The Journal of Neuroscience, August 15, 1998, *18*(16):6599–6607

Interleukin-1 Induces Slow-Wave Sleep at the Prostaglandin D_2-Sensitive Sleep-Promoting Zone in the Rat Brain

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To determine the site of action of the sleep-promoting effect of interleukin-1 (IL-1), we continuously infused (between 11 P.M. and 5 A.M.) murine recombinant IL-1β into seven different locations in the ventricular and subarachnoid systems of the brain in freely moving rats. When IL-1 was infused at 10 ng/6 hr into the subarachnoid space underlying the ventral surface of the rostral basal forebrain, which previously was defined as the "prostaglandin (PG) D_2-sensitive sleep-promoting zone" (PGD_2-SZ), the total amount of slow-wave sleep (SWS) increased by 110.7 min (IL-1 was 208.1 $\pm$ 14.3 min vs control at 97.4 $\pm$ 9.3 min; $n = 8$; $p < 0.01$ by paired Student's t test) from the baseline control level obtained under continuous infusion of saline vehicle. The hourly SWS during the infusion period reached the level of daytime SWS, the physiological maximum, whereas paradoxical sleep (PS) was decreased transiently. This site of action for the SWS promotion was dissociated from the site in the third ventricle sensitive to the IL-1-mediated PS suppression, fever, and anorexia. The SWS increase caused by IL-1 infusion into the PGD_2-SZ was blocked completely by coadministered diclofenac, a nonselective cyclooxygenase (COX) inhibitor. Pretreatment of rats with NS-398 or piroxicam (3 mg/kg of body weight, i.p.), which are said, respectively, to possess high and relative specificity for the COX-2 enzyme, also blocked the SWS-promoting effect of IL-1. We present a hypothesis that IL-1 induces SWS, at least in part, via COX-2-mediated PG production in the PGD_2-SZ.

Key words: interleukin-1; prostaglandin D_2; slow-wave sleep; subarachnoid space; ventral surface of the rostral basal forebrain; COX-2

A variety of studies has demonstrated the important role of interleukin-1β (IL-1) in the sleep–wake phenomena under both physiological and pathological conditions: (1) administration of IL-1 induced excess sleep, especially slow-wave sleep (SWS), in rabbits, rats, cats, and monkeys (Krueger et al., 1984) (for review, see Borbély and Tobler, 1989; Krueger and Majde, 1994); (2) inhibition of endogenous IL-1 by an IL-1 receptor antagonist (Opp and Krueger, 1991) and by anti-IL-1 antibodies (Opp and Krueger, 1994) inhibited the occurrence of spontaneous sleep in rabbits; (3) anti-IL-1 antibodies suppressed the sleep rebound subsequent to sleep deprivation (Opp and Krueger, 1994); and (4) production of IL-1 varied along with sleep–wake behavior and increased during sleep deprivation in various mammalian species, including humans (Lue et al., 1988; Moldofsky et al., 1989). The site of action of IL-1 with respect to this SWS-enhancing effect was investigated by Walter and colleagues (1989), but they failed to define any parenchymal site in the basal forebrain or in the brainstem. Instead, they noticed that the injection of IL-1 into the aqueduct of Sylvius led to an excess of SWS.

On the other hand, a prostaglandin (PG) D_2-sensitive sleep-promoting zone (PGD_2-SZ) was demarcated in the rat within the ventral surface of the rostral basal forebrain, where PGD_2 applied into the subarachnoid space of the zone during the night increased the SWS of this nocturnal animal up to the daytime level, the physiological maximum (Matsumura et al., 1994). The level of PGD_2 in the CSF of rats exhibited a significant alteration between its high during the daytime, i.e., the rest phase of the animal, and its low during the night, the behaviorally active phase (Pandey et al., 1995). The CSF level of PGD_2 in rats increased during sleep deprivation and tended to become higher along with increasing propensity toward sleep and deepening of sleep under a nonsleep-deprived normal condition (Ram et al., 1997).

Thus, both IL-1 and PGD_2 possess sleep-promoting potency. It may be noted generally that IL-1 and PGs share some biological effects such as induction of fever (Dinarello et al., 1983) and anorexia (Hellerstein et al., 1989), as well as activation of both the sympathetic nervous system (Terao et al., 1995) and the hypothalamic–pituitary–adrenal system (Katsuura et al., 1988). However, little is known about the possible interactions between IL-1 and PGs, especially in terms of sleep regulation. Interestingly, neither the site of action for the SWS-promoting effect of IL-1 (Walter et al., 1989) nor that for PGD_2 (Matsumura et al., 1994) was demonstrated in the parenchymal region of the brain.

In this study we searched in freely moving rats for the site of

Received Jan. 16, 1998; revised June 1, 1998; accepted June 4, 1998.

This work was supported in part by funding from the program Grants-in-Aid for Scientific Research of the Ministry of Education, Science, and Culture of Japan and from the Special Coordination Funds for Promoting Science and Technology of the Science and Technology Agency of the Japanese Government, as well as by Grant 8A-6 from the program Research Grants for Nervous and Mental Disorders of the Ministry of Health and Welfare and by a grant from the Naito Foundation. A.T. was supported by a fellowship of the Japan Society for Promotion of Science. We are grateful to Drs. Kiyoshi Matsumura, Chunyu Cao, Shinsuke Satoh, Yoshihiro Urade, and Osamu Hayaishi (Osaka Bioscience Institute) for valuable discussions and Dr. Larry D. Frye for critical reading of this manuscript. We also thank Tomoko Nakajima, Keiko Kasahara-Orita, Nobuko Konishi, and Miyako Matsumura for their excellent technical assistance.

