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Prostaglandin E_2 Exerts an Awaking Effect in the Posterior Hypothalamus at a Site Distinct from That Mediating Its Febrile Action in the Anterior Hypothalamus

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The precise sites for prostaglandin E_2 (PGE_2)-related activity responsible for the promotion of wakefulness and elevation of brain temperature were determined in several regions of the monkey brain. PGE_2 was administered through a microdialysis probe into 11 brain loci mainly in the preoptic area/anterior hypothalamic region (POA/AH) and the tuberomammillary region in the posterior hypothalamus (TuM-PH). Administration of PGE_2 into the POA/AH resulted in a marked and dose-dependent febrile response. When a low dose (15 pmol/min) of PGE_2 was administered into the POA/AH, brain temperature increased significantly up to a 0.6°C rise, but sleep behavior and amounts of time in wakefulness, slow wave sleep (SWS), and REM sleep during the administration period were not significantly different from those of the control monkeys. Doses of PGE_2 above 150 pmol/min elevated the brain temperature and heart rate more markedly and suppressed sleep. The degree of inhibition of sleep by PGE_2 was closely correlated with the changes in these autonomic responses. On the other hand, when a low dose of PGE_2 was administered into the TuM-PH, the time spent awake during the administration period increased up to 3.5-fold and the amount of time spent in SWS decreased to 50% of that of the control level, with negligible changes in brain temperature and heart rate. The awaking response of PGE_2 in the TuM-PH was also dose dependent, but was not correlated with the change in brain temperature. Among 11 brain regions tested, the hyperthermic effect of PGE_2 was most potent in the POA, while its awaking effect was most pronounced in the TuM-PH close to the mammillary complex. These findings demonstrate that the site of action of PGE_2 for the regulation of sleep-wakefulness is clearly distinct from that for the temperature regulation. PGE_2 may be involved in the neurochemical mechanism of wakefulness mediated in a specific site in the PH.

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Prostaglandins D_2 and E_2 (PGD_2, PGE_2) are the major arachidonic acid metabolites in neurons and glial cells of the mammalian brain (Narumiya et al., 1982; Ogorochi et al., 1984, 1987; Urade et al., 1987; Tsubokura et al., 1991). These PGs play roles in a variety of neurophysiological functions including the regulation of body temperature (Milton, 1976; Ueno et al., 1982), neuroendocrine function (Harms et al., 1973; Kinoshita et al., 1982), and sleep-wakefulness (Dzoijic, 1978; Ueno et al., 1982; Inoué et al., 1984; Krueger, 1985; Matsumura et al., 1988; Onoe et al., 1988). Our previous results revealed that PGD_2, acting as a sleep inducer, and PGE_2, acting as a wakefulness promoter, jointly regulate the generation of sleep and wakefulness in the mammalian brain (Hayaishi, 1988, 1991). The sleep-inducing action of PGD_2 was observed when PGD_2 was microinjected into the preoptic area (POA) (Ueno et al., 1982), in which the PGD_2 receptor(s) was reported to be highly concentrated (Yamashita et al., 1983; Watanabe et al., 1989). Therefore, the site of induction of sleep by PGD_2 was assumed to be located in the POA. However, although numerous studies have been performed to unveil the action of PGE_2 in the CNS, there has been no report that establishes the exact site of PGE_2 for the wakefulness promotion.

The hypothesis by von Economo (1926) that the POA/anterior hypothalamic region (AH) plays a crucial role in the regulation of sleep and that the posterior hypothalamus (PH) does so in the regulation of wakefulness has been confirmed by numerous transection or lesion experiments, and by electrophysiological studies in rats, cats, and monkeys (Ranson, 1939; Nauta, 1946; Sterman and Clemente, 1962; McGinty, 1969; McGinty and Sterman, 1968; Swett and Hobson, 1968; Nakata and Kawamura, 1986; Szymusiak and McGinty, 1989; Szymusiak et al., 1989). Furthermore, a number of neuropharmacological studies have demonstrated that several kinds of neurotransmitters, neurohormones, and sleep substances, including 5-HT, histamine, muramyl dipeptide, and PGD_2, are involved in the regulation of sleep and wakefulness in these regions (Ueno et al., 1982; García-Arrarás and Pappenheimer, 1983; Schwartz et al., 1986; Denoyer et al., 1989). The POA has also been considered to be a center of regulation of body temperature since thermosensitive neurons are highly concentrated in the medial portion of this region (Reaves and Hayward, 1979; Boulant and Dean, 1986). These thermosensitive neurons, especially the cold-sensitive ones, have been shown to mediate the febrile response to PGE_2 induced by exogenous and endogenous pyrogens such as lipopolysaccharide and cytokines (Dinarello et al., 1988). Recent studies from our laboratory showed that PGE_2 promoted wakefulness when it was administered into the POA or third

ventricle of the rat (Matsumura et al., 1988, 1989a) and suppressed sleep when it was infused into the lateral ventricle of the monkey (Onoe et al., 1988). In both cases, an intense febrile response occurred concomitantly with the PGE_2 administration. Changes in thermoregulatory mechanisms have been reported to affect the regulation of slow wave sleep (SWS) and of REM sleep in several mammalian species (Parmeggiani, 1977; McGinty and Szymusiak, 1990). However, the relationship between induction and/or maintenance of wakefulness and the regulation of body temperature by PGE_2 has not been described in detail.

Recent *in vitro* autoradiographic studies carried out in our laboratory (Watanabe et al., 1988; Matsumura et al., 1990) on ^{3}H-PGE_2 binding sites revealed a high density of ^{3}H-PGE_2 binding sites, presumably PGE_2 receptors, in the anterior wall of the third ventricle surrounding the organum vasculosum laminae terminalis, the median/medial POA, dorsomedial hypothalamic nucleus, supramammillary nucleus, and dorso- and ventrolateral parts of the mammillary complex in the rat and monkey. These findings strongly suggest that a site possibly involved in the mediation of the febrile response or the promotion of wakefulness exists in the region of the hypothalamus with high density of ^{3}H-PGE_2 binding sites.

In this study, for the purpose of determining precisely the sites responsible for the febrile and the awaking actions of PGE_2, we administered PGE_2 into various hypothalamic and other brain regions. We found that the POA/AH and the tuberomammillary region in the PH (TuM-PH) were responsible for mediation of, respectively, the febrile and the awaking effects of PGE_2.

Materials and Methods

Although many difficulties lie in experiments using primates, the behavioral and electrophysiological situations of the sleep stages and the spatial resolution of the infusion loci are more informative in these animals than in rodents. We therefore selected the rhesus monkey (*Macaca mulatta*) for the present studies.

