Pharmacology Biochemistry & Behavior, Vol. 35, pp. 35–39. © Pergamon Press plc, 1990. Printed in the U.S.A. 0091-3057/90 $3.00 + .00

Free-Running Circadian Activity Rhythms During Long-Term Clonidine Administration in Rats

ALAN M. ROSENWASSER

Department of Psychology, 360 Little Hall
University of Maine, Orono, ME 04469

Received 17 March 1989

ROSENWASSER, A. M. *Free-running circadian activity rhythms during long-term clonidine administration in rats.* PHARMACOL BIOCHEM BEHAV **35**(1) 35–39, 1990.—Experimental and clinical studies indicate that the alpha-adrenergic agonist clonidine can alter mood and activity. However, the behavioral effects of this agent are complex and appear to depend on duration of treatment. Recent work from this laboratory demonstrated that clonidine systematically alters the period, amplitude, and level of free-running circadian activity rhythms in rats. The present study confirms and extends previous observations by employing a longer duration of clonidine treatment. The results show that chronic clonidine administration reversibly shortens the free-running period and reduces the amplitude of the free-running rhythm in constant light. Furthermore, clonidine treatment can increase or decrease the level of activity, depending on baseline activity level, and these effects are not consistently reversed following the termination of treatment. These observations support the hypothesis that noradrenergic systems influence both the circadian periodicity and the level of spontaneous activity, and that clonidine may influence these two parameters by acting at different neural or neuronal loci.

Circadian rhythms Locomotor activity Clonidine Rats

CLONIDINE is a clinically useful antihypertensive agent and alpha-adrenergic agonist, with preferential action at central alpha$_2$ receptors (9). Behavioral studies indicate that acute clonidine administration has sedative, antinociceptive, and anxiolytic effects, similar to those seen with opiates (10, 17, 18). However, the psychopharmacological effects of both clonidine administration and clonidine withdrawal may depend on complex interactions with the dose and duration of treatment, as well as with the clinical status of the research subjects. For example, clonidine has been reported to exacerbate psychotic symptoms in schizophrenia (8), and chronic administration can induce anxiety and depression in hypertensive patients (19). Preliminary clinical observations in affective disorder patients suggest that clonidine may have both antidepressant (8) and antimanic effects (28), and that clonidine withdrawal can precipitate mania (1,8). In animals, acute clonidine administration has been reported to both decrease (13) and increase (7) immobility time in the "forced swim test," a commonly used animal model that detects antidepressant and depressogenic treatments. Similarly, both anxiolytic and anxiogenic effects can be seen, depending on dose (20). Although there is relatively little experimental data on the behavioral effects of chronic clonidine administration, Hoffman and Weiss (7) have reported that termination of clonidine treatment in rats results in the emergence of a long-lasting, desipramine-reversible behavioral depression in the swim test.

Affective disorders have been associated with alterations in the period, phase, and amplitude of circadian rhythms (6, 24, 25). In animals, chronic antidepressant administration (5, 26, 27) and repeated exposure to stress (21,22) have both been reported to lengthen the free-running period of circadian activity rhythms. While these observations may seem paradoxical in light of the depression-promoting effects of stress (2,12), parallel behavioral effects of exposure to stress and antidepressant agents have been noted previously (4); such observations may indicate that stress promotes the generation of an "endogenous antidepressant" response (4,22). In a recent study, chronic clonidine administration consistently and reversibly shortened the free-running period and reduced the amplitude of the circadian wheel-running activity rhythm in rats maintained in constant light (15). While clonidine had previously been shown to reduce the amplitude of daily rhythmicity as expressed under light-dark cycles (11), this was the first study to demonstrate effects on free-running rhythms.

In addition to its effects on free-running rhythmicity, chronic clonidine resulted in complex, but systematic alterations in the level of behavioral activity (15): activity levels were reduced in those animals initially showing relatively high levels of activity, and increased in those animals initially showing relatively low activity levels. Indeed, clonidine-induced alterations in both free-running period and activity level were found to be significantly correlated with individual differences in baseline period and activity level, respectively. Numerous prior studies have reported reduced locomotor activity following acute clonidine administration (10). However, most such studies measured activity in novel environments, and there is some precedent for clonidine-induced increases in activity when animals are tested in familiar environments (3).

