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Effect of Chronic Pentobarbital Treatment on the Development of Cross-Tolerance to Ethanol and Barbital

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KHANNA, J. M., A. D. LÊ, A. GOUGOS AND H. KALANT. *Effect of chronic pentobarbital treatment on the development of cross-tolerance to ethanol and barbital.* PHARMACOL BIOCHEM BEHAV **31**(1) 179–186, 1988.—Recently, we reported that a chronic regimen of ethanol by intubation, which produced clear tolerance to ethanol-induced hypothermia, ataxia and sleep, produced only a marginal degree of cross-tolerance to these effects of pentobarbital. The present experiments were designed to test the reverse process by examining cross-tolerance to ethanol following chronic pentobarbital treatment, also by gastric intubation. In contrast to the minimal cross-tolerance to pentobarbital after chronic pretreatment with ethanol, chronic pentobarbital treatment by gavage conferred clear cross-tolerance to both barbital- and ethanol-induced hypothermia, ataxia and sleep. In a separate experiment, cross-tolerance to barbital- and ethanol-induced hypothermia and ataxia was demonstrated over a wide range of test doses. Determination of ethanol blood levels as well as a complete time course of absorption, distribution and elimination of ethanol suggested that pharmacokinetic alterations may play a role in the development of cross-tolerance to ethanol in pentobarbital-treated subjects. The asymmetry of cross-tolerance raises the possibility that pentobarbital and ethanol invoke tolerance by mechanisms that are not wholly identical. This possibility requires further exploration. Conceivably the actions of ethanol which mediate the measured effects form a subset of a larger range of pentobarbital actions that could provide a stronger stimulus to tolerance development.

Cross-tolerance Pentobarbital Barbital Ethanol Rat

THERE is disagreement among investigators as to whether or not chronic barbiturate treatment confers cross-tolerance to ethanol. In an earlier study from our laboratory [10], we reported cross-tolerance to the motor impairing effect of ethanol on the moving belt test in rats treated chronically with 50 mg/kg of pentobarbital daily for 22 days. However, this pentobarbital treatment failed to produce cross-tolerance to the hypnotic effect of ethanol [17]. Commissaris and Rech [5] were also unable to find cross-tolerance to ethanol in rats chronically treated with pentobarbital and tested on the rotarod. The chronic pentobarbital treatment was 2 mg/g of chow in addition to 30 mg/kg IP twice daily for six days. This is supported by an earlier study in which the same authors found only a marginal degree of functional cross-tolerance to the same effect of ethanol in rats that had been fed a diet of powdered chow containing 1–4 mg of pentobarbital per g of chow (for 23 days), but had not received chronic pentobarbital injections [4]. This is perhaps not surprising, because these investigators found that tolerance to pentobarbital itself, after one week of daily pentobarbital ingestion, was entirely pharmacokinetic in nature [6].

In other studies, chronic treatment with barbiturates was reported to confer cross-tolerance to ethanol. Carney *et al.* [3] reported that administration of 100 mg/kg IP of barbital daily for 30 days resulted in a dose-dependent functional cross-tolerance to the depressant effect of ethanol on operant behavior. There was development of tolerance to barbital itself but to a lesser extent than cross-tolerance to ethanol. Similarly, preexposure of goldfish to pentobarbital for 24 hr produced cross-tolerance to ethanol that was greater than their tolerance to pentobarbital [14]. Preexposure to pentobarbital for six hours, however, produced the same degree of tolerance and cross-tolerance [14]. In another study, a definite cross-tolerance to ethanol was seen in thiopental-tolerant animals using the tilting plane test, but ethanol-tolerant animals showed only marginal tolerance to thiopental-induced sleep [24]. A lack of appreciable cross-tolerance to pentobarbital-induced hypothermia, ataxia and sleep was also observed in a recent study from this laboratory in rats given ethanol chronically by gavage, which produced clear tolerance to these effects of ethanol [13]. Since we examined cross-tolerance in only one direction (i.e., cross-tolerance to barbiturates following chronic ethanol treatment) and there is not complete agreement on

cross-tolerance to ethanol following barbiturate treatment, it is important to reexamine this issue and compare cross-tolerance in both directions. Furthermore, the effect of varying the test dose and the contribution of dispositional factors in cross-tolerance were not assessed in previous investigations.

