The Role of Adenosine in Sleep-Wake Regulation

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Sleep is one of the few experiences in life that all of humanity can relate to on some level. Sleep, or lack thereof, is a prevalent part of our lives, whether it comes in the form of a good night's rest, the power-nap, or the holiday dinner food coma. However, for all of our experiences and observations on the subject, relatively little is known regarding the precise mechanism of sleep. The process isn't as readily evident as the digestive process may be, or even the regulation of oxygen transfer in the blood. The process of sleep is a hidden and subtle thing, residing deep within the convoluted meshes of neurons and chemical pathways that are our brains. We are not completely without guidance though. The study of neurochemistry has shed some light on our most enigmatic organ over the course of decades, and sleep study has also been brought to light. Scientists have identified key structures in the brain whose activity correlate with differing levels of sleep and arousal, structures such as the basal forebrain, the perifornical-lateral hypothalamic area, and the ventrolateral preoptical region. These structures all share something in common, particularly regarding their method of signaling. They all make use of a chemical known as adenosine. This molecule can affect the onset or delay of sleep depending on specific concentration and location. Adenosine and its receptors also play a role in the interactions with common substances, such as alcohol and caffeine.

Adenosine is a nucleoside that plays several roles in biochemical functions throughout the body. It is a key component of adenosine triphosphate (ATP), the molecule critical in energy transfer within cells. Adenosine is also found as a nucleotide in DNA. However, this paper focuses on the role of adenosine as a neurotransmitter.
Neurotransmitters (NTs) are molecules responsible for the transfer of biochemical signals across neural pathways. NTs are paired-up with different receptors, and bind specifically to those receptors. There are four different adenosine-specific receptors: A1, A2A, A2B, and A3 (Zhang et al., 2013). While receptors normally interact with their specific neurotransmitter, they can also bind with substrates that share similar structures as the intended NT. These molecules are known as agonists and antagonists, and they differ in their effect upon the receptor. Agonists behave in a way analogous to the NT, activating the NT receptor. Conversely, antagonists inhibit the activation of a receptor, thereby hindering neural signaling associated with that receptor. The caffeine found in coffee and theophylline present in teas are examples of common adenosine antagonists. The presence of adenosine in the extra-cellular matrix can be attributed to the release of ATP by astrocytes, glial cells found in the brain. Once ATP is released, it is quickly metabolized into its component adenosine, which then interacts with its various receptors, promoting signals throughout the body, such as the propensity for sleep (Fellin et al., 2012).

Adenosine is present in many areas of the brain, one of which is the basal forebrain (BF). This structure plays an active role in the sleep-wake cycle. Researchers tested the effects of the adenosine concentration in the BF on the sleep-wake cycles of cats. Measurements of AD (recovered via an in vitro microdialysis probe) were taken from cats during periods of wakefulness and during non-rapid eye movement sleep (NREM sleep) (Porkka-Heiskanen & Kalinchuk, 2011)(Porkka 1997). The concentration of AD was found to be much higher during the waking state, as NREM concentrations at only 75 % to 80% of the waking values. AD concentrations in the BF were also measured over the course of six hours of sleep deprivation (SD) and compared with concentrations in other sleep-
regulating areas of the brain (Porkka-Heiskanen & Kalinchuk, 2011). At the end of the SD period, adenosine levels in the cats' BF had doubled. AD levels also rose in the cortex, though not to the extent as seen in the BF. Other sleep-regulatory structures, such as the thalamus and preoptic area saw no increase in AD concentration. Recovery sleep in the cats showed a decrease in AD levels in the BF after two to three hours (Porkka-Heiskanen & Kalinchuk, 2011). These findings demonstrate a link between adenosine levels in the BF and the sleep-wake cycle.

