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Neuropharmacology of Sleep and Wakefulness: 2012 Update

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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

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molecule alters ligand binding, neurotransmitter synthesis, release, reuptake, and 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 is an update of a précis[1] of a book chapter[2] and interested readers are referred elsewhere for detailed reviews on sleep.[3–10]

γ-AMINOBUTYRIC ACID (GABA)

GABA is the major inhibitory neurotransmitter in the brain. Although GABA transporters[11] and $GABA_B$[12] receptors can modulate sleep and wakefulness, most research into GABAergic regulation of behavioral arousal focuses on the $GABA_A$ receptor. 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 in[13]) and these subtypes are differentially located throughout the brain (reviewed in[14]). 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.[14] 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,[13] information is lacking about which of the many possible subtypes actually are expressed in specific brain regions,[15–17] and which subtypes are localized synaptically verses extrasynaptically.[18] Extrasynaptically localized $GABA_A$ receptors possess a delta subunit and have particular relevance for sleep medicine.[19, 20]

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 GABAmimetic drugs promotes sleep, sedation, or general anesthesia, enhancing GABAergic transmission with 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.[21–24] Similarly, pharmacologically increasing the concentration of endogenous GABA within the pontine reticular formation increases the time required for isoflurane to induce general anesthesia.[25] Consistent with this finding are data showing that endogenous GABA levels in the pontine reticular formation are greater during wakefulness than during REM sleep[26, 27] (Figure 2) or during the loss of wakefulness caused by isoflurane.[25] Inhibiting GABAergic signaling at $GABA_A$ receptors within the pontine reticular formation causes an increase in REM sleep and a decrease in wakefulness.[22, 23, 28, 29] Likewise, decreasing extracellular GABA levels in the pontine reticular formation of rat decreases wakefulness and increases sleep,[24] and shortens the time required for isoflurane to induce loss of consciousness.[25] Furthermore, blocking $GABA_A$ receptors in the pontine reticular formation increases time needed to regain wakefulness after isoflurane anesthesia.[22] 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 raphé

nucleus (Fig. 1; DRN), tuberomamillary nucleus of the posterior hypothalamus (Fig. 1; TMN), medial preoptic area (Fig. 1; MPO), and ventrolateral periaqueductal gray[30] (for reviews see[8, 31, 32]).

ACETYLCHOLINE

Acetylcholine is distinguished as being the first identified neurotransmitter. Although the first neurochemical theory of sleep[33] 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 (M_1–M_5) of the muscarinic receptor have been identified,[34] and the M2 subtype plays a key role in the generation of REM sleep.[35]

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 in[36]). 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 in[3]). This finding helps explain how acetylcholine can promote both wakefulness and REM sleep. LDT/PPT neurons project to numerous wakefulness-promoting brain regions.[3] Cholinergic terminals in the pontine reticular formation arise from the LDT/PPT,[3] and muscarinic receptors are present in the pontine reticular formation.[35, 37, 38] 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[3, 36]). Electrically stimulating the LDT/PPT increases acetylcholine release in the pontine reticular formation[39] and increases REM sleep.[40] The release of endogenous acetylcholine in the pontine reticular formation is significantly greater during REM sleep than during wakefulness or NREM sleep.[41–43] 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.[44] Intravenous administration of eszopiclone prevented the REM phase of sleep, increased EEG delta power, and decreased acetylcholine release in rat pontine reticular formation (Figure 3).[44] 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.[45] 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.[45] 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.[46, 47]

Cholinergic neurons originating in the basal forebrain project throughout the entire cerebral cortex (reviewed in[48]). Acetylcholine release in the basal forebrain is highest during REM

sleep, lower during quiet wakefulness, and lowest during NREM sleep.[49] Cortical acetylcholine release is increased during wakefulness[48, 50, 51] and REM sleep[50] 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.[52] This finding provides direct support for the hypothesis that sleep serves a restorative function.[53]

Four subtypes of adenosine receptors, A_1, A_{2A}, A_{2B}, and A_3, have been identified and are distributed widely throughout the brain. Adenosine A_1 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.[54] Furthermore, morning caffeine ingestion has been shown to decrease sleep efficiency and overall sleep during the subsequent night.[55] 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.[56]

