REFERENCES


Basal Forebrain and Saporin Cholinergic Lesions: The Devil Dwells in Delivery Details.

Anna V. Kalinchuk, PhD1,2; Tarja Porkka-Heiskanen, MD, PhD2; Robert W. McCarley, MD1

1Department of Psychiatry, Boston VA Healthcare System-Harvard Medical School, Brockton, MA; 2Department of Physiology, Institute of Biomedicine, University of Helsinki, Finland

THE CHOLINERGIC BASAL FOREBRAIN (CBF) AND ADENOSINE AS A HOMEOSTATIC SLEEP FACTOR ARE CURRENT “HOT TOPICS”, AS DEMONSTRATED BY THE very active work going on in several research groups. This comment places the just-published article by Blanco-Centurion et al.1 in the context of the work of other research groups, much of which has been presented at APSS and other meetings and now is in preparation for publication. We here comment on the similarities of findings and attempts to suggest reasons for the differences.

First, a bit of background: The CBF complex includes the medial septum (MS) rostrally to the nucleus basalis magnocellularis caudally and provides widespread afferent cholinergic, GABAergic, and glutamatergic innervation to the cortical mantle, hippocampus, and amygdalae. A recent commentary in Sleep2 outlined the connection with adenosine effects in inhibiting wakefulness promoting CBF neurons, and the role of the CBF and adenosine in mediating recovery sleep.

Disclosure Statement
Dr. Porkka-Heiskanen has participated in a speaking engagement supported by Expert Input Forum. Drs. Kalinchuk and McCarley have indicated no financial conflicts of interest.

Address correspondence to: Robert W. McCarley, MD, Department of Psychiatry, Boston VA Healthcare System-Harvard Medical School, 940 Belmont Street, Brockton, MA 02301; Tel: (774) 826-3723; Fax: (508) 586-0894; Email: robert_mccarley@hms.harvard.edu

SLEEP, Vol. 29, No. 11, 2006

Recent advances in selective destruction of CBF cholinergic neurons include using the immunotoxin 192 IgG-Saporin. This consists of a ribosome inactivating enzyme conjugated with monoclonal antibody targeted to the low affinity p75 nerve growth factor receptor expressed only on cholinergic neurons. A chronological list of 192 IgG-saporin experiments and sleep effects on sleep,1-3,5,8,10 although overall reduction in EEG amplitude can be found.5,10 But: When saporin is administered intracerebroventricularly (ICV) there are very small or no effects on sleep,1-3,5,8,10 although overall reduction in EEG amplitude can be found.5,10 But: When saporin is administered locally into the CBF, two separate groups found, in studies 2-4 weeks post-injection, that spontaneous sleep is decreased, and recovery sleep and delta activity are both profoundly reduced.6,7 These similar results from local injections by two independent groups mitigate against technical error causing these findings. Thus, there seem to be notable differences between ICV-induced and localized cholinergic lesions.

Why the difference between ICV and local injections? There are several possibilities, although we can’t be certain of the mechanism without further work. First, the rapid destruction of terminals with ICV lesions may provoke compensation from other systems not seen with local lesions where terminal destruction takes place over several days. Indeed, ICV injections have been shown to promote an increase in noradrenaline,11 serotonin,12 dopamine metabolites,13 and glycogen synthase kinase,14 perhaps attributable to inhibition of the ribosomal machinery and subsequent acute destruction of the synaptic and axonal protein synthetic machinery.15,16 These altered systems may alter the response to sleep
deprivation. Sleep and wakefulness are regulated by many overlapping systems in the brain, and when one of them is lesioned, others may compensate. A second, nonexclusive possibility for absence of ICV effects is the role of the cholinergic MS neurons, completely eradicated with ICV but preserved with local BF lesions. Srvidiya et al. found total MS lesions (NMDA) decreased slow wave sleep.

An extensive series of experiments conducted by the Finnish authors have demonstrated the relationship between recovery sleep and adenosine levels in the basal forebrain. When using nonlesioned animals or animals with local saporin lesions they have found that changes in CBF adenosine levels and changes in recovery sleep are tightly connected: recovery sleep is always preceded by adenosine increases, and when there is no recovery sleep, CBF adenosine levels stay low. There have been no exceptions to this rule in a wide-ranging series of experiments inducing excess sleep that resembles recovery sleep or manipulating recovery sleep induced by sleep deprivation; methods included energy depletion, nitric oxide induction and depletion, and glutamate agonists and antagonists.

Several other studies support the hypothesis that recovery sleep is mediated through adenosine. In CBF adenosine effects are mediated through the A1 receptor. The US investigators demonstrated, using both in vivo and in vitro techniques, that adenosine inhibits wake-active neurons in this area via the adenosine A1 receptor, suggesting that sleep deprivation-induced increased levels of adenosine mediate sleepiness by inhibiting the activity of these neurons. Prolonged waking upregulates A1 receptor expression, thus accentuating the inhibitory effects of extracellular adenosine leading to increased sleepiness. Further strong supporting evidence for the role of adenosine and its A1 receptor in sleep homeostasis is the demonstration by Thakkar et al. that an antisense-induced transient knockdown of the CBF A1 receptors obliterated both the post-deprivation recovery sleep delta activity increase as well as the % sleep increase seen in controls. These experiments were not cited by Blanco-Centurion et al., although they agree on the effects of the A1 agonists in BF in inducing sleep. It is not surprising that A1 agonists promote sleep even with cholinergic lesions, since we have demonstrated that adenosine inhibits both cholinergic and noncholinergic BF neurons. A note: the impression of Blanco-Centurion et al. that we stated recovery sleep is mediated exclusively through cholinergic mechanisms is incorrect; we do postulate a main role for cholinergic mechanisms in mediation of the A1 receptor increase and the effects of long-term sleep deprivation, but not in the acute response to increased adenosine levels. We agree on the importance of the cholinergic system for increased adenosine following deprivation.

