

BRES 19216

Intravenous administration of inorganic selenium compounds, inhibitors of prostaglandin D synthase, inhibits sleep in freely moving rats

Ryuichi Takahata^a, Hitoshi Matsumura^b, Sachi Sri Kantha^b, Etsuko Kubo^b,
Kumiko Kawase^b, Toshiaki Sakai^a and Osamu Hayaishi^b

^a Department of Neuropsychiatry, Osaka Medical College, Daigakumachi, Takatsuki, Osaka (Japan) and ^b Osaka Bioscience Institute, Furuedai, Suita, Osaka (Japan)

(Accepted 20 April 1993)

Key words: Electroencephalogram; Catheterization; Continuous intravenous infusion; Brain temperature; Food intake; Water intake

Prostaglandin (PG) D₂ has been postulated to be an endogenous sleep-promoting factor. Biosynthesis of PGD₂ is catalyzed by PGD synthase (prostaglandin-H₂ D-isomerase, EC 5.3.99.2), the activity of which is inhibited by inorganic selenium compounds such as SeCl₄ and Na₂SeO₃. We recently examined the effect of intracerebroventricular administration of these selenium compounds on sleep in rats, and demonstrated time- and dose-dependent sleep inhibition. To establish whether this effect of selenium is also produced when the compound is administered systemically, we devised a procedure for intravenous catheterization and examined the effect of these selenocompounds on sleep-wake activity in freely moving rats (*n* = 35). Each test compound was administered into the inferior vena cava continuously between 11.00 and 17.00 h on the experimental day. SeCl₄ time- and dose-dependently inhibited sleep at infusion rates of 5, 7.5, 10 and 20 nmol/μl per min. During the SeCl₄ infusion at 20 nmol/μl per min, slow-wave sleep and paradoxical sleep were reduced to 63% and 50% of their respective baseline values. Na₂SeO₃ exhibited a similar sleep inhibition, though Na₂SO₃ was ineffective. Infusion of SeCl₄ at 10 nmol/μl per min or below produced no consistent changes in the mean brain temperature, or food and water intake during the infusion period. During the nocturnal period subsequent to SeCl₄ infusion, sleep was increased by a rebound phenomenon, while a decrease in brain temperature and inhibition of food and water intake dose-dependently occurred. We conclude that systemic administration of these PGD synthase inhibitors has a sleep-reducing potency.

INTRODUCTION

The significance of selenium as an essential nutrient to rats was first demonstrated³⁴ in 1957. Since then, its essentiality as a trace mineral to humans has also been recognized^{19,24,39,41,42,43}. The established biochemical function of selenium is its role as a constituent of the enzyme glutathione peroxidase^{18,32,37}. Burk et al.⁶ reported recently that more than 60% of plasma selenium is contained in selenoprotein P, the function of which is still unclear. The anticarcinogenic^{14,15,19,20,22,35} and antiviral¹ actions of selenium are also receiving active attention among the researchers of diverse disciplines. Regarding the physiological role of selenium in brain functions, Behne et al.³ reported that under conditions leading to inadequate selenium intake in rats, the brain received a priority supply of the ele-

ment. Two other reports suggest the importance of selenium in functions associated with brain iodothyronine deiodinase² and protection of polyunsaturated fatty acids in synapses against peroxidation¹⁰.

Prostaglandin (PG) D₂ has been postulated to be an endogenous sleep-promoting factor in rats⁴⁰, monkeys³⁰ and probably humans^{16,17}. In an *in vitro* study, Islam et al.²¹ demonstrated that the activity of PGD synthase, the enzyme primarily responsible for producing PGD₂ from PGH₂, was inhibited by quadrivalent inorganic selenium compounds. Therefore, we postulated that selenium administration to the brain could inhibit the synthesis of sleep-promoting PGD₂ and showed previously in an *in vivo* study²⁶ that intracerebroventricular administration of these selenium compounds to freely moving rats via a microdialysis probe resulted in a time- and dose-dependent sleep inhibition. The objec-

tive of the present study was to find out whether the sleep-inhibiting effect of selenium demonstrated previously in rats by the intracerebroventricular administration is reproducible when administered systemically.

Administering test chemical compounds by classical routes such as acute oral administration, peritoneal injection, and acute intravenous injection results in intense and untoward stress to the experimental animals. As a consequence, the sleep-wake activity and other physiological phenomena become distorted and unstable. Chronic infusion techniques, which were previously described and reviewed by Burt et al.⁷, also possess imperfections for examining sleep-wake activities. For example, Salford et al.³³ reported that unilateral clamping of the carotid artery occluded regional blood flow and affected metabolism in the rat brain. Intravenous catheterization via the jugular vein^{5,12,29,38} is also likely to obstruct the blood flow to the brain. Venous catheterization through a tail vein¹³ is susceptible to leakage due to biting by the rat as a consequence of the irritation and stress to the animal. Therefore, we chose a location along the inferior vena cava near the renal blood vessels for chronic catheterization, as Lestage et al.²³ had adopted in their study.

