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BASIC MECHANISMS OF SEDATIVE/ HYPNOTICS

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THEORIES OF HYPNOTIC ACTION

How compounds of a wide variety of chemical classes can have relatively similar effects in inducing sleep is an intriguing pharmacologic question. Probably the dominant theory—that these compounds exert their actions by altering the physical properties of lipids in neuronal membranes—comes from the alcohol and anesthetic literature (1). Although there are many viewpoints about which specific aspect of lipid properties is affected (i.e., fluidity, thickness, or surface tension), it is largely a physicochemical approach. Ultimately, it was found inadequate for a number of reasons; perhaps the most important is that there are very little or no detectable changes in lipid bilayers at the concentrations at which these compounds induce sleep or anesthesia (1). Ultimately, interest has turned to more specific mechanisms; by far the most satisfying one (and the focus of this chapter) is the notion that altering neurotransmitter-gated receptor channels induces sleep and anesthesia (2).

Another approach to understanding sedative/hypnotics has been to hypothesize that sleep results from drug-induced reduction in energy metabolism; barbiturates, for instance, decrease cerebral glucose metabolic rate in human positron emission tomography (PET) scan studies (3). On the other hand, the results of animal studies have been more variable, such that barbiturates may (4) and benzodiazepines may not (5) decrease cerebral metabolic rate of oxygen (CMRO2). A more cogent argument against the notion that hypnotics induce sleep by lowering metabolic rate is that it stems from a view of sleep as being a very passive process, which seems to contradict the more contemporary understanding of sleep as a multifaceted, actively regulated process (6). Indeed, at doses that induce sleep (and prior to achieving anesthetic doses), patients receiving most hypnotic medications demonstrate the alternating ultradian rhythm of NREM and REM sleep, which indicates an active regulatory mechanism. It seems more parsimonious, then, to hypothesize that sedative/hypnotics act at specific sites involved in sleep regulation, rather than producing a nonspecific "slowing" of the nervous system.

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THE MOLECULAR LEVEL: THE CENTRAL $GABA_A$–BENZODIAZEPINE RECEPTOR COMPLEX

Background and Description

A major insight into the action of hypnotics began in the 1970s with the discovery of high affinity, stereospecific receptors for benzodiazepines in the central nervous system (7,8). $GABA_A$-benzodiazepine receptors are the most abundant inhibitory receptor system in the central nervous system (CNS) (9). They can be viewed as representatives of a large family of hetero-oligomeric ligand-gated ion channels (e.g., nicotinic acetylcholine, glycine, and serotonin-3 receptors) (10). Functionally, they contain three distinct but interacting moieties: recognition sites for GABA and benzodiazepines and a chloride ionophore (11). Binding of a benzodiazepine agonist to its recognition site results in increased chloride ion flux, which in turn hyperpolarizes the postsynaptic membrane at a level below that at which spike generation is possible.

Molecular cloning data indicate that the $GABA_A$–benzodiazepine receptor complex is comprised of at least five subunits; these in turn may have various isoforms (9). Each subunit is comprised of four membrane-spanning regions. The intracellular loops of some subunits contain phosphorylation sites, which have been hypothesized to be a locus of receptor modulation. It is possible, for instance, that the receptor system might respond to GABA differently in the phosphorylated versus dephosphorylated state. A- and γ-Subunits need to be present in order to be responsive to benzodiazepines, and a complete system of α, β, and γ are

needed for a fully responsive receptor (12). Recent data using murine gene targeting techniques indicate that the α-1 isoform is not required for the anxiolytic actions of benzodiazepines, but may mediate sedation and ataxic effects; in principle, this might make it possible to develop anxiolytics with a more benign side effect profile than those currently available (13). The γ-1 subunit, which in some senses may be viewed as a marker of the subgroup of GABA receptors representing the $GABA_A$–benzodiazepine receptor complex, is found in 60% to 90% of GABA receptors (14). $GABA_A$-benzodiazepine receptors have also been divided into Type I and II (sometimes referred to as ο-1 and -2), differing on the particular α subunit isoform; whether this distinction has pharmacologic significance in terms of effects of drugs that selectively bind to the Type I receptor is still under investigation. There are peripheral and "Valium-insensitive" receptors that appear not to be involved in sleep-related processes in addition to the central receptors (the focus here); hence, they are beyond the scope of this chapter.

