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Tolerance to Ethanol and Cross-Tolerance to Pentobarbital and Barbital in Four Rat Strains

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KHANNA, J. M., H. KALANT, G. SHAH AND A. CHAU. *Tolerance to ethanol and cross-tolerance to pentobarbital and barbital in four rat strains.* PHARMACOL BIOCHEM BEHAV **39**(3) 705–709, 1991.—Chronic ethanol treatment by gastric intubation conferred tolerance to ethanol-induced motor impairment and hypnosis in four different rat strains: Fischer 344, Long-Evans, Sprague-Dawley, and Wistar. Cross-tolerance to barbital was also observed in all strains after chronic treatment with ethanol. However, chronic ethanol treatment failed to produce cross-tolerance to pentobarbital-induced motor impairment and hypnosis in any of the four strains. The demonstration of cross-tolerance to barbital and the lack of it to pentobarbital after chronic ethanol treatment confirms and extends recent observations on the specificity of the site and/or mechanism of action of sedative-hypnotic drugs that differ in lipid solubility.

Tolerance Cross-tolerance Rat strains Pentobarbital Barbital

RECENTLY, we reported that chronic administration of ethanol by gavage, which produced marked tolerance to ethanol-induced hypothermia, ataxia and loss of righting reflex, produced only a minimal degree of cross-tolerance to these effects of pentobarbital. However, cross-tolerance to another barbiturate, barbital, was observed after this chronic ethanol treatment regimen (3).

Similar findings were reported in two studies by another group of investigators. Newman et al. (11) found that chronic ethanol intake, sufficient to produce tolerance to alcohol, also resulted in cross-tolerance to diazepam, but not to thiopental. In a second study (2), these authors found that cross-tolerance to barbiturates in alcohol-treated rats was not uniform. Clear cross-tolerance to longer-acting barbiturates (barbital and phenobarbital) was seen, whereas cross-tolerance to shorter-acting barbiturates (thiamylal, methohexital and secobarbital) was negligible. These authors, however, did find cross-tolerance to pentobarbital, another relatively short-acting barbiturate.

Although these findings are in general agreement with ours and suggest a degree of specificity in the actions of ethanol and other sedative-hypnotics (3,9), the results of Newman et al. with pentobarbital (11) are puzzling. Furthermore, given the widely accepted belief that cross-tolerance occurs among ethanol and other barbiturates, it is important to investigate further the question of differential cross-tolerance to barbital and pentobarbital, so as to permit firm conclusions. Although selected lines such as Long sleep (LS) and Short sleep (SS) mice, which have been selected for differences in ethanol sensitivity, can provide meaningful data with lesser variability than the unselected strains, we chose outbred strains because most selected lines have been bred for difference in ethanol preference or drinking, and not for difference in sensitivity. Therefore, not enough appropriately selected lines are available for the purpose of the present work. Furthermore, comparison of four or more outbred strains can provide a broader spectrum of different degrees of sensitivity than comparison between one pair of selected lines. We have, therefore, investigated the generality of this phenomenon by examining cross-tolerance in four different rat strains.

METHOD

Animals

Male rats of four different strains (F344, Sprague-Dawley, Wistar and Long-Evans), weighing 150–200 g, were obtained from Charles River breeding laboratories (Montreal, Quebec). They were housed singly and fed a standard laboratory rat chow in a daily ration that was individually adjusted to maintain comparable body weights in two different treatment groups throughout the study. Tap water was available at all times. The temperature of the colony room was maintained at 21 ± 1°C, and lights were on from 7 a.m. to 7 p.m. daily.

Test Procedures

Motor impairment. The tilting-plane test was used to measure motor impairment (1,3). The apparatus consists of a plane which can be rotated about a pivot at one end by means of a cord and pulley arrangement and a motor that moves the plane at a fixed angular velocity through a range of 55° from the horizontal axis. The animal is placed on the slightly roughened surface of the plane, which is then tilted until the animal slides from the starting position. The test measure is the angle at which the animal begins to slide. The sliding angle was measured before and at 30, 60 and 90 min after the injection of ethanol (2.6 g/kg), pentobarbital (25 mg/kg) or barbital (132 mg/kg). The degree of postdrug ataxia was assessed as the percentage change in sliding angle, compared to the same animal's preethanol

value. Maximum impairment, regardless of the time of its occurrence, was employed as the measure of drug effect.

