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Inhibition of sleep in rats by inorganic selenium compounds, inhibitors of prostaglandin D synthase

(electroencephalogram/microdialysis/dithiothreitol/brain temperature/food and water intake)

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ABSTRACT **Prostaglandin (PG) D_2 has been postulated to be an endogenous sleep-promoting factor in rats, and $SeCl_4$ and Na_2SeO_3 recently have been shown to inhibit the PGD synthase (prostaglandin-H_2 D-isomerase, EC 5.3.99.2) activity of rat brain. The effect of these selenium compounds on sleep–wake activities was examined in freely moving rats along with their effects on brain temperature, food and water intake, and behavior. Test substances were administered for 6 hr into the third ventricle of rats, using a microdialysis technique. $SeCl_4$, time- and dose-dependently, inhibited sleep at perfusion rates of 60 pmol/0.2 μl per min and higher, and the inhibition was almost complete at rates >200 pmol/0.2 μl per min. The effect was reversible and was followed by a rebound. Na_2SeO_3 exhibited similar effects, but Na_2SO_3 did not show any effect on sleep. Simultaneous administration of dithiothreitol eliminated the sleep-inhibiting effects of these selenium compounds. These findings indicate that the decrease in sleep is due to inhibition of the PGD synthase activity in the brain by $SeCl_4$ as well as Na_2SeO_3. During the inhibition of sleep, the rats in general showed an activation of behavior with moderate elevation of brain temperature and a detectable increase in food and water intake, suggesting that the sleep-inhibited state of the rats was similar to the physiological state of wakefulness and that the inhibitory effect was not due to the general toxicity of selenium.**

Prostaglandins (PGs) D_2 and E_2, which are the major PGs in the brain of mammals, have been postulated to be endogenous factors for regulating sleep-wake activities in the rat and monkey (1, 2)—the former promoting sleep (3, 4) and the latter augmenting wakefulness (5–7).

Naito *et al.* (8) reported the effects of intracerebroventricularly and systemically administered cyclooxygenase inhibitors such as indomethacin and diclofenac sodium on the sleep–wake activities of rats. However, these inhibitors are not specific for PGD_2 but prevent the synthesis of PGH_2 from arachidonate, which in turn affects the consequent production of various eicosanoids, including PGD_2 and E_2. The rat brain PGD synthase (prostaglandin-H_2 D-isomerase, EC 5.3.99.2) was purified to homogeneity and characterized by Urade *et al.* (9, 10). More recently, Islam *et al.* (11) demonstrated in an *in vitro* study that inorganic quadrivalent selenocompounds, such as $SeCl_4$ and Na_2SeO_3, inhibited the activity of the rat brain PGD synthase whereas other enzymes in the arachidonate cascade were not affected. Therefore, these selenocompounds may serve as useful probes for elucidating the role of endogenous PGD_2 in the physiological sleep–wake regulation.

In the present study, the effects of selenocompounds on sleep–wake activities, brain temperature, food and water intake, and behavior were examined in freely moving rats by administration of these compounds into the third ventricle via a microdialysis probe. The results obtained add further support to the hypothesis that PGD_2 is involved in the promotion of sleep under physiological conditions.

MATERIALS AND METHODS

Experimental Animals and Surgical Procedures. Thirty-nine male rats of the Sprague–Dawley strain (Japan SLC, Hamamatsu City, Japan), 8 weeks of age (≈250 g), were acclimated to environmental conditions of 25°C, 60% relative humidity, and a 12-hr light (0800–2000 hr)/12-hr dark (2000–0800 hr) cycle for 12–13 days prior to the surgical operation.