Presynaptic Effects: Alterations in Calcium Channel Function

Although most work on the effector mechanism of benzodiazepine receptor agonists focuses on the postsynaptic mechanism of alterations in chloride ionophore function, it should be noted that there are also presynaptic actions involving calcium ion flux that have been less fully explored but that may have relevance to sedative/hypnotic properties. In rats, for instance, the dihydropyridine calcium channel blocker nifedipine given intraventricularly blocks the sleep-inducing property of systemically administered flurazepam, whereas BAY-K-8644, which facilitates calcium ion flux in dihydropyridine-sensitive channels, greatly augments the hypnotic effects of flurazepam (15).

The Role of GABA Agonists

One intriguing set of issues that has not been clearly resolved is that more specific $GABA_A$ agonists are relatively weak in their hypnotic effects, do not necessarily augment the effects of benzodiazepines, and may indeed have very different actions. The $GABA_A$ agonist muscimol when given IP to rats, for instance, has mild effects on reducing sleep latency and does not alter total sleep time (16). Analogously, the $GABA_A$ antagonist bicuculline slightly increases sleep latency without altering total sleep in the rat; neither interact with intraperitoneally administered triazolam (16). Muscimol has also been found to have effects on sleep different from midazolam in rats; the former increased both NREM and REM sleep, whereas midazolam increased NREM, decreased REM, and produced opposite effects on low-frequency EEG activity (17). Again, in contrast to triazolam, microinjection of muscimol into the medial preoptic area has been reported to have no effect on sleep in rats (18). The reasons for these differences are not clear; among the possibilities are that in the intraperitoneal administration studies muscimol produced nonspecific actions at GABA receptors throughout the brain, or that muscimol enters the CNS poorly (19); the lack of effects on sleep after microinjection directly into the MPA would seem to rule these possibilities out. It also appears that muscimol may alter chloride channel function in a manner different from GABA, activating channels for greater durations (20). It also may be that muscimol indiscriminately activates GABA receptors, whereas in contrast benzodiazepines may only alter function of receptors with specific compositions (21). Although this is still being assessed, the differences in effects of muscimol and benzodiazepines provide a cautionary note that it may be important not to equate the hypnotic actions of benzodiazepines with simple GABAergic effects.

The $GABA_A$–Benzodiazepine Receptor Complex as a Common Site for Diverse Pharmacologic Classes of Hypnotics

The affinities of various benzodiazepines were found to correlate well with their anxiolytic, anticonvulsant, and muscle relaxant properties in the early reports on central $GABA_A$–benzodiazepine receptors (7). Later evidence indicated that the receptor complex mediates the hypnotic actions of benzodiazepines as well. This role was clarified by studies of the inverse agonist 3-hydroxymethyl-β-carboline (3-HMC), which induced awakening and decreased sleep in rats, an effect prevented by the benzodiazepine receptor blocker CGS 8216 (15). Similarly, the hypnotic properties of the clinically used benzodiazepine flurazepam were blocked by a low dose of 3-HMC that had minimal effects when given alone (22). The stereospecificity of the site was demonstrated by studies of the benzodiazepine B-10 enantiomers, in which the (+) compound induced sleep, whereas the (−) compound increased wakefulness (15). It became clear, then, that it was indeed interaction with this receptor complex that mediates the effects of benzodiazepines on sleep and waking.

The $GABA_A$–benzodiazepine receptor complex contains modulatory sites not only for benzodiazepines, but also for a number of other types of sedating compounds, including barbiturates, neurosteroids, and ethanol (23,24), etomidate (25), and the anesthetic propofol (26). The newer nonbenzodiazepine hypnotics zolpidem, zopiclone, and zaleplon bind to the type I benzodiazepine recognition site as well. The end result is thought to be an enhancement of chloride ion flux, as described. These compounds may achieve this effect by different means, nonetheless; hence, benzodiazepines may increase the frequency of channel opening (27), whereas barbiturates, for instance, may increase the duration of opening (28). Ethanol facilitates GABA-stimulated chloride flux at low concentrations, and directly enhances flux

at higher concentrations (29). Similarly, some cytokines such as interleukin-1 enhance GABA-dependent chloride influx into synaptoneurosomes (30).