Surgical procedures. Four young adult males weighing 6–8 kg were used. The monkeys were anesthetized with pentobarbital (35 mg/kg) following initial sedation with ketamine HCl (5 mg/kg of body weight). Under anesthesia, surgical operations were aseptically performed by a method previously described (Onoe et al., 1988). In brief, for recordings of EEGs, stainless steel screw electrodes were implanted on the dura of the frontal, parietal, and occipital cortices. Similar electrodes were placed across the outer canthi for recording of the electrooculogram (EOG). Stainless steel wire EMG electrodes were inserted subcutaneously into the posterior cervical muscles. For the administration of PGE_2 into various regions in the monkey brain, a microdialysis technique (Tossmann and Ungerstedt, 1986) was used as described below. The position of the ventricular system of the monkey brain was identified by means of x-ray monitoring of Angio-Conray (sodium iothalamate) contrast medium, which was injected via a cannula into the lateral ventricle, by a method previously described (Onoe et al., 1988). Using the obtained x-ray photograph and the stereotaxic atlases of Snider and Lee (1961) and Bleier (1984) for guidance, we then implanted a guide cannula (0.70 mm i.d., 1.10 mm o.d., 30.0 mm in length) into which a microdialysis probe was inserted to a depth of 15.0 mm below the dura matter. The positions of the guide cannula tip necessary for insertion of the probe into the POA/AH and TuM-PH differed from monkey to monkey (POA/AH: A, 21.0 ± 2.0; L, 1.5; TuM-PH: A, 16.0 ± 2.0; L, 1.5). A thermistor, 0.7 mm in outer diameter (Takara Instruments Co., Ltd.), was implanted into the caudate nucleus. The electrodes mentioned above and the thermistor were affixed to the skull with dental acrylic resin and were connected to a polygraph and a data logger (Takara Instruments Co., Ltd., model K722), respectively, with a flexible cable through a slip-ring system as described below. After the surgery, the monkeys were treated with antibiotics and antiinflammatory drugs for 2 weeks.

Experimental protocols. The monkeys were maintained and handled in accordance with recommendations by the U.S. National Institutes of Health and also our institutional guidelines. After a postsurgical recovery period at least 1 month long, during which time the animals were kept in individual cages, each monkey was carefully adapted and acclimated, over a 3 week period prior to the experimental recording, to monkey chairs that allowed free movement of limbs. The room having the monkeys was maintained at 25 ± 1°C and 55 ± 5% humidity on a 12 hr light/12 hr dark cycle. Light was gradually turned on from 7:00 to 8:00 and turned off from 19:00 to 20:00. To prevent mental stress for the monkeys, which might have resulted in a disturbance of circadian sleep patterns, two monkeys were placed in the experimental room. Food was given twice a day (at 9:00 and 17:30), and water was available ad libitum. The behavior of the animals was continuously monitored using a video camera with infrared light sensitivity and recorded using a video system during experiments. For the electrocardiogram (ECG), electrodes, a lead aV_F (augmented voltage of a foot), were introduced noninvasively from a honeycomb copper electrode covering the seat of the monkey chair. Brain temperature was recorded at 15 min intervals over the course of the experimental session.

One day before the experimental period, the subject monkey was slightly anesthetized by intramuscular injection with ketamine HCl (5 mg/kg) and then was seated on the monkey chair in the experimental room. During anesthesia, a microdialysis probe, which had been sterilized with ethylene oxide gas and which was continuously perfused with artificial cerebrospinal fluid (aCSF) (Krebs-Henseleit solution, pH 7.4), was slowly inserted via the guide cannula into various brain regions. The probe was fixed to the guide cannula with the aid of a special attachment and was continuously perfused with aCSF at a rate of 2 μl/min. Since small but significant modifications of sleep behavior were generally observed during the first experimental night with continuous perfusion of aCSF, we tested the effect of either aCSF or a PGE_2 solution on sleep of the animal the following night (the second experimental day). PGE_2 solution was administered continuously for 4 hr (22:30–2:30) in place of aCSF by manual switching of a liquid swivel (Carnegie Medicin AB, CMA/122) 30 min before the beginning and end of the administration period. We calculated the amounts of wakefulness and sleep during the administration period from 22:30 to 2:00 (210 min) because the sleep was disturbed by the entrance of the experimenter at 22:00 and 2:00. On the third experimental day, 2 d after the probe insertion, the microdialysis probe was carefully withdrawn; after the guide cannula had been sealed and covered with a stainless steel cap, the monkey was returned to his own cage without anesthesia. For each animal, the dose–response relationships for PGE_2 in each region were determined with at least a 5 d interval between experiments; the control values were estimated from two separate determinations at the same probe position. Two of the four monkeys (D801 and K802) were further used to examine the effects of PGE_2 in other brain regions.

Administration of PGE_2 by microdialysis. PGE_2 (Ono Central Research Institute, Osaka, Japan) was dissolved in ethanol (1 mg/ml), stored at −20°C until used, freshly redissolved in aCSF following removal of the ethanol under an N_2 gas stream, and then loaded into an air-proof microsyringe (Hamilton Co., Ltd.). Administration of PGE_2 was done by the microdialysis technique. Microdialysis probes (Carnegie Medicin AB, CMA/10) with a dialysis membrane (2 mm in length, 0.45–0.5 mm o.d.) were used with a perfusion rate of 2 μl/min. At this perfusion rate, on the basis of an *in vitro* recovery experiment using ^{3}H-PGE_2 at 37.5°C, 18.69 ± 1.56% (n = 6) of the PGE_2 in the perfusate was calculated to diffuse into the region outside the membrane. The inlet and outlet perfusion tubes of the microdialysis probe, connected to an infusion pump (Carnegie Medicin AB, CMA/100) and a fraction collector (Carnegie Medicin AB, CMA/140), respectively, were supported together with an electric cable by a slip-ring system (Air Precision, Inc.) combined with a liquid swivel (Instech Laboratories, Inc.). Thus, when the monkeys were seated on the chair, body revolutions and limbs movements were not restricted in this system.

Quantitative analyses of the wake and sleep stages of the rhesus monkey. The EEG, EMG, EOG, and ECG were continuously recorded with a polygraph at a paper speed of 15 mm/sec. Based upon these polygraphic features, and upon behavior as visually judged for each 20 sec epoch, we classified behavior by sleep-wake stage according to previously described criteria (Onoe et al., 1988). In brief, A, stage "awake," was characterized by low-voltage, high-frequency EEG activity (16–18 cps), with active EOG and EMG signals; d, stage "drowsy," was characterized by a quiet, sedate manner and exhibition of a typical sleeping animal posture (i.e., a relaxed body posture with eyes closed) and by the presence of burstic high-voltage spindles (of about 100 μV) ranging

from 12 to 18 cps and theta waves on mixed frequency in the EEG; L, stage "light slow wave sleep," was characterized by a high-voltage, low-frequency EEG composed of theta and delta waves, an almost flat EOG, and an EMG of generally low amplitude; D, stage "deep slow wave sleep," was characterized by continuous high-voltage slow waves (0.5–3.0 cps) on EEG with little low-voltage and high-frequency activities; R, stage "REM sleep," was defined polygraphically by the presence of a low-voltage EEG that was a little slower and more rhythmic than that of the awake state and by a completely flat EMG together with occasional eye movements. The analysis of EEG signals was also performed continuously by use of a fast Fourier transform system equipped with a signal processor (NEC San-ei, model 7T18), which helped us to characterize each sleep stage.

