



CIRCADIAN RHYTHMICITY AND THE "LITTLE PURPLE PILL"

by Regina Patrick RPSGT

Phosphodiesterase (PDE) enzymes are found in many body tissues. In the suprachiasmatic nuclei (SCN), the biochemical actions of PDE enzymes may play a role in circadian rhythmicity. Recently, scientists have found that the PDE-5 inhibitor drug sildenafil could induce an advanced-phase shift in hamsters. Scientists hope that this effect may be useful in diminishing the effects of jet lag and in treating dysfunctions of circadian rhythmicity.

PDE enzymes play a role in the degradation of nucleotides. A nucleotide is a basic unit of a DNA (deoxyribonucleic acid) or RNA (ribonucleic acid) molecule strand. It consists of a nucleic acid base (e.g., adenine, guanine, uracil, cytosine, or thymine) linked with a phosphate group (PO₄²⁻, a phosphorus atom linked to four oxygen atoms; the molecule can donate 2 electrons hence its 2- charge) and either a molecule of ribose (a five-carbon ringed sugar) or deoxyribose (a

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ribose lacking one oxygen atom). A DNA molecule strand contains deoxyribose nucleotides and an RNA molecule strand contains ribose nucleotides.

A nucleotide's phosphate group can bind with the ribose molecule in such a way as to form a ring with it. The result is a cyclic nucleotide. Examples of cyclic nucleotides are cyclic guanosine monophosphate (cGMP) and cyclic adenosine monophosphate (cAMP). cGMP and cAMP are found in the SCN (a group of neurons in the anterior portion of the hypothalamus). These neurons act as the master pacemaker for circadian rhythmicity.

Some research indicates that cGMP and cAMP exert their effects on SCN neurons in antiphase to each other (that is, at opposite points in the circadian rhythm). Rachel A. Prosser et al. investigated rhythmicity in the neuronal activity of SCN neurons that had been taken from mice raised in an environment of 12 hours light and 12 hours dark. Brain slices of the animals' SCN neurons were kept in constant illumination while undergoing various treatments. The neurons' firing rate was monitored for 3 days. They noted that the firing rate of untreated neurons peaked about 7 hours after the subjective "lights on." (Subjective lights on means the time that "lights on" had occurred in the intact animal; subjective lights off is the time that "lights off" had occurred in the intact animal). When the neurons were bathed in cGMP agonists midway during the subjective "lights off" period, the cells' peak firing rate occurred about 30 minutes after subjective "lights on." This represents a 6 hour advance. When cGMP was applied mid-

way in the subjective "lights on" period, the peak firing rate of the neurons occurred normally without a phase advance. This indicated that cGMP can only exert its phase-advancing effect during the night but not during the day. By contrast, Prosser found that when a cAMP agonist is applied to brain slices midway into the subjective "lights on" period, the neurons' peak firing rate occurs 2 hours after "lights on" the following day. This represents a phase advance of 4.5 hours. When cAMP treatment is applied midway during the subjective "lights off" period, the neurons' peak firing activity continues to occur at 7 hours after lights on (i.e., no phase advance). This indicated that cAMP can only exert its phase-advancing effect during the day but not during the night.

From these results, Prosser et al. concluded that cGMP may play a role in light-induced phase shifts since the SCN neurons were more sensitive to stimulation of cGMP agonists during the night (i.e., "lights off" period) but not during the day (i.e., "lights on" period). They also concluded that the two nucleotides may stimulate separate biochemical pathways that result in the differential response of the SCN neurons to each nucleotide at specific times in the circadian rhythm.

The SCN has neural input from the retina by way of the retinohypothalamic tract (RHT). It may be this pathway which allows light to be a strong zeitgeber (an external cue that entrains one's circadian rhythm). When light enters the eye, photic energy causes retinal cells to depolarize and release glutamate. The depolarization is then relayed from cell to cell throughout the RHT. At the synapse between the RHT cells and the SCN cells, the glutamate travels across the synapse to bind with receptors on the surface of the adjoining SCN cell. Once bound, calcium floods into the SCN cell. This stimulates the enzyme nitric oxide synthase (NOS) within the SCN cell to synthesize the molecule nitric oxide (NO). NO then stimulates the synthesis of cGMP. When cGMP levels rise sufficiently, PDE enzymes interact with the molecule to convert it into GMP thereby blocking the actions of cGMP. Hypothetically, if the degradation of cGMP could be controlled, advanced phases could be induced at desired times. PDE-5 inhibitor drugs makes investigating this hypothesis possible since the drugs prevent the degradation of cGMP.

