Proc. Natl. Acad. Sci. USA
Vol. 80, pp. 1735–1737, March 1983
Neurobiology

Prostaglandin D_2, a cerebral sleep-inducing substance in rats

(slow wave sleep/paradoxical sleep/intraventricular infusion)

RYUJI UENO*, KAZUKI HONDA†, SHOJIRO INOUÉ†, AND OSAMU HAYAISHI*

*Department of Medical Chemistry, Kyoto University Faculty of Medicine, Sakyo-ku, Kyoto 606, Japan; and †Institute for Medical and Dental Engineering, Tokyo Medical and Dental University, Kanda-Surugadai, Chiyoda-ku, Tokyo 101, Japan

Contributed by Osamu Hayaishi, December 8, 1982

ABSTRACT **We continuously monitored the circadian sleep patterns of unrestrained rats for more than 96 hr and infused various prostaglandins into their third ventricles for 10 hr to study the effects on inducing sleep. Prostaglandin D_2 at 6 fmol/min had no effect on either slow wave sleep or paradoxical sleep. However, prostaglandin D_2 at as little as 60 fmol/min caused a significant amount of excess slow wave sleep as compared with the control level during saline infusion. Paradoxical sleep was induced by prostaglandin D_2 at doses greater than 600 fmol/min. Prostaglandin D_2 (600 fmol/min) increased slow wave sleep by 33% and paradoxical sleep by 56%. Although prostaglandin $F_{2\alpha}$ (600 fmol/min) increased the amount of slow wave sleep, its activity was less than that of the same amount of prostaglandin D_2. Prostaglandin E_2 (600 fmol/min) had no effect on increasing the amounts of both slow wave sleep and paradoxical sleep. During the infusion of prostaglandin D_2, rats were easily aroused by clap sound stimulation and their sleeping and waking postures remained normal. Further, their sleep was episodic, as observed in the physiological sleep of rats.**

Prostaglandin D_2 (PGD_2) has been identified as a natural constituent in brains of various mammals (1–4) and its synthesis and degradation in brain have been investigated in detail (2, 5, 6). Recently, Ueno *et al.* (4, 7, 8) reported that the use of the intracerebral microinjection technique to administer nmol doses of PGD_2 elicits several central actions. One of the prominent actions of PGD_2 was the induction of sleep (7, 8). A site of action of PGD_2 on inducing sleep was the preoptic area and 0.3 nmol of PGD_2 induced excess slow wave sleep (SWS) (7). However, the PGD_2 content in the preoptic/hypothalamic area in rat brain was 4–6 pmol/g (wet weight) of tissue (3, 4), indicating that even the doses we used before were not physiologically feasible. In the present study, we continuously infused small amounts of various PGs into the third ventricles of conscious rats and found that PGD_2 at as little as 60 fmol/min induced excess sleep.

MATERIALS AND METHODS

Experimental Animals. Male Sprague–Dawley rats, 300–350 g, 70–80 days old were used. Rats were housed in a 12-hr light (08:00–20:00)/dark (20:00–08:00) cycle for at least 2 wk prior to surgery. Under pentobarbital anesthesia (50 mg/kg), rats were mounted on a stereotaxic instrument with the head fixed according to the coordinate system of Pellegrino *et al.* (9). For electroencephalogram (EEG) recordings, three silver ball electrodes were placed on the cortex and a silver plate over the frontal skull served as the indifferent electrode. The electromyogram (EMG) electrodes were inserted into the neck muscle. For intracerebral infusion to the third ventricle, a stainless steel cannula (o.d., 0.35 mm) was placed 3.4 mm lateral and 0.6 mm anterior from the bregma and inserted about 9 mm from the surface of the cortex at an angle of 20° from the midsagittal plane. The position of the cannula was determined by x-ray photographs. The electrodes and cannula were fixed by using dental acrylic resin.

