Pharmacology Biochemistry and Behavior, Vol. 57, Nos. 1/2, pp. 101–110, 1997

0091-3057/97 $17.00 + .00

PII S0091-3057(96)00163-3

Effect of Lipid Solubility on the Development of Chronic Cross-Tolerance Between Ethanol and Different Alcohols and Barbiturates

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Received 9 October 1995; Accepted 3 April 1996

KHANNA, J. M., A. D. LÊ, H. KALANT, A. CHAU AND G. SHAH. *Effect of lipid solubility on the development of chronic cross-tolerance between ethanol and different alcohols and barbiturates.* PHARMACOL BIOCHEM BEHAV **57** (1/2) 101–110, 1997.—Tolerance to ethanol and cross-tolerance to other alcohols (*n*-propanol, *n*-butanol, *t*-butanol, isobutanol, *t*-amyl alcohol, *n*-amyl alcohol, and benzyl alcohol) and barbiturates (pentobarbital, secobarbital, amobarbital, thiopental, barbital and phenobarbital) that differ in lipid:water partition coefficient was examined in rats after chronic pretreatment with ethanol. Tolerance and cross-tolerance were studied with three different measures (hypothermia, tilt-plane, and rotarod). Tolerance to ethanol resulted in significant cross-tolerance to alcohols with low lipid solubility (*n*-propanol and *t*-butanol), whereas no cross-tolerance was seen with alcohols of high lipid solubility (isobutanol, *n*-amyl alcohol, *t*-amyl alcohol and benzyl alcohol). Cross-tolerance to *n*-butanol (which has intermediate lipid solubility) appeared to be metabolic rather than functional. Tolerance to ethanol also resulted in significant cross-tolerance to barbital and phenobarbital, but not to pentobarbital, secobarbital, amobarbital or thiopental. These studies suggest that lipid solubility is an important factor in relation to specificity of cross-tolerance to alcohols and barbiturates. © 1997 Elsevier Science Inc.

Tolerance, chronic Cross-tolerance Alcohols Barbiturates Lipid solubility

THE relative potencies of a wide variety of depressant drugs have been known, for nearly a century, to be roughly proportional to their relative lipid : water partition coefficients (6,24–26). This relationship led to the widely held hypothesis that such drugs produced their pharmacological effects by a non-specific physicochemical interaction with the lipid bilayer of the cell membrane (3,26). In recent years, however, it has become apparent that at non-lethal concentrations these drugs exert relatively selective effects on certain constituents of the membrane, such as GABA-activated chloride channels, NMDA receptor-linked cation channels, adenosine receptors and others. Nevertheless, the subject of lipid-solubility has not lost its relevance, because drugs differing in lipid-water partition coefficient might have differential effects at the interfaces between such protein inclusions and specific membrane lipids in their respective microenvironments.

A selectivity in action among sedative-hypnotic drugs, based on differences in their relative lipid solubilities, was first proposed by Howerton et al. (10,11), who showed that mice of the SS (short sleep, i.e., ethanol-resistant) and LS (long sleep, i.e., ethanol-sensitive) lines did not differ with respect to loss of righting reflex produced by pentobarbital or *n*-butanol. Similarly, Marley et al. (23) showed that the ED60 values in the SS and LS lines were closely similar with drugs of high lipid solubility, but differed markedly with less lipid-soluble agents.

The demonstration of cross-tolerance to a water-soluble barbiturate, barbital, and the lack of it to a highly lipid-soluble barbiturate, pentobarbital, after chronic ethanol treatment in rats also suggested some type of specificity or selectivity in the site and/or mechanism of CNS action of apparently similarly acting sedative-hypnotic drugs (8). Among drugs which act on membranes in general, this specificity could involve selective actions on different parts of the lipid bilayer or its protein inclusions (7), depending on the lipid : water partition coeffi-

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cient of the drug. Presumably, if the lipid solubility of a drug determines its site or mechanism of action, cross-tolerance would be more likely to occur between those drugs which have similar lipid solubilities.

Support for this hypothesis is provided in a report by Curran et al. (4), who found cross-tolerance to barbital and phenobarbital (i.e., barbiturates with low lipid:water partition coefficients) after chronic ethanol treatment, but negligible cross-tolerance to the highly lipid-soluble barbiturates, thiamylal, methohexital, secobarbital and thiopental. These authors, however, did find cross-tolerance to pentobarbital, another highly lipid-soluble barbiturate.

