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Prostaglandin D_2-sensitive, sleep-promoting zone defined in the ventral surface of the rostral basal forebrain

(microdialysis/continuous infusion/subarachnoid space/slow-wave sleep/paradoxical sleep)

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ABSTRACT **The site of action for the sleep-promoting effect of prostaglandin (PG) D_2 was extensively examined in the brain of adult male rats (n = 231). PGD_2 was administered at 100 pmol/0.2 μl per min for 6 hr (2300–0500 hr) through chronically implanted microdialysis probes or infusion cannulae. Among the administrations of PGD_2 by dialysis probes (n = 176), only those (n = 8) to a ventro-rostral part of the basal forebrain by the probes implanted on the midline consistently increased slow-wave sleep (SWS), by 51 ± 6 min (mean ± SEM) above the baseline value (111 ± 11 min). Since this area is separated by a cleft into right and left regions, the results were interpreted to mean that, through this cleft, PGD_2 diffused in the subarachnoid space over the adjacent ventral surface, where it had the effect of promoting sleep. When PGD_2 was directly infused into the subarachnoid space (n = 55), extraordinary increases exceeding 90 min were consistently attained for the SWS at sites located between 0.5 and 2 mm rostral to the bregma and between 0 and 1.2 mm lateral to the midline defined according to the stereotaxic coordinates adopted from the brain atlas of Paxinos and Watson [Paxinos, G. & Watson, C. (1986) *The Rat Brain in Stereotaxic Coordinates* (Academic, San Diego)]. Thus, we demarcated a "PGD_2-sensitive, sleep-promoting zone" within this region in the ventral surface of the rostral basal forebrain. During the bilateral infusion of PGD_2 into the subarachnoid space of this zone, the hourly mean SWS level of the nocturnal animals (n = 6) in the night reached the maximum at the second hour of the infusion period; this maximum hourly SWS level, corresponding to the daytime level of the same animals, lasted until the end of PGD_2 infusion.**

Prostaglandin D_2 (PGD_2) has been postulated as one of the endogenous sleep-promoting substances in rats and other mammals including humans (1). PGD_2 has been implicated in the physiological regulation of sleep by the fact that sleep in rats was markedly suppressed by intracerebroventricular (2) or intravenous (3) administration of inorganic selenium compounds, which are inhibitors of PGD synthase (EC 5.3.99.2), the enzyme responsible for the synthesis of PGD_2 in the rat brain (4).

The preoptic area (POA) has long been proposed as a sleep center since the experimental study by Nauta in 1946 (5). The site of action for the sleep-promoting effect of PGD_2 has been postulated to be located in or near the POA since an increase in the amount of sleep was first demonstrated with PGD_2 in 1982 by a microinjection study (6). Subsequent studies in monkeys (7) and rats (8) also supported the assumption that the site of action is located in a rostral and ventral region, adjacent to the third cerebral ventricle; however, the exact site of action has not yet been clearly defined.

In this paper, the site most effective in promoting sleep with PGD_2 administration was extensively studied by use of the microdialysis technique and the continuous infusion method. The results clearly define the site of action within a restricted area in the ventral surface of the rostral basal forebrain.