The present experiment was designed to confirm and extend these observations, and differed from the earlier study in two

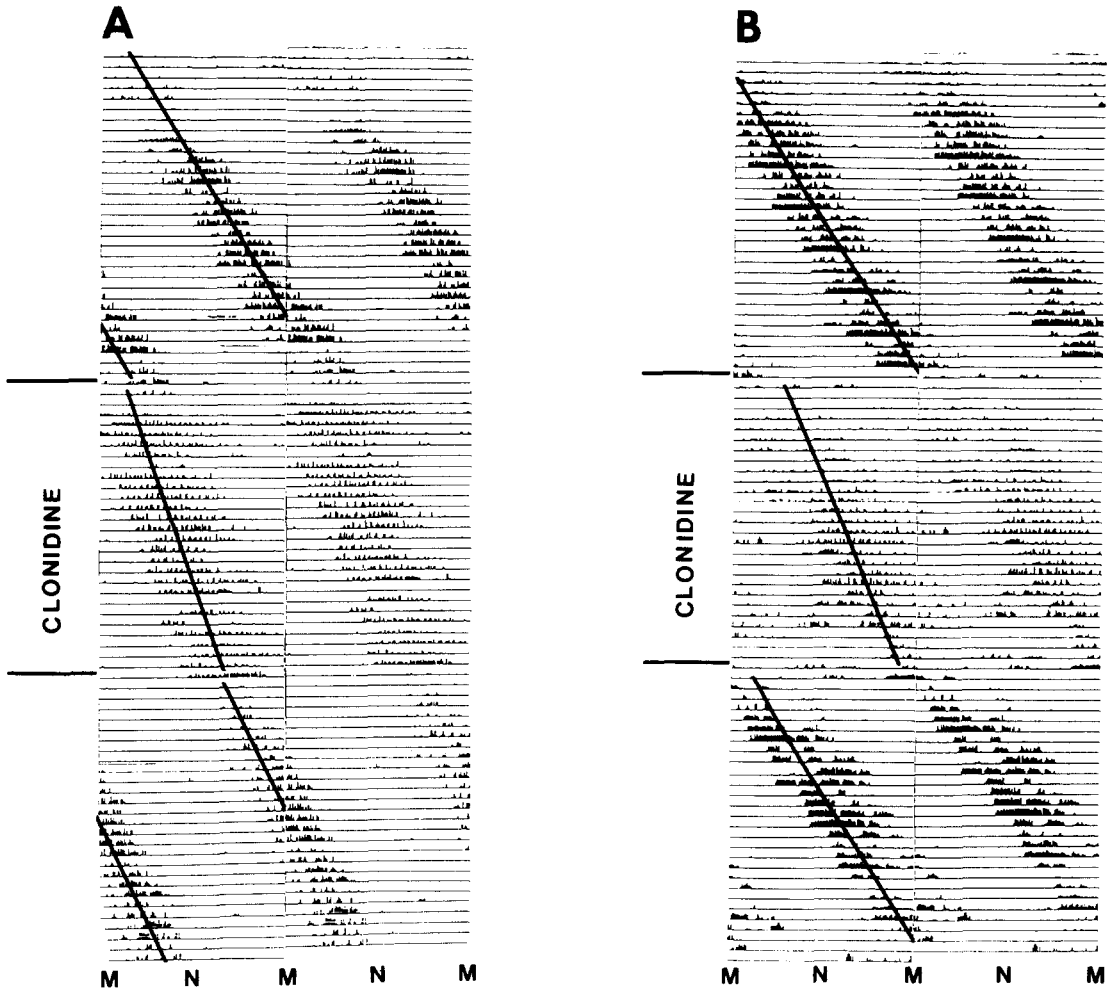

FIG. 1. Double-plotted circadian actograms obtained from two representative animals throughout the entire duration of the experiment. A histogram bar is plotted for each 10-minute time bin with height proportional to the number of wheel-turns in that bin. Time of day is represented along the horizontal axis (48-hr span) and consecutive days from top to bottom along the vertical axis. The days of clonidine administration are indicated along the left margin of each plot. The solid lines superimposed over the data on the left side of each plot indicate the period and phase of the free-running rhythm, separately determined for each segment of the experiment by least-squares spectral analysis. M: midnight; N: noon.

important ways: First, clonidine administration was continued for four weeks, rather than two weeks as in the previous study; and second, fluid intake was monitored during clonidine administration in order to estimate the actual drug dosages delivered to individual animals. The first of these modifications was employed to test the reversibility of clonidine effects following a more extended treatment protocol, while the second modification was designed to determine whether the dependence of the effects of clonidine on baseline activity parameters observed in the previous study could be accounted for by individual differences in drug intake.