GENERAL METHOD

Animals

Male Sprague-Dawley rats weighing 150–175 g were purchased from Charles River (Montréal, Québec). They were housed singly and fed a standard rat chow diet. Tap water was available at all times. They were allowed to adapt to their environment for a period of two weeks. Initial dose-response curves to barbital and ethanol were completed during the next week. Purina Rat Chow was given ad lib until body weights reached approximately 250 g and chronic treatment was initiated. Thereafter, each animal was restricted to 4 chow pellets (18–20 g) daily to maintain comparable body weights. On ad lib diet, the results could have been confounded by variability in weight gain between the two groups. The temperature of the colony room was maintained at 21±1°C and lights were on from 7 a.m. to 7 p.m. throughout the experiment.

Drugs

Drugs used were 95% (w/v) ethanol, twice distilled, sodium pentobarbital (BDH) and sodium barbital (BDH). All drug solutions were prepared in isotonic saline on the day they were used.

Test Procedures

All tests were done 24 hr after the previous treatment dose in order to minimize overlapping drug effects.

Hypothermia and motor-impairment studies. These two measures were made in each animal on the same test day after test doses of ethanol or barbital. For measuring the hypothermic effect, a 5-cm-long thermistor probe was inserted into the rectum and left until a stable temperature recording was obtained (approximately 30 sec) on a Yellow Springs Instrument electrical thermometer. This was done before and at successive 30 min intervals after the IP test injection (i.e., at 30, 60, 90 and 120 min) until the temperature began to return to normal. This generally occurred 60–90 min after injection of ethanol and 90–120 min after barbital. The hypothermic effect was quantified as the maximal drop in temperature over this time period.

The motor-impairment effect was measured after the hypothermia test. The tilting plane was used as a measure of motor impairment [1]. The apparatus consists of a plane hinged at one end, about which it can be rotated at a fixed angular velocity through a range of 55° above the horizontal. The animal is placed on a slightly roughened surface of the plane, which is then tilted until the animal begins to slide from the starting position. The test measure is the angle at which this occurs. The sliding angle was measured before and at 30, 60, 90 and 120 min after the injection of the drug. The degree of postdrug ataxia was expressed as the percentage change in the sliding angle, compared to the predrug value. Maximum impairment, regardless of the time of its occurrence (usually 30–60 min after ethanol and 60–90 min after barbital), was employed as the measure of the drug's effect.

Tail blood samples (50 μl for ethanol and 100 μl for barbital) were taken immediately after the last motor-impairment measurement (i.e., at 120 min after the injection of the drug).

Measurement of sleeping time. Rats were injected IP with solutions of ethanol (17.5% v/v), or barbital (0.75 w/v) in physiological saline. The time between the injection and the loss of righting reflex was recorded as the sleep onset time. Sleeping time was recorded as the interval between the loss and return of righting reflex. Recovery was verified by again placing the rat on its back and the initial measurement was accepted only if the animal again righted itself within one minute. The observer who measured the time intervals did not know the identities of the groups. Blood samples (50 μl for ethanol measurement, 100 μl for barbital) were taken from the tail-tip of each rat at awakening.

Ethanol metabolism. Ethanol absorption, distribution and elimination were studied in Experiment II on Day 67 after the IP injection of a test dose of ethanol (2.6 g/kg). Tail vein blood samples (50 μl) were obtained from each animal at 20, 40, 60, 80, 100, 120, 180, 240 and 300 min after the administration of ethanol. 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/hr was calculated as described by Khanna and Kalant [16].

Drug analysis. Blood ethanol was analyzed by the enzymatic method described previously [15]. Barbiturates were analyzed by gas-liquid chromatography by means of an on-column methylation procedure previously described [20].

Statistical analysis. The data were subjected to analysis of variance using statistical computer package programme BMDP-2V (Statistical Software Inc., University of California). When appropriate, the group means at individual time points were compared by the Newman-Keuls test [25]. Group means obtained on individual test days (i.e., sleep time data) were compared by means of Student's *t*-test. Probability (*p*) values equal to or lower than 0.05 were considered to indicate statistical significance.

Procedure

Experiment I. The effect of chronic pentobarbital treatment by gastric intubation on the development of cross-tolerance to barbital and ethanol. In Experiment I, two groups of rats (n=30 each) were tested for their hypothermia and motor-impairment responses to either ethanol (2.6 g/kg) or barbital (142 mg/kg), administered IP. Each group was then subdivided into two subgroups matched with respect to their maximum hypothermia and motor impairment responses. One subgroup was designated to serve as the treated group and the other as the control. Treated animals received pentobarbital chronically and the control groups received water. An initial dose of 50 mg/kg as a 5% pentobarbital solution in water was administered by intubation twice a day. This was increased by 10 mg/kg/administration every 16–18 days to a maximum of 90 mg/kg twice a day.