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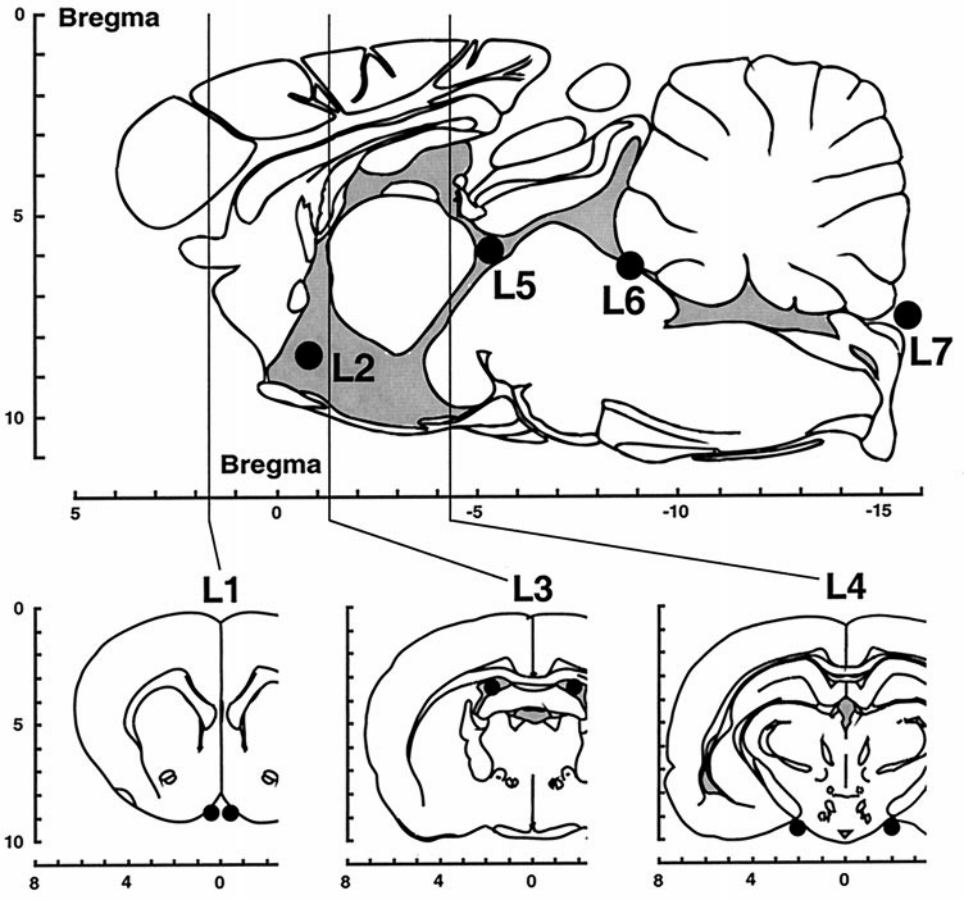

Figure 1. Schematic sagittal and coronal drawings indicating the seven locations (*L1* through *L7*) for infusing IL-1 into the ventricle and subarachnoid space in the rat brain. *Numerals* indicate the distance from bregma, the reference point, in the stereotaxic coordinates. *L1*, In the subarachnoid space of the PGD_2-SZ; *L2*, in the 3V, apposed to the medial preoptic area; *L3*, in the lateral ventricle; *L4*, in the subarachnoid space underlying the posterior hypothalamus; *L5*, in the 3V, near the aqueduct of Sylvius; *L6*, between the aqueduct of Sylvius and the fourth ventricle, apposed to the locus coeruleus; *L7*, in the cisterna magna.

action for the SWS-promoting effect of IL-1 and found that IL-1 increased SWS in the most pronounced manner when it was applied to the PGD_2-SZ. This site of action was dissociated from the site(s) responsible for other effects of IL-1, such as suppression of paradoxical sleep (PS) and induction of fever and anorexia. Furthermore, inhibitors of cyclooxygenase (COX), which is said to be the rate-limiting enzyme for the synthesis of PGs, clearly were demonstrated to block the SWS promotion caused by IL-1. We hypothesize that IL-1 promotes SWS via activated synthesis of PGs in the PGD_2-SZ.

MATERIALS AND METHODS

Animals. One hundred and sixty-eight male rats of the Sprague Dawley strain (Japan SLC, Hamamatsu City, Japan), weighing between 300 and 380 gm (9–10 weeks old), were used. The animals were housed before experimentation in a soundproof chamber at an ambient temperature of 25°C and 60% relative humidity. The chamber was maintained on a 12 hr light/12 hr dark cycle (lights on at 8 A.M.), and standard laboratory rat chow and water were supplied *ad libitum*. All animal use procedures were approved by the Animal Care and Use Committee of Osaka Bioscience Institute.

Surgical operation. Under pentobarbital anesthesia (50 mg/kg of body weight) each animal was chronically implanted with electrodes for recording the electroencephalogram and electromyogram as well as with a thermistor probe for monitoring brain temperature, as described previously (Matsumura et al., 1991). Seven locations were chosen for placing the stainless steel cannulae (0.35 mm outer diameter) in the ventricular and subarachnoid systems. Figure 1 illustrates the schematic sagittal and coronal sections of the rat brain showing the infusion locations. Locations of the tip of the respective infusion cannulae, based on the stereotaxic coordinates adopted from the atlas of Paxinos and Watson (1986) with bregma as the reference point, were as follows (shown as mm): location 1 (L1), in the subarachnoid space of the PGD_2-SZ [anteroposterior distance from bregma (A), 1.7; lateral distance from the midline (L), 0.4; dorsoventral distance from the horizontal plane passing through bregma on the surface of the skull (D), 8.7; bilateral]; L2, in the third ventricle (3V), apposed to the medial preoptic area (A, −0.8; L, 0.0; D, 8.5; midline); L3, in the lateral ventricle (A, −1.3; L, 1.8; D, 3.4; bilateral); L4, in the subarachnoid space underlying the posterior hypothalamus (A, −4.3; L, 2.0; D, 9.4; bilateral); L5, in the 3V, near the aqueduct of Sylvius (A, −5.3; L, 0.0; D, 5.9; midline); L6, between the aqueduct of Sylvius and the fourth ventricle, apposed to the locus coeruleus (A, −8.8; L, 0.0; D, 6.2; midline); L7, in the cisterna magna (A, −15.7; L, 0.0; D, 7.6; midline). For locations 1, 3, and 4 a pair of cannulae was implanted bilaterally, whereas a single cannula was directed to the respective midline position for locations 2, 5, 6, and 7. For implantation of a catheter into the cisterna magna, a burr hole was made on the midline 5.0 mm posterior to the lambda (15.7 mm from the bregma), and the catheter was inserted 7.0 mm deep toward the cisterna magna, as described previously (Pandey et al., 1995).

Materials. Murine recombinant IL-1β (specific activity, 2.2×10^7 U/mg protein, based on the IL-1β-dependent proliferation of D10 cells) was purchased from Genzyme (Cambridge, MA). The manufacturer indicated a molecular weight of 18 kDa and purity >98%, with an endotoxin contamination <0.013 ng/μg protein. IL-1 was diluted just before use at the desired concentration in sterile physiological saline containing 0.01% bovine serum albumin.

Diclofenac sodium (Sigma, St. Louis, MO) was dissolved in saline and continuously and bilaterally infused at 46 μg (145 nmol) per 6 hr in total (between 11 P.M. and 5 A.M.) into the PGD_2-SZ, together with IL-1. Piroxicam (Sigma) and NS-398 (Cayman Chemicals, Ann Arbor, MI) were dissolved separately in dimethyl sulfoxide (DMSO) and further diluted in saline (1:1) before use. Either one (500 μl) was injected intraperitoneally at 3 mg (~9 μmol) per kilogram of body weight 3.2 hr before the commencement of the administration of IL-1.

