it should be noted that DOPA-containing peptides were co-localized with halocyamines in the same hemocytes. Like DOPA-containing peptides and halocyamines of *H. roretzi*, tunichromes of *A. nigra* and *A. ceratodes* have been reported to be mainly localized in one type of their hemocytes [11].

Several biological roles have been proposed for DOPA-containing proteins or peptides. DOPA-containing protein of the marine mussel, *Mytilus edulis*, functions as an adhesive protein through its many repeating DOPA-containing sequences [1, 2]. Ferriascidin of the ascidian, *P. stolonifera*, is assumed to be an iron-binding protein [3, 4]. Tunichromes of the ascidians are potent reductants and are proposed as vanadium-binding and iron-binding compounds in the vanadium-sequestering ascidian, *A. nigra*, and the iron-sequestering one, *M. manhattensis*, respectively [5, 6]. Halocyamines of the ascidian, *H. roretzi*, show antibacterial and cytotoxic activity [7]. We made a preliminary examination of the antibacterial activity of DOPA-containing peptides isolated in this study against *Bacillus subtilis*, by the paper disc method, but could not detect any. It has been reported that vanadium is accumulated at a high concentration in hemocytes of ascidians of the phlebobranch, whereas hemocytes of *H. roretzi* of the stolidobranch contain iron in an amount higher than that of vanadium [12, 13]. Our preliminary experiments showed that halocyamines can reduce ferric iron to ferrous iron. Thus, DOPA-containing peptides including halocyamines isolated from *H. roretzi* hemocytes may function in binding iron, like the ferriascidin of *P. stolonifera* hemocytes and tunichromes of *M. manhattensis* hemocytes.

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Pentobarbital-induced phase shifts of circadian rhythms of locomotor activity are not mediated through stimulated activity in mice

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Summary. The possibility that phase shifts of circadian rhythms of locomotor activity induced by pentobarbital injections are mediated through hyperactivity after recovery from the sedative condition was tested in DBA/2 mice. The mice were restrained for 3 h in a tube immediately after injections of pentobarbital at either CT 9 or CT 0. The results indicated that immobilization did not block the phase shifts, suggesting that pentobarbital-induced phase shifts are not due to increasing the level of activity.
Key words. Circadian rhythms; pentobarbital; phase shifts; mice; immobilization; hyperactivity.

It has been generally accepted that the circadian clock is independent of changes in the external and internal environment and the clock itself is not affected by clock-controlled events such as changes of the level of locomotor activity. However, recent studies in hamsters have indicated that in certain circumstances the clock may be susceptible to feedback from overt activity. Mrosovsky's group has clearly indicated that induced wheel-running brought about by cage-changing and social interactions can induce phase shifts in the circadian clock of the hamster [1, 2]. These findings raised the possibility that the phase-shifting effects reported for some drugs may be mediated by changes of behavioral events, not by direct effects of the drugs on the circadian system. In fact, phase shifts induced by triazolam, which causes hyperactivity in the hamster, are totally blocked by immobilization of the animal during treatment [3].

We have reported that γ-aminobutyric acid (GABA)-active drugs including triazolam, pentobarbital and muscimol induce phase-dependent phase shifts of the circadi-

an rhythm of locomotor activity in SK/Nga mice, and that the phase response curve (PRC) after pentobarbital injections is similar in shape to that obtained in hamsters by stimulated activity such as that resulting from cage-changing[4, 5]. The injection of these drugs does not lead to hyperactivity in mice; in fact they induce inactivity or sedation. However, it is still possible that the level of activity may increase after recovery from the sedative condition[6, 7]. Therefore in the present study we tested this possibility using the same technique as the study of immobilization in hamsters injected with triazolam[3].

As we have reported, in mice there are large strain differences in the response of the circadian system to GABA-active drugs[4, 5]. In the experiment, SK/Nga mice were found to shift their circadian rhythm after injection of the GABA-active drugs, but no effects of these drugs on the circadian system were observed in C57BL/6 mice. Since there are some difficulties in breeding SK/Nga mice, we have tried to look for other strains whose circadian system is able to respond to GABA-active drugs. After screening several strains (C57BL/6, DBA/2, C3H/He, DDD, IVCS, CS), DBA/2 mice were found to show phase shifts induced by pentobarbital injections (Shimizu et al., unpublished results). Therefore, in the present study we used male DBA/2 mice (6–10 weeks at the beginning of the experiment, purchased from Shizuoka Laboratory Animal Center) instead of SK/Nga mice. The first experiment was carried out to obtain the PRC generated by pentobarbital injections, and in the next experiment effects of immobilization on pentobarbital-induced phase shifts were studied.