Using an improved surgical technique for chronic intravenous infusion, we examined the effects of inorganic selenium compounds on sleep-wake activity and other related physiological parameters in freely-moving rats. The data obtained showed that inorganic selenium compounds such as SeCl_4 and Na_2SeO_3 , which were shown to inhibit the activity of PGD synthase in vitro, also time- and dose-dependently inhibited sleep of rats, which results are consistent with our previous

findings by intracerebroventricular administration of these compounds.

MATERIALS AND METHODS

Experimental animals

Thirty-five male rats of the Sprague-Dawley strain (Japan SLC, Hamamatsu City, Japan), 8 weeks of age (250–260 g), were acclimated to an environment of 25°C, 60% relative humidity, and a 12-h light (08.00–20.00 h)/12-h dark (20.00–08.00 h) cycle for 12–13 days prior to the surgical operation. Animals were permitted free access to food (selenium content $\approx 500 \mu\text{g}/\text{kg}$ diet) and water. Each rat received two surgical operations. At 9–10 weeks of age (310–380 g), electrodes for recordings of electroencephalogram (EEG) and electromyogram (EMG) were implanted in the skull and neck muscles, respectively, and a thermistor probe for recording the brain temperature was inserted into the brain, under pentobarbital anesthesia (50 mg/kg of body weight) as described previously²⁶. One week following this operation, intravenous catheterization of the inferior vena cava was performed.

Surgical procedure of intravenous catheterization

Under pentobarbital anesthesia (50 mg/kg of body weight), a midline laparotomy was performed. The intestines were displaced from the abdominal cavity and covered with saline-soaked gauze to avoid dehydration. The inferior vena cava was exposed by clearing of the overlying connective tissue.

A catheter for chronic infusion was prepared in three steps as follows (Fig. 1A): (1) a polyethylene tubing (PE10, Intramedic, Clay Adams, Parsippany, NJ) was thread with a small glass bead to 2 cm from its extremity where the bead was fixed by bonding agent; (2) the tubing was set in a cyclical loop (one-and-a-half times) at the portion more distal from the extremity with the aid of two additional glass beads, to prevent any stress on the catheter made by the tension of the tubing formed due to the mobility of the rats; and (3) the tubing was then filled with physiological saline containing heparin (50 units per ml). The catheter was inserted into the inferior vena cava at the level distal to the renal vein and fixed in place with a small piece of patch (Tower DualPeel Tubing 6", American Convertors, Evanston, IL) which had been sterilized and soaked with bonding agent (Vetbond, 3M, St. Paul, MN). One ligature was made around the catheter distally 0.5–1.0 cm from the fixed small bead and anchored to the major psoas muscle. The intestines were then placed back into

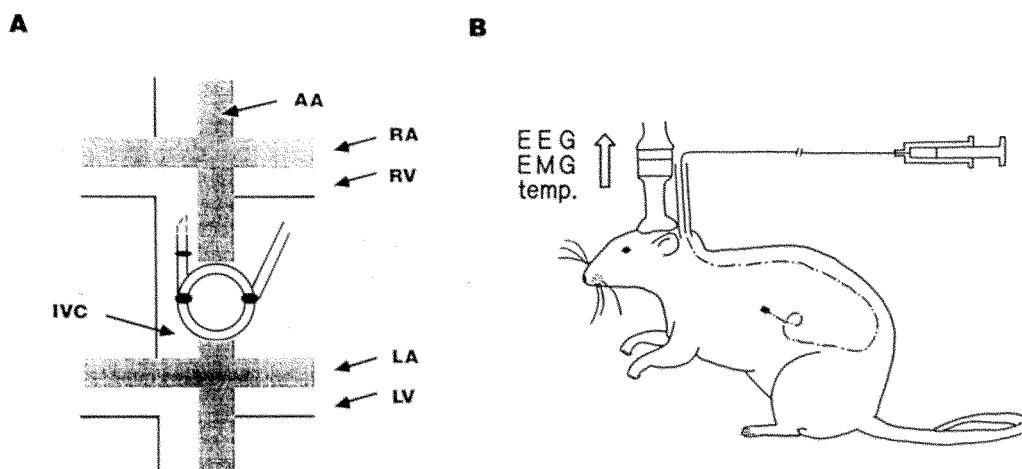


Fig. 1. A: schematic drawing of the catheter placement. AA, abdominal aorta; IVC, inferior vena cava; RA, renal artery; RV, renal vein; LA, lumbar artery; LV, lumbar vein. B: schematic drawing of an experimental rat implanted with electrodes for EEG and EMG recordings, a brain thermistor probe, and the venous catheter.

the abdominal cavity. Subsequently, the free end of the catheter was brought through the subcutaneous space to the neck region and released through a cut in the skin (Fig. 1B). The external portion of the catheter was covered with Teflon tubing, which was fixed to the posterior portion of the skull with dental acrylic resin. The tip of the catheter was then sealed by heating.