METHOD

Female Long-Evans rats (Charles River Labs.) were maintained in standard running-wheel cages with attached home-cage compartments (Lafayette Instr.). The running wheels were housed within ventilated, light-shielded enclosures with either 1, 2, or 4 cages per enclosure, and each enclosure was equipped with an individually programmable light source. Each wheel revolution closed a microswitch mounted on the cage, and switch closures were continuously monitored and stored on the hard disk of a Zenith 158 computer in 10-min time bins using a commercial interface system (Dataquest III; Mini-Mitter Co.). Food (Agway Prolab 3000) and filtered deionized water were freely available.

The activity data were plotted in "actogram" format (e.g., Figs. 1 and 2) using software supplied with the Dataquest system. Statistical parameters of free-running rhythms were estimated using least-squares spectrum analysis, a common approach to the analysis of biological rhythm data. This analysis iteratively determines the amplitude, phase, and mean level (i.e., "mesor") of the best-fitting sinusoidal function for each of a family of prespecified periods, and also determines the "goodness of fit" (i.e., the proportion of data variance accounted for) at each selected period. In the present study, continuous 21-day data samples were fit by sinusoidal functions with periods ranging from 3 to 30 hr in 0.10 hr increments. For each sample, the best-fitting period within this range was taken as the free-running period of the circadian activity rhythm. All period estimates reported in this paper represent fit functions that accounted for sufficient variance to satisfy a 0.001

