Pharmacology Biochemistry & Behavior, Vol. 10, pp. 361–368. Printed in the U.S.A.

Diurnal Rhythms in Noradrenaline Turnover and Motility after Reserpine and 6-Hydroxydopamine[1,2]

BJÖRN LEMMER AND KLAUS WENDA

Centre of Pharmacology, J. W. Goethe-University
Theodor-Stern-Kai 6, D-6000 Frankfurt/M 70
German Federal Republic

(Received 12 July 1978)

LEMMER, B. AND K. WENDA. *Diurnal rhythms in noradrenaline turnover and motility after reserpine and 6-hydroxydopamine*. PHARMAC. BIOCHEM. BEHAV. 10(3) 361–368, 1979.—Experiments were performed in male Wistar rats synchronized by controlled conditions of light (0700–1900 hr) and of darkness (1900–0700 hr). Separately in each photoperiod the effects of reserpine or 6-OHDA on the cardiac noradrenaline, the diurnal variations in the respective turnover and in the motor activity were investigated. Initial depletion of the cardiac noradrenaline after acute application of either drug was significantly greater when injected at 2000 hr compared to 0800 hr. In both photo-periods the cardiac turnover of noradrenaline was increased after peripheral chemical sympathectomy with 6-OHDA as well as after amine depletion with reserpine. Inhibition of the protein synthesis had no effect, ganglionic blockade by chlorisondamine on the other hand abolished the rhythm in the cardiac noradrenaline turnover. Whereas peripheral chemical sympathectomy did not greatly affect the diurnal rhythm in the motor activity, subacute treatment with reserpine differently affected motor activity in both photo-periods, depending on the time of drug application within 24 hr of a day. The results show that diurnal variations in the levels of neuronal and of motor activity are able to influence drug effects and have thus to be taken into account in animal studies.

Diurnal rhythms Cardiac NA turnover Motor activity Reserpine 6-OHDA Chlorisondamine Actinomycine D

THERE is an increasing body of evidence demonstrating the existence and importance of biorhythms for the effects of drugs in man as well as in experimental animals (reviewed in [24]). However, few data are available with respect to chronopharmacological effects of reserpine [2,19] and 6-OH dopamine (6-OHDA) [19]. These drugs were widely used as experimental tools for the investigation of the adrenergic nervous system. In most studies rodents like mice and rats were used, which are known to be night-active animals [1]. Pharmacological experiments on the other hand are mainly performed at day-time, i.e. in the resting phase of the animals and not in their activity period, i.e. during darkness. In rodents not only daily fluctuations in the concentrations of biogenic amines and in the motor activity were described [4, 20, 21, 23, 25, 29], it was also shown that the turnover of noradrenaline in the rat heart and of dopamine in the rat brain exhibit significant daily variations [16,21].

Both reserpine and 6-OHDA have in common a noradrenaline depleting effect brought about, however, by different kinds of mechanisms [13, 14, 27]. Both drugs also differ in the sites of action as after systemic administration penetration of 6-OHDA in contrast to reserpine is limited by the blood-brain-barrier in adult animals [5]. As chronopharmacological differences in the effects of other drugs interfering with the adrenergic transmission on the diurnal variations in the cardiac turnover of noradrenaline [18, 20, 22] and in the motor activity [20] were described, it was the aim of this investigation to study the influence of acute and subacute application of reserpine and 6-OHDA on the concentration and turnover of noradrenaline in the rat heart and on the motor activity on a chronopharmacological basis of the experimental design. It is assumed that differences in the levels of neuronal and motor activity, which are present at different times of the day, may also influence the effects of these two drugs. In order to elucidate further the mechanisms responsible for triggering the diurnal rhythm in the cardiac turnover of noradrenaline [16] the effects of ganglionic blockade with chlorisondamine and of inhibition of the protein synthesis with actinomycine were studied.

METHOD

Environmental Conditions

Male Wistar rats (TNO W. 74, SFP; originally bred in the

[1]This work was supported by a grant of the Deutsche Forschungsgemeinschaft.
[2]Part of this work was presented at the Spring Meeting of the German Pharmacological Society, Mainz, 1976 [19].

"TNO-Institut for Breeding of Laboratory Animals" in Zeist, Netherlands, now derived and bred by Fa. Winkelmann, Borchen-Kirchborchen, W. Germany) of about 100–150 g were used. Groups of 10 animals were housed in a separate room under a controlled lighting regimen of 12 hr of light (L:0700–1900 hr) alternating with 12 hr of darkness (D:1900–0700 hr). The room temperature was 23 ± 1°C, the relative humidity 45% and the animals had free access to food and tap water during the experiments. The animals were allowed to live under these standardized conditions for at least 5 days before being used. Twenty-four hours before starting the experiments the rats were placed in groups of 5 animals in plastic cages (Makrolon®, 500×350×200 mm).

Biochemical Studies

In the acute experiments reserpine (0.1 and 3 mg/kg) or 6-OHDA (1 and 25 mg/kg) was injected either at 0800 or at 2000 hr. Groups of 5 animals were sacrificed 0, 1.5, 3.5, 6.5 and 9.5 hr thereafter. After decapitation the hearts were removed, rinsed in fresh 0.9% saline solution, blotted on filter-paper, weighed and stored in liquid nitrogen for determination of noradrenaline the next day. After homogenizing the hearts in acidified n-butanol the procedure of extraction, absorption and delusion from aluminia, and the spectrofluorometric determination of noradrenaline was performed according to the method of Chang [3] as modified previously [16]. In each experiment internal standards were run in parallel, which allowed correction of assay values.