Experimental protocol

After a recovery period of 3–4 days from the surgery for catheterization, each rat was moved to a cage set inside the experimental chamber (Medical Agent, Kyoto) described previously²⁶. The catheter was connected to a Hamilton gas-tight syringe via a slip ring, and continuous infusion of saline was initiated into the inferior vena cava of the rats. After an acclimatization period of ca. 60 h, the baseline-day recording was begun and continued for 24 h commencing at 08.00 h; thus each rat served as its own control. The following day was designated as the experimental day. Saline infusion was substituted with an infusion of a test solution between 11.00 and 17.00 h on the experimental day. The infusion rate was maintained at 1 $\mu\text{l}/\text{min}$ for the infusion of saline and test solution.

Test solutions examined in this study were prepared by dissolving the following compounds in sterile saline: (1) SeCl_4 (Mitsuwa Chemicals, Osaka), (2) $\text{Na}_2\text{SeO}_3 \cdot 5\text{H}_2\text{O}$ (Merck), and (3) Na_2SO_3 (Fluka). The pH of the solutions was adjusted to between 6.5 and 7.0 by incremental additions of 1 M NaOH or 1 M HCl, when necessary.

Slow-wave sleep (SWS), paradoxical sleep (PS), and wakefulness were scored visually on the EEG and EMG recordings, as described previously²⁵. The minimal scoring interval for each vigilance state was set at 15 s. Paired *t*-test was used for the comparison of parameters obtained on the experimental and baseline days.

RESULTS

Sleep in the rats was markedly inhibited by the continuous infusion of SeCl_4 into the inferior vena cava at the rate of 10 $\text{nmol}/\mu\text{l}$ per min (Fig. 2). The inhibition of SWS and PS was first noticeable after two hours following the commencement of selenium infusion. It appeared, thus, that at least two hours were needed for the initiation of sleep inhibition by this intravenous infusion. The maximal inhibition of SWS and PS occurred at the end of four hours from the commencement of the infusion. No inhibitory effect on SWS and PS was noticed later than one hour following the termination of selenium infusion. The hourly

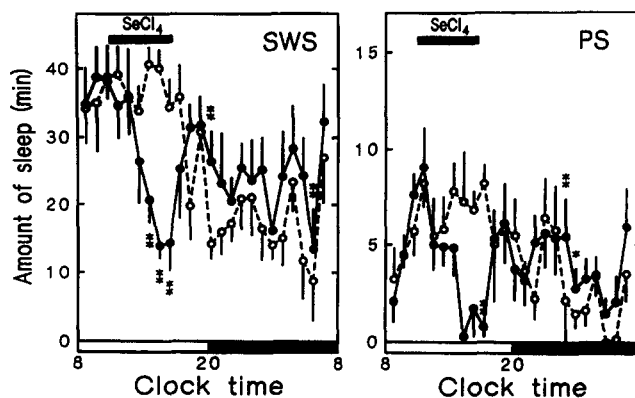


Fig. 2. The effect of SeCl_4 infusion into the inferior vena cava on sleep in rats ($n = 5$). Open circles represent hourly changes in SWS and PS on baseline day, obtained by continuous infusion of saline solution. Closed circles represent the hourly changes on the experimental day. On the experimental day, SeCl_4 solution was infused at the rate of 10 $\text{nmol}/\mu\text{l}$ per min between 11.00 and 17.00 h (represented by horizontal bars); otherwise, continuous saline infusion was performed. Vertical bars represent S.E.M. * $P < 0.05$; ** $P < 0.01$; (by paired *t*-test).

amounts of SWS following one hour into the post-infusion period remained higher than the baseline level throughout the rest of the day. Such a rebound increase was also seen for PS during the later period of the night. A sleep-inhibitory effect of similar magnitude was also obtained in rats infused with $\text{Na}_2\text{SeO}_3 \cdot 5\text{H}_2\text{O}$ at 10 $\text{nmol}/\mu\text{l}$ per min. However, Na_2SO_3 showed no inhibitory effect on sleep at the same infusion rate (Table I).