MATERIALS AND METHODS

Animals and Surgical Operation. Two-hundred and thirty-one male Sprague–Dawley rats (Japan SLC, Hamamatsu City, Japan), 8 weeks of age (250–260 g), were acclimated to the experimental environment of 25°C, 60% relative humidity, and a 12-hr light (0800–2000 hr)/12-hr dark (2000–0800 hr) cycle for 7–10 days prior to the surgical operation. Under pentobarbital anesthesia (50 mg/kg of body weight), each rat was implanted with electrodes for recording electroencephalogram (EEG) and electromyogram (EMG) along with a thermistor probe for monitoring brain temperature. According to the stereotaxic coordinates of Paxinos and Watson (9), each rat was also implanted with a microdialysis probe(s) (EiCOM, Kyoto) or stainless-steel cannula(e) in its skull for the administration of PGD_2, following one of the modalities described below. The bregma was used as the reference point for indicating the anteroposterior position, with positive numbers for the ones rostral to, and negative numbers for those caudal to, the bregma. *Experiment 1*: Paired microdialysis probes were bilaterally implanted in the brain of each rat (n = 115). A coaxial probe used was covered by a semipermeable dialysis membrane (length, 3.0 mm; o.d., 0.22 mm). The "cutoff" value for the membrane was 50 kDa. The sites examined covered almost all brain regions from the anteroposterior level of 4.0 mm to −12.3 mm (see Fig. 1). *Experiment 2*: One to four dialysis probes were implanted on the midline of the brain, being arranged in the anteroposterior direction (see Fig. 2*A*) in each rat (n = 29). *Experiment 3*: Two pairs of dialysis probes were bilaterally implanted at two different anteroposterior levels in the ventro-medial region (not on the midline) of the basal forebrain (see Fig. 2*B*) of each rat (n = 8). *Experiment 4*: For the efficient supply of PGD_2 to the ventral surface layer of the rostral basal forebrain, paired U-shaped dialysis probes were bilaterally implanted in each rat (n = 24). The anteroposterior position for each pair of probes was in the range between 3.2 and −1.3 mm. The length of the dialysis membrane was 1 mm. The cutoff value was the same as described above. *Experiment 5*: Two stainless-steel cannulae (o.d., 0.35 mm for each) for the continuous infusion of PGD_2 were implanted in the skull of each rat (n = 40), aiming at a site in the subarachnoid space surrounding the brain surface (see Fig. 3*A*) or in the anterior horn of the lateral ventricle. *Experiment 6*: A single stainless-

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Abbreviations: SWS, slow-wave sleep; PS, paradoxical sleep; EEG, electroencephalogram; EMG, electromyogram; PGD_2, prostaglandin D_2; POA, preoptic area; aCSF, artificial cerebrospinal fluid.

steel cannula (o.d., 0.35 mm) was implanted in each rat (n = 15), aiming at a midline position in the subarachnoid space under the rostral basal forebrain (see Fig. 3).

Experimental Protocol. After 10–13 days of their recovery from the surgery, the rats were housed in specially devised experimental cages (Osaka Microsystems), which permit multiple routes for infusion/perfusion and simultaneous multichannel electrical recordings in a freely behaving animal (10). Each rat was further acclimated to the perfusion of artificial cerebrospinal fluid (aCSF; Na^+ = 155.0 mM, Ca^{2+} = 1.1 mM, K^+ = 2.9 mM, Cl^- = 132.76 mM, Mg^{2+} = 0.83 mM, pH 7.2) through an implanted dialysis probe(s) for 2 days or to the continuous infusion of saline through an implanted cannula(e) for 3 days. The "baseline" recordings were taken for 24 hr, beginning at 2000 hr. On the following "experimental" day, the perfusion/infusion of vehicle was replaced by PGD_2 solution (aCSF solution for dialysis and saline solution for continuous infusion, respectively) during the period between 2300 and 0500 hr. The perfusion/infusion speed was maintained at 0.2 μl/min for the vehicle and PGD_2 solution. Significant increases in sleep were observed with continuous infusion of PGD_2 into the third cerebral ventricle at rates between 60 fmol/min and 6 pmol/min in a previous study (11) and also at 1, 10, and 100 pmol/min in a recent study (8); thus, we set the rate at 100 pmol/0.2 μl per min for the PGD_2 perfusion through each dialysis probe or infusion by each cannula in this study. The location of the probes and cannulae was verified histologically after completing each experiment. Rats were allowed to have free access to food and water throughout the experiment. Slow-wave sleep (SWS), paradoxical sleep (PS), and wakefulness were scored visually on the EEG and EMG recordings as described (12), with the minimal scoring interval set at 15 sec. Details were described elsewhere (2). Data are expressed as mean ± SEM.

RESULTS

Among the 115 administrations in experiment 1, only 6 resulted in increases in the SWS by >30 min, as indicated by the solid rectangles in Fig. 1. The POA, which has been postulated as the site of action for the sleep-promoting effect of PGD_2, is located in the anteroposterior level between 0.5 and −1.4 mm. When compared with the POA, a ventral and medial region in the basal forebrain, rostral to the POA, appeared to be a more feasible site of action, where five of the six solid rectangles are seen. If all administrations to the same or adjacent area, including these five, are combined, the mean SWS increment is 23 ± 4 min (vehicle, 111 ± 7 min; PGD_2, 134 ± 6 min; n = 15; t = 5.94; $P < 0.001$ by paired t test).