Recently, we reported that chronic treatment with ethanol resulted in cross-tolerance to the loss of righting reflex induced by *n*-propanol and *t*-butanol but not by *n*-butanol and pentobarbital. Since ethanol, *n*-propanol and *t*-butanol have low degrees of lipid solubility, we suggested that the development of cross-tolerance among these sedative-hypnotic drugs might be related to the similarity of their relative lipid:water solubilities (16).

As most of the work presented above dealt with loss of righting reflex, it is not possible to state whether the role of lipid solubility in cross-tolerance might be dependent on the specific test (i.e., hypnosis) employed or is a general phenomenon independent of the test measure used. The present studies were therefore undertaken to examine cross-tolerance between ethanol and other alcohols and barbiturates differing in lipid solubility, as studied with three different measures: hypothermia, tilt-plane and rotarod.

TABLE 1

LOG OCTANOL/H_20 PARTITION COEFFICIENT FOR VARIOUS ALCOHOLS AND BARBITURATES

		Partition Coefficient*
(a)	Alcohols	
	ethanol	−0.32
	n-propanol	0.34
	t-butanol	0.32
	iso-butanol	0.65
	n-butanol	0.88
	t-amyl alcohol	0.89
	benzyl alcohol	1.1
	n-amyl alcohol	1.4
(b)	Barbiturates	
	barbital	0.65
	phenobarbital	1.42
	amobarbital	2.07
	pentobarbital	2.03
	thiopental	2.96
	secobarbital	2.34*

*Values shown are logs of octanol/water partition coefficients, except that for secobarbital, which is the log of the (50% ether/50% DMF)/H_20 partition coefficient.

MATERIALS AND METHODS

Animals

Male Sprague–Dawley rats weighing 150–200 g were obtained from Charles River Laboratories (Montreal, Quebec). They were housed singly and fed a standard laboratory rat chow in a daily ration which was individually adjusted to bring them all to a body weight of 250–280 g before any training or testing was carried out. Thereafter, each animal received five standard pellets daily for the balance of this 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 0700 to 1900 daily throughout the experiment.

Drug Analysis

Blood ethanol was analyzed by the enzymatic method described previously (9). Other alcohols were analyzed by the gas-liquid chromatographic procedure described by LeBlanc (19). Barbiturates were also analyzed by gas-liquid chromatography, by an on-column methylation procedure (14).

Drugs

All drug solutions were freshly prepared in saline except for *n*-amyl alcohol (pentanol-1) which was dissolved in propylene glycol as a 50% v/v solution. Benzyl alcohol was used directly from pure stock. Table 1 lists the drugs used, and their respective octanol/water partition coefficients as given by Leo et al. (22). All drugs or saline control solution were given by intraperitoneal (i.p.) injection, in a volume of 0.3–1.5 ml/l00 g B.W., depending on the dose.

Test Procedures

Accelerod Test. An accelerating rotarod apparatus similar to that described by Bogo et al. (2) was used. The speed of rotation of the rod accelerated linearly with time, from 0 to 110 rpm, at approximately 1 rpm/s. The dimensions of the Plexiglas box were 13 × 16 × 20 inches (width × depth × height). Floor grid bars were located 7.5 inches below the rotating rod itself. Shock delivery (scrambled with respect to bars) was constant at 30 volts (3 ma) but could be doubled to 60 volts (6 ma) by a push-button control switch. This was done (only during training) with rats that repeatedly jumped off the rod as soon as they were placed on it. The rotating rod was a cylinder made of Plexiglas (9.5 inches long, 1.75 inches in diameter) and wrapped in wire mesh to provide a better foothold for the rats. The cylinder was mounted concentrically on a half-inch diameter axle fitted into the drive socket of a gearmotor mounted on the side of the enclosure.

Initially, the subjects were required to stay on the stationary rod for 30 s. Then they were required to maintain balance on the rod for a minute at a constant speed of 5 rpm. A dismount in either of the preceding phases resulted in an immediate shock that usually lasted for about 0.5–1 second until the subject was picked up and placed on the rod once again. After 2 to 3 trials on day 1, they were ready for training in the accelerating mode on the following day. Rats were given at least 3 runs on the first day with minimal shaping, and then on every second day until they all were able to stay on the rod at rotary speeds of 35 rpm or better. The time they remained on the rotarod in each trial was noted. Usually a total of five days training was involved.