At 9–10 weeks of age (310–380 g), each rat was anesthetized by intraperitoneal injection of pentobarbital (50 mg/kg of body weight), mounted on a stereotaxic instrument (Narishige, Tokyo), and subjected to surgery. Through a hole bored at 0.8 mm posterior from bregma and 0.9 mm left lateral from the midline, a guide cannula (o.d., 0.9 mm), for locating a microdialysis probe at the third ventricle, was inserted into the brain 8.05 mm deep from the level of bregma at an angle of 6° from the sagittal plane according to the stereotaxic coordinates of Paxinos and Watson (12). Five Teflon-coated, stainless steel wire electrodes for electroencephalogram (EEG) recordings, bared at the tip, were attached to the skull with their tips inserted in the frontal cortex of the brain, three on the right and two on the left hemisphere. A stainless steel screw attached to the frontal region of the skull served as the earth electrode. A thermistor probe (o.d., 0.75 mm; Technol Seven, Yokohama) was inserted into the right hemisphere through a hole bored in the skull with the sensor portion 2 mm below the surface of the brain. The cannula, electrodes, and thermistor probe were affixed to the skull with dental acrylic resin and four stainless steel screws anchored to the skull. Two stainless steel wire electrodes for electromyogram (EMG) recordings were inserted into the neck muscles.

Experimental Protocol. After a recovery period of 8–10 days in individual cages, each rat was moved to a cage set inside the experimental chamber (Medical Agent, Kyoto), as depicted in Fig. 1, and acclimated for 4 days in the experimentally controlled environment. A microdialysis probe for administration of a test substance(s) into the third ventricle was inserted via the guide cannula. Prior to insertion, the microdialysis probe was perfused with artificial cerebrospinal fluid (a-CSF), which was continued until the end of the experiment excluding the 6-hr experimental period during which the test solution was perfused. Baseline day recordings began at 0800 hr, 39–42 hr following the insertion of the microdialysis probe. The experimental period was assigned from 1100 to 1700 hr on the day following the baseline day. Recordings were continued until 48 hr elapsed following the experimental day.

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Abbreviations: SWS, slow-wave sleep; PS, paradoxical sleep; EEG, electroencephalogram; EMG, electromyogram; PG, prostaglandin; DTT, dithiothreitol; a-CSF, artificial cerebrospinal fluid.

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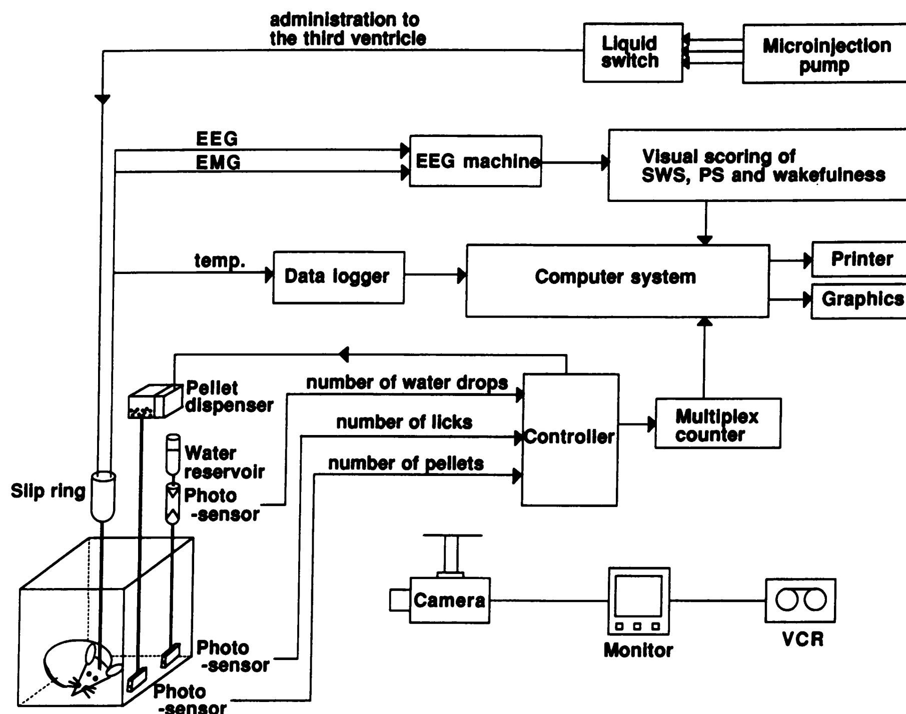

FIG. 1. Flow diagram of the experimental system for administration of test substances into the third ventricle of the brain and recordings of sleep–wake activities, brain temperature, food and water intake, and behavior in the freely moving rat. VCR, video-cassette recorder; SWS, slow-wave sleep; PS, paradoxical sleep.

