wake-On/REM-Off) (reviewed in²). This finding helps explain how acetylcholine can promote both wakefulness and REM sleep. LDT/PPT neurons project to numerous wakefulness-promoting brain regions.² Cholinergic terminals in the pontine reticular formation arise from the LDT/PPT,² and muscarinic receptors are present in the pontine reticular formation.^{30, 32, 33} Many studies have administered cholinomimetics to the pontine reticular formation and have demonstrated that cholinergic transmission in the pontine reticular formation induces REM sleep (reviewed in^{2, 31}). Electrically stimulating the LDT/PPT increases acetylcholine release in the pontine reticular formation³⁴ and increases REM sleep.³⁵ The release of endogenous acetylcholine in the pontine reticular formation is significantly greater during REM sleep than during wakefulness or NREM sleep.^{36–38} Taken together, these data demonstrate that cholinergic projections from the LDT/PPT to the pontine reticular formation promote REM sleep.

Recent in vivo data obtained from normal rats demonstrate that the sedative/hypnotics zolpidem, diazepam, and eszopiclone differentially alter acetylcholine release in the pontine reticular formation.³⁹ Intravenous administration of eszopiclone prevented the REM phase of sleep, increased EEG delta power, and decreased acetylcholine release in rat pontine reticular formation.³⁹ These data provide the first functional evidence for a heterogeneous distribution of GABA_A receptor subtypes within the pontine reticular formation. The different effects of GABA_A receptor agonists on sleep have been attributed to brain region-specific distributions of GABA_A receptors and differences in sedative/hypnotic affinities for GABA_A receptor subtypes.⁴⁰ These preclinical data can be contrasted with human psychopharmacology where there has been no study convincingly demonstrating differential GABA_A subtype binding among benzodiazepine and non-benzodiazepine sleeping medications.⁴⁰ To date, the non-benzodiazepine, benzodiazepine-receptor agonist eszopiclone remains the only sleeping medication for which the long-term (6 months) effects have been characterized.^{41, 42}

Cholinergic neurons originating in the basal forebrain project throughout the entire cerebral cortex (reviewed in⁴³). Acetylcholine release in the basal forebrain is highest during REM sleep, lower during quiet wakefulness, and lowest during NREM sleep.⁴⁴ Cortical acetylcholine release is increased during wakefulness^{43, 45, 46} and REM sleep⁴⁵ as compared to NREM sleep. These support the interpretation that cholinergic transmission from the basal forebrain promotes cortical activation during wakefulness and REM sleep.

ADENOSINE

Adenosine is a breakdown product of adenosine triphosphate (ATP). Increases in endogenous adenosine levels in a specific brain region during a period of prolonged wakefulness indicate that the region has been metabolically active. Direct biochemical measures show that ATP levels increase during sleep in areas of the brain that are most active during wakefulness.⁴⁷ This finding provides direct support for the hypothesis that sleep serves a restorative function.⁴⁸

Four subtypes of adenosine receptors, A₁, A_{2A}, A_{2B}, and A₃, have been identified and are distributed widely throughout the brain. Adenosine A₁ and A_{2A} receptors are antagonized by caffeine and the idea that adenosine promotes sleep is supported by the ubiquitous consumption of caffeine to maintain wakefulness and enhance alertness. In humans, oral administration of caffeine prior to nocturnal sleep increases sleep latency and reduces sleep efficiency.⁴⁹ Furthermore, morning caffeine ingestion has been shown to decrease sleep efficiency and overall sleep during the subsequent night.⁵⁰ No adenosine agonists are presently available to promote sleep. Adenosine, however, is relevant for sleep medicine, as insomnia can be caused by consumption of caffeine or by the respiratory stimulant theophylline. Interestingly, adenosine can have analgesic effects and this action shows promise for clinical use.⁵¹

Adenosinergic transmission in brain regions that regulate sleep and wakefulness has been extensively investigated (reviewed in^{2, 52–54}). Activating adenosine A₁ receptors causes neuronal inhibition, and A₁ is the most abundant adenosine receptor subtype in brain. This section highlights selected studies supporting the interpretation that adenosine promotes sleep, at least in part, by inhibiting neurons in several key wakefulness-promoting brain areas.