Each group was tested for cross-tolerance to the hypothermic and motor-impairing effects of ethanol (2.6 g/kg) or barbital (142 mg/kg), approximately every 10 days during the first 40 days of treatment. Blood samples were taken immediately after the last motor-impairment measurement and stored at 4°C until the analysis was done. Ethanol (3.5 g/kg, IP) or barbital (200 mg/kg, IP) sleep time was determined on Day 56 of treatment. Tail blood samples were

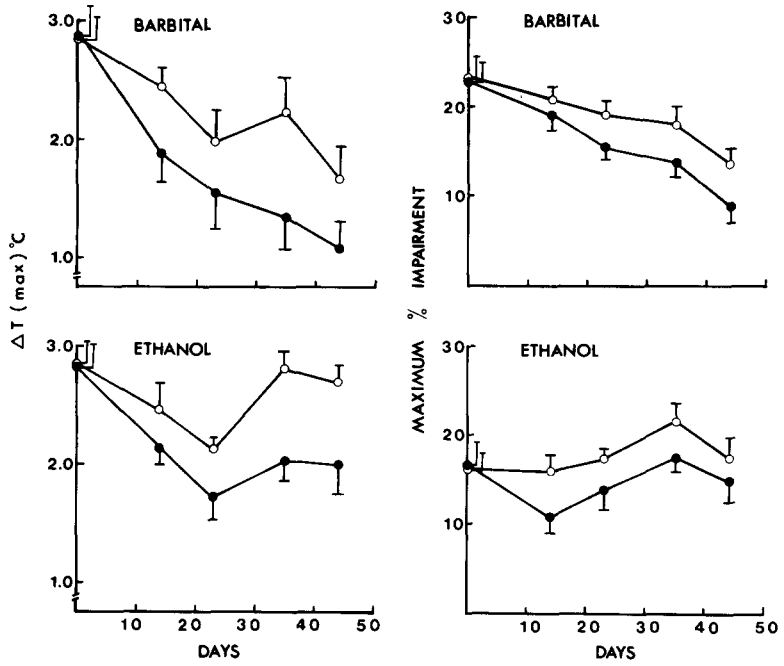

FIG. 1. The effect of chronic pentobarbital treatment by gastric intubation on the hypothermia and motor-impairment responses to barbital and ethanol. Two groups of rats were tested approximately every 10 days with either 2.6 g/kg IP of ethanol or 142 mg/kg IP barbital. Chronic pentobarbital (●) vs. water (○). Results shown are means±SEM with n=10–15 animals per group.

taken upon awakening. On Day 64, barbital sleep time was determined in the group which had previously been tested with ethanol only. On Day 73, the group which had been tested previously with barbital was tested for ethanol sleep time.

Experiment II. Dose-response study of cross-tolerance to barbital and ethanol after chronic pentobarbital treatment. Sixty-four rats were used in this study. Initial dose-response curves for the hypothermia and motor-impairment effects of ethanol (1.9, 2.2 and 2.6 g/kg) and barbital (91, 110 and 132 mg/kg) were carried out in all animals. Since only 32 animals could be tested in one day, the dose-response curve for each drug was completed in 2 days. The barbital dose-response curve experiment was done one week following the ethanol tests. After completion two groups of rats (n=32 each) were given either chronic pentobarbital or water by gastric intubation for approximately 70 days. A single dose of 50 mg/kg (0.5% w/v) pentobarbital was administered for 3 days. Thereafter, two daily intubations (9 a.m. and 5 p.m.) were used and the dose for each intubation was also increased by 10 mg/kg/intubation every two days until a dose of 100 mg/kg/intubation was reached. This dose was then maintained for the remainder of the chronic treatment period. On Day 48, one half of each group was randomly divided into 3 groups (n=5–6), each one receiving a different dose of barbital (91, 110, 132 mg/kg). The same was done with the other half of each group on Day 49. The results from the two days were pooled. The same procedure was carried out for ethanol (1.9, 2.2, 2.6 g/kg) on Days 60 and 61 of treatment.

RESULTS

Experiment I

The effect of chronic pentobarbital treatment on barbital- and ethanol-induced hypothermia is shown in Fig. 1 (left panels). An analysis of variance indicated that chronic pentobarbital treatment significantly diminished the hypothermic effect of barbital, $F(1,27)=5.9$, $p<0.02$, and of ethanol, $F(1,23)=10.7$, $p<0.003$. The effect of pentobarbital treatment on barbital hypothermia was probably less striking than it would have been if tolerance to barbital had not developed in the water-treated group as a result of the repeated tests, $F(4,55)=3.1$, $p<0.02$. There was no significant interaction of time and treatment for either barbital or ethanol, $F(3,81)=0.4$, $p>0.7$ and $F(3,69)=0.7$, $p>0.5$, respectively, but there was a significant main effect of time for both barbital, $F(3,81)=5.2$, $p<0.006$, and ethanol, $F(3,69)=7.3$, $p<0.001$.

