Vol. 109, No. 2, 1982 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
November 30, 1982 Pages 576-582

PROSTAGLANDIN D_2 INDUCES SLEEP WHEN MICROINJECTED INTO THE PREOPTIC AREA OF CONSCIOUS RATS

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Received October 20, 1982

Prostaglandin D_2, when microinjected into the preoptic area of conscious rats, increased the amount of slow wave sleep dose-dependently from 0.3 to 1.25 nmol/rat. The amount of slow wave sleep induced by the injection of 2.5 nmol prostaglandin D_2 increased about six fold as compared with that by the injection of sterile saline in the experimental session of 3 hrs. Injections of prostaglandin D_2 into the posterior hypothalamus had no effect on inducing sleep. Although prostaglandin E_2 and $F_{2\alpha}$ induced the high voltage delta waves on the recordings of electroencephalogram, the effect was much smaller than that of prostaglandin D_2. These results demonstrated that prostaglandin D_2, acting on the preoptic area, may play an important role in inducing sleep as a chemical mediator.

In the central nervous system of rats, prostaglandin (PG) D_2 is identified as the major compound among various endogenous PGs and thromboxanes (1-4). The highest activity of PGD synthetase among various tissues of rats is found in the brain, especially in the hypothalamus (4,5). In the previous report, we demonstrated that the microinjection of PGD_2 into the preoptic area of rat brain caused a fall of the colonic temperature (6). During

Abbreviations used: PG, prostaglandin; EEG, electroencephalogram; LSWS, light slow wave sleep; DSWS, deep slow wave sleep.

0006-291X/82/220576-07$01.00/0

the experiments, we also found that the injection of PGD_2 appeared to induce sleep. In the present study, the sleep inducing effect of PGD_2 is described based on the recordings of EEG, heart rate and colonic temperature.

MATERIALS AND METHODS

Male Wistar rats weighing 380-420 g were used. Under pentobarbital anesthesia (50 mg/kg), the guide tube was implanted to the preoptic area (anterior, A:7.4 mm, lateral, L:1.0 mm, horizontal, H:-0.5 mm) from the stereotaxic zero point (7) or to the posterior hypothalamus (A:4.8 mm, L:1.5 mm, H:-1.0 mm) of the left half of the brain. The injection cannula was adjusted to protrude 1.0 mm beyond the guide cannula. A skull screw, over the frontal sinus, served as the indifferent electrode. A surface silver ball electrode was placed on the cortex (A:3.0 mm, L:2.0 mm) and depth bipolar stainless steel electrodes in the dorsal hippocampal formation (A:4.0 mm, L:2.0 mm, H:2.0 mm) for recordings of EEG. Cannula and electrodes were cemented to the skull. The animals were permitted to recover from surgery for at least 5 days before experiments. Food and water were available ad libitum except during the experimental session.

In each experimental session, the rat was removed from its home cage at 10 a.m. and placed on an unsteady small platform (8) set in a sound-proof and electrically sealed room maintained at 25 $\pm$ 2°C. The colonic temperature at 7 cm from the anus was measured by means of thermocouples and a recording potentiometer. A needle electrode was inserted into the leg for recording of electrocardiogram. We observed the behavior of the rat through a small window and did not enter the room except during the injection period. PGs (gifts from Ono Central Research Institute) were dissolved in sterile saline and 3 µl of the solution were microinjected in 1 min (6). Animals used once were not used again. After the experiments, pontamine sky blue was microinjected and the sites of the injections were verified histologically. The amount of sleep and heart rates were expressed as mean $\pm$ SD. EEG was recorded (2 cm/sec) and scored in 20 sec segments, each segment being assigned to sleep stages (awake, LSWS, DSWS) that had the longest duration during that period of time. The criteria used for identification of various sleep stages were almost identical with those used by Ursin (9).

RESULTS and DISCUSSION

PGD_2 (2.5 nmol), PGE_2 (2.5 nmol) or saline was microinjected to the preoptic area of the conscious rats. The typical patterns of sleep stage alterations after the injection are illustrated in Fig. 1. While the control rat kept awake in most of the experimental session of 200 min on the small high platform (8), the administration of PGD_2 markedly induced slow wave sleep.

