Sleep-Promoting Factor Isolated

Glycopeptide from human urine causes a 50 percent increase in deep sleep in animals

O sleep! O sleep! Do not forget me. . . . O, I am tired!

—JEAN INGELOW

This plaintive cry of the 19th-century poet is still heard today. An estimated one-third of all Americans suffer from occasional sleep disorders and as many as one in every ten use medication to help them sleep. Over-the-counter pharmaceuticals are of limited value in helping these individuals, however, and prescription drugs often have undesirable side effects. The recent isolation from humans of a glycopeptide that induces safe, normal sleep in laboratory animals thus represents a significant breakthrough toward both the development of more natural, more effective sleeping aids and a better understanding of the sleep process. It will clearly be many years before the glycopeptide or a modification will be available for use in humans, but the promise is present.

The sleep-promoting substance, known as factor S, was isolated from human urine by James M. Krueger, now at the Chicago Medical School, and John R. Pappenheimer and Manfred L. Karnovsky of the Harvard Medical School. The isolation and purification of about 30 micrograms of the glycopeptide from more than 4.5 tons of urine from healthy males was reported in the *Journal of Biological Chemistry* [**257**, 1664 (1982)] and at a recent seminar at Harvard.

Factor S is composed of the amino acids glutamic acid, alanine, and diaminopimelic acid and the sugar muramic acid in the molar ratio of 2:2:1:1 with a mass of 922 daltons. When infused into the brain of a rabbit at a concentration of about 5 picomoles per kilogram of body weight, factor S induces a 50 percent increase in what is known as slow wave sleep, a deep, dream-free sleep that occurs in animals and humans after sleep deprivation, and is normal as judged by various criteria.

Pappenheimer, Karnovsky, and their colleagues had previously isolated sleep-promoting substances from the cerebrospinal fluid and the brain of sleep-deprived goats. These substances have not been completely purified and characterized, but preliminary evidence suggests that they are similar, if not identical, to each other and to factor S from humans. These substances induce excess slow wave sleep in rats, rabbits, and cats. Koji Uchizono and his colleagues at the Tohoku University School of Medicine in Japan have partially purified and characterized a sleep-promoting factor from the brain stems of sleep-deprived mice, and this is also similar or identical to factor S.

In contrast, Marcel Monnier and his colleagues at the University of Basel in Switzerland have isolated and characterized a nonapeptide sleep-promoting factor by dialysis from the blood of sleeping rabbits. This substance, called delta-sleep-inducing-peptide or DSIP, causes a transitory increase in slow wave and delta wave (another type of brain wave associated with sleep) activity at concentrations (20 nanomoles per rabbit) substantially higher than required with factor S. The Basel group demonstrated last year that synthetic DSIP could produce deeper sleep with fewer awakenings in six individuals with severe, chronic insomnia. Synthetic DSIP has no activity, however, in their laboratory assay, says Karnovsky.

> The sleep-promoting agent may be derived from bacterial degradation products.

The identification of the consituents of factor S has recently been confirmed by mass spectrometry by Klaus Biemann and his associates at the Massachusetts Institute of Technology. Biemann will soon begin studying fragmentation patterns and hopes to have the sequence within a year. Meanwhile, Karnovsky and Pappenheimer are trying to develop a sensitive assay that could be used to monitor factor S at the low concentrations found in biological fluids. Among other things, they would like to know whether the concentration of factor S increases in individuals deprived of sleep and whether the concentration is abnormal in individuals with sleep disorders.

The structure of factor S is rather unusual. Glutamic acid and alanine both are common amino acids, but both muramic acid and diaminopimelic acid are more typically associated with the cell walls of bacteria. After extensive studies, the Harvard investigators are convinced that factor S does not arise from bacterial contamination during their isolation procedure. This suggests, says Pappenheimer, either that mammals can synthesize peptides containing muramic acid in quantities too small to have been detected previously, or that the sleep factor is derived from bacterial products absorbed through the gut. The factor, says Pappenheimer, would thus "be akin to any of the essential amino acids or vitamins which cannot be synthesized by mammalian cells." Some bacterial degradation products, in fact, have sleep-antagonist properties, suggesting that they have different structures but share a common receptor.

While isolating and characterizing factor S, Pappenheimer and Karnovsky have been exploring its mechanism of action with some synthetic analogs. Edgar Lederer of the National Center for Scientific Research at Gif-sur-Yvette, France, has synthesized more than 300 muramyl peptides in a search for immunostimulants that could serve as adjuvants or potency increasers for vaccines. Pappenheimer and Karnovsky have studied many of these to develop structure-activity correlations. One of the most potent of these in rabbits is *N*-acetylmuramyl-L-alanyl-D-isoglutamine, also known as muramyl dipeptide or MDP. It produces about a 50 percent increase in slow wave sleep when infused into the brain of rabbits at concentrations about ten times higher than that required for factor S. The same effect is observed when a millionfold higher dose is administered orally.

In addition to the potential for assisting individuals with sleep disorders, factor S could open the door to a much better understanding of the biochemical nature of sleep—an area where almost nothing is now known. Once its structure is determined, it will be possible to synthesize it in a radioactively labeled form that could be used to locate receptors in the brain, to determine how the glycopeptide binds to the receptor, and to trace the chain of chemical commands that are subsequently issued.

—THOMAS H. MAUGH II

0036-8075/82/0625-1400$01.00/0