Histological examination of the position of the implanted probes. After the last experiment, in order to determine the sites of administration, we perfused 2% pontamine sky blue in 0.5 M sodium acetate at the rate of 2 μl/min for 10 min through the microdialysis probe with the animal in deep pentobarbital anesthesia. The brains were then perfused with 20 mM PBS, pH 7.4, followed by 10% formalin in PBS, through the left ventricle of the heart by means of a rotary infusion pump. The brains were removed and postfixed by immersion in 10% formalin in PBS followed by a cytoprotection step in which they were submerged in 30% sucrose in PBS. They were then frozen and sectioned at a 50 μm thickness, and the sections were stained with cresyl violet.

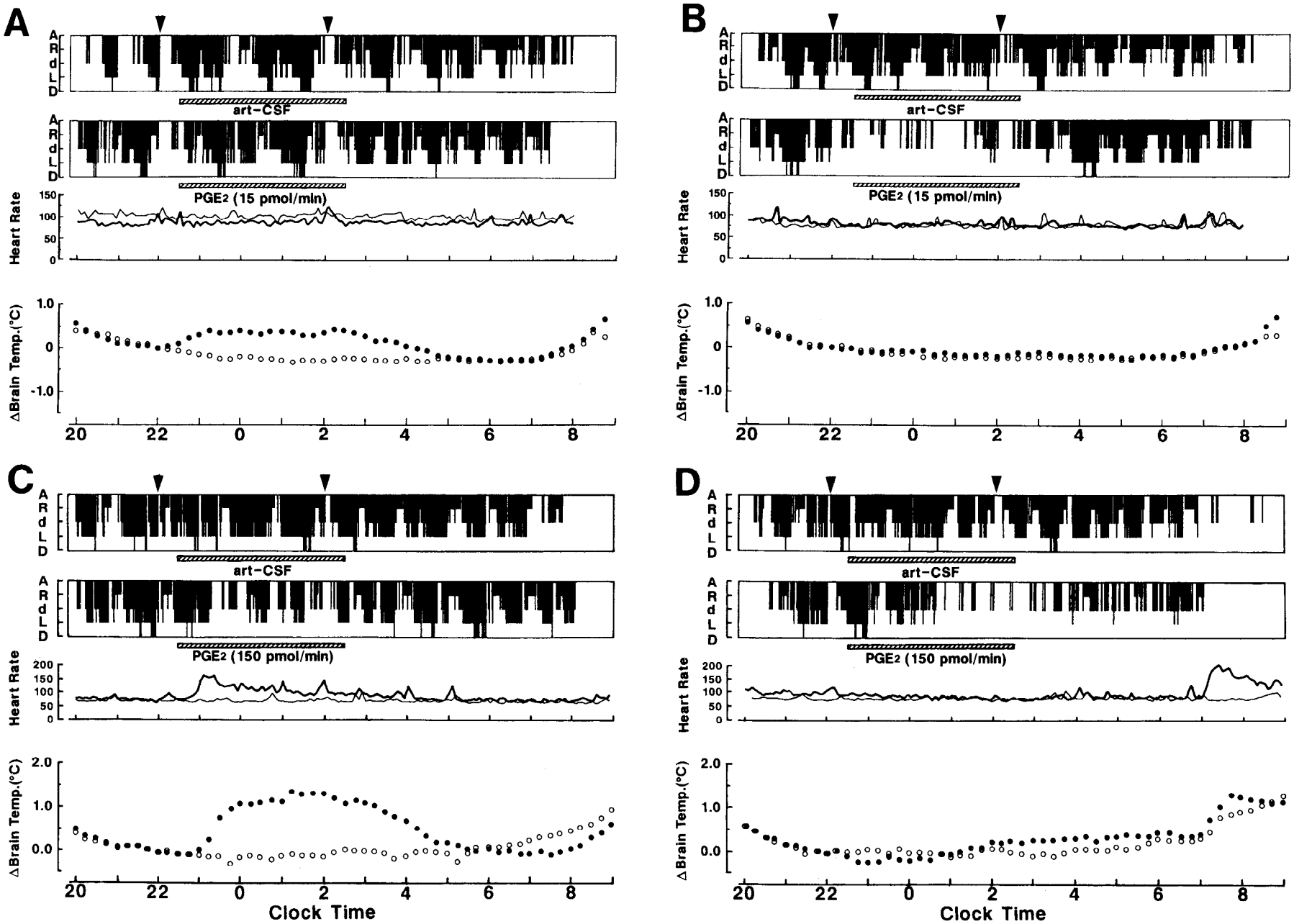
Statistics. All data are expressed as means ± SEM. Results from studies of the dose dependency of PGE_2 in various brain regions were tested by two-way analysis of variance and the Friedman test followed by the estimation of the homogeneity of variance within groups (Bartlett test). The values at each dose of PGE_2 were compared with the value of the aCSF control by Student's t test for paired data. An obtained $p < 0.05$ was taken to indicate statistical significance.

Results

Sleep-wake stage, brain temperature, and heart rate alterations induced by the administration of PGE_2 into the POA/AH or TuM-PH. Typical results of administration of PGE_2 into the POA/AH and TuM-PH for two monkeys (Y901 and K802) are shown in Figure 1. Judging from the results of the histological examination, as described in Materials and Methods, the probes inserted into the POA/AH were found to have been positioned at the junction of the medial and lateral POA, and those inserted into the TuM-PH, in the region of the dorsolateral and ventrolateral parts of the mammillary complex. Unilateral administration of PGE_2 (15 pmol/min) into the POA/AH resulted in a significant increase in brain temperature (approximately 0.4°C above the prelevel), but did not significantly change the amount or pattern of sleep stages, as shown in Figure 1*A*. However, when PGE_2 was administered in a larger dose (150 pmol/min) into the POA/AH, sleep was disturbed by the frequent occurrence of episodes of wakefulness, and the durations of SWS episodes were thus shortened by PGE_2 administration (Fig. 1*C*). In addition, following the administration period, a small increase in SWS, especially in deep SWS, occurred and lasted until dawn; these findings were similar to those of sleep "rebound" (Fig. 1*C*). At this higher PGE_2 level, the heart rate increased maximally, up to twofold, above the control level (about 150 beats/min during PGE_2 treatment, compared with about 70 beats/min in the control study) within the first 30 min of the beginning of PGE_2 administration, and then gradually decreased to the control level. The brain temperature also began to increase 30 min after PGE_2 administration and reached its maximum level (about 1.3°C above the prelevel) within 2 hr. In addition, stretching behavior and autonomic syndrome–related behavior such as shivering and polypnea were observed in association with hyperthermia during the administration period. The brain temperature plateaued during the administration period and decreased to the control level after the conclusion of PGE_2 administration. Marked hyperthermia and hyperrhythmia with slight but significant sleep disturbance were observed in all four monkeys tested at this dose of PGE_2 administered into the POA/AH.