Experimental procedures. After surgery each rat was allowed a minimum 9 d recovery period in an individual cage before being placed in our originally developed experimental cage (Matsumura et al., 1995), which

permitted multiple routes for infusion into, and simultaneous multichannel polygraph recordings from, a freely moving animal (Osaka Microsystem, Osaka, Japan). A length of tubing, which had been attached to each cannula implanted in rats, then was connected with another tube coming from an infusion syringe; we commenced continuous infusion of sterile physiological saline into the brain at a speed of 0.2 μl/min. After an acclimation period of 3 d, baseline recordings were taken in each animal for 24 hr, beginning at 8 P.M., which served as the control for the same animal. On the next experimental day the infusion of saline was replaced by a test solution during the period between 11 P.M. and 5 A.M. Sleep–wakefulness states, brain temperature, and food intake were monitored for a period of 72 hr, which comprised baseline, experimental, and recovery days. When an experiment was over, each rat was killed with an overdose of pentobarbital sodium and injected through the implanted cannula(e) with a microquantity of pontamine sky blue dye solution (0.5% w/v) to verify the site of infusion. The excised brains were fixed in 10% formalin solution, and the location of the track of each cannula was ascertained histologically.

In the first series of experiments ($n = 120$) a solution of IL-1 was infused into one of the seven locations described above. The total dose of IL-1 that was infused ranged from 0.1 to 100 ng/6 hr, which corresponded to the IL-1 concentration of the test solution from 4.0×10^{-11} to 4.0×10^{-8} M for the bilateral infusion or from 8.0×10^{-11} to 8.0×10^{-8} M for the midline single infusion. In the second series of experiments ($n = 48$) the effect of COX inhibitors (diclofenac, NS-398, and piroxicam) on the changes produced by IL-1 was examined, where IL-1 was infused into the subarachnoid space of the PGD_2-SZ at 10 ng/6 hr.

Analysis of data. Duration of wakefulness, SWS, and PS was measured on the generated polygraph recordings, based on the visual judgment by an expert of the sleep–wakefulness states. The minimal scoring interval was set at 0.25 min of recording time. All results were expressed as the mean ± SEM. Statistics were performed by the use of paired Student's *t* test or ANOVA, depending on the experimental design. The Student–Neuman–Keuls multiple comparison test was used as the *post hoc*. Differences were considered to be significant at $p < 0.05$.

RESULTS

Effects of IL-1 at the seven locations in the brain

To determine the site of action of IL-1, we infused 10 ng of IL-1 continuously for 6 hr (between 11 P.M. and 5 A.M.) into seven different locations of the rat brain (Fig. 1). Total amounts of SWS, PS, and food intake plus the average brain temperature during the 6 hr period of IL-1 infusion were compared with corresponding baseline values. The deviations of the experimental values obtained by the IL-1 infusion from corresponding baseline levels were calculated and averaged (Fig. 2).

Regardless of the site of infusion, the total amounts of SWS that appeared during the IL-1 infusion period were increased significantly over their corresponding baseline levels. However, the magnitude of these SWS increments differed greatly, in a site-dependent manner. When IL-1 was infused into L1, an extraordinary increase in SWS by 110.7 min (IL-1 was 208.1 ± 14.3 min vs control at 97.4 ± 9.3 min; $n = 8$; $p < 0.01$ by paired Student's *t* test) was attained. In contrast, infusion into any of the other six locations produced only mildly increasing responses (24.1–61.9 min). Thus, the increment attained at L1 was significantly larger than the increments at the other six locations ($p < 0.01$ by Student–Neuman–Keuls multiple comparison test following one-way ANOVA; $F = 6.460$; $p = 0.0001$).

Infusion of IL-1 into L2 caused a significant decrease in the total amount of PS (IL-1 was 7.0 ± 2.1 min vs control at 19.6 ± 1.8 min; $n = 8$; $p < 0.01$ by paired Student's *t* test), whereas infusion into the other locations resulted in marginal changes.

Infusion of IL-1 also caused fever and anorexia at all seven locations that were examined. Fever was induced most severely at L2 among the seven locations ($p < 0.01$ by Student–Neuman–Keuls multiple comparison test following one-way ANOVA; $F = 11.345$; $p = 0.0001$). The degree of anorexia caused by the infusion of IL-1 into L2 was also significantly more severe than that seen under infusion into L1 or L7 ($p < 0.05$ by Student–Neuman–Keuls multiple comparison test following one-way ANOVA; $F = 11.345$; $p = 0.0167$).

