Brain Research, 444 (1988) 265–272
Elsevier

BRE 13385

Awaking effect of PGE_2 microinjected into the preoptic area of rats

Hitoshi Matsumura[1], Yasumasa Goh[2], Ryuji Ueno[2], Toshiaki Sakai[1] and Osamu Hayaishi[2]

[1]*Department of Neuropsychiatry, Osaka Medical College and* [2]*Hayaishi Bioinformation Transfer Project, Research Development Corporation of Japan, c/o Osaka Medical College, Takatsuki (Japan)*

(Accepted 8 September 1987)

Key words: Prostaglandin E_2; Sleep–wake activity; Temperature; Preoptic area; Microinjection; Electroencephalogram; Electromyogram

We examined the effect of prostaglandin (PG)E_2 on the sleep–wake activity and on body temperature by microinjecting PGE_2 into the preoptic area of rats that had been chronically implanted with guide cannulae and electrodes for the recordings of electroencephalogram and electromyogram. PGE_2 at doses of 2.5×10^{-13}, 2.5×10^{-11}, and 2.5×10^{-9} mol reduced the time of slow wave sleep (SWS) to 75%, 61%, and 59% and that of paradoxical sleep (PS) to 73%, 50%, and 25% of the controls, respectively. The SWS and PS reductions were mainly due to the shortening of the SWS episode and the less frequent occurrence of PS episodes. The sleep reduction was accompanied by increased behavioral movement. The maximum increases of rectal temperature at doses of 2.5×10^{-11} and 2.5×10^{-9} mol of PGE_2 were 1.3 °C and 2.7 °C, respectively. At a dose of 2.5×10^{-13} mol of PGE_2, the time of SWS and that of total sleep (sum of SWS and PS) decreased significantly, but the change in body temperature was negligible. This may imply that the effect of PGE_2 on the sleep–wake activity is not caused by the hyperthermia produced by PGE_2. Injections of PGE_2 at a dose of 2.5×10^{-15} mol and saline control induced alteration in neither sleep–wake activity nor body temperature. PGD_2 at a dose of 2.5×10^{-9} mol slightly elevated the rectal temperature (0.5 °C), but did not produce any change in the sleep–wake activity. On the basis of these findings, we conclude that PGE_2 acts at the preoptic area of rats to increase the wakefulness by inhibiting the maintenance of SWS and consequently decreasing the occurrence of PS.

INTRODUCTION

Prostaglandin (PG)E_2, endogenously formed and released in the brain tissue of several species, is well known to have various central actions such as hyperthermia, anticonvulsant effects (cf. ref. 38) and induction of behavioral sedation[5,24]. Concerning the sleep–wake activity, it was reported that PGE_1 and E_2, applied into the third cerebral ventricle and into the hypothalamus, induced sleep in fowls[20,21]. In rats, contrary to the result in fowls, centrally applied PGE_1 decreased the time that animals spent in sleep. However, even in rats, the intracisternal injection of PGE_1 decreased the time of paradoxical sleep (PS)[17], whereas the lateral ventricular administration of PGE_1 was shown to decrease the time of slow wave sleep (SWS)[3]. Thus, the results obtained are complicated to interpret and the effect of PGE on the sleep–wake activity has not yet been clarified.

The anterior hypothalamic preoptic area is well known to be responsible for PGE-induced hyperthermia (e.g. refs. 4, 16, 28, 30, 37). Since the preoptic area has been generally recognized to be a center of sleep regulation[19,29] as well as a center of temperature regulation, it is possible that PGE_2 has some effect on the sleep–wake activity in addition to its thermal effect in the preoptic area. Incidentally, this area has recently been shown in monkeys to contain a binding protein of PGE_2, presumably its receptor, in high concentration[36]. This finding further makes it in-

Correspondence: O. Hayaishi, Hayaishi Bioinformation Transfer Project, Research Development Corporation of Japan, c/o Osaka Medical College, 2-7 Daigakumachi, Takatsuki, Osaka 569, Japan.

0006-8993/88/$03.50 © 1988 Elsevier Science Publishers B.V. (Biomedical Division)

teresting to investigate the effect of PGE_2 in the preoptic area. In this experiment, therefore, we microinjected PGE_2 into the preoptic area of rats, and investigated the effect of PGE_2 on the sleep–wake activity together with its effect on body temperature.