The animals were maintained in individual cages equipped with a running wheel and were exposed to a light-dark cycle (LD 12:12 h) for at least one week. The animals were then blinded by bilateral enucleation under ether anesthesia. The mice were returned to the cages and allowed to free-run under the LD condition. The cages were not isolated individually, but placed on shelves side by side in a room where the temperature was set at about 24 °C. Food and water were provided ad libitum and replenished once a week. Wheel-running behavior was continuously recorded by an event recorder. After establishing a stable free-running rhythm, each mouse received injections of pentobarbital. In the experiment for the PRC, most of the mice received several injections with at least a 2-week interval between injections. All injections were intraperitoneal. Pentobarbital (Abbott, 50 mg/kg) dissolved in sterile water containing 8% propylene glycol and 2.1% ethanol was injected at various circadian times (CTs) and phase shifts of circadian locomotor activity rhythms were measured. Sterile water containing 8% propylene glycol and 2.1% ethanol was used for control injections. Phase shifts were determined by measuring the phase difference between eye-fitted lines connecting the onsets of activity for approximately 10 days before and after injections. Transients occurring before a steady-state phase shift was achieved were not taken into account to determine phase shifts. For immobilization of the mice, 60 ml polypropylene syringes (29 mm diameter) with holes for respiration were used. The mice were injected with pentobarbital (50 mg/kg) or vehicle (0.15 ml) at either CT 9 or CT 0 and immediately after the injection they were restrained in the tube for 3 h. The phase shifts were calculated and compared between the restrained and the unrestrained mice.

Although injections of vehicle alone had no obvious effects on the circadian rhythm of locomotor activity, single injections of pentobarbital in DBA/2 mice induced phase shifts of the activity rhythm whose directions were dependent on the circadian time of the injections. Phase advances were observed at CT 8, 9, 10 (CT 12 is the onset of locomotor activity) and phase delays were observed from CT 12 to CT 3. The magnitude of the phase shifts was much larger in phase advances (about 1.5 h) than in phase delays (about 0.5 h). The transient for phase advances was observed, but it was not clear in phase delays (fig. 1, upper half; fig. 2).

Immobilization for 3 h did not block the phase-shifting effects of pentobarbital (fig. 1, lower half). Both phase advances and phase delays were observed in the re-

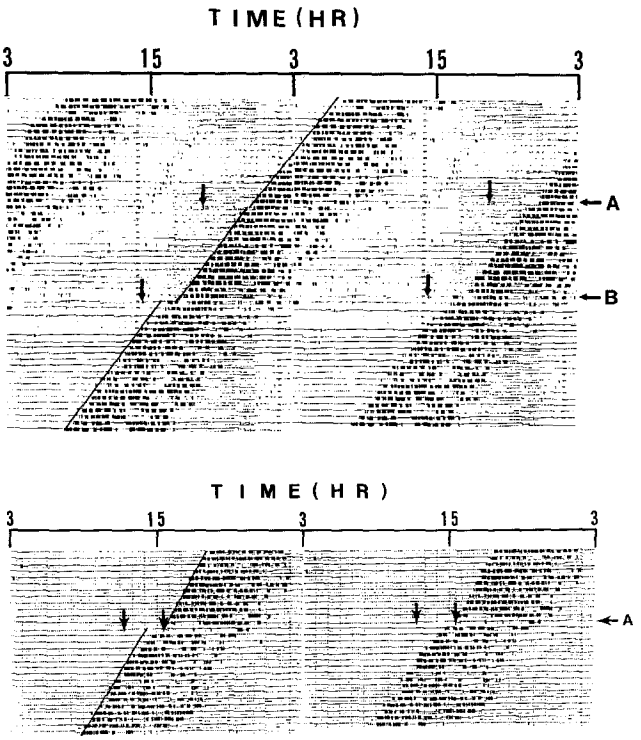

Figure 1. *Upper half*: Effects of a single injection of vehicle (A) or pentobarbital (B) on the phase of the circadian rhythm of locomotor activity in DBA mice. Arrows in the activity records indicate CT 9 when injections were given. Mice were blinded and allowed to free-run. Each horizontal line represents the activity record which is placed sequentially from top to bottom. Double-plot records on a 48-h time scale are shown. Onset of activity, defined as CT 12, was used as a phase reference. *Lower half*: Effects of immobilization on pentobarbital-induced phase shifts in DBA mice. Mice were injected with pentobarbital (50 mg/kg) at CT 9 (the 1st arrow) and immediately after the injection they were restrained for 3 h (until the 2nd arrow). A: the day of the treatment.

