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HOMEOSTATIC, ENTRAINMENT AND PACEMAKER EFFECTS OF DRUGS THAT REGULATE THE TIMING OF SLEEP AND WAKEFULNESS

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SUMMARY

The timing of wakefulness and sleep in humans, and other diurnal primates such as the squirrel monkey (*Saimiri sciureus*), is influenced not only by the duration of prior wakefulness or prior sleep, but also by the phase of the circadian timing system. In continuous, round-the-clock operations, or with transportation between time zones, conflicts frequently occur between these determinants of arousal state. The predictive circadian component favors wakefulness and sleep at phases consistent with the recent history of environmental and internal time cues. On the other hand, the reactive homeostatic component is principally determined by the length of prior wakefulness on the particular day in question.

Investigations of pharmacological agents which influence the timing of sleep and wakefulness indicate they may exert their effects directly on the neuronal/humoral mechanisms responsible for the generation of sleep (homeostatic effect), or by altering the phase of the circadian system. The circadian effects may either be achieved by resetting the phase of the circadian pacemaker (pacemaker effects) or may act by influencing the interaction between environmental light-dark cycles and circadian pacemakers (entrainment effect). Examples of drugs which appear to have predominantly homeostatic effects (e.g. muramyl dipeptide), pacemaker effects (e.g. sodium valproate) or entrainment effects (e.g. diazepam) will be discussed. An appropriate strategy for the management of alert wakefulness at any hour of day and night must use the appropriate pharmacological tools to manage circadian and homeostatic components of wakefulness and sleep.

LIST OF SYMBOLS

τ = Circadian Period

ϕ = Phase of Circadian Rhythm

$+\Delta\phi$ = Phase Advance

$-\Delta\phi$ = Phase Delay

In recent years pharmacological strategies have been extended into another dimension--that of time. Not only do many drugs have different efficacies and effects depending on the time of day when they are administered (1), but also certain pharmacological agents can reset the timing of circadian pacemakers (2). Once fully characterized, the clock-resetting properties of such drugs can be exploited to adapt humans to changing time schedules. If ignored, these agents could even act against their intended purpose. A sleeping pill that induces sleep might also reset in the wrong direction the neurophysiological pacemakers that govern the natural timing of the circadian sleep-wake cycle.

In view of these potentially conflicting effects, it is essential to differentiate between the direct effects of a drug on a physiological function from the effects induced by altering the timing of the biological pacemaker which generates rhythms in that physiological function. It is the purpose of this talk to present a system for classifying drugs in terms of "homeostatic" (H), "entrainment" (E) and "pacemaker" (P) effects and to discuss examples of drugs which illustrate each of these properties.

Nowhere are these distinctions more important than in the regulation of sleep and wakefulness. As we move into a twenty-four-hour-a-day global community, in which continuous operations, rotating shifts, night work, and travel across time zones become common, our innate timekeeping mechanisms come into conflict with our artificial schedules. It has become essential to develop a set of pharmacological tools for manipulating the natural timing of the sleep-wake cycle as the situation demands.

To appreciate the basis for our proposed classification scheme, it is first necessary to review briefly the factors that determine the timing, duration and quality of sleep. It is increasingly apparent that these characteristics of sleep and sleep stages are influenced not only by the duration of prior wakefulness but also by the phase of the circadian timing system. The relative importance of these factors is important to quantify since conflicts may at times occur between the predictive circadian component, which favors sleep at a phase consistent with the recent history of environ-

mental and internal time cues, and the reactive homeostatic response to prior wakefulness (3).

Homeostatic Regulation of Sleep

It has long been recognized that sleep serves a recovery function, allowing the conservation, storage and restoration of depleted energy and other physiological requirements (4,5,6). The conceptualization of sleep as a homeostatic regulatory response to deficits accumulated during wakefulness derives largely from the fact that sleep deprivation results in an increased probability sleep onset and the lost sleep is, at least in part, compensated for in subsequent sleep episodes. In the extreme case, sleep appears to be essential for life since prolonged sleep deprivation results in severe pathology and death (7). Recently evidence has been presented that certain peptides accumulate during wakefulness and are metabolized during sleep, and may act as sleep-promoting factors (e.g. factor S) (8,9).