Adenosinergic transmission in brain regions that regulate sleep and wakefulness has been extensively investigated (reviewed in[3, 57–60]). Activating adenosine A_1 receptors causes neuronal inhibition, and A_1 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,[61, 62] and increases adenosine A_1 receptor binding in human[63] and rat[64] brain. Pharmacologically increasing adenosine levels in the basal forebrain[65] or administering adenosine A_1 receptor agonists to the basal forebrain[59] causes an increase in sleep. Intravenous administration of buprenorphine decreases adenosine levels in the basal forebrain and increases wakefulness.[66] Inactivating adenosine A_1 receptors in the basal forebrain decreases EEG delta power and NREM sleep time,[67] and immunohistochemical studies reveal that the basal forebrain contains A_1 receptors, but not A_{2A} receptors.[68] 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 A_1 receptors.[69] Adenosine indirectly inhibits wakefulness-promoting hypocretin (orexin)-containing neurons in the lateral hypothalamus (Fig. 1; LH) by activating A_1 receptors.[70] Blocking adenosine A_1 receptors in the lateral hypothalamus causes an increase in wakefulness and a decrease in sleep.[71] Histaminergic neurons in the tuberomammillary nucleus (Fig. 1; TMN) express adenosine A_1 receptors, and activating those receptors increases NREM sleep.[72] 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 mouse[73] have shown that adenosine acting at A_1 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 A_1 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 A_1 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,[74] increases acetylcholine release,[74, 75] and increases the amount of time spent in NREM sleep[75] and REM sleep.[75, 76] 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.[76] 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, fentanyl and buprenorphine, decrease adenosine levels in the pontine reticular formation[66, 77] {added one reference} and disrupt REM sleep[66] (also reviewed in[78]) 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 raphé 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 in[3]). Dopaminergic neurons, by contrast, do not show major changes in firing rates across the sleep-wakefulness cycle.

Serotonin

Serotonin release in the dorsal raphé nucleus[79] and preoptic area[80] of rat is highest during wakefulness. Furthermore, electrical stimulation of the dorsal raphé nucleus increases wakefulness.[81] Serotonin receptors are divided into seven families ($5HT_1$–$5HT_7$).[82] Systemic administration of agonists for $5HT_{1A}$, $5HT_{1B}$, $5HT_{2A/2C}$ or $5HT_3$ receptors causes an increase in wakefulness and a decrease in sleep (reviewed in[7]). Local administration of a $5HT_{1A}$ receptor agonist to the dorsal raphé nucleus increases wakefulness in rat[83] but increases REM sleep in cat.[84] Microinjection of a $5HT_{2A/2C}$ receptor agonist into rat dorsal raphé nucleus also decreases REM sleep with no significant effect on wakefulness.[85] 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 $5HT_6$ receptor to rat during the dark phase of the light/dark cycle (active period) decreases wakefulness, increases NREM sleep, and has no effect on REM sleep.[86] 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 $5HT_{1A}$[87] or $5HT_{1B}$[88] receptor showed an increase in REM sleep. Administration of a $5HT_{1A}$[87, 89], a $5HT_{1B}$[88], or a $5HT_{2A/2C}$[90] 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 forgoing 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 in[91]). Noradrenergic receptors include α_1-, α_2-, and β-adrenergic subtypes.[92] Administration of noradrenaline or α- and β-receptor agonists to the medial septal area[93, 94] or the medial preoptic area[95, 96] increases wakefulness. Stimulation of locus coeruleus neurons increases noradrenaline in the prefrontal cortex of anesthetized rat,[97, 98] 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.[99] 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 tuberomamillary nucleus of the posterior hypothalamus have diffuse projections throughout the brain (reviewed in[100, 101]). Data from posterior hypothalamic lesion studies and from single unit recordings indicate that the tuberomamillary nucleus promotes wakefulness.[100, 101] Three histaminergic receptors, denoted H_1, H_2, and H_3, are present in the brain (for review see[102]). First generation H_1 receptor antagonists, such as diphenhydramine, cause drowsiness (sedation) and impaired performance in humans[103] and rats.[104] 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.[105] Systemic administration of the H_1 receptor antagonists mepyramine[106] and cyproheptadine[107] 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[108, 109] and cat.[106] 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.[110–114]

