The REM Sleep-Inducing Action of a Naturally Occurring Organic Bromine Compound in the Encéphale Isolé Cat*

Shizuo Torii, Kenji Mitsumori, Shikio Inubushi, and Isamu Yanagisawa

Departments of Physiology and Biochemistry, Toho University School of Medicine, Tokyo, Japan

Received September 5, 1972

Abstract. The acute effects on the sleep-wakefulness cycle of 2-octyl-γ-bromoacetoacetate (γ-Br), an organic bromine compound which occurs naturally in the brain and cerebrospinal fluid (CSF) of mammals, were studied in the encéphale isolé cat preparation under artificial respiration without fixation.

In unmedicated preparations, the percentage of the three states, wakefulness, NREM sleep and REM sleep during the 3 h recording period was 32.8 ± 15.59, 62.9 ± 16.81 and 4.3 ± 3.81, respectively.

Various doses of synthetic γ-Br (0.1, 1.0 and 5.0 mg/kg) were injected intravenously. No central depressant effect was seen after injection of γ-Br up to 5 mg/kg. The effect of γ-Br on the sleep-wakefulness cycle was to increase REM sleep and to slightly decrease wakefulness or NREM sleep. Increase in REM sleep was significant (p < 0.05) and its effective dose was 0.1 mg/kg. REM sleep-increasing effect appeared 5 to 15 min after injection. This coincided closely with the time of appearance of γ-Br in the CSF after intravenous injection.

The relationship between γ-Br and short chain fatty acids as well as the possible mechanism related to REM sleep induction by γ-Br are discussed.

Key words: Sleep-Wakefulness Cycle — REM Sleep — Organic Bromine Compound — Short Chain Fatty Acids.

During the course of investigation on bromide poisoning it was found that, in addition to exogenous bromide, a bromine containing substance occurs naturally in human cerebrospinal fluid (CSF) (Yanagisawa and Yoshikawa, 1968). The major portion of this bromine was demonstrated to exist in the form of an organic compound, which was isolated and identified as 2-octyl-γ-bromoacetoacetate (γ-Br) (Yanagisawa and Yoshikawa, in preparation). An almost identical bromo-substance was also found in the CSF of cats. Because of the therapeutic use of bromide, a

* This study was supported in part by a grant from the Ministry of Education of Japan. A preliminary report of this research was presented at the meeting of the Association for the Psychophysiological Study of Sleep in Santa Monica, California, U.S.A., April 6—9, 1967.
hypnotic effect of this new bromo-substance was then suspected. The present study, which aimed to examine the effect of γ-Br on the sleep-wakefulness cycle in cats, was further stimulated by the observations that the short chain fatty acids such as n-butyrate or γ-hydroxybutyrate produced REM sleep (Jouvet et al., 1961; Matsuzaki et al., 1964; Winter and Spooner, 1965), and that the short chain fatty acids could be metabolically converted into γ-Br in vivo (Yanagisawa and Torii, unpublished data).

Since the discovery that sleep consists of two different states, that is, REM sleep and NREM sleep (Jouvet, 1967), it has become necessary to reconsider methods of testing hypnotics which depend mainly on observation of righting reflexes or EEG tracings. In animal experiments, polygraphic observations of the sleep-wakefulness cycle for 24 h is recommended for more critical evaluation of hypnotics (Wallach et al., 1969). In the case of γ-Br, this type of experiment has failed to yield reproducible results so far, because this substance is water-insoluble and easily decomposed into toxic substances such as octanol. Consequently water-soluble and chemically stable related compounds of γ-Br have been synthesized and their effects on the 24-h sleep-wakefulness cycle have been studied in chronically prepared cats (Torii et al., 1969). With γ-Br itself, relatively short periods of polygraphic observations, about 4—6 h, have been made, using the encéphale isolé preparation in which REM sleep appears periodically as will be shown in this paper. The encéphale isolé preparation is also useful for study of the blood-cerebrospinal fluid barrier of synthetic γ-Br, which is particularly important in the evaluation of drug action in the central nervous system when administered intravenously.

Methods

Twenty adult cats were used. Under ether anesthesia the head of the cat was fixed on a stereotaxic apparatus (TodaiNoken type) and the encéphale isolé preparation was made by cutting the upper spinal cord at the C₆ level (Torii and Wikler, 1966). A dorsal midline incision in the scalp was made from the forehead to the occiput. Both temporal muscles were deflected, the periosteum was scraped on the exposed region of the skull, and the frontal sinus was exposed by removal of its dorsal wall. A stainless steel screw was placed into the calvarium overlying the pericruciate cortex for EEG recording with reference to a lead provided by another screw in the frontal bone. A pair of screw electrodes were placed into the orbital roofs on both sides for the electrooculogram (EOG). Bipolar needle electrodes, about 1 cm apart, were inserted into the posterior neck muscle for the electromyogram (EMG), and the same type of electrodes into the chest wall for the electrocardiogram (ECG).

After completion of the surgical procedure, the cat was released from fixation in the stereotaxic apparatus and was placed with the left side of the body upwards. The rectal temperature was kept at 36—38°C by a heating pad. Artificial respiration was maintained at rates of 32—38 per min. Penicillin (100,000 U) was injected