Conversely, the administration of PGE_2 (15 pmol/min) into the TuM-PH, in this case the dorsal region of the tuberomammillary nucleus, resulted in no significant changes in brain temperature or heart rate, while it markedly increased the time of wakefulness (Fig. 1*B*). Within 10 min of the beginning of PGE_2 administration, SWS was eliminated and the monkey exhibited vigilance. The A state observed in the monkey during PGE_2 administration into the TuM-PH was a calm and active waking state with desynchronization, and with eyes kept open and grooming behavior, features similar to those observed in normal awake monkeys. No excited, aggressive, or hyperactive behavior was observed. Notably, 1 hr after the administration was concluded, an increase in the amount of time spent in SWS was observed, which continued until dawn as a "rebound" following the reduction in sleep induced by PGE_2. Observations similar to these were made for each of the other monkeys tested. For example, a monkey in which the probe in the TuM-PH was later found to have been located near the lateral mammillary nucleus awoke in response to the larger dose of PGE_2 (150 pmol/min) with a rather long latency (Fig. 1*D*), probably due to the fact that the distance from this site to the active point was much greater than that from the former site (Fig. 1*B*). In this case, the waking effect of PGE_2 continued even after the end of the administration, and no significant rebound of sleep was observed. In the morning after the experimental session, all monkeys administered PGE_2 into either the POA/AH or TuM-PH region, except for the markedly febrile ones that had been given high doses of PGE_2 into the POA/AH, exhibited normal behavioral responses to food and water.

Dose dependency and time course of effects of PGE_2 on the amount of each sleep stage and on the brain temperature in the POA/AH or TuM-PH. Three different perfusion rates of PGE_2 (15, 150, and 500 pmol/min) were examined with the microdialysis probes implanted into four sites in the POA/AH and TuM-PH, individually. The patterns and amounts of time in the waking state and in each sleep stage of the control monkey with a microdialysis probe implanted into the TuM-PH were similar to those of the normal monkey without implantation of a probe (data not shown). However, the implantation of the probe into the POA/AH slightly increased the amounts of time in awake and drowsy states and decreased the amount of SWS compared with the normal case. PGE_2 administered into the POA/AH increased the brain temperature with as low a dose as 15 pmol/min in a dose-dependent manner. The amounts of time in wakefulness and SWS during the administration period were not changed significantly at the lowest dose of PGE_2 (Fig. 2*A*). However, high doses of PGE_2 increased the amount of time in wakefulness, decreased the amount of SWS, and also resulted in marked hyperthermia of more than 1.0°C above the control level. A significant reduction in the amount of time in REM sleep to approximately 30% of the control level was observed at the highest dose of PGE_2. The time course of changes in hourly amount of wakefulness induced by PGE_2 (Fig. 3*A*) was very similar to that of changes in the brain temperature as shown in Figure 1, *A* and *C*. Small changes in the hourly amount of wakefulness and SWS were observed during the administration period of PGE_2 at 15 pmol/min. When higher doses of PGE_2 were administered into the POA/AH, hourly amounts of wake-


A
B
C
D
ARdLD
art-CSF
PGE2 (15 pmol/min)
PGE2 (150 pmol/min)
Heart Rate
ΔBrain Temp.(°C)
Clock Time
20
22
0
2
4
6
8