The compensatory increase in sleep duration that follows sleep loss is rarely complete--a result which was originally considered inconsistent with the idea that sleep was homeostatically regulated. A solution to this problem, however, came with the realization that sleep involves an intensity dimension in addition to duration. Studies in several species show an increase in the proportion of recovery sleep spent in deep sleep (characterized by high amplitude slow waves in the cortical EEG and corresponding to stages 3 and 4 in humans) and, in some cases, in REM sleep, at the expense of light (or stage 2) sleep (10,11,12,13,14,15,16,17,18,19,20). Slow-wave sleep also increases in training athletes after strenuous exercise, although apparently not in other subjects (21,22). Slow-wave sleep decreases following extended sleep (23) or afternoon naps (24). Delta waves, in fact, appear to be homeostatically conserved in that nocturnal delta-wave parameters are reduced in proportion to the amount of delta activity exhibited during a daytime nap (25). Quantification of the EEG pattern obtained in NREM sleep has revealed increases in the density, duration and/or amplitude of delta waves (0-0.5 hz to 3-4 hz) or in EEG power density in that frequency range following sleep deprivation (26,13,27), as well as a decrease in these parameters following extended sleep (28).

Circadian Regulation of Sleep

Most organisms, including humans, have endogenous neural pacemakers which generate circadian (approximately 24-hour) rhythms in a wide range of physiological functions including sleep. In mammals the suprachiasmatic nuclei (SCN) of the hypothalamus act as the circadian pacemaker. Through a phase modulation by neural inputs responding to the illumination of the retina, the SCN maintain the sleep-wake on a daily schedule appropriate to the environmental timing of night and day. These pacemaker rhythms are self-generated; when an individual is isolated from environmental time cues, the sleep-wake cycle will "free run" at its natural period, which often differs from the twenty-four hour day. Humans, for example, have an endogenous circadian period of typically twenty-five hours. The sleep-wake cycle may be entrained to other periods close to its natural period, usually by the appropriate light-dark schedule. Hence we are normally entrained to a twenty-four hour period by the twenty-four hour alternation of night and day. However, the pacemaking system has significant inertia. An abrupt change in the environmental schedule may require several cycles before reentrainment occurs, leading to the difficulties that people experience in changing work shifts or time zones.

The mechanism of entrainment to light-dark cycles has been extensively characterized in mammals (although not yet directly in humans). Brief light pulses phase-shift the free-running activity rhythm of animals living in constant darkness by differing amounts and direction depending on when (i.e., at what phase) the stimulus was given. By measuring such phase shifts in response to light pulses given at different phases of the circadian cycle, a phase response curve (PRC) can be constructed. Such PRCs have been recognized as a universal feature of the mechanism of entrainment to all effective stimuli in a wide variety of species, from unicellular algae to primates (29,30,31). Furthermore, PRCs for light pulses in all species, whether nocturnal or diurnal, share the following general properties:

1. Phase delay shifts ($-\Delta\phi$) occur when the stimulus is early in the subjective night of the animal.

2. Stimuli late in the subjective night cause phase advance shifts ($+\Delta\phi$).

3. The response system is relatively insensitive (no phase shifts) during most of the subjective day.

These curves describe the capacity of the system to phase advance or phase delay under free-running conditions. Entrainment of circadian rhythms to a 24-hour day is

accomplished by periodic stimuli which cause a phase shift each day equal in amount to the difference between the natural period of the pacemaker (τ) and 24 hours. Entrainment to other day lengths is necessarily limited to a range of values around τ, called the range of entrainment (ROE), which is related to the maximum resetting capacity of the system in each direction as described by the PRC. Since the amplitude and exact shape of the PRC vary between species and among individuals (32), the ROE does as well.

In man, the synchronized circadian system can be entrained to period lengths ranging from about 23.5 to 26.5 hours. This means that there is only a very limited capability for resetting circadian rhythms in any one 24-hour period. This explains the lack of tolerance that humans show to schedules which require acute shifts in the timing of sleep and duty hours.