MATERIALS AND METHODS

Animals and surgical procedure

Male Wistar rats weighing 300–350 g were anesthetized with intraperitoneal injections of pentobarbital sodium (50 mg/kg b. wt.). A guide cannula (o.d. 0.7 mm) was inserted stereotaxically into the left half of the brain through the burr hole created on the skull and positioned 1.0 mm above the site of injection. Since the injection cannula (o.d. 0.35 mm), inserted into the guide cannula, was adjusted to protrude 1.0 mm beyond the tip of the guide cannula, the site of injection was to be located 0.6 mm posterior to the bregma, 8.5 mm ventral to it, and 1.0 mm lateral to the midline in the preoptic area according to the coordinate system of Paxinos and Watson[23]. The accuracy of the site was verified histologically by the microinjection of Pontamine sky blue (0.5% w/v) after the experiment was completed. The diffusion of the dye, microinjected at a volume of 3 μl, was observed to have remained within the anterior hypothalamic preoptic area. The guide cannula was plugged with a stylet, which was replaced with the injection cannula at the experimental sessions described below. A pair of electrodes for bipolar derivation of the electroencephalogram (EEG) were put on the frontoparietal cortex at a position 2 mm anterior to the bregma and 2 mm lateral to the midline. Electrodes for the recordings of electromyogram (EMG) were introduced into the neck muscles and fixed to them. Another electrode in the frontal bone of the skull served as an indifferent electrode. The guide cannula, EEG electrodes, and the indifferent electrode were mounted on the skull with dental cement. After surgery, the rats were housed in individual cages in a sound-proof room maintained at a constant temperature of 25 °C and 55% relative humidity on a 12-h light (08.00–20.00 h)/dark (20.00–08.00 h) cycle. Food and water were available ad libitum. The animals were permitted to recover from surgery for two weeks prior to experimentation.

Experimental design

During a one-week period after the recovery, each rat was habituated to the experimental procedure described below. During this period, each rat received 4 microinjections of pyrogen-free physiological saline. By the habituation procedure, the amount of total sleep reached a plateau that was around 50% of the 90-min recording period. In the experiment, rats were divided into 6 groups: 4 for PGE_2 at the doses of 2.5×10^{-15}, 2.5×10^{-13}, 2.5×10^{-11}, and 2.5×10^{-9} mol; one for PGD_2 at a dose of 2.5×10^{-9} mol; and one for the saline control. Rats of each group received 3 microinjections at 48-h intervals: the first was saline for control recordings of each group; then a dose of PGE_2 or D_2 or saline was given for the experiment; and finally saline was injected for the examination of the long-term influence by the PG applications. The results obtained by the second and the third injections were compared with that obtained by the first injection and the data were statistically analyzed within each group using the paired *t*-test, unless otherwise indicated.

Experimental procedure

In an experimental session, rats were placed in small boxes made of transparent acrylic plates, specially designed for this experiment, between 11.30 and 12.30 h. To record the temperature, a thermistor probe (Takara, Yokohama, Japan) was inserted into the rectum to a constant depth of 6 cm from the anus. After 37–43 min of acclimation to the box and the conditions, the microinjection was started. The stylet in the guide cannula was removed and replaced with the injection cannula, which was connected by polyethylene tubing to a microsyringe. Then, PG dissolved in pyrogen-free physiological saline, or the saline alone, was injected in a volume of 3 μl unilaterally into the preoptic area within 3 min. In the same room described above, with the rats partially restrained in the boxes, behavior was monitored through a video recording system, and EEG, EMG, and the rectal temperature were recorded simultaneously for 90 min after the beginning of the injection. The injection cannula was not removed until the end of the experimental session. The periods of wakefulness, SWS, and PS were determined with the criteria used by Honda et al.[10]. The minimal scoring interval was 12 s of recording time. After the experi-

mentation, the accuracy of the injection site and the absence of any overt abnormalities of the brain tissue except the injuries made by the insertions of cannulae, were histologically verified. PG's were supplied by Ono Pharmaceutical Company.