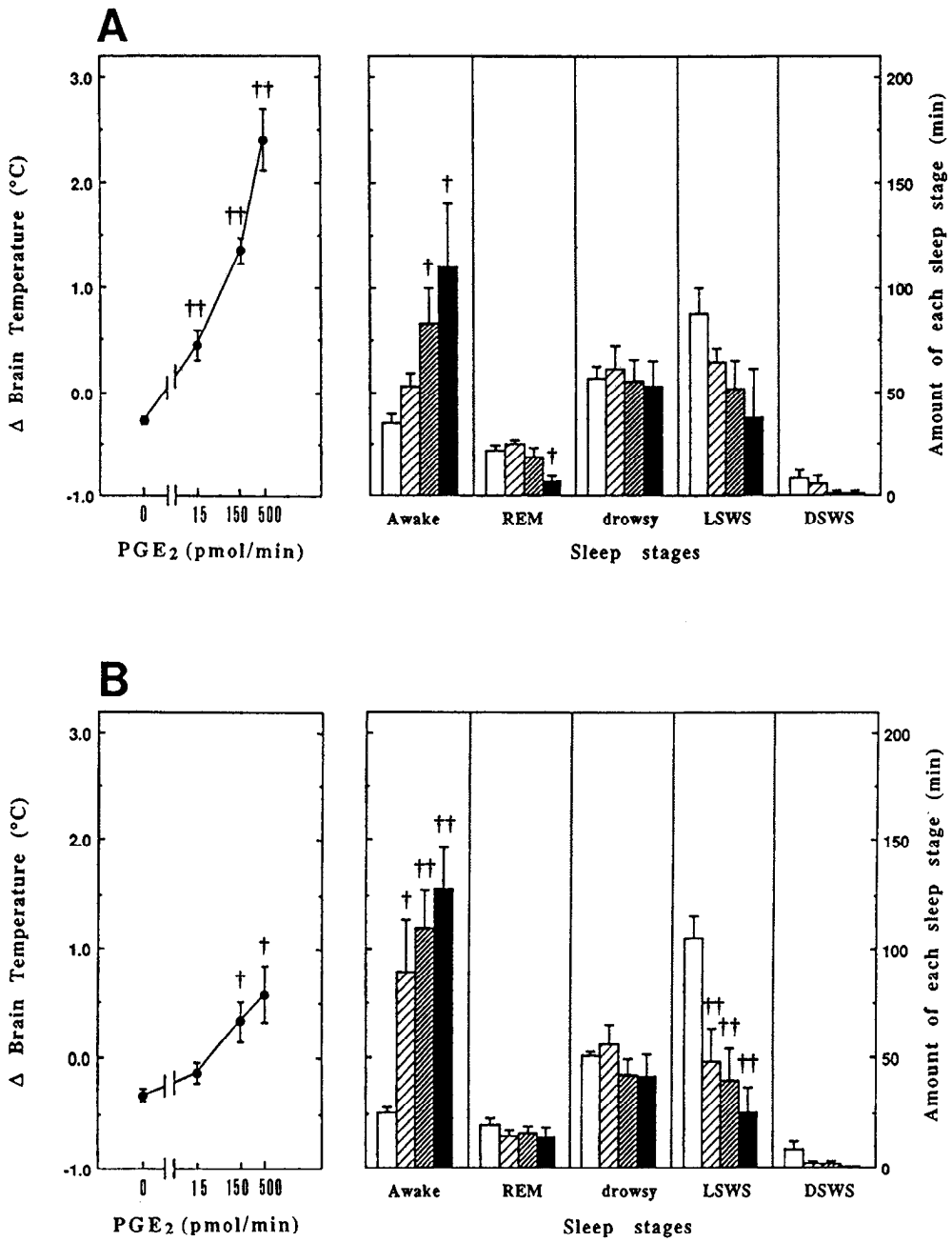


Figure 2. Dose dependency of the effect of PGE_2 on the amounts of wakefulness, each sleep stage, and brain temperature during the perfusion period into the POA/AH (*A*) and TuM-PH (*B*) of monkeys. Data were obtained from the results at four sites in the POA/AH (positions b–e) and in the TuM-PH (positions f–i), as shown in Table 1 and Figure 5. Each value represents the mean ± SEM of four monkeys. Significant differences from the aCSF control (PGE_2 at 0 pmol/min) are indicated with *single daggers* ($p < 0.05$) and *double daggers* ($p < 0.01$) (paired *t* test). *open bars,* aCSF; *light hatched bars,* PGE_2 at 15 pmol/min; *dark hatched bars,* PGE_2 at 150 pmol/min; *solid bars,* PGE_2 at 500 pmol/min.

fulness and SWS increased and decreased, respectively, within 1 hr after the beginning of administration and returned to the control level within 2–3 hr after the end of the administration period. Furthermore, a high dose (500 pmol/min) of PGE_2 reduced the hourly amounts of REM sleep during and after the administration period, while lower doses had no significant effect.

In contrast, only a small (approximately 0.5°C) increase in

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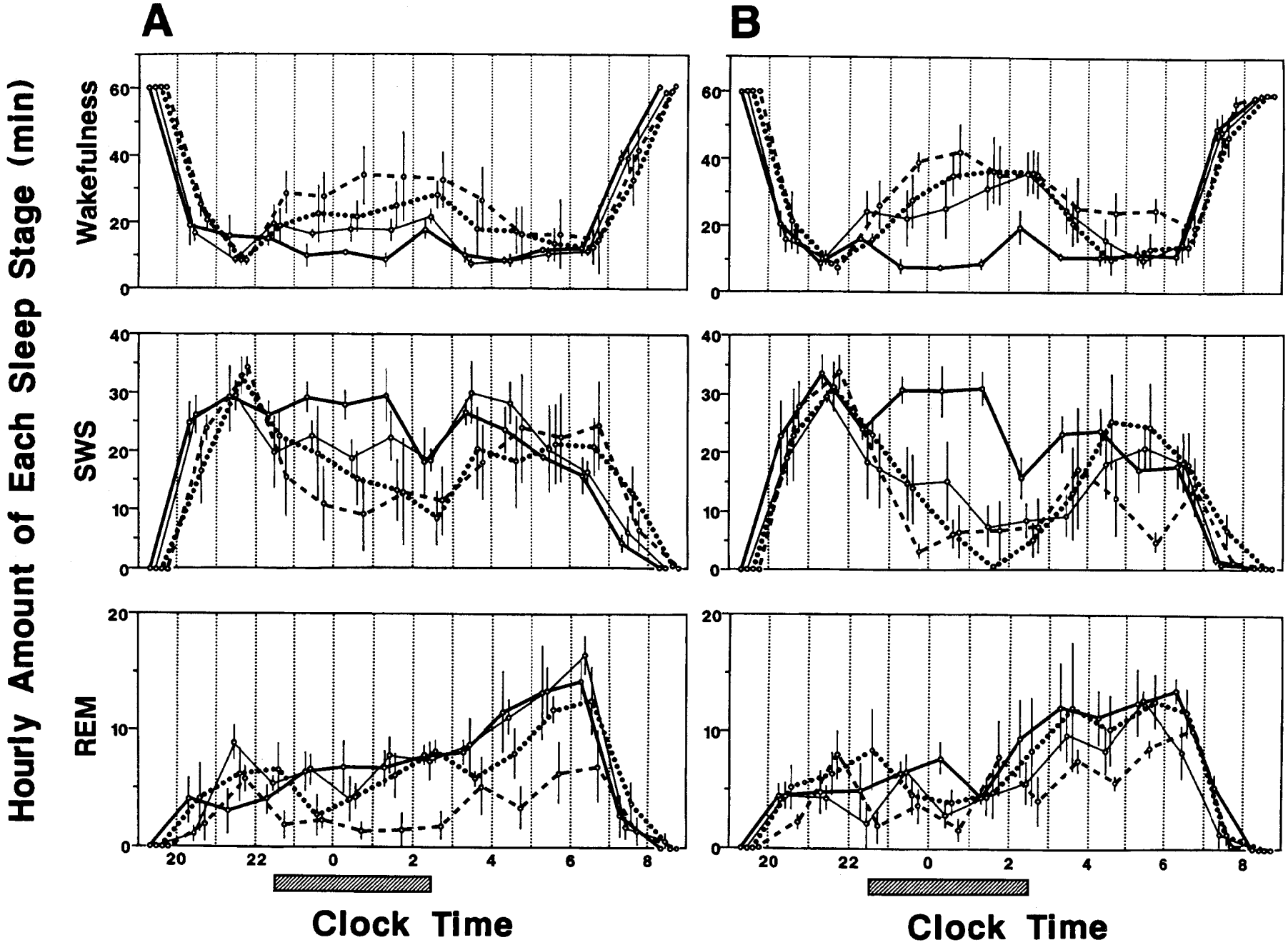
Figure 1. Effects of PGE_2 on sleep, brain temperature, and heart rate in the POA/AH (*A* and *C*) and TuM-PH (*B* and *D*) of two monkeys. *A* and *B,* Administration of PGE_2 at the rate of 15 pmol/min into positions d and f, respectively, of the monkey Y901, as shown in Figure 5. *C* and *D,* Administration of PGE_2 at 150 pmol/min into positions c and h, respectively, of the monkey K802, as shown in Figure 5. *Upper panels* of each figure show hypnograms of the night experimental session for the aCSF control and PGE_2-administered monkey. Sleep-wake stages are classified into *A,* awake; *R,* REM sleep; *d,* drowsy; *L,* light SWS; and *D,* deep SWS. *Hatched bars* in the *upper panels* indicate the administration period (22:30–2:30). *Middle* and *lower panels* indicate the heart rate, which is shown in beats per minute, and the change in the brain temperature with reference to the temperature 30 min before the start of administration (22:00), respectively. The data of the heart rate and brain temperature were obtained at 5 min and 15 min intervals, respectively. *Thin lines* and *open circles,* control sessions; *heavy lines* and *solid circles,* PGE_2 sessions. *Arrowheads* indicate the entrance of the experimenter (H.O.) into the experimental room to change the swivel switch for the beginning (22:00) and ending (2:00) of the administration period. Using the results of the *in vitro* recovery experiments described in Materials and Methods, we calculated that the rate of 15 pmol/min corresponds to approximately 3 pmol (1.05 ng of PGE_2)/min in the tissue just outside the dialysis membrane.