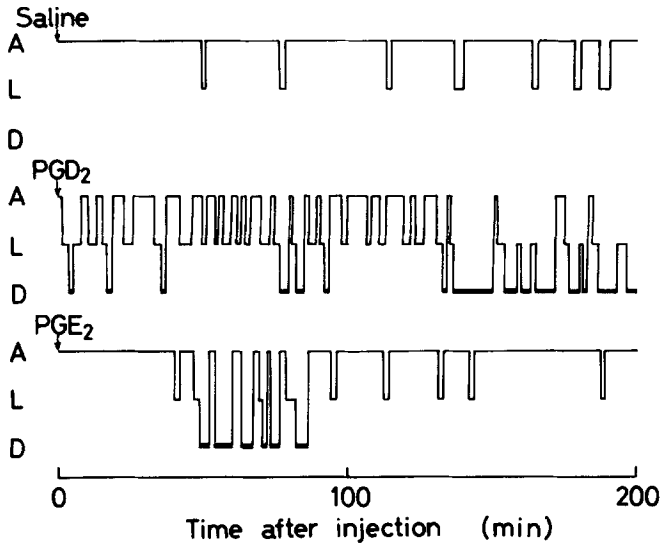

Fig. 1. Sleep stage alterations after the preoptic injections of sterile saline (upper), PGD_2 (middle) and PGE_2 (lower). The amount of PG administered was 2.5 nmol/rat. A, Awake; L, LSWS; and D, DSWS.

Within 1 min after the injection, PGD_2 caused a relaxed body extension of rats with head lowered and eyes closed. Short bursts of high voltage activity (sleep spindle) on EEG developed in 3 min being followed by high voltage 0.5-3 cycles/sec delta waves (DSWS). The effect of PGD_2 on inducing slow wave sleep was

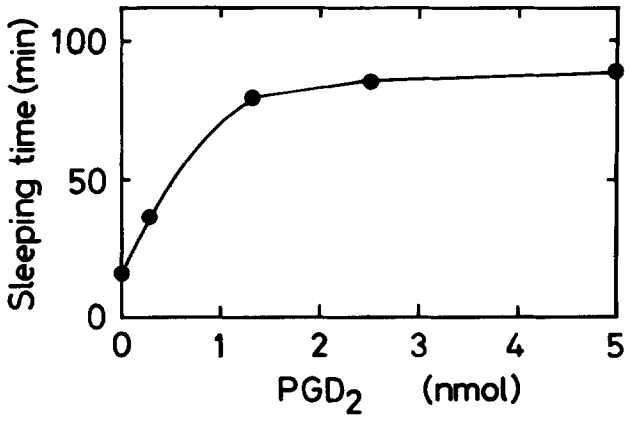

Fig. 2. The dose response curve of PGD_2 on inducing sleep. PGD_2 was microinjected into the preoptic area of conscious rats. The ordinate shows the amount of sleeping time (LSWS + DSWS) during the experimental session of 3 hrs. The volume of the PGD_2 solution injected was 3 µl except the injection of 5 nmol PGD_2 (6 µl).

maintained through the experimental session of 200 min (Fig. 1). The sleep patterns of rats (n = 4) returned to the control level in 220-350 min after the injection of PGD_2.

On the other hand, the rats, injected PGE_2 into the preoptic area, kept awake with curled posture until their colonic temperature attained the maximum. The synchronized patterns of EEG recordings were observed in the limited periods (30-80 min post-injection) when the colonic temperature was maintained 1-2°C above the initial level (see below). The preoptic injection of $PGF_{2\alpha}$ also induced sleep in the limited periods. Hyperthermia synchronizes the cortical EEG (10-12). Thus, it was not certain whether the effect of PGE_2 and $PGF_{2\alpha}$ on inducing delta waves was a direct effect or the secondary one caused by the high body temperature. The dose response curve of PGD_2 on inducing sleep is shown in Fig. 2. The microinjection of PGD_2 into the preoptic area increased the amount of sleep (LSWS + DSWS) dose-dependently from 0.3 to 1.25 nmol/rat in the experimental session of 3 hrs. The injection of 0.3 nmol PGD_2 increased the amount of sleep twice as much as that by control injection of saline. The injection of PGD_2 above 1.25 nmol did not cause further increase in the sleeping time. Injection of PGD_2 (2.5 nmol) made the amount of sleep about 5.7 times longer (86.4 $\pm$ 15.0 min/180 min, n = 4) as compared with the control values (15.1 $\pm$ 6.4 min/180 min, n = 4). Further, Fig. 3 shows that about 50% of the sleeping time is identified as DSWS characterized by delta waves on EEG recordings. Injection of PGD_2 (2.5 nmol) into the posterior hypothalamus caused neither DSWS nor significant increase in the amount of sleep indicating that preoptic area is a site of action of PGD_2 on inducing sleep. These results demonstrated that the sleep inducing effect of PGD_2 was comparable to those of Factor S (13) and delta sleep inducing peptide (14). Although PGE_2 and

