induced by dopamine. Domperidone did not influence the relaxations induced by dopamine (fig. 2d). These findings indicate that the relaxations induced by dopamine in the human pulmonary arteries are associated with activation of dopamine receptors and the subtype of dopamine receptors appears to be DA_1 receptors. This conclusion regarding the subtype of dopamine receptors agrees with the results with other human [7] and animal [2-4] arteries.

This is the first study demonstrating dopamine-induced relaxation in the human pulmonary arteries. Dopamine and its analogues may be useful therapeutic drugs for certain disorders in pulmonary circulations such as increased pulmonary vascular resistance or right heart failure [9-11].

** To whom reprints requests should be addressed.

1 Goldberg, L. I., and Toda, N., Circ. Res. *36* and *37*, suppl. I (1975) I97.
2 Goldberg, L. I., Glock, D., Kohli, J. D., and Barnett, A., Hypertension *6*, suppl. I (1984) I25.
3 Brodde, O.-E., Freistühler, J., and Meyer, F.-J., J. cardiovasc. Pharmac. *3* (1981) 828.
4 Ohlstein, E. H., Zabko-Potapovich, B., and Berkowitz, B. A., J. Pharmac. exp. Ther. *229* (1984) 433.
5 Ueda, S., Yano, S., and Sakanashi, M., J. cardiovasc. Pharmac. *4* (1982) 76.
6 Toda, N., Experientia *39* (1983) 1131.
7 Forester, C., Drew, G. M., Hilditch, A., and Whalley, E. T., Eur. J. Pharmac. *87* (1983) 227.
8 Hoshino, Y., Obara, H., and Iwai, S., Life Sci. *39* (1986) 2525.
9 Holloway, E. L., Polumbo, R. A., and Harrison, D., Br. Heart J. *37* (1975) 482.
10 Goldberg, L. I., and Rajfer, S., Circulation *72* (1985) 245.
11 Young, J. B., Leon, C. A., Pratt, C. M., Suarez, J. M., Aronoff, R. D., and Roberts, R., J. Am. Coll. Cardiol. *6* (1985) 792.
12 Muramatsu, I., Oshita, M., and Yamanaka, K., J. Pharmac. exp. Ther. *227* (1983) 194.
13 Toda, N., and Goldberg, L. I., Cardiovasc. Res. *9* (1975) 384.

0014-4754/89/020150-03$1.50 + 0.20/0

Single injections of triazolam, a short-acting benzodiazepine, lengthen the period of the circadian activity rhythm in golden hamsters

J. E. Joy, S. Losee-Olson and F. W. Turek

Department of Neurobiology and Physiology, Northwestern University, Evanston (Illinois 60208, USA)
Received 29 August 1988; accepted 26 October 1988

Summary. Single injections of the benzodiazepine, triazolam, induce phase shifts and cause a lengthening of the circadian activity rhythm in the golden hamster. The effect of triazolam on period depends on the phase of injection, but is not dependent on the direction of the phase shifts. Triazolam injections caused increases in period that were associated with phase advances as well as phase delays in the activity rhythm. This relationship between triazolam-induced phase shifts and changes in period is different from the relationship between light-induced phase shifts and period changes.
Key words. Circadian rhythms; benzodiazepines; golden hamsters.

Since the components of circadian oscillators remain unknown, the only two parameters of biological rhythms that provide meaningful information about underlying pacemakers are phase and period [1]. Many different agents are known that can change either phase or period of circadian rhythms, but very little is known about how changes in phase can alter period and vice versa. Light is the only agent for which changes in both phase and period have been described following a single, discrete stimulus. One-hour light pulses that cause phase advances in the circadian activity rhythms of golden hamsters and mice, also cause a shortening of the free-running activity period [2, 3]. In mice, a 1-h light pulse that causes a phase delay also causes a lengthening of the free-running period [2].