A
B
Hourly Amount of Each Sleep Stage (min)
Wakefulness
SWS
REM
60
40
20
0
40
30
20
10
0
20
10
0
20
22
0
2
4
6
8
Clock Time
Clock Time

the brain temperature was evident even when PGE_2 was administered into the TuM-PH at higher doses (Fig. 2*B*, left), presumably as a consequence of behavioral activation. The amounts of time in wakefulness and SWS during the administration of PGE_2 into the TuM-PH markedly increased and decreased, respectively, in a dose-dependent manner (Fig. 2*B*, right). At a perfusion rate of 15 pmol/min of PGE_2, at which dose PGE_2 failed to change the brain temperature significantly, the amount of wakefulness increased, almost maximally, up to 3.5-fold over that of control, and the amount of SWS was reduced by 50%. Hourly analysis indicated that these effects were significant as early as the second hour after the beginning of administration, and that the maximum effect was reached after approximately 4, 3, and 2 hr of perfusion of PGE_2 at the rates of 15, 150, and 500 pmol/min, respectively (Fig. 3*B*). The quantity of REM sleep was slightly affected by PGE_2 administration, but no significant change in the total amount of REM sleep was observed during the administration period at any of the doses tested compared with that of the control session (Figs. 2*B*, right; 3*B*). However, when the highest dose of PGE_2 was administered, an increase in time awake and decreases in SWS time and REM sleep time were evident until 3 hr after the end of the administration period (Fig. 3*B*).

As shown in Figure 4*A*, the elevation of brain temperature during the PGE_2 administration into the POA/AH was closely related to the shortening effect it had on time in sleep ($r = 0.685$; $p < 0.01$). The awaking index, calculated from the changes in the amounts of time of wakefulness and SWS, increased in parallel with the intensity of fever. In contrast, the intensity of the awaking effect of PGE_2 administered into the TuM-PH (Fig. 4*B*) did not correlate with that of its hyperthermic activity ($r = 0.255$).

Site study of PGE_2 actions responsible for the elevation of brain temperature and promotion of wakefulness. Eleven brain regions, mostly in the hypothalamus, were tested for sensitivity to the awaking and hyperthermic activity of PGE_2, and the results are summarized in Figure 5 and Table 1. The site responsible for the febrile activity of PGE_2 was clearly distinguishable from that responsible for its awaking effect. The latter effect of PGE_2 was most intense in the region of the PH close to the mammillary complex (Fig. 5, Table 1, positions f–h). Administration of PGE_2 into the POA/AH was somewhat effective in increasing the time spent awake, but was less so in the other rostral part of the hypothalamus and in regions caudal to the TuM-PH. On the contrary, the febrile activity induced by PGE_2 was most intense in the region of the POA (Fig. 5, Table 1, positions b–d). PGE_2 was somewhat effective in inducing febrile responses when administered at the level of the dorsomedial hypothalamic nucleus (Fig. 5, Table 1, position e), while slight hyperthermia was observed following administration at the level of the basal forebrain region close to the diagonal band of Broca (Fig. 5, Table 1, position a). Caudally, near the subthalamic nucleus and the substantia nigra, and also in the mesencephalic reticular formation, PGE_2 had far less effect on both febrile and awaking responses (Fig. 5, Table 1, positions j, k). Some individual differences in the sleep pattern, behavior, and amount of time in each sleep stage were observed for the monkeys tested. Such individual differences may also affect the sensitivity to PGE_2 administered into the various brain regions. However, the result from the region nearby in different monkeys (Fig. 5, Table 1, positions c and d, or g and h) exhibited similar febrile and waking responses to various doses of PGE_2. Further, a difference in response to PGE_2 was evident for points as little as 3 mm apart rostrocaudally within the same monkey brain (Fig. 5, Table 1, positions a and b, or h and j). These results indicate that the majority of differences in the febrile and waking responses to PGE_2 observed in individual monkey brain regions reflect differences in the sensitivity to the effects of PGE_2.

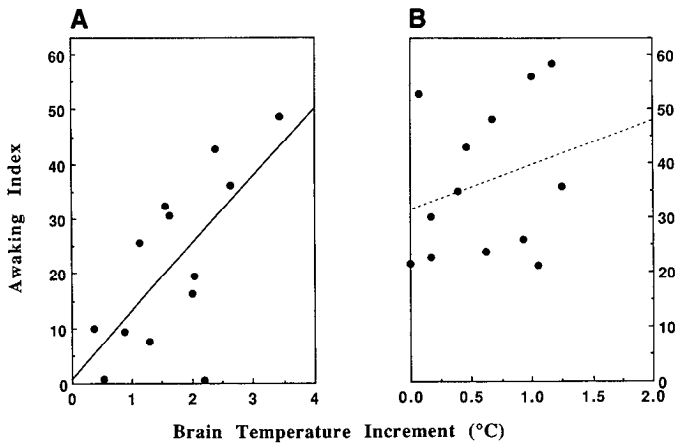


Figure 4. Relationship between the awaking index and the increment of brain temperature produced by PGE_2 administration into the POA/AH (*A*) and TuM-PH (*B*) of monkeys. Awaking index was calculated using the following equation: awaking index (%) = $100(a + b)/2c$, where a = increased amounts of wakefulness (min) from the control value, b = decreased amounts of SWS (min) from the control value, and c = duration of the administration period (210 min). Increased brain temperature is given as the maximal change in the brain temperature during PGE_2 administration from that during the control session. These values were calculated from the data of Table 1. A positive correlation was found only in the POA/AH when the data were subjected to linear regression analysis ($r = 0.685$; $P < 0.01$, $n = 13$).