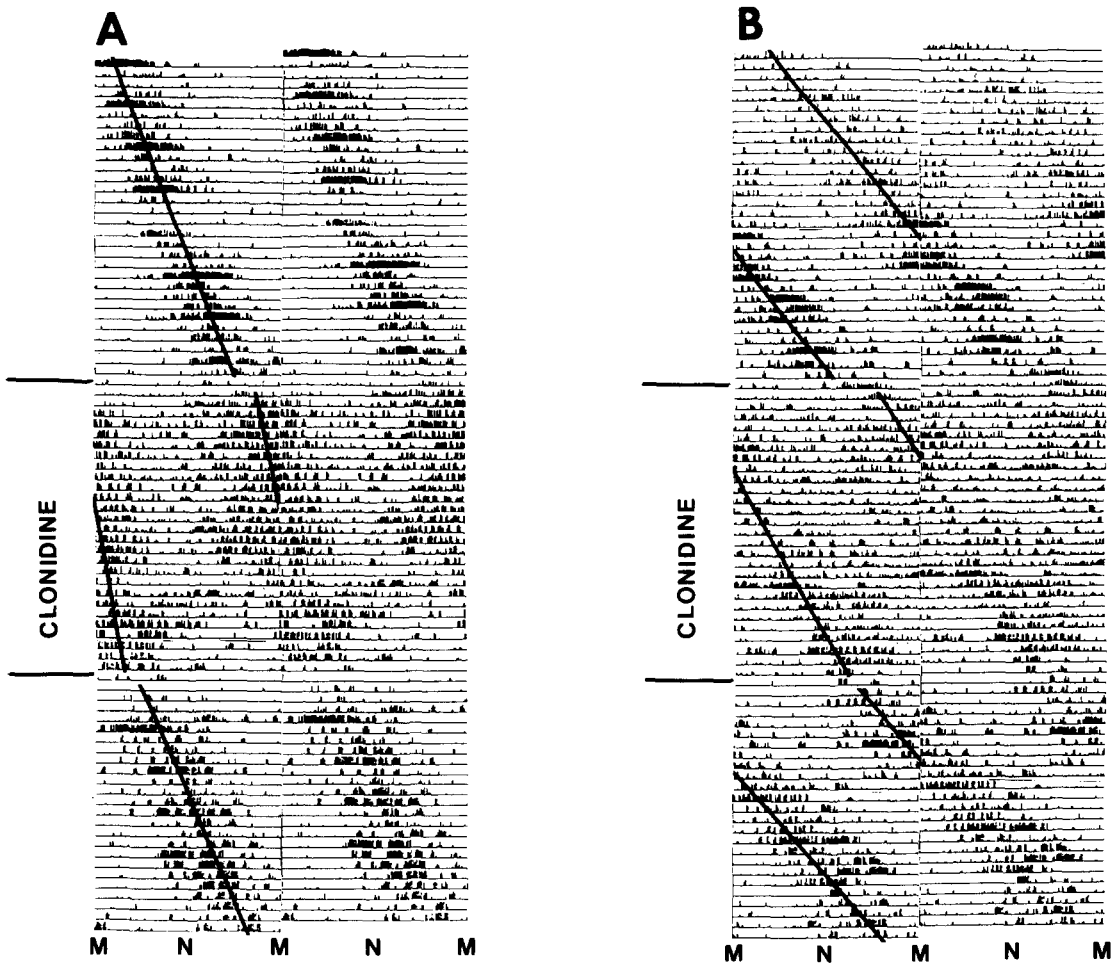

FIG. 2. Same as Fig. 1 for two additional animals. All conventions as in Fig. 1, except that the histogram bars are plotted proportional to the logarithm of the number of wheel-turns in that bin. This transformation improves the representation of data from animals with relatively low levels of activity.

significance level. These estimates are highly correlated with estimates based on visual inspection [cf. (15)]. Since the amplitude of the best-fit sinusoidal function is strongly correlated with its mesor, "amplitude ratios" were computed by dividing each amplitude by its mesor; this transformation provides a measure of rhythm amplitude that is uncorrelated with level of activity, and thereby allows unconfounded amplitude comparisons between animals, or between conditions with different activity levels. Finally, activity levels are reported as wheel-revolutions per 24 hr.

All animals were maintained under constant light of moderate intensity ("LL"; approximately 100–200 lux, depending on cage position) throughout the experiment. The experiment consisted of three phases: a 28–32-day predrug baseline; a 28-day drug administration protocol, during which clonidine hydrochloride (Sigma) was added to the drinking water (5.0 μg/ml); and a 28-day postdrug recovery period. The free-running period, amplitude ratio, and level of activity were determined for each animal for each of the three conditions; the last three weeks of each condition were used in the analyses to avoid including transient activity disruptions that frequently accompanied the introduction and termination of drug treatment.

RESULTS

During baseline conditions all ten animals displayed clear free-running activity rhythms, with periods ranging from 24.5 to 25.5 hr (Figs. 1–3). During clonidine administration, eight of the ten animals displayed shorter free-running periods than during baseline (Figs. 1 and 2), one animal showed a period identical to that seen during baseline, and one animal failed to display detectable rhythmicity. In the eight animals that displayed period shortening during clonidine, period changes ranged from 0.2 to 0.9 hr. Following the termination of clonidine treatment, clear rhythmicity re-emerged in the one animal that failed to display rhythmicity during clonidine treatment, and periods lengthened in eight of the remaining nine animals (Figs. 1 and 2), including the one animal that did not show period shortening during clonidine treatment. One-way repeated-measures analysis of variance (excluding the one case of apparent arrhythmicity) revealed that period changes across conditions were significant for the group, $F(2,16) = 9.91$, $p < 0.01$ (Fig. 3). Post hoc pairwise comparisons using the Scheffé test revealed that free-running periods observed during clonidine administration were significantly shorter than both predrug and postdrug periods, and that predrug and postdrug periods were not significantly different.

Amplitude ratios were reduced during clonidine treatment in all nine animals showing detectable rhythmicity, and increased following the termination of clonidine administration in eight of those nine animals. These changes across conditions were significant for the group, $F(2,16) = 25.77$, $p < 0.001$ (Fig. 3), again excluding the one apparently arrhythmic animal. Pairwise Scheffé tests revealed