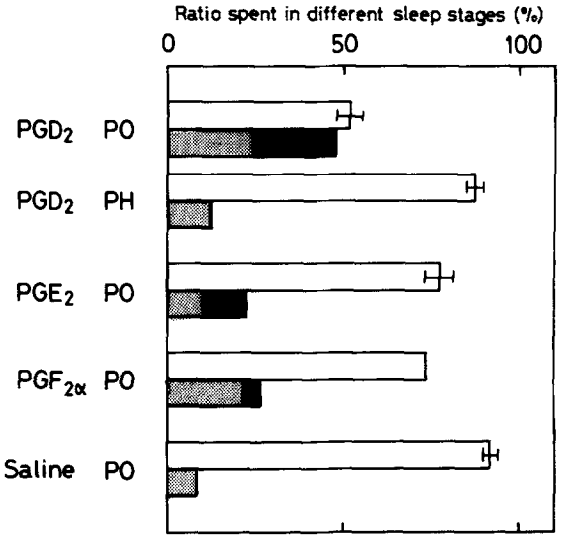

Fig. 3. The ratios spent in different sleep stages after the injection of PGs. PGs (2.5 nmol) were injected into PO, preoptic area and PH, posterior hypothalamus. The ratios were expressed by mean (%) ± SD (n = 4) during the experimental session of 3 hrs. The ratio for $PGF_{2\alpha}$ was the mean (%) of the 2 observations. Open bars, awake; half tone bars, LSWS; and solid bars, DSWS.

$PGF_{2\alpha}$, when injected into the preoptic area, evoked delta waves on the recordings of EEG, the effect was less than half as much as that by PGD_2 (Fig. 3). Paradoxical sleep was not observed throughout these experiments. The lack of the periods of paradoxical sleep is either due to restricted posture on the platform (8) or due to the possibility that the center of paradoxical sleep is located elsewhere (15).

The preoptic injection of PGD_2 caused bradycardia as well as hypothermia (Fig. 4A). About 1 hr after the beginning of the sleep caused by PGD_2, the heart rate decreased by 20% (100 ± 2.9 beats/20 sec, n = 4) from the initial level (126 ± 7.7 beats/20 sec, n = 4) with the fall of the colonic temperature (-0.9°C) and these changes returned to the initial levels with the arousal of the experimental rats. Although PGE_2 is an isomer of PGD_2, the preoptic injection of PGE_2 produced fever as described (16-19) and furthermore it caused tachycardia prior to the rise of the colonic temperature (Fig. 4B). The increase in the heart rate

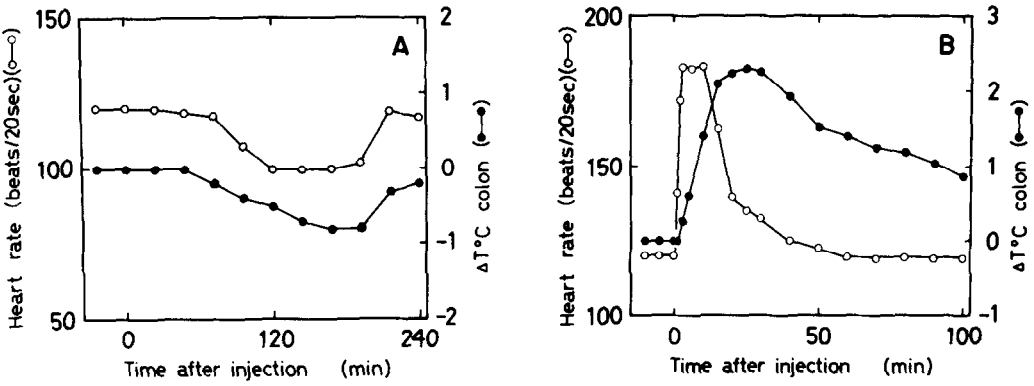

Fig. 4. Effect of PGD_2 and PGE_2 on heart rate and colonic temperature. 2.5 nmol of PGD_2 (A) and PGE_2 (B) were micro-injected to the preoptic area at an ambient temperature of 25 ± 2°C. The heart rate (o) and colonic temperature (●) were measured.

was observed immediately after the injection and within 3 min the heart rate attained its maximal change (183 ± 5.6 beats/ 20 sec, n = 5). The peak of the colonic temperature (+ 2.4°C) was observed when the heart rate had almost returned to the initial level. The injection of PGD_2, PGE_2 or saline into the posterior hypothalamus or that of saline into the preoptic area had little effect on the heart rate. The injection of $PGF_{2\alpha}$ (2.5 nmol) into the preoptic area (n = 2) also increased the heart rate to 170 beats/20 sec prior to the rise in the colonic temperature. These results demonstrated that the preoptic area is again the site of action of PGs on regulating the heart rate of rats.

Judging from the patterns of EEG, the sleep induced by the preoptic injection of PGD_2 was not discriminated from the physiological sleep. The sleep remained episodic and rats were easily aroused by noise. Further, the colonic temperature and heart rate decreased as observed during the physiological slow wave sleep (20-24). PGD_2 is the natural constituent of the brain in various animal species (1,2) and is actively synthesized and metabolized in the hypothalamic regions (4,5,25). From these

results, we propose a hypothesis that PGD_2 plays an important role in inducing slow wave sleep as a chemical mediator.

ACKNOWLEDGMENTS

We acknowledge useful discussions and advices of Dr. T. Shimizu and Dr. S. Narumiya of Kyoto University and Miss K. Imai of Osaka University. This work was supported in part by Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan.

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