The test solutions examined were prepared by dissolving the following compounds in a-CSF: (*i*) $SeCl_4$ (Mitsuwa Chemicals, Osaka), (*ii*) $Na_2SeO_3 \cdot 5H_2O$ (Merck), (*iii*) Na_2SO_3 (Fluka), (*iv*) $SeCl_4$ plus dithiothreitol (DTT) (Wako Pure Chemical, Osaka), and (*v*) $Na_2SeO_3 \cdot 5H_2O$ plus DTT. The purity of the two inorganic selenium compounds and the sulfur compound was >99%; DTT was at least 97% pure. The pH of the solutions was adjusted to 7.2 by bubbling a mixture of 95% CO_2/5% O_2 or by addition of 0.2 M NaOH. SWS, PS, and wakefulness were scored on EEG and EMG recordings by visual determination, as described (6). The minimal scoring interval for each vigilance state was set at 15 sec. On completion of each experiment, the accuracy of the location of the microdialysis probe was verified by histological examination of brain sections.

Experimental Conditions. Rats were kept at an ambient temperature of 25°C and relative humidity of 60% while in the acclimation cabinets during pre- and postsurgical phases and while in the experimental chambers (Medical Agent, Kyoto). Soundproof cabinets and experimental chambers were used, which were maintained on a 12-hr light (0800–2000 hr)/12-hr dark (2000–0800 hr) cycle. Illuminance at the bottom center of each cage was kept at 30–50 lux during the light cycle. The interior of the experimental chambers was electromagnetically shielded. Rats had free access to food and water throughout the period of experimentation.

Experimental Devices. Each cage in the experimental chamber was equipped with a slip ring through which EEG and EMG signals were led to an EEG machine (model 1A98A, NEC San-ei, Tokyo) and signals on brain temperature were led to a thermistor-data logger (type K722, Technol Seven). Each cage was equipped with an automatic monitoring system for food and water intake of the rat (Osaka Microsystem, Osaka, and Medical Agent). The automatic monitoring system (Fig. 1) counted the number of food pellets (45 mg per pellet) (Bio-Serv, Frenchtown, NJ) and drops of water (50 μl per drop) consumed by the rat. Data on brain temperature and food and water intake were stored in a computer system (Respy Laboratory, Osaka). Behavior of the rat was monitored using video cameras set within the experimental chambers.

Microdialysis. Test substances were administered using a microdialysis technique that minimized the influence of vehicle on physiological milieu of the rat brain. The microdialysis probe had a semipermeable membrane 1 mm long and 0.22 mm o.d. with a 50-kDa "cut-off" value (EiCOM, Kyoto). The a-CSF used as vehicle (Na^+ = 155.0 mM, Ca^{2+} = 1.1 mM, K^+ = 2.9 mM, Cl^- = 132.76 mM, Mg^{2+} = 0.83 mM, D-glucose = 5.9 mM, pH = 7.2) was derived from the Karolinska Institute formulation (13). Hamilton gastight syringes were used to perfuse the vehicle and test solutions with the aid of microinjection pumps (Carnegie Medicin, Stockholm). Two solutions were alternated with the aid of a liquid switch (Carnegie Medicin). The flow speed of the solution was fixed at 0.2 μl/min.

RESULTS

Sleep in rats was markedly inhibited by the administration of $SeCl_4$ into the third ventricle. Perfusion of $SeCl_4$ at a rate of 400 pmol/0.2 μl per min between 1100 and 1700 hr began to inhibit SWS and PS about 2 hr after the commencement of the perfusion (Fig. 2). Maximal inhibition commenced 3 hr after the beginning of selenium perfusion and lasted until 2 hr after the cessation of perfusion. During this maximal inhibition, the hourly amounts of SWS and PS were about 10 min or less and 0 min, respectively. Sleep inhibition manifested itself at least 3 hr after the cessation of selenium perfusion. Thereafter, the hourly amount of SWS, followed by PS, switched to recovery phases, resulting in noticeable increases in these values beyond baseline levels. Thus, inhibition of sleep occurred between 1300 and 2000 hr.