In the subacute experiments reserpine was injected either at 0800 hr or at 2000 hr on three consecutive days. Chemical sympathectomy was achieved by injecting 2×25 mg/kg 6-OHDA at the first day either at 0800 hr and at 1800 hr (light) or at 2000 hr and at 0600 hr (darkness) with an additional dose of 100 mg/kg on the 5th day at 0800 hr or at 2000 hr. After both dosage regimens the cardiac turnover of noradrenaline was determined 24 hr after the last dose from the logarithmic deline of ^{3}H-(–)-noradrenaline (spec. activity 7.8–8.2. Ci/mmole, Radiochemical Centre, Amersham) after IV injections of tracer doses (0.2 μg/kg) of the labelled amine either at 0830 hr or at 2030 hr. The concentrations of the endogenous and the ^{3}H-noradrenaline in the rat hearts were determined 1, 3, 6 and 9 hr after injection of the labelled amine. ^{3}H-Noradrenaline was specifically determined in 0.5 ml samples of the alumina eluate (see above) by liquid scintillation counting and the cpm of ^{3}H-(–)-noradrenaline/g heart (cpm/g) were then calculated after volume correction as previously described [16]. Groups of saline injected control animals were investigated in parallel in order to exclude possible seasonal variations.

In additional experiments the cardiac turnover and release of noradrenaline were determined in both photoperiods following ganglionic blockade with chlorisondamine (10 mg/kg, IP injected 30 min before ^{3}H-noradrenaline, i.e. either at 0800 hr or at 2000 hr) or after inhibition of the protein synthesis with actinomycine D (0.8 mg/kg SC, dissolved in isotonic saline containing 10% ethanol) 24 hr before IV injections of ^{3}H-(–)-noradrenaline either at 0830 hr or at 2030 hr.

Studies on Motor Activity

Motor activity in groups of 5 rats was determined using a 2-channel Animex motimeter (Farad AB, Stockholm). Total motor activity was registered on channel A at a sensitivity of 47 μA and the mainly running activity was registered at a sensitivity setting of 10 μA on channel B as differentiated in earlier experiments [20]. The printing counter of the Animex was placed in a separate room and the printing interval was 30 min. In order to minimize registration of gross exploratory and social behaviour in a new environment the animals were housed and tested with the same subjects. As significant seasonal variations in the motor activity exist (unpublished results), the experiments on motor activity were performed within 4 weeks in October.

Statistical Analysis

In the acute experiments the unpaired two-tailed Student's *t*-test was used to evaluate statistical significance in the initial noradrenaline depletion 1.5 hr after application of the drugs obtained either during light or during darkness. In the turnover experiments the regression lines of the logarithmic decline of the cpm ^{3}H-noradrenaline/g were calculated by the method of least squares, and linearity of the regression functions was tested by the F-distribution. Significance between the regression lines obtained from control and drug treated animals was tested by the unpaired two-tailed Student's *t*-test.

RESULTS

1. Acute Experiments

As can be seen from Fig. 1 acute injection of reserpine (0.1 or 3 mg/kg) resulted in a dose-dependent decrease of the cardiac noradrenaline concentrations in both photo-periods. After application of the lower dose of reserpine the initial noradrenaline depletion 1.5 hr later was significantly greater ($p<0.025$) when the drug was injected at the beginning of the dark period (65.4% of initial value) than at the beginning of the light period (84.2% of initial value). No significant light-to-darkness differences in the initial noradrenaline depletion were observed after 3 mg/kg of reserpine (Fig. 1). During darkness motor activity was also depressed dose-dependently by reserpine (Fig. 1) when compared to saline injected control animals (Fig. 2). During light, however, a slight increase in motor activity was observed after the low dose of 0.1 mg/kg of reserpine. The cumulative activity counts registered in each investigation period of 9 hr duration are compiled in Table 1.

Figure 3 shows that an acute injection of 6-OHDA (1 or 25 mg/kg IV) also reduced the cardiac noradrenaline concentration in both photo-periods dose-dependently. At the low dose of 1 mg/kg 6-OHDA the initial noradrenaline depletion 1.5 hr after drug injection at 2000 hr (53.4% of initial value) was not only significantly greater ($p<0.025$) but also longer lasting than when the same dose was injected at 0800 hr which lead to a decrease in the cardiac noradrenaline by about 30% 1.5 hr later. At the high dose of 6-OHDA no light-to-darkness differences were observed. Immediately after injections of either dose of 6-OHDA sympathomimetic reactions (piloerection, protrusion of eyes, etc.) were observed. When compared to controls (Fig. 2) the pattern of the motor activity during darkness was more markedly influenced by either dose of 6-OHDA than the cumulative activity counts (Table 1). In resting phase no clearcut effects of 6-OHDA on motor activity could be observed (Fig. 3, Table 1). The pronounced initial reduction in motor activity during darkness by 25 mg/kg 6-OHDA (Fig. 3) can possibly be re-

