

mixture of **3** and 10% Pd/C in a long glass tube was heated at 285°C for 4 min. The product deposited on the tube wall was purified by HPLC to give a purple crystalline compound (yield: 73%) which was identical with linderazulene (**1**) in all respects. In addition to moderate antitumor activity, compounds **1–3** are antifungal against *Candida albicans*, **3** being most active. Compounds **1** and **3** also show immunostimulatory activity at low concentrations, while **2** exhibits immunodepressant activity.

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- 6 Low resolution EIMS m/z 212 (M^+ , 100), 197 (60), 182 (22), 169 (19), 165 (9), 154 (11), 153 (11), 141 (8), 128 (8), 115 (8), and 106 (6 rel. %). ^{13}C NMR (acetone- d_6) δ 166.23 (s), 146.14 (s), 134.16 (s), 132.68 (s), 131.97 (d), 129.54 (d), 126.75 (s), 124.47 (s), 115.74 (d), 109.53 (d), 77.93 (t), 39.96 (d), 24.40 (q), 20.68 (q), and 12.83 (q).
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Circadian fluctuation of susceptibility to haloperidol under constant conditions

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Summary. An endogenous circadian rhythm of the sedative and antiapomorphine effects of haloperidol was observed under constant conditions for 7 days as well as under an entrained light-dark cycle.

Key words. Haloperidol; circadian rhythm; sedation; antiapomorphine effect; constant condition.

A circadian rhythm of susceptibility to haloperidol¹, chlorpromazine², tetrabenazine³ and apomorphine⁴ has been shown to exist. The rhythm is synchronized to the light-dark (LD) cycle, since it is reversed following the reversal of the cycle².

But this alone does not clarify whether this rhythm is due to endogenous causes or is merely a response to the external light-dark cycle. This can be determined by studying whether it persists when conditions such as light, temperature, and humidity, are kept constant. We carried out such a study of the sedative and additionally antiapomorphine effects of haloperidol.

Method. Male S.D. rats weighing 300–400 g were used. They were kept in a quiet dark animal house under controlled lighting conditions with 12 h of artificial light between 19.30 h and 07.30 h and 12 h of complete darkness. For at least 5 weeks before the experiment the temperature of the room was maintained at 25±1°C, humidity at 55%. Food and water were supplied ad libitum. A dim red light was used during injection.

1) Circadian fluctuation of the sedative effect of haloperidol under constant conditions. The activity of one rat placed in a completely dark sound-proof room was measured with Animex DS (AB FARARD). Haloperidol 0.5 mg/kg (0.05% solution) at a temperature of 24°C was administered i.p. with the utmost care not to excite the rats. After the administration the sedation period was assessed by the method of Nagayama et al.³. The sedation period is defined as the time from the beginning of sedation due to the administration of haloperidol to the time the Animex reads 90 counts/10 min. Haloperidol was administered 0–168 h after rats were placed under constant conditions at one of the following times: 01.30, 07.30, 13.30, 19.30 h. Each rat was used only once in the experiment.

With the control group, under 12:12 LD conditions, a similar experiment was conducted in the sound-proof room. At each administration of haloperidol or saline, a group of 4 rats were used.

2) Circadian fluctuation of the antiapomorphine effect of haloperidol under constant conditions. Under the same conditions as in (1), haloperidol 0.5 mg/kg was administered i.p. to one group, and to the other saline i.p. To both groups, apomorphine 5 mg/kg was administered s.c. 1 h later and the duration of AISB (apomorphine-induced stereotyped behavior) assessed according to the method of Nagayama et al.⁴.

Haloperidol or saline was administered 42–60 h after rats were placed under constant conditions at one of the following times: 01.30, 07.30, 13.30, 19.30 h. Each rat was used only once in the experiment (N = 6/group). The AISB duration was compared between the saline- and haloperidol-treated groups, to calculate the AISB inhibition percentage, which was used as an index of the antiapomorphine effect of haloperidol.