RESULTS

In the control group, saline injections induced no alteration in behavior, rectal temperature, or sleep–wake activity (Fig. 1A). PGD_2 at a dose of 2.5×10^{-9} mol also had no influence on behavior or sleep–wake activity, but did slightly elevate the rectal temperature during the recording time. The difference between the mean rectal temperature for over 90 min after PGD_2 application and that after saline control

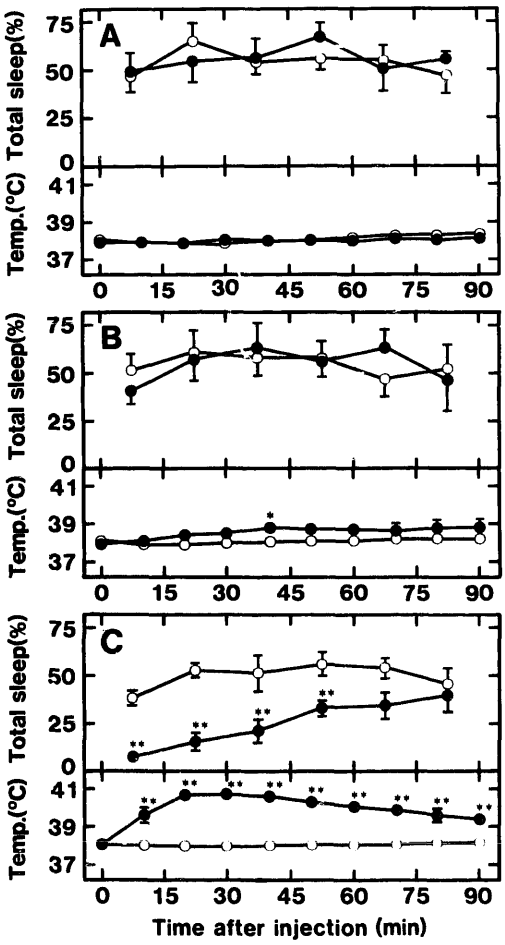

Fig. 1. Effects of PGD_2 and PGE_2 on the sleep–wake activity and rectal temperature. Individual points are means (± S.E.M.). Vertical bars represent the S.E.M.; where absent, the S.E.M. is smaller than the symbol. The time of total sleep (sum of slow wave sleep and paradoxical sleep) is expressed as a percentage of each 15-min recording time. A: rats (n = 6) received two saline microinjections (open and closed circles) at 48-h intervals. B: rats (n = 5) received saline control injection (open circles) and injection of PGD_2 at a dose of 2.5×10^{-9} mol (closed circles) instead of the second saline injection. C: rats (n = 6) received saline control injection (open circles) and injection of PGE_2 at a dose of 2.5×10^{-9} mol (closed circles). $^*P < 0.05$, $^{**}P < 0.01$ by paired t-test.

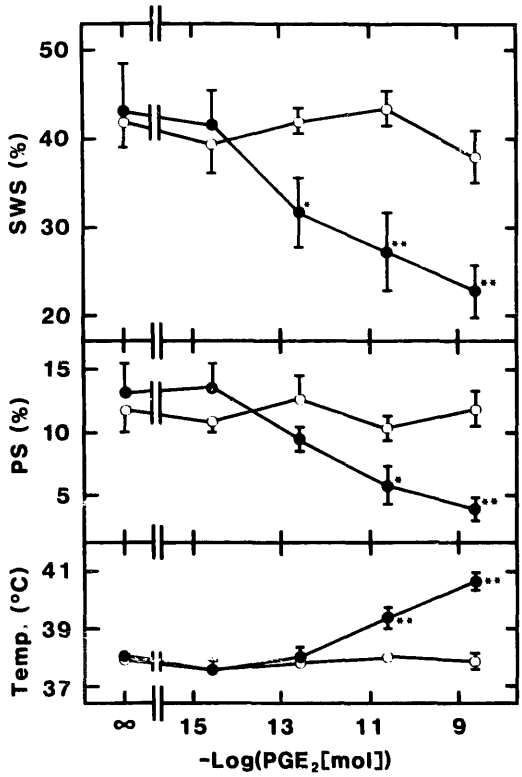

Fig. 2. Dose–response effect of PGE_2 microinjected into the preoptic area of rats on slow wave sleep (SWS), paradoxical sleep (PS) and rectal temperature (Temp.). Individual points are means (± S.E.M.). Vertical bars represent the S.E.M.; where absent, the S.E.M. is smaller than the symbol. SWS and PS are expressed as percentages of the recording time of 90 min after the microinjections. The magnitude of the effect of PGE_2 on rectal temperature is represented by the mean values at 30 min after microinjection, when the rise in the rectal temperature from the paired saline control reached the maximum. A similar dose–response curve was obtained by representing the magnitude of the effect using the mean temperature for over a 90-min period after microinjection. The doses of PGE_2 microinjected were 2.5×10^{-15} (n = 4), 2.5×10^{-13} (n = 5), 2.5×10^{-11} (n = 5), 2.5×10^{-9} (n = 6) mol. ∞ represents the saline control group (n = 6). Rats in the saline control group received two saline microinjections (open and closed circles) at 48-h intervals. Rats in an experimental group received saline control injection (open circles) and a dose of PGE_2 (closed circles) instead of the second saline injection. The results are compared within each group by paired t-test. $^*P < 0.05$, $^{**}P < 0.01$.

injection was 0.5 ± 0.1 °C (mean ± S.E.M., $P < 0.01$) (Fig. 1B).