Prolonged wakefulness increases adenosine levels selectively in the basal forebrain (Fig. 1; BF) and cortex,⁵⁵ and increases adenosine A₁ receptor binding in human⁵⁶ and rat⁵⁷ brain. Pharmacologically increasing adenosine levels in the basal forebrain⁵⁸ or administering adenosine A₁ receptor agonists to the basal forebrain⁵⁴ causes an increase in sleep. Inactivating adenosine A₁ receptors in the basal forebrain decreases EEG delta power and NREM sleep time,⁵⁹ and immunohistochemical studies reveal that the basal forebrain contains A₁ receptors, but not A_{2A} receptors.⁶⁰ Cholinergic neurons in the basal forebrain project to the cortex and contribute to the EEG activation characteristic of wakefulness and REM sleep. Adenosine directly inhibits cholinergic neurons in the basal forebrain by activating A₁ receptors.⁶¹ Adenosine indirectly inhibits wakefulness-promoting hypocretin (orexin)-containing neurons in the lateral hypothalamus (Fig. 1; LH) by activating A₁ receptors.⁶² Blocking adenosine A₁ receptors in the lateral hypothalamus causes an increase in wakefulness and a decrease in sleep.⁶³ Histaminergic neurons in the tuberomammillary nucleus (Fig. 1; TMN) express adenosine A₁ receptors, and activating those receptors increases NREM sleep.⁶⁴ These complementary data suggest that adenosine promotes sleep by inhibiting wakefulness-promoting neurons localized to the basal forebrain, lateral hypothalamus, and tuberomammillary nucleus.

Adenosine also exerts sleep-promoting effects by actions at the level of the prefrontal cortex (Fig. 1; PFC) and the pontine reticular formation (Fig. 1; PnO, PnC). In vivo microdialysis experiments in mouse⁶⁵ have shown that adenosine acting at A₁ receptors in the prefrontal cortex inhibits traits that characterize wakefulness (including acetylcholine release in the prefrontal cortex and activation of the EEG), as well as the state of wakefulness. Activation of adenosine A₁ receptors in the prefrontal cortex also causes a decrease in the release of acetylcholine in the pontine reticular formation. These findings demonstrate that in the

prefrontal cortex, adenosine A₁ receptors mediate a descending inhibition of wakefulness-promoting systems. Within the pontine reticular formation, activation of adenosine A_{2A} receptors increases time needed to recover from general anesthesia,⁶⁶ increases acetylcholine release,^{66, 67} and increases the amount of time spent in NREM sleep⁶⁷ and REM sleep.^{67, 68} The increase in REM sleep may be a result of the A_{2A}-mediated increase in acetylcholine release, because coadministration of a muscarinic receptor antagonist with the A_{2A} agonist blocks the REM sleep increase.⁶⁸ Studies examining the effects on sleep of adenosine receptor antagonists are required in order to conclude that endogenous adenosine within the pontine reticular formation modulates sleep. The finding that clinically used opioids, such as morphine and fentanyl, decrease adenosine levels in the pontine reticular formation⁶⁹ and disrupt REM sleep (reviewed in⁷⁰) suggests the possibility that adenosinergic transmission within the pontine reticular formation participates in REM sleep generation.

BIOGENIC AMINES

The monoamines have long been known to promote wakefulness. Serotonin (5-hydroxytryptamine; 5HT)-containing neurons of the dorsal raphe nucleus (Fig. 1; DRN), norepinephrine-containing neurons of the locus coeruleus (Fig. 1; LC), and histamine-containing neurons of the tuberomammillary nucleus (Fig. 1; TMN) discharge at their fastest rates during wakefulness, slow their firing in NREM sleep, cease discharging prior to and during REM sleep, and resume firing prior to the onset of wakefulness (reviewed in²). Dopaminergic neurons, by contrast, do not show major changes in firing rates across the sleep-wakefulness cycle.