In experiment 2 (Fig. 2*A*), the magnitude of the SWS increase was clearly correlated with the anteroposterior position of the implanted probes, when the probes were situated in the ventral part of the brain (ventro-median in Fig. 2*A*). The SWS increased markedly by 51 ± 6 min when at least one dialysis probe was located between 0.7 mm and 2.7 mm (exclusive) rostral to the bregma (vehicle, 111 ± 11 min; PGD_2, 162 ± 15 min; n = 8; t = 8.96; $P < 0.001$ by paired t test); otherwise, the SWS increase ranged between −19 and 36 min. However, if the probes were situated in a dorsal position (dorso-median in Fig. 2*A*), the SWS increase became marginal even when the probes were located at anteroposterior positions between 0.7 and 2.7 mm.

In experiment 3 (Fig. 2*B*), the SWS increase did not exceed 34 min even when a pair of probes was located at an anteroposterior level between 0.7 mm and 2.7 mm. Also in experiment 4 (data not shown), administrations increased SWS only up to 29 min except for one, in which an increment of 69 min was attained by administration to a site 1.2 mm rostral to the bregma and touching the medial margin of the respective right and left regions separated by a cleft on the midline.

In experiment 5 (Fig. 3*A*), with bilateral infusion of PGD_2 into the subarachnoid space surrounding the ventral surface of the rostral basal forebrain, increments exceeding 30 min or even 60 min were obtained for SWS at various sites rostral to the bregma and medial to the lateral olfactory tracts. Furthermore, SWS increments consistently exceeded 90 min at the six sites located between 0.5 and 2 mm rostral to the bregma and between 0 and 1.2 mm lateral to the midline. In contrast, SWS increases appeared varied, smaller, or marginal when the infusion into the subarachnoid space was performed at more caudal or lateral locations (Fig. 3*A*), at sites under the posterior hypothalamus (39 and 84 min) and around the pineal gland (17 and −11 min), or when the infusion was carried out into the anterior horn of the lateral

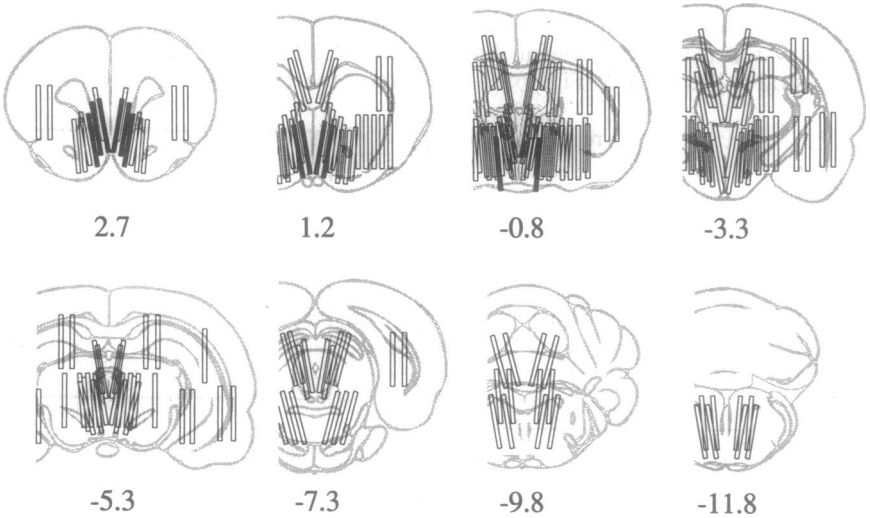

FIG. 1. Responses as the amount of SWS to PGD_2 administration continued for 6 hr (2300–0500 hr) through paired microdialysis probes bilaterally implanted at various sites in the brain. Each rectangle indicates the position and size of the dialysis membrane (length, 3.0 mm; o.d., 0.22 mm). The position of each paired dialysis membrane is superimposed on the nearest one among the coronal drawings, under which the anteroposterior distance of the plate from the bregma is indicated with positive numbers for levels rostral to, and with negative numbers for those caudal to, the bregma, according to the brain atlas of Paxinos and Watson (9). Filled rectangles show the sites where the SWS increase above the baseline exceeded 30 min during the PGD_2 administration; otherwise, the sites are shown by open rectangles.

