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Influence of Ambient Temperature on the Development and Maintenance of Tolerance to Ethanol-Induced Hypothermia

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LE, A. D., H. KALANT AND J. M. KHANNA. *Influence of ambient temperature on the development and maintenance of tolerance to ethanol-induced hypothermia.* PHARMACOL BIOCHEM BEHAV **25**(3) 667–672, 1986.—The development of tolerance to the hypothermic effect of ethanol was examined during chronic ethanol treatment (5 g/kg PO daily) at various ambient temperatures (Ta). Tolerance to the hypothermic effect of ethanol, monitored at five-day intervals for 25 days, developed rapidly when ethanol treatment was carried out at 4°C. On the other hand, rats receiving ethanol treatment at a Ta of 36°C, at which they did not experience hypothermia, acquired tolerance more slowly, but achieved the same level of tolerance as other groups after 25 days of treatment. This cannot be accounted for by the repeated testing at 21°C at five-day intervals, since it was also observed under a non-repeated testing condition. Once tolerance to the hypothermic effect of ethanol was acquired, termination of ethanol treatment resulted in the loss of tolerance, but mere prevention of the hypothermic effect of ethanol did not. These results suggest that tolerance still developed even though the organisms did not experience hypothermia during ethanol treatment. Therefore there appears to be a component of tolerance, that depends upon a direct cellular action of the drug, as distinct from the physiological consequences of that action. However, variation in the degree of physiological disturbance (hypothermia) during drug exposure can modulate the rate of development of this tolerance.

Ambient temperature Tolerance Ethanol Hypothermia

AT normal ambient temperature (Ta), administration of ethanol produces a dose-dependent drop in body temperature in a variety of experimental animals (for reviews see [9,13]). Because ethanol-induced hypothermia is easily measurable, it has been employed quite extensively in the past decade as a dependent variable to study various aspects of ethanol tolerance. For example, ethanol-induced hypothermia has been employed by a number of investigators [6, 18, 26] to study the involvement of Pavlovian conditioning in ethanol tolerance or cross-tolerance between ethanol and pentobarbital [3]. Similarly, the development of cross-tolerance between ethanol and morphine has been demonstrated in hypothermia studies [15,25]. In addition, knowledge of the involvement of neurotransmitters such as norepinephrine [27,28] or serotonin [17,26] or the pituitary peptide hormone vasopressin and its desglycinamide derivative [11,16] in ethanol tolerance has been derived in part from studies of tolerance to ethanol-induced hypothermia.

At any given dose, however, the hypothermic effect of ethanol is a function of ambient temperature [13]. The degree of hypothermia induced by ethanol is more pronounced at lower Ta and diminished at higher Ta [8, 22, 23, 27]. At Ta ranging from 34–37°C ethanol has been shown to produce hypothermia in the mouse [21,28] and rat [27]. Little attention [1], however, has been paid to the essentially poikilothermic effect of ethanol in the study of tolerance. Although the production of tolerance to the hypothermic effect of ethanol by chronic ethanol treatment at normal room Ta is well documented, as pointed out above, it is important to study tolerance development to the hypothermic effect of ethanol during chronic treatment at higher and lower Ta. Such studies would further our understanding of the role of the drug effect *per se*, in comparison with that of learning and other factors, in ethanol tolerance [5, 12, 30].

In the present study, we examined the effect of chronic treatment with ethanol at different ambient temperatures on the rate of acquisition of tolerance to ethanol-induced hypothermia. In addition, the effect of preventing the hypothermic effect of ethanol on the maintenance of an already acquired tolerance to ethanol-induced hypothermia was also investigated.

METHOD

Male Wistar rats weighing 200–250 g were obtained from Charles River Laboratories (Quebec, Canada). They were housed singly and fed with a standard rat chow diet with food and water available ad lib. The temperature of the colony

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room was maintained at 21±1°C, and lights were on from 7 a.m.–7 p.m. throughout all experiments.

In all studies, rectal temperature was measured by inserting a teleprobe 5 cm into the rectum for 30 sec or longer until a stable reading was obtained on a Yellow Spring Instrument electrical thermometer. No external restraint was applied for this procedure, the rats being permitted to rest on the experimenter's arm. The hypothermic effect of ethanol was determined by taking the rectal temperature prior to, and at 30, 60 and 90 minutes after, the administration of ethanol at Ta of 21°C. The hypothermic effect was quantified as the maximal drop in temperature over this time period.