Serotonin

Serotonin release in the dorsal raphe nucleus⁷¹ and preoptic area⁷² of rat is highest during wakefulness. Furthermore, electrical stimulation of the dorsal raphe nucleus increases wakefulness.⁷³ Serotonin receptors are divided into seven families (5HT₁–5HT₇).⁷⁴ Systemic administration of agonists for 5HT_{1A}, 5HT_{1B}, 5HT_{2A/2C} or 5HT₃ receptors causes an increase in wakefulness and a decrease in sleep (reviewed in⁷⁵). Local administration of a 5HT_{1A} receptor agonist to the dorsal raphe nucleus increases wakefulness in rat⁷⁶ but increases REM sleep in cat.⁷⁷ Microinjection of a 5HT_{2A/2C} receptor agonist into rat dorsal raphe nucleus also decreases REM sleep with no significant effect on wakefulness.⁷⁸ These incongruent findings may be due to species differences, or may indicate that in addition to promoting wakefulness, serotonin plays a permissive role in the generation of REM sleep. Systemic administration of antagonists for the 5HT_{2A} receptor or the 5HT₆ receptor to rat decreases wakefulness, increases NREM sleep, and has no effect on REM sleep.⁷⁹ These data are consistent with the view that serotonin is wakefulness-promoting. Genetically modified mice also have been used to explore the role of serotonin in sleep and wakefulness. Mice lacking the genes for the 5HT_{1A}⁸⁰ or 5HT_{1B}⁸¹ receptor showed an increase in REM sleep. Administration of a 5HT_{1A}^{80, 82}, a 5HT_{1B}⁸¹, or a 5HT_{2A/2C}⁸³ receptor agonist decreased REM sleep in rodent and human. These data indicate that serotonin acting at 5HT_{1A}, 5HT_{1B}, and 5HT_{2A/2C} receptors plays a role in suppressing REM sleep. The foregoing data underlie the fact that insomnia can be secondary to the use of selective serotonin reuptake inhibitors (SSRI) or serotonin, norepinephrine reuptake inhibitors (SNRI).

Norepinephrine

Noradrenergic cells of the locus coeruleus inhibit REM sleep, promote wakefulness, and project to a variety of other arousal-regulating brain regions (Fig. 1) including the hypothalamus, thalamus, basal forebrain, and cortex (reviewed in⁸⁴). Noradrenergic

receptors include α_1 -, α_2 -, and β -adrenergic subtypes.⁸⁵ Administration of noradrenaline or α - and β -receptor agonists to the medial septal area^{86, 87} or the medial preoptic area^{88, 89} increases wakefulness. Stimulation of locus coeruleus neurons increases noradrenaline in the prefrontal cortex of anesthetized rat,^{90, 91} and contributes to cortical activation. These data are consistent with the view that noradrenaline promotes wakefulness. However, bilateral microinjection of an α_1 -antagonist (prazosin), an α_2 -agonist (clonidine), or a β -antagonist (propranolol) into the pedunculopontine tegmental nucleus increases REM sleep with little to no effect on NREM sleep or wakefulness.⁹² The arousal-regulating effects of noradrenaline are brain-region specific. The treatment of hypertension with blockers of α - and/or β -adrenergic receptors can disrupt normal sleep.

Histamine

Histaminergic cell bodies, which are located in the tuberomammillary nucleus of the posterior hypothalamus have diffuse projections throughout the brain (reviewed in⁹³). Data from posterior hypothalamic lesion studies and from single unit recordings indicate that the tuberomammillary nucleus promotes wakefulness.⁹³ Three histaminergic receptors, denoted H_1 , H_2 , and H_3 , are present in the brain (for review see⁹⁴). First generation H_1 receptor antagonists, such as diphenhydramine, cause drowsiness (sedation) and impaired performance in humans⁹⁵ and rats.⁹⁶ Newer antagonists that are relatively selective for the H_1 histamine receptor, such as the potent antagonist doxepin, improve subjective and objective measures of sleep in insomnia patients without causing sedation or psychomotor impairments the next day.⁹⁷ Systemic administration of the H_1 receptor antagonists mepyramine⁹⁸ and cyproheptadine⁹⁹ caused a significant increase in NREM sleep in cat and rat, respectively. Decreasing brain histamine levels by inhibiting synthesis significantly decreases wakefulness and increases NREM sleep in rat^{100, 101} and cat.⁹⁸ These data suggest that histaminergic signaling via the H_1 receptor promotes wakefulness. New therapies for sleep disorders and for maintaining vigilance include H_3 receptor antagonists and inverse agonists.^{102–104}

