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Rapid Tolerance and Crosstolerance to Motor Impairment Effects of Benzodiazepines, Barbiturates, and Ethanol

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KHANNA, J. M., H. KALANT, A. CHAU AND G. SHAH. *Rapid tolerance and crosstolerance to motor impairment effects of benzodiazepines, barbiturates, and ethanol.* PHARMACOL BIOCHEM BEHAV **59**(2) 511–519, 1998.—Motor impairment (tilt-plane test) test was used to assess the phenomenon of rapid tolerance and crosstolerance to benzodiazepines, barbiturates, and ethanol. The motor impairment responses to benzodiazepines (chlordiazepoxide and diazepam) and to various barbiturates (pentobarbital, phenobarbital, and barbital) were significantly reduced on day 2 in rats that had been treated on day 1 with benzodiazepines and barbiturates, respectively, compared to the control group treated with saline on day 1. Benzodiazepine treatment on day 1 resulted in rapid crosstolerance to the motor impairment effects of ethanol on day 2. Benzodiazepine treatment, however, did not result in rapid crosstolerance to the three barbiturates (pentobarbital, barbital, and phenobarbital) tested. In contrast to the lack of rapid crosstolerance to barbiturates after treatment with benzodiazepines, barbiturate treatment clearly conferred rapid crosstolerance to benzodiazepines and to ethanol. This asymmetry of rapid crosstolerance raises the possibility that benzodiazepines and barbiturates invoke tolerance by mechanisms that are not wholly identical. Therefore, tolerance to the broad range of actions of barbiturates would include crosstolerance to the effects of benzodiazepines, whereas tolerance to benzodiazepines would include only a weak or partial crosstolerance to some of the effects of barbiturates. © 1998 Elsevier Science Inc.

Rapid tolerance Crosstolerance Barbiturates Benzodiazepines Ethanol

CROSSTOLERANCE among ethanol, barbiturates, and benzodiazepines is generally assumed, but in fact is not documented unequivocally. Chan (5) and Khanna and Mayer (25) have reviewed both the clinical and animal studies on crosstolerance between benzodiazepine and other sedative-hypnotic drugs, and have found apparently contradictory evidence from different investigators. Although most studies do provide some indication of crosstolerance to benzodiazepines after chronic intake of ethanol (7,19,33,35), there is disagreement among investigators as to whether or not chronic benzodiazepine treatment confers crosstolerance to ethanol and other sedative-hypnotics. Cesare and McKearney (4) reported lack of crosstolerance to pentobarbital in pigeons treated chronically with chlordiazepoxide and tested on a food-reinforced task with either FI or FR schedules. Rosenberg et al. (37) also reported that chronic treatment with flurazepam produced a high degree of tolerance to diazepam-induced ataxia, but a much smaller degree of crosstolerance to ethanol and pentobarbital.

Chan et al. (6) found that chronic ingestion of ethanol by mice, which produced tolerance to ethanol on four different tests, led to a comparable degree of crosstolerance to chlordiazepoxide (CDP) on the hypothermia and horizontal dowel tests, partial crosstolerance on the runway test, and no crosstolerance on the head-dipping test. Gent et al. (13) observed crosstolerance between the anticonvulsant effects of all the benzodiazepines, but not between those of benzodiazepines and phenobarbital. In a recent study by Wolffgramm et al. (39), chronic administration of diazepam to mice induced tolerance to diazepam on a series of tests of motor coordination, body temperature, and locomotion, but produced a sensitization to secobarbital-induced motor incoordination, and chronic ad-

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ministration of secobarbital sensitized the mice to the sedative and hypothermic effects of acute diazepam.

In contrast to these studies, Lê et al. (33) reported that chronic treatment of rats with chlordiazepoxide conferred full crosstolerance to ethanol and pentobarbital. Similarly, McMillan and Leander (34) reported symmetrical crosstolerance between chlordiazepoxide and pentobarbital in rats, with respect to the rate-decreasing effects of these drugs on unpunished responding for food on a fixed-interval schedule. In drug discrimination studies (2) lorazepam-trained rats did not show generalization to pentobarbital initially, but after chronic treatment with pentobarbital at doses that produce tolerance to pentobarbital they did show lorazepam-like responding to pentobarbital.

Recently, a rapid tolerance model (20,21) similar to that described by Crabbe et al. (9) was used to investigate rapid tolerance to ethanol and pentobarbital and crosstolerance between them. Our results on rapid crosstolerance mimicked the results obtained by us and others in chronic tolerance and crosstolerance studies reported earlier (14,24), and suggested that rapid tolerance and crosstolerance can be used as predictors of chronic tolerance and crosstolerance. Chan et al. (7) also reported a similar degree of rapid crosstolerance to chlordiazepoxide in mice pretreated with ethanol 24 h earlier compared to mice chronically treated with ethanol on a liquid diet for 15 days. Surprisingly, chlordiazepoxide-pretreated mice (30 mg/kg; 24 h earlier) did not show rapid crosstolerance to ethanol. These authors concluded that different rates of tolerance development or different mechanisms of actions between chlordiazepoxide and ethanol, rather than differences in initial dosage between ethanol and chlordiazepoxide, may explain these findings.

Recently, we compared rapid crosstolerance to various benzodiazepines after an acute administration of ethanol with the results obtained after chronic ethanol treatment (21). Our findings on rapid crosstolerance showed good agreement with such studies on chronic crosstolerance. Because we examined crosstolerance in only one direction (i.e., crosstolerance to benzodiazepines following acute or chronic ethanol treatment), and there is not complete agreement on crosstolerance to ethanol following benzodiazepine treatment, it is important to reexamine this issue and compare crosstolerance in both directions. It remained to be determined whether drugs such as barbiturates also show crosstolerance to benzodiazepines and display a similar or different profile than ethanol.

METHOD

Animals

Male Sprague–Dawley rats obtained from Charles River Canada, Ltd. (Montreal, Quebec) had initial body weights of 175–200 g. They were individually housed in a colony room maintained at 21 ± 1°C, with lights on from 0700–1900 h. Water was available at all times. Purina Rat Chow was given ad lib for 1 week. Thereafter, the daily ration was restricted and individually adjusted to maintain comparable body weights in the various groups.

Test Procedures

The tilt-plane test was used as a measure of motor impairment (1). The apparatus consists of a plane hinged at one end, around which it can be inclined at a fixed angular velocity through a range of 55° above the horizontal. The animal was placed on the slightly roughened surface of the plane, which was then tilted until the animal began to slide from the starting position. The test measure used was the angle at which this sliding occurred. The sliding angle was measured before and 30, 60, and 90 min after the IP injection of ethanol (E) or various other drugs. The degree of postdrug ataxia was expressed as the percentage change in the sliding angle, compared to the predrug value for the same animal. Maximum impairment, regardless of the time of its occurrence, was employed as the measure of E or drug effect. This generally occurred about 30–60 min after injection of all the drugs except phenobarbital and barbital, which produced their peak effects at 60–90 min.