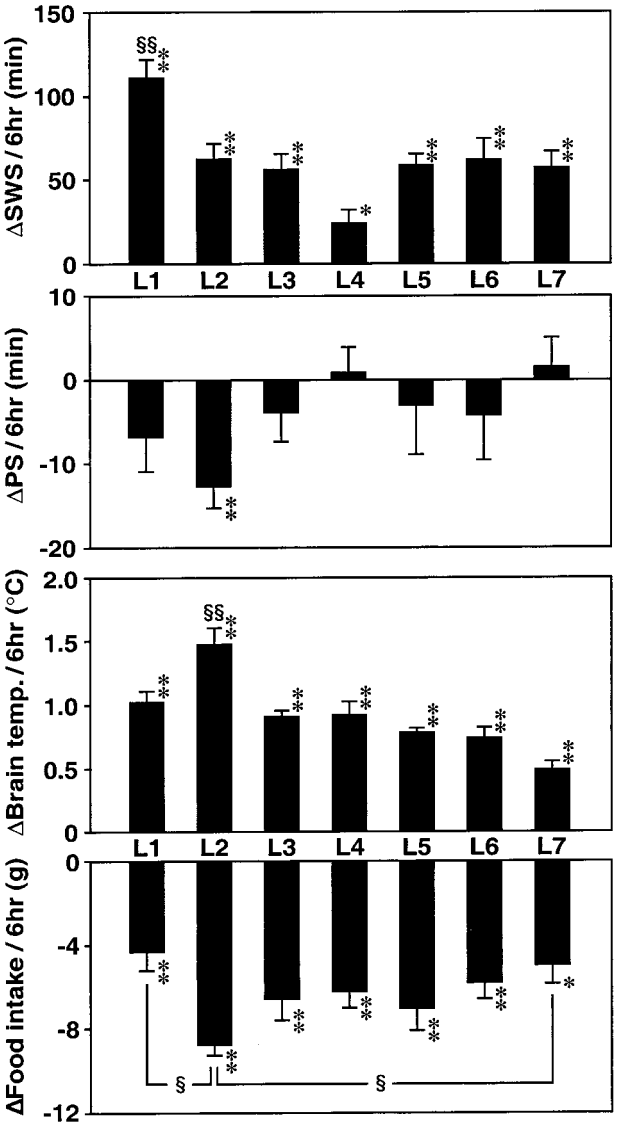

Figure 2. Changes in SWS, PS, brain temperature, and food intake from respective baseline levels caused by the 6 hr (from 11 P.M. to 5 A.M.) infusion of IL-1. The difference between the experimental level by the IL-1 infusion and the baseline level (control) under the infusion of saline vehicle was calculated for each rat; each *column* indicates the mean ± SEM of the differences obtained in this manner in a group of rats ($n = 8$ each) ascribed to the experiment for one of the seven locations. $^{*}p < 0.05$, $^{**}p < 0.01$ by paired Student's *t* test, compared between the IL-1 experiment and vehicle baseline. $^{\S}p < 0.05$, $^{\S\S}p < 0.01$ compared among the changes for the seven locations by Student–Newman–Keuls test following one-way ANOVA, wherein the mark *above L1* in the panel for ΔSWS and that *above L2* in the panel for Δbrain temperature (Δ*Brain temp.*) indicate the significant differences over the changes for the other six locations.

Comparisons of the effects between L1 and L2

Because SWS was promoted most effectively by IL-1 at L1, whereas L2 was the site for the most severe fever and anorexia, dose-dependent (Fig. 3) and time-dependent (Fig. 4) responses were examined further at these two locations.

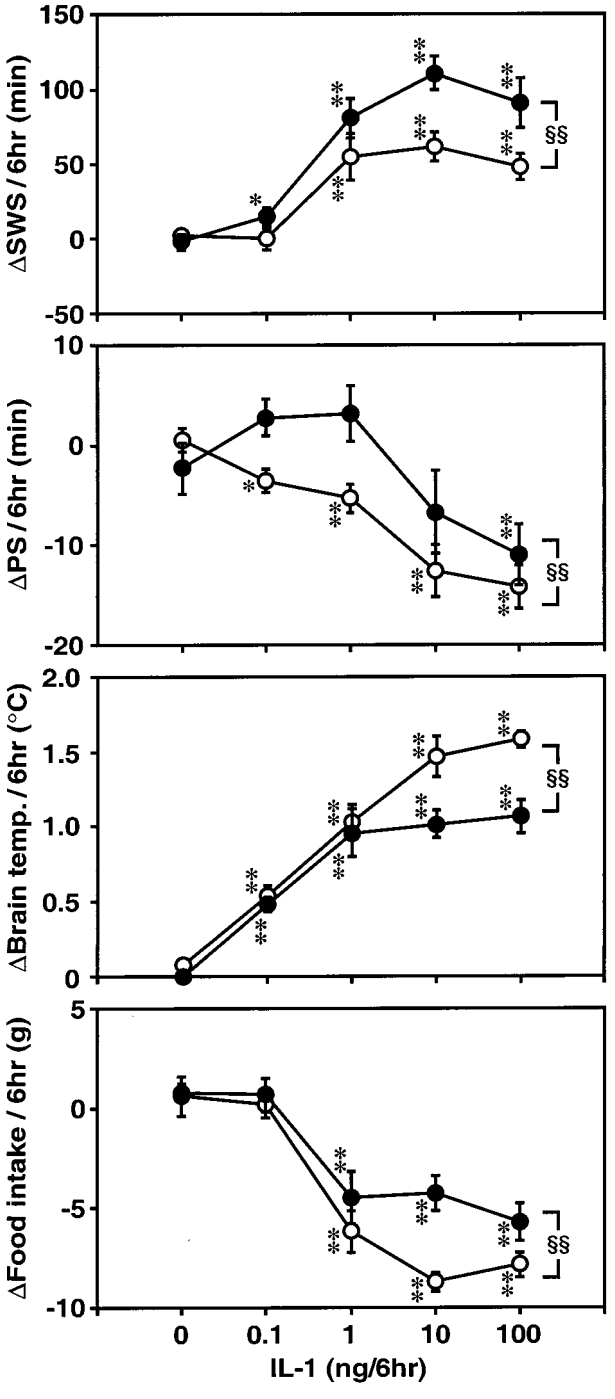

Figure 3. Changes in SWS, PS, brain temperature, and food intake caused by the 6 hr infusion of IL-1 at different doses from respective baseline (control) levels. See Figure 2 for the method of calculation. Each *datum point* represents the mean ± SEM of eight rats. ○, Results by IL-1 infused into L2; ●, results by IL-1 infused into L1. $^{*}p < 0.05$, $^{**}p < 0.01$ by paired Student's *t* test, compared between the IL-1 experiment and the baseline under saline infusion. $^{§§}p < 0.01$ by repeated two-way ANOVA between L1 and L2.

Dose-related responses

Infusion of IL-1 into L1 and L2 caused similar dose-dependent increases in SWS, with respective maximum levels at 10 ng/6 hr of infusion (Fig. 3). However, the magnitude of the overall responses was significantly larger at L1 when compared with that at L2 ($F = 13.932$; $p = 0.0004$ by repeated two-way ANOVA). Furthermore, at a rate of 0.1 ng/6 hr the infusion of IL-1 into L1 increased SWS from the baseline by 15.1 min with statistical significance ($p < 0.05$ by paired Student's *t* test), whereas infusion into L2 caused no significant change.

The infusion of IL-1 into L1 apparently increased PS at 0.1–1 ng/6 hr but decreased it at 10–100 ng/6 hr, with statistical significance at 100 ng/6 hr ($p < 0.01$ by paired Student's *t* test). On the other hand, PS was dose-dependently suppressed at 0.1 ng/6 hr and above when IL-1 was infused into L2. These profiles of PS for L1 and L2 differed significantly from each other ($F = 4.342$; $p = 0.0409$ by repeated two-way ANOVA).

Infusion of IL-1 also raised the brain temperature at 0.1 ng/6 hr and above, regardless of the site of infusion at L1 or L2. However, site L2 appeared to be more sensitive than L1 in raising the temperature ($F = 14.704$; $p = 0.0003$ by repeated two-way ANOVA).

Significant decreases in food intake were observed under the infusion of IL-1 into both L1 and L2 at 1 ng/6 hr and above. However, the dose-dependent decrease for L2 was significantly more severe than that for L1 ($F = 10.733$; $p = 0.0017$ by repeated two-way ANOVA).