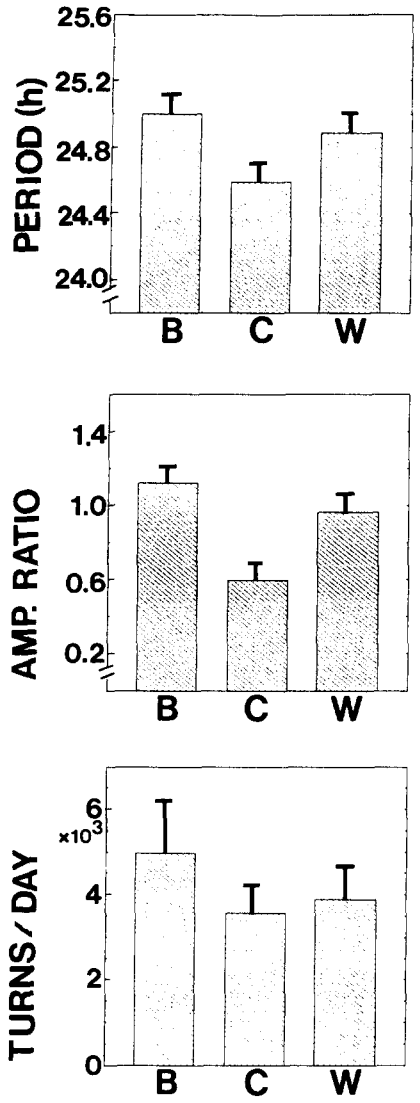

FIG. 3. Group means and standard errors of free-running period (top), amplitude ratio (middle), and activity level (bottom), for the three segments of the experiment; baseline ("B"), clonidine administration ("C"), and following clonidine withdrawal ("W").

that amplitude ratios were significantly reduced during clonidine treatment compared to both predrug and postdrug conditions, and that predrug and postdrug values were not significantly different. Therefore, clonidine administration resulted in consistent and reversible alterations in the period and amplitude of free-running activity rhythms.

The results for daily activity level were more complex. There was considerable interindividual variability in baseline activity (range: 192.9 to 11,521.4 wheel revolutions per day), and both increases and decreases were seen during and following clonidine treatment (Figs. 1 and 2). Eight of the ten animals showed at least some reduction of activity during clonidine administration. However, only four of those eight subsequently displayed increased activity following the termination of treatment, while the other four showed further reductions. Of the two animals that increased activity during clonidine treatment, one showed a decrease and one showed a further increase following drug termination. For the group, activity levels did not differ significantly across conditions, $F(2,18)=2.15$, $p=0.15$, n.s. (Fig. 3). Correlation analysis revealed that individual differences in the effect of clonidine on activity level were systematically related to differences in baseline activity, $r(8)=.92$, $p<0.01$, indicating that clonidine administration tended to reduce activity only in animals showing relatively high baseline activity levels.

In addition to these effects on the period, amplitude, and level of activity, clonidine administration also appeared to alter the patterning of activity over the course of the cycle. Inspection of the activity records (Figs. 1 and 2) suggests that the duration of the active segment of the cycle ("alpha") was increased in most animals during clonidine treatment. Since activity levels were reduced in most animals, this expansion of activity was accomplished mainly by an increase in the "dispersal" of activity over the cycle. While this effect is partially reflected in the significant reduction in the amplitude ratio, no attempt was made to directly quantify the relative duration of the activity and rest phases of the circadian cycle.

Fluid intake increased significantly over the four weeks of drug administration, $F(3,27)=10.95$, $p<0.001$; during the first week of clonidine treatment fluid intake averaged 20.4 ml/day/animal, but during the last week of treatment fluid intake averaged 30.1 ml/day/animal. Previous research has shown that animals will self-administer clonidine (10), and the increases in fluid intake seen in the present study may reflect the reinforcing potential of this drug. However, total fluid intake over the four weeks was not significantly correlated with clonidine-induced alterations in any of the other dependent measures. Indeed, no significant pairwise correlations could be detected among clonidine-induced changes in free-running period, amplitude ratio, and activity level.

DISCUSSION

The results of this study are generally consistent with and serve to extend previous work from this laboratory. Clonidine administration resulted in shortening of the free-running period and reduced amplitude of the circadian activity rhythm of animals maintained in constant light, and these changes were reversed following the termination of drug treatment. While the effects seen with two-week (15) and four-week (present study) treatment protocols were quite similar, there was some suggestion of a differential response to drug termination: animals in the previous study showed an apparent "rebound" effect after drug termination, in that free-running periods were significantly longer after clonidine treatment than during the predrug baseline, while no such difference was seen in the present study. Indeed, in the present study free-running periods following clonidine treatment were actually somewhat shorter than those observed during baseline conditions. Of course, strict quantitative comparisons across studies are inappropriate, and additional studies would be needed to confirm any relationship between treatment duration and response to treatment termination.

The dependence of the activity-altering effect of clonidine on baseline activity level was also consistent with earlier results. While clonidine administration reduces daily activity in most animals, such effects are most pronounced in animals showing relatively high levels of baseline activity. Furthermore, the effects of clonidine on activity level are not consistently reversible upon treatment termination, in contrast to the effects on circadian period and amplitude described above. Measurement of fluid consumption during drug administration showed that the dependence of the activity-altering effects of clonidine on baseline activity level was not mediated by individual differences in drug intake that could have been associated with differences in baseline activity level. However, it is difficult to completely exclude the possibility that changes in activity level were due to "regression toward the mean." This issue might have been circumvented by inclusion of

a nondrug-treated control group to evaluate the extent of spontaneous changes in activity level, but such a group was not included.