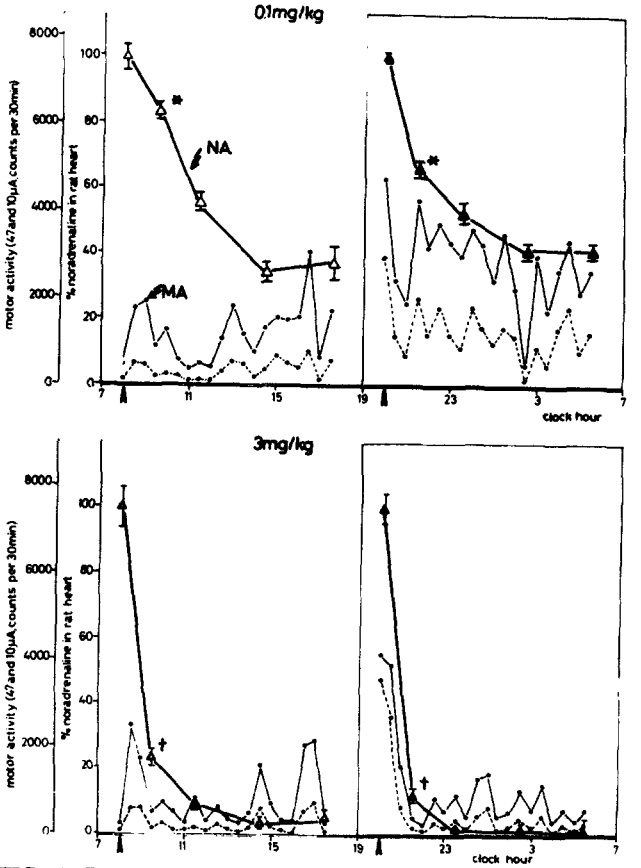

FIG. 1. Reserpine (0.1 or 3 mg/kg, SC) was injected into synchronized rats (light: 0700 hr–1900 hr, darkness 1900–0700 hr) either at 0800 hr or at 2000 hr as indicated by the arrows. NA (triangles) represents the cardiac noradrenaline concentrations as percent of initial values (0.87–0.95 $\mu g \cdot g^{-1}$); depicted are the means ± SEM of 5 rats each. Significance between the initial noradrenaline depletion obtained 1.5 hr after drug injection in either photoperiod, i.e. at 0930 hr or at 2130 hr: $*p<0.025$, $\dagger p>0.05$. MA represents the motor activity of groups of 5 rats with a two-channel Animex motimeter, thereby differentiating between total motor activity (●——●) and the running activity (● – – – ●). For controls in motor activity see Fig. 2.

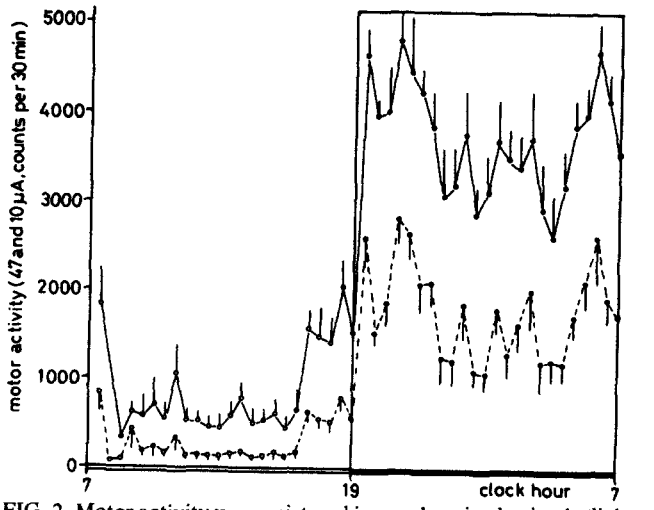

FIG. 2. Motor activity was registered in synchronized animals (light: 0700 hr–1900 hr, darkness: 1900–0700 hr) with a two-channel Animex motimeter, thereby differentiating between total motor activity (channel A: 47 μA, solid line) and the mainly running activity (channel B: 10 μA). Counting interval was 30 min, mean values ± SEM of 8 groups of 5 rats each. Experiments were performed in October.

lated to the marked indirect sympathomimetic effects of this drug, mainly an increase in blood pressure [8,14].

2. Subacute Experiments:

2.1 Turnover studies. Twenty-four hours after a three days treatment with reserpine (3 mg/kg/d) body weight of the rats was significantly reduced below initial values (Table 2). The mean heart weight of the reserpine treated animals was also significantly reduced by about 25%. In both photoperiods the initial uptake of ^{3}H-(-)-noradrenaline in the hearts of the reserpine treated rats was diminished by about 50% (Fig. 4), though the endogenous noradrenaline concentrations were reduced to about 10% of control (Table 2). Figure 4 shows that treatment with reserpine resulted in an increased release of tritiated noradrenaline from the rat heart in both photo-periods. As steady-state conditions were present the increased release of ^{3}H-noradrenaline represents an increase in the cardiac turnover of noradrenaline. Thus, pretreatment with reserpine in the period of light reduced the half-life of the cardiac turnover of noradrenaline from 9.7 to 4.5 hr and from 5.6 hr to 3.4 hr in the period of darkness (Fig. 4).

Chemical sympathectomy with 6-OHDA in contrast to reserpine did not reduce the body weight of the rats below initial values, however, chemical sympathectomy significantly reduced the physiological increase in body weight (Table 2). The mean heart weight of the 6-OHDA treated rats was slightly but significantly reduced to 88% of controls (Table 2). Peripheral chemical sympathectomy also greatly reduced the initial uptake of ^{3}H-noradrenaline in the rat heart to about 10% as can be seen from Fig. 4 and the endogenous cardiac noradrenaline concentrations were equally reduced (Table 2). Within both photoperiods the half-lives of the cardiac turnover of noradrenaline were reduced by chemical sympathectomy to the same degree as already observed after daily treatment with reserpine, i.e. during light from 10.8 to 4.2 hr and during darkness from 6.7 hr to 3.5 hr (Fig. 5).