Experiment 1(a)

Forty rats were employed for this study. Following two weeks of acclimation to the housing conditions at a normal Ta of 21°C, the hypothermic responses to a challenge dose of 3 g/kg of ethanol IP (15% w/v solution in saline) were determined in all animals. The rats were divided into 4 groups (n=10 each) matched with respect to their maximal hypothermic responses, and were designated to receive ethanol or sucrose treatment. Three groups of rats were treated daily with 5 g/kg of ethanol (25% w/v solution in tap water) by gavage. Following ethanol administration, animals in Group I were placed immediately into a chamber kept at 36°C, which offset ethanol-induced hypothermia. They were left there for 12 hr to ensure complete elimination of ethanol, so that they could not experience hypothermia when returned to their home cages. The animals in Group II were placed in individual cages in a cold room kept at 4°C, which enhanced the hypothermic effect of ethanol. They could not be left there for more than 6 hr because of the risk of irreversible hypothermia. The third group received daily ethanol treatment in individual cages at normal Ta (21°C). An additional control group received daily equicaloric sucrose at room temperature. To monitor the development of tolerance to the hypothermic effect of ethanol, the hypothermic responses to a challenge dose of 3 g/kg IP at room Ta were examined in all animals at five-day intervals. On day 29, the hypothermic responses to the same test dose of ethanol were assessed in all animals at Ta of 4°C.

Experiment 1(b)

Because of limited accommodation in the environmental chambers, it was not possible to include all the appropriate control groups in Experiment 1(a). Therefore the possibility remained that repeated exposure to low and high Ta might by itself alter the hypothermic response to a test dose of ethanol. This question was addressed in a separate experiment. Forty-two rats were acclimatized to a Ta of 21°C and were then tested with ethanol and divided into five matched groups of 8 or 9 per group, as in the preceding experiment. Two groups (n=9) then received daily ethanol exposures at 4°C or 21°C, as for groups II and III of Experiment 1(a). The remaining three groups (n=8 each) received daily intubations of sucrose, at environmental temperatures of 36°, 21° and 4°C respectively, for the same duration as in Experiment 1(a). All groups were then tested under ethanol (3 g/kg IP) at 21°C, on the same schedule as in the preceding experiment.

Experiment 2

This experiment was carried out to examine whether the development of tolerance to the hypothermic effect of ethanol in the group treated with ethanol at Ta of 36°C in Experiment 1 was due to the repeated testing at five-day intervals at room Ta. Three groups of rats (n=10 each) were employed for this study. The first two groups received daily ethanol treatment (5 g/kg) at Ta of 21°C and 36°C respectively, as described above. The remaining group received equicaloric sucrose treatment at 21°C. After 25 days of chronic treatment, the hypothermic responses to the test dose of 3 g/kg were determined in all animals at Ta of 21°C.

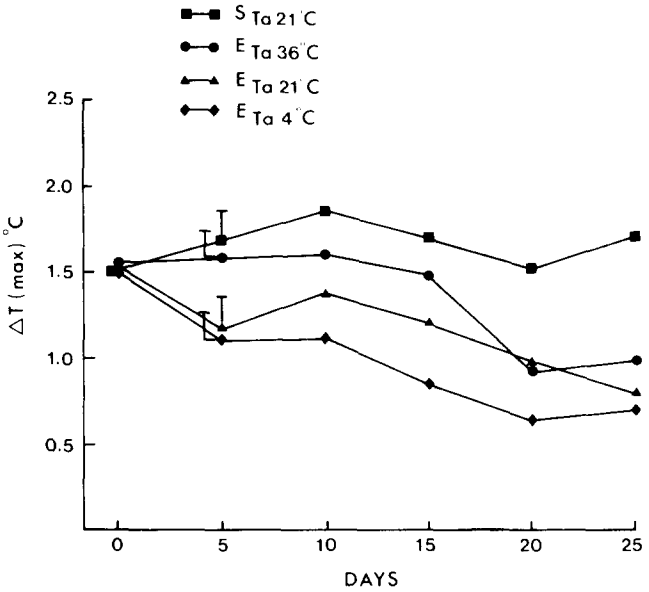

FIG. 1. Effects of daily ethanol treatment at various ambient temperatures on the acquisition of tolerance to ethanol-induced hypothermia. ΔT (max) is the maximum fall in rectal temperature after a dose of 3 g/kg IP at Ta of 21°C. Tests were conducted before and at various times during chronic daily administration of 5 g/kg ethanol (E) and equicaloric sucrose (S) by gavage. Vertical bars represent positive half of the largest S.E.M. for each group; n=10 per group.