The effect of infusion rate of SeCl_4 on sleep parameters is shown in Fig. 3. During the 6-h infusion period (11.00–17.00 h), the amounts of SWS and PS showed a dose-dependent decrease. The apparent ID_{50} for the inhibition of SWS and PS was approximately 6 $\text{nmol}/\mu\text{l}$ per min. The magnitudes of the SWS and PS inhibition at ID_{50} were close to 15% and 25%, respectively. After the cessation of SeCl_4 infusion, SWS and PS showed a dose-dependent increase during the dark period

TABLE I

Effects of seleno- and sulfur compounds on sleep-wake activities of rats

Each compound was administered into the inferior vena cava of rats between 11.00 and 17.00 h on the experimental day. Cumulated amounts of SWS, PS, and wakefulness observed during the infusion period are the mean (\pm S.E.M.) of five rats. Control values were obtained for the same period on the baseline day during which saline was continuously infused at the rate of 1 $\mu\text{l}/\text{min}$.

Treatment	Infusion rate, $\text{nmol}/\mu\text{l}$ per min	<i>n</i>	SWS, min	PS, min	Wakefulness, min
No. 1		5			
Control			224.0 \pm 12.7	41.4 \pm 5.2	94.7 \pm 13.3
SeCl_4	10		145.7 \pm 9.1 *	25.9 \pm 2.6 *	188.5 \pm 9.0 *
No. 2		5			
Control			227.8 \pm 3.5	40.7 \pm 2.5	91.5 \pm 2.6
Na_2SeO_3	10		164.8 \pm 10.5 *	20.8 \pm 3.1 *	174.5 \pm 12.8 *
No. 3		5			
Control			230.1 \pm 6.5	40.7 \pm 4.6	89.2 \pm 7.4
Na_2SO_3	10		242.9 \pm 8.1	41.8 \pm 4.4	75.3 \pm 11.2

* $P < 0.01$ by paired *t*-test.

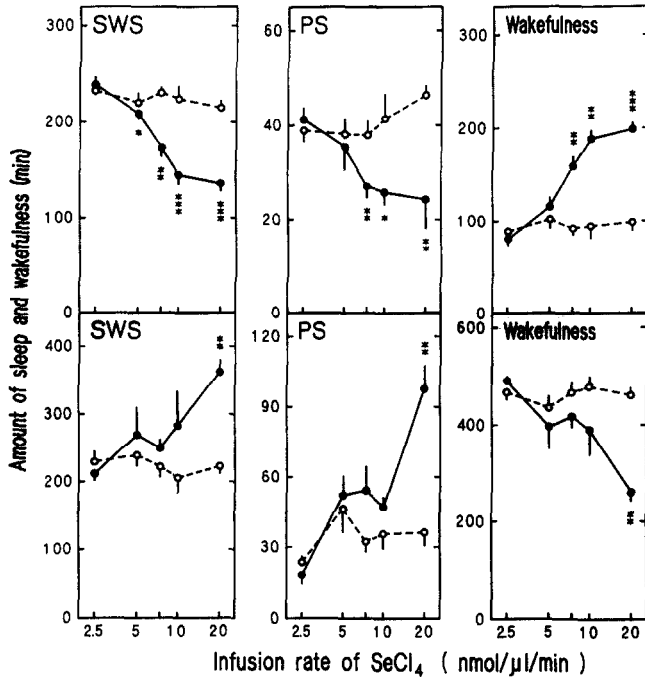


Fig. 3. The effect of SeCl_4 infusion rate on sleep parameters. Upper panels: cumulated amounts of SWS, PS, and wakefulness observed in the 6-h infusion period between 11.00 and 17.00 h are the mean values of five rats for each infusion rate (closed circles). Lower panels: cumulated amounts of SWS, PS and wakefulness observed in the dark period (20.00–08.00 h) subsequent to selenium infusion, are the mean values of five rats for each infusion rate (closed circles). Open circles represent control values obtained from the respective baseline-day recordings. Vertical bars represent S.E.M. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ (by paired t -test).

(20.00–08.00 h), revealing that the inhibition of sleep produced by selenium is reversible.

Contrary to the results obtained by the intracerebroventricular administration of selenium reported previously by us²⁶, the intravenous infusion of selenium lowered the brain temperature rather than elevated it. Whereas change in brain temperature hardly occurred during the first five hours of SeCl_4 infusion at the rate of 10 $\text{nmol}/\mu\text{l}$ per min, thereafter, the brain temperature showed a decreasing profile for the subsequent 16 h (Fig. 4). Selenium administration at the rate up to 10 $\text{nmol}/\mu\text{l}$ per min exhibited minimal effect on the mean brain temperature during the infusion period (Fig. 5, upper panel). But, SeCl_4 infusion at 20 $\text{nmol}/\mu\text{l}$ per min decreased the brain temperature by 0.7°C. During the post-infusion period, this temperature-lowering phenomenon was observed dose-dependently at the infusion rates of 7.5 $\text{nmol}/\mu\text{l}$ per min and above (Fig. 5, lower panel).