In additional turnover experiments ganglionic blockade was performed by administration of chlorisondamine (10 mg/kg, 30 min prior ^{3}H-noradrenaline) and the protein synthesis was inhibited by pretreatment with actinomycine D (0.8 mg/kg, 24 hr prior ^{3}H-noradrenaline) and the decline of IV injected ^{3}H-(-)-noradrenaline in the rat hearts was studied thereafter in both photo-periods. The results of these experiments are summarized in Table 3. It can be seen that inhibition of the protein synthesis did not influence the parameters of the cardiac turnover of noradrenaline in either photoperiod, and this drug had, therefore, no influence on the daily rhythm. Ganglionic blockade, on the other hand, greatly reduced the increase in the turnover normally observed in control animals during darkness (Table 3). The half-life of the ^{3}H-noradrenaline decline increased from 6.6 hr in control rats to 43.7 hr after chlorisondamine. During light, i.e. during the resting phase of the animals, no signifi-

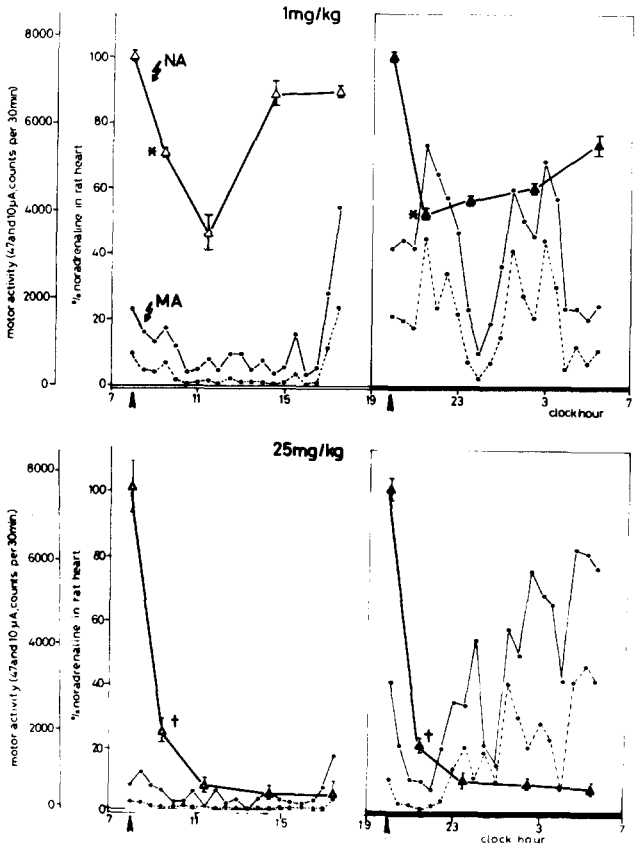

FIG. 3. 6-OHDA (1 or 25 mg/kg, IV) was injected into synchronized rats (light: 0700–1900 hr, darkness: 1900–0700 hr) either at 0800 hr or at 2000 hr as indicated by the arrows. NA (triangles) represents the cardiac noradrenaline concentrations as percent of initial values (0.92–0.99 $\mu g \cdot g^{-1}$), depicted are the means ± SEM of 5 rats each. Significance between the initial noradrenaline depletion obtained 1.5 hr after drug application in either photoperiod, i.e. at 0930 hr or at 2130 hr: $^{*}p<0.025$, $\dagger p>0.05$. MA represents the motor activity of groups of 5 rats as registered with a two-channel Animex motimeter, thereby differentiating between total motor activity (●——●) and the running activity (● – – – ●). For controls in motor activity see Fig. 2.

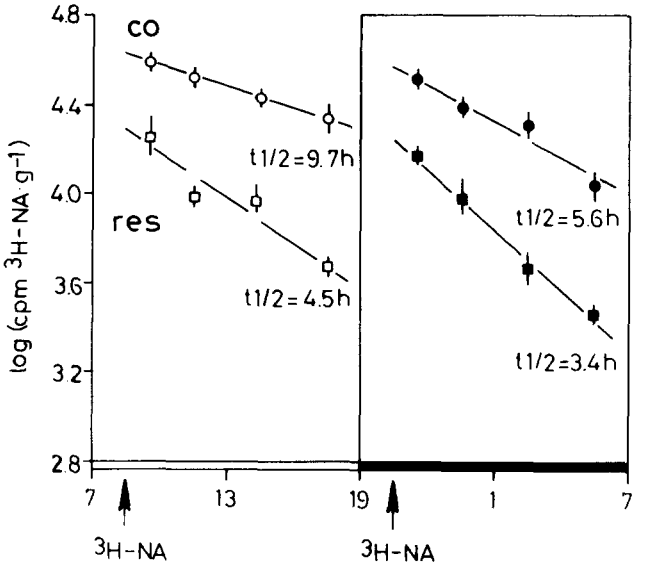

FIG. 4. Reserpine was injected at a dose of 3 mg/kg/day for 3 consecutive days either always at 0800 hr or at 2000 hr. The decline of ^{3}H-(–)-noradrenaline per g rat heart was determined 24 hr after the last dose in either photoperiod after IV injection of a tracer dose of ^{3}H-(–)-noradrenaline (arrows). Co=control animals, res=reserpine treated rats. In the period of light the slopes of the regression lines control to reserpine were significantly different ($p<0.005$).