Sleep Time

Rats of the different strains were injected IP with ethanol (3.5 g/kg), pentobarbital (40 mg/kg) or barbital (175 mg/kg). The time between the injection and loss of righting reflex was recorded as the induction time. Sleep time was the interval between loss and return of righting reflex. Recovery was verified by immediately placing the rat on its back again and observing a second successful righting response within 1 min.

Ethanol Metabolism

Ethanol elimination was studied in separate groups of rats in three of these strains (n=20 for each strain) before and at 7 weeks after chronic ethanol or sucrose treatment. After the administration of a test dose of ethanol (2.5–3 g/kg), tail vein blood samples (50 μl) were obtained from each animal at half-hourly intervals until 2 h, and thereafter at hourly intervals for 4 h more. The disappearance rate of blood ethanol was calculated from the slope of the linear descending portion of each curve, and the rate of ethanol metabolism in mg/kg/h was calculated as described previously (8).

Pentobarbital Metabolism

Pentobarbital metabolism was studied after 11 weeks of chronic ethanol or sucrose treatment in the same animals previously used for ethanol metabolism. For this purpose, rats were injected IP with pentobarbital (25 mg/kg), and tail vein blood samples (100 μl) were obtained from each animal every 15 min until 105 min after injection. The samples were collected into glass or polypropylene tubes containing 1 μg amobarbital in 0.25 ml water as internal standard.

Chronic Ethanol Treatment

Rats of each of the four different strains were separated into two matched subgroups based on their initial motor impairment response to ethanol. One subgroup received ethanol. The other was used as control and received isocaloric sucrose. Ethanol was initially given by gavage at 5 g/kg daily in the morning. An additional dose of 2 g/kg at 5 p.m. daily was introduced after three weeks of treatment and increased to 2.5 g/kg in 5 days. The solution strength started at 17.5% w/v and was increased by 2.5% w/v every 4 days, to a maximum of 25% w/v, in order to avoid large increases of volume due to the growth of the rats. On the day before a test day, the afternoon dose was omitted.

Drug Analysis

Blood ethanol was analyzed by the enzymatic method described previously (5). Barbiturates were analyzed by gas-liquid chromatography, by an on-column methylation procedure (8).

Experimental Procedure

Eighteen rats from each strain were tested for initial sensitivity to ethanol-induced motor impairment on the tilt-plane test. They were then divided into two subgroups matched with respect to their maximum motor impairment response, as noted above. After the second, third and fifth week of chronic ethanol treatment, the motor impairment response on the tilt-plane was again measured to assess tolerance to ethanol. The duration of sleep induced by ethanol was measured at 6 weeks. Pentobarbital and barbital sleep parameters were measured at 7 and 11 weeks respectively. Tolerance to the motor impairment effect of pentobarbital and barbital was studied at 8 weeks and 13 weeks respectively. Tolerance to the motor impairment effect of ethanol was again assessed at 9 weeks to verify its stability; the degree of tolerance was unchanged from that found at 5 weeks.

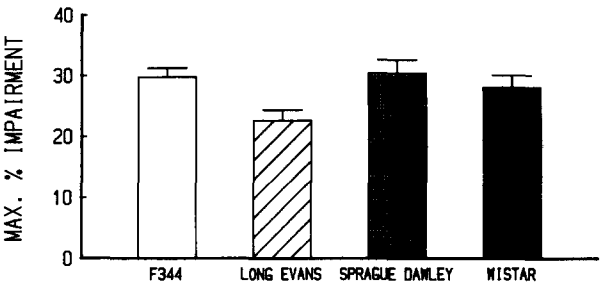

FIG. 1. Maximum percentage impairment (tilt-plane test) by ethanol (2.5 g/kg) on day 1 (before start of chronic treatment) for Fischer 344, Long-Evans, Sprague-Dawley and Wistar rats. N=18 animals in each strain. Vertical lines indicate positive half of standard error of each mean.

RESULTS

Figure 1 presents results of maximum percentage impairment (i.e., day 1, prior to start of chronic treatment with ethanol) on the tilt-plane test in the four strains. A one-way ANOVA of between strain variation showed significant difference for percent impairment, $F(3,34)=3.958$, $p<0.01$. Post hoc multiple comparison of means by Scheffe's method revealed that Fischer 344 and Sprague-Dawley rats had significantly greater maximum percentage impairment ($p<0.01$) than Long-Evans rats. All other comparisons failed to show any significant difference.