Intravenous infusion of SeCl_4 also influenced the food and water intake by the rats. Whereas intracerebroventricular administration of selenium increased food intake²⁶, intravenous infusion of SeCl_4 resulted in decreased mean food intake, although the decrease

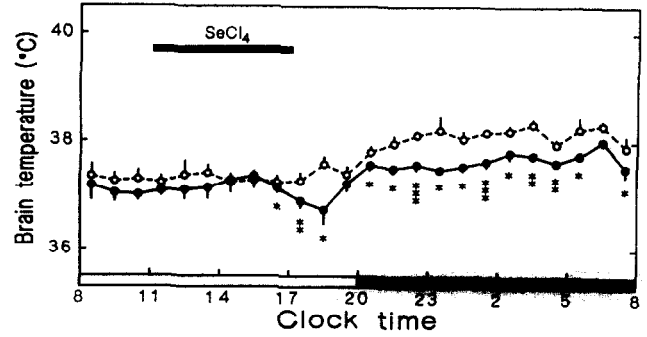


Fig. 4. Hourly alteration in the brain temperature due to SeCl_4 infusion between 11.00 and 17.00 h (shown by horizontal bar) at the rate of 10 $\text{nmol}/\mu\text{l}$ per min. The temperature values recorded at an interval of 3 min during each hour were averaged for every rat, and the resulting value was defined as the temperature during that period in the rat. Each circle represents the mean value of five rats. Open circles, baseline day; closed circles, experimental day. Vertical bars represent S.E.M. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ (by paired t -test).

was not significant (Fig. 6, upper panel); some of the rats, in which selenium was infused at 7.5, 10, or 20 $\text{nmol}/\mu\text{l}$ per min, showed an increment in their food intake during the infusion period. The mean water intake in rats infused with doses of selenium above 5 $\text{nmol}/\mu\text{l}$ per min was relatively higher than the baseline values, though not significantly, and the water intake was reduced at the infusion rate of 2.5 $\text{nmol}/\mu\text{l}$

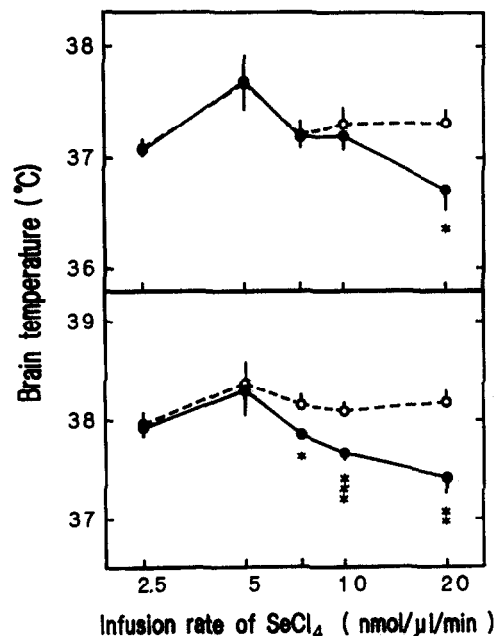


Fig. 5. The effect of SeCl_4 infusion rate on the mean brain temperature of rats (closed circles) during the 6-h infusion period between 11.00 and 17.00 h (upper panel), and during the dark period (20.00–08.00 h) subsequent to selenium infusion (lower panel). Each circle represents the mean value obtained from five rats at each infusion rate. Open circles represent control values obtained from the respective baseline-day recordings. Vertical bars represent S.E.M. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ (by paired t -test).

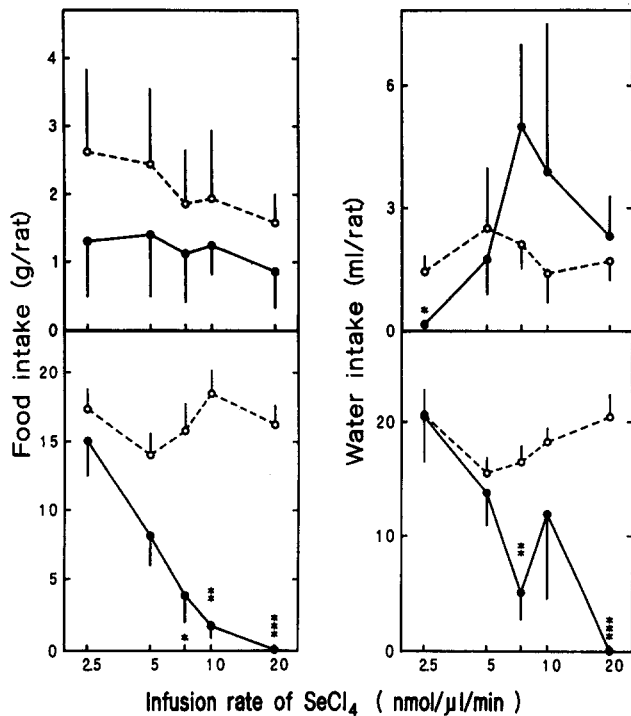


Fig. 6. The effect of SeCl_4 infusion rate on food and water intake in rats during the 6-h infusion period between 11.00 and 17.00 h (upper panels), and during the dark period (20.00–08.00 h) subsequent to selenium infusion (lower panels). Each circle represents the mean value obtained from five rats at each infusion rate. Open circles represent control values obtained from respective baseline-day recordings. Vertical bars represent S.E.M. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ (by paired t -test).

per min (Fig. 6, upper panel). The inhibition of food and water intake appeared to be significant and dose dependent during the post-infusion period (Fig. 6, lower panels).

