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Circadian Variation in Tolerance to the Hypothermic Action of CNS Drugs

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WILLIAMS, R. L., K. F. A. SOLIMAN AND K. M. MIZINGA. *Circadian variation in tolerance to the hypothermic action of CNS drugs.* PHARMACOL BIOCHEM BEHAV 46(2) 283–288, 1993.—Male SAF mice (30–35 g) or male Sprague–Dawley rats (180–250 g) were used to study the circadian variation in tolerance to the hypothermic action of ethanol, apomorphine, and nicotine. Animals were treated for 2 or 3 consecutive days during the light phase (1000, 1400, or 1800 h) or the dark phase (2200, 0200, or 0600 h) and hypothermia produced measured. In one experiment, repeated injections of 20% ethanol (3 g/kg, IP) to mice resulted in varying degrees of hypothermia depending upon the time of injection. Tolerance to hypothermic action was observed only in animals treated during the light phase. On the contrary, the hypothermic response in animals treated during the dark phase increased. In another experiment, apomorphine (15 mg/kg, IP) was used and tolerance to apomorphine-induced hypothermia observed following repeated injections during the light phase with maximum tolerance noticed at 1400 h. In the third experiment, nicotine (2 mg/kg, IP) was repeatedly administered and resulted in tolerance development when given during the light phase. These results indicate that the rapid development of tolerance to CNS drugs studied is a diurnally controlled phenomenon.

Ethanol Apomorphine Nicotine Hypothermia Tolerance Diurnal

TOLERANCE is defined as a diminished response to drug administration after repeated exposure to that drug. Tolerance is evident when increasingly larger doses of a given drug must be administered to obtain the same magnitude of pharmacological effect observed with the original dose (10). Others have shown that acute tolerance to ethanol-induced hypothermia can be developed (3). In rats, it has been demonstrated that a high degree of tolerance to pentobarbital can be accelerated by continuous oral exposure (16). Tolerance may involve either a change in the disposition of a drug (metabolic tolerance) or the development of resistance to the effects of a drug at the cellular level in the CNS (functional tolerance). In addition, tolerance has been classified as "conditional" or "environmental" under certain circumstances (4).

Acquired tolerance is known to occur to a wide variety of chemical substances of which ethanol, apomorphine, and nicotine are but classical examples. Drug tolerance has been viewed traditionally as a homeostatic response to a direct chemical action of the agent on the neuron (7). The study of tolerance to CNS drugs is important for two reasons. In relation to alcoholism and other forms of drug dependence, tolerance and physical dependence may contribute to the strength of the addictive process (2). Therefore, knowledge about the mechanisms of tolerance may facilitate the development of a rational approach for treating addiction and preventing its relapse (7). A second and more general reason is that tolerance to drugs is a form of neuroadaptation, and elucidation of its mechanisms and controlling factors may contribute greatly to our understanding of CNS adaptive mechanisms in general (7).

Several studies demonstrated that the effect of drugs acting on the CNS follows a circadian rhythm pattern. We have shown that the hypothermic action of ethanol follows specific circadian rhythm (15). Previously, we reported that the rapid development of tolerance to ethanol and morphine is related to the brain cholinergic system (12,13). Earlier, we also established that there is a circadian variation in the brain cholinergic activity (11). Therefore, it was of interest to investigate the circadian variation in the rapid development of tolerance to the hypothermic effect of ethanol, apomorphine, and nicotine.

METHOD

In these experiments, SAF male mice purchased from the Southern Animal Farm (Prattville, AL) weighing 30–35 g or male Sprague–Dawley rats weighing 180–250 g also purchased from Southern Animal Farm were used. Animals were housed in groups of six in clear plastic cages and kept in a controlled environment with room temperature at 21 ± 1°C and a 12 L : 12 D (LD) cycle (lights on at 0700 h). Feed and water were

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provided ad lib. Animals were allowed a 2-week acclimation period before experimentation.

In the first experiment, male mice were randomly assigned to six groups (n = 6/group) and injected with 20% ethanol (3 g/kg, IP) for 3 consecutive days at 0600, 1000, 1400, 1800, 2200, or 0200 h, respectively. At each specified hour of the LD cycle, body temperature was measured immediately before ethanol administration and 30 min later.

In the second experiment, male rats were randomly assigned to six groups (n = 8/group) and injected with apomorphine (15 mg/kg, IP) for 2 consecutive days at 0600, 1000, 1400, 1800, 2200, or 0200 h, respectively. At each specified hour of the LD cycle, body temperature was measured immediately before apomorphine administration and at 30 and 60 min after drug administration.