One unexplained difference between the present and previous results concerns individual differences in the effect of clonidine on free-running period. In two separate earlier experiments, the magnitude of clonidine-induced period change was significantly correlated with baseline period, while no such correlation was seen in the present data. While it is possible that this apparent discrepancy is somehow related to the different treatment durations employed, further studies would be needed to clarify the conditions under which this relationship is or is not observed.

Although delivery of drugs via the drinking water is convenient, such a procedure also potentially creates a complex situation including the possibility of drug self-administration through drinking behavior. This situation may be particularly complex when the agents administered possess reinforcing or other psychoactive properties that might interact with and modify the normal circadian rhythmicity of fluid intake. In light of recent observations that behavioral arousal may produce "feedback" that can modulate the expression of circadian rhythmicity, dissimilar drug effects might be seen using drug administration protocols that remove the possibility of such behavioral feedback. Therefore, it would be worthwhile to determine whether the effects of clonidine seen in the present study could be replicated using continuous drug administration via an implanted osmotic pump.

In conclusion, the results of this study support the hypothesis that noradrenergic systems influence both the periodicity and the level of spontaneous activity, and also suggest that the effects of clonidine on circadian rhythmicity and on activity level may be mediated by distinct neural mechanisms. These results contribute to an emerging neuropsychopharmacology of circadian rhythmicity. Previous studies have implicated serotonergic, cholinergic, peptidergic, and amino acid neurotransmitter systems in the control and expression of rhythmicity (14, 16, 23, 26), and the present results indicate that noradrenergic mechanisms may also play a role in the circadian system. Finally, these results may be relevant to the understanding of relationships between circadian rhythmicity and both normal and abnormal manifestations of behavioral state.

ACKNOWLEDGEMENTS

Supported by a Faculty Research Fund Award, University of Maine. The author wishes to thank J. Zoidis, L. Plante and S. Gerkin for technical assistance.