Figure 2 shows the effect of chronic ethanol treatment after 5, 8 and 13 weeks, on the motor impairment response (maximum percentage impairment) to ethanol, pentobarbital and barbital respectively. A multifactorial ANOVA was performed on maximum percentage impairment data for overall analysis with strain, treatment and drug as main effects. It showed a significant strain difference, $F(3,159)=16.16$, $p<0.0001$, a significant effect of treatment, i.e., ethanol vs. sucrose treatment, $F(1,159)=29.71$, $p<0.0001$, and a significant difference between drugs, i.e., ethanol, pentobarbital and barbital, $F(2,159)=17.02$, $p<0.0001$. The strain × treatment interaction was not significant, $F(3,159)=0.16$, $p>0.93$, indicating that the effect of chronic ethanol treatment was similar in all strains. A significant strain × drug interaction, $F(6,159)=5.01$, $p<0.0001$] and drug × treatment interaction, $F=(2,159)=8.01$, $p<0.0005$, suggested that the test drugs have significantly different effects on motor impairment response among different strains after ethanol treatment.

A further breakdown analysis was made between cross-tolerance response to pentobarbital and barbital. This also showed that strains were significantly different, $F(3,100)=17.65$, $p<0.0001$. A significant effect of ethanol vs. sucrose treatment, $F(1,100)=5.40$, $p<0.022$, and a significant difference between drugs, i.e., pentobarbital vs. barbital motor impairment, $F(1,100)=8.48$, $p<0.0044$, was observed. The strain × treatment interaction was not significant, $F(3,100)=0.09$, $p>0.97$, and there was no strain × drug interaction, $F(3,100)=1.33$, $p>0.271$. These results showed that the effect of chronic ethanol treatment was

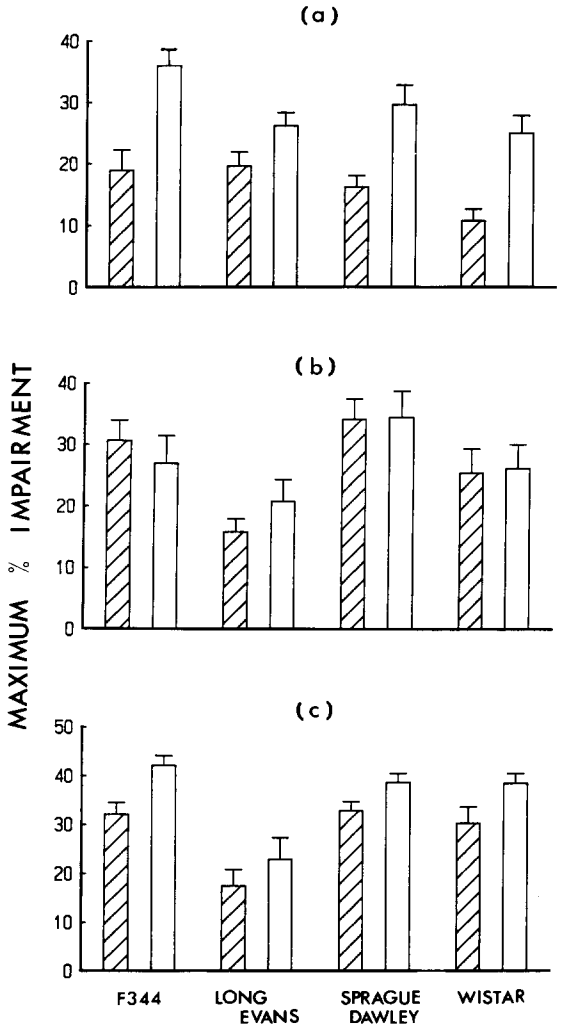

FIG. 2. Effect of chronic ethanol treatment on the motor impairment response to ethanol (2.5 g/kg), pentobarbital (25 mg/kg) and barbital (132 mg/kg) in the four strains of rats. (a) ethanol test after 5 weeks of chronic ethanol, (b) pentobarbital test after 8 weeks of ethanol, and (c) barbital test after 13 weeks of ethanol. Hatched bars, chronic ethanol-treated; plain bars, control. Vertical lines indicate positive half of standard error of each mean. N=9 animals in each group, except Fischer 344, for which n=6 animals each.