In the third experiment, male rats were randomly assigned to six groups (n = 6/group) and injected with nicotine (2 mg/kg, IP) for 2 consecutive days at 0600, 1000, 1400, 1800, 2200, or 0200 h, respectively. At each specified hour of the LD cycle, body temperature was measured before nicotine administration and at 30 and 60 min posttreatment.

In all experiments, body temperature was recorded using a telethermometer (Yellow Spring Instrument Co., Yellow Springs, OH) by inserting a lubricated temperature probe approximately 3 cm into the rectum of the animal. Data were subjected to one-way analysis of variance and differences among the means were detected by Duncan's multiple-range test.

RESULTS

Figure 1 presents the effect of repeated administration of ethanol on the development of tolerance to the hypothermic action of ethanol. At all time points studied, the first as well as the subsequent doses of ethanol resulted in significant, $F(15, 84) = 2.91$, $p < 0.001$, hypothermia. Following the first dose of ethanol, the hypothermic response was greatest, $F(5, 36) = 5.45$, $p < 0.004$, at 1800 (light phase) and lowest at 0200 and 0600 h (middle and end of the dark phase). The data indicate that tolerance development to ethanol was evident when ethanol was given during the light phase. Animals treated at 1800 h developed tolerance to ethanol after one

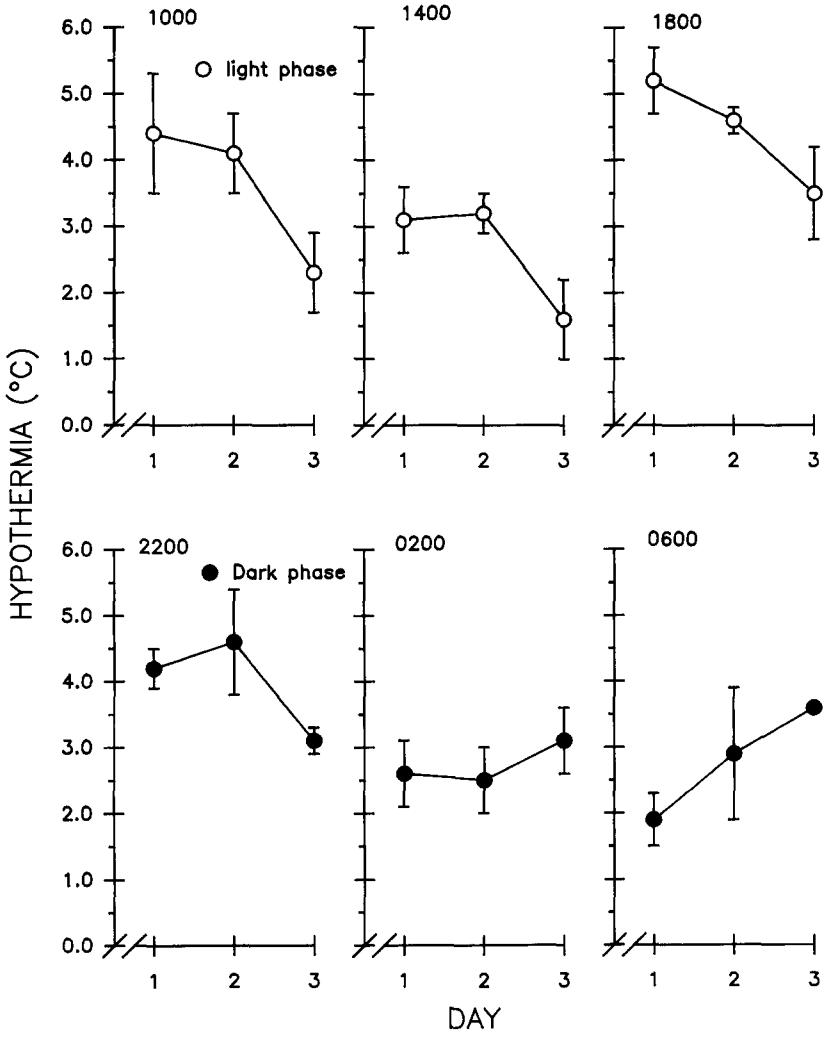

FIG. 1. Effect of repeated ethanol administration on the hypothermic action (means ± SE) of ethanol.