The amounts of SWS, PS, and wakefulness scored between 1300 and 2000 hr were clearly related to the perfusion rate of $SeCl_4$ (Fig. 3). At perfusion rates of 60 pmol/0.2 μl per min and above, SWS and PS decreased, and wakefulness increased, in a dose-dependent manner. The dose–response curves also showed that PS was more strongly inhibited than

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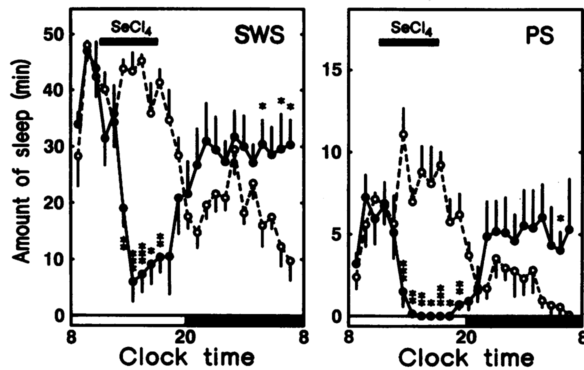

FIG. 2. Effect of $SeCl_4$ perfusion into the third ventricle on sleep in rats ($n = 4$). ○, Hourly changes in SWS and PS on baseline day, obtained by continuous perfusion of a-CSF through a microdialysis probe chronically implanted in the brain of freely moving rats. ●, Hourly changes on the experimental day. On the experimental day $SeCl_4$ was dissolved in a-CSF and perfused through the probe at a rate of 400 pmol/0.2 μl per min between 1100 and 1700 hr (represented by the horizontal bar); otherwise, a-CSF was continuously perfused. Vertical bars represent SEM. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$ (by paired t test).

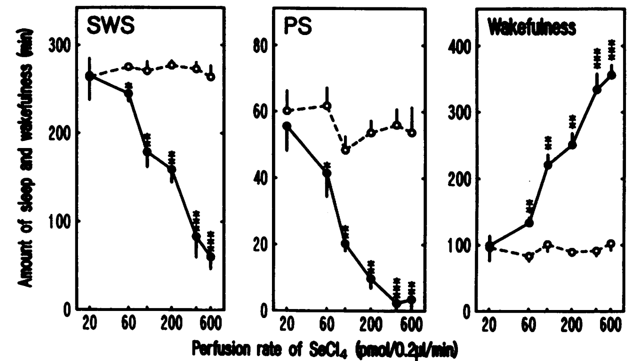

FIG. 3. Relationship between amounts of SWS, PS, and wakefulness and the rate of $SeCl_4$ perfusion. Amounts of SWS, PS, and wakefulness observed between 1300 and 2000 hr are represented separately: ○, baseline day; ●, experimental day. The perfusion rates were 20 ($n = 4$), 60 ($n = 3$), 100 ($n = 4$), 200 ($n = 4$), 400 ($n = 5$), and 600 ($n = 5$) pmol/0.2 μl per min. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$ (by paired t test). For further details, see the text and the legend to Fig. 2.

SWS. Perfusion with Na_2SeO_3 resulted in a similar response to that of $SeCl_4$ in the sleep–wake activities of rats (Table 1). However, Na_2SO_3 did not have any effect on sleep–wake activities when perfused, even at a rate as high as 600 pmol/0.2 μl per min (Table 1).

Islam et al. (11) have shown that DTT added at concentrations about 50 times or higher than the concentration of selenium prevented the selenium-produced inhibition of PGD synthase activity *in vitro*. The present finding that simultaneous administration of DTT at concentrations 50 or 100 times higher than either $SeCl_4$ or Na_2SeO_3 produced no inhibition of sleep (Table 1) is consistent with this observation.