Discussion

Recently, we showed that the intraventricular infusion of PGD_2 and PGE_2 induced and reduced sleep, respectively, in the monkey (Onoe et al., 1988, 1990). On the basis of EEG, EMG, EOG, other polygraphic features, and body temperature, the sleep induced by PGD_2 was considered indistinguishable from "physiological" sleep. However, the promotion of wakefulness by PGE_2 occurred concomitantly with a significant febrile response. It has been widely accepted that the control of sleep and wakefulness is closely related to the regulation of body temperature (Parmeggiani, 1977; McGinty and Szymusiak, 1990). However, we have made the following findings in the present study: (1) PGE_2 could promote and maintain the wakeful state without generation of hyperthermia when administered into the TuM-

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Figure 3. Time courses of the hourly amounts of wakefulness, SWS, and REM sleep before, during, and after the administration of PGE_2 into the POA/AH (*A*) or TuM-PH (*B*). The values were obtained from the results at four sites in the POA/AH (positions b–e) and in the TuM-PH (positions f–i), as shown in Table 1 and Figure 5. Each *point* represents mean ± SEM of four monkeys. The *hatched bars* represent the administration period of PGE_2 (22:30–2:30). *Thick solid line,* aCSF was continuously administered as a control; *thin solid line,* PGE_2 was administered at the rate of 15 pmol/min; *dotted line,* PGE_2 at 150 pmol/min; *dashed line,* PGE_2 at 500 pmol/min.

Table 1. Effects of various doses of PGE_2 on the amounts of wakefulness, SWS, and REM sleep and on the brain temperature change during the administration period in 11 monkey brain loci tested as shown in Figure 5

Probe position	Monkey	PGE_2 (pmol/min)	Amount of each sleep stage (min)			Awaking index (%)	Δ Brain temp. (°C)
			Awake	TSWS[a]	REM		
a	D801	0	40.7	101.0	15.7	0	−0.65
		150	35.3	72.0	21.3	5.6	+0.11
b	D801	0	41.5	83.3	24.4	0	−0.37
		15	43.7	46.0	27.3	9.4	+0.51
		50	83.0	43.0	23.3	19.5	+2.04
		150	61.7	35.0	31.7	16.3	+1.63
		500	131.0	21.3	0.3	36.2	+2.27
c	K802	0	23.6	127.7	19.0	0	−0.31
		15	72.6	69.0	22.7	25.7	+0.83
		150	87.9	56.4	10.7	32.3	+1.25
		500	100.7	25.7	4.3	42.7	+2.09
d	Y901	0	44.2	75.2	27.9	0	−0.24
		15	47.7	91.0	20.0	0.9	+0.39
		150	128.0	30.3	18.7	30.7	+1.44
		500	176.0	25.3	8.7	48.6	+3.25
e	I703	0	31.1	98.6	15.3	0	−0.21
		15	49.1	95.0	29.8	9.9	+0.16
		150	54.3	90.0	12.9	7.6	+1.10
		500	33.6	108.0	14.3	0.6	+2.00
f	Y901	0	28.3	99.3	26.3	0	−0.24
		15	156.0	5.3	14.3	52.8	−0.17
		150	149.7	18.7	15.0	48.1	+0.43
		500	176.7	2.7	4.0	58.3	+0.93
g	D801	0	21.7	119.3	20.3	0	−0.50
		15	66.3	64.3	11.7	23.8	+0.12
		50	48.0	36.0	29.3	26.1	+0.43
		150	117.0	34.0	25.0	43.0	−0.04
		500	150.0	13.0	20.3	55.9	+0.50
h	K802	0	30.3	107.0	21.9	0	−0.28
		15	87.0	66.4	10.3	22.8	−0.11
		150	108.6	36.4	12.0	35.1	+0.11
		500	88.5	36.6	23.7	30.2	−0.11
i	I703	0	21.7	129.4	11.3	0	−0.25
		15	47.7	65.3	22.6	21.5	−0.30
		150	61.3	80.3	12.0	21.2	+0.80
		500	95.6	52.5	8.0	35.9	+1.00
j	K802	0	28.0	111.6	26.7	0	−0.27
		15	36.0	110.7	15.0	2.1	−0.24
		150	29.6	120.7	29.0	−1.8	−0.22
		500	47.7	83.3	23.3	11.4	−0.10
k	D801	0	26.0	97.0	26.3	0	−0.44
		150	12.7	109.4	41.0	−6.1	−0.45

The control values (PGE_2 at 0 pmol/min) for the POA/AH (b–e) and TuM-PH (f–i) represent the average of two determinations, as described in Materials and Methods.

[a] TSWS, total amounts of SWS (light SWS and deep SWS).

PH, and (2) the site of the febrile effect of PGE_2 is in the POA/AH, being clearly distinct from that mediating promotion of wakefulness.

Several kinds of techniques including microinjection and push-pull perfusion have been used for the administration of neuroactive substances into discrete brain regions. However, a considerable hyperthermia occurs with use of most of these techniques, probably because of mechanical tissue damage and subsequent production of neurochemical and inflammatory factors (reviewed by Cooper, 1987). PGE_2 is known to be released from injured tissues and to produce hyperthermia as part of an inflammatory response (Rudy et al., 1977). Therefore, when testing the effect of PGE_2 administration, it is of paramount importance to employ a technique that minimizes artifactually induced hyperthermia (neurogenic hyperthermia). The microdialysis technique has been developed and used for extended

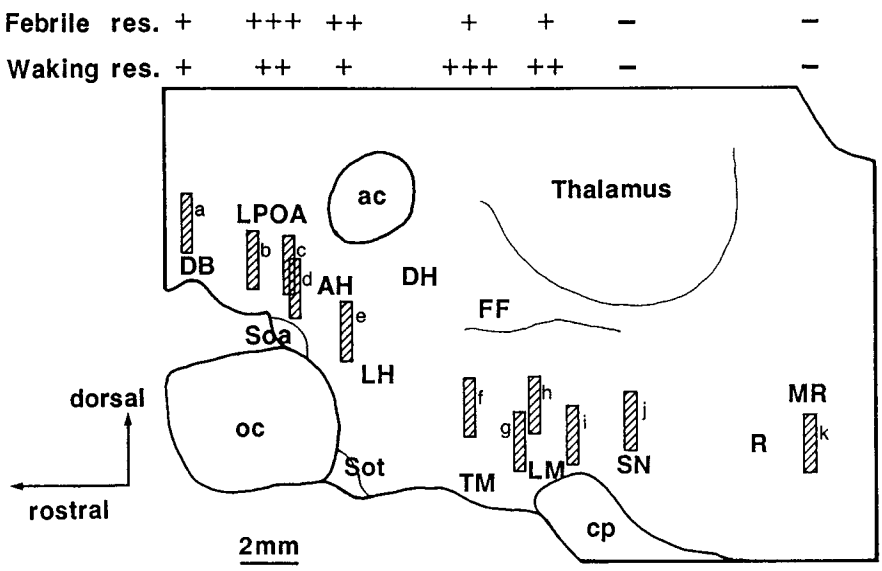

Figure 5. Summary of the febrile and waking responses of PGE_2 in 11 brain loci (*a–k*). Histological examinations were schematically drawn in the sagittal plane of 1.5 mm lateral (2.5 mm in the midbrain part) to the midline of the monkey brain. *Denotements* shown at the *top* represent changes in the brain temperature and awaking index from each control value during the administration of PGE_2 at the rate of 150 pmol/min as follows: −, <0.5°C; +, 0.5–1.0°C; ++, 1.0–1.5°C; +++, >1.5°C for the febrile response; −, <5%; +, 5–20%; ++, 20–40%; +++, >40% for the waking response. *Columns* express the position of implanted probes, and *a–k* next to the columns correspond to those as shown in Table 1. *ac,* anterior commissure; *AH, LH,* and *DH,* anterior, lateral, and dorsal hypothalamic area, respectively; *cp,* cerebral peduncle; *DB,* nucleus of the diagonal band of Broca; *FF,* fields of Forel; *LM,* lateral mammillary nucleus; *LPOA,* lateral preoptic area; *MR,* mesencephalic reticular nucleus; *oc,* optic chiasm; *SN,* substantia nigra; *Soa* and *Sot,* supraoptic nucleus, anterior and tuberal portion, respectively; *TM,* tuberomammillary nucleus; *R,* red nucleus.