PDE-5 inhibitor drugs were initially developed in the 1980s with the hope of being used to treat high blood pressure and angina (thoracic pain resulting from insufficient blood flow to the heart). The drugs raise the levels of cGMP which has a smooth muscle relaxant effect. Smooth muscle relaxation in blood vessels results in vasodilation and increased bloodflow. In 1994, sci-

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sildenafil. For approximately 10 days the hamsters were on a 14:10 light-dark cycle (that is, 14 hours light and 10 hours dark). On the day of the phase shift, the hamsters were given either saline or sildenafil six hours after lights off. They were then monitored several days for changes in their activity level.

Based on locomotor activity (i.e., wheel-running), the activity of the sildenafil group more quickly adjusted to the new earlier time than did the saline group. Additionally, the response was dose-dependent. On average it took about 12 days for the saline group to re-entrain to the new schedule but 8 days for the sildenafil group on a moderate (3.5 mg/kg) dose of the drug. A low dose of the drug (1.0 mg/kg) did not re-entrain the rhythm but a high dose (10 mg/kg) more quickly re-entrained the hamsters (6 days). (However, the high dose resulted in erections.) The researchers concluded that sildenafil could more quickly re-entrain animals to a new schedule by inducing a phase advance.

In another aspect of the study to investigate the effect of PDE inhibition on the circadian effects of light pulses, Agostino et al. subjected the hamsters 6 hours after lights off to a low level (50 lux) light pulse lasting 15 minutes. Sildenafil (3.5 mg/kg) or saline was injected at 15, 45, or 90 minutes before the light pulse. With the saline group, the onset of dark phase activity occurred approximately 76.0 minutes earlier the next night. When the animals were given sildenafil 45 minutes before the pulse, the onset of dark phase activity occurred about 150 minutes earlier the next night. When the animals were given sildenafil 90 minutes before the light pulse, the onset of activity was not significantly different from that of the saline group. The researchers also found no significant difference between the groups when the animals were injected 15 minutes before the light pulse. Sildenafil is the most studied PDE-5 inhibitor drug. Other PDE enzyme classes besides the PDE-5 class may play a role in circadian rhythmicity. (PDE enzymes encompass 11 classes.) The PDE-6 class of enzymes are found in the photoreceptive cells (i.e., rods and cones) of the retina and interact with cGMP in the retina. The PDE-9 class also interacts with cGMP and is found in the SCN. The role of these two classes of PDE enzymes have not yet been investigated for their roles in circadian rhythmicity.

The phase-advancing effect of sildenafil has not yet been examined in humans. Current treatments for circadian rhythm disorders (e.g., delayed sleep-phase syndrome) or circadian rhythm dysfunctions caused by sleep disruptions (e.g., shift work, jet lag) may involve chronotherapy, bright light therapy, or melatonin. Altering the levels of cGMP may offer another treatment strategy. If one were able to control phase-advances, this could potentially improve safety, alertness, and even the health of people who work jobs involving a counter-clock-wise rotating shift (i.e., working third-second-first shift) or flying/driving frequently from west-to-east. For people with circadian rhythm disorders, altering cGMP levels in the SCN could potentially improve one's entrainment to environmental cues (particularly light).

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entists at Pfizer laboratories in England had much hope for the compound UK-92,480. However, clinical studies proved it was ineffective as a treatment for high blood pressure or angina. Interestingly, during clinical studies subjects had frequently reported improved erections as a side effect. This shifted scientific focus on using UK-92,480 to treat impotence. In 1996, Pfizer patented UK-92,480 as sildenafil and on March 27, 1998 sildenafil became the first PDE-5 inhibitor drug approved by the United States Food and Drug Administration (FDA) for the treatment of impotence. Since that time, two other PDE-5 inhibitor drugs have been approved for treating impotence: vardenafil (Levitra), and tadalafil (Cialis). Scientists believe the ability of the PDE-5 inhibitor drugs to cause erections appears to be mediated by nitric oxide. NO stimulates the production of cGMP in penile tissues. With sildenafil blocking the degradation of cGMP, the increased levels of the nucleotide allow it to more easily exert its vasorelaxant effect in penile tissues. Blood vessels in the penis fill with blood thereby resulting in an erection.

Argentinian neuroscientist Patricia V. Agostino et al. studied the effect of PDE-5 inhibition on resynchronization in hamsters that had undergone a 6 hour advance in the light-dark cycle. They used the PDE-5 inhibitor drug