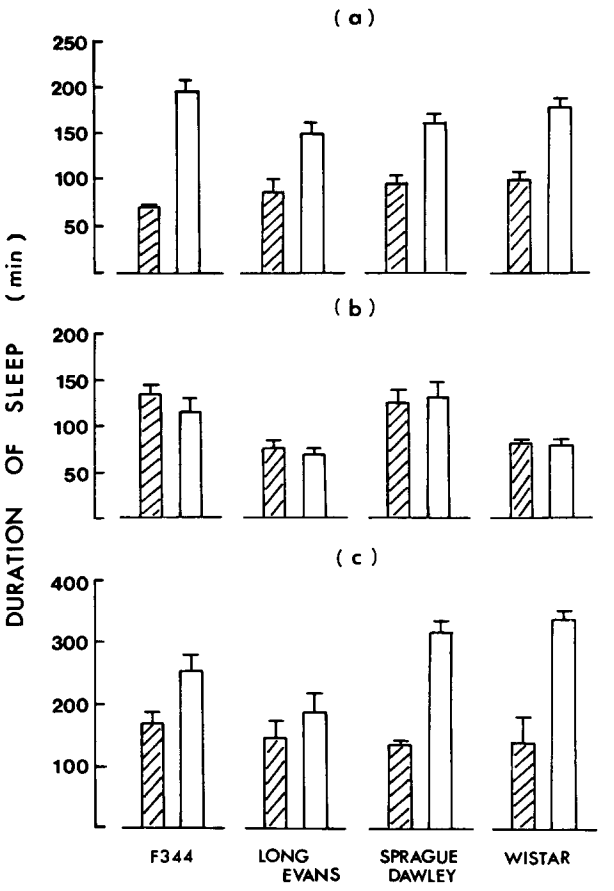

FIG. 3. Effect of chronic ethanol treatment on duration of sleep induced by ethanol (3.5 g/kg), pentobarbital (40 mg/kg) and barbital (175 mg/kg) in the four strains of rat: (a) ethanol test after 6 weeks of chronic ethanol (b) pentobarbital test after 7 weeks of ethanol (c) barbital test after 11 weeks of ethanol. Cross-hatched bars, chronic ethanol-treated; plain bars, control. Vertical lines indicate standard errors. N=9 animals in each group, except Fischer 344, for which n=6 animals each.

similar in all strains, and the effect of a particular drug was similar for different strains. The significant treatment × drug interaction, $F(1,100)=3.95$, $p<0.05$, showed that the effect of chronic ethanol treatment was significantly less for pentobarbital than for barbital motor impairment.

The effect of chronic ethanol treatment on the duration of sleep induced by ethanol, pentobarbital and barbital is shown in Fig. 3. A multifactorial ANOVA showed significant main effects of strain, $F(3,133)=4.71$, $p<0.004$, treatment, $F(1,133)=97.16$, $p<0.0001$, and drug, $F(2,133)=93.87$, $p<0.0001$. This indicates that the different strains of rats had significantly different durations of hypnotic effect after chronic ethanol treatment, and that this duration was significantly different for ethanol, pentobarbital and barbital. A significant strain × treatment × drug interaction, $F(6,133)=3.60$, $p<0.0025$, indicated that the changes in effects of the three drugs after chronic ethanol treatment are significantly different among different strains. A further breakdown analysis was carried out between cross-tolerance response to pentobarbital and barbital. This analysis also showed all main effects to be significant: strain, $F(3,84)=4.06$, $p<0.0097$, treatment, $F(1,84)=41.24$, $p<0.0001$, and drug, $F(1,84)=151.94$, $p<0.0001$. There was also a significant strain × treatment × drug interaction, $F(3,84)=3.25$, $p<0.027$. A significant treatment × drug interaction, $F(1,84)=49.01$, $p<0.0001$, suggested that the effect of chronic ethanol treatment (i.e., tolerance) is significantly smaller for pentobarbital than for barbital tests.

Table 1 shows the calculated rates of ethanol metabolism at 0 week and after 7 weeks of chronic ethanol or sucrose treatment. One-way ANOVA showed a significant effect of strain, $F(2,51)=13.47$, $p<0.0001$, and a Newman-Keuls test showed that this was due to the Long-Evans rats having a significantly higher rate than the other two strains ($p<0.01$ in each case). In contrast, after 7 weeks of chronic treatment with either ethanol or equicaloric sucrose, only the treatment effect (ethanol vs. sucrose) was significant, $F(1,50)=57.03$, $p<0.00001$, but neither strain nor strain × treatment interaction was significant.