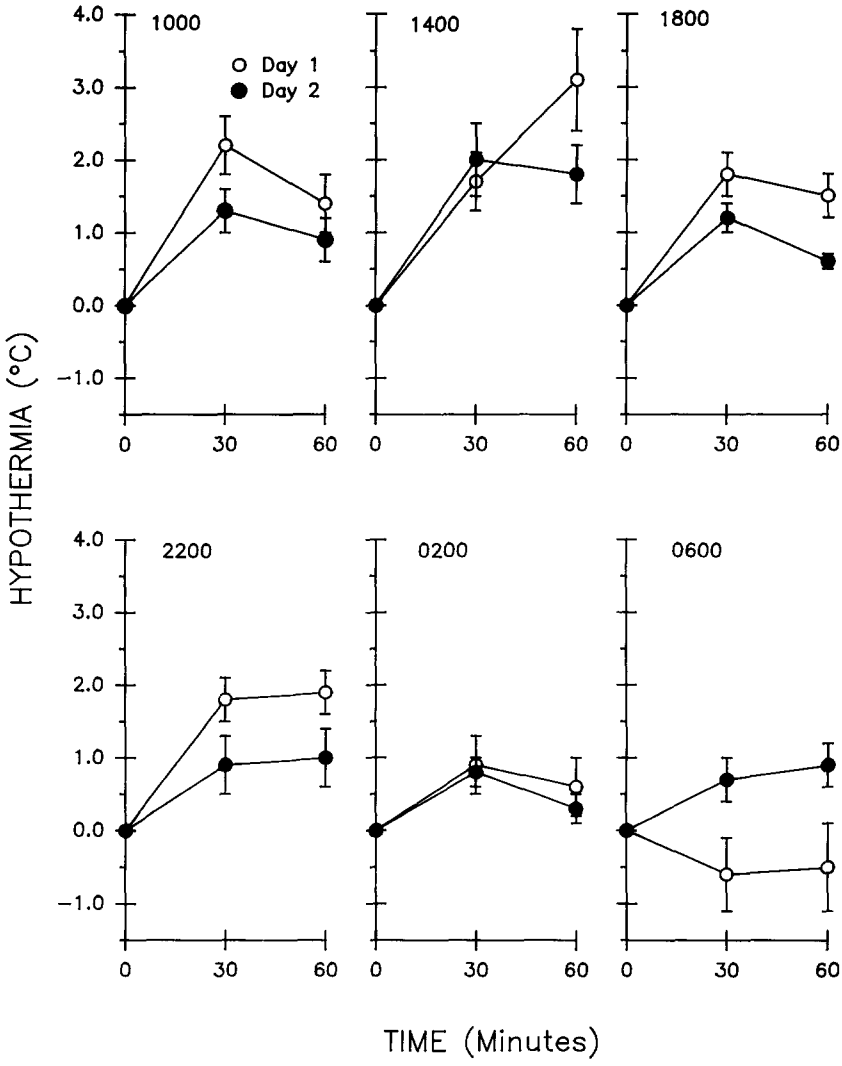

FIG. 2. Circadian variation in the hypothermic effect (means ± SE) of apomorphine.

injection. Meanwhile, tolerance to ethanol developed after the second injection when drug treatment was at 1000 and 1400 h. All animals developed tolerance by the third injection during the light phase. On the other hand, repeated administration of ethanol during the dark phase produced variable results. At the beginning of the dark phase, there was no tolerance development established. During the middle of the dark phase (0200 h), there was a significant increase in the amount of hypothermia produced by ethanol by the third injection (hypersensitivity). Moreover, toward the end of the dark phase (0600 h) the degree of hypersensitivity was found to be accelerated and was evident by the second injection of ethanol.

The results of the second experiment (presented in Fig. 2) indicate that there was a circadian variation in the degree of hypothermia produced in response to apomorphine administration. The first injection of apomorphine given at 0600 h resulted in statistically insignificant, mild hyperthermia. However, the second injection of apomorphine at 0600 h resulted in decreased, $F(5, 42) = 2.70$, $p < 0.034$, body temperature at 30 and 60 min posttreatment. Marked apomorphine-induced hypothermia occurred, $F(10, 84) = 5.26$, $p < 0.0001$, following the first dose given during the light and early dark phases, specifically at 1000, 1400, 1800, and 2200 h, whereas no significant hypothermia was noted at 0200 h. Tolerance was evident following administration of the second dose of apomorphine at 1000, 1400, 1800 (light phase), and 2200 h (early dark phase). The greatest hypothermic response to the first dose of apomorphine as well as maximal tolerance development occurred during the light phase at 1400 h.