It is not known if this relationship between changes in phase and changes in period is a fundamental property of circadian organization or is, instead, a specific property of light-induced phase shifts. Therefore, we decided to explore the interaction between changes in phase and changes in period using an agent other than light. The benzodiazepine, triazolam, is an ideal agent to contrast with the effects of light on different parameters of circadian rhythms. Triazolam is a short-acting benzodiazepine with a half-life in the golden hamster of about 30 min [4]. Like light, triazolam causes both phase advances and phase delays, depending on when it is given to the animal [5]. However, the relationship between the time of the triazolam treatment and the direction of the phase shifts is quite different from the relationship for light pulses. For example, injections of triazolam given to hamsters 6 h before the onset of activity cause maximum phase advances of 90 min, whereas light pulses given at this time cause no phase shifts. Light pulses given 6–8 h after the onset of activity cause phase advances of about 2 h [6, 7], whereas triazolam given at this time causes phase delays of about 30 min [5].

We have previously suggested that triazolam may act on circadian rhythms through a pathway other than the light input pathway [5]. It is also possible that light and triazolam may act on the circadian system in fundamentally different ways. This study shows that the relationships between phase shifts and period changes differ, depending on whether light or triazolam is used to probe the circadian system.

Materials and methods. Adult male golden (Syrian) hamsters, *Mesocricetus auratus* [LAK:LVG(SYR)] were initially group housed under a 14:10-h light/dark cycle before being transferred to constant darkness. Upon transfer to constant darkness, the animals were placed in individual cages equipped with a running wheel connected to an event recorder to allow for continuous monitoring of running wheel activity.

Beginning after two weeks in constant darkness, 92 animals received an intraperitoneal injection of either 2.5 mg of triazolam dissolved in 0.1 ml dimethyl sulfoxide (DMSO) or DMSO alone. This dose of triazolam causes maximum phase advances when injected at the appropriate phase of the activity cycle [8]. All injections were made in the dark with the aid of an infrared viewer (FJW Industries). Some animals received more than one injection. In these cases, consecutive

injections were separated by at least two weeks. Animals were injected at different phases of the circadian activity cycle, with the time of activity onset defined as circadian time (CT) 12.

Free-running activity periods (tau) were estimated using eye-fit regression lines drawn through successive activity onsets [2]. Tau before the injections was calculated using the seven days immediately before the injections. Tau after the injections was calculated for the seven days beginning with the fourth day after the injection. The first three days immediately following the injection were excluded to avoid possible bias due to transient cycles [1].

To verify the consistency of this method of estimating period, an additional method was used on a subset of the data. Period was estimated by calculating the linear regression for the times of successive activity onsets. Period estimates of the two methods agreed within 0.01 h.

Phase shifts were measured as the difference between the post-injection and the pre-injection regression lines for the day immediately after the injection, i.e., as the difference between post-injection activity onsets observed after an injection and the predicted activity onset based on activity onsets prior to an injection. Positive values indicate advances in the rhythm; negative values indicate phase delays.

Unless stated otherwise, differences between experimental groups were tested using t-tests for independent samples. Means are given ±SEM.

Results and discussion. The free-running period of activity was lengthened by triazolam injections given at some phases, but not at others (fig. 1 a). Figure 1 b shows the phase shifts caused by the same injections shown in 1 a. Increases in tau were associated with phase delays, as well as phase advances. Statistically significant increases in tau were seen with triazolam injections at CT 3 and CT 6 (mean increases = 0.16 ± 0.04 h and 0.07 ± 0.02 h, respectively; $p < 0.01$) and also with injections at CT 18 and CT 21 (mean increases = 0.11 ± 0.04 h and 0.15 ± 0.04 h, respectively; $p < 0.01$). The mean change in tau following the vehicle injections across all injection times was 0.01 ± 0.01 h ($n = 33$), which was not a statistically significant change ($p > 0.05$, paired t-test). Figure 2 shows representative samples for triazolam injections that induced phase shifts and/or changes in period.

Comparison of figures 1 a and 1 b shows the relationship between phase shifts and period changes following triazolam injections. In general, phase advances as well as phase delays were associated with increases in period. There is considerable overlap in the times at which triazolam has no effect on either phase or period, although injections at CT 18 are a notable exception. Triazolam injections at this time caused relatively large increases in tau.