Statistical Methods

Results in the various experiments were analyzed by one-, two-, or three-way ANOVA as required, using the GLM-ANOVA program in the NCSS statistical package for PCs. Post hoc comparisons were carried out by the Newman–Keuls range test.

EXPERIMENTAL PROCEDURE AND RESULTS

Experiment 1: Effect of Different Day 1 Treatment Doses of Chlordiazepoxide (CDP) on Rapid Tolerance Development to CDP

Thirty-two rats were randomly divided into four equal groups ($n = 8$). On day 1, one group was injected IP with saline (S), while the other groups received 9, 13, or 18.5 mg/kg CDP, respectively. Before the injections and at successive 30-min intervals up to 90 min after CDP or S, the degree of motor impairment was assessed (tilt-plane test) in all animals. At 120 min after the initial injections, each animal was given an identical second dose of CDP or S, respectively, to make a total day 1 dose of 18, 26, or 37 mg/kg CDP or only S. This procedure of giving CDP in two doses rather than as one single dose was employed because preliminary experiments (26) had shown that (a) a total dose of 37 mg/kg was sufficient to produce rapid tolerance on day 2, and (b) we wanted to determine the dose–response curve for CDP on day 1 and use a dose in the linear part of the dose–reponse curve to test rapid tolerance on day 2. Rats were then returned to their home cages. On the basis of the day 1 responses, 18.5 mg/kg CDP was selected as the day 2 dose for testing rapid tolerance in all rats.

The results of this experiment are shown in Fig. 1a. The day 1 maximum percent impairment values were subjected to a multiple regression analysis. There was a positive correlation ($r = 0.6102$) for log dose vs. maximum percent impairment. This was confirmed by a one-way ANOVA, which showed a significant effect of dose, $F(1, 22) = 13.05$, $p < 0.002$. The day 2 maximum percent impairment values, after injection of 18.5 mg/kg CDP in all groups, showed a decreasing response in inverse proportion to the day 1 dose. A one-way ANOVA again showed a significant main effect of groups, $F(3, 28) = 8.15$, $p < 0.0005$. The post hoc Newman–Keuls range test showed that the control group had significantly higher ($p < 0.05$) maximum percent impairment than the 26 and 37 mg/kg CDP day 1 dose groups. There were no other significant intergroup differences. These results suggested that only the 26 and 37 mg/kg CDP-treated groups developed rapid tolerance to CDP.

Experiment 2: Effect of Different Day 1 Treatment Doses of Diazepam (DZ) on Rapid Tolerance Development to DZ

Forty-eight rats were randomly divided into four equal groups ($n = 12$). On day 1 one group was injected IP with sa-

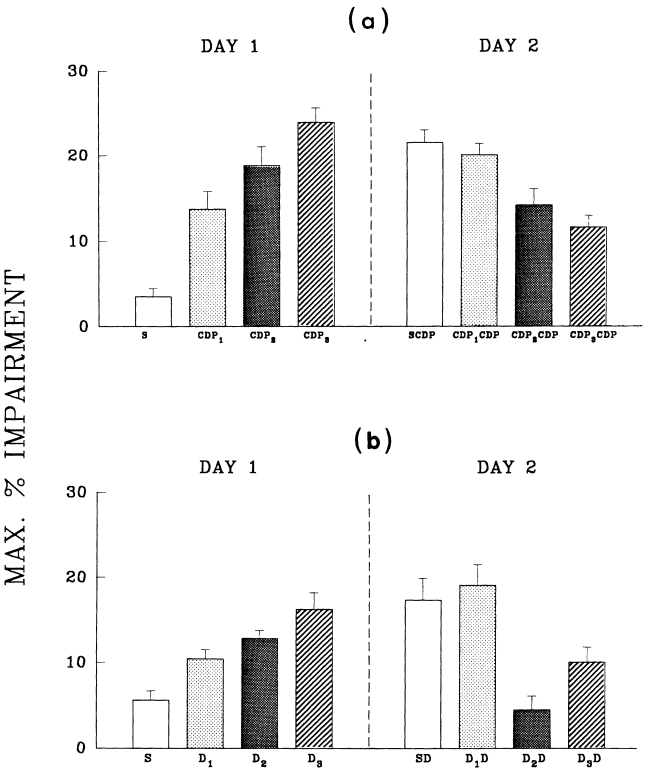

FIG. 1. (a) Effect of three different treatment doses of chlordiazepoxide (CDP) on the development of rapid tolerance on day 2. Maximum percentage impairment (tilt-plane test) in rats given on day 1 saline, S (open column), CDP_1 (9 mg/kg; dotted column), CDP_2 (13 mg/kg; shaded column), or CDP_3 (18.5 mg/kg; striped column). Rapid tolerance to CDP (18.5 mg/kg; IP) was assessed in groups on day 2. Results shown are means ± SEM (n = 8 animals per group). (b) Effect of three different treatment doses of diazepam (DZ) on the development of rapid tolerance on day 2. Maximum percentage impairment (tilt-plane test) in rats given on day 1 saline, S (open column), DZ_1 (1.5 mg/kg; dotted column), DZ_2 (2.1 mg/kg; solid column), or DZ_3 (3.0 mg/kg; striped column). Rapid tolerance to DZ (2.9 mg/kg; IP) was assessed in groups on day 2. Results shown are means ± SEM (n = 8 animals per group).

line (S), while the other groups received 1.5, 2.1, or 3.0 mg/kg diazepam (D), respectively. Before injections and at successive 30-min intervals up to 90 min after D or S injections, the degree of motor impairment was assessed in all animals. At 120 min after the initial injections all animals were given supplementary IP doses of either S or D 1.5, 4.3, or 10.6 mg/kg respectively, to make day 1 total doses of 3.0, 6.4, or 13.6 mg/kg D. Rats were then returned to their home cages. On day 2 all rats were challenged with 2.9 mg/kg D to assess rapid tolerance to D.

The results of this experiment are shown in Fig. 1b. The day 1 maximum percent impairment values were subjected to a multiple regression analysis. There was a positive correlation (r = 0.5365) for log dose vs. maximum percent impairment. One-way ANOVA showed a significant effect of groups, $F(1, 34) = 8.89$, $p < 0.007$. These results confirmed that there was a good dose–response effect for day 1 DZ doses. The day 2 maximum percent impairment values were also subjected to one-way ANOVA. The main effect of groups was again significant, $F(3, 44) = 9.83$, $p < 0.0001$. The post hoc Newman–Keuls range test showed that the control group had signficantly ($p < 0.05$) higher maximum percent impairment than the 6.4 and 13.6 mg/kg DZ dose groups. These results suggested that treatment with the two higher doses of DZ on day 1 resulted in rapid tolerance to DZ on day 2.