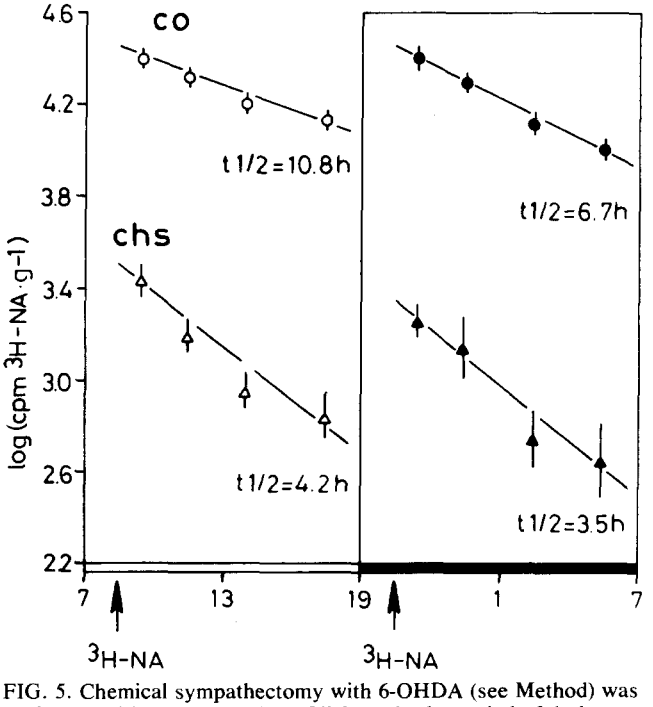

FIG. 5. Chemical sympathectomy with 6-OHDA (see Method) was performed either in the period of light or in the period of darkness. The decline of ^{3}H-(–)-noradrenaline per g rat heart was determined 6 days later in either photoperiod after injection of a tracer dose of ^{3}H-(–)-noradrenaline (arrows). Co=control animals, chs=chemical sympathectomy. In the period of light the slopes of the regression lines control to chemical sympathectomy were significantly different ($p<0.025$).

cant effect of chlorisondamine on the half-life was observed. However, the endogenous cardiac noradrenaline concentration was significantly increased by chlorisondamine in the period of light but not during darkness. Such an increase was described earlier [10] from experiments which were apparently performed during the light cycle.

2.2 Experiments on motor activity. During the subacute experiments with reserpine and 6-OHDA motor activity of groups of 5 rats was continuously recorded with a two-channel Animex motimeter. As can be seen from Figs. 6 and 7, the daily variations in the motor activity were quite differently affected by reserpine, depending on whether the drug was applied at the beginning of the light period or at the beginning of the period of darkness. Figure 6 shows that injections of reserpine (3 mg/kg) 1 hr after the onset of darkness almost suppressed the physiological increase in the motor activity thereafter.

TABLE 1

CUMULATIVE MOTOR ACTIVITY IN SYNCHRONIZED RATS UNDER VARIOUS TREATMENTS

	Light		Darkness	
	A	B	A	B
Control (8)	12780 ± 842	3813 ± 363	68355 ± 2840	30446 ± 2764
6-OHDA				
1 mg/kg	16764	4179	57965	29378
25 mg/kg	8007	2155	60798	25639
Chemical Sympathectomy	9065	2172	74756	42673
Reserpine				
0.1 mg/kg (2)	19717	6862	45537	18162
3.0 mg/kg (3)	10879 ± 2924	2765 ± 883	15806 ± 1460	4909 ± 640
Subacute Treatment (with 3 × 3 mg/kg/d)	23256	9466	51071	17946

Cumulative motor activity (activity counts/9 hr) was registered in groups of 5 rats with a two-channel Animex motimeter. The animals were synchronized by alternating spans of light (0700 hr–1900 hr) and darkness (1900–0700 hr). A represents the total motor activity (channel setting 47 μA) and B represents the mainly running activity (channel setting 10 μA). Shown are the mean activity counts/9 hr ± SEM, obtained after various treatments in either photo-period, (n)=number of experiments. For time-schedule of drug treatment see Material and Methods.

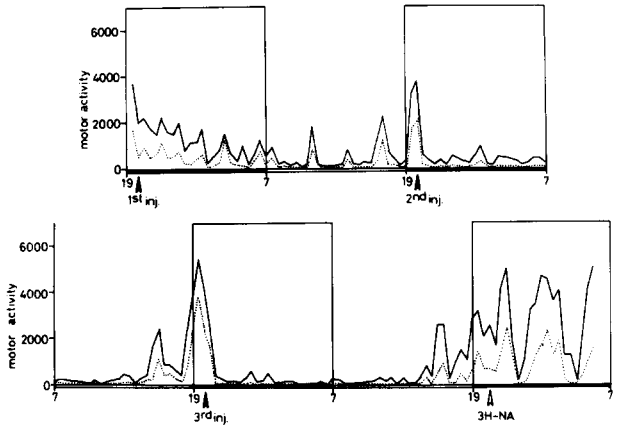

FIG. 6. Reserpine (3 mg/kg, black arrows) was injected into synchronized rats at 2000 hr on three consecutive days. Motor activity of a group of 5 rats was registered continuously with a two-channel Animex motimeter, thereby differentiating between total motor activity (47 μA, solid line) and running activity (10 μA, dashed line). Cardiac noradrenaline turnover was determined on the 4th night by means of IV injection of ^{3}H-(–)-noradrenaline (open arrow).

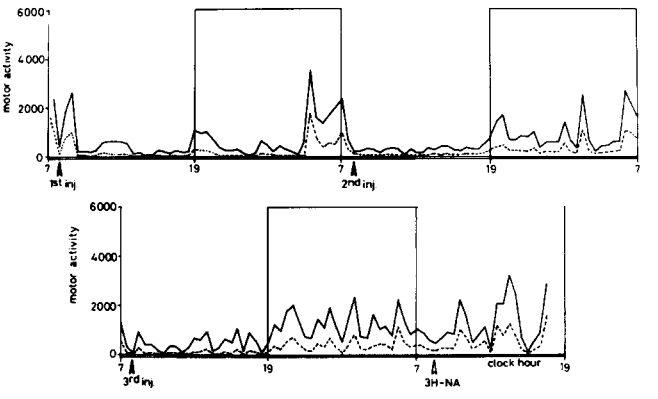

FIG. 7. Reserpine (3 mg/kg, black arrows) was injected into synchronized rats at 0800 hr on three consecutive days. Motor activity of a group of 5 rats was registered continuously with a two-channel Animex motimeter, thereby differentiating between total motor activity (47 μA, solid line) and running activity (10 μA, dashed line). Cardiac noradrenaline turnover was determined on the 4th day by means of IV injection of ^{3}H-(–)-noradrenaline (open arrow).