Experiment 3

This experiment was designed to examine the influence of Ta on the maintenance of tolerance to ethanol-induced hypothermia. Rats were rendered tolerant to the hypothermic effect of ethanol by daily gavage with 5 g/kg of ethanol for 28 days at Ta of 21°C. On day 29, they were challenged with a test dose of 3 g/kg of ethanol IP, and were divided into three groups (n=10 each) matched with respect to their hypothermic responses to this dose. Groups I and II received continuing ethanol treatment at Ta of 21°C and 36°C respectively. Ethanol treatment was discontinued in the remaining group. The ethanol-induced hypothermia responses in all three groups were measured at three-day intervals for 9 days with the same test dose of ethanol, at Ta of 21°C.

RESULTS

The development of tolerance to the hypothermic effect of ethanol, in groups receiving ethanol chronically at different ambient temperatures, is shown in Fig. 1. An overall analysis of variance shows a significant Groups effect, $F(3,36)=22.3$, $p<0.001$, and Groups × Trials effect,

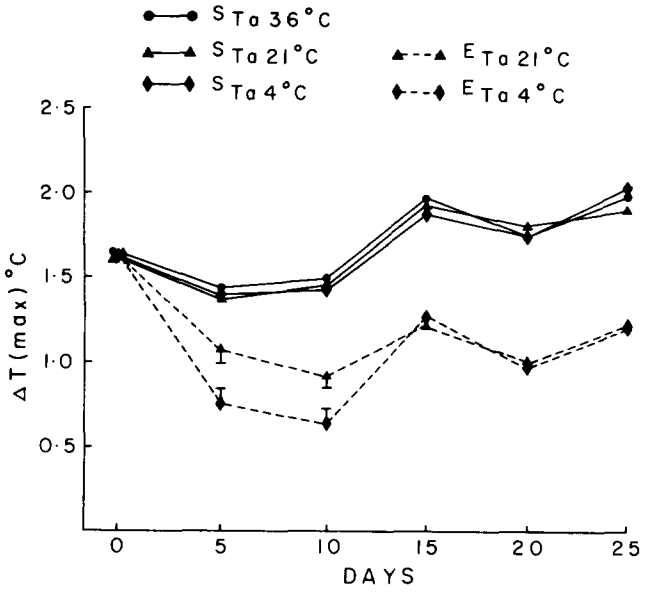

FIG. 2. Effects of daily ethanol treatment at various ambient temperatures on the acquisition of tolerance to ethanol-induced hypothermia. ΔT (max) is the maximum fall in rectal temperature after a dose of 3 g/kg IP at Ta of 21°C. Tests were conducted before and at various times during chronic daily administration of 5 g/kg ethanol (E) and equicaloric sucrose (S) by gavage. Vertical bars represent positive or negative half of the largest S.E.M. for each group; n=8–9 per group.

$F(5,180)=3.11$, $p<0.001$. Simple effect tests show that the degree of hypothermia in all ethanol-treated groups changed significantly with the duration of treatment, $F(5,32)=9.3$, $p<0.001$ for ethanol-treated group at 36°C; $F(5,32)=10.7$, $p<0.001$ for ethanol at 21°C, and $F(5,32)=13.1$, $p<0.001$ for ethanol at 4°C; this indicated that tolerance developed to the hypothermic effect of ethanol in all ethanol-treated groups. The hypothermic responses in the sucrose-treated group, however, remained unchanged over the duration of the experiment, $F(5,32)=0.83$, $p>0.05$.