The 24 hr profiles

In the time-dependent profiles of SWS (Fig. 4) the amount of SWS during the first hour of the IL-1 infusion was not increased greatly from the corresponding baseline levels in either L1 or L2 profile; i.e., the duration of latency before the increase in SWS was not greatly different between the two locations. However, from the second hour of IL-1 infusion the hourly mean SWS reached its maximum level in the profile for L1, whereas the maximum level was attained only at the last hour of the infusion in the profile for L2. It is notable that, when IL-1 was infused into L1, the hourly SWS level increased to a level comparable to that of daytime SWS, which is the physiological maximum.

Incidentally and interestingly, the rebound decrease in SWS appeared to be more severe in the profile for L2 during the light period after the IL-1 infusion, in contrast to the marginal rebound in the profile for L1, in which significant rebound was observed transiently only between 9 and 10 A.M. Thus, in the profile for L2 in comparison with that for L1, the smaller increase in SWS by IL-1 was followed by a larger decreasing rebound.

With regard to PS, brain temperature, and food intake (Fig. 4), their responses were more outstanding in the profiles for L2 than in those for L1 from the viewpoints of latency before the onset of marked responses and the magnitude of the responses. In these profiles no rebound phenomena were noticeable, at least within the time duration shown in Figure 4.

Analyses of SWS episodes

The state of SWS is composed of episodes, with each duration varying from several seconds up to several minutes. The duration of all SWS episodes was measured, and the values were averaged on an hourly basis (Fig. 5*L1-A,L2-A*). Under the IL-1 infusion into L1 (Fig. 5*L1-A*) the average episode duration for the second hour of the infusion period was prolonged significantly when compared with the corresponding average value calculated from the baseline-day recording (mean duration of SWS episodes for IL-1 experiment was 2.21 ± 0.17 min vs baseline at 1.48 ± 0.20 min; $n = 8$; $p < 0.01$ by paired Student's *t* test). A histogram comprising the numbers of SWS episodes with lengths within respective ranges is shown for this hour (Fig. 5*L1-B*). It indicates

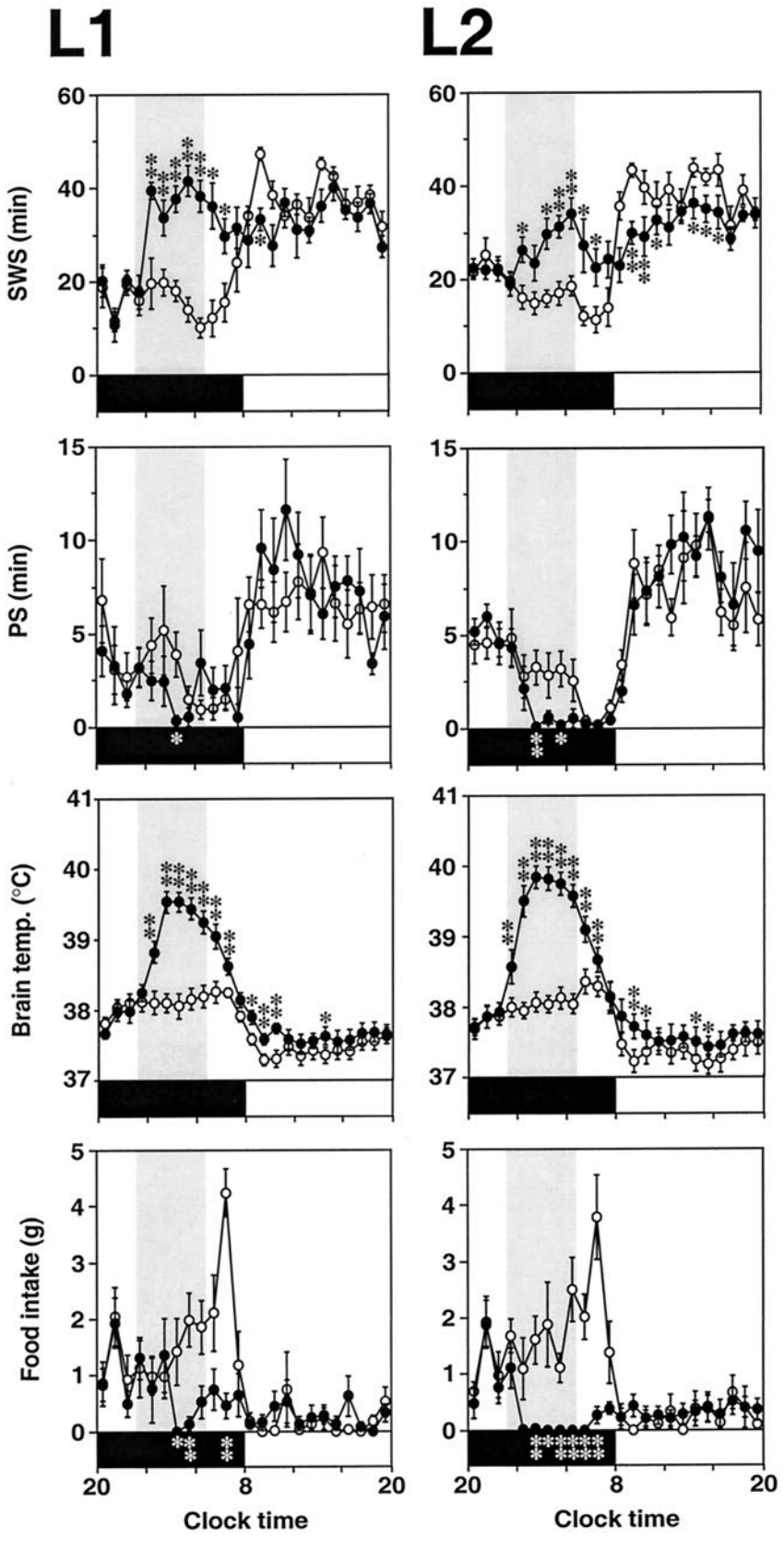

Figure 4. The 24 hr profiles of SWS, PS, brain temperature, and food intake in rats that received IL-1 into *L1* and *L2*. ○, Baseline; ●, experimental day; each *datum point* represents the mean ± SEM of eight values. IL-1 was infused into each rat between 11 P.M. and 5 A.M. (indicated by the *shaded area*) on the experimental day at 10 ng/6 hr. $*p < 0.05$, $**p < 0.01$ by paired Student's *t* test, compared between the experimental datum point and the corresponding baseline point.