Experimental Devices for Sleep Analyses (Fig. 1). After surgery, each rat was housed in a cage in a sound-proof room maintained at 25 ± 1 °C and 50 ± 10% relative humidity on the 12:12 hr light/dark cycle. The electrodes and cannula were connected to amplifiers and an infusion pump (B. Braun, Melsungen, Federal Republic of Germany), respectively, with a slip ring that allowed unrestrained movement of the rat. Food and water were available ad lib. Locomotor activities of the rat were detected by a vibration transducer and the electrical signals were recorded on a polygraph. At the same time, the electrical signals above a specified voltage were chosen by a comparator and summarized every hour on a digital counter and printer. EEGs and EMGs were recorded on polygraphs. The behavior of the experimental animals was monitored by a video recording system throughout day and night. The amounts of SWS and paradoxical sleep (PS) were determined by polygraphic recordings of cortical EEG, neck EMG, and locomotor activities. SWS is identified as a high-amplitude slow-wave EEG with moderate EMG and no locomotion. PS is defined as a low-amplitude fast-wave EEG with lack of EMG but slight locomotion of short duration. The whole sleep scores over 96 hr were visually analyzed in each rat by using a digitizer (MOP, Kontron, München, Federal Republic of Germany). These digital data were computed and stored by a central processing unit (Nippon Data General, Tokyo) for further statistical analyses. Details are described elsewhere (10).

Infusion into the Third Ventricle. Sterile saline was infused continuously at a rate of 20 μl/hr to the third ventricle from the 3rd day after the operation except during the period of PG infusion. Recovery of the rats from surgery, which required at least 8 days, was determined by their daily sleep rhythm. The continuous infusion of saline did not essentially affect the sleep patterns of the rats (11). PGs (gifts from Ono Central Research Institute, Osaka, Japan) stored at −20°C were weighed and dissolved in sterile saline 30 min before infusion. The sample (200 μl) containing the indicated amount of PG was set in a sample chamber at 16:00 hr and infused to the third ventricle of a conscious rat for 10 hr (19:00–05:00) at a rate of 20 μl/hr. Throughout the infusion period, rats were housed in the same cage without any restriction of their movement. Rats that had been infused with PG once were used again with an interval of at least 5 days if their sleep rhythm returned to the control level.

RESULTS

Effects of PGD_2 on Induction of Sleep. The circadian sleep patterns of saline-infused rats (control) are shown in Fig. 2. The

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

Abbreviations: PG, prostaglandin; SWS, slow wave sleep; PS, paradoxical sleep; EEG, electroencephalogram; EMG, electromyogram.