Our results indicate that single injections of triazolam can cause a lengthening of the circadian activity rhythm in the golden hamster. The effect of triazolam on period depends on the phase of injection, but is not dependent on the direction of the phase shifts. Increases in tau were associated with both phase advances and phase delays, indicating that the change in tau is not dependent on the phase shifts themselves. Thus a single stimulus can alter either phase or period or both.

This relationship between triazolam-induced phase shifts and changes in period is different from the relationship between light-induced phase shifts and period changes in several important ways. First, the magnitude of the period change is much greater with triazolam injections than with 1-h light pulses. The mean change in period following 1-h light pulses that cause phase advances in golden hamsters is about 0.04 h [1, 3]. If the mean changes for triazolam-induced phase advances are combined (i.e., for injections at CT 3, 6 and 9), the mean period change is 0.09 h. Second, the relationship between phase shifts and period changes is different. Light

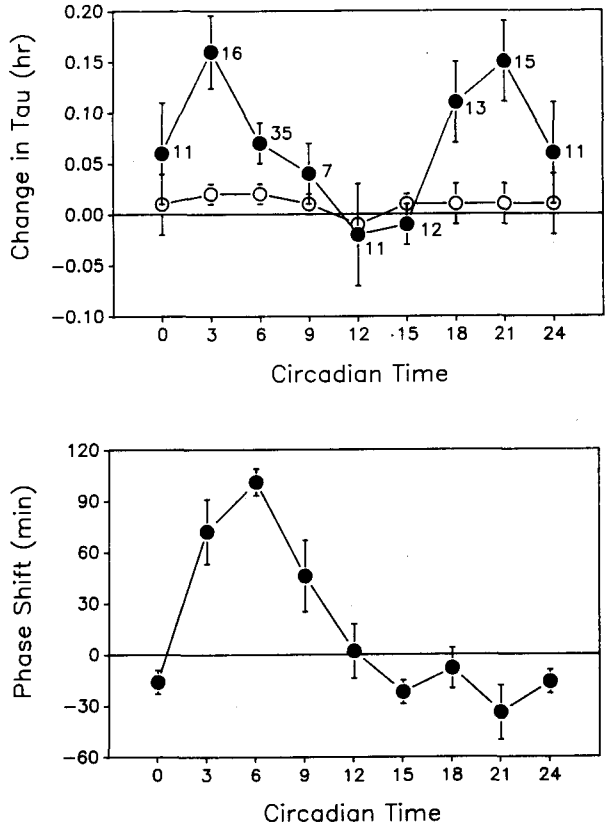

Figure 1. The upper panel (a) shows the mean (±SEM) effect of single triazolam (●) or vehicle (○) injections on the period of the free-running rhythm of activity (tau). Sample sizes are indicated for the triazolam-injected groups. Thirty-three animals received vehicle injections. The lower panel (b) shows the mean (±SEM) phase shifts in the activity rhythm for the same injections as shown in the upper panel.

pulses that cause phase advances are associated with a shortening of period; whereas triazolam injections that cause phase advances are associated with a lengthening of period. Triazolam-induced phase delays are also associated with a lengthening of period. Although light-induced phase delays are associated with increases in period for white-footed mice and house mice, they do not result in significant period changes for golden hamsters or deermice [1].

Triazolam has now been shown to affect circadian rhythms in the hamster in a number of different ways. Endocrine rhythms, as well as behavioral activity rhythms can be regulated by triazolam [9]. Triazolam can cause phase shifts [5], changes in period (present study), changes in the phase relationship between activity and lighting schedules [10], and can affect rates of re-entrainment to altered lighting schedules [11]. Finally, daily injections of triazolam can entrain activity rhythms [12].

It is becoming increasingly apparent that, like light, many drugs affect different parameters of circadian rhythms, with effects on both phase and period. Agents such as lithium, ethanol, clorgyline and deuterium oxide cause a lengthening of period when administered chronically, but have not been shown to cause phase shifts [13–17]. However, chronic treatments with lithium or clorgyline that increase period length in hamsters also influence the effect of light pulses on hamster circadian rhythms [18–20]. Lithium and clorgyline are both used in the treatment of depressive disorders and it has been suggested that some of their therapeutic value is due to their effects on circadian rhythmicity [21–23].