$SeCl_4$ perfusion at the rate of 400 pmol/0.2 μl per min also resulted in an increase in brain temperature after 1300 hr (Fig. 4), but the minimal increase observed between 1300 and 1400 hr cannot explain the profound inhibition of SWS and PS during this period (Fig. 2). After reaching a peak of 39.6°C between 1800 and 1900 hr, the brain temperature declined slowly to the baseline level.

The mean brain temperature during the period of sleep inhibition by $SeCl_4$ from 1300 to 2000 hr was elevated at perfusion rates of 60 pmol/0.2 μl per min and higher (Fig. 5). However, the magnitude of the temperature increase was less dependent on perfusion rates when compared with the changes seen in the amounts of SWS, PS, and wakefulness (Fig. 3).

Food and water intake of the rats was apparently altered during the period of sleep inhibition from 1300 to 2000 hr (Fig. 6). The mean food intake increased correspondingly with an increase in perfusion rates of 60 pmol/0.2 μl per min and higher, whereas the dose–response curve for water intake showed a bell-shape pattern. The apparent changes in the mean value seem less significant, since the magnitude of the response to selenium varied greatly in individual rats. However, 19 of the 21 rats that received $SeCl_4$ perfusion at 60 pmol/0.2 μl per min and higher ate more pellets, and all 13 rats that received $SeCl_4$ perfusion at 100, 200, and 400

Table 1. Effects of seleno- and sulfur compounds on sleep–wake activities of rats

Treatment	Seleno- or sulfur compound perfusion rate, pmol/0.2 μl per min	n	SWS, min	PS, min	Wakefulness, min
No. 1		2			
Control			281.7 ± 11.9	51.8 ± 7.7	86.5 ± 4.3
Na_2SeO_3	200		158.3 ± 25.7	8.8 ± 3.6	252.8 ± 29.3
No. 2		2			
Control			257.3 ± 5.7	58.0 ± 4.3	104.8 ± 10.0
Na_2SeO_3	600		41.7 ± 5.7	1.9 ± 1.9	376.4 ± 3.8
No. 3		3			
Control			267.3 ± 10.3	58.8 ± 3.9	94.0 ± 9.7
Na_2SO_3	600		282.1 ± 8.3	56.7 ± 6.4	81.2 ± 14.1
No. 4		3			
Control			274.7 ± 16.6	46.9 ± 4.6	98.4 ± 14.6
Na_2SeO_3 + DTT*	200		273.1 ± 12.2	47.2 ± 5.5	99.8 ± 11.8
No. 5		3			
Control			274.2 ± 13.7	53.5 ± 3.0	92.3 ± 16.2
$SeCl_4$ + DTT*	400		268.6 ± 6.2	59.6 ± 5.5	91.7 ± 1.7

Test substances were administered to the third ventricle between 1100 and 1700 hr on the experimental day by microdialysis. The compounds were dissolved in a-CSF. Each value (mean ± SEM) shows the amount of SWS, PS, or wakefulness observed during the 7-hr period (1300–2000 hr) during which effects of selenocompounds on sleep–wake activities were commonly induced. Control values are the amounts of SWS, PS, and wakefulness scored during the same period on the baseline day, during which a-CSF was continuously perfused at a rate of 0.2 μl/min.
*DTT perfusion rate, 20,000 pmol/0.2 μl per min.

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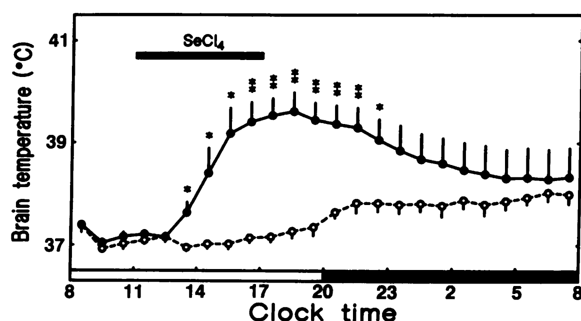