Similarly, the half-life values revealed no differences in pentobarbital metabolism between ethanol-treated and control rats in

TABLE 1

ETHANOL METABOLISM AT 0 AND 7 WEEKS AND PENTOBARBITAL METABOLISM AT 11 WEEKS IN THREE DIFFERENT RAT STRAINS AT VARIOUS WEEKS AFTER CHRONIC ETHANOL (E) OR ISOCALORIC SUCROSE (S) TREATMENT*

Strains		Ethanol Metabolism (mg/kg/h) 0 Week		7 Week		Pentobarbital Half Life (Min) 11 Week
Wistar	E	236.0 ± 9.5	E	362.3 ± 9.6	E	77.5 ± 7.6
	S	244.0 ± 7.4	S	303.5 ± 10.6	S	68.0 ± 4.1
Sprague-Dawley	E	226.2 ± 13.2	E	346.8 ± 17.8	E	145.7 ± 12.9
	S	241.7 ± 6.6	S	276.4 ± 16.0	S	146.3 ± 14.0
Long-Evans	E	286.2 ± 15.2	E	385.0 ± 11.1	E	53.7 ± 3.4
	S	304.2 ± 17.4	S	278.5 ± 9.9	S	64.0 ± 6.6

*n = 8–10 animals in each group.

any of the strains, although the strains differed markedly from each other. Therefore, it seems unlikely that the differences among strains after chronic ethanol treatment in the present work could be attributed to differences in dispositional tolerance.

DISCUSSION

Tolerance to the motor impairment effect of ethanol was observed in all strains chronically treated with ethanol (Fig. 2a). The tolerance was approximately of the same magnitude (45–50% reduction of effect at the test dose used), except for the Long-Evans strain, in which the difference was small and only marginally significant ($t = 2.042$; for $p < 0.05$, $t = 2.11$). The reason for this is not clear. In contrast to a lack of clear tolerance to ethanol in Long-Evans on the motor impairment task, this strain showed significant tolerance to the hypnotic effect of ethanol (Fig. 3a). The extent of ethanol tolerance was approximately similar in all strains (40% reduction of effect) except for Fischer 344, in which it appears somewhat higher (64%). The presence of clear tolerance to the hypnotic effect of ethanol in the Long-Evans rats, despite the marginal result in the tilt-plane test, underscores the fact that tolerance is not a unitary phenomenon and can differ substantially in degree for different effects of ethanol in the same animal (7). Although ethanol metabolism in these strains was not examined in this study, we did compare it in another study in three of these strains (Long-Evans, Sprague-Dawley and Wistar), both in the naive state and after seven weeks of a similar chronic ethanol treatment (Table 1). It would seem unlikely that the differences among strains after chronic ethanol treatment in the present work could be attributed to differences in dispositional tolerance.

In spite of clear evidence of tolerance to ethanol, chronic treatment with ethanol failed to produce significant cross-tolerance to pentobarbital-induced motor impairment and loss of righting reflex in any of the strains. Again, Long-Evans rats (both control and ethanol-treated) showed significantly lower motor impairment and sleep time scores than the other three strains. Examination of pentobarbital metabolism in three of these strains after 11 weeks of a similar regimen of chronic intubation with ethanol in another study revealed no differences in pentobarbital metabolism between ethanol-treated and control rats in any of the strains, although the strains differed markedly from each other. The observed differences in motor impairment and sleep time among the three strains are in the same rank order as the differences in pentobarbital metabolism. However, before one can attribute the differences in response to those of metabolism, it will be necessary to have full concentration-response curves in all four strains.

In contrast to the lack of cross-tolerance to pentobarbital, cross-tolerance to barbital was observed in all strains after chronic treatment with ethanol, although the differences were somewhat less clear in Long-Evans rats. But again, as with ethanol, the basal values in the Long-Evans control group were markedly lower than in the other strains.

The assessment of tolerance to barbital motor impairment was conducted on rats that had previously been administered pentobarbital for the sleep and motor impairment tests and barbital for the sleep test. The two previous pentobarbital exposures probably did not contribute to the barbital cross-tolerance, because both control and ethanol-treated animals received the challenge doses of pentobarbital and did not differ significantly in their responses to them. Therefore, one would not expect the pentobarbital exposures to have any differential effect on the later barbital tests. Furthermore, due to the fact that a three-week interval was left between the second pentobarbital test and the barbital sleep test, it is possible to rule out any residual influence of pentobarbital testing. Ideally, we should have also left an even longer gap between the two barbital tests. Therefore, it is not possible to rule out some confounding influence of barbital sleep-time test on barbital motor impairment.