Cross-tolerance to the motor-impairing effect of barbital and ethanol following chronic pentobarbital treatment was also observed (Fig. 1, right panels). An analysis of variance indicates that pentobarbital treatment had an attenuating effect on barbital- and ethanol-induced ataxia [for barbital, $F(1,27)=6.8$, $p<0.01$; for ethanol, $F(1,23)=5.5$, $p<0.02$. Again, there was tolerance to barbital in the water-treated group as a result of the repeated tests, $F(4,70)=3.6$, $p<0.009$, and there was a significant main effect of time for both barbital, $F(3,81)=11.1$, $p<0.001$, and ethanol, $F(3,69)=5.9$, $p<0.001$. There was, however, no significant interaction of time and treatment for either barbital or ethanol, $F(3,81)=0.3$, $p>0.3$; and $F(3,69)=0.2$, $p>0.8$, respectively.

The blood barbital and ethanol concentrations measured at the end of each test session are shown in Fig. 2. Analysis of variance indicated no significant difference between treated and control groups with respect to blood barbital concentration, $F(1,27)=0.9$, $p>0.4$. There was a significantly lower ethanol concentration in the treated group compared to the controls, $F(1,21)=15.3$, $p<0.001$, but no significant

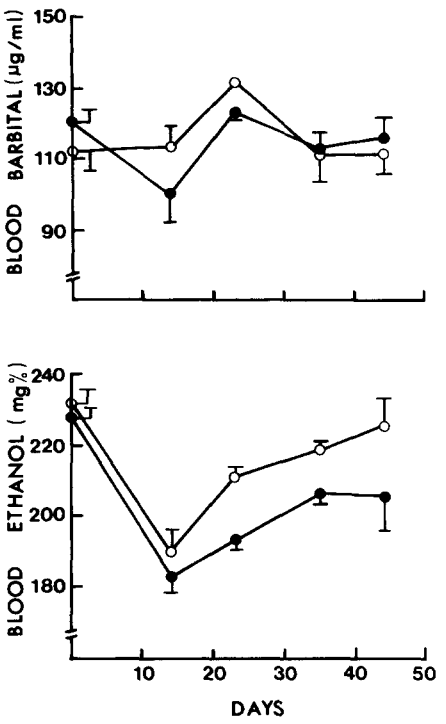

FIG. 2. The concentration of barbital or ethanol in the blood of rats chronically treated with pentobarbital by gastric intubation. The doses administered were 2.6 g/kg IP of ethanol and 142 mg/kg IP barbital. Samples were taken at the end of each test sesssion (120 min after injection). Chronic pentobarbital (●) vs. water (○). Results shown are means±SEM with n=10–14 animals per group.

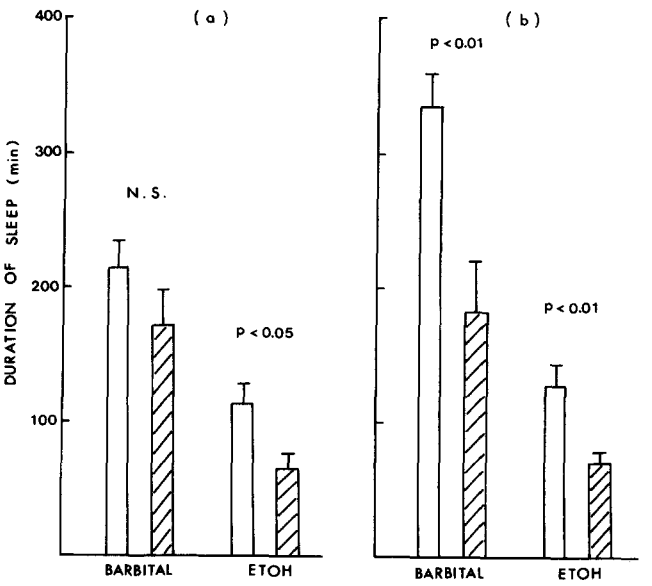

FIG. 3. Effect of chronic treatment by gastric intubation with pentobarbital (hatched bars) or water (open bars) on the duration of sleep induced by barbital (200 mg/kg IP) or ethanol (3.5 g/kg IP). (a) Two groups of rats were tested on Day 56 of chronic treatment. Each group had been tested repeatedly for hypothermic response to the same drug used now for sleep induction. (b) The same groups as shown in (a) were now crossed over and tested under the other drug: barbital test on Day 64 in previously ethanol-tested rats, and ethanol test on Day 73 in previously barbital-tested rats. Results shown are means±SEM; n=8–10 per group.