The neural pathways involved in this entrainment by environmental light-dark cues utilize a specialized group of retinal ganglion cells and a monosynaptic "retinohypothalamic tract" (RHT) from the retina to the suprachiasmatic nuclei. The SCN respond to the level of incident illumination on the retinae rather than the patterns utilized in visual perception. Light at dawn and dusk, in particular, by falling on the photosensitive portion of the PRC achieves the daily modulation of the phase of the circadian suprachiasmatic pacemaker, and therefore the sleep-wake cycle.

Mechanisms of Actions of Drugs That Influence the Timing of Sleep

From the above discussion, it can be seen that the timing of sleep could be manipulated using pharmacological agents which affect any one (or more) of the various mechanisms that influence the timing, duration or quality of sleep. In broad terms, a drug may influence either the neuronal centers responsible for the generation of sleep and its homeostatic function or the circadian pacemaking system which influences the timing of the endogenous sleep-wake cycle. Furthermore, drugs which manipulate the phase of the circadian system may either directly reset the phase of the circadian pacemaker, or may alternatively influence the interaction between the light-dark cycle and the pacemaker.

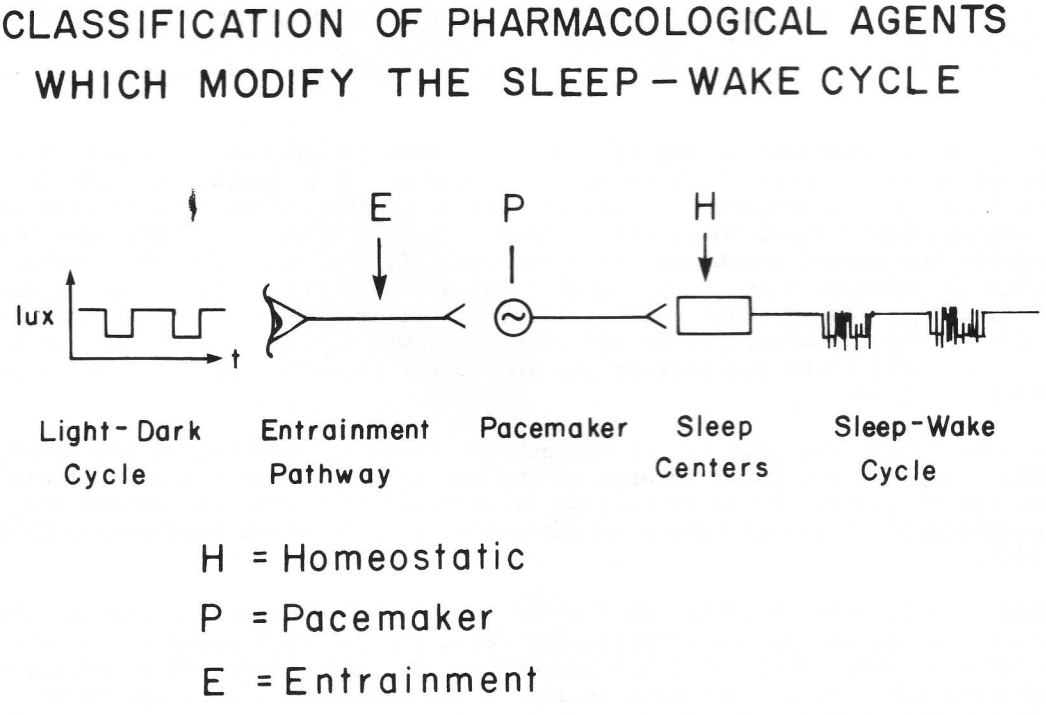

FIG. 1

Figure 1 summarizes the types of pharmacological actions that could result in an alteration in the timing of sleep and wakefulness. Drugs which induce pure "H" effects on the homeostatic sleep processes may induce sleep at an altered time of day, but on cessation of treatment, the sleep-wake cycle will instantaneously resume at its original circadian phase. Drugs with "P" effects directly on the phase of the circadian pacemaker, however, will cause a phase-resetting of the sleep-wake cycle which will persist after treatment (depending on the resultant phase achieved). Finally, drugs with "E" effects on the entrainment mechanisms will have phase-resetting effects on the circadian pacemaker that will depend on the light or dark stimulus being applied at that time.