Significant differences between the hypothermic response of the sucrose-treated group and those of the ethanol-treated groups at 4° and 21°C were observed after 10 days of ethanol treatment ($p<0.05$ in both cases, Newman-Keuls test). However, the test responses of the group receiving ethanol at 36°C did not differ significantly from those of the sucrose group until day 20 of the experiment. Similar comparisons also revealed that the group tested with ethanol at 4°C showed a significantly smaller degree of hypothermia on day 15 than the group tested with ethanol at 21°C ($p<0.05$). However, by day 25 the three ethanol-treated groups did not differ significantly from one another with respect to their hypothermia test responses. Thus, although chronic ethanol treatment produced tolerance to the hypothermic effect in all the groups, the onset of such tolerance and the duration of treatment required to attain maximal tolerance were dependent on the ambient temperature at which the ethanol treatment was carried out.

When tested at an ambient temperature of 4°C on day 29, the sucrose-treated group showed a drop in body temperature of 3.5±0.25°C, while drops of 1.48±0.18°, 1.55±0.15° and 1.8±0.16° were observed for the groups which had been treated with ethanol at 4, 21 and 36°C respectively. Duncan multiple range t-tests revealed that the degrees of hypothermia in the three ethanol-treated groups were indistinguishable from one another; they were, however, all significantly smaller than that of the sucrose-treated group.

The results of Experiment 1(b) are shown in Fig. 2. Consistent with the visual impression, analysis of variance revealed no significant differences among the three sucrose-treated groups, $F(2,20)=0.7$, $p>0.9$. This indicated that daily exposure to Ta of 4°C or 36°C following sucrose treatment for 25 days did not alter the hypothermic response to ethanol measured at Ta of 21°C. A comparison of the ethanol and sucrose-treated groups at Ta of 21°C and 4°C revealed no significant effect of Ta, $F(1,30)=0.63$, $p>0.4$. This analysis of variance, however, revealed a highly significant effect of ethanol treatment, $F(1,30)=39.6$, $p<0.001$, as well as a significant ethanol × days interaction, $F(5,150)=8$, $p<0.001$, which indicated that chronic ethanol treatment resulted in the development of tolerance to the hypothermic effect of ethanol. A comparison of two ethanol-treated groups from day 0 to 15 showed that the interaction between days and Ta approached significance, $F(2,32)=2.8$, $p=0.07$. Simple effect tests revealed that the degree of hypothermia in the ethanol-treated groups at Ta of 4°C were significantly smaller than that of the ethanol-treated group at Ta of 21°C on day 5, $t(16)=2.4$, $p<0.028$, and day 10, $t(16)=2.1$, $p<0.05$, but not on all the subsequent test days. These analyses thus indicated that ethanol treatment at Ta of 4°C resulted in a facilitation in the rate of tolerance acquisition.

The results of the second experiment are shown in Fig. 3. Both ethanol-treated groups showed a significantly lesser degree of ethanol-induced hypothermia than the sucrose group, $t(15)=4.2$, $p<0.01$ for ethanol at 21°C, and $t(16)=4.4$, $p<0.01$ for ethanol at 36°C, compared to sucrose. There was no significant difference in the degree of hypothermia between the two ethanol-treated groups. These results indicate that chronic treatment with ethanol at 21° or 36°C produced tolerance to its hypothermic effect, even without repeated testing.

The effect of ambient temperature on the maintenance of tolerance to the hypothermic effect of ethanol is shown in Fig. 4. An overall analysis of variance showed a significant group effect, $F(2,27)=7.9$, $p<0.005$, as well as a significant interaction between groups × trials, $F(6,81)=3.9$, $p<0.003$; these results indicate that the hypothermic responses were different among the three groups examined, and that such differences varied as a function of time. Tests of simple effects within groups across trials showed that the hypothermic responses in the ethanol-withdrawn group changed significantly with time, $F(3,25)=12.4$, $p<0.001$. Similar tests, however, showed that the hypothermic responses in the two groups still receiving ethanol treatment either at 36°C Ta, $F(3,23)=2.3$, $p>0.10$, or at 21°C, $F(3,23)=1.5$, $p>0.24$, remained unchanged over the testing period. These results thus indicate that tolerance to the hypothermic effect of ethanol was maintained by continuation of ethanol treatment at Ta either of 21° or 36°C but decayed only if ethanol treatment was terminated.