interaction of treatment with time, $F(3,63)=0.4, p>0.8$. Post hoc tests (Newman-Keuls), however, indicated no significant difference in blood ethanol levels between pentobarbital-treated and control rats on any single test days. There was a significant main effect of time for both barbital, $F(3,81)=6.5, p<0.001$, and ethanol, $F(3,63)=10.8, p<0.0001$.

The effect of chronic pentobarbital treatment on the duration of sleep induced by barbital and ethanol is shown in Fig. 3. There was no effect of pentobarbital treatment on the duration of barbital-induced sleep (Fig. 3a). This may be due to the development of tolerance to the test dose of barbital which was administered every 10 days, thereby minimizing the expected difference between the treated and untreated groups. This is supported by the observation (Fig. 3b) that control rats previously tested with only ethanol showed a significantly longer duration of barbital-induced sleep than control rats previously tested repeatedly with barbital ($p<0.01$). The mean barbital sleep times of the pentobarbital-treated groups were similar in the two tests; therefore, variation in the response from test to test cannot explain this finding. Furthermore, the response to ethanol of the group previously tested with barbital only (Fig. 3b) was comparable to the response of the group previously tested with ethanol only (Fig. 3a). Ethanol sleep time was significantly shorter in pentobarbital-treated rats compared to water-treated rats ($p<0.05$, t-test) in both tests (Fig. 3a,b), indicating that cross-tolerance occurred to the hypnotic effect of ethanol. Blood barbital and ethanol concentrations taken at the time of awakening were not significantly different as a result of chronic pentobarbital vs. water treatment in either of the tests (Fig. 4).

Experiment II

The effect of chronic pentobarbital treatment on the hypothermia and motor-impairment effects of various doses of barbital is shown in Fig. 5 (top panels). Pentobarbital-treated animals showed significantly diminished hypothermia and ataxia responses to barbital compared to controls over the given dose range [$F(1,50)=8.24, p<0.006$ for hypothermia; $F(1,50)=8.48, p<0.005$ for motor impairment]. A significant effect of dose was observed for both hypothermia, $F(2,50)=13.88, p<0.0001$, and motor impairment, $F(2,50)=17.69, p<0.0001$, but there was no significant interaction between treatment and test doses in either test [$F(2,50)=0.43$, $p>0.43$ for hypothermia; $F(2,50)=0.48$, $p>0.62$ for motor impairment]. These results are consistent with the observations that chronic pentobarbital treatment resulted in the development of tolerance to barbital and that there is a parallel shift of the Dose-Response (D-R) curve to the right following chronic pentobarbital treatment.

Figure 5 also shows the results obtained with ethanol (bottom panels) in animals chronically treated with pentobarbital or water. An analysis of variance shows that pentobarbital treatment significantly reduced the hypothermia, $F(1,48)=16.29, p<0.002$, and motor-impairment response, $F(1,48)=6.34, p<0.01$, to ethanol, indicating the development of cross-tolerance to ethanol. The same analysis of

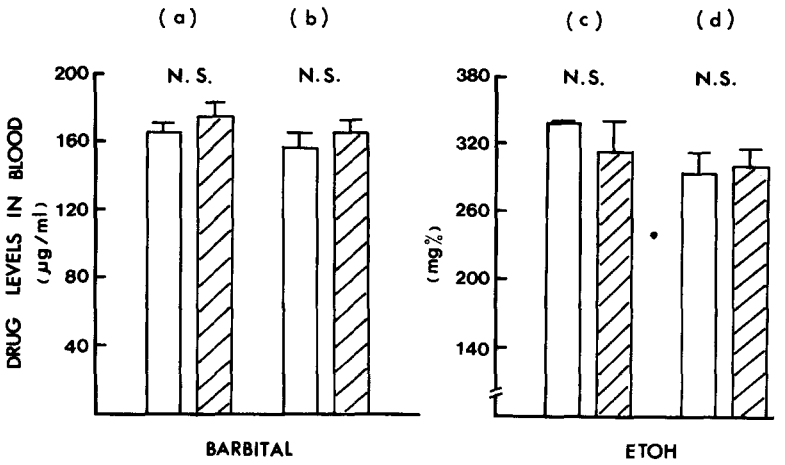

FIG. 4. Drug levels in blood on recovery of righting reflex, in rats chronically treated with pentobarbital (hatched bars) or water (open bars). (a) and (c) show blood barbital and ethanol concentration respectively, in rats chronically treated with pentobarbital or water intubation for 56 days and tested with either barbital or ethanol every 10 days. The same groups as shown in (a) and (c) were now crossed over and tested under the other drug: (b) barbital concentration on Day 64 in previously ethanol-tested rats, and (d) ethanol concentration on Day 73 in previously barbital-tested rats. Results shown are means±SEM; n=8–10 per group.