It should be emphasized that these are operational rather than anatomical definitions. It will be important for therapeutic purposes to determine whether agents are dependent on the incident light intensity for their effect. However, a drug with an "E" effect may be acting on the retina, RHT, or even in the SCN on neurons which are responsive to RHT inputs. One way of distinguishing these criteria more rigorously will be to compare PRCs derived in LL, DD and optically-enucleated animals. In the latter the RHT degenerates and if the PRCs in LL and optically-enucleated animals are similar, it is safe to conclude that the drug has a "P" effect rather than an "E" effect. No two PRCs will be absolutely identical; for one thing, the environmental light intensity influences the circadian period of the pacemaker. However, phase-advance and phase-delay sections of a PRC which are of comparable timing and magnitude can be accepted.

Drugs With Predominantly "H" Effects

An example of a drug that effects sleep without affecting its circadian timing is muramyl dipeptide (MDP), a factor S analog as well as an immunoadjuvant and pyrogen (33). In squirrel monkeys individually housed in temporal isolation in constant light, we have studied the effect of 50 nmol of synthetic MDP injected either one hour after wake-up time (subjective day) or just before sleep time (subjective night). At both phases, decreases in percent time awake (relative to saline controls) were observed. After administration of MDP early in the subjective day, the animals exhibited alert wakefulness only 47.4% of the subjective day, compared with a mean 86.7% of subjective day after a saline control injection. MDP given at the circadian late subjective day resulted in sleep and transitional episodes occupying 84% of the subjective night vs. 73% of time asleep after control injection. Despite the marked influences of MDP on the sleep-wake pattern over the 24-hour period after administration, the circadian timing system demonstrated no consistent shifts in phase. MDP in squirrel monkeys thus appears to modify sleep-wake states by mechanisms that do not require phase resetting the circadian timing system, and hence by our classification would act with an "H" effect.

Drugs with Circadian ("P" or "E") Effects

Pharmacological agents which shift the phase of the circadian pacemaker thereby manipulate the neurophysiologic mechanisms that generate circadian rhythms, and not just the expression of the sleep-wake cycle on the day of treatment. A pacemaker-resetting drug is characterized by its lasting effects on the circadian rhythms of the individual; after discontinuation of the drug, the rhythms (depending on the phase of treatment) would typically not revert to their previous phase, but would be subsequently reset to a new initial phase.

A variety of pharmacological agents have been shown to influence the period or the phase of the circadian system. They include such chemicals as deuterium oxide (heavy water) and lithium, which lengthen the natural period of the circadian pacemaker in a variety of species from plants to mammals (34,35). Agents where full PRCs have been documented include the methyl xanthines, theophylline (36) and caffeine (37); certain protein synthesis inhibitors, such as chloramphenicol (38); and puromycin (39,40,41,42), the ionophore valinomycin (43,44), and ethanol (45,44). It is interesting that some of these agents are present in the beverages we commonly drink while flying. However, we normally take these with no regard to the time of day and do not consider their potential effect on our biological clocks.

Agents which alter the activity of the neurotransmitter GABA are of particular interest. GABA receptors are found throughout the brain, but in their highest concentrations in the hypothalamus. It is of particular interest that the SCN contain the highest concentration of the glutamate dehydrogenase enzyme, which synthesizes GABA from glutamate (46).

One GABA agonist with an effect on the primate circadian system is sodium valproate which enhances GABA synthesis and inhibits its degradation. Oral doses of valproate comparable per unit body weight to those prescribed for human psychiatric therapy were given to squirrel monkeys, free-running in individual cages under constant light. Within days, valproate consistently caused either a lengthening or a shortening of period in individual animals; the squirrel monkeys could be divided consistently into two groups of equal size, those that lengthened and those that shortened their period. Valproate is used in the treatment of acute mania, particularly in lithium-non-responders, and inter-individual differences in circadian response may well account for the differing therapeutic efficacy in different individual patients (46).

Distinguishing Between "P" and "E" Effects

We have discussed the significance of distinguishing between pacemaker (P) and entrainment (E) effects. Drugs with each type of effect will ultimately reset the phase of the SCN and will therefore influence the phase of the circadian sleep-wake cycle.