Dopamine

Stimulants such as amphetamine, cocaine, and methylphenidate increase wakefulness and counter hypersomnia by increasing levels of endogenous dopamine (reviewed in¹⁰⁵). In vivo imaging studies suggest that sleep deprivation increases dopamine levels in human brain.¹⁰⁶ The cell bodies of dopaminergic neurons that regulate arousal reside in the ventral tegmental area (Fig. 1; VTA) and the substantia nigra pars compacta.⁷⁵ These dopaminergic neurons project to the dorsal raphe nucleus, basal forebrain, locus coeruleus, thalamus, and LDT (reviewed in¹⁰⁷). There are also dopaminergic neurons in the ventrolateral periaqueductal gray that are active during wakefulness and have reciprocal connections with sleep-regulating brain areas.¹⁰⁸

Five dopaminergic receptors have been cloned (D1–D5). Dopaminergic neurons of the substantia nigra and ventral tegmental area do not change firing rates as a function of states of sleep and wakefulness (reviewed in²). Dopamine does promote wakefulness and dopamine-transporter-knockout mice display increased wakefulness and decreased NREM sleep compared to controls.¹⁰⁹ Systemic administration of D1 receptor agonists or antagonists causes an increase or decrease, respectively, in wakefulness.¹¹⁰ Intracerebroventricular administration of a D1 or D2 receptor agonist to rat increases wakefulness.¹¹¹ Systemic administration of a D2 receptor agonist causes biphasic effects with low doses decreasing wakefulness and high doses increasing wakefulness.^{112, 113} Systemic administration of D-amphetamine to rat increases wakefulness and decreases NREM sleep and REM sleep.¹¹⁴ The mechanisms by which modafinil counters excessive

daytime sleepiness remain to be specified. There is evidence that modafinil enhances synaptic release of dopamine and norepinephrine.¹¹⁵

GLUTAMATE

Glutamate is the main excitatory neurotransmitter in the brain and acts at α -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA), kainate, and *N*-methyl-D-aspartate (NMDA) ionotropic receptors. Surprisingly, little is known about glutamatergic regulation of sleep and wakefulness. Sleep state-dependent changes in levels of endogenous glutamate change differentially across the brain (see Table 8 of¹¹⁶). For example, glutamate levels in some areas of rat cortex show increases in concentration during wakefulness and REM sleep, and decreases during NREM sleep.¹¹⁷ Microinjection and electrophysiological studies provide evidence that glutamate acts within the laterodorsal tegmental nucleus and pedunculopontine tegmental nucleus^{118–120} (Fig. 1; PPT), the pontine reticular formation^{121–123} (Fig. 1; PnO, PnC), and medial portions of the medullary reticular formation^{122, 124} to modulate traits and states of arousal. Glutamatergic neurons are present in rat pontine reticular formation¹²⁵ and neurons in the pontine reticular formation are capable of synthesizing glutamate for use as a neurotransmitter.¹²⁶ Glutamate elicits excitatory responses from pontine reticular formation neurons,^{127, 128} and glutamatergic and cholinergic transmission in the pontine reticular formation interact synergistically to potentiate catalepsy.¹²⁹ Given individually, agonists for AMPA, kainate, and NMDA receptors evoke excitatory responses from pontine reticular formation neurons.¹²¹ Dialysis delivery of the NMDA receptor antagonists ketamine or MK-801 to cat pontine reticular formation decreases acetylcholine release in the pontine reticular formation and disrupts breathing.³⁸

PEPTIDES

Many peptides are known to modulate sleep (reviewed in¹³⁰). The present chapter focuses on hypocretin (orexin), leptin, and ghrelin because of their relevance for sleep disorders medicine.