REFERENCES

1. Adler, L. E.; Bell, J.; Kirch, D.; Friedrich, E.; Freedman, R. Psychosis associated with clonidine withdrawal. Am. J. Psychiatry 139:110–112; 1982.
2. Anisman, H. Vulnerability to depression: contributions of stress. In: Post, R. M.; Ballenger, J. C., eds. Neurobiology of mood disorders. Baltimore: Williams and Wilkins; 1984:407–431.
3. Bednarczyk, B.; Vetulani, J. Stimulatory and inhibitory actions of clonidine on the locomotor activity in the rat. Pol. J. Pharm. Pharmacol. 29:219–229; 1977.
4. Danysz, W.; Kostowski, W.; Archer, T. Some aspects of stress and depression. Prog. Neuropsychopharmacol. Biol. Psychiatry 12:405–419; 1988.
5. Duncan, W. C.; Tamarkin, L.; Sokolove, P. G.; Wehr, T. A. Chronic clorgyline treatment of Syrian hamsters: an analysis of effects on the circadian pacemaker. J. Biol. Rhythms 3:305–322; 1988.
6. Gillin, J. C.; Sitaram, N.; Wehr, T. A.; Duncan, W.; Post, R.; Murphy, D. L.; Mendelson, W. B.; Wyatt, R. J.; Bunney, W. E., Jr. Sleep and affective illness. In: Post, R. M.; Ballenger, J. C., eds. Neurobiology of mood disorders. Baltimore: Williams and Wilkins; 1984:157–189.
7. Hoffman, L. J.; Weiss, J. M. Behavioral depression following clonidine withdrawal: a new animal model of long-lasting depression? Psychopharmacol. Bull. 22:943–949; 1986.
8. Jimerson, D. C.; Post, R. M.; Stoddard, F. J.; Gillin, J. C.; Bunney, W. E., Jr. Preliminary trials of the noradrenergic agonist clonidine in psychiatric patients. Biol. Psychiatry 15:45–57; 1980.
9. Kobinger, W. Central alpha-adrenergic systems as targets for hypotensive drugs. Rev. Physiol. Biochem. Pharmacol. 81:39–100; 1978.
10. Lal, H.; Shearman, G. T. Psychotropic actions of clonidine. In: Lal, H.; Fielding, S., eds. Psychopharmacology of clonidine. New York: A. R. Liss Inc.; 1981:99–146.
11. Lewis, S. J.; Jarrott, B. The effects of continuous clonidine infusion on the circadian rhythms of arterial blood pressure, heart rate and spontaneous locomotor activity in normotensive Wistar-Kyoto rats. In: Redfern, P. H.; Campbell, I. C.; Davies, J. A.; Martin, K. F., eds. Circadian rhythms in the central nervous system. Weinheim, F.R.G.: VCH Pubs.; 1985:213–216.
12. Maier, S. F. Learned helplessness and animal models of depression. Prog. Neuropsychopharmacol. Biol. Psychiatry 8:435–446; 1984.
13. Malick, J. B. Clonidine: Antidepressant potential? In: Lal, H.; Fielding, S., eds. Psychopharmacology of clonidine. New York: A. R. Liss Inc.; 1981:165–176.
14. Meijer, J. H.; Rietveld, W. J. The neurophysiology of the suprachiasmatic circadian pacemaker in rodents. Physiol. Rev. 69:671–707; 1989.
15. Rosenwasser, A. M. Effects of chronic clonidine administration and withdrawal on free-running circadian activity rhythms. Pharmacol. Biochem. Behav. 33:291–297; 1989.
16. Rosenwasser, A. M. Behavioral neurobiology of circadian pacemakers: a comparative perspective. In: Epstein, A. N.; Morrison, A. R., eds. Progress in psychobiology and physiological psychology. vol. 13. New York: Academic Press; 1988:155–226.
17. Redmond, D. E., Jr. Clonidine and the primate locus coeruleus: Evidence suggesting anxiolytic and anti-withdrawal effects. In: Lal, H.; Fielding, S., eds. Psychopharmacology of clonidine. New York: A. R. Liss Inc.; 1981:147–164.
18. Sandyk, R.; Gillman, M. A.; Iacono, R. P.; Bamford, C. R. Clonidine in neuropsychiatric disorders: a review. Int. J. Neurosci. 35:205–215; 1987.
19. Simpson, F. O. Hypertension and depression and their treatment. Austr. N. Z. J. Psychiatry 7:133–137; 1973.
20. Soderpalm, B.; Engel, J. A. Biphasic effects of clonidine on conflict behavior: Involvement of different alpha-adrenoceptors. Pharmacol. Biochem. Behav. 30:471–477; 1988.
21. Stewart, K. T.; Rosenwasser, A. M.; Adler, N. T.; Volpicelli, J. R. Circadian activity rhythms in an animal model of depression. Soc. Neurosci. Abstr. 12:597; 1986.
22. Stewart, K. T.; Rosenwasser, A. M.; Volpicelli, J. R.; Hauser, H. H.; Adler, N. T. Circadian rhythms and escape learning in rats exposed to shock. Soc. Neurosci. Abstr. 13:607; 1987.
23. Turek, F. W. Pharmacological probes of the mammalian circadian clock: use of the phase-response curve approach. Trends Pharmacol. Sci. 8:212–217; 1987.
24. Wehr, T. A.; Gillin, J. C.; Goodwin, F. K. Sleep and circadian rhythms in depression. In: Chase, M.; Weitzman, E. D., eds. Sleep disorders: Basic and clinical research. New York: Spectrum Pubs.; 1983:195–226.
25. Wehr, T. A. Biological rhythms and manic-depressive illness. In: Post, R. M.; Ballenger, J. C., eds. Neurobiology of mood disorders. Baltimore: Williams and Wilkins; 1984:190–206.
26. Wirz-Justice, A.; Groos, G. A.; Wehr, T. A. The neuropharmacology of circadian timekeeping in mammals. In: Aschoff, J.; Daan, S.; Groos, G., eds. Vertebrate circadian systems: Structure and physiology. Berlin: Springer-Verlag; 1982:183–193.
27. Wirz-Justice, A.; Campbell, I. C. Antidepressant drugs can slow or dissociate circadian rhythms. Experientia 38:1301–1309; 1982.
28. Zubenko, G. S.; Cohen, B. M.; Lipinski, J. F.; Jones, J. M. Clonidine in the treatment of mania and mixed bipolar disorder. Am. J. Psychiatry 141:1617–1618; 1984.