We are beginning to gain insight into the problem we posed originally: how administration of sedative/hypnotic compounds from such diverse pharmacologic classes can result in sleep induction. It appears that most or all of them produce pharmacologic effects by altering the function of various moieties of the $GABA_A$–benzodiazepine receptor complex.

NEUROANATOMIC STUDIES

Microinjection of Hypnotics into the CNS

In some ways, it has been more challenging to determine the neuroanatomic sites of action of hypnotics than to assess their actions at a molecular level. The most parsimonious neuroanatomic approach would seem to be that hypnotics act at sites thought to be involved in physiologic sleep regulation, on the basis of lesion or stimulation studies. Using this reasoning, the author's laboratory set out some years ago to administer benzodiazepines into such sites, many of which were chosen on the basis of the classical studies of Hernandez-Peon (31). The compound chosen for these studies was the benzodiazepine triazolam, which was the most widely administered clinical hypnotic. Perhaps the most surprising finding was the absence of effects on sleep following triazolam microinjection into many loci thought to be involved in sleep regulation, including the locus ceruleus, horizontal limb of the diagonal band of Broca, lateral preoptic area, basomedial nucleus of the amygdala (32), and ventrolateral preoptic area (33). In contrast, microinjection at several sites—notably the dorsal raphe nuclei and medial preoptic area— profoundly altered sleep and waking. We examine these in turn.

The Dorsal Raphe Nuclei

The dorsal raphe nuclei, tubular structures in the upper pons and lower midbrain that contain most of the forebrain serotonin (34) send fibers via the medial forebrain bundle to a various areas including the hypothalamus, basal forebrain, septal area, striatum, hippocampus, and cerebral cortex (35, 36). The heaviest innervation in the hypothalamus is in the medial preoptic area, suprachiasmatic nucleus, and dorsal and ventral premammillary nuclei (37), as well as in histamine-containing neurons of the tuberomamillary nucleus (38). Stimulation of the dorsal raphe has been demonstrated by the 2-deoxyglucose method to increase glucose utilization in the somatosensory cortex, thalamus, hypothalamus, and extrapyramidal system (39). Ascending fibers innervate the suprachiasmatic nucleus (34), raising the possibility that they influence circadian rhythm function. The dorsal raphe nuclei in turn receive descending fibers originating from the preoptic area (40), suggesting a kind of reciprocal innervation discussed in the following. The ventrolateral preoptic area (VLPO), which has been postulated to play a role in sleep initiation (41), sends GABAergic and galaninergic descending fibers to the dorsal and median raphe nuclei as well as the locus ceruleus, which might result in inhibitory action on ascending monoaminergic systems (42). In addition, dorsal raphe cells receive inhibitory serotonergic input from other local dorsal raphe neurons (43–46) as well as from the median raphe (43). Interestingly, microinjections of triazolam into the dorsal raphe *deceased* sleep in the rat—sleep latency was significantly increased and total sleep time was significantly reduced (47).

The Medial Preoptic Area

There has been considerable work on the role of the hypothalamus and adjacent structures in the regulation of sleep and waking, ever since pathological studies following the epidemic of encephalitis lethargica in the 1920s (48). Later lesion studies by Hess (49) and Nauta (50) suggested that the anterior hypothalamus is involved in sleep maintenance, whereas a more caudal area might promote wakefulness. Stimulation of a basal forebrain area, including the medial preoptic area (MPA), enhances (51) and lesions decrease (52) sleep in cats. Lesions of the medial preoptic area acutely decrease sleep in the rat, albeit subject to effects of ambient temperature (53). The MPA receives visual and auditory inputs, and contains neurons sensitive to glucose and steroids (54), osmotic and cardiovascular measures (55), and temperature (56). It is also a thermoregulatory site, and it may coordinate many processes in homeostatic and reproductive functions (36).