Studies of cross-tolerance to pentobarbital and barbital were performed after different periods of ethanol exposure, with the barbital cross-tolerance always being tested near the end of the study. This was done to avoid any complications due to the long half-life of barbital compared to that of pentobarbital. It could be argued that cross-tolerance was seen with barbital because it had more time to develop than with pentobarbital. However, this is not a convincing explanation since no cross-tolerance was seen when short-acting barbiturates were tested on the tilt-plane test after 17–19 weeks of a similar chronic ethanol treatment (Khanna et al., unpublished work).

The sleep durations following barbital were considerably longer than after pentobarbital. The possibility that these baseline differences in sleep durations could have accounted for cross-tolerance differences between barbital and pentobarbital would be compatible with the suggestion that the proportion of time during which the CNS is exposed to the drug is a critical factor in determining the development of functional tolerance. This could explain the differential development of cross-tolerance to barbital and not to pentobarbital. The present studies do not provide any further insight into this, and further studies are required to address this issue with respect to the sleep measure. However, this explanation does not apply to the motor impairment task. Cross-tolerance to barbital and the lack of it to pentobarbital, after chronic ethanol treatment, were seen when equipotent test doses of barbital and pentobarbital were used, resulting in the production of equivalent motor impairment (3).

The demonstration of cross-tolerance to barbital and the lack of it to pentobarbital, after chronic treatment with ethanol in four different strains, are consistent with the notion of considerable specificity in the site and/or mechanism of action of sedative-hypnotic drugs that differ in lipid solubility [for references, see (3, 6, 9)]. Marley et al. (10) also reported recently that LS and SS mice differ with respect to sleep induced by water-soluble barbiturates (phenobarbital and barbital), but not to that induced by the lipid-soluble barbiturates (pentobarbital and secobarbital). In other recent work, Harris and Allan (4) reported differential sensitivity to augmention by ethanol and phenobarbital, but not by pentobarbital, of muscimol-stimulated chloride flux.

Since the initial sensitivities to the selected doses of the three drugs were not strikingly different in any of the strains, the dif-

ference in cross-tolerance to pentobarbital and to barbital in all four strains also raises the question of possible differences in mechanisms responsible for direct tolerance to the two barbiturates. This warrants further study.

REFERENCES

1. Arvola, A.; Sammalisto, L.; Wallgren, H. A test for level of alcohol intoxication in the rat. Q. J. Stud. Alcohol. 19:563–572; 1958.
2. Curran, M. A.; Newman, L. M.; Becker, G. L. Barbiturate anesthesia and alcohol tolerance in a rat model. Anaesth. Analg. 67:868–871; 1988.
3. Gougos, A.; Khanna, J. M.; Lê, A. D.; Kalant, H. Tolerance to ethanol and cross-tolerance to pentobarbital and barbital. Pharmacol. Biochem. Behav. 24:801–807; 1986.
4. Harris, R. A.; Allan, A. M. Alcohol intoxication: Ion channels and genetics. FASEB J. 3:1689–1695; 1989.
5. Hawkins, R. D.; Kalant, H.; Khanna, J. M. Effect of chronic intake of ethanol on rate of ethanol metabolism. Can. J. Physiol. Pharmacol. 44:241–257; 1966.
6. Howerton, T. C.; O'Connor, M. F.; Collins, A. C. Lipid solubility is correlated with hypnotic and hypothermic responses of long-sleep (LS) and short-sleep (SS) mice to various depressant drugs. J. Pharmacol. Exp. Ther. 227:389–393; 1983.
7. Kalant, H.; Khanna, J. M. Methods for the study of tolerance. In: Adler, M. W.; Cowan, A., eds. Modern methods in pharmacology, testing and evaluation of drugs of abuse, vol. 6. Philadelphia: Wiley-Liss, Inc.; 1990:43–66.
8. Khanna, J. M.; Lê, A. D.; Kalant, H.; Kim, C. Differential sensitivity to ethanol, pentobarbital and barbital in spontaneously hypertensive (SH) and normotensive Wistar Kyoto (WK) rats. Psychopharmacology (Berlin) 86:296–301; 1985.
9. Khanna, J. M.; Mayer, J. M. An analysis of cross-tolerance among ethanol, other general depressants and opioids. Subst. Alcohol Actions Misuse 3:243–257; 1982.
10. Marley, R. J.; Miner, L. L.; Wehner, J. M.; Collins, A. C. Differential effects of central nervous system depressants in long-sleep and short-sleep mice. J. Pharmacol. Exp. Ther. 238:1028–1033; 1986.
11. Newman, L. M.; Curran, M. A.; Becker, G. L. Effects of chronic alcohol intake on anesthetic responses to diazepam and thiopental in rats. Anesthesiology 65:196–200; 1986.