All rats received a practice run on the day before the test day. On the test day, two trials were given to each rat prior to any drug injections and the average score on these two trials was the baseline of the rat's performance on the accelerod. If the two pre-drug scores differed by more than 20%, the animal was not used in the experiment. The performance duration was checked again at 2.5, 7.5, 12.5 and 17.5 min after drug injection and calculated as a percentage of the animal's own

pre-test value. The lowest percentage performance, regardless of the time at which it was found, was used to quantify the maximum percentage impairment. This occurred between 2.5 and 7.5 min. after injection in all cases.

Tilt-Plane Test. The tilting-plane test was also used as a measure of motor impairment (1,8). The apparatus consists of a plane which can be inclined at a fixed angular velocity through a range of 55° above 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 various alcohols (including ethanol), barbiturates and benzodiazepines. For more rapidly acting alcohols (*n*-propanol and *n*-butanol), the sliding angle was measured at 15, 30, 45 and 60 min after injection. The degree of post-drug ataxia was assessed as the percentage change in sliding angle, compared to the same animal's pre-drug value. Maximum impairment, regardless of the time of its occurrence, was employed as the measure of drug effect. This generally occurred about 30 min after injection, except for *n*-propanol and *n*-butanol, which produced their peak impairment at 15 min.

Hypothermia. A 4-cm-long thermistor probe was inserted into the rectum and left until a stable temperature recording was obtained (approximately 30 s) on a Yellow Springs Instrument electrical thermometer. This was done before and at 30, 60, 90 and 120 min (except for *n*-propanol and *n*-butanol, for which temperature measurement was done at 15, 30, 45 and 60 min) after the intraperitoneal test injection, until the temperature began to return to normal. This occurred about 60 min after injection of all drugs except *n*-propanol and *n*-butanol, which produced their peak effect earlier (30 min). The hypothermic effect was expressed as the maximal drop in temperature (from baseline) over the period of observation.

EXPERIMENTAL DESIGN

Preliminary Dose-Response Studies

Groups of well-trained rats were used for preliminary log-dose response studies of various alcohols and barbiturates on the accelerod test. Each group was divided, on the basis of their initial scores, into four or five balanced subgroups. Each subgroup (n = 5 or 6) received an assigned dose of the drug to be tested. The rats were then tested on the accelerod at 2.5, 7.5, 12.5 and 17.5 min after drug injection. Dose-response curves for the drugs examined in this way are shown in Fig. 1. The same groups of animals were used repeatedly, for testing each drug in turn. In order to minimize the possibility of drug interactions or of gradual development of cross-tolerance as a result of the repeated tests, intervals of at least 10 days were left between tests. On the basis of the dose-response curves, an appropriate dose of each drug, that gave 50–70% of maximum impairment, was selected as the test dose for the cross-tolerance study. These doses are indicated by arrows in Fig. 1.

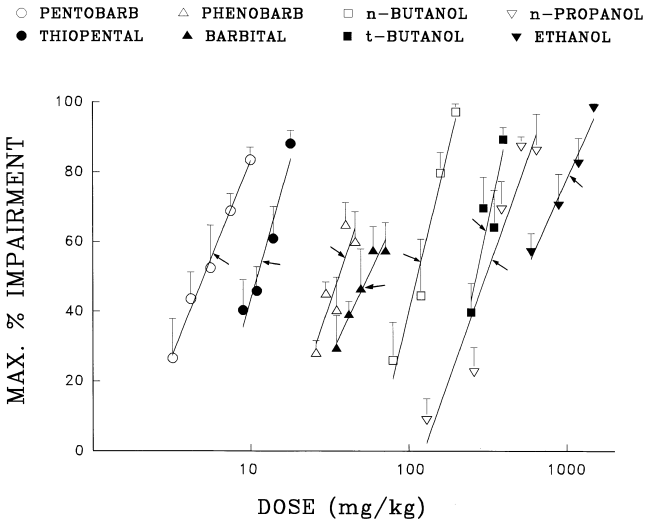

FIG. 1. Dose-response curves for the effects of various alcohols and barbiturates on accelerod performance of trained rats. See text for details of methods. Arrows indicate the respective doses selected for use in cross-tolerance tests after chronic treatment with ethanol.

Experiment 1: Effect of Chronic Ethanol Treatment on the Development of Tolerance to Ethanol and Cross-Tolerance to Other Alcohols and Barbiturates (Accelerod Test)

Rats were trained on the accelerod apparatus until they all reached criterion. Two groups of well-trained rats (n = 30 each) were then subdivided into two subgroups each. One subgroup received ethanol chronically and the other served as the isocaloric sucrose control.