Cell bodies and fibers in the MPA cross react in immunohistochemical studies with a wide variety of neurotransmitters such as substance P and neuropeptide Y (57). Push-pull cannula studies of the MPA have documented release of catecholamines, GABA, and glutamate (58). GABA is uniformly found throughout the hypothalamus, and its synthetic enzyme GAD has very high concentrations in the preoptic area in particular (59). The MPA is also rich in some forms of the $GABA_A$–benzodiazepine receptor complex, suggesting that benzodiazepine hypnotic compounds might bind there (14). Neurons that become more active during NREM and REM sleep are inside the MPA (60). Its projections travel throughout the forebrain and brainstem (61) to the median and dorsal raphe nuclei, and possibly to the locus ceruleus (40). Stimulation of the MPA influences firing rates in the midbrain reticular formation (62,63). In summary, the preoptic area/basal forebrain appears to play an important role in the regulation of sleep and its integration with other processes; thus, it seems a likely candidate to be influenced by compounds that alter sleep and waking. Studies in the author's laboratory indicate that microinjections of pentobarbital (64), triazolam (65),

and propofol (66) into the MPA result in enhanced sleep in rats. Other groups have found similar results after microinjection of ethanol (67), adenosine (68), and prostaglandin D2 (69). Interestingly, the ability of adenosine to induce sleep when infused into the MPA is prevented by the benzodiazepine receptor blocker flumazenil (70).

Lesion Studies

One question that arises as a result of these studies is whether the MPA is the only site at which these compounds act to induce sleep; phrased somewhat differently, we know from the microinjection studies that the MPA is *sufficient,* but not whether it is *necessary,* for the hypnotic action of benzodiazepines. One way to approach this is to lesion the MPA, and then determine whether peripherally administered triazolam will still induce sleep. In order to assess this, we induced ibotenic acid lesions of the preoptic area in rats, and allowed 7 to 9 days of recovery in order for sleep to return to prelesion levels. We then administered triazolam .8 mg per kg intraperitoneally, and found that it still potently reduced sleep latency and increased total sleep (71). To put this finding in context, many of the studies of sleep physiology have suggested the presence of redundant mechanisms, which perhaps reflects the importance to the CNS of maintaining sleep/wake processes. One classic example, for instance, is that after anatomic or pharmacologic lesions of the dorsal raphe nuclei, sleep is initially greatly reduced, but then slowly returns to normal amounts (72,73). It seems likely, then, that although the MPA represents a common area at which a wide range of hypnotic compounds may act, there remain additional redundant mechanisms for sleep regulation, at which these agents can alter sleep in the absence of an intact MPA.

Do Hypnotics Induce Sleep by Altering Brain Temperature?

As we have described, the MPA contains warm- and cold-sensitive neurons, and is involved in thermoeffector activities (56). Typically, brain temperature decreases in NREM sleep compared to waking (74), and preoptic temperature drops in behaviorally defined sleep (75). It is known that peripherally administered benzodiazepines reduce rodent core (76) and brain (77) temperature. Thus, one interesting issue is whether the hypnotic effects of drugs such as triazolam are owing to "direct" effects on sleep regulatory mechanisms, or alternatively, whether the observed effects on sleep are secondary to drug-induced changes in temperature. In general, both rectal (78) and peritoneal (65) temperatures rise briefly following microinjection of triazolam into the MPA, but they do so equally in animals injected with vehicle. This transient rise in peripheral temperature, then, appears to be a nonspecific response related to the mechanics of injecting a fluid into the MPA. It is not clear whether brain temperature itself is altered by this microinjection procedure. In a recent study, cerebral brain temperature was measured following microinjection of triazolam .25 μg or vehicle into the MPA. Although sleep latency and waking time were significantly reduced, and total sleep increased in the 2 hours after injection, there were no changes in mean temperature in the first or second hour, temperature at sleep onset, or mean change from preinjection baseline (79). This suggests that the effects of triazolam microinjections into the MPA on sleep are not secondary to alterations in brain temperature, and raise the interesting possibility that pharmacologic and physiologic sleep initiation may differ in their relationship to temperature. Whether benzodiazepines induce subtler changes in hypothalamic temperature and play a role in sleep initiation remain to be determined.