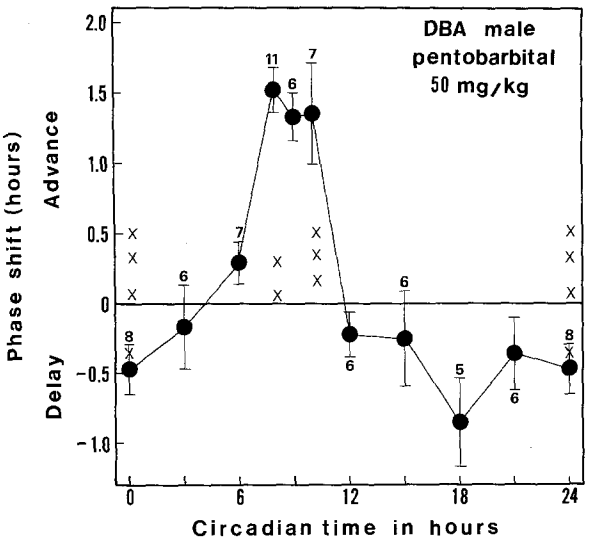

Figure 2. Phase response curve for pentobarbital (50 mg/kg) injections in DBA/2 mice. Each point represents mean (± SEM) phase shifts (h). The values beside each data point indicate the number of animals tested. CT 12 is the time of onset of activity. Values at CT 0 and CT 24 are the same date. x: vehicle injections.

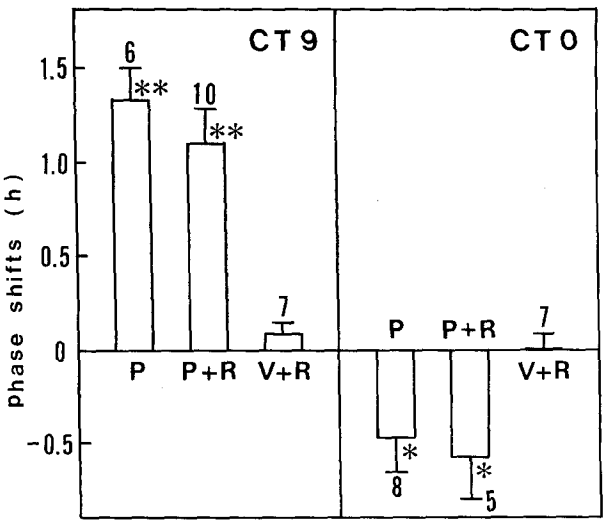

Figure 3. Mean (± SEM) phase shifts (h) of circadian rhythms of locomotor activity in each group at CT 9 and CT 0. The phase shifts are shown by positive values for phase advances and negative values for phase delays. P, pentobarbital (50 mg/kg); P + R, restraint for 3 h after pentobarbital injections (50 mg/kg); V + R, restraint for 3 h after vehicle injections. The values in each group are the number of animals tested. Asterisks indicate significance as compared with control (V + R) ($^{**}p < 0.01$, $^{*}p < 0.05$, Student's t-test).

strained mice with the same amplitude of phase shifts as the unrestrained mice (fig. 3). The transient for phase advances was also observed in the restrained mice. Immobilization without pentobarbital injections at CT 9 and CT 0 had no effects on the phase of the circadian rhythm of locomotor activity.

Pentobarbital injections induce phase-dependent phase shifts of the circadian rhythm of locomotor activity in DBA/2 mice. The PRC for pentobarbital injections obtained in DBA mice is quite similar in shape to that reported in SK/Nga mice[4]. In both strains, large phase advances (1.5–2.0 h) in the late subjective day and small phase delays (0.5–0.8 h at the maximum) in the late subjective night or early subjective day are observed. Although pentobarbital has anesthetic effects, total activity may increase after recovery from the anesthesia[6,7]; recovery from the anesthesia occurs within 1 h after injections. However, our results in the present study show that the restraint does not block the phase shifts, suggesting that the phase shifts induced by pentobarbital in DBA mice are not due to increasing the level of activity.

Other studies have also indicated that an increase in activity is not necessary for inducing phase shifts. Unlike its effects on hamsters, triazolam inhibits activity in mice and squirrel monkeys. Nevertheless, a similar PRC to that observed in hamsters injected with triazolam is obtained in squirrel monkeys[8] and phase advances are induced by an injection of triazolam in the late subjective day in mice[5]. Muscimol, the GABA agonist, also suppressed locomotor activity, but the PRC for microinjections of muscimol into the suprachiasmatic nucleus (SCN) is similar in shape to that for triazolam in hamsters[9] and phase advances in the late subjective day and phase delays in the late subjective night are induced by intraperitoneal injections of muscimol in mice[5]. However, at present we cannot exclude the possibility that phase shifts are mediated by chemically-induced changes in some other factors such as body temperature, hormonal levels and the state of arousal[2].