However, 24 hr later motor activity again increased at the onset of darkness and was immediately depressed again by the following dose of reserpine. When reserpine was omitted in the 4th night (Fig. 6) motor activity was not totally depressed in this photo-period: The cumulative activity counts were reduced to 75% (total motor activity) and 59% (running activity) of controls (Table 1). After injection of reserpine at the beginning of the light period (Fig. 7) motor activity was always reduced in the consecutive period of darkness. However, 24 hr after the last dose of reserpine (Fig. 7) the cumulative activity counts were 2–3 times above controls normally registered in the period of light (Table 1).

TABLE 2

EFFECTS OF RESERPINE AND PERIPHERAL CHEMICAL SYMPATHECTOMY WITH 6-OHDA ON BODY WEIGHT, HEART WEIGHT AND CARDIAC NORADRENALINE IN SYNCHRONIZED RATS

Photoperiod	Control				Drug			
	Body Weight (g)		Heart Weight (g)	Noradrenaline ($\mu g.g^{-1}$)	Body Weight (g)		Heart Weight (g)	Noradrenaline ($\mu g.g^{-1}$)
	before	after			before	after		
Reserpine								
L	122.9 ± 0.9	138.1 ± 1.1	0.457 ±0.007	0.94 ±0.03	122.5 ± 1.0	95.5*† ± 1.6	0.337* ±0.006	0.06 ±0.004
D	91.2 ± 2.1	106.2 ± 2.3	0.402 ±0.011	0.92 ±0.03	93.2 ± 2.3	75.6*† ± 2.1	0.302* ±0.008	0.04* ±0.004
6-OHDA								
L	116.7 ± 1.7	144.0 ± 1.9	0.514 ±0.006	0.90 ±0.02	120.1 ± 1.2	129.0*† ± 1.3	0.453 ±0.007	0.05* ±0.003
D	104.4 ± 1.2	133.1 ± 1.8	0.465 ±0.006	0.83 ±0.03	105.7 ± 1.0	123.3*† ± 1.4	0.408* ±0.006	0.09* ±0.006

Reserpine was injected at a dose of 3 mg/kg SC for 3 consecutive days either 1 hr after the beginning of the light period (L:0700–1900 hr) or 1 hr after the onset of darkness (D:1900–0700 hr). Thirty-three hr after the last dose the rats were killed. Chemical sympathectomy with 6-OHDA (see Material and Methods) was also performed separately in either photo-period. Rats were killed on the 6th day. Mean values ± SEM of 20–24 rats.

*p to controls <0.001,two-tailed Student's t-test.

†p before to after <0.001, two tailed Student's t-test.

TABLE 3

EFFECTS OF ACTINOMYCINE D AND CHLORISONDAMINE ON THE CARDIAC TURNOVER OF NORADRENALINE

	Light			Darkness		
	Half Life $t_{1/2}$ (hr)	Rate Constant $k(h^{-1})$	Noradrenaline ($\mu g.g^{-1}$)	Half-Life $t_{1/2}$ (hr)	Rate Constant $k(h^{-1})$	Noradrenaline ($\mu g.g^{-1}$)
Control	13.1	0.0528	0.82 ± 0.03	8.3	0.0839	0.86 ± 0.04
Actinomycine D	15.8	0.0457	0.86 ± 0.04	7.4	0.0938	0.77 ± 0.04
Control	10.1	0.0684	0.94 ± 0.04	6.6	0.1055	0.89 ± 0.05
Chlorisondamine	15.2	0.0456	1.29 ± 0.05*	43.7	0.0158†	0.92 ± 0.03

In synchronized rats (light: 0700 hr—1900 hr, darkness: 1900–0700 hr) the parameters of the cardiac turnover of noradrenaline were determined separately in each photo-period from the logarithmic decline of IV injected ^{3}H-(-)-noradrenaline. Actinomycine D (0.8 mg/kg SC) was injected 24 hr before and chlorisondamine (10 mg/kg IP) 0.5 hr before the labelled amine. The noradrenaline concentrations are mean values ± SEM of 10–15 animals.

*p to control<0.001, two-tailed Student's t-test.

†Slope of regression line significantly (p<0.001) different from that of control.

In contrast to reserpine peripheral chemical sympathectomy with 6-OHDA performed either during light or during darkness did not greatly affect the diurnal variations in the motor activity of the rat (Fig. 8). Indirect sympathomimetic effects of 6-OHDA were only observed after the initial injections of the drug in either photo-period, after the third injection no such effects were observed. A slight increase in the cumulative running activity was registered during darkness 24 hr after the last dose of 6-OHDA (Table 1).

DISCUSSION

The experiments performed in male rats synchronized by alternating spans of light and darkness clearly show that biochemical and behavioural effects of reserpine and 6-OHDA are greatly influenced by the time-point of application within 24 hr of a day.

Earlier investigations had already shown that the turnover of noradrenaline in the rat heart in vivo was significantly enhanced in the period of darkness when compared with the period of light [16], results which were reproduced in the present experiments. This study shows that also the degree of initial noradrenaline depletion on acute application of reserpine or 6-OHDA was significantly greater when the drugs were injected shortly after the onset of darkness. In whole rat brain a similar daily rhythm in noradrenaline depletion by