Hypocretin-1 and -2

Numerous lines of evidence support a role for hypocretin-1 and -2 (also called orexin A and B) in the maintenance of wakefulness. The cell bodies of hypocretin-producing neurons are localized to the dorsolateral hypothalamus^{131, 132} and send projections to all the major brain regions that regulate arousal.^{133, 134} Hypocretinergic neurons discharge with the highest frequency during active wakefulness and show almost no discharge activity during sleep.^{135, 136} Hypocretin-1 levels in the hypothalamus of cat are greater during wakefulness and REM sleep than during NREM sleep.¹³⁷ Dogs displaying a narcoleptic phenotype have a mutation of the hypocretin receptor-2 gene,¹³⁸ and hypocretin mRNA and peptide levels are greatly reduced in human narcoleptic patients.^{139, 140} Patients presenting with narcolepsy-cataplexy also have greatly reduced levels of hypocretin in their cerebrospinal fluid compared to controls.¹⁴¹ Preclinical studies have demonstrated that selective lesions of hypocretin-containing neurons^{142, 143} or genetic removal of the peptide¹⁴⁴ result in a narcoleptic phenotype. By what mechanisms might hypocretin enhance wakefulness?

Two receptors for the hypocretin peptides have been identified. Hypocretin-1 and -2 receptors have been localized to the LDT/PPT, pontine reticular formation, dorsal raphe nucleus, and locus coeruleus.^{145–149} Electrophysiological studies demonstrate that hypocretin-1 and/or hypocretin-2 excite neurons in these same brain regions.^{150–158} Hypocretin-1 and -2 also excite tuberomammillary neurons^{159, 160} and cholinergic neurons of the basal forebrain.¹⁶¹

Intracerebroventricular administration of hypocretin-1 increases wakefulness and decreases NREM sleep and REM sleep in rat.^{162, 163} When administered into the lateral preoptic area,¹⁶⁴ the LDT,¹⁶⁵ pontine reticular formation,^{20, 166} or basal forebrain,^{167, 168} hypocretin-1 causes an increase in wakefulness. In cat, microinjection of hypocretin-1 into the pontine reticular formation increases REM sleep if delivered during NREM sleep,¹⁵² but suppresses REM sleep if delivered during wakefulness.¹⁶⁶ The wakefulness-promoting effect of hypocretin in the pontine reticular formation is further supported by evidence that delivery of antisense oligonucleotides against the hypocretin-2 receptor to the pontine reticular formation of rat enhance REM sleep and induce cataplexy.¹⁶⁹

Measuring the effect of hypocretin-1 on the release of other arousal-regulating transmitters may provide insight into how hypocretin-1 promotes wakefulness. Microinjection of hypocretin-1 into the basal forebrain of rat increases cortical acetylcholine release.¹⁷⁰ Intracerebroventricular delivery of hypocretin-increases histamine in rodent frontal cortex¹⁷¹ and anterior hypothalamus.¹⁷² Microinjection of hypocretin-1 into the ventricles or the ventral tegmental area increases dopamine release in rat prefrontal cortex.¹⁶³ Hypocretin-1 delivered to rat dorsal raphe nucleus increases serotonin release in the dorsal raphe nucleus,¹⁷³ and dialysis delivery of hypocretin-1 to rat pontine reticular formation increases acetylcholine release¹⁷⁴ and GABA levels²⁰ in the pontine reticular formation. The increase in wakefulness produced by microinjecting hypocretin-1 into the pontine reticular formation is prevented by blocking GABA_A receptors.¹⁷⁵ This finding suggests that hypocretin may increase wakefulness, in part, by increasing GABA levels in the pontine reticular formation. Considered together, these data support the classification of hypocretin-1 as a wakefulness-promoting neuropeptide.