Dopamine

Stimulants such as amphetamine, cocaine, and methylphenidate increase wakefulness and counter hypersomnia by increasing levels of endogenous dopamine (reviewed in[115]). In vivo imaging studies suggest that sleep deprivation increases dopamine levels in human brain.[116] The cell bodies of dopaminergic neurons that regulate arousal reside in the ventral tegmental area (Fig. 1; VTA) and the substantia nigra pars compacta.[117] These dopaminergic neurons project to the dorsal raphé nucleus, basal forebrain, locus coeruleus, thalamus, and LDT (reviewed in[118]). There are also dopaminergic neurons in the ventrolateral periaqueductal gray that are active during wakefuless and have reciprocal connections with sleep-regulating brain areas.[119]

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 in[3]). Dopamine does promote wakefulness and dopamine-transporter-knockout mice display increased wakefulness and decreased NREM sleep compared to controls.[120] Systemic administration of D1 receptor agonists or antagonists causes an increase or decrease, respectively, in wakefulness.[121] Intracerebroventricular administration of a D1 or D2 receptor agonist to rat increases wakefulness.[122] Systemic administration of a D2 receptor agonist causes biphasic effects

with low doses decreasing wakefulness and high doses increasing wakefulness.[123, 124] Systemic administration of D-amphetamine to rat increases wakefulness and decreases NREM sleep and REM sleep.[125] 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.[126]

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 of[127]). For example, glutamate levels in some areas of rat cortex show increases in concentration during wakefulness and REM sleep, and decreases during NREM sleep,[128] and glutamate concentrations in rat pontine reticular formation are higher during wakefulness than during NREM sleep and REM sleep.[27] Sleep deprivation increases glutamate concentrations in rat dorsal hippocampus and medial thalamus.[129] Microinjection and electrophysiological studies provide evidence that glutamate acts within the laterodorsal tegmental nucleus and pedunculopontine tegmental nucleus[130–132] (Fig. 1; PPT), the pontine reticular formation[31, 133, 134] (Fig. 1; PnO, PnC), the medial preoptic area,[135] the insular cortex,[136] and medial portions of the medullary reticular formation[137, 138] to modulate traits and states of arousal. Glutamatergic neurons are present in rat pontine reticular formation[139] and neurons in the pontine reticular formation are capable of synthesizing glutamate for use as a neurotransmitter.[140] Glutamate elicits excitatory responses from pontine reticular formation neurons,[141, 142] and glutamatergic and cholinergic transmission in the pontine reticular formation interact synergistically to potentiate catalepsy.[143] Given individually, agonists for AMPA, kainate, and NMDA receptors evoke excitatory responses from pontine reticular formation neurons.[133] 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.[43]

PEPTIDES

Many peptides are known to modulate sleep (reviewed in[144]). 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[145, 146] and send projections to all the major brain regions that regulate arousal.[147, 148] Hypocretinergic neurons discharge with the highest frequency during active wakefulness and show almost no discharge activity during sleep.[149, 150] Hypocretin-1 levels in the hypothalamus of cat are greater during wakefulness and REM sleep than during NREM sleep.[151] Dogs displaying a narcoleptic phenotype have a mutation of the hypocretin receptor-2 gene,[152] and hypocretin mRNA and peptide levels are greatly reduced in human narcoleptic patients.[153, 154] Patients presenting with narcolepsy-cataplexy also have greatly reduced levels of hypocretin in their cerebrospinal fluid compared to controls.[155] Preclinical studies have demonstrated that selective lesions of hypocretin-containing neurons[156, 157] or genetic removal of the peptide[158] 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 raphé nucleus, and locus coeruleus.[159–163] Electrophysiological studies demonstrate that hypocretin-1 and/or hypocretin-2 excite neurons in these same brain regions.[164–172] Hypocretin-1 and -2 also excite tuberomamillary neurons[173, 174] and cholinergic neurons of the basal forebrain.[175] Studies using intracerebroventricular injection in wild-type and knock-out mice ($OX_1R^{-/-}$, $OX_2R^{-/-}$, and $OX_1R^{-/-};OX_2R^{-/-}$) suggest a differential regulation of arousal state via each hypocretin receptor subtype.[176]