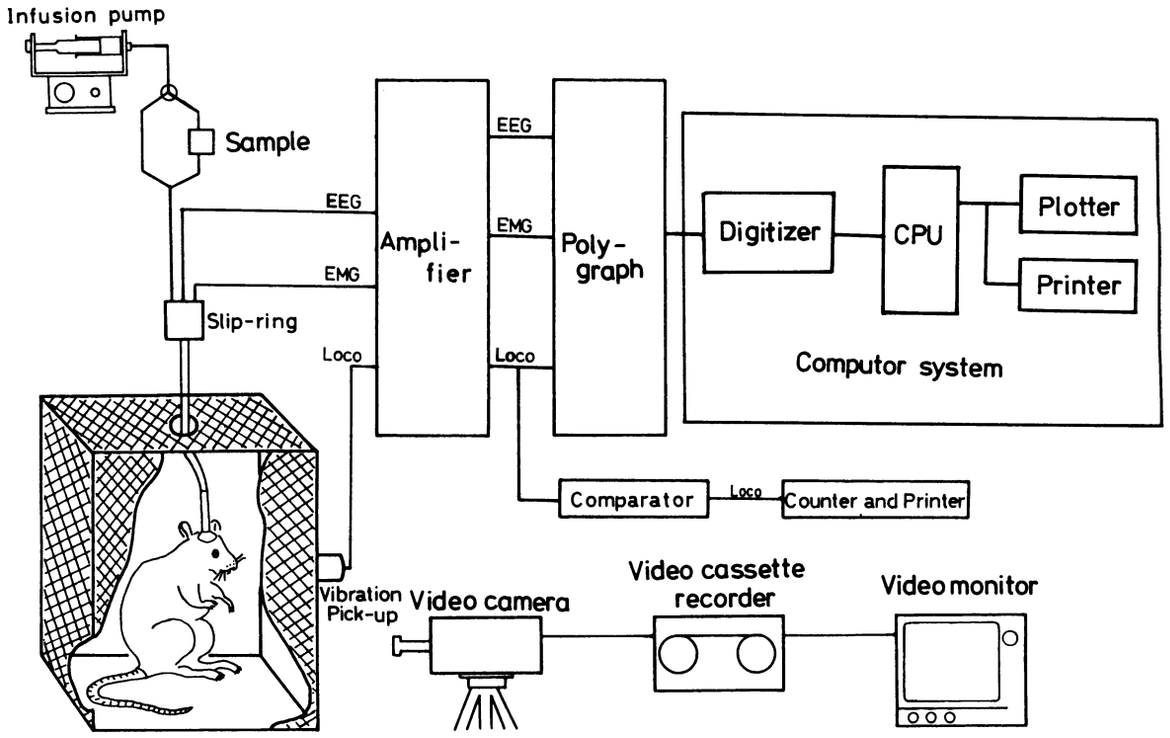

FIG. 1. Circadian bioassay systems for analysis of sleep of rats. CPU, central processing unit; Loco, locomotor activities.

amounts of both SWS and PS in the light period were about twice as much as those observed in the dark, when the rats were active. PGD_2 (6 pmol/min) in sterile saline was infused for 10 hr (19:00 to 05:00) to the third ventricle of the experimental rat 1 hr prior to the beginning of the active period. The action of PGD_2 in inducing both SWS and PS started within 1 hr after the initiation of PGD_2 infusion and disappeared within 2 hr after the termination of PGD_2 infusion (Fig. 2). These observations were reproducible in all four rats tested (Table 1). By 1 day after termination of PGD_2 infusion the sleep patterns returned to the control level and no withdrawal effect of PGD_2 was observed. The effects of different doses of PGD_2 on inducing sleep are shown in Table 1. In the experimental series, we used 26 rats. During the dark period, the amounts of SWS and PS of saline-infused rats were 228.1 ± 5.8 min/12 hr and 35.0 ± 4.1 min/12 hr (mean ± SEM), respectively. Intraventricular infusion of PGD_2 at 6 fmol/min for 10 hr failed to increase the amounts of either SWS or PS. However, PGD_2 at as little as 60 fmol/min induced excess SWS. PGD_2 at 600 fmol/min increased the amount of SWS by 33% ($P < 0.001$) from the control level together with

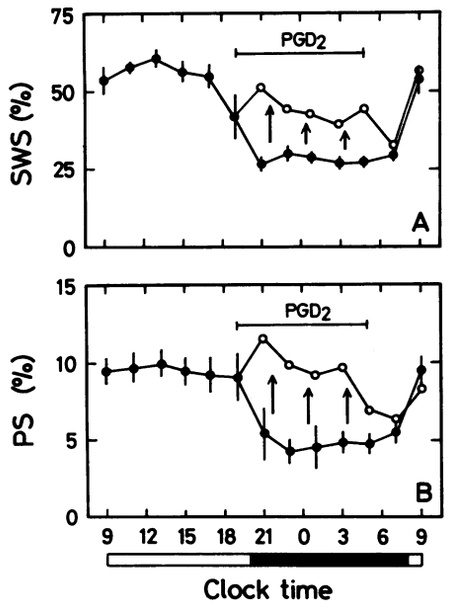

FIG. 2. Effects of PGD_2 on sleep patterns of rats. Amounts of SWS (*A*) and PS (*B*) in 2-hr (± 1 hr of the indicated clock time) periods are plotted. The sleep pattern of the control rat (●) under continuous saline infusion into the third ventricle was obtained for 5 consecutive days before PGD_2 infusion. Results are mean ± SEM for five daily recordings with a single rat. ○, PGD_2 in sterile saline (3.6 nmol/200 μl) was infused to the rat for 10 hr (19:00 to 05:00) at a rate of 6 pmol/min. Throughout the infusion period, the rat was housed under the same conditions as the control rat without any restriction of its movement. Arrows indicate increments from control levels. Open and closed bars indicate light and dark periods.