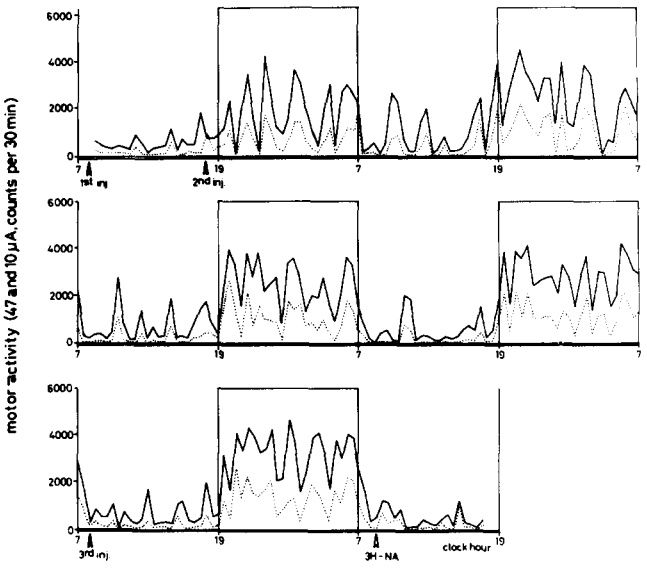

FIG. 8. 6-OHDA was injected into synchronized rats in the light period. First and second dose: 25 mg/kg IV, third dose 100 mg/kg IV (black arrows). Motor activity of a group of 5 rats was registered continuously with a two-channel Animex motimeter, thereby differentiating between total motor activity (47 μA, solid line) and running activity (10 μA, dashed line). Cardiac noradrenaline turnover was determined on the sixth day by means of IV injection of ^{3}H-(-)-noradrenaline (open arrow).

reserpine has been reported [2]. The results indicate that the daily variations in neuronal activity as represented by daily variations in the amine turnover are able to modify also drug-induced noradrenaline release. Furthermore, the experimental results obtained after ganglionic blockade with chlorisondamine indicate that daily variations in the central impulse flow are mainly responsible for triggering the daily variations in the release and turnover of noradrenaline from the rat heart. As earlier data had shown that the reserpine-induced noradrenaline depletion is partly dependent on sympathetic reflex activity [9–12, 15, 26] the present results extend these findings in as demonstrating the importance of the physiological daily variations in impulse flow for the action of noradrenaline depleting drugs. However, it cannot be excluded that also the sensitivity of the storage vesicles to reserpine-induced amine release exhibits daily variations as also daily variations in the granular vesicle count have been reported [28]. From these histochemical studies it was assumed that under conditions of increased activity the formation of vesicles is facilitated and the mobilization of newly formed vesciles is enhanced [28].

In contrast to ganglionic blockade inhibition of the protein synthesis by actinomycine D had no effect on the diurnal rhythm of the cardiac turnover of noradrenaline. This indicates that variations in the central mediated transmitter release are of greater importance than daily variations in the synthesis of enzymes responsible for the biosynthesis of catecholamines.

Though the mechanisms of the noradrenaline depletion by reserpine and by 6-OHDA are different [13, 14, 27] peripheral chemical sympathectomy as well as daily administration of reserpine lead to an identical decrease in the half-lives of the cardiac turnover of noradrenaline in either photo-period. After both treatments the cardiac noradrenaline concentrations were reduced to about 10% of control. Interestingly, after a 21 days treatment with guanethidine, which also decreased the cardiac noradrenaline concentration by about 90%, a half-life of the cardiac turnover of noradrenaline of 4 hr was found in the period of light [17]. Thus, three different drugs with different sites of action brought about an identical decrease in the half-life of the cardiac noradrenaline turnover in the period of light. These data support the hypothesis that a reflex mediated increase in sympathetic activity on peripheral noradrenaline depletion is of greater importance for drug-induced increase in the cardiac turnover of noradrenaline in vivo than the mechanisms by which this depletion is brought about. Furthermore, the present data show that concomitant depletion of central amine stores by reserpine had no additional effect on the half-life of the cardiac noradrenaline when compared with the effects of peripheral chemical sympathectomy.

Whereas the central and/or peripheral sites of action of reserpine and 6-OHDA, resp. were not reflected in the turnover experiments, clear-cut differences in the effects of these two drugs were observed in the experiments on motor activity. Aside from the initially observed short lasting decrease in motor activity, which can be possibly related to the indirect sympathomimetic effects of 6-OHDA [8,14], peripheral chemical sympathectomy did not greatly influence the diurnal rhythm in the spontaneous motor activity of the rat,—independent from the time of drug application.

Daily administration of reserpine on the other hand greatly affected the levels and rhythm of the motor activity with additional influences resulting from the time of application of this drug. Nevertheless, depletion of central and peripheral amine stores by reserpine did not completely inhibit rhythmic changes in motor activity. The motility studies show that a time interval of about 24 hr has to elapse after the last reserpine injection so that darkness can trigger an increase in motor activity, though the endogenous amine stores are almost completely depleted.

Earlier investigations had already shown that a small functional transmitter pool is able to restore the neuronal function in central noradrenergic neurons [6] and it was concluded that the reappearance of adrenergic function after reserpine treatment is closely connected with the transport of newly formed amine storing granules, which have the greatest capacity to take up and retain catecholamines [7]. The half-life of this capacity in the young granules was calculated to be about 12 hr [7]. These data are in close relation to the findings observed in the present study on motor activity. In addition, it has to be taken into account that the turnover of the central noradrenaline and dopamine with half-lives in the range of 2.3–4.2 hr, depending on the time of the day [21], is much faster than that of the cardiac noradrenaline [16], so that the effects of a small functional pool in the central nervous system will reappear much earlier. Thus, the effects of reserpine observed on the diurnal variations in the motor activity of the rat greatly support the hypothesis of the importance of a small functional pool for various physiological functions.

In conclusion, the biochemical and behavioural findings described in the present study clearly show temporal dependencies in the effects of noradrenaline depleting drugs in synchronized animals.

ACKNOWLEDGEMENT

We are thankful to Ciba-Geigy AG for the generous gift of chlorisondamine-HCl. The authors wish to thank Mrs. G. Winklmaier for her excellent assistance. This work is part of the M.D.-thesis of K.W.