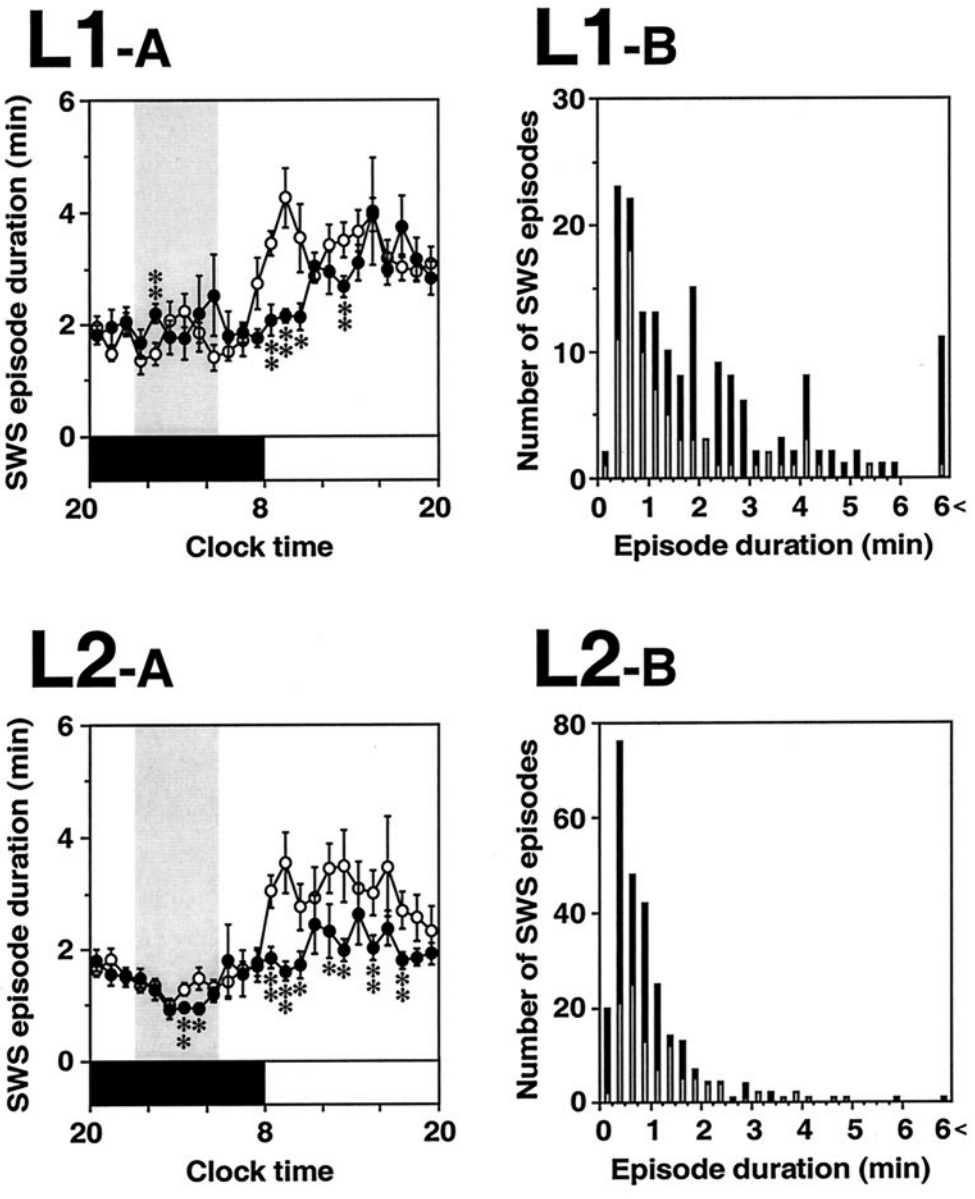

Figure 5. Changes in the hourly mean of SWS episode duration caused by the IL-1 infusion. *L1-A*, The 24 hr profiles of the hourly mean of the SWS episode duration on the baseline day (○) and on the experimental day (●), which were obtained for rats that received IL-1 infusion into L1; *L1-B*, Histogram indicating the changes in the number of SWS episodes of various length ranges, which was for the SWS episodes that appeared between 12 and 1 A.M. on the baseline day (*open column*) and on the experimental day (*open + closed column*) in *L1-A*. *L2-A*, The 24 hr profiles of the hourly mean of the SWS episode duration in rats that received the IL-1 infusion into L2; *L2-B*, Histogram for the SWS episodes that appeared between 2 and 3 A.M. in *L2-A*. SWS episode duration is expressed as the mean ± SEM of eight rats. IL-1 was infused between 11 P.M. and 5 A.M. (indicated by the *shaded area*) at 10 ng/6 hr through implanted cannulae. $^{*}p < 0.05$, $^{**}p < 0.01$ by paired Student's *t* test.

that the numbers of episodes were increased irrespective of their length. It is notable that the number of SWS episodes with a duration >3 min or even the number of those episodes >6 min was increased greatly by IL-1 and that the number of SWS episodes of shorter duration also was increased. In contrast, the infusion of IL-1 into L2 shortened the average duration of SWS episodes during the fourth and fifth hour after the commencement of IL-1 infusion (Fig. 5*L2-A*). The histogram for the SWS episodes during the fourth hour (mean duration of SWS episodes for IL-1 experiment was 0.96 ± 0.08 min vs baseline at 1.29 ± 0.11 min; $n = 8$; $p < 0.01$ by paired Student's *t* test) indicates that the numbers of episodes <2 min were increased but that episodes with longer duration were suppressed mainly by the infusion of IL-1 into L2 (Fig. 5*L2-B*).

Incidentally, it is also notable that the average duration of SWS episodes was shortened markedly during the light period that followed the IL-1 infusion. This shortening of SWS episodes appeared to be longer lasting in the profile for L2 (Fig. 5*L2-A*) than in that for L1 (Fig. 5*L1-A*). It is likely that the rebound decreases in SWS in Figure 4 were caused, at least in part, by the shortening of SWS episodes.

Effect of COX inhibitors on the SWS promotion

Coadministration of the nonselective COX inhibitor diclofenac (46 μg/6 hr) with IL-1 (10 ng/6 hr) to L1 resulted in the disappearance of the SWS-promoting effect of IL-1 (Figs. 6*A* vs 4*L1*) as well as in that of its effects on brain temperature and food intake (data not shown). The rebound decrease in SWS during the light period that followed the IL-1 infusion (Fig. 4*L1*) was not visible, either, after this coadministration. Neither the elongation of the average SWS episode during the second hour of the IL-1 infusion period nor the shortening of SWS episodes during the rebound phase after the IL-1 infusion (Fig. 5*L1-A*) became visible (Fig. 6*B*). Thus, those changes in SWS and SWS episodes produced by IL-1 infusion completely disappeared by the addition of diclofenac.