Figure 3 depicts the rapid development of tolerance to nicotine-induced hypothermia (NIH). The first dose of nicotine resulted in significant, $F(5, 30) = 7.61$, $p < 0.0001$, hypothermia at all time points studied. The hypothermic response to the first dose of nicotine was greatest, $F(5, 30) = 7.61$, $p < 0.0001$, at 1800 h (light phase) and lowest at 2200 and 0200 h (dark phase). The second dose of nicotine resulted in significant, $F(5, 29) = 4.46$, $p < 0.025$, hypothermia at all time points except 1800 h. The results of this experiment demonstrate a circadian variation in the hypothermic effect of nicotine, with a significant, $F(1, 10) = 9.76$, $p < 0.011$, peak during the light phase at 1800 h and a diminished effect during

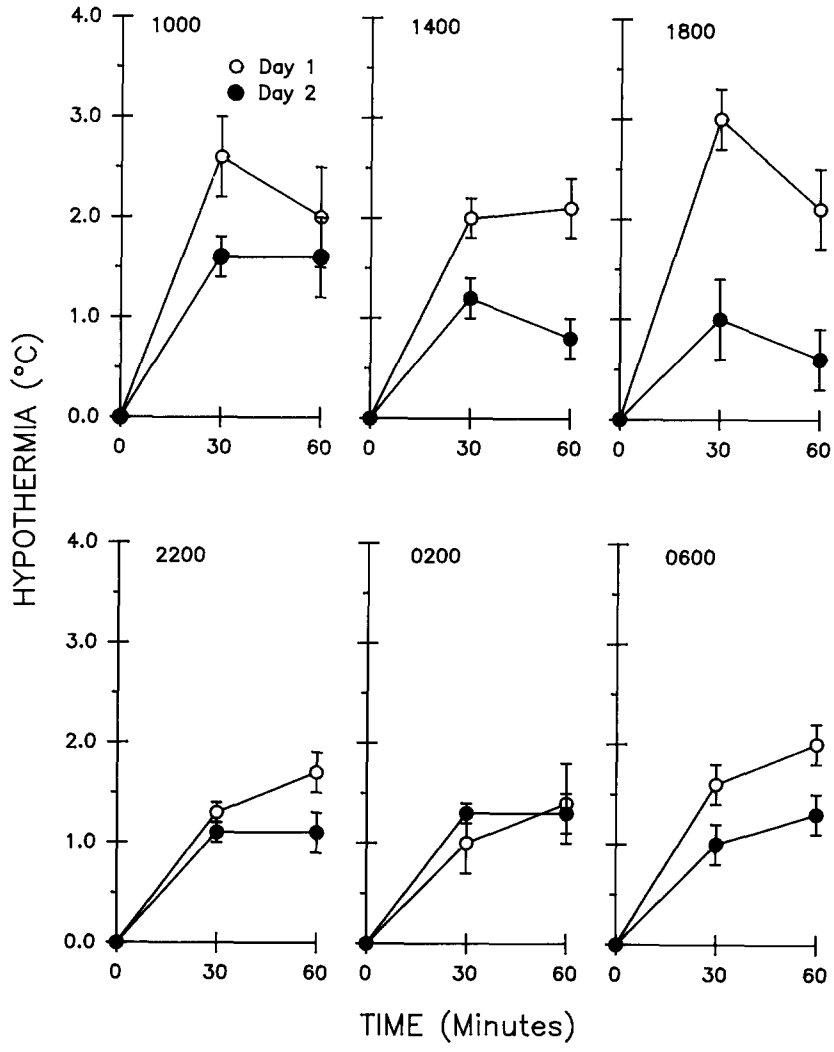

FIG. 3. Circadian variation in the hypothermic effect (means ± SE) of nicotine.

the dark phase at 0200 h. Tolerance to NIH was noticed in animals injected during the light phase, namely, at 1000, 1400, and 1800, and during the dark phase at 0600 h. Maximum NIH tolerance developed at 1800 with minimum NIH at 1000 h. However, there was no tolerance developed to nicotine in groups of animals injected at 2200 or 0200 h.