REFERENCES

1. Aschoff, J. Die Tagesperiodik licht- und dunkelaktiver Tiere. *Rev. Suisse Zool.* **71:** 528–558, 1964.
2. Black, J. B., L. Parker and J. Axelrod. A daily rhythm in the rate of depletion of brain norepinephrine by reserpine. *Biochem. Pharmac.* **18:** 2688–2691, 1969.
3. Chang, C. C. A sensitive method for spectrofluometric assay of catecholamines. *Int. J. Neuropharmac.* **3:** 643–649, 1964.
4. Friedman, A. H. and C. A. Walker. Circadian rhythms in rat mid-brain and caudate nucleus biogenic amine levels. *J. Physiol.* **197:** 77–85, 1968.
5. Garver, D., J. Cedarbaum and J. Maas. Blood-brain barrier to 6-OH dopamine: Uptake by heart and brain. *Life Sci.* **17:** 1081–1084, 1975.
6. Häggendahl, J. and M. Lindquist. Disclosure of labile monoamine fractions in brain and their correlation to behaviour. *Acta Physiol. scand.* **60:** 351–357, 1964.
7. Häggendahl, J. and A. Dahlström. The recovery of the capacity for uptake-retention of ^{3}H-noradrenaline in rat adrenergic nerves after reserpine. *J. Pharm. Pharmac.* **24:** 565–575, 1972.
8. Häusler, G. Early pre- and postjunctional effects of 6-hydroxydopamine. *J. Pharmac. exp. Ther.* **178:** 49–62, 1971.
9. Hertting, G., J. Axelrod and R. W. Patrick. Actions of bretylium and guanethidine on the uptake and release of ^{3}H-noradrenaline. *Br. J. Pharmac.* **18:** 161–166, 1962a.
10. Hertting, G., L. T. Potter and J. Axelrod. Effect of decentralization and ganglionic blocking agents on the spontaneous release of ^{3}H-norepinephrine. *J. Pharmac. exp. Ther.* **136:** 289–292, 1962b.
11. Holzbauer, M. and M. Vogt. Depression by reserpine of the noradrenaline concentration in the hypothalamus of the cat. *J. Neurochem.* **1:** 8–11, 1956.
12. Iggo, A. and M. Vogt. Preganglionic sympathetic activity in neuronal and reserpine-treated cats. *J. Physiol., Lond.* **150:** 114–133, 1960.
13. Iversen, L. L. *The Uptake and Storage of Noradrenaline in Sympathetic Adrenergic Nerves.* London: Cambridge University Press, 1967.
14. Kostrzewa, R. M. and D. M. Jacobowitz. Pharmacological actions of 6-hydroxydopamine. *Pharmac. Rev.* **26:** 199–288, 1974.
15. Kroneberg, G. and H. J. Schümann. Die Wirkung des Reserpins auf den Hormongehalt des Nebennierenmarks. *Arch. exp. Path. Pharmak.* **231:** 349–360, 1957.
16. Lemmer, B. and R. Saller. Influence of light and darkness on the turnover of noradrenaline in the rat heart. *Naunyn-Schmiedeberg's Arch. Pharmac.* **282:** 75–84, 1974.
17. Lemmer, B., R. Saller and H. Grobecker. Increased cardiac turnover of noradrenaline after chronic administration of guanethidine in the rat. *Experientia* **30:** 379–380, 1974.
18. Lemmer, B. and R. Saller. Effect of propranolol on the diurnal rhythm of the cardiac turnover of noradrenaline in the rat. Second Congr. Hung. Pharmacol. Soc., Publishing House of the Hung. Acad. Sci., pp. 173–176, 1976.
19. Lemmer, B. and K. Wenda. Chronopharmacological investigations on the effects of 6-OH dopamine and reserpine on the motor activity and the cardiac content and turnover of noradrenaline in the rat. *Naunyn-Schmiedeberg's Arch. Pharmac.* Suppl. **293:** 18, 1976.
20. Lemmer, B. and T. Berger. Diurnal variations in the motor activity of the rat: Effects of inhibitors of the catecholamine synthesis. *Naunyn-Schmiedeberg's Arch. Pharmac.* **303:** 251–256, 1978a.
21. Lemmer, B. and T. Berger. Diurnal rhythm in the central dopamine turnover in the rat. *Naunyn-Schmiedeberg's Arch. Pharmac.* **303:** 257–261, 1978b.
22. Lemmer, B. and A. Charrier. Daily variations in the effects of α- and β-blockers on cardiac turnover of noradrenaline. *Chronobiologia* **5:** 190–191, 1978.
23. Quay, W. B. Circadian and estrus rhythms in the pineal and brain serotonin. *Brain Res.* **8:** 61–63, 1964.
24. Reinberg, A. and F. Halberg. Circadian pharmacology. *Ann. Rev. Pharmac.* **11:** 455–492, 1971.
25. Scheving, L. E., H. Harrison, P. Gordon and J. E. Pauly. Daily fluctuation (circadian and ultradian) in biogenic amines of the rat brain. *Am. J. Physiol.* **214:** 166–173, 1968.
26. Sedvall, G. C. and I. J. Kopin. Influence of sympathetic denervation and nerve impulse activity on tyrosine hydroxylase in the rat submaxillary gland. *Biochem. Pharmac.* **16:** 39–46, 1967.
27. Stitzel, R. E. The biological fate of reserpine. *Pharmac. Rev.* **28:** 179–205, 1976.
28. Walker, C. A., S. G. Speziale and A. H. Friedman. The influence of drug treatment on norepinephrine levels and ultrastructure of the rat hypothalamus and caudate nucleus during a programmed light-dark-cycle. *Neuropharmacology* **10:** 325–334 1971.
29. Wurtman, R. J. and J. Axelrod. A 24-hours rhythm in the content of norepinephrine in the pineal and salivary glands of the rat. *Life Sci.* **5:** 655–669, 1969.