Rats were intubated in their home cages with 3 g/kg ethanol (15% w/v in tap water) or isocaloric sucrose solution, but the dose was gradually increased (0.5 g/kg every 3 days) to 5 g/kg ethanol daily. One group of 30 rats was tested with ethanol and the other with *n*-propanol at 5 weeks. These tests did not reveal significant tolerance, and an afternoon dose of 2 g/kg was therefore added, and increased by 0.5 g/kg every 3 days until the total daily dose reached a maximum of 8 g/kg. The solution strength was gradually increased from 15% to 25% (w/v) in order to avoid excessively large volumes at the higher doses. Control animals received equal volumes of isocaloric sucrose solutions. The two groups were again tested for tolerance to ethanol (1 g/kg IP) and cross-tolerance to *n*-propanol (0.35 g/kg) respectively, at 7 weeks. Cross-tolerance to *n*-butanol (0.12 g/kg, IP) was tested at 10 weeks in the first group of rats. Cross-tolerance to *t*-butanol (0.3 g/kg IP) was tested at 10 weeks in the second group of 30 rats. In the intervals between cross-tolerance tests, chronic treatment with ethanol was continued at the same dosage. Since all intubations were carried out in the home cages, and no behavioral tests were carried out after intubation, this can be regarded as a non-learning or minimal-learning paradigm of tolerance.

Another group of rats trained on the rotarod (n = 28) received either ethanol or isocaloric sucrose as described above, except that the afternoon dose was introduced after the second week. As a result, these animals showed clear tolerance to ethanol after 4 weeks of daily ethanol treatment. Cross-tolerance to pentobarbital, thiopental, phenobarbital and barbital was then assessed in the same rats at approximately 10-day intervals.

Experiment 2: Chronic Ethanol Tolerance and Cross-Tolerance to Other Alcohols and Barbiturates (Hypothermia and Tilt-Plane Test)

Two groups of 24 rats were used in these studies. Before the rats were put on chronic ethanol or sucrose treatment, an

initial response to the hypothermic effect of ethanol (2.2 g/kg) was assessed on the first group of 24 rats. Similarly, an initial response on the tilt-plane test after a test dose of ethanol (2.5 g/kg) was measured on the second group of rats. According to the initial response scores on both tests, rats were matched into pairs and one of each pair was given ethanol and the other isocaloric sucrose by intubation daily. The dose of ethanol was 3 g/kg to begin with, and was increased every 3 days by 0.5 g/kg until a daily maximum of 5 g/kg was reached. After three weeks on this regimen the rats were tested for tolerance to ethanol; since significant tolerance was found, the dose was not increased further. Cross-tolerance to other drugs was tested in the same animals at 10-day intervals to minimize the possibilities of drug interactions. A 50 μl tail blood sample was taken from each rat at the end of each experiment. Tests of cross-tolerance on both hypothermia and tilting-plane tests, to *n*-propanol, *n*-butanol and *t*-butanol were conducted within the first 8 weeks of the chronic ethanol treatment. To avoid prolonged and repeated use of the rats, cross-tolerance to barbiturates was subsequently assessed on the tilting-plane test only.

Another group of rats ($n = 26$) was similarly treated with ethanol or isocaloric sucrose ($n = 13$ each) as described above. These animals were used for testing tolerance to ethanol and cross-tolerance to the hypothermic effect of various barbiturates.

An additional two separate groups of 30 rats each received the same chronic ethanol or sucrose treatment as described above. They were used for testing cross-tolerance to the hypothermic and motor-impairment effects of other alcohols, i.e. isobutanol, *t*-amyl alcohol, *n*-amyl alcohol and benzyl alcohol.

As in Experiment 1, chronic ethanol treatment was continued in the intervals between cross-tolerance tests.

Statistical Analysis

For initial comparisons of group data shown in Figs. 3–5, the test results for maximum effect, in control and chronic ethanol groups, were compared for each drug separately, by means of a *t*-test for unpaired data, since the animals had not been treated in a strictly paired design. If the *t* value was significant, no further analysis was done. However, if a marginally significant difference in either direction was found, the results at all test times were analyzed by a two-way ANOVA (times, treatment groups) with repeated measures, using the BMDP-2V statistical package for PC. This is analogous to comparing the total areas under the time × effect curves of the two groups.