FIG. 4. Changes in brain temperature with time due to perfusion of $SeCl_4$ at a rate of 400 pmol/0.2 μl per min. Temperature values recorded at an interval of 3 min during every 60 min are averaged in each rat, and the obtained value is defined to be the temperature during that period for that rat. Each circle represents the mean of the values obtained from separate rats (n = 4) by this procedure. ○, Baseline day; ●, experimental day. Vertical bars represent SEM. The horizontal bar represents the period of $SeCl_4$ perfusion. *, $P < 0.05$; **, $P < 0.01$ (by paired t test). For further details, see the text and the legend to Fig. 2.

pmol/0.2 μl per min drank more water compared with the food and water intake during the same period on the baseline day.

No evidence for selenium intoxication was observed during and after $SeCl_4$ administration, except for two of the six rats that received $SeCl_4$ perfusion at the highest rate of 600 pmol/0.2 μl per min. The general posture of these two rats looked worse after the selenium administration. Although their food intake on the experimental day was 1.5 and 2.8 times higher, respectively, than on the baseline day, showing no difference in their behavior from other rats, food intake on the second day following the experimental day was much less than on the baseline day and water intake on the days following the experimental day was almost nil. Deterioration in their body condition developed probably later in time after the period of sleep inhibition produced by selenium administration.

DISCUSSION

The administration of $SeCl_4$ and Na_2SeO_3 into the third ventricle of rats clearly showed a time- and dose-dependent inhibition of sleep. Further, selenium administration at perfusion rates >200 pmol/0.2 μl per min produced an almost complete inhibition of sleep. Islam *et al.* (11) have recently demonstrated that inorganic quadrivalent and divalent selenium compounds inhibit the activity of the PGD synthase from the rat brain. PGD_2 has been postulated to be an

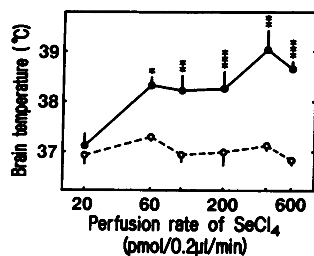

FIG. 5. Relationship between mean brain temperature during the period of sleep inhibition and the perfusion rate of $SeCl_4$. Temperature recorded at a 3-min interval during the period from 1300 to 2000 hr was averaged in each rat. Each circle represents the mean of the values obtained from separate rats by this procedure. Vertical bars represent SEM. The numbers of rats used for $SeCl_4$ perfusion at 20, 60, 100, 200, 400, and 600 pmol/0.2 μl per min were 4, 3, 4, 4, 4, and 5, respectively. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$ (by paired t test).

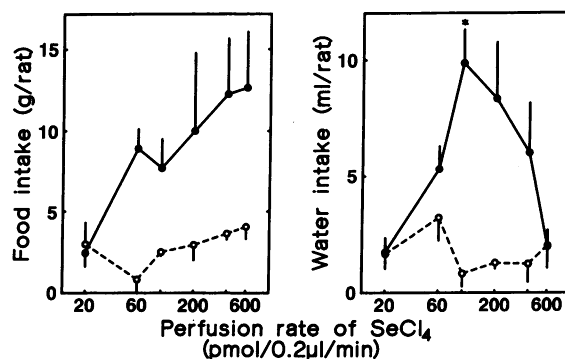

FIG. 6. Effect of $SeCl_4$ perfusion on food and water intake. Each circle represents the mean food and water intake during the sleep-inhibition period (1300–2000 hr). ○ and ●, intake during the same period on the baseline day and experimental day, respectively. Vertical bars represent SEM. The numbers of rats used for $SeCl_4$ perfusion at 20, 60, 100, 200, 400, and 600 pmol/0.2 μl per min were 4, 3, 4, 4, 5, and 5, respectively. *, $P < 0.05$ (by paired t test).

endogenous sleep-promoting factor (1–4). Hence, our present results support the hypothesis that the PGD synthase activity in the brain can be inhibited by administration of selenocompounds and that the inhibition of PGD synthase activity, which leads to a reduction in the PGD_2 levels, results in sleep inhibition.