With the control group, under 12:12 LD condition, a similar experiment was conducted in the same sound-proof room.

Results and discussion. A significant diurnal fluctuation in the sedative effects of haloperidol was observed under 12:12 LD with the peak at 19.30 and nadir at 07.30 h. A similar rhythm was also observed daily for 7 days under constant conditions (fig. 1).

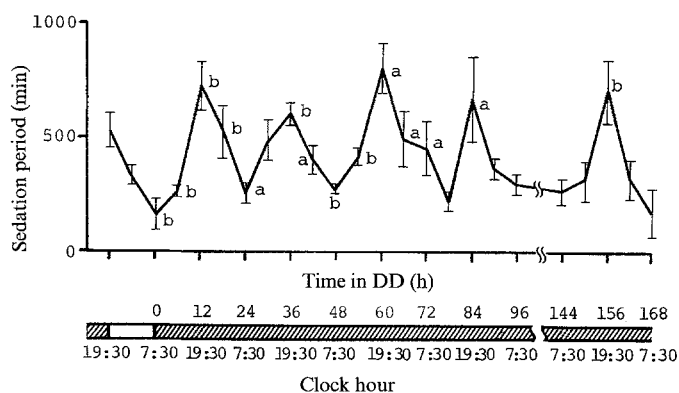


Figure 1. Rhythm of sedative effect of haloperidol under constant conditions. Significant differences were observed in 24 h covering 5 administration times centering on a ($p < 0.05$) or b ($p < 0.01$) (one-way ANOVA).

The antiapomorphine effect of haloperidol after 42–60 h under constant conditions has a circadian rhythm with almost the same pattern as under an LD cycle (fig. 2). In other words, rhythmic susceptibility to haloperidol persisted in the absence of time cues, showing that this rhythm is endogenous.

This conclusion does not agree in many points with previous reports. Davis et al.⁵ and Holcslaw et al.⁶ reported the disappearance of a rhythm of susceptibility to drugs under constant conditions; Scheving et al.⁷ reported that a rhythm with two peaks or

irregular patterns appeared. In these experiments, however, animals seem to have been placed under constant conditions for too long a period. Since each animal's rhythm is expected to free-run with its own period, placing animals under constant conditions for too long would bring about apparent disappearance of the rhythm. This may explain the data reported by Davis et al. and Holcslaw et al., which were obtained from only two measurements a day. Furthermore, in the experiments described by Scheving et al., rats were not raised individually and social synchronizers may have modified the results.

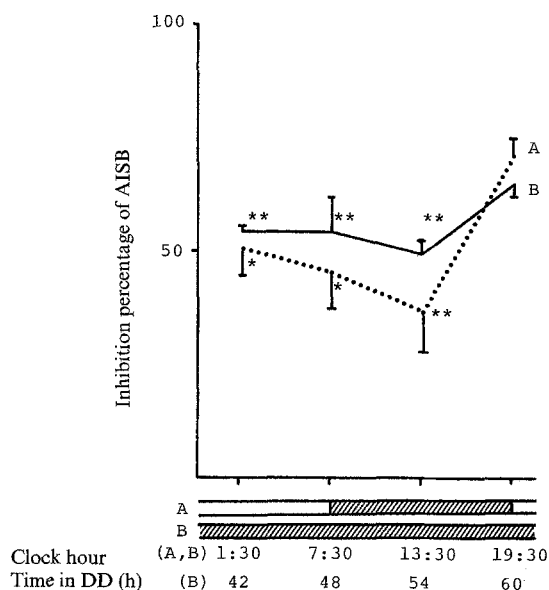


Figure 2. Rhythm of antiapomorphine effect of haloperidol under constant conditions. *A* Group under controlled lighting conditions. *B* Group under constant conditions. Significant difference from 19.30 (ANOVA). $p < 0.05$, $**p < 0.01$.

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