Intracerebroventricular administration of hypocretin-1 increases wakefulness and decreases NREM sleep and REM sleep in rat.[177, 178] When administered into the lateral preoptic area,[179] the LDT,[180] pontine reticular formation,[24, 181] or basal forebrain,[182, 183] 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,[166] but suppresses REM sleep if delivered during wakefulness.[181] The wakefulness-promoting effect of hypocretin in the pontine reticular formation is further supported by evidence that delivery of antisense oligionucleotides against the hypocretin-2 receptor to the pontine reticular formation of rat enhance REM sleep and induce cataplexy.[184]

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.[185] Intracerebroventricular delivery of hypocretin-increases histamine in rodent frontal cortex[186] and anterior hypothalamus.[187] Microinjection of hypocretin-1 into the ventricles or the ventral tegmental area increases dopamine release in rat prefrontal cortex.[178] Hypocretin-1 delivered to rat dorsal raphé nucleus increases serotonin release in the dorsal raphé nucleus,[188] and dialysis delivery of hypocretin-1 to rat pontine reticular formation increases acetylcholine release[189] and GABA levels[24] 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.[190] 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.[151] Hypocretinergic neurons also have very low firing rates during quiet wakefulness (without movement) compared to active wakefulness.[149, 150] Oral administration of the hypocretin-1 and -2 receptor antagonists ACT-078573, DORA-22 or MK-6096 increases NREM sleep and/or REM sleep in mouse,[191] rat,[191, 192] dog,[191, 192] and human,[192] 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.[193, 194] Obese patients with obstructive apnea sleep syndrome (OSAS) have increased plasma levels of leptin[195] and ghrelin[196] compared to age-matched obese patients without OSAS. Obese patients with OSAS and excessive day time sleepiness have significantly lower levels of ghrelin and a trend for lower plasma levels of leptin compared

to obese patients with OSAS but without excessive day time sleepiness.[197] These data suggest that there is a complex relationship between leptin, ghrelin, obesity, and sleep disruption that warrants further investigation. {New paragraph break}

Rodent models that may increase the understanding of the link between metabolic syndrome, leptin, and sleep disorders include *ob/ob* mice (obese mice with reduced levels of leptin) and *db/db* mice (which are also obese but are resistant to leptin). Leptin deficient mice have attenuated responses to certain drug treatments when compared to control species. When dialyzed into the prefrontal cortex of mouse, the atypical antipsychotic olanzapine increases acetylcholine release in the prefrontal cortex.[198] The increase in acetylcholine release is significantly greater in C57BL/6J mice than in leptin-deficient mice. However, when leptin is restored to leptin-deficient mice, the olanzapine-induced increase in acetylcholine release is the same as that in the C57BL/6J mouse (Figure 4). Similarly, leptin deficient mice have a reduced antinociceptive response to supraspinal administration of neostigmine (an acetylcholinesterase inhibitor that increases levels of acetylcholine) when compared to C57BL/6J mice.[199] Leptin replacement restores the antinociceptive responses of the leptin-deficient mice to that of the C57BL/6J mice. These data indicate that a possible link between leptin and cholinergic signaling within the prefrontal cortex and the pontine reticular formation – two brain areas that play a role in the regulation of sleep and wakefulness (see Figure 1).

The sleep of *ob/ob* mice is characterized by an increase in number of arousals and a decrease in the duration of sleep bouts compared to wild type controls.[200] The *ob/ob* mice also have an impaired response to the cholinergic enhancement of REM sleep.[201] Similarly, *db/db* mice 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.[202] Local administration of ghrelin into rat lateral hypothalamus, medial preoptic area, or paraventricular nucleus increases wakefulness, decreases NREM sleep, and increases food intake.[203] 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[204–206] and increases the dose of opioids required for successful pain management (reviewed in[77, 78, 206]). Clinically relevant doses of opioids given to naïve rats[66] or to otherwise healthy humans (reviewed in[207]) disrupt sleep. 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.[208] A nighttime dose of morphine or methadone also decreases stages 3 and 4 NREM sleep while increasing stage 2 NREM sleep.[209] Constant infusion of analgesic doses of remifentanil overnight decreases REM sleep in healthy volunteers.[210] Patients receiving methadone treatment for opioid dependence experience sleep disturbances including insomnia, decreases in total sleep time, slow wave sleep, and sleep efficiency as well as increases in the number of awakings.[211–213] 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.[214]