This hypothesis is further supported by the following two observations. (*i*) Na_2SO_3 had no effect on the sleep of rats in the present study, consistent with the finding by Islam *et al.* (11) that the sulfur compound does not inhibit the activity of PGD synthase. These results reveal that, although sulfur and selenium belong to the same group in the periodic table and possess similar chemical properties, only selenium affects sleep and PGD synthase activity. (*ii*) Simultaneous perfusion of DTT at rates 50 or 100 times higher completely neutralized the sleep-inhibiting effect of selenium in the present study, and DTT at concentrations about 50 times or higher eliminated the inhibitory effect of selenium on PGD synthase *in vitro* (11). It was also shown that the inhibitory effect of inorganic selenocompounds was specific for PGD synthase, and other enzymes involved in the arachidonate cascade were not affected by the inorganic selenocompounds (11). PGD_2, possessing a sleep-promoting effect, is actively synthesized by PGD synthase in the brain of rat (9). The temperature elevation elicited by selenocompounds in this study can also be explained by the inhibition of PGD synthase in the brain, because PGD_2 was reported to decrease the body temperature of rats (14). Thus, the inhibition of PGD synthase may provide a plausible explanation for the selenium-produced inhibition of sleep.

Although there are many reports on selenium toxicity (15, 16), it seems unlikely that the sleep inhibition was caused by general toxicity of the selenocompounds. Two of the six rats that received $SeCl_4$ perfusion at the highest rate of 600 pmol/0.2 μl per min lost their appetite for food and water and also showed posture distortion in the post-sleep-inhibition phase. However, other rats, including those exhibiting profound sleep inhibition, did not show any manifestation of this kind. Furthermore, during the sleep-inhibition period, all rats showed normal behavior similar to that observed during the natural awaking phase. These results indicate that the sleep inhibition should not be attributed to selenium intoxication.

Neither the increase in food and water intake nor the temperature elevation was a likely cause for the sleep inhibition, because the changes in food and water intake were less significant and the temperature elevation did not correspond well with the degree of sleep inhibition. It is more likely

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that the increased activity during the sleep-inhibition period influenced the food and water intake and brain temperature.

Sleep inhibition began to occur about 2 hr after the commencement of selenium administration. This time lag may be explained by the time needed for the selenocompounds to diffuse to the site of action and accumulate there in sufficient concentration to inhibit PGD synthase. Even when the synthase is inhibited, sleep may be maintained by the PGD_2 already present in the brain until it reaches a level too low to be effective. Some *in vitro* studies reported previously provide data suggesting that selenocompounds react with thiols such as glutathione and DTT to form products that in turn play a vital role in many metabolic reactions (17–20). Islam *et al.* (11) also showed in their *in vitro* study that small amounts of DTT were required for the inhibition of PGD synthase by selenocompounds. It is, therefore, possible that the selenocompounds administered to the brain react with endogenous sulfhydryl compounds to form biologically active adduct(s) and that the resulting adduct(s) inhibits the activity of PGD synthase. The time required for this adduct formation may also contribute to some extent to the time lag seen between the administration of selenocompounds and the induction of sleep inhibition.

During the sleep-inhibition phase, the rats generally ate and drank well and exhibited active behavior. After the termination of selenium administration, the sleep–wake activities switched to a recovery phase, which resulted in a noticeable increase in SWS and PS beyond the baseline level. This indicates that the sleep-inhibiting effect of the selenocompounds is reversible. Furthermore, the rebound manifestation implies that the changes occurred along homeostatic alteration of sleep–wake activities as seen under physiological conditions (21). These results indicate that the selenocompounds produced a state similar to that of physiological awaking.

Selenium has been identified as an important trace mineral for many species, including rats and humans (22, 23), and its deficiency has been reportedly associated with skeletal and cardiac muscle degeneration, hepatosis, and so forth (24–26). The present findings, as well as those of other research groups (27), suggest that selenium is also involved in the physiology and pathology of the brain function. Thus, selenium appears to be a good probe for future research in the field of sleep–wake regulation.

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