On the other hand, PGE_2 dose-dependently reduced the amounts of both SWS and PS as shown in Fig. 2. PGE_2 at a dose of 2.5×10^{-9} mol induced marked alteration in the behavior, rectal temperature, EMG activity, and sleep–wake activity. The behavioral movement of rats was stimulated during the recording period, in contrast with the behavior of saline- or PGD_2-injected rats, which slept silently and did not move so vigorously even when they were awake. As for EMG recordings, a remarkable increase in the amplitude was observed within 20 min after the injection. The rectal temperature increased markedly also within 20 min, attained the maximum elevation from the paired control value (2.7 ± 0.2 °C; mean ± S.E.M.) at 30 min after injection, and returned gradually to the initial level thereafter (Fig. 1C). Total sleep (TS; sum of SWS and PS) during the 90-min recording period was significantly reduced by this dose of PGE_2 (25.4 ± 3.9 for PGE_2 vs 49.6 ± 4.2 for saline; mean ± S.E.M. % of recording time, $P < 0.01$). TS time in the experiment was 51% of that in the control. The reduction in TS was due to reductions in both SWS (22.4 ± 3.0 for PGE_2 vs 37.9 ± 3.2 for saline; mean ± S.E.M. % of recording time, $P < 0.01$) and PS (2.9 ± 1.0 for PGE_2 vs 11.8 ± 1.4 for saline; mean ± S.E.M. % of recording time, $P < 0.01$). Time occupied by SWS and that by PS in rats injected with PGE_2 at a dose of 2.5×10^{-9} mol were 59% and 25% of those in the control, respectively (Fig. 2). As shown in Fig. 1C, TS time in every 15-min recording period was reduced during the first 60 min of the recording period, and the reduction was observed to last after the rectal temperature had passed its maximum. The action of PGE_2 on the SWS and PS episodes was, therefore, examined in this period of 60 min and is shown in Table I. The significant reduction in total time of SWS during this period was due to the shortening of SWS episodes, whereas the number of episodes was not altered. A significant reduction in total time of PS was also observed, which was due to both the decreased number and the shortening of PS episodes. Duration of PS episode was subjected to the Cochran–Cox test instead of the paired *t*-test, since PS did not appear in 4 of 6 rats of this group. The effects of PGE_2 observed in this experiment were all transient. By saline injection 48 h after PGE_2 application, no change from the saline control was observed in the behavior, rectal temperature, EMG activity, and sleep–wake activity. Therefore, PGE_2 injection may neither cause irreversible damage nor have long-term influence on these parameters.

At a dose of 2.5×10^{-11} mol, PGE_2 elicited similar effects but with less magnitude than those observed at 2.5×10^{-9} mol on the behavior, rectal temperature, EMG activity, and sleep–wake activity. The maximum elevation of rectal temperature from the paired control value (1.3 ± 0.2 °C; mean ± S.E.M.) was almost half of that by 2.5×10^{-9} mol (Fig. 2). TS in the 90-min recording period was significantly reduced (31.6 ± 5.8 for PGE_2 vs 53.5 ± 2.2 for saline; mean ± S.E.M. % of recording time, $P < 0.01$). TS time in the experiment was 59% of that in the control, which was accounted for by the reduction in SWS (26.8 ± 4.7 for PGE_2 vs 43.7 ± 2.0 for saline; mean ± S.E.M. % of recording time, $P < 0.01$) and

TABLE I

Effect of PGE_2 on SWS and PS episodes

Each value represents mean duration or number of episodes (± S.E.M.) during the post-injection period of 60 min.