If such chemically induced changes are not involved for phase shifts, then the drugs must affect the circadian system directly. In hamsters, phase shifts of circadian rhythms are induced by direct effects of muscimol on the SCN, the putative circadian pacemaker[9]. We have also examined the effects of local administration of pentobarbital (1 mM, 10 mM) to the SCN in DBA mice, but no phase shifts have been observed (Ebihara and Hayakawa, unpublished results). The SCN receives multiple afferent connections (e.g. from the midbrain raphe, the intergeniculate leaflet of the lateral geniculate body etc.)[10], therefore it is conceivable that pentobarbital affects the input pathways to the SCN.

In C57BL mice, no phase shifts of circadian rhythms are induced by any GABA-active drugs so far tested[5]. Therefore comparing C57BL mice with DBA mice may be valuable for further understanding of the mechanisms of drug-induced phase shifts of circadian rhythms.

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Effects of selective dopamine D_1 and D_2 antagonists on male rat sexual behavior [1]

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Summary. The effects of selective dopamine (DA) D_1 and D_2 antagonists on male rat sexual behavior were investigated. The D_1 antagonist (+)SCH-23390, 25–100 μg kg^{-1} s.c. – 20 min, and the D_2 antagonist raclopride, 0.1–1.6 mg kg^{-1} s.c., – 20 min, decreased both the number of mounts and intromissions preceding ejaculation. No statistically significant effects in the time up to ejaculation or in the time up to the first intromission were noted, whereas both compounds produced a statistically significant increase in the post-ejaculatory interval. The effect can generally be characterized as psychomotor inhibition, and no evidence was obtained for a specific role of DA D_1 or D_2 receptors in the mediation of male rat sexual behavior.
Key words. Male rat; sexual behavior; dopamine.

It is well known that the administration of drugs that enhance brain dopamine (DA) neurotransmission, like apomorphine, N-*n*-propyl-norapomorphine, *d*-amphetamine or *L*-DOPA, results in a facilitation of male rat sexual behavior, as evidenced by a decrease in the number of intromissions preceding ejaculation and in the time up to ejaculation [4]. These effects, generally small, are probably due to psychomotor stimulation; an inhibition of brain DA neurotransmission, as produced by DA receptor blocking agents, monoamine depleting drugs like tetrabenazine or reserpine, or by lesions of ascending dopaminergic pathways, does not markedly effect the performance of male rat sexual behavior, except in the higher dose range, where the number of animals initiating copulation is diminished [4–7]. Brain DA neurotransmission is mediated via two types of DA receptors: D_1, positively coupled to adenylate cyclase formation, and D_2, not (or negatively) coupled to adenylate cyclase formation [8]. In recent years a number of selective agonists and antagonists which have their effect at these two types of brain DA receptors have been developed.

The results described above, on the role of brain DA in the mediation of male rat sexual behavior, are based on the use of treatments with poor selectivity for the DA D_1 and D_2 receptor subtypes. Therefore in the present experiments we investigated the effects of selective inhibition of brain DA D_1 and D_2 receptors by use of the selective antagonists (+)SCH-23390 [9] and raclopride [10], respectively.

Materials and methods

Animals. Adult male (350–400 g) and female (240–260 g) Wistar rats were used (Möllegaard, Vejle, Denmark). The animals were housed, 5 per cage, under controlled conditions of temperature, humidity and light-dark cycle (12:12 h, lights off 10.00 h). Food (R3, Ewos, Södertälje) and tap water was available ad libitum in the home cage. The animals arrived in the laboratory at least 2 weeks before the experiments to be described below.

Drugs. Raclopride tartrate (Astra, Södertälje, Sweden) and (+)SCH-23390 HCl (RBI, Natick, MA) were used. Both drugs were dissolved in physiological saline and injected subcutaneously in a constant volume of 2 ml kg^{-1}. Doses refer to the form given above.

Behavioral observations. Male rats were presented with a female brought into estrous by sequential treatment with estradiol benzoate (12.5 μg rat^{-1}, i.m. in sesame oil, – 54 h), and progesterone (0.5 mg rat^{-1}, i.m. in sesame oil, – 6 h). The following items of the male rat sexual behavior were recorded: Mounts (M): number of mounts without penile intromission; Intromissions (I): number of mounts with penile intromission; Intromission latency (IL): time from the presentation of the female to the first intromission; Ejaculation Latency (EL): time from the first intromission until ejaculation; Postejaculatory interval (PEI): time from ejaculation until the following intromission. The observations were terminated at the first intromission following ejaculation, but also if the IL was