However, agents with "E" effects can block or inhibit stimuli derived from light input, thus appearing like a dark pulse administered in constant light. However, in constant darkness or an optically-enucleated animal, where there is no light input to be modulated, the phase response characteristics would be significantly different. In contrast, a "P" compound will display a similar PRC irrespective of the animal's lighting conditions. A clear operational distinction between "P" and "E" effects can thus be made if the full PRCs for both constant light and constant darkness are known.

Some headway has been made in distinguishing between entrainment and pacemaker effects in the investigations that we and others have conducted with benzodiazepines. The benzodiazepines, including diazepam (valium), flurazepam (dalmane) and triazolam (halcion), are GABA agonists and potent, widely-prescribed hypnotics. Recently diazepam and triazolam also have been discovered to have circadian resetting properties, but where are they acting? GABA receptors are not only found in the SCN, but also in the retina and the lateral geniculate nuclei, both components of the pathways through which light information is conveyed to the SCN. Thus the benzodiazepines could have either "P" or "E" effects (or both).

Many of the studies of benzodiazepine circadian phase-resetting actions have been conducted in hamsters. The "H" effects of benzodiazepines seem to be minimal in this species even at relatively large doses. Within minutes of treatment they are running on their wheels again. The first reports by Ralph and Menaker showed that large intraperitoneal injections of diazepam in hamsters free-running in constant darkness could block light-pulse-induced phase advances, but not light-induced phase delays (47). However, at the two times of the cycle they studied, insignificant phase shifts were caused by diazepam alone. This suggests that diazepam has an entrainment effect, but only during late subjective night when light pulses cause phase advances. Ralph and Menaker speculated that diazepam acted on retinal GABA receptors, but that light input followed two separate neural pathways to the SCN for delays and advances, and only the advances passed through GABA-inhibited neurons.

Figuring that if diazepam blocked light pulses it would simulate the application of dark pulses if animals were treated in constant light, we gave the same dosage of diazepam I.P. as Ralph and Menaker to free-running hamsters in constant light, and derived a PRC very comparable to a dark pulse PRC at all phases of the day. Although this could imply that diazepam has an "E" effect rather than the "P" effect, the other explanation (i.e., that the PRC represents a direct action of diazepam on the pacemaker irrespective of the light-dark schedule) could not be ruled out without determining the PRC to diazepam in constant darkness.

Such a test of benzodiazepine action has been provided by Turek and Losee-Olsen who determined the PRC for a large dosage of triazolam (halcion, a more potent but shorter-lived hypnotic benzodiazepine) in two groups of hamsters, one free-running in constant light, and the other in constant darkness (48). While those in constant light displayed a PRC comparable to that of diazepam and dark pulses, those in constant darkness also displayed a similar PRC. Obviously a PRC in darkness could not be produced by simulated dark pulses, so the benzodiazepines appear to act directly on the pacemaker. Ralph and Menaker's blockage of light pulses can thus be interpreted as the light-induced phase advances being nullified by diazepam-induced phase delays, resulting in no net effect. However, close inspection of the two PRCs indicates there are still small differences between the constant light and constant dark PRCs; potentially benzodiazepines therefore act, in light, both on light input and the SCN. Further studies, in which diazepam or triazolam is applied intracranially or to optically-enucleated hamsters, may help settle this question.

Of course, benzodiazepines also have homeostatic effects on sleep; this is the basis for the millions of prescriptions that are written every year. By determining the "P" and "E" effects of benzodiazepines, we can increase treatment efficiency by formulating the best parameters for therapy. The pacemaker effects can be applied to complement rather than antagonize the hypnotic effects; knowing the entrainment effects, if any, we can compensate for environmental lighting conditions. Although some agents may have all three effects, it is useful to distinguish between them to ensure that the appropriate therapeutic strategy is used. Such a scheme, we believe, will facilitate further research into the mechanisms and therapeutic regimens for controlling sleep and wakefulness at times required by operational considerations.

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Discussion

Spencer, GE
You said that the pathway between the retina and the suprachiasmatic nucleus was monosynaptic and then proceeded to try and see if it is possible to find out if drugs are having a direct effect there. Wouldn't the problem be somewhat simpler if you know what the neurotransmitter of this pathway is, and if you do, what is it?