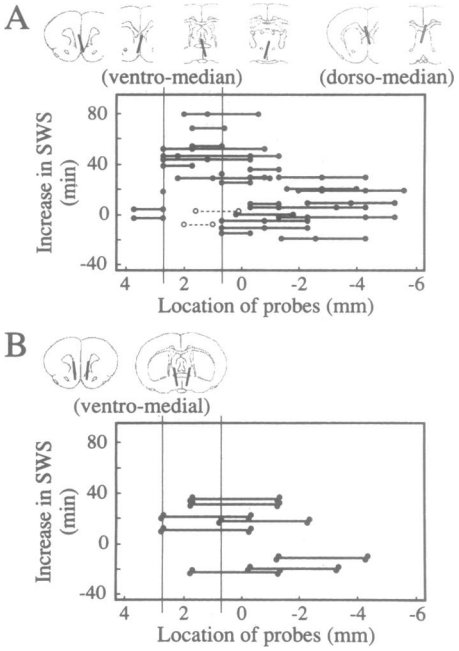

FIG. 2. Increases in the amount of SWS during PGD_2 administration continued for 6 hr (2300–0500 hr) through dialysis probe(s). (*A*) PGD_2 solution was perfused through one to four dialysis probe(s), which were implanted in the brain along the midline as shown in the upper illustrations. Each horizontal line (or single circle) represents an individual rat; the circles connected by it represent the probes implanted. Solid circles with a solid line or a single solid circle indicate the SWS increment above the baseline (min) on the ordinate against the anteroposterior position(s) (on the abscissa) of the dialysis probe(s) implanted in the ventral region ("ventro-median"). Open circles with a broken line indicate the SWS increment (ordinate) against the anteroposterior position (abscissa) of the probes implanted in the dorsal region ("dorso-median") as shown in the upper right illustration. (*B*) The SWS increment on the ordinate is shown against the anteroposterior positions (abscissa) of two pairs of probes bilaterally implanted in the ventro-medial region. Vertical lines indicate anteroposterior levels at 0.7 and 2.7 mm rostral to the bregma (see text for explanations).

ventricle at a level of 1.7 mm rostral to the bregma (26 and 47 min).

In experiment 6, the anteroposterior extension where SWS increments exceeded 90 min was also defined on the midline between 0.5 and 1.5 mm rostral to the bregma (Fig. 3).