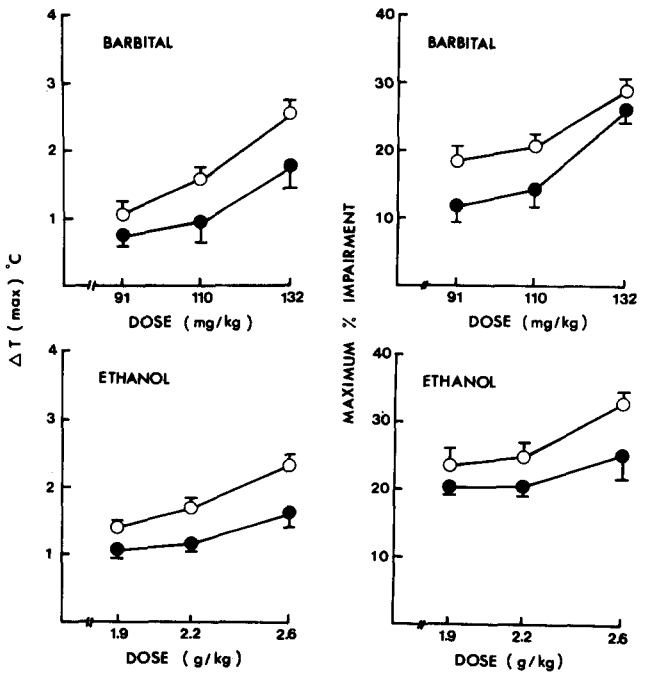

FIG. 5. The hypothermia and motor-impairment responses to various doses of barbital and ethanol in rats receiving pentobarbital intubation chronically (Experiment II). Barbital and ethanol testing was done on Days 48 and 49 and ethanol testing was done on Days 60 and 61 of treatment. Chronic pentobarbital (●) vs. water (○). Results shown are means±SEM with n=7–11 animals per group.

variance also showed a significant effect of dose for both hypothermia, $F(2,48)=12.33$, $p<0.0001$, and motor impairment, $F(2,48)=5.18$, $p<0.009$, but no significant interaction between treatment and test doses [$F(2,48)=0.82$, $p>0.82$ for hypothermia; $F(2,48)=0.54$, $p>0.58$ for motor impairment]. The lack of significant interaction is consistent with a parallel shift of the D-R curve to the right following chronic pentobarbital treatment.

Blood barbital and ethanol levels taken at the end of each test period are shown in Fig. 6. The blood barbital or ethanol concentrations are similar in treated and control groups, $F(1,49)=0.63$, $p>0.44$ and $F(1,48)=0.17$, $p>0.69$, respectively, although there was a significant effect of dose for each drug, i.e., the higher the dose, the higher the blood level [for barbital, $F(2,49)=48.14$, $p<0.0001$; and for ethanol, $F(2,48)=86.11$, $p<0.0001$.

Blood ethanol absorption and disappearance curves after a test dose of ethanol (2.6 g/kg) are shown in Fig. 7. Ethanol blood levels are not significantly different in the treated group compared with control at any times except at 20 min, when a lower blood ethanol level was observed in the treated group ($p<0.05$). The two groups did not differ in calculated rates of ethanol metabolism (mg/kg/hr), which were 219±10 for the chronic pentobarbital group and 218±4 for the controls.

DISCUSSION

Cross-tolerance to barbital hypothermia, ataxia and hypnosis was observed in pentobarbital-treated animals. Cross-tolerance to the hypothermic, hypnotic and ataxic effects of ethanol was also observed in subjects chronically treated with pentobarbital. Furthermore, cross-tolerance to both hypothermia and ataxia could be demonstrated over a wide range of doses.