While we do not exclude the possibility that, in non-CBF areas, particularly in the VLPO, adenosine could induce sleep through A2a receptors, neither we nor anyone have found any evidence of A2a receptor mediated effects in CBF. On the contrary: in the CBF, A1 receptor agonists induce sleep, A1 but not A2a receptor

**Table 1—Chronological list of 192 IgG-saporin experiments.**

<table>
<thead>
<tr>
<th>Authors</th>
<th>Route of 192 IgG-saporin administration</th>
<th>Dose/strain of rats</th>
<th>Effect of BF cholinergic lesion on sleep</th>
<th>Effect of BF adenosine on recovery sleep after SD</th>
<th>Effect of BF A1 agonist on sleep after SD</th>
<th>Effect of total BF lesion on sleep (ibotenate or other)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bassant et al., 1995</td>
<td>icv</td>
<td>4 µg; Sprague Dawley</td>
<td>Transient decrease in REM. SD not done.</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Kapas et al., 1996</td>
<td>icv</td>
<td>4 µg; Sprague Dawley</td>
<td>None on spontaneous sleep or recovery sleep after SD</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Gerashchenko et al., 2001</td>
<td>icv</td>
<td>4 µg; Sprague Dawley</td>
<td>None on spontaneous sleep or recovery sleep after SD</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Kalinchuk et al., 2005</td>
<td>local</td>
<td>0.23 µg; Wistar</td>
<td>Transient effect on spontaneous sleep; decrease in recovery sleep after SD</td>
<td>No adenosine rise with SD</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Kaur et al., 2005 and personal communication</td>
<td>local</td>
<td>0.26 µg; Wistar</td>
<td>Transient effect on spontaneous sleep; decrease in recovery sleep after SD</td>
<td>NA</td>
<td>NA</td>
<td>None on spontaneous sleep; decrease in recovery sleep after SD</td>
</tr>
<tr>
<td>Mason et al., 2005</td>
<td>icv</td>
<td>4 µg; Sprague Dawley</td>
<td>Transient decrease in REM. SD not done.</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Lu et al., 2006</td>
<td>icv</td>
<td>1 µg; Sprague Dawley</td>
<td>None on spontaneous sleep or recovery sleep after SD</td>
<td>NA</td>
<td>NA</td>
<td>No wakefulness</td>
</tr>
<tr>
<td>Blanco-Centurion et al., 2006</td>
<td>icv</td>
<td>6 µg; Sprague Dawley</td>
<td>Transient effect on spontaneous sleep; decrease in recovery sleep after SD</td>
<td>No adenosine rise with SD</td>
<td>Increase in sleep</td>
<td>NA</td>
</tr>
<tr>
<td>Porka-Heiskanen et al., and Kalinchuk et al., unpublished</td>
<td>icv</td>
<td>4 µg; Wistar</td>
<td>Transient effect on spontaneous sleep; decrease in recovery sleep after SD</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

*NA = data not available
antagonists decrease recovery sleep (unpublished results), while $A_2$ agonists induce sleep, not in CBF, but in the subarachnoidal space.\textsuperscript{25}

Finally, other differences between the Finnish authors’ experiments and those by Blanco-Centurion et al.\textsuperscript{1} include different rat strains, possible differences in adaptation, in handling the animals and performing the deprivation, different circadian times, and different durations of the sleep deprivation.

We welcome the results of our colleagues as a valuable addition to the knowledge regarding the effects of the saporin lesion on sleep and adenosine levels, but we think the reader would be well advised to be aware of the devil dwelling in delivery details and take the conclusions from this ICV study in the context of other work in the field.

**ACKNOWLEDGMENT**

This work was supported by Academy of Finland and Finska Läkaresällskapet, the Sigrid Juselius Foundation (T.P.H.) NIMH grant MH 39683 (R.W.M.)

**REFERENCES**


21. Wigren H-K, Matto V, Schepens M, Kalinchnuk A, Stenberg D, Porkka-Heiskanen T. Excitation of cells in the basal forebrain results in subsequent increase in sleep which resembles recovery sleep induced by sleep deprivation. Sleep 2006;28(suppl).


**Adenosine and Sleep Homeostasis in the Basal Forebrain: Final Comment.**

Carlos Blanco-Centurion, PhD; Priyattam J. Shiromani, PhD

*Harvard Medical School and the West Roxbury VA Medical Center, West Roxbury, MA*

**Disclosure Statement**

Drs. Blanco-Centurion and Shiromani have indicated no financial conflicts of interest.

Address correspondence to: Priyattam J. Shiromani, PhD, West Roxbury VA, 1400 VFW Parkway, Room 2C109, West Roxbury, MA 02132; Tel: (617) 323-7700; Fax: (617) 363-5717; E-mail: pshiromani@hms.harvard.edu

**SLEEP, Vol. 29, No. 11, 2006**

**WE THANK THE EDITORS FOR THIS FORUM AND THE COMMENTATORS FOR THEIR DISCUSSION OF OUR WORK.\textsuperscript{1} WE AGREE WITH THE COMMENTATORS that adenosine (AD) is an important molecule in regulating sleep drive.** The interest in AD began with Radulovacki’s research in the early 1980s, some of which he reviewed in his commentary.\textsuperscript{2} Then, Rainnie et al.\textsuperscript{3} provided a theoretical framework of how AD might...