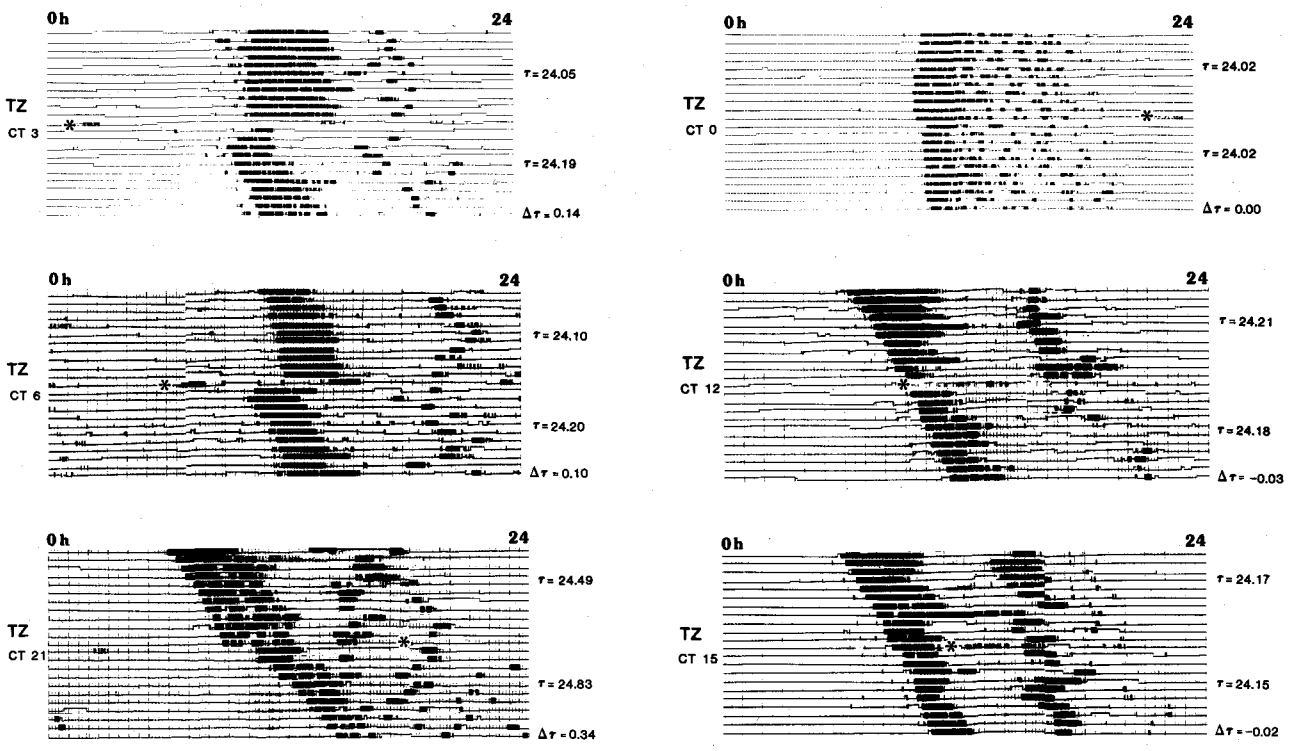

Figure 2. Representative sections of activity records are shown for hamsters before and after triazolam (TZ) injections. Each horizontal line represents 24 h, with successive days plotted from top to bottom. The time of the triazolam injections is indicated by the asterisks and the phase (i.e., circadian time [CT]) of the injection is indicated in the left margin of each activity record. Lengths of free-running periods (τ) before and after the triazolam injections are shown to the right of each activity record, with the change in period ($\Delta\tau$) shown at the bottom right of each record.

It is interesting to note that among the many agents that have been shown to affect either phase or period, triazolam and light are the only agents in which a single, discrete stimulus has been shown to cause phase shifts as well as long-term effects on period. Benzodiazepines are widely prescribed as anxiolytics and for sleep disorders and it is possible that some of their therapeutic effects are due to effects on circadian rhythms. Understanding the various ways that drugs can affect different parameters of circadian systems may be useful in developing strategies for the treatment of mental disorders [24].