Treatment	*n*	*Slow wave sleep*			*Paradoxical sleep*		
		Total time (s)	*Duration of episode (s)*	*Number of episodes*	*Total time (s)*	*Duration of episode (s)*	*Number of episodes*
PGE_2 (2.5×10^{-9} mol)	6	660 ± 71**	115 ± 14**	7.0 ± 0.6	45 ± 31**	44 ± 3†	0.7 ± 0.5**
Paired control	6	1386 ± 119	260 ± 27	7.1 ± 0.3	396 ± 43	114 ± 10	3.3 ± 0.5
PGE_2 (2.5×10^{-11} mol)	5	849 ± 145**	161 ± 28**	6.4 ± 0.4	106 ± 38*	95 ± 21	1.0 ± 0.3*
Paired control	5	1662 ± 96	284 ± 22	7.4 ± 0.5	314 ± 46	109 ± 10	2.6 ± 0.3

*$P < 0.05$, **$P < 0.01$ by paired *t*-test. †$P < 0.01$ by Cochran–Cox test. This test was used because of the absence of PS episodes in 4 out of 6 rats.

that in PS (4.9 ± 1.3 for PGE_2 vs 9.8 ± 1.1 for saline; mean ± S.E.M. % of recording time, $P < 0.05$). The time of SWS and that of PS in the experiment were 61% and 50% of those in the control, respectively. Similar to the result by 2.5×10^{-9} mol of PGE_2, significant reductions in TS were found in 15-min recording periods during the first 60 min. The reduction in the total time of SWS during this period was due to the shortening of duration of SWS episodes, whereas the number of episodes was not altered. The reduction in the total time of PS during this period was due to the reduction in the number of PS episodes; and in one of five rats of this group, PS did not appear. Dissimilar to the result by 2.5×10^{-9} mol, episode duration of PS, as analyzed by the Cochran–Cox test, was not changed at 2.5×10^{-11} mol of PGE_2 (Table I).

At a dose of 2.5×10^{-13} mol of PGE_2, a significant reduction in TS was observed during the 90-min recording period (40.5 ± 4.0 for PGE_2 vs 54.4 ± 1.7 for saline; mean ± S.E.M. % of recording time, $P < 0.05$). TS time in the experiment was 74% of that in the control. The reduction in SWS (31.4 ± 3.9 for PGE_2 vs 41.9 ± 1.4 for saline; mean ± S.E.M. % of recording time, $P < 0.05$) was significant, but that in PS (9.1 ± 1.1 for PGE_2 vs 12.5 ± 2.0 for saline; mean ± S.E.M. % of recording time) was not significant (Fig. 2). The time of SWS and that of PS in the experiment were 75% and 73% of those in the control, respectively. The sleep reduction was accompanied by slightly stimulated behavioral movement. No remarkable increase in amplitude of EMG recording or elevation of rectal temperature was observed.

Microinjection of PGE_2 at a dose of 2.5×10^{-15} mol did not affect the behavior, rectal temperature, EMG activity, or the sleep–wake activity (Fig. 2).

DISCUSSION

The results of the present study demonstrated that microinjections of PGE_2 at doses between 2.5×10^{-13} and 2.5×10^{-9} mol reduced the amount of SWS and PS and that the degree of the reduction was dependent upon the amount of PGE_2 injected. The sleep reduction by PGE_2 was mainly due to the shortening of SWS episodes and the decrease in the occurrence of PS episodes, suggesting that PGE_2 does not disturb the initiation but interrupts the maintenance of SWS, which in turn may lead to the less frequent occurrence of PS episodes. It was reported in rats that intracisternal injection of PGE_1 at the dose almost equivalent to 140 nmol/kg decreased the amount of PS[17], while the lateral ventricular application of PGE_1 at almost 280 nmol/kg reduced the amount of SWS without significant reduction of PS[3]. In our experiment, we used PGE_2 instead of PGE_1 and found that PGE_2 injected into the preoptic area at much lower doses effectively decreased the amounts of both SWS and PS. Although there exist structural differences between PGE_2 and PGE_1, our results demonstrated that the preoptic area, a center of sleep regulation, may be one of the sites of PGE_2 action on the sleep–wake activity. This is further supported by the recent observation that the preoptic area is enriched with a binding protein or receptor of PGE_2 (ref. 36).

On the other hand, PGD_2, a chemical isomer of PGE_2, was ineffective in inducing any alterations in the sleep–wake activities by diurnal injection into the preoptic area under the experimental conditions described in this paper. In the previous report of our group, the microinjection of PGD_2 into the preoptic area of rats at doses of 0.3–5 nmol induced sleep under the experimental conditions which made control rats remain awake during the period recorded[34]. It was further reported that the continuous infusion of PGD_2 during the night into the third cerebral ventricle of freely moving rats increased the amount of sleep; the infusion of PGD_2 during the night, i.e. during the period when rats spent time chiefly in being awake, increased the amount of sleep[11,33]. However, the similar infusion of PGD_2 during the daytime, i.e. during the period when rats spent time chiefly in being asleep, failed to increase the amount of sleep above the control level[12]. In addition, other endogenous sleep substances such as delta-sleep-inducing peptide and uridine also failed to increase 'diurnal' sleep[12], in contrast with their sleep-promoting effects noted following 'nocturnal' infusion[11]. These data, including our results suggest that the effect of PGD_2 on sleep may be altered by the circadian time of PGD_2 administration or by the arousal state of rats.