CONCLUSION

Integrating Pharmacology of Hypnotics with Physiologic Sleep Regulatory Mechanisms

How should we picture the results of these microinjection and lesion studies in the context of what is known about the regulation of sleep? One of the most intriguing questions is: How does triazolam, which has a potent hypnotic effect when taken systemically, *reduce* sleep when injected into the dorsal raphe nuclei? The most likely explanation is that triazolam effectively inhibits raphe nuclei function. Benzodiazepines given systemically or iontophoretically have been reported to potentiate GABA inhibition of the dorsal raphe (80). Moreover, both depletion of serotonin by PCPA (81) or anatomic lesions of the dorsal raphe (82) greatly decrease sleep. Thus, it seems likely that the reduction in sleep seen after microinjection of triazolam mimics the effects of lesions of this structure.

As described, ascending fibers originating in the dorsal raphe travel via the medial forebrain bundle to various areas, including the hypothalamus (particularly the MPA and tuberomamillary nucleus), basal forebrain, septal area, striatum, hippocampus, and cerebral cortex (35). Descending fibers, in turn, travel to the dorsal raphe from the preoptic area. The dorsal and median raphe and the locus ceruleus also receive GABAergic and galaninergic innervation from the ventrolateral preoptic area (VLPO), which has an inhibitory influence on ascending monoaminergic systems (42).

Just as the MPA sends descending fibers to these specific monoaminergic brainstem nuclei, it also profoundly alters function of the reticular formation. This diffuse ascending system originates in the upper brainstem thalamic core, and projects largely through the intralaminar and other thalamic nuclei to the cortex, where it induces arousal (83). The main neurotransmitters involved in this process appear to be acetylcholine and glutamate (84). Stimulation of the MPA evokes inhibitory field potentials and suppresses neuronal

activity in the midbrain reticular formation, or MRF (62). Other stimulation data suggest that it induces an initial excitation followed by postexcitatory discharge suppression, suggesting that some actions of the basal forebrain on sleep might be mediated by alterations in activity in the MRF (63,85). In summary, we have described a system in which ascending monoaminergic pathways innervate the preoptic area, which appears to be an integrative center for sleep and a variety of physiologic processes, including thermoregulation and cardiovascular function, and at which microinjections of a wide range of classes of hypnotic compounds induce sleep. In turn, descending fibers from the preoptic area provide an inhibitory influence on both specific aminergic nuclei such as the dorsal raphe, as well as the midbrain reticular formation. We theorize that benzodiazepine hypnotics such as triazolam act by altering function of this reciprocal system of innervation between the hypothalamus/basal forebrain and brainstem structures.

Future Directions

Obviously, there are many potential areas to explore as possible sites of action of hypnotics. One possibility that has received little attention has been that classical hypnotics, such as benzodiazepines or barbiturates, might alter the ascending histaminergic arousal system, which is presumably the mechanism by which antihistamines produce sedating effects. Certainly one area of interest is the tuberomamillary nucleus, which lies adjacent to the mamillary bodies, just above the ventral surface of the hypothalamus (38). It is a histamine-producing cell group thought to be part of the ascending arousal system, with fibers going to the amygdala, hippocampus, and cortex (86). Data using the retrograde tracer cholera toxin subunit B indicate that the ventrolateral preoptic area (VLPO), which has been postulated to be involved in physiologic sleep initiation, innervates the histaminergic neurons in the tuberomamillary nucleus, via a GABAergic pathway (41,85,87). In principle, benzodiazepine or other hypnotic compounds might act by enhancing GABAergic inhibition of the tuberomamillary nucleus, decreasing its arousing effects. As described, the tuberomamillary nucleus also receives ascending serotonergic input from the dorsal raphe nuclei (38); therefore, it is conceivable that effects of hypnotics on sleep might also be mediated by their actions on the dorsal raphe, which in turn affect this structure.

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