An alternative hypothesis is that a primary function of hypocretin is to enhance activity in motor systems and the increase in wakefulness is secondary. This hypothesis is supported by data showing that hypocretin-1 concentrations in the cerebrospinal fluid are significantly greater during active wakefulness with movement than during quiet wakefulness with no movement.¹³⁷ Hypocretinergic neurons also have very low firing rates during quiet wakefulness (without movement) compared to active wakefulness.^{135, 136} Oral administration of the hypocretin-1 and -2 receptor antagonist ACT-078573 increases NREM sleep and REM sleep in rat, dog, and human,¹⁷⁶ suggesting a direct, wakefulness-promoting effect of endogenous hypocretin.

Leptin and Ghrelin

Due to the ongoing epidemic of obesity and the association between metabolic syndrome and sleep disorders, many studies aim to understand the sleep-related roles of leptin and ghrelin. Decreased levels of leptin (a hormone that suppresses appetite) and increased levels of ghrelin (a hormone that stimulates appetite) are associated with short sleep duration in humans.^{177, 178} The sleep of *ob/ob* mice (obese mice with reduced levels of leptin) is characterized by an increase in number of arousals and a decrease in the duration of sleep bouts compared to wild type controls.¹⁷⁹ The *ob/ob* mice also have an impaired response to the cholinergic enhancement of REM sleep.¹⁸⁰ Similarly, *db/db* mice (which are also obese but are resistant to leptin) have significant alterations in sleep architecture compared to wild type control mice that include, but are not limited to, increases in NREM sleep and REM sleep during the dark phase and decreases in wakefulness and NREM sleep bout duration.¹⁸¹ Local administration of ghrelin into rat lateral hypothalamus, medial preoptic area, or paraventricular nucleus increases wakefulness, decreases NREM sleep, and increases food intake.¹⁸² Together, these findings suggest that leptin and ghrelin, hormones that are important for appetite regulation, significantly influence sleep and are significantly modulated by sleep.

OPIOIDS

Opioids are the major class of drugs used to treat acute and chronic pain, and one side effect of opioids is sleep disruption. Sleep disruption, in turn, exacerbates pain^{183, 184} and increases the dose of opioids required for successful pain management (reviewed in^{69, 70}). Clinically relevant doses of opioids given to otherwise healthy humans disrupt sleep (reviewed in¹⁸⁵). For example, a single intravenous infusion of morphine in healthy volunteers decreases stages 3 and 4 NREM sleep, decreases REM sleep, and increases stage 2 NREM sleep.¹⁸⁶ A nighttime dose of morphine or methadone also decreases stages 3 and 4 NREM sleep while increasing stage 2 NREM sleep.¹⁸⁷ Constant infusion of analgesic doses of remifentanyl overnight decreases REM sleep in healthy volunteers.¹⁸⁸ The cycle of opioid-induced sleep disruption leading to increased pain and increased opioid requirement is recognized as a significant clinical problem that must be addressed at the mechanistic level.¹⁸⁹

Opioid-induced disruption of REM sleep is mediated, at least in part, by decreasing acetylcholine release in the pontine reticular formation.⁷⁰ Opioids also decrease adenosine levels in the basal forebrain and in the pontine reticular formation,⁶⁹ two brain regions where adenosine has sleep-promoting effects. Local administration of morphine into the pontine reticular formation of cat¹⁹⁰ or rat¹⁹¹ increases wakefulness and decreases REM sleep.