Adenosine performs an inhibitory role in the structure of the brain known as the perifornical-lateral hypothalamic area (PF-LHA). The PF-LHA is known to play a role in behavioral arousal, and includes a certain wake-active neuron known as hypocretin neurons (HCRT) (Rai et al., 2010). It is hypothesized that inhibition of the HCRT neurons at the A1 adenosine receptor could induce a sleep state, while activation of the said receptor could lead to a state of arousal. To test this hypothesis, researchers ran experiments that exposed the PF-LHAs of rats concentrations of the adenosine agonist N<sub>6</sub>-cyclopentyladenosine (CPA), and an antagonist of adenosine, 1,3-dipropyl-8-phenylxanthine (CPDX) (Rai et al., 2010). 34 Sprague–Dawley male rats were fitted with surgically implanted EEG and electromyogram electrodes to record sleep-wake states via a polygraph machine. A microdialysis assembly was used to administer the drugs, just above the PF-LHA. Baseline neuronal discharge rates of the HCRT were recorded for nine isolated neurons in the PF-LHA, and then compared to rates corresponding to the administration of CPA. It was found that seven out of nine of the neurons were suppressed, experiencing a decrease in discharge rate of over 25% (Rai et al., 2010). This suppression occurred during waking and NREM sleep. The researchers ran another experiment, which tested for
expression of the c-Fos gene, a gene often expressed during neuronal activity. The experiment was conducted in two sets. The first set was conducted during waking nighttime hours for the rats. A control group was administered artificial cerebrospinal fluid (aCSF), while the experimental group received doses of the adenosine agonist CPA. This would test for the agonist’s effect on a usual waking state. The second set was ran during daytime sleeping hours. Once again, the control group was given aCSF, while the experimental group was given doses of the adenosine antagonist CPDX. Afterwards, the rats were euthanized, and slices of the each rat's PF-LHA were immunostained for c-Fos expression, then stained once again to highlight HCRT neurons. Staining showed that the rats treated with CPA exhibited less c-Fos expression that the control. Conversely, rats treated with CPDX showed an increase in c-Fos expression compared to the control (Rai et al., 2010). By associating c-Fos expression with HCRT neuron activation, these results indicate that adenosine has an inhibitory effect on the PF-LHA. This is a fitting result; given previous mention of the PF-LHA’s role in behavioral arousal, it follows that inhibition of this structure's neuronal cells would facilitate a sleeping state (Rai et al., 2010).

The ventrolateral preoptic area (VLPO) is a structure in the brain connected to the regulation of sleep. This makes the VLPO a candidate for study regarding its interaction with adenosine. Researchers sought to test this relationship by studying the sleep-wake cycles of rats when given injections of adenosine, an AD agonist (CHA), an AD antagonist (CPT), and an AD transporter inhibitor (NBTI) (Zhang et al., 2013). The adenosine receptor tested in this experiment was the A1R receptor. Rats were fitted with electrodes above the VLPO to record EEG and EMG signals. These signals were used to differentiate between different sleep-wake stages, specifically NREM, REM, and wakefulness. Rats in the control
group were given injections of artificial cerebrospinal fluid, while experimental rats were set apart in five separate groups characterized by administered drug: AD, NBTI, CHA, CPT, and CPT + AD (Zhang et al., 2013). The results were unexpected. Rats given injections of AD, CHA, and NBTI, all of which would promote activation of the A1R receptor, actually spent more time awake and less time in REM and NREM sleep when compared to the control group. Conversely, rats that received doses of CPT spent more time in both sleep stages than the control. (Zhang et al., 2013) Adenosine's interaction with the A1R receptors of the VLPO is a notable one, because it differs from the relationship shown in other adenosinergic areas, like the basal forebrain or the perifornical-lateral hypothalamic area. Activation of the A1R receptor in the BF and PF-LHA produced an inhibitory effect on the system, promoting sleep. In the VLPO, the opposite is true: the activation of the A1R receptor is excitatory, promoting arousal. This shows that activation of the A1R receptor plays a crucial role in sleep regulation throughout the brain, though that role is not universal.