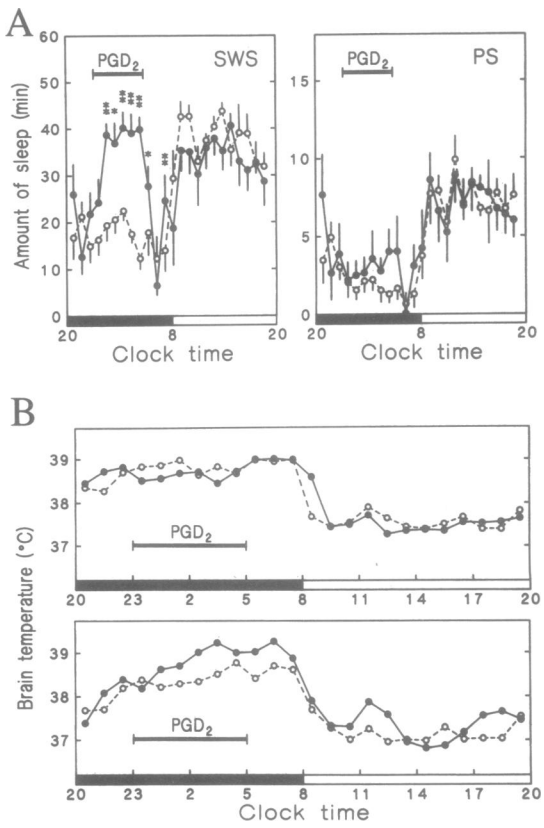

FIG. 4. Hourly changes in the SWS, PS, and brain temperature caused by bilateral PGD_2 infusion to the core region of the "PGD_2-sensitive, sleep-promoting zone" in the subarachnoid space surrounding the ventral surface of the rostral basal forebrain. (*A*) Twenty-four-hour profiles (mean ± SEM) of the six rats that received bilateral PGD_2 infusion at sites between 0.5 and 2 mm rostral to the bregma and between 0 and 1.2 mm lateral to the midline (see Fig. 3*A*). *, $P < 0.05$; **, $P < 0.01$ by paired *t* test. (*B*) Typical profiles in the brain temperature observed in two among the same group of rats. ○, Baseline day; ●, experimental day, on which PGD_2 was infused between 2300 and 0500 hr (indicated by horizontal bars) at 100 pmol/0.2 μl per min through each implanted cannula.

Hourly SWS and PS changes in the above-mentioned six rats that showed SWS increases exceeding 90 min during the bilateral PGD_2 infusion (Fig. 3*A*) are shown in Fig. 4. In these rats, the total SWS increment during the 6-hr infusion of

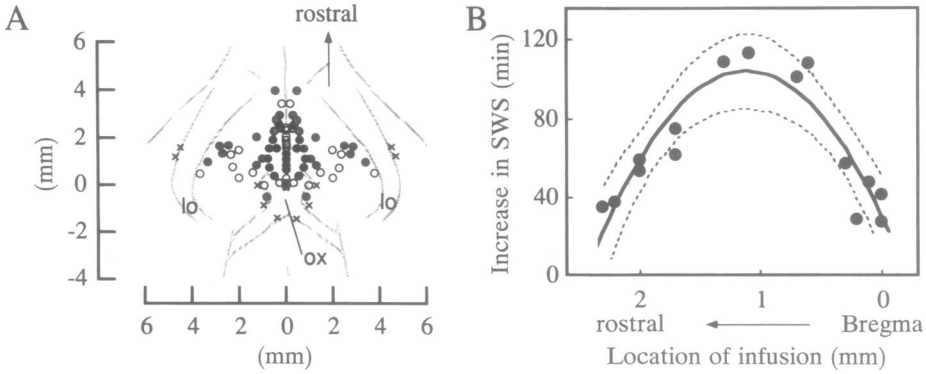

FIG. 3. SWS responses to PGD_2 infusion continued for 6 hr (2300–0500 hr) to various sites in the subarachnoid space surrounding the ventral surface of the rostral basal forebrain. (*A*) Each circle indicates a site of the PGD_2 infusion. The infusion was conducted through a bilaterally implanted pair of cannulae or a single cannula aimed at a position on the midline. Solid circles indicate the site where the SWS increase above the baseline exceeded 90 min; shaded and open circles, increases exceeding 60 and 30 min, respectively; ×, the increase less than 30 min. ox, Optic chiasm; lo, lateral olfactory tract. (*B*) The increment (ordinate) obtained with PGD_2 infusion through a single cannula aimed at a midline position is plotted against the anteroposterior position (abscissa) of the infusion. Solid line, second-order polynomial regression curve; broken lines, 99% confidence limits for regression.

PGD_2 was 110 ± 7 min (vehicle, 109 ± 4 min; PGD_2, 219 ± 10 min; $n = 6$; $t = 15.2$; $P < 0.001$ by paired t test). Although the mean SWS level began to increase over the baseline during the first hour, the mean total SWS amount for the first hour was not largely increased from the baseline amount due to varied latency for the beginning of the SWS increase among the rats. At the second hour of the PGD_2 infusion, the hourly mean SWS reached its maximum, which lasted until the end of the PGD_2 infusion. This maximum hourly SWS level was comparable to the level of the daytime SWS of the same rats. The total SWS amount during this maximum elevation between 0000 and 0500 hr was 214% ± 15% of the baseline (vehicle, 92 ± 6 min; PGD_2, 195 ± 11 min; $n = 6$; $t = 9.63$; $P < 0.001$ by paired t test), showing that the SWS occupied 65% of the total recording time of 5 hr. The mean episode duration of SWS during this period was 157 ± 16 sec, which did not significantly differ from the value of 177 ± 12 sec calculated for the daytime-SWS episode in the same rats ($t = 1.65$). During the period of this sleep promotion, SWS appeared episodically, alternating with, in general, a short episode of wakefulness. Sometimes these rats were also observed to take food pellets and water, spending a relatively long time awake. Thus, the state brought about by PGD_2 infusion was indistinguishable from the physiological state of sleep.