Experiment 3: Effect of Different Day 1 Treatment Doses of Pentobarbital (P) on Rapid Tolerance Development to P

Forty rats were randomly divided into five equal groups (n = 8). On day 1, one group was injected IP with saline (S), while the other groups received 13.5, 17.5, 23, or 30 mg/kg IP pentobarbital (P), respectively. Before the S or P injections and at successive 30-min intervals up to 90 min after that, the degree of motor impairment was assessed in all animals. At 120 min after the initial injections all animals were given an identical second dose of S or P, respectively, to make total day 1 doses of 27, 35, 46, or 60 mg/kg of P. Rats were then returned to their home cages. On the basis of the regression line for doses vs. maximum percent impairment responses on day 1, 23 mg/kg P was selected as the day 2 test dose for rapid tolerance testing in all rats.

The results of this experiment are shown in Fig. 2a. The day 1 maximum percent impairment values were subjected to a multiple regression analysis. There was a positive correlation (r = 0.6611) for log dose vs. maximum percent impairment. One-way ANOVA also showed a very significant effect of groups, $F(1, 30) = 23.29$, $p < 0.001$. The day 2 maximum percent impairment values after a dose of P (23 mg/kg in all groups) were also subjected to one-way ANOVA. There was again a significant main effect of groups, $F(4, 34) = 4.39$, $p < 0.0057$. The post hoc Newman–Keuls range test showed that the control group had significantly ($p < 0.05$) higher maximum percent impairment than the 46 and 60 mg/kg P dose groups, but did not differ from the lower dose groups. These results suggested that only the groups treated with P 46 and 60 mg/kg on day 1 showed rapid tolerance to P on day 2.

Experiment 4: Effect of Different Day 1 Treatment Doses of Barbital (B) on Rapid Tolerance Development to B

Thirty-two rats were randomly divided into four equal groups (n = 8). On day 1 one group was injected IP with saline (S), while the other groups received barbital (B) 80, 100, or 125 mg/kg IP, respectively. Before the S or B injections and at successive 30-min intervals up to 120 min after them, the degree of motor impairment was assessed in all animals. Rats were then returned to their home cages. Because of the long half-life of B, no supplementary posttest doses were given. On the basis of the regression line for day 1 doses vs. maximum percent impairment responses, 100 mg/kg B was selected as the day 2 dose for rapid tolerance test in all groups. On day 2, an identical procedure was followed.

The results of this experiment are shown in Fig. 2b. A multiple regression analysis showed a strong positive correlation (0.8268) for log dose vs. maximum percent impairment on day 1. One-way ANOVA showed a very significant effect of groups, $F(1, 22) = 47.54$, $p < 0.001$. These results showed a good dose–response relationship for day 1 B doses. The day 2 maximum percent impairment values, after a dose of B 100 mg/kg in all groups, were subjected to one-way ANOVA, which again showed a significant main effect of groups, $F(3, 28) = 5.91$, $p < 0.003$. The post hoc Newman–Keuls range test showed that the control group had significantly ($p < 0.05$)

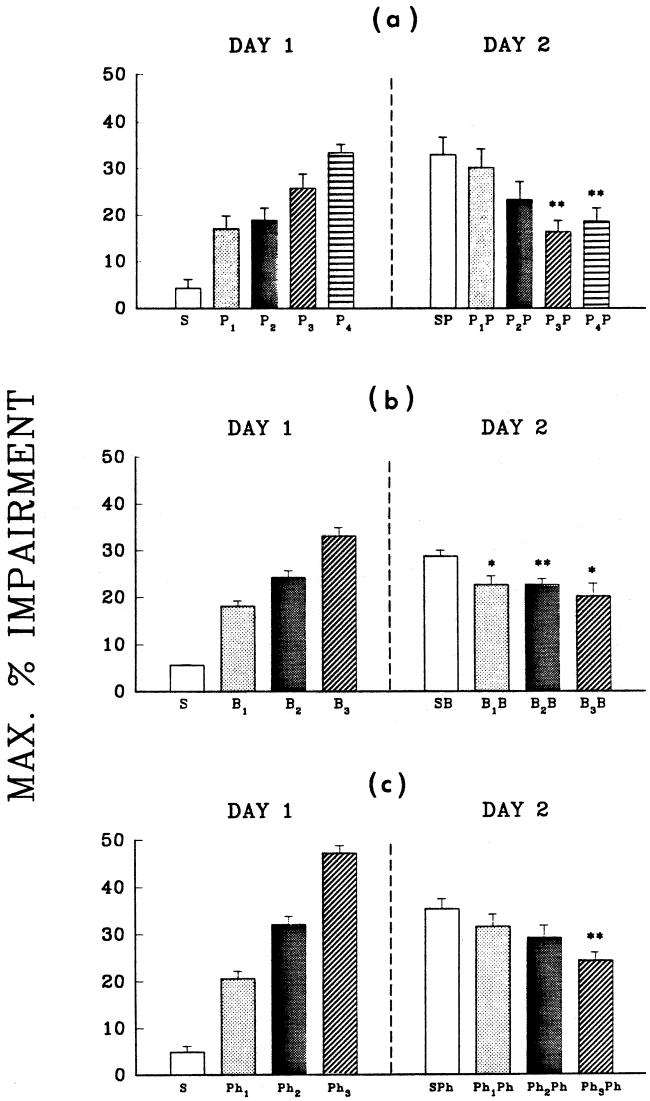

FIG. 2. (a) Effect of four different treatment doses of pentobarbital (P) on the development of rapid tolerance on day 2. Maximum percentage impairment (tilt-plane test) in rats given on day 1 saline, S (open column), P_1 (13.5 mg/kg; dotted column), P_2 (17.5 mg/kg; solid column), or P_3 (23 mg/kg; striped), or P_4 (30 mg/kg; horizontal striped column). Rapid tolerance to P (23 mg/kg; IP) was assessed in groups on day 2. Results shown are means ± SEM (n = 8 animals per group). (b) Effect of three different treatment doses of barbital (B) on the development of rapid tolerance on day 2. Maximum percentage impairment (tilt-plane test) in rats given on day 1 saline; S (open column), B_1 (80 mg/kg; dotted column), B_2 (100 mg/kg; solid column), or B_3 (125 mg/kg; striped column). Rapid tolerance to B (100 mg/kg; IP) was assessed in groups on day 2. Results shown are means ± SEM (n = 8 animals per group). (c) Effect of three different treatment doses of phenobarbital (Ph) on the development of rapid tolerance on day 2. Maximum percentage impairment (tilt-plane test) in rats given on day 1 saline, S (open column), Ph_1 (50 mg/kg; dotted column), Ph_2 (75 mg/kg; solid column) or Ph_3 (112 mg/kg; striped column). Rapid tolerance to Ph (80 mg/kg; IP) was assessed in groups on day 2. Results shown are means ± SEM (n = 8 animals per group).

higher maximum percent impairment than all three B dose groups. These results indicated that all B dose groups had developed rapid tolerance to B.