Opioid-induced disruption of REM sleep is mediated, at least in part, by decreasing acetylcholine release in the pontine reticular formation.[78] Opioids also decrease adenosine levels in the basal forebrain and in the pontine reticular formation,[77] two brain regions

where adenosine has sleep-promoting effects. Local administration of morphine into the pontine reticular formation of cat[215] or rat[216] 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.[217, 218] 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 noted[218] "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 actions[45] when they are activated in some brain regions, yet $GABA_A$ receptors enhance wakefulness when activated selectively in the posterior hypothalamus[219] or pontine reticular formation.[22, 23, 25] 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.*[3] 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.*[220] Future progress is most likely to come from a systems biology approach that seeks to integrate genomic, cellular, network, and behavioral levels of analysis.[221] 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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Key Points

1. 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.
2. 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.
3. This chapter reviews recent advances in research aiming to elucidate the neurochemical mechanisms regulating sleep and wakefulness.

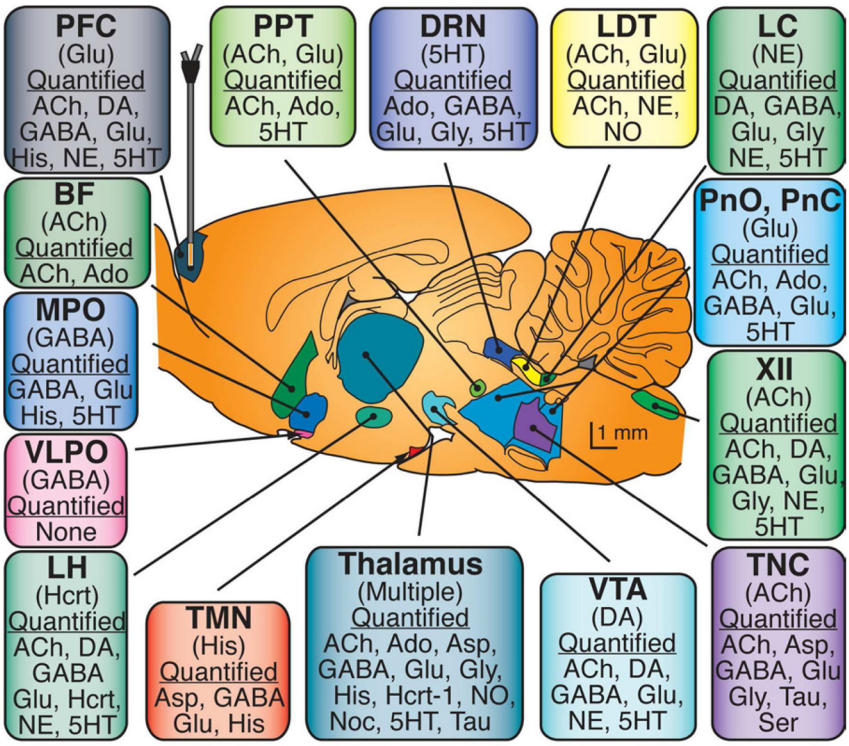

Figure 1. Brain regions modulating sleep and wakefulness

Sagittal drawing of the rat brain (modified from[222]) 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 raphé 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 – tuberomamillary 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; Hcrt – hypocretin; NE – norepinephrine; NO – nitric oxide; Noc – nociceptin; Ser – serine; 5HT – serotonin; Tau – taurine. Figure reprinted from Watson et al., 2010[2] with permission.