monitoring of the dynamics of neurotransmitters in discrete regions of the brain (reviewed by Benveniste, 1989). Recently, Quan and Blatteis (1989) showed that the microdialysis technique may be utilized for the administration of drugs into specific brain loci without induction of neurogenic hyperthermia. This finding is in agreement with one of the results of our present study that no significant change in brain temperature was induced during experimental sessions with continuous perfusion of aCSF (Fig. 1). Using the microdialysis technique, we could accurately assess the relationship between the awaking response and febrile response to the administration of PGE_2.

A large number of reports indicate that bilateral damage to or experimental lesioning of the POA induces a state of insomnia, while that of the PH induces a state of somnolence or hypersomnia in man, monkey, rat, and cat (von Economo, 1926; Ranson, 1939; Swett and Hobson, 1968; McGinty, 1969; Sallanon et al., 1989). In addition, the medial POA also has an important role in the regulation of body temperature, and damage to cells in this region results in dysfunction of the control of both sleep-wakefulness and body temperature in cats (McGinty and Sterman, 1968; Szymusiak et al., 1991). Furthermore, the thermosensitive neurons in the POA are known to change their sensitivities to the brain temperature in association with changes in the sleep-wakefulness alteration (Glotzbach and Heller, 1984; Parmeggiani et al., 1987). These findings indicate that the regulation of body temperature in the POA is closely related to the sleep-wakefulness control mechanism. The hyperthermia induced by PGE_2 was considered to be mediated by the activation of the thermosensitive and PGE_2-sensitive neurons located in the medial POA that modulate both behavioral and autonomic febrile responses (McGinty and Szymusiak, 1990). Although a small but statistically insignificant sleep reduction was observed associated with a low-dose administration of PGE_2 in the POA/AH, a febrile response was apparent at the same dose of PGE_2. Reduction in sleep time occurred with larger doses of PGE_2 administered there, and was consistently associated with marked autonomic responses including the elevation of heart rate and brain temperature (Figs. 2, 4). Therefore, it is possible that the sleep reduction observed in the POA/AH may be secondary to the activation of thermoregulatory mechanisms. In addition, the finding of a significant correlation between the awaking index and the increase in brain temperature (Fig. 4*A*) also indicates the coupling of the sleep reduction with the febrile response induced by PGE_2 administration into the POA/AH. On the contrary, the mechanism of wakefulness and/or EEG desynchronization induced by PGE_2 in the TuM-PH was not associated with the thermoregulating action since the administration of PGE_2 into this region significantly increased the amount of time awake without causing any febrile and autonomic responses (Fig. 1*B,D*). Recently, Matsumura et al. (1989b) showed that infusion of AH6809, a PGE_2 antagonist, into the third ventricle of the rat decreased the amount of wakefulness but failed to affect the brain temperature. Therefore, these findings suggest that the thermo-independent arousal-controlling function of PGE_2 in the TuM-PH may be physiologically involved in the regulation of sleep-wakefulness.

The awaking response following PGE_2 administration was largest in the portion of the PH close to the mammillary complex among the various regions tested. This was especially evident in the case of monkey Y901, in which the probe was implanted into the region closest to the tuberomammillary nucleus, for this monkey responded maximally to the lowest dose of PGE_2 tested with a minimal latency (Figs. 1*A,* 5; Table 1, position f). Recently, histaminergic, histamine-immunoreactive, and histidine decarboxylase–immunoreactive neurons have been found and shown to be exclusively localized in the TuM-PH of the mam-

malian brain (reviewed by Schwartz et al., 1991). Furthermore, numerous pharmacological and physiological studies have shown that these histaminergic and histaminoreceptive neurons (via H1 receptors) participate in the arousal mechanism mediated in the TuM-PH (Schwartz et al., 1986; Reiner and McGeer, 1987; Lin et al., 1988; Szymusiak et al., 1989). In addition, Lin et al. (1989) suggested that the neural activity of the TuM-PH was important for maintaining both behavioral and EEG wakefulness since bilateral microinjection of muscimol, a GABA agonist, into the ventrolateral part of the mammillary region of the PH potently reduced the awake time in cats. Our group recently reported that a high density of binding sites of ^{3}H-PGE_2 is localized in the supramammillary nucleus and in the medial and ventrolateral parts of the mammillary complex in rats and monkeys, but not in the midbrain reticular formation (Watanabe et al., 1988; Matsumura et al., 1990). These results strongly indicate that PGE_2 may be one of the endogenous wakefulness-controlling factors that regulate the arousal systems in the TuM-PH of the mammalian brain.

Intraventricular administration of PGE_2 strongly inhibits not only SWS but also REM sleep in rats (Matsumura et al., 1988). Further, cataplexy of the narcoleptic dog, a condition associated with the REM sleep disorders, was also inhibited by systemic administration of PGE_2 (Nishino et al., 1989). However, in the present study, the amount of REM sleep was not significantly affected by the administration of PGE_2 into any regions examined, except for that of the highest dose (500 pmol/min) into the POA/AH (Fig. 2). Since the intraventricular infusion of PGE_2 also inhibited REM sleep in monkeys (H. Onoe, Y. Watanabe, K. Ono, and O. Hayaishi, unpublished observations), the site responsible for this action may exist in another brain region(s) outside the hypothalamus. Interestingly, Masek et al. (1976) showed that the intracisternal injection of PGE_1 markedly reduces the generation of both SWS and REM sleep in rats. These results indicate that the site of the inhibition of REM sleep by PGE_2 may exist in the brainstem in which REM sleep-related neurons have already been shown to exist (reviewed by Siegel, 1989). Indeed, more recent study in our laboratory has shown that PGE_2 causes a large reduction in REM sleep when infused into the aqueduct of the monkey (H. Onoe, K. Ono, Y. Watanabe, and O. Hayaishi, unpublished observations). We, therefore, speculate that both the TuM-PH and a certain nucleus of the brainstem play an important role in the arousal-controlling mechanism of PGE_2.

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