DISCUSSION

The results of Experiment 1 confirmed the prediction that the development of tolerance to the hypothermic effect of ethanol would vary according to the environmental temperature at which the ethanol effects were experienced. This variation was not due to the repeated exposure to different Ta

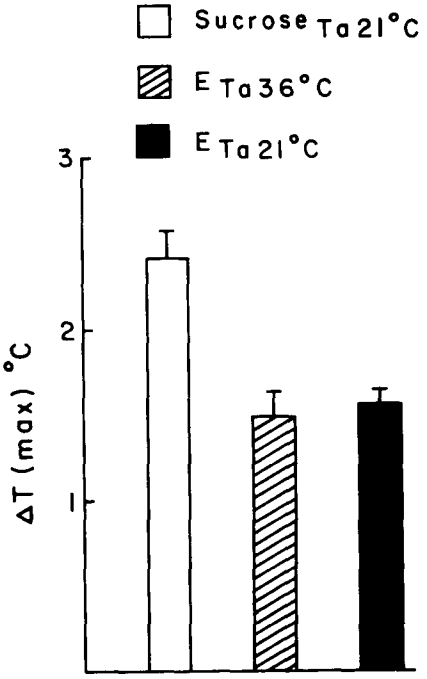

FIG. 3. Maximum fall in body temperature (ΔT max) in response to an IP injection of ethanol (3 g/kg) at 21°C Ta in animals which had been chronically treated with ethanol at Ta of 21°C and 36°C, and with sucrose at 21°C. Single test was performed after 25 days of treatment. Vertical bars indicate positive half of S.E.M. for each group; n=10 per group.

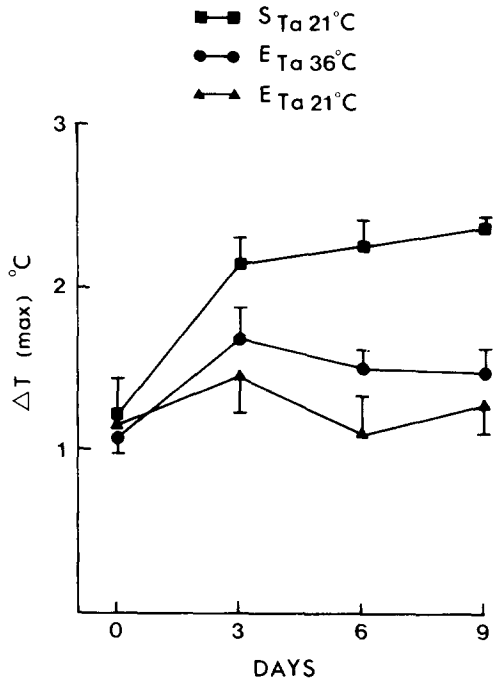

FIG. 4. The hypothermic responses to ethanol (3 g/kg IP, at Ta of 21°C) at various intervals following termination of chronic ethanol treatment (ˢTa 21°) or during continuing ethanol (E) treatment at 21° and 36°C Ta. Vertical bars indicate positive or negative half of S.E.M. at each test; n=10 per group.

per se, since the control groups at different temperatures were not affected.

A most important finding derived from these studies is that tolerance to the hypothermic effect of ethanol still developed following chronic treatment with ethanol at Ta of 36°C. In other words, tolerance to the hypothermic effect of ethanol developed despite the absence of the hypothermic experience during chronic treatment. A longer duration of chronic treatment days, however, was required to produce significant tolerance to ethanol-induced hypothermia in the group treated at Ta of 36°C than in those treated at 21° or 4°C (20 vs. 10 and 10 days respectively). It could be argued that the observed tolerance in the group treated with ethanol at Ta of 36°C was attributable to the hypothermia experience resulting from the repeated testing at five-day intervals [30]. Such a possibility, however was ruled out completely by the results of the second experiment, in which a non-repeated testing design was employed. Moreover, it should be pointed out that if the test doses alone were sufficient to produce tolerance, one would expect that tolerance should develop equally in both the sucrose-treated and ethanol-treated group at Ta of 36°C.

Although blood ethanol levels were not determined in the present study, it is unlikely that changes in pharmacokinetic factors would play a critical role in the observed effects of Ta on tolerance. Acclimation to cold [29] or exposure to Ta of 34°C [7] has been reported to cause minimal or no change in the rate of ethanol elimination in the rats. In our experiments, even though chronic ethanol treatment was carried out at various Ta's, tolerance tests were always carried out at room temperature with the exception of the last test day in Experiment 1. With the test dose and the concentration of ethanol employed in our experiments, maximum hypothermia occurred usually at 30–60 min after ethanol administration, at which time the effect of metabolic tolerance on the blood alcohol level is still quite small [10]. It is difficult to assess this point accurately, because the taking of blood samples during the experiment would itself probably disturb body temperature.