Moore-Ed, US
. . (not recorded). . the neurotransmitters in that pathway, there is some evidence for several transmitters being involved. The issue, however that we have been trying to tackle, has been if we can study in hamsters, optically enucleated animals, we can remove that by degeneration, that entire pathway can degenerate. Really we're looking for an operational definition, namely, does the impact of light per se influence the effect the drug has as a pacemaker resetter. And so that our direction right now is to look for that interaction and offer a new set of definitions, an operational set of definitions, rather than a neuron chemical set of definitions. That's our first approach to this. Subsequently, we are going to want to understand the neurochemistry, but I think before we understand the rather complex neurochemistry there are an awful lot of transmitters lurking in the SCN that we need to understand operationally as to which drugs influence or are interactive with the resetting properties of light-dark cycles.

Jones, US
Do you intend to see if the effect of light on the body, other than through the eye, might also have through the skin a direct photon stiumlation of the brain?

Moore-Ede, US
. . (not recorded). . one or two other species have extraretinal photoreceptors. To all intents and purposes it is not possible to demonstrate, in mammals, their existence. That optically enucleated animals, for mammals are for all intents and purposes insensitive to light. That's really not proved to be data that had been replicated outside some lizards and some birds. But in mammals it does not appear to be a problem.

Van Den Biggelaar, NE
Dr. Moore, on one of your graphs you showed us the minimum reaction times or maximum sleepiness times of aircrew. These were, as I recall, between 3 or 4 o'clock in the afternoon. . .

Moore-Ede, US
No, in the morning.

Van Den Biggelaar, NE
Oh, in the morning. OK, I'm sorry. That answers my question.

Moore-Ede, US
It is those early hours before dawn. As anybody in operational situations can well-attest, it is the hours before dawn.

Terrian, US
I believe you've shown quite clearly that determining the phase-response curves for pharmacological agents in this free-running condition can be a productive and meaningful approach to this research, but I hope I misunderstood you, you don't believe that it is premature to begin to try to dissect out the neurochemistry of the suprachiasmatic nucleus. And that it is a potentially fruitful and complimentary approach to this subject for your own research.

Moore-Ede, US
Oh, absolutely. The answer to the question previously was to say, that the definition, because of the problems of defining the differences between these drugs, because of the purposes of definitions at this time we use an operational definition. Does

light and the drug interact? Now that actually is of practical value in the first steps for us toward practical use of those agents. However, there is an enormously important area of research which is the neurochemistry of the SCN, the understanding of the interaction of those transmitters. That will be our route in the future towards the identification of drugs. And we may have to wait for drugs that have the effects we desire at doses which are tolerable to the individual. There is a very big dose problem which is unresolved as yet in this area.

Terrian, US

Well, as a neurochemist I appreciate that answer and I wonder if I could follow. I think there really are two transmitters that may code for light in central nervous system that are most promising. Certainly, acetylcholine, historically, and now with the data Menaker will present at the forthcoming neurosciences meeting where they have stimulated the optic fibers and have shown that non- and NMDA antogonists block the ability to stimulate the SCN, glutamate becomes a viable candidate. Don't you think that working with phase shift curves as they have been done classically in the past where they are light-induced and then look for pharmacological means of manipulating that, that would be a promising way of dissecting out the chemistry of the SCN?

Moore-Ede, US

It is certainly a promising way to dissect out the chemistry, but the challenge we've directly taken on is trying to look operationally at drugs that are being used and try to understand which of those you might have to be concerned about the interaction between sunlight and the drug at certain phases. Now, I think this is really a question in the aims of the institute we are forming associated with Harvard is to address both basic and applied questions in parallel because in fact the fruitful interaction will come in attacking both approaches. There are some things we have to do right now because people quite frankly are starting to prescribe drugs like halcyon and some of these other agents quite freely and we need to understand some of those interactions as soon as we can because there may be some undesirable effects that are lurking in there that not only interfere with the pilot's individual performance but may also cloud the interpretation of the data. But at the same time, I would be, I'm rarely accused of not putting a pitch in for basic research at the same time.