The SWS-promoting effect of IL-1 (Fig. 6*C*) as well as its effects on brain temperature and food intake (data not shown) also were

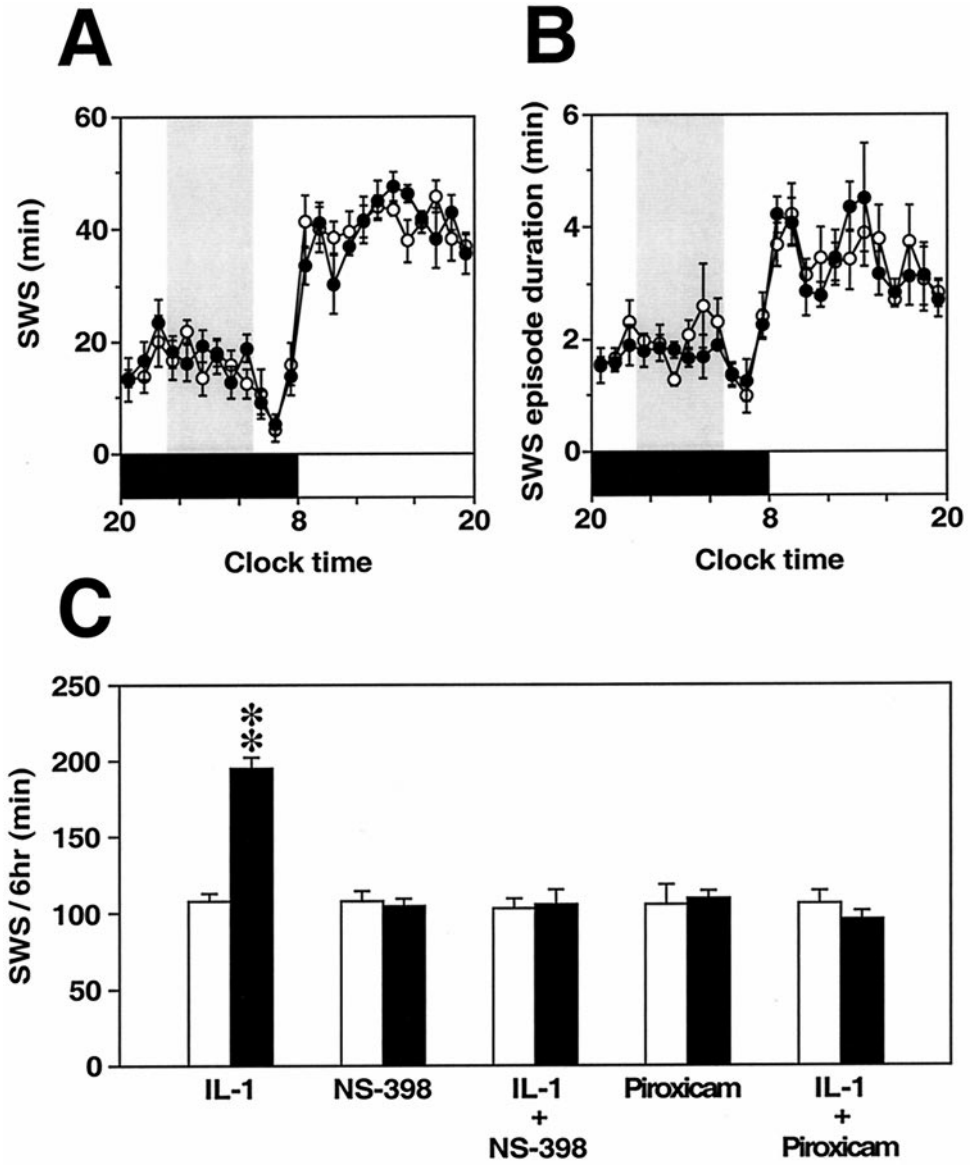

Figure 6. Effect of COX inhibitors on the SWS-promoting effect of IL-1 in L1. Shown are the 24 hr profiles of the hourly amount of SWS (*A*) and those of the hourly mean of the SWS episode duration (*B*) in rats that received the coinfusion into L1 of IL-1 (10 ng/6 hr) and diclofenac sodium (46 μg/6 hr) between 11 P.M. and 5 A.M. (indicated by the *shaded area*). ○, Baseline day; ●, experimental day. *C*, Piroxicam (3 mg/kg), NS-398 (3 mg/kg), or vehicle (for the *column* marked *IL-1*) was injected intraperitoneally at 500 μl, 3.2 hr before the commencement of the continuous infusion of IL-1 (or saline vehicle) into L1 between 11 P.M. and 5 A.M. at 10 ng/6 hr. Each *datum column* represents the mean ± SEM of eight rats. $^{**}p < 0.01$ by paired Student's *t* test between the experimental result (*filled column*) and the corresponding control result (*open column*).

blocked by NS-398 and by piroxicam, which are said, respectively, to possess high and relative specificity for the COX-2 enzyme (Futaki et al., 1994; Engelhardt et al., 1996; Riendeau et al., 1997). They were injected intraperitoneally at 3 mg/kg of body weight 3.2 hr before the commencement of continuous infusion of IL-1. These COX inhibitors had been dissolved in 500 μl of vehicle composed of saline and DMSO at 50:50% (v/v), and the intraperitoneal injection of this vehicle alone exhibited no effect on SWS or on PS, brain temperature, and food intake (data not shown). The intraperitoneal injection of NS-398 or piroxicam did not affect SWS by itself, whereas the combination of the intraperitoneal injection of vehicle and the continuous infusion of IL-1 into L1 produced a significant increase in SWS, by 89.1 min, from the baseline level.

DISCUSSION

Site of action

Among the seven locations that were examined, L1 appeared to be the most effective in promoting SWS (see Fig. 2), a region that previously was reported by our group to be the site of action for the SWS-promoting effect of PGD_2 (Matsumura et al., 1994). At other locations IL-1 also increased SWS, but the magnitude of those increments was much smaller than that produced by IL-1 infusion into L1. Walter and colleagues (1989) searched for the site of action of IL-1 in rabbits but failed to find any specific location in the brain parenchyma. These findings may suggest that IL-1 acts on some tissues/cells existing within or adjacent to the subarachnoid space underlying the rostral basal forebrain to promote SWS.

In contrast to the SWS response, other parameters were found to be affected most markedly by the infusion of IL-1 into L2 among those sites that were examined (see Fig. 2). The PS response varied according to the site of infusion from L1 through L7, but a statistically significant change from the baseline was attained only at L2. Thus, it is likely that IL-1 inhibited PS by acting at the region adjacent to the 3V.

The pyrogenic and anorectic effects of IL-1 also were outstanding when IL-1 was infused into L2. The anorectic effect was concordant with those previous results that were demonstrated with continuous infusion of IL-1 into the 3V (Plata-Salamán,

1994; Plata-Salamán et al., 1996). In addition, latency before the onset of fever and anorexia that followed IL-1 infusion into L2 was much shorter than that after infusion into L1 (see Fig. 4). IL-1 generally is considered as a biological mediator of fever (Dinarello et al., 1983) and anorexia (Hellerstein et al., 1989; Plata-Salamán, 1994; Plata-Salamán et al., 1996), and emphasis has been placed on the predominant roles of the hypothalamus in these phenomena (Sellami et al., 1995). We infer that the 3V side, rather than the subarachnoid side, of the organum vasculosum of the lamina terminalis (OVLT) plays crucial roles in these effects of IL-1.