In this present study, PGD_2 slightly increased the rectal temperature of rats under the experimental condition which made control rats remain sedate or asleep during the period recorded. The increase of rectal temperature has been reported by PGD_2 injec-

tion into the cerebral ventricle of urethane-anesthetized rats[6,27]. On the other hand, the previous study of our group reported that PGD_2 microinjected into the brain caused hypothermia under the experimental condition which made control rats remain awake during the period recorded[34]. By the injection into the lateral ventricle of conscious rats using the method of Herman[9], biphasic effect on rat temperature was obtained by PGD_2; PGD_2 at a dose of 20 μg increased temperature, while PGD_2 at a dose of 2 μg decreased it[5]. These results including our data suggest that the effect of PGD_2 on body temperature may be altered by the arousal state or sedate–excited condition of rats as well as by the amount of PGD_2 injected.

Concerning the effect of PGE_2 on body temperature, our results are similar to previously published observations[5,25,28]. Microinjection of PGE_2 at doses of 2.5×10^{-11} and 2.5×10^{-9} mol induced high-voltage recordings of EMG during the first 20 min after microinjection, when the rectal temperature was markedly increasing. The increased muscular tension may be related to the heat production caused by PGE_2, and is probably responsible for the 'shivering' described previously[18].

It is not likely that the change in body temperature caused the sleep reduction for the following reasons: (1) pyrogenic substances such as muramyl peptides[13], interleukin-1 (refs. 14, 32, 35), lipopolysaccharide[15], and lipid A[15] have been shown to be simultaneously somnogenic, or SWS-inducing, (2) the central heating of the preoptic area reportedly elicited a non-REM sleep-like EEG pattern[1,26,31], and (3) PGE_2 at a dose of 2.5×10^{-13} mol decreased the time of sleep without altering the rectal temperature in this experiment. Thus, it is conceivable that the effect of PGE_2 on the sleep–wake activity is independent of its thermal effect.

Sedation, catalepsy, and enhancement of the anesthetic or sleep-inducing effect of hypnotic drugs like pentobarbital were reported to be induced in rats (e.g. refs. 2, 5, 7, 8, 24) as well as in other species by peripherally and intracerebroventricularly administered PGE_1 and E_2. In our experiment, however, increased behavioral movements were observed with sleep reduction caused by PGE_2. The difference between the previous reports and ours may be due to the difference in the experimental procedure; e.g. in some previous studies, PG's were injected into the cerebral ventricle by puncturing the skull shortly before the experiments according to Herman's method[9]. In addition, it has been pointed out that reduction in blood flow to the brain caused by peripheral and intracerebroventricular administrations of PGE's may contribute to the sedation (cf. ref. 38). Therefore, it is possible that the stimulating effect on the behavior may be due to the action of PGE_2 in the preoptic area. It should also be ascertained whether PGE_2 induces sedation at sites outside the preoptic area in the brain. Incidentally, the striatum was reported to be the site responsible for $PGF_{2\alpha}$-induced cataleptic behavior in rats[22].

In this study, the SWS amounts of the control were around 40% of the recording time, and they are relatively low for the diurnal SWS amount of rats. However, it is unlikely that there was such serious damage in the brain as to specifically alter the basal state of the rats in this experiment, for the following reasons: (1) by the stereomicroscopic examination after the experiment, no overt histological damage was observed in the sections of the rat brains, except the injuries caused by the insertion of injection cannulae and guide cannulae; (2) the responsiveness of rectal temperature to the microinjections of PGE_2 at the doses administered in this way was not attenuated, compared with the results in the previously published reports[5,16,25,28,37]. Therefore, it is most likely that the low control amounts of SWS were due to the experimental conditions under which the rats were recorded, i.e. the rats were partially restrained during the period recorded.

As shown in this experiment, the microinjection of extremely low doses of PGE_2 into the preoptic area elicited sleep reduction, which was accompanied by the activated behavioral movements. The sleep reduction was transient and reversible. Furthermore, the increase in the body temperature was concomitantly observed, but it may not cause the sleep reduction. These findings suggest that PGE_2 has a central action to increase wakefulness via its effect on the preoptic area, i.e. a sleep center. Since wakefulness and the concomitant elevation of body temperature are commonly observed in humans and experimental animals during the physiological circadian cycle, it is interesting to further investigate the central role of PGE_2 under physiological conditions.