During the first hour after the cessation of the PGD_2 infusion, the SWS began to decrease; thereafter, showing some fluctuations, it went into a phase of rebound decrease in the subsequent daytime (0800–2000 hr: baseline, 447 ± 13 min; experimental, 394 ± 24 min; $n = 6$; $t = 3.50$; $P < 0.05$ by paired t test). However, since this decrease was relatively small when compared with the increase that occurred during the PGD_2 infusion, the total amount of SWS for the whole experimental day (2000–2000 hr) was significantly larger than that for the baseline day (baseline, 652 ± 18 min; experimental, 732 ± 38 min; $n = 6$; $t = 3.65$; $P < 0.05$ by paired t test). The SWS and PS amounts, and their circadian profiles on the subsequent day following the day of PGD_2 infusion, were not different from those on the baseline day (data not shown), thus indicating that such an extraordinary effect of PGD_2 on sleep is reversible without long-lasting influences on sleep.

The hourly mean PS of these six rats also showed an apparent increase between 0200 and 0600 hr, but it was not significant (Fig. 4*A*). In two of them, the PS amounts between 0300 and 0600 hr reached their respective daytime levels.

The mean brain temperature during the PGD_2 infusion was lower than that for the baseline, in two animals, by 1.32°C and 0.82°C (the latter case, shown in Fig. 4*B*, upper panel), whereas in three animals, it was higher by a range from 0.86°C to 2.18°C (one example, shown in Fig. 4*B*, lower panel).

DISCUSSION

The results of experiments 1 and 2 suggested that the site of action for the SWS-promoting effect of PGD_2 is located somewhere in the ventro-rostral basal forebrain. However, SWS increases exceeding 30 min were attained only in limited administration sites tested in experiments 1, 3, and 4, contradicting the postulation that the site of action exists in the parenchyma of the same forebrain region. In contrast, when the administration was performed through the probes implanted between 0.7 and 2.7 mm rostral to the bregma on the midline in experiment 2, consistent increases by 51 ± 6 min were attained for the SWS. Since the rostral part of the basal forebrain is separated by a cleft into right and left regions at an anteroposterior level of 1 mm and rostral to it, we suppose that PGD_2 administered through the dialysis probes on the midline or in touch with the midline diffused, through the cleft, in the subarachnoid space over the adjacent ventral surface, where it brought about sleep promotion.

In experiments 5 and 6, marked SWS increases exceeding 60 min were attained by infusions to a relatively restricted region spreading rostral to the bregma and medial to the lateral olfactory tracts. Furthermore, more pronounced increases exceeding 90 min were exclusively observed in this region. According to the consistent responses exceeding 90 min observed within a confined area, we define the core region of the PGD_2-sensitive, sleep-promoting zone within an area between 0.5 and 2 mm rostral to the bregma and between 0 and 1.2 mm lateral to the midline based on the stereotaxic coordinates adopted from the brain atlas of Paxinos and Watson (9). Thus, by taking these results together with those of the dialysis experiments, we define the site of action for the SWS-promoting effect of PGD_2 within the above-mentioned core region in the ventral surface of the rostral basal forebrain.

In contrast, even though some PGD_2 infusions could also produce a considerable increase in PS, such increases appeared only from 3 or 4 hr after the commencement of the PGD_2 infusion. Several explanations for this, as described below, should be investigated for their validity in a separate series of studies: (*i*) promotion of PS is not a direct effect of PGD_2, but a kind of compensatory response in the brain, which subsequently occurs following such an extraordinary increase in SWS; (*ii*) the site where PGD_2 promotes PS is located elsewhere; therefore, a long lapse of time was needed for the PGD_2 administered to reach and accumulate there; and (*iii*) PGD_2 inhibits PS acting on a different site, which counteracted for a period of time the promotion of PS brought about by PGD_2 administered to the ventral SWS-promoting region.