Effect of IL-1 on SWS episodes

During the increase in SWS that was caused by IL-1 infusion into L1, the number of SWS episodes increased regardless of the length range of the episodes (see Fig. 5*L1-B*); i.e., both long and short episodes increased in number. This feature was strikingly different from the feature observed in the SWS increase brought about by IL-1 infusion into L2 (see Fig. 5*L2-B*), wherein episodes with short duration increased but those with long duration were suppressed. Because IL-1 activates both the hypothalamic–pituitary–adrenal system (Sapolsky et al., 1987) and the sympathetic nervous system (Terao et al., 1994), the shortening of SWS episodes caused especially by IL-1 infusion into L2 might be correlated with the activation of these two systems. Even with the infusion into L1, the activation of these two systems may not have been negligible because of diffusion toward the L2 region. This might explain why the elongation of SWS episodes, which is a characteristic of physiological daytime sleep, occurred only during the second hour of the IL-1 infusion period and not thereafter at L1 (see Fig. *5L1-A*).

Involvement of COX in the effects of IL-1

It is postulated that COX in the brain plays a critical role in the central actions of IL-1 such as fever (Dinarello et al., 1983) and anorexia (Hellerstein et al., 1989) induction and is critically involved in the sympathetic nervous system (Terao et al., 1995) and hypothalamic–pituitary–adrenal system (Katsuura et al., 1988). On the other hand, COX-mediated promotion of sleep has been doubtful until now (Krueger et al., 1982).

In the current study, however, not only IL-1-induced fever and anorexia (data not shown) but also SWS promotion were all blocked completely by COX inhibitors (see Fig. 6). The blocking of the SWS-promoting effect of IL-1 by the COX inhibitor also became manifest from the finding that, not only during the IL-1 infusion but also during the expected rebound phase after IL-1 infusion, IL-1-induced changes in the length of SWS episodes were abolished by the addition of diclofenac (see Figs. 6*B* vs 5*L1-A*).

We infer that in the study of Krueger and colleagues (1982), the antipyretic drug may not have been fully supplied to the ventral surface, sleep-promoting zone of the rostral basal forebrain defined as the site of action of PGD_2 and IL-1. We believe that the current results have provided solid evidence for the crucial role of COX in the SWS-promoting effect of IL-1.

Diclofenac is a general inhibitor of COX regardless of subtypes of the enzyme, i.e., COX-1 and COX-2, whereas inhibitors NS-398 and piroxicam, respectively, are said to possess high and relative specificity for the COX-2 (Futaki et al., 1994; Engelhardt et al., 1996; Riendeau et al., 1997). Because these COX-2 inhibitors themselves were sufficient to block completely the promotion of SWS by IL-1 (see Fig. 6*C*), it is likely that COX-2 rather than COX-1 plays the major role in this promotion of SWS. COX-2 generally is thought to be an inducible enzyme; however, it also was observed to be expressed in the CNS of the rat even under the normal, nonstimulated condition (Yamagata et al., 1993; Breder et al., 1995; Cao et al., 1996). Therefore, it remains to be clarified whether the promotion of SWS produced by the sequence of IL-1 and COX-2 action is exclusively pathological or even active under the physiological condition.

There was a time lag between the commencement of IL-1 infusion and the onset of the increase in SWS, which was calculated to be ~55 min. In a previous study of our group (Matsumura et al., 1994) the time lag between the commencement of infusion of PGD_2 and the onset of the SWS increase was assessed to be 35 min. The infusion of IL-1 in the present study and that of PGD_2 in our previous study were both made into the same site, i.e., L1. Expression of mRNA of COX-2 was shown to be induced by intraperitoneal injection of IL-1 by use of an *in situ* hybridization technique (Cao et al., 1996). These findings lead us to postulate the following: (1) IL-1 induced the synthesis of COX-2, which subsequently catalyzed the formation of PGH_2 from arachidonate, resulting in the formation of PGD_2 and other PGs and in the promotion of SWS; and/or (2) IL-1 increased the availability of arachidonate, which facilitated the production of PGH_2 from arachidonate, resulting in the formation of PGD_2 and other PGs to promote SWS.

Role of PGs in the promotion of SWS

At this moment there is no report on whether PGD_2 production is augmented by an IL-1 challenge *in vivo*, but the PGE_2 level reportedly rose in response to intravenous injection of IL-1 in some brain areas, including the OVLT (Komaki et al., 1992). In cultured rat astrocytes IL-1 stimulated the production of PGE_2 (Katsuura et al., 1989) and PGD_2 (Yamamoto et al., 1988). Because PGD synthase is abundant in leptomeninges and CSF (Urade et al., 1993; Watanabe et al., 1994), it is highly probable that PGD_2 is synthesized actively in the ventral surface zone of the rostral basal forebrain after the induction of COX-2 and/or the increased availability of arachidonates, both of which might be caused by IL-1.

It was shown previously that PGE_2 possessed a wakefulness-promoting potency in rats and monkeys, which was brought about by its action in the diencephalon (Matsumura et al., 1988; Onoe et al., 1992; Gerozissis et al., 1995). However, the same PGE_2 promoted SWS when it was infused into the subarachnoid space of the PGD_2-SZ (Ram et al., 1997). In this zone $PGF_{2\alpha}$ also promoted SWS in the same study. Thus, these three PGs were all SWS-promoting in the zone; therefore, it is plausible without any contradiction that hyperproduction of PGH_2 is an important step in the promotion of SWS triggered by the infusion of IL-1 into this zone of the rostral basal forebrain.

Type 1 IL-1 receptor (Ericsson et al., 1995), COX-2 (Cao et al., 1996), PGD synthase (Urade et al., 1993), and PGD receptor (Oida et al., 1997) were all shown to be expressed predominantly in the leptomeninges as well as, in some cases, in blood vessels and other tissues/cells. These findings support the idea that the leptomeninges and CSF in the PGD_2-SZ of the rostral basal forebrain play critical roles in the mediation of SWS promotion. Because CSF levels of IL-1 activity vary in phase with sleep–wake cycles (Lue et al., 1988) and because the expression of mRNA of IL-1 exhibited a diurnal variation (Taishi et al., 1997), a sequence of events including IL-1 and PGs may be responsible,

at least in part, for physiological sleep–wakefulness phenomena as well as for certain kinds of pathological processes.

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