Experiment 5: Effect of Different Day 1 Treatment Doses of Phenobarbital (Ph) on Rapid Tolerance Development to Ph

Thirty-two rats were randomly divided into four equal groups (n = 8). On day 1, one group was injected IP with saline (S), while the other groups received phenobarbital (Ph) in doses of 50, 75, or 112 mg/kg, respectively. Before the injections and at successive 30-min intervals up to 120 min after, the degree of motor impairment was assessed in all animals. Rats were then returned to their home cages. No supplementary dose of Ph was given at the end of the test. On the basis of the regression line for day 1 doses vs. maximum percent impairment responses, 80 mg/kg Ph was selected as the day 2 dose for rapid tolerance test in all groups. The procedure was identical to that followed on day 1.

The results of this experiment are shown in Fig. 2c. A multiple regression analysis for day 1 values showed a strong positive linear correlation (r = 0.9251) for log dose vs. maximum percent impairment. One-way ANOVA also showed a very significant effect for groups, $F(1, 22) = 130.65$, $p < 0.001$, indicating a good dose–response relationship for Ph on day 1. The maximum percent impairment value on day 2, after a Ph dose of 80 mg/kg in all groups, were subjected to a one-way ANOVA, which showed a significant main effect of groups, $F(3, 28) = 4.02$, $p < 0.017$. The post hoc Newman–Keuls range test showed that the control group had a significantly ($p < 0.05$) higher maximum percent impairment score than the 112 mg Ph day 1 dose group, but did not differ significantly from the other dose groups. These results suggested that only the group treated with 112 mg/kg on day 1 developed rapid tolerance to Ph.

Experiment 6: Rapid Crosstolerance From Benzodiazepines to Ethanol

Twenty-four rats were randomly divided into three equal groups (n = 8). On day 1 one group was injected with CDP (26 mg/kg, IP), one group was injected with DZ (6.4 mg/kg, IP) and one group was injected with saline. Before the injections and at 30, 60, and 90 min after them the degree of motor impairment was assessed in all animals. Rats were then returned to their home cages. In other ongoing studies in which CDP was given as a single dose (26 mg/kg) rather than in two doses of 13 mg/kg each on day 1, the extent of tolerance on day 2 produced by the single dose on day 1 was similar to that resulting from the divided doses. Similar results were previously reported with ethanol. Therefore, we administered the entire dose on day 1 as a single injection for all crosstolerance studies. On day 2, an identical procedure was followed after a challenge dose of E (2.3 g/kg, IP) in all three groups to test for rapid crosstolerance to E. The test dose of E was selected to give responses in the middle portion of the E dose–response curve as determined in previous studies in this laboratory (20,21).

On day 1 rats injected with CDP or DZ showed their expected motor impairment responses (Fig. 3). The day 2 maximum percent impairment values after a challenge dose of E were also subjected to a one-way ANOVA. The main effect of group was significant, $F(2, 21) = 15.62$, $p < 0.0001$. The post hoc Newman–Keuls range test showed that the S-E group was significantly ($p < 0.05$) different from the CDP-E and DZ-E groups. These results indicate that both CDP and

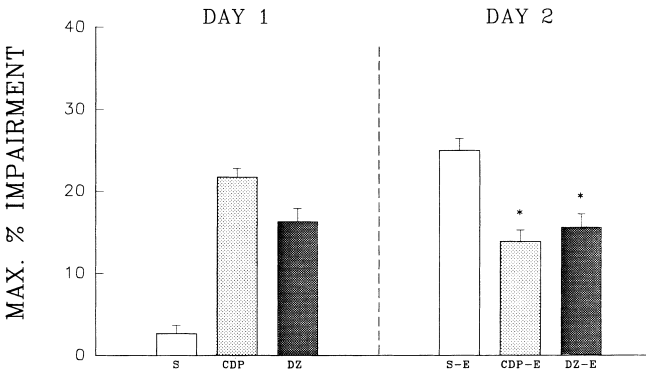

FIG. 3. Rapid crosstolerance from chlordiazepoxide (CDP) and diazepam (DZ) to ethanol (E). Maximum percentage impairment (tilt-plane test) in rats given on day 1 saline, S (open column) or CDP (26 mg/kg; dotted column) or DZ (6.4 mg/kg; solid column). Rapid crosstolerance to E (2.3 g/kg; IP) was assessed in all groups on day 2. Results shown are means ± SEM (n = 8 animals per group).

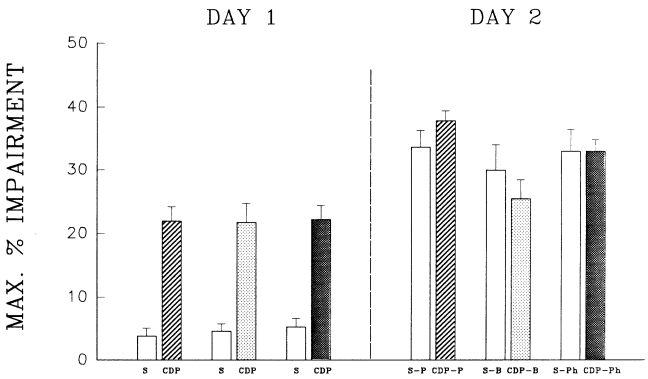

FIG. 4. Rapid crosstolerance from CDP to barbiturates. Maximum percentage impairment (tilt-plane test) in rats given on day 1 saline, S (open column) or CDP (26 mg/kg). Rapid crosstolerance to pentobarbital (P, 23 mg/kg; striped column), barbital (B, 100 mg/kg; dotted column), or phenobarbital (Ph, 80 mg/kg; solid column) was assessed in all groups on day 2. Results shown are means ± SEM) n = 8 animals per group).

DZ treatment on day 1 resulted in crosstolerance to the motor impairing effects of E on day 2.