Table 1. Effects of PGs on induction of excess sleep

Treatment	Rate, fmol/min	*n*	SWS, % control	PS, % control
Saline (control)		20	100	100
PGD_2	6	3	96.5 ± 2.3*	89.1 ± 7.9*
	60	4	122.4 ± 3.6†	110.3 ± 11.9*
	600	8	133.3 ± 3.9†	156.2 ± 16.0‡
	6,000	4	125.5 ± 4.8†	178.3 ± 12.1†
$PGF_{2\alpha}$	600	6	115.2 ± 6.0‡	100.4 ± 16.3*
PGE_2	600	8	96.3 ± 5.1*	104.3 ± 11.7*

PGs were infused for 10 hr (19:00 to 05:00) and amounts of SWS and PS in the dark period (20:00 to 08:00) were determined. Results are mean ± SEM. SWS, 228.1 ± 5.8 min/12 hr; PS, 35.0 ± 4.1 min/12 hr. Significance was calculated by *t* test.
*Not significant ($P > 0.05$).
†$P < 0.001$.
‡$P < 0.05$.

that of PS by 56% ($P < 0.05$). Although increasing the dosage to 6 pmol/min failed to further increase the amount of SWS, it increased the amount of PS to almost twice that of the control level. However, the larger increments in PS were statistically less significant because of larger individual differences and because the amount of PS was smaller as compared with that of SWS. Judging from the recordings of EEG, EMG, and locomotor activities, the SWS and PS during PGD_2 infusion were normal and no alteration from the state of waking to PS without SWS was observed. During the sleep caused by PGD_2, rats were easily aroused by clap sound stimulation. Their sleep was episodic, as observed in the controls, and their posture remained normal.

To compare the effects with that of PGD_2, we also infused $PGF_{2\alpha}$ (600 fmol/min) and PGE_2 (600 fmol/min) into the third ventricle of conscious rats. As shown in Table 1, $PGF_{2\alpha}$ at this dose increased the amount of SWS by 15% without changing the amount of PS. Although the effect of $PGF_{2\alpha}$ on inducing SWS was weaker than that of PGD_2 (600 fmol/min), the increase of SWS was statistically significant ($P < 0.05$). On the other hand, PGE_2 (600 fmol/min), which is an isomer of PGD_2, had no effect on the amounts of either SWS or PS.

DISCUSSION

In the present study, we used a circadian bioassay system for sleep analysis and found that PGD_2 exerted the strongest action among the PGs tested. The effective dose of PGD_2 for induction of sleep was as little as 60 fmol/min, which might be physiologically feasible, because the preoptic/hypothalamic area, the site of action of PGD_2 in inducing sleep (7), contained 4–6 pmol of PGD_2/g (wet weight) of tissue after sacrifice of the rats by microwave radiation (3, 4). It has been reported that monoamines (12) and several peptides such as δ-sleep-inducing peptide (13), factor S (14, 15), and muramyl peptides (16) induce excess sleep. However, factor S from brain is not yet purified and muramyl peptides probably originate from bacteria. Recently, the effects of δ-sleep-inducing peptide and of muramyl peptide have been investigated using the bioassay system described here (unpublished data). It was found that muramyl peptide at 200 fmol/min fails to induce excess sleep in rats and that a dose of 2 pmol/min is required to elicit an effect. δ-Sleep-inducing peptide at a dose of 4 pmol/min was effective on inducing sleep. In comparison with these substances, PGD_2 appears to be a far more potent sleep-inducing compound under our experimental conditions. Although it has been reported that acetaminophen, a weak inhibitor of PG synthetase (17, 18), reduces the fever caused by a muramyl peptide without affecting sleep, the minimal doses of PGs effective in inducing sleep may be so low that the amount of sleep is not affected significantly by acetaminophen treatment.