RESULTS

Preliminary Dose-Response Studies

As shown in Fig. 1, the dose-response curves for the four alcohols (ethanol, *n*-propanol, *n*-butanol, *t*-butanol) and four barbiturates (barbital, phenobarbital, pentobarbital and thiopental) studies in detail were essentially parallel. It was therefore possible to estimate, by simple interpolation in the respective dose-response graphs, the dose of each drug that would produce a 60% impairment response (ED60). The ED60 was found to be inversely related to the octanol/water partition coefficient for the four alcohols and three of the four barbiturates (Fig. 2). Only thiopental, with ED60 = 12.8 mg/kg and partition coefficient = 795, fell far off the common regression line for the other drugs. The seven points in Fig. 2 yielded a standardized correlation coefficient of -0.9616 ($p < 0.0005$).

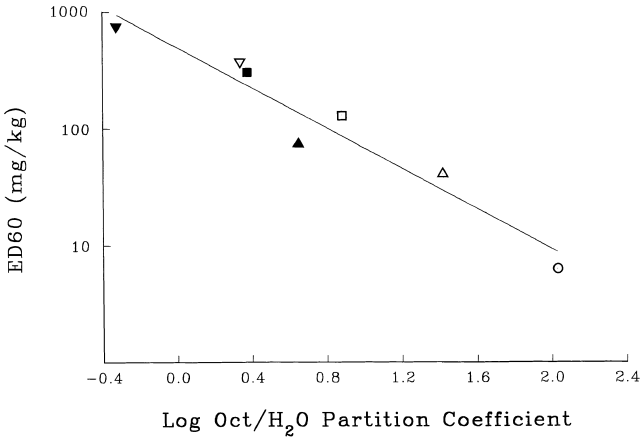

FIG. 2. Negative correlation between ED60 and octanol/water partition coefficient for a series of lower aliphatic alcohols and barbiturates, in rats tested acutely in the accelerating rotarod procedure. The drugs are identified by the same symbols as in Fig. l. Thiopental did not fall on the same regression line as the other drugs: its ED60 was 12.8 mg/kg despite a partition coefficient of nearly 800.

Experiment 1: Effect of Chronic Ethanol Treatment on the Development of Tolerance to Ethanol and Cross-Tolerance to Other Alcohols, and Barbiturates

Accelerod test. Figure 3a shows the results of tests of chronic tolerance to ethanol and cross-tolerance to other alcohols on the accelerod test. After 7 weeks of chronic treatment, the maximum percentage impairment produced by a test dose of ethanol (1 g/kg) was significantly lower in chronically ethanol-treated rats than in sucrose controls ($t = 3.58$, $df = 26$, $p < 0.01$). Ethanol treatment significantly reduced motor impairment by *n*-butanol (0.12 g/kg) at 10 weeks ($t = 4.252$, $df = 26$, $p < 0.001$) i.e., cross-tolerance to *n*-butanol was evident. A separate group of chronically ethanol-treated rats demonstrated significant cross-tolerance to *n*-propanol (0.35 g/kg) at 7 weeks ($t = 2.979$, $df = 26$, $p < 0.01$) and to *t*-butanol (0.3 g/kg) at 10 weeks ($t = 2.870$, $df = 26$, $p < 0.01$).

Figure 3b shows the results of the tests of chronic tolerance to ethanol and cross-tolerance to various barbiturates. Again, chronic ethanol tolerance (test dose = 1.0 g/kg) was verified at 4 weeks ($t = 5.289$ vs. sucrose controls, $df = 25$, $p < 0.001$). There was a significant difference in maximum impairment between ethanol-treated and control rats after a test dose of phenobarbital (40 mg/kg) at 9 weeks ($t = 3.00$, $df = 25$, $p < 0.01$) and of barbital (50 mg/kg) at 10 weeks ($t = 4.152$, $df = 25$, $p < 0.00$l). No cross-tolerance was seen to pentobarbital (6 mg/kg) at 6 weeks ($t = 1.742$, $df = 25$, NS) or thiopental (12 mg/kg) at 8 weeks ($t = 1.635$, $df = 25$, NS) when maximum impairment scores were compared. However, an ANOVA of percentage impairment of performance at all test times within the session (data not shown) showed a marginally significant main effect of groups, suggestive of cross-tolerance to pentobarbital ($F = 4.22$, $df = 1,25$, $p < 0.051$), but not to thiopental ($F = 2.75$, $df = 1,25$, $p > 0.11$).