Experiment 7: Rapid Crosstolerance From Benzodiazepines to Barbiturates

Forty-eight rats were randomly divided into six equal groups (n = 8). On day 1, three groups were injected with CDP (26 mg/kg, IP) and the other three groups were injected with saline (S). Before the injections and at 30, 60, and 90 min after them the degree of motor impairment was assessed in all animals. Rats were then returned to their home cages. On day 2, an identical procedure was followed, except that one S and one CDP day 1 group were challenged with either P (23 mg/kg), B (100 mg/kg), or Ph (80 mg/kg) to test for rapid crosstolerance development. A separate batch of 48 animals was injected with S or DZ (6.4 mg/kg, IP) on day 1 and the rest of the day 1 and day 2 procedures and drug doses employed were identical to those described above, to test for crosstolerance development to barbiturates.

The results for the CDP experiment are shown in Fig. 4, and those for the DZ experiment in Fig. 5. On day 1 rats injected with CDP (Fig. 4) or DZ (Fig. 5) showed their expected motor impairment responses. The day 2 maximum percent impairment values after challenge doses of the different barbiturates were subjected to one-way ANOVA comparisons between the control group (that had received S on day 1) and the benzodiazepine-treated group. The ANOVA results for the crosstolerance test from CDP to P, $F(1, 14) = 1.84$, $p > 0.197$, to B, $F(1, 14) = 0.77$, $p > 0.396$, and to Ph, $F(1, 14) = 0.00$, $p > 0.985$, were not significant (Fig. 4). The ANOVA results for crosstolerance for DZ to P, $F(1, 14) = 0.73$, $p > 0.406$, B, $F(1, 14) = 0.74$, $p > 0.405$, and Ph, $F(1, 14) = 0.55$, $p > 0.471$, were also not significant (Fig. 5). These results show that there was no rapid crosstolerance development from benzodiazepines (CDP and DZ) to the different barbiturates at the doses employed here.

Experiment 8: Rapid Crosstolerance From Barbiturates to Ethanol (E)

Thirty-two rats were randomly divided into four equal groups (n = 8). On day 1, each group was injected IP with either saline (S), P (23 mg/kg), B (125 mg/kg), or Ph (112 mg/kg). Before the injections and at 30, 60, and 90 min after them, the degree of motor impairment was assessed in all animals. At 120 min after the initial P injections a second dose of P (23 mg/kg) was given to rats receiving P, but the other drug groups (B and Ph) did not receive any additional injections. Rats were then returned to their home cages. On day 2, an identical procedure was followed except that all animals received a challenge dose of E (2.3 g/kg) to test for rapid cross tolerance to E.

The results of this experiment are shown in Fig. 6 . On day 1 rats injected with different barbiturates showed their expected motor impairment responses. The day 2 maximum percent impairment values after a challenge dose of E were subjected to a one-way ANOVA. The main effect of group was

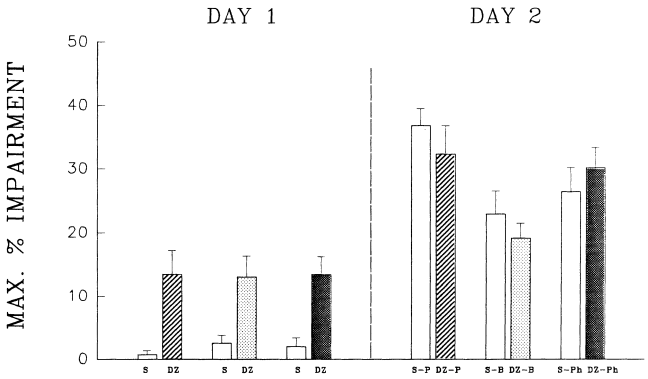

FIG. 5. Rapid crosstolerance from DZ to barbiturates. Maximum percentage impairment (tilt-plane test) in rats given on day 1 saline, S (open column) or DZ (6.4 mg/kg). Rapid crosstolerance to pentobarbital (P, 23 mg/kg; striped column), barbital (B, 100 mg/kg; dotted column), or phenobarbital (Ph, 80 mg/kg; solid column) was assessed in all groups on day 2. Results shown are means ± SEM (n = 8 animals per group).

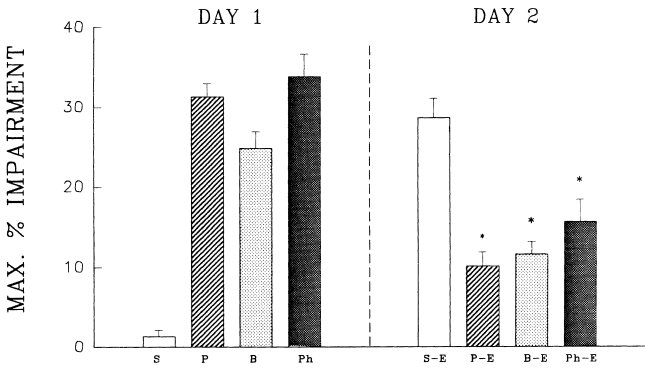

FIG. 6. Rapid crosstolerance from barbiturates to ethanol. Maximum percentage impairment (tilt-plane test) in rats given on day 1 saline, S (open column), P (23 mg/kg; striped column), B (125 mg/kg; dotted column), or Ph (112 mg/kg; solid column). Rapid crosstolerance to E (2.3 g/kg; IP) was assessed in all groups on day 2. Results shown are means ± SEM (n = 8 animals per group).

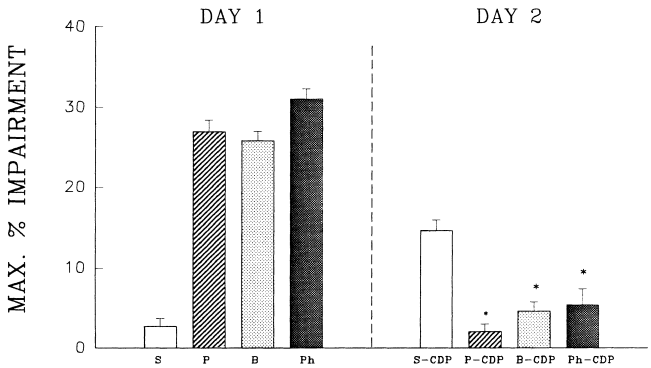

FIG. 7. Rapid crosstolerance from barbiturates to chlordiazepoxide. Maximum percentage impairment (tilt-plane test) in rats given on day 1 saline, S (open column), P (23 mg/kg; striped column), B (125 mg/kg; dotted column), or Ph (112 mg/kg; solid column). Rapid crosstolerance to CDP (16 mg/kg; IP) was assessed in all groups on day 2. Results shown are means ± SEM (n = 8 animals per group).

significant, $F(3, 28) = 14.37$, $p < 0.0001$. The post hoc Newman–Keuls range test showed that the S-E group differed significantly ($p < 0.05$) from all the barbiturate pretreated groups (P-E, B-E, and Ph-E). These results suggest that day 1 treatment with all three barbiturates resulted in crosstolerance to the motor-impairing effects of E.