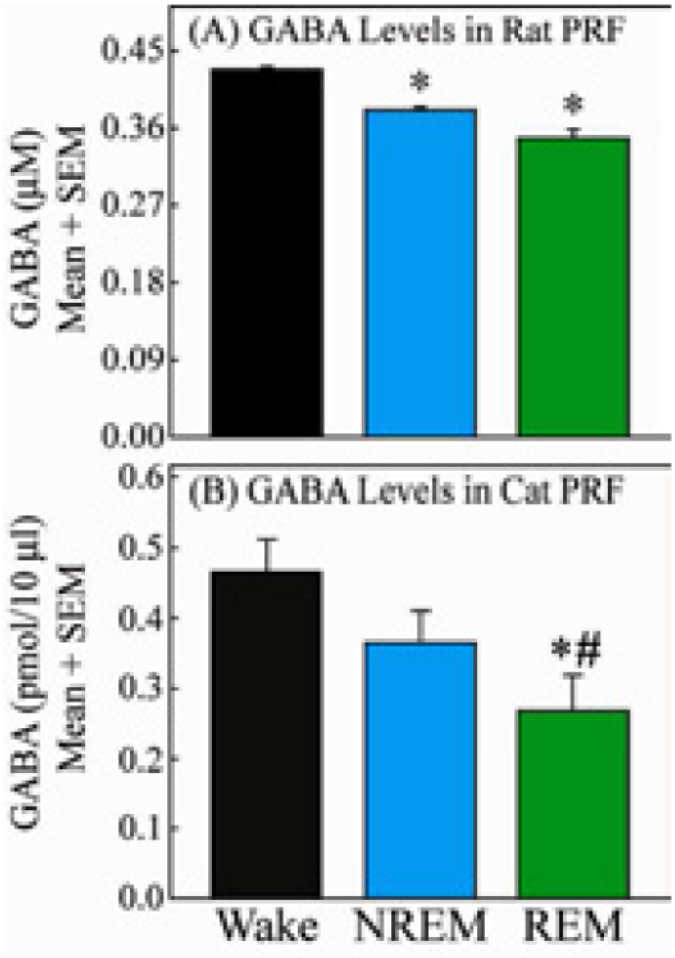

Figure 2. GABA levels in pontine reticular formation during wakefulness, NREM sleep, and REM sleep

These comparative data illustrate two key points. First, that in both rat (A) and cat (B) there are parallel, state-dependent changes in GABA levels. In rat and cat GABA levels are significantly lower in REM sleep than during wakefulness. Second, methodological differences in the collection of GABA preclude direct comparison of GABA levels between these two species. GABA levels shown in A and B reflect differences in microdialysis flowrate (0.4 μL/min for rat and 2.0 μL/min for cat), molecular weight cut-off of the microdialysis probe membrane (18000 Daltons for rat and 6 Daltons for cat) and possibly membrane material (regenerated cellulose for rat and cuprophane for cat). Figures modified from Watson et al., 2011[27] and Vanini et al., 2011[26] with permission.

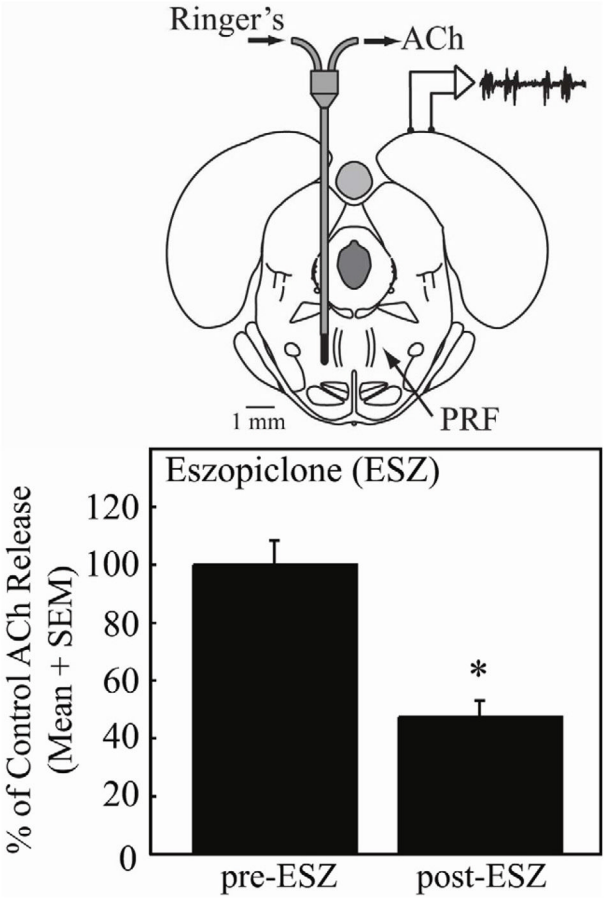

Figure 3. Intravenous administration of eszopiclone to intact, behaving rats decreases acetylcholine (ACh) release in the pontine reticular formation (PRF)

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., 2010[44] with permission.