DISCUSSION

The method of continuous intravenous administration described here seems useful for detecting the effects of systemic administration of chemical compounds on physiological phenomena, such as sleep. The merits of this improved method include (a) relative ease in the preparation of the catheter, and (b) quickness of the catheterization procedure, which can be completed within 40 min. In order to prevent withdrawal of the catheter, instead of fixing the catheter by making ligatures around the vessel, the catheter was connected to the psoas muscle by a thread, since direct ligatures around a vessel blocks blood flow completely. The catheter was filled with heparinized saline and retained until the commencement of continuous saline infusion at the beginning of the experiment; thus, this modification did not require additional procedures such as daily flushing of the catheter with high concentra-

tion of heparin, to avoid clotting in the catheter^{5,7}. Furthermore, the cannula was retained in the vein without any hinderance throughout the long experimental period, despite the intense physical activity of the rats during the nocturnal period. A few aspects of this described procedure resemble those techniques developed by Lestage et al.²³ and Crane and Porrino¹¹. However, we believe that the modifications adopted here are of much practical value to physiological investigations related to the sleep–wake activity.

The continuous infusion of saline into the inferior vena cava of rats under the freely-moving condition did not noticeably distort the normal circadian rhythms of sleep–wake activity, brain temperature, food and water intake in the animals. Saline infusion at rates up to 1 ml/6 s did not reportedly affect blood pressure or heart rate⁵. The infusion rate of saline at 1 $\mu\text{l}/\text{min}$ used in this study was thus extremely mild, producing no untoward responses in the rats. Based on food and water intake, it has been reported that rats require a period of at least 48 h to recover from anesthesia before the beginning of experimentation⁴. Rats in the present study are believed to be sufficiently recovered since they were allowed a recovery period of more than 12 days following the first surgical operation and 5 days following the second operation. Thus, it appears that the described method is optimal for testing the effect of compounds on sleep–wake activity and other physiological parameters.

Under the experimental conditions described above, an intravenous infusion of selenium in rats time- and dose-dependently inhibited sleep. The sleep inhibition by selenium became prominent following the first two hours of the infusion at 10 $\text{nmol}/\mu\text{l}$ per min or lower. Hourly SWS and PS entered the recovery phase one hour after or later following the cessation of selenium infusion. These results corroborate the results obtained in our previous study with intracerebroventricular administration of selenium²⁶. The elevation of brain temperature and the increase in food and water intake that occurred during the sleep inhibition produced by intracerebroventricular administration of selenium in our previous study²⁶ were not observed in this study. Thus, the parameter consistently altered by either intravenous infusion or intracerebroventricular administration is sleep inhibition alone, suggesting that the sleep inhibition occurred due to a central action independently of other changes caused by selenium administration.

Literature reports on selenium toxicity in livestock animals suggest that the deleterious effects of selenium are dependent on the supplied compound, its dose and route of entry, as well as difference in species³¹. Excess

intake of selenium leads to toxicity symptoms such as depression, ataxia, dyspnea, frequent urination, elevated body temperature, increased pulse and respiratory rates, pulmonary congestion and edema^{8,28}, and even to death³⁶. Symptoms such as lack of appetite and refusal to drink water were also observed during the incipient stage of intoxication produced by intraperitoneal injection of selenium in horses²⁷. Thus, temperature decrease and inhibition of food and water intake observed during the post-infusion period in this study may be related to selenium intoxication. However, since the sleep inhibition appeared before such probable intoxication symptoms manifested and sleep was increased as a rebound phenomenon when such symptoms were apparent during the post-infusion period, the sleep inhibition was unlikely a secondary effect of such symptoms.

Clausen¹⁰ showed in rats that ⁷⁵Se, when subcutaneously injected as selenite, was incorporated into myelin, light synaptosomal, and vesicular fractions of a brain homogenate. While the sleep-inhibitory rate of intravenous infusion of selenium was 5–20 nmol/ μ l per min in the current study, selenium perfusion through a microdialysis probe at 60–600 pmol/ μ l per min inhibited sleep when the probe was located in the third cerebral ventricle in our previous study²⁶. Considering the fact that only a portion of the perfused selenium can enter the brain tissue through a microdialysis membrane, we estimate that 0.1–1% of the intravenously infused selenium entered the brain through the blood–brain barrier and caused the sleep inhibition.