Experiment 9: Rapid Crosstolerance From Barbiturates to Benzodiazepines

For these studies a procedure identical to that used in experiment 8 was followed on day 1 in two separate batches of 32 animals each. On day 2 one batch was challenged with CDP (16 mg/kg, IP) and the other batch was challenged with DZ (2.9 mg/kg, IP) to test for rapid crosstolerance to benzodiazepines. The results are shown in Figs. 7 and 8, respectively.

On day 1, rats injected with different barbiturates showed their expected motor-impairment responses (Figs. 7 and 8). The day 2 maximum percent impairment values after a challenge dose of CDP (Fig. 7) were subjected to a one-way ANOVA. The main effect of group was significant, $F(3, 28) = 14.55$, $p < 0.001$. The post hoc Newman–Keuls range test showed that the S-CDP group was significantly ($p < 0.05$) different from all three barbiturate-treated groups (P-CDP, B-CDP, and Ph-CDP). Similarly, a one-way ANOVA of the day 2 results in the DZ experiment (Fig. 8) showed a significant main effect of group, $F(3, 28) = 25.39$, $p < 0.001$. The post hoc Newman–Keuls range test showed that the S-D group was significantly different from all three barbiturate-treated groups (P-DZ, B-DZ, and Ph-DZ). These results suggest that all three barbiturates (P, B, Ph) produced crosstolerance to the motor-impairing effects of CDP and of DZ.

DISCUSSION

The results of these studies show that treatment with either CDP or DZ resulted in rapid crosstolerance to the motor-impairment effects of ethanol. These results confirm and extend our previous observations of rapid crosstolerance to ethanol after treatment with benzodiazepines (26). In other studies, we and others have reported clear evidence of rapid and chronic crosstolerance to benzodiazepines after ethanol treatment (7,19,21,33,35). In contrast to these findings with ethanol, treatment with either CDP or DZ failed to produce rapid crosstolerance to the three barbiturates (pentobarbital, barbital, and phenobarbital) tested.

Various explanations must be considered for these differences in crosstolerance, including possible pharmacokinetic as well as pharmacodynamic explanations. Both diazepam and chlordiazepoxide have active metabolites that need to be considered in studies of this nature. Among the pharmacokinetic factors to be considered are the elimination $t_{1/2}$ values for the various drugs studied, and the possibility of enzyme induction by the day 1 treatment doses. Most of the drugs tested are known to have short $t_{1/2}$ for both the parent compounds and their active metabolites. Numerous studies have shown a mean $t_{1/2}$ of 0.9–1.15 h for diazepam and 39 min to 1.66 h for

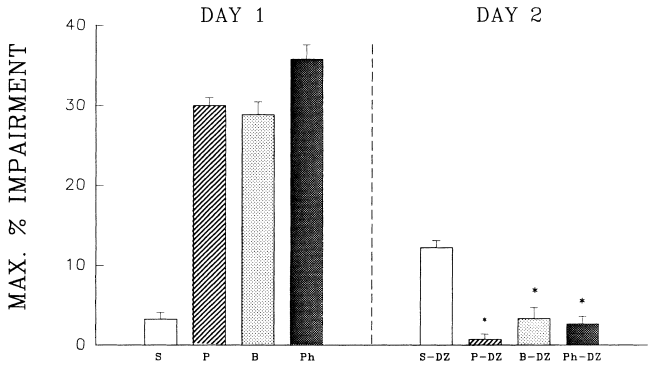

FIG. 8. Rapid crosstolerance from barbiturates to diazepam. Maximum percentage impairment (tilt-plane test) in rats given on day 1 saline, S (open column), P (23 mg/kg; striped column), B (125 mg/kg; dotted column), or Ph (112 mg/kg; solid column). Rapid crosstolerance to DZ (2.9 mg/kg; IP) was assessed in all groups on day 2. Results shown are means ± SEM (n = 8 animals per group).

desmethyldiazepam (10,12,28). Chlordiazepoxide has a $t_{1/2}$of about 4 h in the rat (29). Pentobarbital also has a short $t_{1/2}$ of less than an hour in the rat (17,23,38). Therefore, no residual amount of any of these drugs from the day 1 doses would have been present to contribute to the measured effect on day 2.

In contrast, the longer acting barbiturates barbital and phenobarbital have $t_{1/2}$ values in the rat of about 13 h (11,18). Thus, significant residual amounts of these two barbiturates could well have been present on day 2 and contributed to the measured impairment. Despite this, rapid tolerance was clearly seen to both of these barbiturates, as well as crosstolerance between them and ethanol, diazepam, and chlordiazepoxide. Moreover, for each of these drugs the degree of crosstolerance produced by pentobarbital was very similar to that produced by barbital or phenobarbital (Figs. 6–8). Similarly, neither diazepam ($t_{1/2}$ = l h) nor chlordiazepoxide ($t_{1/2}$ = 4 h) produced crosstolerance to any of the barbiturates (Fig. 4 and 5), although they produced good tolerance to themselves (Fig. l) and crosstolerance to ethanol (Fig. 3). Thus, $t_{1/2}$ does not appear to be a significant factor in relation to rapid tolerance and crosstolerance.

With respect to the possibility of induction of biotransforming enzymes, there is virtual unanimity in the literature that induction does not occur under our experimental conditions. Much larger single doses of benzodiazepines did not cause induction of metabolism of the benzodiazepines themselves (16,30), of ethanol (26), nor of barbiturates and other drugs (3,15). Ethanol given on day 1 in the rapid tolerance paradigm was previously shown to have no effect on the blood levels of ethanol itself (20,27) and even chronic ethanol treatment at a dose of 5 g/kg by gavage did not alter the elimination of any of the three barbiturates (22). A single dose of pentobarbital on day 1 did not act as an inducer for the day 2 metabolism of either pentobarbital or ethanol (20,21). Finally, even phenobarbital, which is a potent inducer of cytochrome P450, caused only negligible increase in the metabolism of hexobarbital 24 h after a single dose of 80 mg/kg (36).

All of the foregoing data on $t_{1/2}$, and on the lack of significant induction by single doses of these drugs, are fully consistent with previous demonstrations that rapid tolerance in the present paradigm is not pharmacokinetic in nature (7–9,20,21,27).