We propose that PGD_2 in the brain may be an endogenous regulator of physiological sleep, at least in the rat, for the following reasons. (*i*) PGD_2 is actively synthesized (2–5) and metabolized (6) in the brain. (*ii*) Sleep induced by PGD_2 is indistinguishable from physiological sleep as judged by EEG, EMG, and the locomoter activities and behavior of the rat. (*iii*) PGD_2 at as little as 60 fmol/min causes excess sleep and the concentration of endogenous PGD_2 in the brain (3, 4) appears sufficient to induce sleep. (*iv*) PGD_2 is not pyrogenic and it slightly decreases body temperature (4, 7) as observed in physiological sleep (19–23). In support of this hypothesis, Krueger *et al.* (16) have reported that indomethacin, a potent inhibitor of PG synthetase (17, 18), blocks normal sleep.

This work was supported in part by a grant-in-aid for scientific research from the Ministry of Education, Science, and Culture of Japan.

1. Abdel-Halim, M. S., Hamberg, M., Sjöquist, B. & Änggård, E. (1977) *Prostaglandins* **14,** 633–643.
2. Shimizu, T., Yamamoto, S. & Hayaishi, O. (1979) *J. Biol. Chem.* **254,** 5222–5228.
3. Narumiya, S., Ogorochi, T., Nakao, K. & Hayaishi, O. (1982) *Life Sci.* **31,** 2093–2103.
4. Ueno, R., Narumiya, S., Ogorochi, T., Nakayama, T., Ishikawa, Y. & Hayaishi, O. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 6093–6097.
5. Shimizu, T., Mizuno, N., Amano, T. & Hayaishi, O. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 6231–6234.
6. Watanabe, K., Shimizu, T., Iguchi, S., Wakatsuka, H., Hayashi, M. & Hayaishi, O. (1980) *J. Biol. Chem.* **255,** 1779–1782.
7. Ueno, R., Ishikawa, Y., Nakayama, T. & Hayaishi, O. (1982) *Biochem. Biophys. Res. Commun.* **109,** 576–582.
8. Ueno, R., Hayaishi, O., Ishikawa, Y., Nakayama, T., Honda, K. & Inoué, S. (1982) *Seikagaku* **54,** 619.
9. Pellegrino, L. J., Pellegrino, A. S. & Cushman, A. J. (1979) in *A Stereotaxic Atlas of the Rat Brain* (Plenum, New York).
10. Honda, K. & Inoué, S. (1978) *Rep. Inst. Med. Dent. Eng.* **12,** 81–85.
11. Inoué, S., Honda, K. & Komoda, Y. (1983) in *Sleep 1982,* ed. Koella, W. P. (Karger, Basel, Switzerland), in press.
12. Jouvet, M. (1969) *Science* **163,** 32–41.
13. Schoenenberger, G. A. & Monnier, M. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 1282–1286.
14. Pappenheimer, J. R., Koski, G., Fencl, V., Karnovsky, M. L. & Krueger, J. M. (1975) *J. Neurophysiol.* **38,** 1299–1311.
15. Krueger, J. M., Pappenheimer, J. R. & Karnovsky, M. L. (1982) *J. Biol. Chem.* **257,** 1664–1669.
16. Krueger, J. M., Pappenheimer, J. R. & Karnovsky, M. L. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 6102–6106.
17. Flower, R. J. & Vane, J. R. (1972) *Nature (London)* **240,** 410–411.
18. Flower, R. J. (1974) *Pharmacol. Rev.* **26,** 33–67.
19. Adams, T. (1963) *Science* **139,** 609–610.
20. Kovalzon, V. M. (1973) *Physiol. Behav.* **10,** 667–670.
21. Hayward, J. N. & Baker, M. A. (1969) *Brain Res.* **16,** 417–440.
22. Roussel, B., Dittmar, A. & Chouvet, G. (1980) *Waking Sleeping* **4,** 63–75.
23. Haskell, E. H., Palca, J. W., Walker, J. M., Berger, R. J. & Heller, H. C. (1981) *J. Appl. Physiol.* **51,** 948–954.