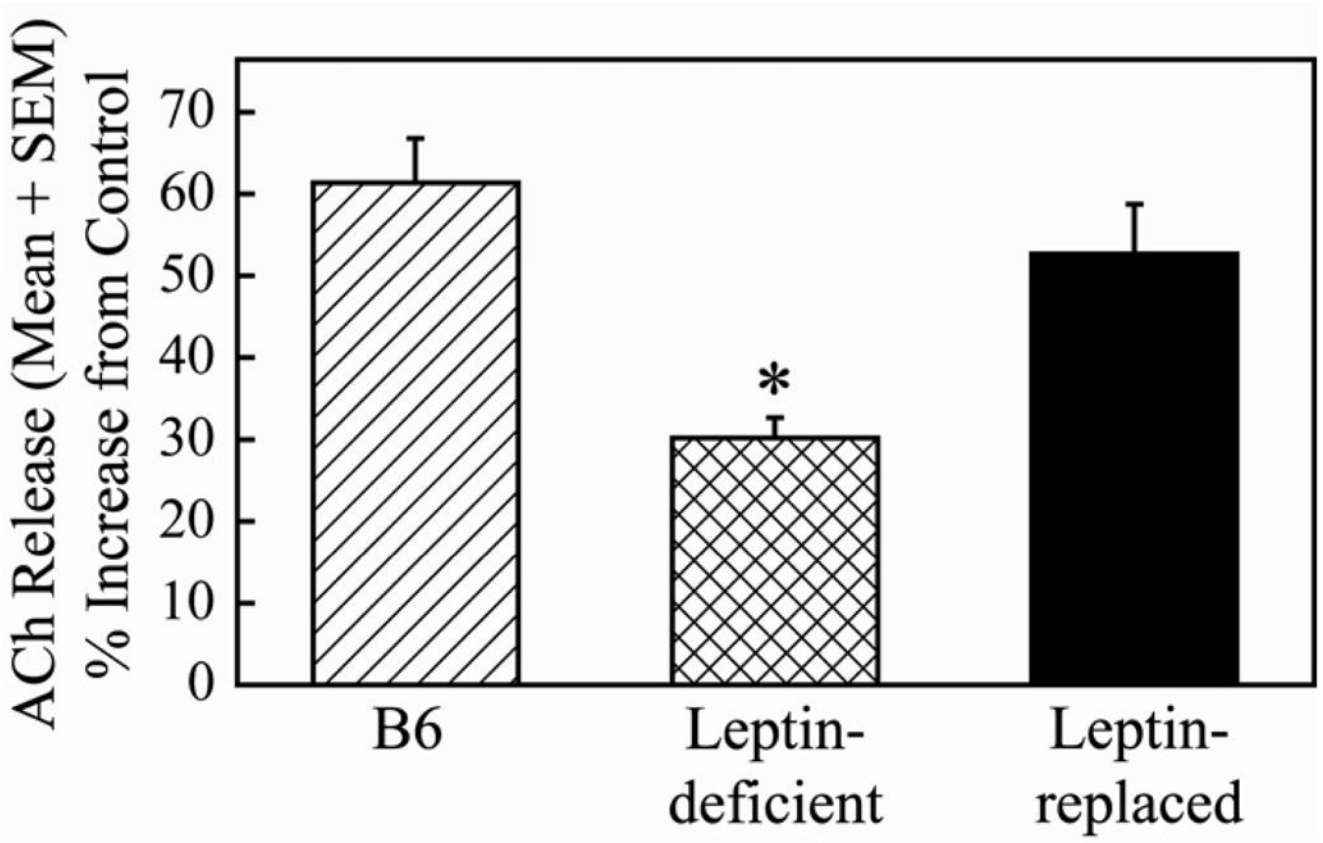

Figure 4. Leptin replacement restores the olanzapine-induced increase of acetylcholine (ACh) release in the prefrontal cortex of leptin-deficient mice

Dialysis administration of olanzapine (100 μM) to the prefrontal cortex of C57BL/6J (B6), leptin-deficient, or leptin-replaced mice caused an increase in ACh release in the prefrontal cortex. The increase in ACh release was significantly smaller in leptin-deficient mice compared to B6 controls. The olanzanpine-induced increase in ACh release was not significantly different between B6 controls and leptin-replaced mice. This suggests that leptin modulates the release of ACh within the prefrontal cortex and may also play a role in the cortical activation that occurs during wakefulness and REM sleep. Data reprinted from Wathen et al., 2012[198] with permission.