We have demonstrated with an improved method that intravenous administration of inorganic selenium compounds inhibits sleep. The current study suggests that a PGD synthase inhibitor that enters the brain and exhibits minimal adverse side effects can be a good agent for the treatment of hypersomnia disorders and sleep–wake schedule disorders in humans.

Acknowledgements. This study was supported in part by a Grant-in-Aid for Scientific Research and a Grant-in-Aid for Developmental Scientific Research from the Ministry of Education, Science, and Culture of Japan and by a grant from the Takeda Science Foundation.

REFERENCES

- Balansky, R.M. and Argirova, R.M., Sodium selenite inhibition of the reproduction of some oncogenic RNA viruses, *Experientia*, 37 (1981) 1194–1195.
- Beckett, G.J., MacDougall, D.A., Nicol, F. and Arthur, J.R., Inhibition of type I and type II iodothyronine deiodinase activity in rat liver, kidney and brain produced by selenium deficiency, *Biochem. J.*, 259 (1989) 887–892.
- Behne, D., Hilmert, H., Scheid, S., Gessner, H. and Elger, W., Evidence for specific selenium target tissues and new biologically important selenoproteins, *Biochim. Biophys. Acta*, 966 (1988) 12–21.
- Birkhahn, R.H., Bellinger, L.L., Bernardis, L. and Border, J.R., The stress response in the rat from harnessing for chronic intravenous infusion, *J. Surg. Res.*, 21 (1976) 185–190.
- Bryan Jr, R.M., Hawkins, R.A., Mans, A.M., Davis, D.W. and Page, R.B., Cerebral glucose utilization in awake unstressed rats, *Am. J. Physiol.*, 244 (1983) C270–C275.
- Burk, R.F., Hill, K.E., Read, R. and Bellew, T., Response of rat selenoprotein P to selenium administration and fate of its selenium, *Am. J. Physiol.*, 261 (1991) E26–E30.
- Burt, M.E., Arbeit, J. and Brennan, M.F., Chronic arterial and venous access in the unrestrained rat, *Am. J. Physiol.*, 238 (1980) H599–H603.
- Caravaggi, C., Clark, F.L. and Jackson, A.R.B., Acute selenium toxicity in lambs following intramuscular injection of sodium selenite, *Res. Vet. Sci.*, 11 (1970) 146–149.
- Chen, X.S., Yang, G.Q., Chen, J.S., Chen, X.C., Wen, Z.M. and Ge, K., Studies on the relations of selenium and Keshan disease, *Biol. Trace Element Res.*, 2 (1980) 91–107.
- Clausen, J., Uptake and distribution in rat brain of organic and inorganic selenium, *Biol. Trace Element Res.*, 28 (1991) 39–45.
- Crane, A.M. and Porrino, L.J., Adaptation of the quantitative 2-[¹⁴C]deoxyglucose method for use in freely moving rats, *Brain Res.*, 499 (1989) 87–92.
- Dalton, R.G., Touraine, J.L. and Wilson, T.R., A simple technique for continuous intravenous infusion in rats, *J. Lab. Clin. Med.*, 74 (1969) 813–815.
- Eve, C. and Robinson, S.H., Apparatus for continuous long-term intravenous infusions in small animals, *J. Lab. Clin. Med.*, 62 (1963) 169–174.
- Greeder, G.A. and Milner, J.A., Factors influencing the inhibitory effect of selenium on mice inoculated with Ehrlich ascites tumor cells, *Science*, 209 (1980) 825–827.
- Harr, J.R., Exon, J.H., Weswig, P.H. and Whanger, P.D., Relationship of dietary selenium concentration; chemical cancer induction; and tissue concentration of selenium in rats, *Clin. Toxicol.*, 6 (1973) 487–495.
- Hayaishi, O., Sleep–wake regulation by prostaglandins D₂ and E₂, *J. Biol. Chem.*, 263 (1988) 14593–14596.
- Hayaishi, O., Molecular mechanisms of sleep–wake regulation: roles of prostaglandins D₂ and E₂, *FASEB J.*, 5 (1991) 2575–2581.
- Hoekstra, W.G., Biochemical function of selenium and its relation to vitamin E, *Fed. Proc.*, 34 (1975) 2083–2089.
- Ip, C., Selenium inhibition of chemical carcinogenesis, *Fed. Proc.*, 44 (1985) 2573–2578.
- Ip, C. and Ganther, H.E., Activity of methylated forms of selenium in cancer prevention, *Cancer Res.*, 50 (1990) 1206–1211.
- Islam, F., Watanabe, Y., Morii, H. and Hayaishi, O., Inhibition of rat brain prostaglandin D synthase by inorganic selenocompounds, *Arch. Biochem. Biophys.*, 289 (1991) 161–166.
- LeBoeuf, R.A. and Hoekstra, W.G., Changes in cellular glutathione levels: possible relation to selenium-mediated anticarcinogenesis, *Fed. Proc.*, 44 (1985) 2563–2567.
- Lestage, P., Vitte, P.A., Rolinat, J.P., Minot, R., Broussolle, E. and Bobillier, P., A chronic arterial and venous cannulation method for freely moving rats, *J. Neurosci. Methods*, 13 (1985) 213–222.
- Levander, O.A., A global view of human selenium nutrition, *Annu. Rev. Nutr.*, 7 (1987) 227–250.
- Matsumura, H., Honda, K., Goh, Y., Ueno, R., Sakai, T., Inoué, S. and Hayaishi, O., Awakening effect of PGE₂ in freely moving rats, *Brain Res.*, 481 (1989) 242–249.
- Matsumura, H., Takahata, R. and Hayaishi, O., Inhibition of sleep in rats by inorganic selenium compounds, inhibitors of prostaglandin D synthase, *Proc. Natl. Acad. Sci. USA*, 88 (1991) 9046–9050.
- Miller, W.T. and Williams, K.T., Minimum lethal dose of selenium, as sodium selenite, for horses, mules, cattle, and swine, *J. Agric. Res.*, 60 (1940) 163–173.