Experiment 2: Tolerance to Ethanol and Cross-Tolerance to Other Alcohols and Barbiturates on Hypothermia and Tilt-Plane Tests

Hypothermia test. The effect of chronic ethanol treatment on the hypothermic response to ethanol and other alcohols

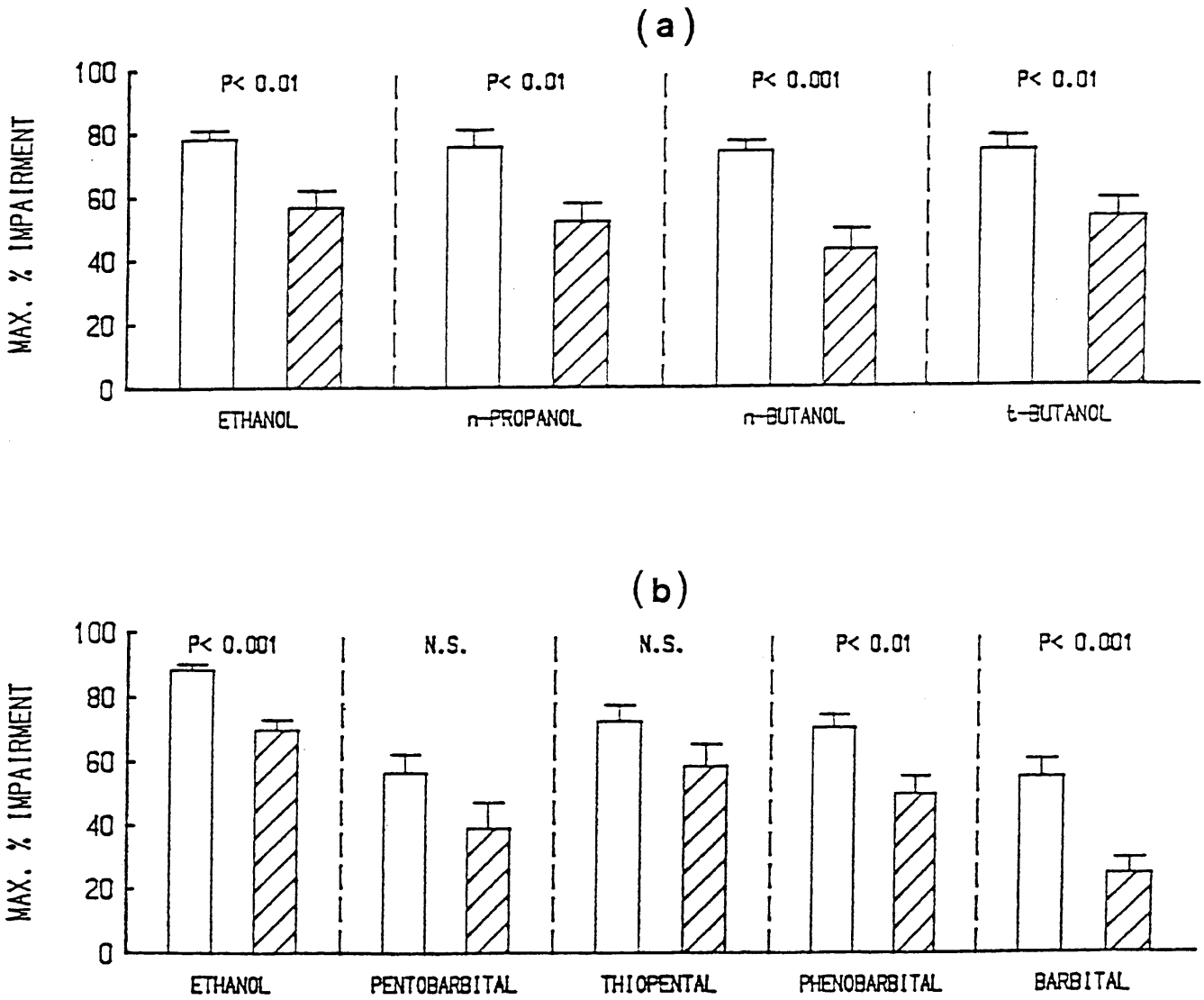

FIG. 3. Tolerance to ethanol and cross-tolerance to other drugs on the accelerod test of motor-impairment, after chronic treatment by gastric intubation with ethanol (hatched bars) or sucrose (open bars): (a) ethanol (1.0 g/kg), *n*-propanol (0.35 g/kg), *n*-butanol (0.12 g/kg) and *t*-butanol (0.3 g/kg); and (b) ethanol (1 g/kg), pentobarbital (6 mg/kg), thiopental (12 mg/kg), phenobarbital (40 mg/kg) and barbital (50 mg/kg). Vertical lines indicate the positive half of each standard error, with n = 13–15 animals per group. The significance of difference between groups was estimated by Student's *t*-test for unpaired data. NS = not significant, $p > 0.05$.

