Novel melatonin receptor molecules make possible therapies to adjust biological clock

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Like breathing or blinking, behaviors regulated by our circadian rhythms, such as digestion and sleep-wake cycles, go unnoticed by most people. But when circadian rhythms malfunction, the result can be any one of a broad range of serious, chronic disorders, from insomnia and depression to obesity, diabetes and bipolar disorder.

A key piece to the puzzle of circadian rhythms and the disorders they're involved in is the hormone melatonin, which the brain produces in the evening, to facilitate falling asleep and fine-tune circadian adjustments.

Now, a team of researchers from UC San Francisco (UCSF), the University of North Carolina at Chapel Hill (UNC) and the University at Buffalo (UB) has discovered through a vast and novel computational library the first molecules that can modulate circadian rhythms by binding with high selectivity to the MT1 melatonin receptor in the biological clock, located in the hypothalamus at the base of the brain.

The research, published Feb. 10 online before print in *Nature*, will greatly facilitate the development of targeted therapies that can either mimic or counteract the actions of melatonin, which is implicated in numerous circadian disorders ranging from depression, blindness, seasonal affective disorder and sleep disorders to difficulties experienced as a result of jet lag and shift work.

Rhythms in sync

"This discovery allows us to now focus on the development of unique new molecules to generate a response that will help bring sleep patterns and other biological rhythms in line with environmental light and dark cycles, providing the sense of well-being that is only experienced when such rhythms are in sync," said Margarita L. Dubocovich, Ph.D., SUNY Distinguished Professor in the Department of Pharmacology and Toxicology in the Jacobs School of Medicine and Biomedical Sciences at UB.

Dubocovich is one of three corresponding authors, with Brian K. Shoichet, Ph.D., professor in the Department of Pharmaceutical Chemistry at UCSF, and Bryan L. Roth, MD, Ph.D., Michael Hooker Distinguished Professor in the UNC School of Medicine.

They note that the new research represents a remarkable confluence of major, complementary achievements and expertise at three institutions. They are all three members of the Clinical and Translational Science Awards Program of the National Center for Advancing Translational Sciences of the National Institutes of Health.

These achievements are:

- The discovery of ligands unrelated to any known melatonin receptor ligands through the computational docking (simulating three-dimensional binding) of more than 150 million diverse, "make-on-demand" molecules (UCSF).
- The discovery that these molecules (UCSF 7447 and UCSF 3384) that never existed before, attach with high strength and selectivity to human or mouse MT1 melatonin receptors. They generate cellular responses opposite to that of melatonin, which provided the rationale for the mouse circadian behavior. (UNC)
- The discovery that the cellular responses seen in vitro translated directly to in vivo function, slowing down adjustment to a new environmental light/dark period in the mouse model of jet lag, with one unexpected finding demonstrating that they mimic melatonin to modulate rhythms in the absence of light cues (UB).

This work was further facilitated by the publication last spring of the first crystal structure of the MT1 receptor, providing the team with the "template" to fit new melatonin molecules into the receptor pocket.

"For us, it was at first exciting to see the novelty of the new ligands that emerged from fitting members of an ultra-large chemical library into the receptor structure," said Shoichet. "That's what one always hopes for in a structure-based program—finding new chemistries not imaginable from knowing the endogenous ligand (here, melatonin). What made this doubly exciting was to see the new chemistries lead to new signaling, in

the Roth lab experiments, and to unexpected animal pharmacology in the Dubocovich lab."

Roth added: "My UNC lab spent more than a year characterizing the pharmacology and drug-like properties of the molecules before we could hand them off for animal testing in the Dubocovich lab at UB. We were all excited to see that the new compounds Brian and I had discovered had interesting properties in mice."

Fifteen-year search

The new research caps what Dubocovich says has been her 15-year search to discover MT1 ligands.

"Ever since we demonstrated that melatonin's effect of resetting biological clocks in vivo circadian models occurs through actions at the MT1 receptors, we have focused through various collaborations on searching for ligands that would better fit the human melatonin receptor," she said. "Our hope has always been to find selective MT1 -type molecules, either one that works to modulate circadian rhythms responses as with melatonin or its opposite as with the molecules discovered in this study."

The ultimate goal, she said, was always to develop drugs that could address all the disorders that disrupted circadian rhythms can cause. "So," she noted, "when Brian Schoichet called to ask about our interest in testing in our circadian mouse models the novel molecules they had identified from his ultra-large library of over 150 million compounds, we were eager to collaborate!"

The availability of the UCSF vast virtual library was a critical aspect of the research. Dubocovich described it as a "gold mine" of millions of molecules with distinct shapes, many of which have never been

synthesized or seen in nature, and all of them available for mining and "docking" (fitting) into the pocket of the targeted receptor. The team advanced the research directly from discovery of these molecules to the assessment of their ability to mimic or oppose the effect of melatonin, to in vivo demonstrations of how these molecules impact the animals' circadian function.

The team found it especially interesting that the two molecules discovered in this study generate two distinct and opposite mouse circadian responses that are dependent on clock time and the environmental light conditions that the animals experience.

In the experiments where the onset of dark is advanced (known as reentrainment or the jet lag model) the molecules slow this reentrainment or adjustment, an effect opposite to that of melatonin. However, when mice were exposed to constant dark, the two molecules demonstrate an effect identical to that of melatonin.

"This could be potentially useful to entrain rhythms to the 24-hour day in populations removed from natural light/dark exposure, including the blind as well as some shift workers, submarine workers or those working in extreme environments, such as polar explorers," she said.

Jet lag and chronopharmocology

"When the body is exposed to an abrupt change in the light/dark cycle, like what we experience when we travel across continents, there isn't sufficient time for the biological clock to adjust upon reaching the destination," Dubocovich explained.

"Giving these new molecules at the appropriate clock time under a light/dark cycle would allow us to decelerate our ability to adjust to the new environment, potentially providing a treatment for certain types of

jet lag and, more importantly, addressing other conditions affected by circadian rhythm disruptions, such as shift work, sleep disorders and depression," she said.

This finding reinforces the increased interest in chronopharmacology, the premise of which is that pharmaceuticals given at the time when a patient's biological clock is ready to receive them—given conditions of a precise time of day and environmental lighting—will produce a more effective outcome.

Dubocovich said the next step will be to identify the molecular and signaling pathways that translate the response exerted by these molecules from the time they interact with the receptors in the biological clock to the ultimate circadian behavior expressed in a mouse or human.

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