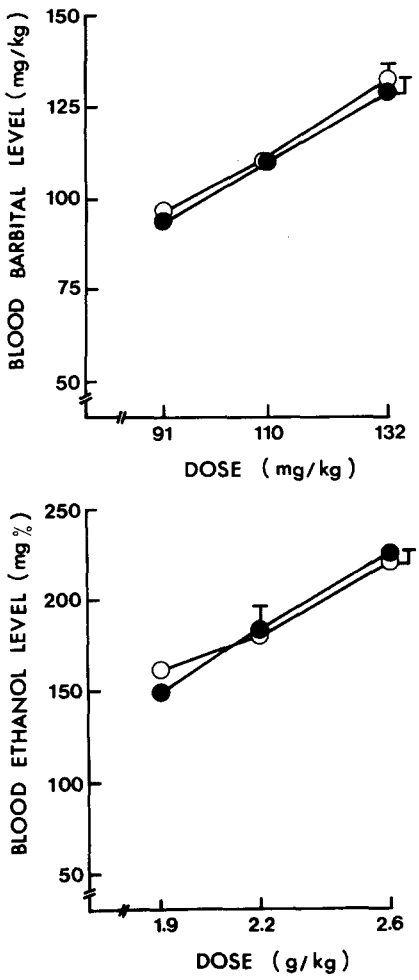

FIG. 6. The concentration of barbital or ethanol in the blood of rats following administration of various doses of barbital and ethanol in rats receiving pentobarbital intubation chronically (Experiment II). Samples were taken at the end of each test session (120 min after injection). Chronic pentobarbital (●) vs. water (○). Results shown are means with n=7–11 animals per group. Only the largest SEM of each group is shown.

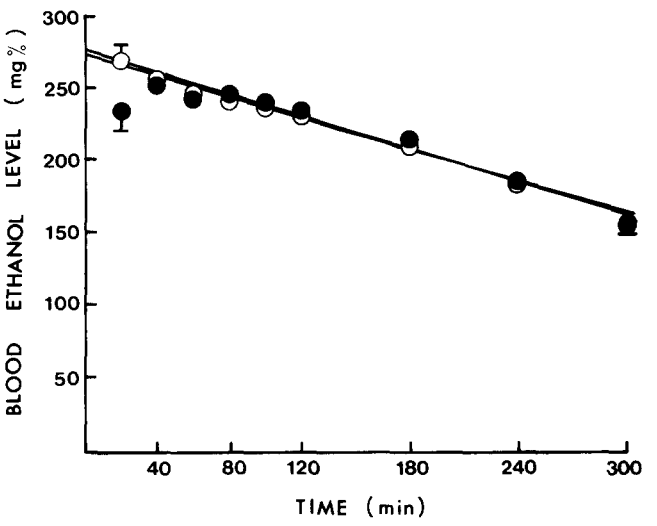

FIG. 7. Blood ethanol disappearance curves after a test dose of ethanol (2.6 g/kg) in pentobarbital-treated (●) and control (○) rats after 9½ weeks of chronic treatment. Points represent means (n=10 animals per group). Vertical lines indicate positive or negative half of the standard error. Only the largest SEM of each group is shown. Regression lines were calculated by the method of least mean squares.

Blood ethanol levels in animals chronically treated with pentobarbital were significantly lower than those of controls over the course of treatment in Experiment I. In contrast, the blood levels of ethanol, measured at the end of Experiment II, showed no intergroup differences. Though these findings appear to be contradictory, there is some evidence that anticipatory arousal induced by the repeated testing in Experiment I may alter the disposition of ethanol [9,22]. On each test day, the animals were transported to the test room in which they were subjected to a variety of disturbing procedures, including measurement of rectal temperature, performance on the tilt plane, and blood sample collection. Over a few test days, the movement to a different environment comes to serve as an effective cue for the animals in anticipation of testing. The autonomic changes associated with this anticipatory arousal state have been suggested as the mechanism of altered ethanol disposition, especially the early changes in absorption. These changes would be more marked in the pentobarbital-treated rats because they were in a partial withdrawal state due to the omission of the afternoon dose. This interpretation is consistent with the fact that the blood ethanol levels in the pentobarbital-treated group did not differ from those of controls on Day 14 but almost attained statistical significance on Days 23 and 44 ($q=3.36$ and 3.92 respectively).

While lower blood ethanol levels in pentobarbital-treated animals may indicate a pharmacokinetic component in ethanol cross-tolerance, its exact contribution cannot be assessed from a single measurement done 30–60 min after the time of peak effect. In Experiment II, where the complete time course of absorption, distribution and elimination was studied, changes in the absorption of ethanol were observed in the pentobarbital-treated rats. Although marginally lower ethanol levels were seen at only 20 min in the time course study and the two curves were practically identical at all other times (Fig. 7), the pharmacokinetic contribution to ethanol cross-tolerance cannot be disregarded. Therefore, the relative contributions of pharmacodynamic vs. pharmacokinetic components in ethanol cross-tolerance require further investigation.