- 28 Morrow, D.A., Acute selenite toxicosis in lambs, *J. Am. Vet. Med. Assoc.*, 152 (1968) 1625–1629.
- 29 Nicolaidis, S., Rowland, N., Meile, M.-J., Marfaing-Jallat, P. and Pesez, A., A flexible technique for long term infusions in unrestrained rats, *Pharmacol. Biochem. Behav.*, 2 (1974) 131–136.
- 30 Onoe, H., Ueno, R., Fujita, I., Nishino, H., Oomura, Y. and Hayaishi, O., Prostaglandin D₂, a cerebral sleep-inducing substance in monkeys, *Proc. Natl. Acad. Sci. USA*, 85 (1988) 4082–4086.
- 31 Painter, E.P., The chemistry and toxicity of selenium compounds, with special reference to the selenium problem, *Chem. Rev.*, 28 (1941) 179–213.
- 32 Rotruck, J.T., Pope, A.L., Ganther, H.E., Swanson, A.B., Hafeman, D.G. and Hoekstra, W.G., Selenium: biochemical role as a component of glutathione peroxidase, *Science*, 179 (1973) 588–590.
- 33 Salford, L.G. and Siesjö, B.K., The influence of arterial hypoxia and unilateral carotid artery occlusion upon regional blood flow and metabolism in the rat brain, *Acta Physiol. Scand.*, 92 (1974) 130–141.
- 34 Schwarz, K. and Foltz, C.M., Selenium as an integral part of Factor 3 against dietary necrotic liver degeneration, *J. Am. Chem. Soc.*, 79 (1957) 3292–3293.
- 35 Shamberger, R.J., Relationship of selenium to cancer. I. Inhibitory effect of selenium on carcinogenesis, *J. Nat. Cancer Inst.*, 44 (1970) 931–936.
- 36 Shortridge, E.H., O'Hara, P.J. and Marshall, P.M., Acute selenium poisoning in cattle, *New Zealand Vet. J.*, 19 (1971) 47–50.
- 37 Stadtman, T.C., Selenium biochemistry, *Annu. Rev. Biochem.*, 59 (1990) 111–127.
- 38 Steffens, A.B., A method for frequent sampling of blood and continuous infusion of fluids in the rat without disturbing the animal, *Physiol. Behav.*, 4 (1969) 833–836.
- 39 Thomson, C.D. and Robinson, M.F., Selenium in human health and disease with emphasis on those aspects peculiar to New Zealand, *Am. J. Clin. Nutr.*, 33 (1980) 303–323.
- 40 Ueno, R., Honda, K., Inoué, S. and Hayaishi, O. Prostaglandin D₂, a cerebral sleep-inducing substance in rats, *Proc. Natl. Acad. Sci. USA*, 80 (1983) 1735–1737.
- 41 Varo, P., Alfthan, G., Ekholm, P., Aro, A. and Koivistoinen, P., Selenium intake and serum selenium in Finland: effects of soil fertilization with selenium, *Am. J. Clin. Nutr.*, 48 (1988) 324–329.
- 42 Whanger, P.D., China; a country with both selenium deficiency and toxicity: some thoughts and impressions, *J. Nutr.*, 119 (1989) 1236–1239.
- 43 Young, V.R., Selenium; a case for its essentiality in man, *New Engl. J. Med.*, 304 (1981) 1228–1230.