Another potential explanation for the differences in crosstolerance described above, which could also be dependent on pharmacokinetic factors, involves differences in the speed of onset and duration of action of various drugs. A drug with slow onset and long duration of action might not be effective in producing rapid crosstolerance to a drug with rapid onset and short duration of action, especially if conditioning of drug-related stimuli is an important mechanism of tolerance. However, the present findings do not offer much support for this suggestion. Pentobarbital, phenobarbital, and diazepam all produced their maximum effects within 30 min, yet pentobarbital and phenobarbital produced crosstolerance to diazepam, whereas diazepam did not produce crosstolerance to either of these barbiturates. Similarly, both barbital and chlordiazepoxide produced their peak effect at 60 min, yet there was crosstolerance from barbital to chlordiazepoxide but not from chlordiazepoxide to barbital. Finally, ethanol produced its peak effects at 30 min, yet there was crosstolerance in both directions between ethanol and all of the other drugs tested.

With respect to possible pharmacodynamic explanations for the asymmetry of crosstolerance, one possibility is that because the day 1 doses of the various drugs did not all produce the same magnitude of impairment, they might have constituted differing stimuli to the production of tolerance. Again, the data are not supportive of such an explanation. For example, the respective doses of barbital, pentobarbital, and chlordiazepoxide (Figs. 4 and 7) on day 1 produced closely similar degrees of effect (25, 27, and 23, respectively), yet both of the barbiturates produced crosstolerance in the ethanol experiments (Figs. 6–8) and marked crosstolerance to chlordiazepoxide, whereas the reverse did not occur. Conversely, diazepam and phenobarbital, in the doses used, give markedly different magnitudes of response on day 1 (22 and 34, respectively), yet they produced identical degrees of crosstolerance to ethanol.

Thus, neither the speed of onset, magnitude of day 1 effect, nor the $t_{1/2}$ of the drug can explain the asymmetry of crosstolerance seen here and in earlier chronic studies (14,24). However, it is not yet possible to rule out an influence of total area under the curve (AUC) of drug effect vs. time. The present data are insufficient to permit calculation of the AUC because testing on day 1 was limited to 2 h, and a second dose was given for the short-acting drugs without subsequent testing. To answer this question, specially designed experiments will be required.

Our results on lack of crosstolerance to barbiturates after benzodiazepine treatment are in agreement with other observations in the literature (4,37,39), described in the introductory paragraphs. However, Lê et al. (33) reported crosstolerance both to ethanol and to pentobarbital in rats treated with chlordiazepoxide. Although the results of the latter study may appear contradictory to those of other reports and to the present findings, Lê et al. (33) used highly learned tasks such as the moving belt test and shuttle-box test, and the animals were given frequent opportunities to practice the tasks while under the influence of the various drugs, because the experimental design included much repeated testing. It is highly likely that the contribution of learning in that study could have accounted for crosstolerance; the presence or absence of crosstolerance between ethanol and pentobarbital (31) or ethanol and hydralazine (32) was previously found to depend on whether opportunities for either operant or associative learning were provided.

A similar explanation may possibly account for most of the apparent disagreements in the literature. The two studies that demonstrated clear crosstolerance from benzodiazepines to pentobarbital (2,34) used experimental designs and techniques that provided repeated (daily) opportunities for task performance under the influence of the treatment drug, so that the learning factor in tolerance would have been very strong. Studies that found very little or no crosstolerance from benzodiazepines to pentobarbital (13,37) used experimental designs that presented no opportunity for learning, because each animal was tested only once, and only under a single drug. Chan et al. (7) used an experimental design that did not include repeat testing, but found crosstolerance on those tests in which the drug effects bear the greatest resemblance to the effects that would be experienced in the home cage, and little or no crosstolerance in those tests for which home-cage drug experience could not provide analogous learning. The most difficult observations to explain are those of Cesare and McKearney (4), whose experimental design offered daily opportunities for learning to perform under drug, yet still revealed no crosstolerance from a benzodiazepine to pentobarbital.

Treatment with barbiturates resulted in crosstolerance both to benzodiazepines (CDP as well as DZ) and to ethanol, yet benzodiazepine treatment resulted in crosstolerance only to ethanol and not to barbiturates. This asymmetry of cross-

tolerance between benzodiazepines and barbiturates is not unlike the asymmetry we reported on crosstolerance between ethanol and pentobarbital (14), i.e., lack of tolerance to pentobarbital after ethanol treatment and clear evidence of tolerance to ethanol after pentobarbital treatment (24). We have suggested that the asymmetry between ethanol and pentobarbital could be due to ethanol effects being a subset of a larger range of actions exerted by pentobarbital, so that pentobarbital pretreatment could generate a stronger stimulus to the development of crosstolerance to ethanol than vice versa. The same explanation can perhaps be invoked here, because benzodiazepines interact with a specific benzodiazepine receptor (32), and on the whole have a more specific range of actions than the barbiturates. Therefore, tolerance to the broad range of actions of barbiturates would include crosstolerance to the effects of benzodiazepines, whereas tolerance to benzodiazepines would include only a weak or partial crosstolerance to the effects of barbiturates.