ACKNOWLEDGEMENTS

We would like to thank Professor Shojiro Inoué of Tokyo Medical and Dental University for useful discussions and suggestions. We also thank Miss Yuriko Shimada for secretarial assistance.

REFERENCES

1 Benedek, G., Obál Jr., F., Szekeres, L. and Obál, F., Cortical synchronization induced by thermal stimulation of the preoptic area in immobilized rats, *Acta Physiol. Acad. Sci. Hung.*, 48 (1976) 65–72.
2 Bodzenta, A. and Wiśniewski, K., The effect of prostaglandin E_1 on central cholinergic mechanisms, *Pharmacology*, 15 (1977) 143–151.
3 Dzoljic, M.R., Prostaglandins and sleep. Awaking effect of prostaglandins and sleep pattern of essential fatty acids deficient (EFAD) rats, *Prostaglandins*, 15 (1978) 317–324.
4 Feldberg, W. and Saxena, P.N., Further studies on prostaglandin E_1 fever in cats, *J. Physiol. (London)*, 219 (1971) 739–745.
5 Förstermann, U., Heldt, R. and Hertting, G., Effects of intracerebroventricular administration of prostaglandin D_2 on behaviour, blood pressure and body temperature as compared to prostaglandins E_2 and $F_{2\alpha}$, *Psychopharmacology*, 80 (1983) 365–370.
6 Förstermann, U., Heldt, R. and Hertting, G., Studies on the mechanism of central cardiovascular and temperature responses to prostaglandin D_2, *Prostaglandins Leukot. Med.*, 18 (1985) 301–308.
7 Gilmore, D.P. and Shaikh, A.A., The effect of prostaglandin E_2 in inducing sedation in the rat, *Prostaglandins*, 2 (1972) 143–149.
8 Haubrich, D.R., Perez-Cruet, J. and Reid, W.D., Prostaglandin E_1 causes sedation and increases 5-hydroxytryptamine turnover in rat brain, *Br. J. Pharmacol.*, 48 (1973) 80–87.
9 Herman, Z.S., The effects of noradrenaline on rat's behaviour, *Psychopharmacologia (Berl.)*, 16 (1970) 369–374.
10 Honda, K., Komoda, Y., Nishida, S., Nagasaki, H., Higashi, A., Uchizono, K. and Inoué, S., Uridine as an active component of sleep-promoting substance: its effects on nocturnal sleep in rats, *Neurosci. Res.*, 1 (1984) 243–252.
11 Inoué, S., Honda, K., Komoda, Y., Uchizono, K., Ueno, R. and Hayaishi, O., Differential sleep-promoting effects of five sleep substances nocturnally infused in unrestrained rats, *Proc. Natl. Acad. Sci. U.S.A.*, 81 (1984) 6240–6244.
12 Inoué, S., Honda, K., Komoda, Y., Uchizono, K., Ueno, R. and Hayaishi, O., Little sleep-promoting effect of three sleep substances diurnally infused in unrestrained rats, *Neurosci. Lett.*, 49 (1984) 207–211.
13 Krueger, J.M., Pappenheimer, J.R. and Karnovsky, M.L., Sleep-promoting effects of muramyl peptides, *Proc. Natl. Acad. Sci. U.S.A.*, 79 (1982) 6102–6106.
14 Krueger, J.M., Walter, J., Dinarello, C.A., Wolff, S.M. and Chedid, L., Sleep-promoting effects of endogenous pyrogen (interleukin-1), *Am. J. Physiol.*, 246 (1984) R994–999.
15 Krueger, J.M., Kubillus, S., Shoham, S. and Davenne, D., Enhancement of slow-wave sleep by endotoxin and lipid A, *Am. J. Physiol.*, 251 (1986) R591–R597.
16 Lipton, J.M., Welch, J.P. and Clark, W.G., Changes in body temperature produced by injecting prostaglandin E_1, EGTA and bacterial endotoxins into the PO/AH region and the medulla oblongata of the rat, *Experientia (Basel)*, 29 (1973) 806–808.
17 Mašek, K., Kadlecová, O. and Pöschlová, N., Effect of intracisternal administration of prostaglandin E_1 on waking and sleep in the rat, *Neuropharmacology*, 15 (1976) 491–494.
18 Milton, A.S. and Wendlandt, S., Effects on body temperature of prostaglandins of the A, E and F series on injection into the third ventricle of unanaesthetized cats and rabbits, *J. Physiol. (London)*, 218 (1971) 325–336.
19 Nauta, W.J.H., Hypothalamic regulation of sleep in rats. An experimental study, *J. Neurophysiol.*, 9 (1946) 285–316.
20 Nisticò, G. and Marley, E., Central effects of prostaglandin E_1 in adult fowls, *Neuropharmacology*, 12 (1973) 1009–1016.
21 Nisticò, G. and Marley, E., Central effects of prostaglandins E_2, A_1 and $F_{2\alpha}$ in adult fowls, *Neuropharmacology*, 15 (1976) 737–741.
22 Ono, N., Saito, R., Abiru, T., Kamiya, H. and Furukawa, T., Possible involvement of prostaglandins in cataleptic behavior in rats, *Pharmacol. Biochem. Behav.*, 25 (1986) 463–467.
23 Paxinos, G. and Watson, C., *The Rat Brain in Stereotaxic Coordinates*, Academic, 1982.
24 Poddubiuk, Z.M., A comparison of the central actions of prostaglandins A_1, E_1, E_2, $F_{1\alpha}$, and $F_{2\alpha}$ in the rat, *Psychopharmacology*, 50 (1976) 89–94.
25 Potts, W.J. and East, P.F., Effects of prostaglandin E_2 on the body temperature of conscious rats and cats, *Arch. Int. Pharmacodyn.*, 197 (1972) 31–36.
26 Roberts, W.W. and Robinson, T.C.L., Relaxation and sleep induced by warming of preoptic region and anterior hypothalamus in cats, *Exp. Neurol.*, 25 (1969) 282–294.
27 Sirén, A., Central cardiovascular and thermal effects of prostaglandin D_2 in rats, *Prostaglandins Leukot. Med.*, 8 (1982) 349–359.
28 Spławiński, J.A., Górka, Z., Zacny, E. and Wojtaszek, B., Hyperthermic effects of arachidonic acid, prostaglandins E_2 and $F_{2\alpha}$ in rats, *Pflügers Arch.*, 374 (1978) 15–21.
29 Sterman, M.B. and Clemente, C.D., Forebrain inhibitory mechanisms: sleep patterns induced by basal forebrain stimulation in the behaving cat, *Exp. Neurol.*, 6 (1962) 103–117.
30 Stitt, J.T., Prostaglandin E_1 fever induced in rabbits, *J. Physiol. (London)*, 232 (1973) 163–179.
31 ten Cate, J., Horsten, G.P.M. and Koopman, L.J., The influence of the body temperature on the EEG of the rat, *EEG Clin. Neurophysiol.*, 1 (1949) 231–235.
32 Tobler, I., Borbély, A.A., Schwyzer, M. and Fontana, A., Interleukin-1 derived from astrocytes enhances slow wave activity in sleep EEG of the rat, *Eur. J. Pharmacol.*, 104 (1984) 191–192.
33 Ueno, R., Honda, K., Inoué, S. and Hayaishi, O., Prosta-