FUTURE DIRECTIONS

This selective overview was completed during the summer of 2010, a date also marking the 20th anniversary of the human genome project. The stunning successes – and unmet hopes – of genomic approaches to medicine were highlighted in the June 12th and 14th issues of *The New York Times*.^{192, 193} These two articles offer a sobering reminder that taking a molecule from pre-clinical discovery to commercially available drug typically requires 15 or more years. This time interval is without any mandate to understand the mechanisms of drug action. As a former director of research and development at Wyeth noted¹⁹³ “Genomics did not speed up drug development. It gave us more rapid access to new molecular targets.” Potential molecular targets can be rapidly interrogated with high throughput screening programs that use a cell line transfected to contain a reporter construct. But identifying potential molecular targets leaves unanswered the question of whether the candidate targets will be druggable in vivo. This complexity is exemplified by sedative/hypnotic medications commonly used in sleep medicine. GABA_A receptors are drug targets that promotes a sleep-like state by unknown actions⁴⁰ when they are activated in some brain regions, yet GABA_A receptors enhance wakefulness when activated selectively in the posterior hypothalamus¹⁹⁴ or pontine reticular formation.^{18, 19, 21} As busy as Fig. 1 may seem, it barely hints at the complexity of data that must be logically integrated if we are to derive a coherent model of the endogenous neurochemical processes that regulate states of sleep and wakefulness.

Recent progress in understanding the basic neuropharmacology of sleep can be appreciated by comparing the 1990 and the 2005 editions of *Brain Control of Wakefulness and Sleep*.² The incorporation of basic neuropharmacology into sleep disorders medicine is readily apparent by comparing the first and most recent editions of *Principles and Practice of Sleep Medicine*.¹⁹⁵ Future progress is most likely to come from a systems biology approach that seeks to integrate genomic, cellular, network, and behavioral levels of analysis.¹⁹⁶ The focus on sleep medications in the Clinics of North America series demonstrates the cross-cutting relevance of sleep for the practice of medicine. The pressing clinical problem of sleep disorders medicine will continue to stimulate advances in understanding the neurochemical regulation of sleep.

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Figure 1. Brain regions modulating sleep and wakefulness

Sagittal drawing of the rat brain (modified from¹⁹⁷) schematizes the location, shape, and size of some brain regions that regulate sleep and wakefulness. The name of each brain region appears in bold print, the major neurotransmitters used for signaling to other brain regions are in parentheses, and neurochemical analytes relevant for arousal-state control that have been measured in that brain region are listed under the header "Quantified". The microdialysis probe is drawn to scale and is shown sampling from the prefrontal cortex. Abbreviations: XII – hypoglossal nucleus; BF – basal forebrain; DRN – dorsal raphe nucleus; LC – locus coeruleus; LDT – laterodorsal tegmental nucleus; LH – lateral hypothalamus; MPO – medial preoptic area; PFC – prefrontal cortex; PPT – pedunculopontine tegmental nucleus; PnC – pontine reticular formation, caudal part; PnO – pontine reticular formation, oral part; TMN – tuberomammillary nucleus; TNC – trigeminal nucleus complex; VLPO – ventrolateral preoptic area; VTA – ventral tegmental area; 5HT – serotonin; ACh – acetylcholine; ADo – adenosine; Asp – aspartate; DA – dopamine; GABA – γ -aminobutyric acid; Glu – glutamate; Gly – glycine; His – histamine; Hcr – hypocretin; NE – norepinephrine; NO – nitric oxide; Noc – nociceptin; Ser – serine; 5HT – serotonin; Tau – taurine. Figure reprinted from Watson et al., 2010¹ with permission.

Figure 2. Pontine reticular formation levels of GABA during wakefulness and isoflurane anesthesia

Consistent results were obtained when measuring GABA during the transition from wakefulness to anesthesia (A) and during the resumption of wakefulness after anesthesia (B). In both cases GABA was significantly decreased during the loss of wakefulness caused by isoflurane anesthesia. Data reprinted from Vanini et al., 2008²¹ with permission.

Figure 3. Intravenous administration of eszopiclone to intact, behaving rats decreases acetylcholine (ACh) release in the pontine reticular formation (PRF)

Top: schematic coronal section of rat brain stem illustrates placement of a microdialysis probe in the PRF. Ringer's solution is pumped into the probe and samples are collected for quantification of ACh. Schematized at top right of brain are electrodes and an amplifier for recording the cortical electroencephalogram (EEG), and a representative trace showing EEG activity after intravenous administration of eszopiclone. Bottom: Histograms summarize the significant decrease in ACh release within the PRF caused by intravenous administration of eszopiclone. Data reprinted from Hambrecht-Wiedbusch et al., 2010³⁹ with permission.