REFERENCES

1. Arvola, A.; Sammalisto, L.; Wallgren, H. A.: A test for level of alcohol intoxication in the rat. Q. J. Stud. Alcohol 19:563–572; 1958.
2. Ator, N. A.; Griffiths, R. R.: Differential generalization to pentobarbital in rats trained to discriminate lorazepam, chlordiazepoxide, diazepam, or triazolam. Psychopharmacology (Berlin) 98: 20–30; 1989.
3. Breckenridge, A.; Orme, M.: Interaction of benzodiazepines with oral anticongulants. In: Garattini, S.; Mussini, E.; Randall, L. O., eds. The benzodiazepines. New York: Raven Press; 1973:647–654.
4. Cesare, D. A.; McKearney, J. W.: Tolerance to suppressive effects of chlordiazepoxide on operant behavior: Lack of cross tolerance to pentobarbital. Pharmacol. Biochem. Behav. 13:545–548; 1980.
5. Chan, A. W. K.: Effects of combined alcohol and benzodiazepine: A review. Drug Alcohol Depend. 13:315–341; 1984.
6. Chan, A. W. K.; Langan, M. C.; Leong, F. W.; Schanley, D. L.; Penetrante, M. L.: Does chronic ethanol intake confer full cross-tolerance to chlordiazepoxide? Pharmacol. Biochem. Behav. 30: 385–389; 1988.
7. Chan, A. W. K.; Schanley, D. L.; Aleo, M. D.; Leong, F. W.: Cross-tolerance between ethanol and chlordiazepoxide. Alcohol 2:209–213; 1985.
8. Chan, A. W. K.; Schanley, D. L.; Langan, M. C.; Leong, F. W.; Penetrante, M. L.: Chronic treatment with ethanol and chlordiazepoxide alters the metabolism of chlordiazepoxide. Pharmacol. Biochem. Behav. 35:363–366; 1990.
9. Crabbe, J. C.; Rigter, H.; Uijlen, J.; Strijbos, C.: Rapid development of tolerance to the hypothermic effect of ethanol in mice. J. Pharmacol. Exp. Ther. 208:128–133; 1979.
10. Diaz-Garcia, J. M.; Oliver-Botana, J.; Fos-Galve, D.: Pharmacokinetics of diazepam in the rat: Influence of a carbon tetrachloride-induced hepatic injury. J. Pharm. Sci. 81:768–772; 1992.
11. Ebert, A. G.; Yim, G. K. W.; Miya, T. S.: Distribution and metabolism of barbital-^{14}C in tolerant and nontolerant rats. Biochem. Pharmacol. 13:1267–1274; 1964.
12. Friedman, H.; Abernethy, D. R.; Greenblatt, D. J.; Shader, R. I.: The pharmacokinetics of diazepam and desmethyldiazepam in rat brain and plasma. Psychopharmacology 88:267–270; 1986.
13. Gent, J. P.; Bentley, M.; Feely, M.; Haigh, J. R. M.: Benzodiazepine cross-tolerance in mice extends to sodium valproate. Eur. J. Pharmacol. 128:9–15; 1986.
14. 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.
15. Greenblatt, D. J.; Shader, R. I.: Benzodiazepines in clinical practice. New York: Raven Press; 1973.
16. Haigh, J. R. M.; Feely, M.: Tolerance to the anticonvulsant effect of benzodiazepines. Trends Pharmacol. Sci. 9:361–391; 1988.
17. Kalant, H.; Khanna, J. M.; Marshman, J.: Effect of chronic intake of ethanol on pentobarbital metabolism. J. Pharmacol. Exp. Ther. 175:318–324; 1970.
18. Khanna, J. M.; Kalant, H.; Lê, A. D.; Mayer, J.; LeBlanc, A. E.: Effect of p-chlorophenylalanine on the acquisition of tolerance to the hypnotic effects of pentobarbital, barbital and ethanol. Can. J. Physiol. Pharmacol. 58:1031–1041; 1980.
19. Khanna, J. M.; Kalant, H.; Shah, G.: Tolerance to ethanol and cross-tolerance to other alcohols, barbiturates and benzodiazepines (abstract 317). Toronto: International Society for Biomedical Research on Alcoholism; 1990.
20. Khanna, J. M.; Kalant, H.; Shah, G.; Weiner, J.: Rapid tolerance as an index of chronic tolerance. Pharmacol. Biochem. Behav. 38: 427–432; 1991.
21. Khanna, J. M.; Kalant, H.; Weiner, J.; Shah, J.: Rapid tolerance and cross-tolerance as predictors of chronic tolerance and cross-tolerance. Pharmacol. Biochem. Behav. 41:355–360; 1992.
22. Khanna, J. M.; Lê, A. D.; Kalant, H.; Chau, A.; Shah, G.: Effect of lipid solubility on the development of chronic cross-tolerance between ethanol and different alcohols and barbiturates. Pharmacol. Biochem. Behav. 57:101–110; 1997.
23. Khanna, J. M.; Kalant, H.; Yee, Y.; Chung, S.; Siemens, A. J.: Effect of chronic ethanol treatment on metabolism of drugs in vitro and in vivo. Biochem. Pharmacol. 25:329–335; 1976.
24. Khanna, J. M.; Lê, A. D.; Gougos, A.; Kalant, H.: Effect of chronic pentobarbital treatment on the development of cross-tolerance to ethanol and barbital. Pharmacol. Biochem. Behav. 31: 179–186; 1988.
25. 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.
26. Khanna, J. M.; Mihic, S. J.; Weiner, J.; Shah, G.; Wu, P. H.; Kalant, H.: Differential inhibition by NMDA antagonists of rapid tolerance to, and cross-tolerance between, ethanol and chlordiazepoxide. Brain Res. 574:251–256; 1992.
27. Khanna, J. M.; Shah, G.; Weiner, J. M.; Wu, P.; Kalant, H.: Effect of NMDA antagonists on rapid tolerance to ethanol. Eur. J. Pharmacol. 230:23–31; 1993.
28. Klockowski, P. M.; Levy, G.: Kinetics of drug action in disease states. XXV. Effect of experimental hypovolemia on the pharmacodynamics and pharmacokinetics of desmethyldiazepam. J. Pharmacol. Exp. Ther. 245:508–512; 1988.
29. Koechlin, B. A.; Schwartz, M. A.; Krol, G.; Oberhansli, W.: The metabolic fate of C^{14}-labeled chlordiazepoxide in man, in the dog, and in the rat. J. Pharmacol. Exp. Ther. 148:399–411; 1965.
30. Lê, A. D.; Khanna, J. M. Dispositional mechanisms in drug tolerance and sensitization. In: Goudie, A. J.; Emmett-Oglesby, M., eds. Psychoactive drugs. Tolerance and sensitization. Clifton, NJ: Humana Press; 1989: 281–351.
31. Lê, A. D.; Khanna, J. M.; Kalant, H.: Effect of chronic treatment with ethanol on the development of cross-tolerance to other alcohols and pentobarbital. J. Pharmacol. Exp. Ther. 263:480–485; 1992.
32. Lê, A. D.; Khanna, J. M.; Kalant, H.: Role of Pavlovian conditioning in the development of tolerance and cross-tolerance to the hypothermic effect of ethanol and hydralazine. Psychopharmacology (Berlin) 92:210–214; 1987.
33. Lê, A. D.; Khanna, J. M.; Kalant, H.; Grossi, F.: Tolerance to and cross-tolerance among ethanol, pentobarbital and chlordiazepoxide. Pharmacol. Biochem. Behav. 24:93–98; 1986.
34. McMillan, D. E.; Leander, J. D.: Chronic chlordiazepoxide and pentobarbital interactions on punished and unpunished behavior. J. Pharmacol. Exp. Ther. 207:515–520; 1978.
35. 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.
36. Remmer, H.: Tolerance to barbiturates by increased breakdown. In: Steinberg, H., ed. Scientific basis of drug dependence. New York: Grune & Stratton; 1969:111–128.
37. Rosenberg, H. C.; Smith, S.; Chiu, T. H.: Benzodiazepine-specific and nonspecific tolerance following chronic flurazepam treatment. Life Sci. 32:279–285; 1983.
38. Siemens, A. J.; Kalant, H.; Khanna, J. M.; Marshman, J.; Ho, G.: Effect of cannabis on pentobarbital-induced sleeping time and pentobarbital metabolism in the rat. Biochem. Pharmacol. 23: 477–488; 1974.
39. Wolffgramm, J.; Mikolaiczyk, C.; Coper, H.: Acute and subchronic benzodiazepine-barbiturate-interactions on behaviour and physiological responses of the mouse. Naunyn Schmiedebergs Arch. Pharmacol. 349:279–286; 1994.