It has been suggested that adenosine and its receptors may play a role in one of the symptoms of alcohol withdrawal. Researchers propose that insomnia due to alcohol withdrawal may be linked to an impairment of the basal forebrain’s (BF) adenosinergic mechanisms via frequent alcohol consumption. To test this hypothesis, they conducted four experiments on ethanol-dependent (ED) rats, as well as a control group of rats. The first experiment was a measure of sleep-wakefulness in the both groups of rats (Sharma et al., 2010). Second, activation of wake-promoting cholinergic neurons (neurons that use acetylcholine as the neurotransmitter to relay signals) in the ED and control rats was measured by examining the level of expression of the c-Fos protein in cholinergic neurons,
which is expressed during cell activation (Sharma et al., 2010). Third, the effect of sleep deprivation on concentrations of extracellular adenosine in the BF of the control and ED rats was measured (Sharma et al., 2010). Finally, the levels of expression of the ENT1 and AR1 genes were measured and compared between the experimental and control group. Gene expression was measured by the extraction and reverse transcription of mRNA responsible for the expression of ENT1 and AR1 genes, which were then quantified. ENT1 codes for a glycoprotein that assists in the reuptake of neurotransmitters. AR1 is an adenosine receptor that regulates the effect of adenosine in the BF (Sharma et al., 2010). In comparison to the control group, the experimental group showed increased wakefulness, increased activation of wake-promoting neurons, no significant increase in AD concentration during sleep deprivation, and reduced expression of ENT1 and AR1. Thus, the researchers concluded that the down-regulation of the ENT1 and AR1 genes and their expression results in the release of less adenosine and lowered adenosinergic inhibition, leading to insomnia-like symptoms in rats with ethanol dependency (Sharma et al., 2010).

The ways in which caffeine interacts with the brain's A1R and A2AR receptors are a bit less straight forward. Caffeine is a drug commonly ingested by the populace, often in the forms of coffee, tea, energy drinks and various sodas. Many people attest to the boost in attentiveness and cognitive performance provided by caffeine. The biochemical reasons for this performance increase aren't known for sure, but there is experimental evidence that provides a strong hypothesis. It is known that cognitive performance has much to do with memory and learning, which can in turn be linked to the plasticity of neurons in the hippocampus (Costenla, Cunha, de Mendonça, 2010). Plasticity, measured by what is called long-term potentiation (LTP), refers to the ability for neurons to make new connections,
which correlate to new memories and learned abilities (Costenla et al., 2010). Caffeine plays a layered role when interacting with the hippocampal neurons, relying on two key variables: the type of adenosine receptor in question, and the level of neuronal stimulation, be it high or baseline. Caffeine is an antagonist for both the A1 and A2A receptors in the hippocampus. Trying to classify the effects of caffeine by receptor type is difficult as the effects oppose one another. A1 antagonists will increase LTP, while A2A antagonists will decrease LTP. Looking at tests run at different levels of neuronal excitation, hippocampal neurons subjected to high-frequency stimulation in the presence of caffeine showed a decrease in LTP, similar to the behavior of an A2A antagonist. Conversely, hippocampal neurons exposed to caffeine and basal-frequency stimulation, behaved in a manner similar to an A1 antagonist, promoting LTP (Costenla et al., 2010). It could be concluded then that caffeine could have a positive effect on cognition and learning, but only when neurons are in a highly stimulated state.

Adenosine has been shown to affect states of sleep and arousal throughout the brain. The effects of A1R receptor activation differ according to the brain structure in which the receptor is located. In regards to wake-promoting neurons, adenosine can be inhibitory (as in the basal forebrain and perifornical-lateral hypothalamic area) or excitatory (as seen in the ventrolateral preoptic area). Ethanol can be seen to play a disruptive role in the sleep-wake cycle by negatively impacting adenosine release mechanisms. Caffeine, by its nature as an adenosine antagonist, has been shown to affect learning and cognition, but only when in the presence of appropriate neuronal excitation. Through its interactions with A1R receptors throughout the brain, adenosine aids in regulating the sleeping patterns of the populace.