Alkana *et al.* [1] reported a lack of tolerance to the hypothermic effect of ethanol in mice following chronic ethanol treatment at Ta of 36°C. This apparent discrepancy between our present finding and that of Alkana *et al.*, however, might be due to the difference in the treatment regimen employed. In the study by Alkana *et al.*, the mice were treated daily with 3.6 g/kg for a duration of 6 days, while in our study animals were treated daily with 5 g/kg for a period of 25 days. Furthermore, we did not observe any significant tolerance until day 20 of the experiment. Therefore, our study would have led to the same conclusion as that of Alkana *et al.* if the experiment had been terminated at an earlier time.

In contrast, chronic ethanol treatment at Ta of 4°C, which augmented its hypothermic effect, facilitated the development of tolerance to ethanol-induced hypothermia. Like ethanol treatment at 36°C, treatment with ethanol at 4°C affected only the rate at which tolerance was acquired, but not its final extent when tested at Ta of 21°C on day 25. More surprising, however, was the observation that, even though the actual hypothermic response was greater at 4°C (day 29) than at 21°C, the three ethanol-treated groups all showed the same degree of tolerance when tested at 4°C. Work by

Lomax and Lee [22] had shown that rats which were acclimated to low Ta (4°C) showed an attenuation of the hypothermic effect induced by various doses of ethanol at 4°C. We have also found that rats exposed to −10°C for 2 hr daily for 10 days were cross-tolerant to ethanol-induced hypothermia as measured at Ta of 21°C (Le *et al.*, unpublished results). Therefore it might have been expected that the group receiving ethanol daily at 4°C would show greater tolerance than the other two groups when tested with ethanol at 4°C. However, the experiment by Lomax and Lee involved continuous acclimation to a Ta of 4°C for 7 days, whereas in the present work the daily exposure to cold was limited to 6 hr. Therefore the findings may not really be in conflict.

Termination of ethanol treatment resulted in the expected loss of the acquired tolerance to the hypothermic effect of ethanol (Experiment 3). In fact, consistent with our previous observation [16], tolerance to the hypothermic effect of ethanol decayed almost completely by 3 days after cessation of ethanol treatment. Once tolerance to the hypothermic effect of ethanol has been acquired, it is also quite clear that such tolerance can be maintained by continuing ethanol treatment even in conditions in which the hypothermic effect of ethanol was antagonized (Ta 36°C). Since a repeated test design was employed in this experiment, the influence of the test dose on the maintenance can not be ruled out. However, it is quite apparent that simply blocking the ethanol-induced hypothermia did not abolish tolerance during the same 3-day period in which cessation of ethanol treatment did eliminate tolerance.

Taken together, these data suggest that ethanol tolerance can not be explained purely as a consequence of learning [4, 5, 30]. According to a learning theory, we should expect an absence of tolerance to the hypothermic effect of ethanol after chronic treatment with ethanol at Ta of 36°C, as well as an extinction of the acquired tolerance when the hypothermic effect of the drug is antagonized. The facilitation or retardation of tolerance development, by respectively augmenting or antagonizing the drug effect, as observed in the present study is consistent with the notion that the degree of functional disturbance induced by the drug is an important factor in modulating tolerance development [12, 14, 18, 21]. However, the eventual development of tolerance in the group receiving ethanol at 36°C suggests that the adaptation to the administration of ethanol might reflect a reduced cellular action of ethanol (e.g., on the membrane), which in turn provides tolerance to ethanol independent of the specific experiences during intoxication (e.g., hypothermia). This is consistent with earlier observations on tolerance to the effects of ethanol on various cognitive and motor performances [19–21]. Recent work by Alkana *et al.* [2] has shown that the sensitivity to ethanol-induced hypnosis varies with body temperature/ambient temperature in accordance with membrane theories of anaesthesia. It would be therefore of interest in future work to examine simultaneously the rate of acquisition of tolerance to both the hypothermic and the hypnotic effects of ethanol under various ambient temperatures to evaluate further the role of the drug effect on tolerance development.

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