is shown in Figs. 4a & b. Chronic tolerance to ethanol was demonstrated at 3 weeks, as the maximum hypothermic response (ΔTmax) to a 2.2 g/kg test dose was significantly lower in ethanol-treated rats than in sucrose controls ($t = 5.933$, $df = 22$, $p < 0.00l$). Chronic ethanol treatment also produced cross-tolerance to the hypothermic effect of other alcohols: *n*-propanol (1 g/kg) at 5 weeks ($t = 4.488$, $df = 21$, $p < 0.001$), *n*-butanol (0.4 g/kg) at 7 weeks ($t = 5.577$, $df = 21$, $p < 0.001$) and *t*-butanol (0.65 g/kg) at 8 weeks ($t = 2.865$, $df = 21$, $p < 0.01$). The hypothermic response to isobutanol (0.4 g/kg) at 6 weeks appeared slightly smaller in ethanol-treated rats than in sucrose controls, but the difference proved not to be significant ($p > 0.1$). No cross-tolerance was seen with respect to *t*-amyl alcohol (0.3 g/kg) at 7.5 weeks ($t = 0.117$, $df = 22$; $p > 0.95$), *n*-amyl alcohol (0.32 g/kg) at 9 weeks ($t = 1.95$, $df = 22$, $p > 0.10$) or benzyl alcohol (0.26 g/kg) at 11 weeks ($t = 0.98$, $df = 21$; $p > 0.40$).

Fig 4c shows the corresponding results for cross-tolerance to the hypothermic effects of various barbiturates. Among the barbiturates tested, no cross-tolerance was seen with respect to pentobarbital (22 mg/kg) at 4 weeks ($t = 1.39$, $df = 24$, NS), thiopental (28 mg/kg) at 5 weeks ($t = 1.41$, $df = 24$, NS), secobarbital (22 mg/kg) at 6 weeks ($t = 1.276$, $df = 24$, NS), or amobarbital (48 mg/kg) at 7 weeks ($t = 0.38$, $df = 24$, NS). However, cross-tolerance was seen with phenobarbital (55 mg/kg) at 13 weeks ($t = 2.219$, $df = 24$, $p < 0.05$) and barbital (100 mg/kg) at 15 weeks ($t = 2.655$, $df = 24$, $p < 0.02$).

Tilt-plane test. The results of the test of chronic tolerance to ethanol and the cross-tolerance tests with other alcohols and barbiturates on the tilting plane test are shown in Fig. 5.