Very little is known about the regulation of circadian periodicity. Resettable phase, whereby a discrete stimulus can cause a phase shift, is a general property of circadian clocks. Although period is known to be labile, depending on environmental conditions as well as environmental history [1], resettable period has not appeared to be a general feature of circadian clocks. Based on our earlier results with triazolam injections in hamsters, Winfree [25] raised the possibility that, in addition to resettable phase, the clock mechanism has resettable periodicity. The present results support this hypothesis.

Our view of circadian organization clearly depends on the agents used to probe the system. If only light is used to probe the circadian system, then it would appear that changes in period are induced by phase shifts, such that phase advances cause a shortening of period and phase delays cause a lengthening of period. However, our results with triazolam suggest that this is not a general property of circadian organization. Phase shifts and changes in period may be regulated in a variety of different ways – each subject to numerous influences that may interact or act independently.

1 Pittendrigh, C. S., and Daan, S., J. comp. Physiol. *106* (1976) 223.
2 Pittendrigh, C. S., and Daan, S., J. comp. Physiol. *106* (1976) 253.
3 Nelson, D., Davis, F. C., Menaker, M., and Takahashi, J. S., unpublished results.
4 Turek, F. W., and Losee-Olson, S., unpublished results.
5 Turek, F. W., and Losee-Olson, S., Nature *321* (1986) 167.
6 Ellis, G. W., McKlveen, R. E., and Turek, F. W., Am. J. Physiol. *242* (1982) R44.
7 Takahashi, J. S., DeCoursey, P. J., Bauman, L., and Menaker, M., Nature *308* (1984) 186.
8 Turek, F. W., and Losee-Olson, S., Life Sci. *40* (1987) 1033.
9 Turek, F. W., and Losee-Olson, S., Endocrinology *122* (1988) 756.
10 Turek, F. W., and Losee-Olson, S., J. biol. Rhythms *2* (1987) 249.
11 van Reeth, O., and Turek, F. W., Am. J. Physiol. *253* (1987) R204.
12 Turek, F. W., and van Reeth, O., Trends Neurosci. (1988) in press.
13 Kripke, D. F., and Wyborney, V. G., Life Sci. *26* (1980) 1319.
14 Daan, S., and Pittendrigh, C. S., J. comp. Physiol. *106* (1976) 267.
15 Wirz-Justice, A., Kafka, M. S., Naber, D., Campbell, I. C., Marangos, P. J., Tamarkin, L., and Wehr, T., Brain Res. *241* (1982) 115.
16 Zucker, I., Rusak, B., King, R. G. Jr., in: Advances in Psychobiology, vol. 3, p. 354. Eds A. H. Riesen and R. F. Thompson. John Wiley and Sons, New York 1976.
17 McEachron, D. L., Kripke, D. F., Hawkins, R., and Haus, E., Neuropsychobiology *8* (1982) 12.
18 Duncan, W. C., Sokolove, P. G., Tamarkin, L., and Wehr, T., Soc. Neurosci. *113* (1987) 606.
19 Han, S.-Z., J. interdiscipl. Cycle Res. *15* (1984) 139.
20 McEachron, D. L., Kripke, D. F., and Wyborney, V. G., Psychiat. Res. *5* (1981) 1.
21 Kripke, D. F., Mullaney, D. J., Atkinson, M., and Wolf, S., Biol. Psychiat. *13* (1978) 335.
22 Wehr, T., and Wirz-Justice, A., Pharmacopsychiatry *15* (1982) 31.
23 Wehr, T., Sack, D., Rosenthal, N., Duncan, W., and Gillin, J. C., Fedn Proc. *42* (1983) 2809.
24 Van Cauter, E., and Turek, F. W., Perspect. biol. Med. *29* (1986) 510.
25 Winfree, A. T., Nature *321* (1986) 114.

0014-4754/89/02152-03$1.50 + 0.20/0