It is unlikely that changes in the brain temperature can be causally implicated in the sleep-promoting phenomenon produced by PGD_2 infusions, because the direction and magnitude of the temperature response varied among rats even though similar SWS increments were consistently attained among the same rats.

We defined the PGD_2-sensitive, sleep-promoting zone within an area in the ventral surface of the rostral basal forebrain. We believe it probable that an endogenous substance could regulate or modulate functions of the central nervous system at a site in the outer surface of the brain, taking into account similar observations reported elsewhere—i.e., (*i*) two chemosensitive areas, which reacted to H^+ ion and drove ventilation (13); (*ii*) the rostral and caudal endothelin-sensitive areas, in which endothelin caused cardiorespiratory effects (14); and (*iii*) the site where drugs known to interact with serotonin 1A receptors, such as 8-hydroxy-2-(di-*N*-propylamino)tetralin (8-OH-DPAT), produced cardiorespiratory effects (15) were all identified in the ventral surface of the medulla.

Although the present results clearly showed that PGD_2 acts on the ventral surface of the rostral basal forebrain to promote sleep, it is still unclear on what kind of cells or structures PGD_2 primarily acts. With regard to cardiorespiratory effects at the ventral surface of the medulla, it was suggested that endothelin acts directly on vasomotor neurons in the rostral ventrolateral medulla and indirectly through the ventral surface of the medulla (16), although precise mechanisms in the surface area still remain obscure. In a separate study, a packed neuron group was observed within the area where cooling initiated suppression of respiration in the rostral ventral medullary surface (17). These findings may suggest that neurons existing within the surface layer of the parenchyma are involved in the mechanisms responsible for the sleep promotion by PGD_2 as well as the above-mentioned cardiorespiratory effects.

Alternatively, we can also postulate that PGD_2 primarily acts on structures, tissues, and cells existing in the surface layer of the brain, such as subarachnoid membrane, pia mater, pia connective tissue space, basal lamina, glia limitans, and vessels (18, 19), wherein some changes in turn

affect adjacent neurons and/or fibers passing nearby to initiate sleep. Recently, by use of *in situ* hybridization and immunohistochemical staining, the mRNA for the rat brain PGD synthase and this enzyme *per se* were shown to be predominantly expressed and localized in the leptomeninges covering the brain surface and large intracerebral vessels as well as choroid plexus and oligodendrocytes in the adult rat brain (20). More recently, β-trace, a major protein of human CSF, was identified as the PGD synthase (21). Our present results together with these previous findings lead us to hypothesize that the PGD_2 synthesized in the leptomeninges covering the ventral surface of the rostral basal forebrain acts at the same or adjacent surface layer(s) to promote sleep.

Adjacent to the core region of the PGD_2-sensitive, sleep-promoting zone are situated the diagonal bands of Broca. Sterman and Clemente (22) demonstrated in cats that the ventral portion of the diagonal band is a site where bilateral electrical stimulation effectively produces sleep. Szymusiak and McGinty (23) reported that this region contains a population of sleep-active neurons and that kainic acid-induced lesions of the basal forebrain including the same region result in a marked suppression of sleep (24). Concurrently, the same region and also the adjacent olfactory tubercle reportedly contain neurons that discharge more frequently during wakefulness than during non-rapid-eye-movement sleep (23, 25). In addition, the septum and orbitofrontal cortex, which were also implicated in the sleep–wake regulating system (26, 27), are located in the surrounding region. Furthermore, cholinergic and GABAergic (GABA = γ-aminobutyric acid) neurons are spread widely along the ventral surface in this vicinity without being organized into a compact nucleus (28). The sum of our present results together with these reported findings lead us to postulate that the PGD_2-sensitive, sleep-promoting zone is correlated with such specific neurons, nuclei, and areas in the regulation of sleep and wakefulness.

In conclusion, we propose that PGD_2 acts at the "PGD_2-sensitive, sleep-promoting, ventral-surface zone" of the rostral basal forebrain as an endogenous factor for triggering and promoting sleep, which subsequently affects the adjacent sleep–wake-related neurons and/or fibers by some unknown regional mechanisms.

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