DISCUSSION

We previously reported that ethanol-induced hypothermia was lowest during the dark phase coincident with the circadian peak in normal body temperature in mice (15). The same study revealed a discordant relationship between plasma ethanol concentrations and the hypothermic response when alcohol was administered at different times of the circadian cycle. Therefore, it was concluded that differential hypothermic action was related to circadian variation in sensitivity of the brain to ethanol (15). Similarly, the basis of the diurnal fluctuation in tolerance development in the present study may be related to the circadian variation in brain sensitivity to these drugs. It is also possible that the circadian variation in the dependent variable, hypothermia, could itself alter the sensitivity of the receptors on which the drug is acting. The development of tolerance to the hypothermic effect of ethanol was most rapid (evident by the second dose) at 1800 h, the time point with the greatest first dose response. Dose dependence of tolerance to ethanol with tolerance observed only at higher doses was reported in mouse lines genetically susceptible (COLD) or resistant (HOT) to acute hypothermic effects of ethanol (9). These and our results suggest that a dynamic threshold in stimulus intensity needs to be attained or exceeded to trigger tolerance. If the increased hypothermic response at 1800 h is indeed associated with increased brain sensitivity to this effect, the attainment of such a stimulus-intensity threshold for inducing tolerance would be relatively easier at that part of the circadian cycle. The attainment of tolerance by the third daily dose for all light-phase time points suggests that the stimulus-intensity threshold for inducing tolerance declined with repeated dosing. Results obtained during the dark phase indicate development of tolerance to ethanol-induced hypothermia at 2200 h contrasted with a progressive increase in sensitivity at 0200 and 0600 h. These observations would be consistent with a progressive increase in brain sensitivity at 0200 and 0600 h (middle and end of the dark phase)

coupled with an elevated or unchanged stimulus-intensity threshold for inducing tolerance. Overall, the stimulus-intensity threshold for ethanol-induced hypothermia probably operates independently of the circadian variation in brain sensitivity and shifts in different directions at various points of the circadian cycle.

The apomorphine-induced hypothermia observed following the first (except at 0600 h) and second doses in the present study is consistent with previous reports (5,17). Others (17) reported that a low dose (0.1 mg/kg, IP) of apomorphine can simultaneously activate a dopamine (DA)-related mechanism, which tends to decrease body temperature, and a serotonin [5-hydroxytryptamine (5-HT)]-related mechanism, which tends to increase body temperature. The results of the same study suggested that the response of the hyperthermia mechanism compared to the hypothermia mechanism saturated at a lower dose of apomorphine, resulting in "masking" of the former mechanism when higher doses (1.0 and 2.0 mg/kg, IP) were administered. In the present study, however, apomorphine was administered at a relatively high dose (15 mg/kg, IP). Therefore, it is interesting that mild hyperthermia was observed at 0600 h following the first dose of apomorphine. In contrast with our study, others (17) may have conducted their experiments at a different time of the circadian cycle compared to our 0600-h time point. Our results suggest that the 5-HT-related hyperthermia mechanism was preponderant over the DA-related hypothermia mechanism at 0600 h (end of the dark phase), which may have been less sensitive at that time. The hypothermic response to the second apomorphine dose at 0600 h may be indicative of increasing brain sensitivity similar to that proposed for ethanol at 0200 and 0600 h in the present study. The apparent circadian synchrony between the occurrence of maximal sensitivity to apomorphine-induced hypothermia and the most rapid development of tolerance at 1400 h may be indicative of the existence of a stimulus-intensity threshold for inducing tolerance.

The nicotine-induced hypothermia observed following the first dose is in agreement with previous studies (1). As with ethanol, tolerance development was of greatest magnitude at 1800 h, coincident with the time point with the highest first dose drug-induced hypothermia. These results may be explained by the same or a similar stimulus-intensity threshold mechanism proposed above for ethanol. Previously, it was found after 14 days of SC injections of nicotine to rats that tolerance developed to locomotor activity depression and the hypothermic effect of this drug (8). Further, this tolerance was not linked to any alteration in the brain cortex, midbrain, or hippocampus nicotinic receptors (8). These authors suggested that tolerance to the hypothermic effect of nicotine may be produced by alterations of other factors in the transmitter system of this receptor.

We previously reported on the circadian differences in the responses to CNS drugs (14,15). The results obtained here not only confirm our previous findings but also provide evidence that tolerance development in the CNS is a diurnally controlled phenomenon. Whether or not the two phenomena (i.e., the circadian rhythm of drug response and circadian rhythm of tolerance development) are related is not known. In the three experiments reported here, drug-induced hypothermia was of a lower magnitude during the dark phase compared to the response during the light phase. Also, the rate of development of tolerance was of high magnitude during the light phase and was not significant during the dark phase. Overall, when the drug effect was minimal no significant tolerance was noticed. The results of these experiments clearly indicate the rapid development of tolerance to the hypothermic effects of various CNS drugs are a diurnally controlled phenomenon. We suggest the use of the term "chronotolerance" to describe this phenomenon.

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