glandin D_2, a cerebral sleep-inducing substance in rats, *Proc. Natl. Acad. Sci. U.S.A.*, 80 (1983) 1735–1737.

34 Ueno, R., Ishikawa, Y., Nakayama, T. and Hayaishi, O., Prostaglandin D_2 induces sleep when microinjected into the preoptic area of conscious rats, *Biochem. Biophys. Res. Commun.*, 109 (1982) 576–582.

35 Walter, J., Davenne, D., Shoham, S., Dinarello, C.A. and Krueger, J.M., Brain temperature changes coupled to sleep states persist during interleukin 1-enhanced sleep, *Am. J. Physiol.*, 250 (1986) R96–103.

36 Watanabe, Y., Watanabe, Y. and Hayaishi, O., Prostaglandin bindings in the central nervous system. In O. Hayaishi and K. Torizuka (Eds.), *Biomedical Imaging*, Academic, N.Y., Tokyo, 1986, pp. 227–238.

37 Williams, J.W., Rudy, T.A., Yaksh, T.L. and Viswanathan, C.T., An extensive exploration of the rat brain for sites mediating prostaglandin-induced hyperthermia, *Brain Research*, 120 (1977) 251–262.

38 Wolfe, L.S., Eicosanoids: prostaglandins, thromboxanes, leukotrienes, and other derivatives of carbon-20 unsaturated fatty acids, *J. Neurochem.*, 38 (1982) 1–14.