It is apparent that tolerance to the barbital test dose developed in control animals over successive test sessions (Experiment I) as shown by the decrease in their response. This is because of the low rate of elimination of barbital ($t^1/_2$ for barbital is approximately 13 hr compared to about 1–2 hr for pentobarbital) [17,18], resulting in a prolonged CNS depression and facilitating the development of tolerance. The ease with which functional tolerance to pentobarbital could be produced in cats ($t^1/_2$ pentobarbital=10–12 hr) [22] compared to rodents ($t^1/_2$=1–2 hr) [18] suggests that duration of exposure of the CNS is a critical factor in the development of cellular tolerance. Since metabolism of barbital is negligible [2, 8, 11], and distribution and elimination remain unchanged

after chronic treatment [3,8], the development of cross-tolerance to barbital from chronic pentobarbital treatment can likely be attributed primarily to adaptive changes leading to decreased sensitivity in the CNS, rather than to increased hepatic metabolism.

Somewhat surprisingly, blood ethanol or barbital levels at awakening (Fig. 4). were not higher in tolerant animals than in controls. This observation does not prove that functional tolerance did not exist, because brain rather than blood ethanol concentrations would be needed to settle this question. Moreover, as shown by Gostomzyk *et al.* [12] and others, during the rising phase of the blood ethanol curve the concentration is lower in venous than in arterial blood, and the difference is even more likely to be marked and prolonged in the tail than in the limbs. However, both the pentobarbital-treated and control rats awoke during the period of almost constant "plateau" value of blood ethanol concentration (see Fig. 7). The same is true of the tests under barbital: because of the very long plasma half-life of barbital, it is likely that the concentration did not change significantly over the duration of sleep in both groups. Therefore, in both the ethanol and the barbital tests the difference between the pentobarbital-treated and control groups with respect to time of awakening probably reflected a difference in within-session tolerance of the CNS. Barbital is absorbed and distributed considerably more slowly than ethanol. The peak effect is not attained until an hour or more after an IP injection [21], and the plateau of maximum blood level after the same dose and route was seen between 2 and 6 hr after injection [17]. This may explain why the blood barbital levels measured at 120 min in Experiment I did not differ significantly between the pentobarbital and water groups.

The results of this study are in agreement with those of a good number of clinical and experimental studies (for references, see [19]) which show the presence of cross-tolerance to ethanol in barbiturate-tolerant subjects. Since the involvement of pharmacokinetic factors cannot be ruled out, it could not be concluded that the observed cross-tolerance was due to a decrease in CNS sensitivity. It is noteworthy that the degree of cross-tolerance seen is relatively small even though prolonged treatment with high doses of pentobarbital was employed. The small extent of cross-tolerance might in fact explain some of the negative findings in the literature. The inability to show cross-tolerance to ethanol in pentobarbital- or methaqualone-treated animals in the studies of Commissaris *et al.* [4,5] may be related to the short duration and/or low dose chronic treatment regimen employed in their work. The authors acknowledge this fact and further indicate that only a slight degree of cellular tolerance to the drug of treatment was evident in their studies. The sensitivity of the test employed for measuring tolerance is also important in relation to the chronic dose employed for treatment. For example, relatively short duration of pentobarbital treatment, or use of a low dose for a longer time (50 mg/kg, PO daily for approximately one month) did not produce CNS cross-tolerance to barbital- and ethanol-induced sleep, whereas this treatment was sufficient to produce clear CNS tolerance on the moving belt test [17]. Furthermore, when the size and frequency of pentobarbital treatment doses were increased (50–80 mg/kg, three times daily), a clear CNS tolerance to barbital was seen [17].

The asymmetry of cross-tolerance (i.e., clear evidence of cross-tolerance to ethanol after chronic pentobarbital treatment compared to lack of cross-tolerance to pentobarbital after ethanol treatment) cannot yet be explained. Coper [7] cites many examples of asymmetry of cross-tolerance among similarly and dissimilarly acting drugs, but offers no ready explanation. One possibility is that the actions of ethanol which subserve the measured effects might form a subset of a larger sphere of pentobarbital actions. Therefore, pentobarbital could be a greater stimulus to the development of cross-tolerance to ethanol than ethanol would be to pentobarbital. This is essentially a pharmacodynamic explanation. The other possibility is the involvement of pharmacokinetic factors in ethanol cross-tolerance in pentobarbital-treated subjects.

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