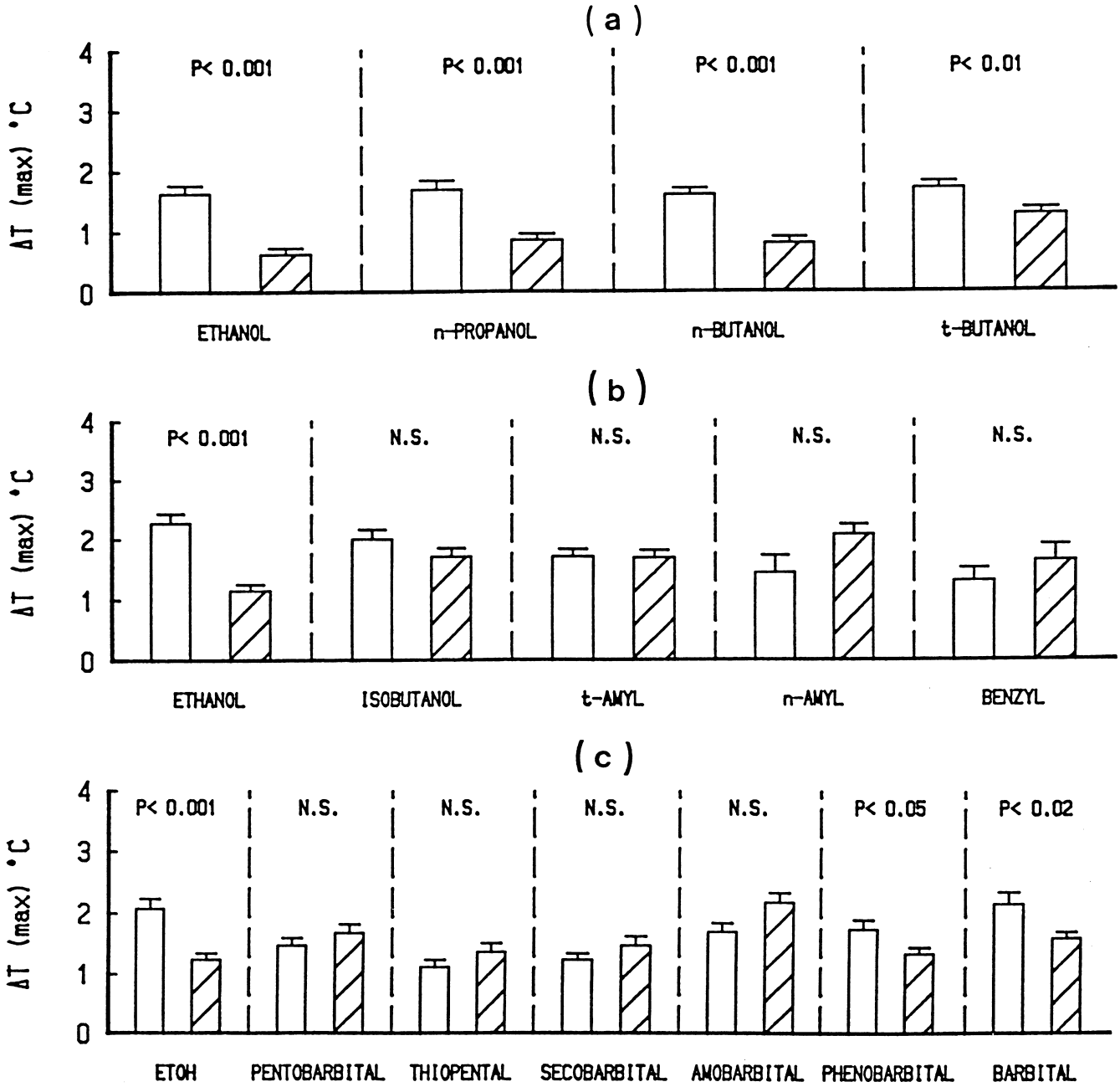

FIG. 4. Effect of chronic treatment by gastric intubation with ethanol (hatched bars) or sucrose (open bars) on the hypothermic response to ethanol and other drugs: (a) ethanol (2.2 g/kg), *n*-propanol (1 g/kg), *n*-butanol (0.4 g/kg) and *t*-butanol (0.65 g/kg); (b) ethanol (2.2 g/kg), isobutanol (0.4 g/kg), *t*-amyl alcohol (0.3 g/kg), *n*-amyl alcohol (0.32 g/kg) and benzyl alcohol (0.26 g/kg), and (c) ethanol (2.2 g/kg), pentobarbital (22 mg/kg), thiopental (28 mg/kg), secobarbital (22 mg/kg), amobarbital (48 mg/kg), phenobarbital (55 mg/kg) and barbital (100 mg/kg). Vertical lines indicate positive half of the standard error, with $n = 12$–13 animals per group. The significance of difference between groups was estimated by Student's *t*-test for unpaired data. NS = not significant, $p > 0.05$.

Comparison of the maximum percentage impairment by ethanol (2.5 g/kg) in the chronic ethanol-treated group with that in the sucrose control group at 3 weeks showed significant tolerance in the ethanol-treated group ($t = 4.99$, $df = 22$, $p < 0.001$). Chronic ethanol treatment also produced cross-tolerance to other short-chain alcohols (Fig. 5a): for *n*-propanol (1.1 g/kg) at 5 weeks ($t = 3.58$, $df = 22$, $p < 0.01$); *n*-butanol (0.45 g/kg) at 6.5 weeks ($t = 3.945$, $df = 22$, $p < 0.001$); and for *t*-butanol (0.75 g/kg) at 7.5 weeks ($t = 2.236$, $df = 22$, $p < 0.05$). Figure 5b shows the results of cross-tolerance tests with other alcohols with higher lipid:water partition coefficients. Significant cross-tolerance was seen only with isobutanol at 6 weeks (0.45 g/kg; $t = 4.34$, $df = 22$, $p < 0.001$). Cross-tolerance did not occur to *t*-amyl alcohol (0.32 g/kg; $t = 1.77$, $df = 22$, $p > 0.10$) at 7.5 weeks, *n*-amyl alcohol (0.38 g/kg; $t = 0.62$, $df = 22$, $p > 0.60$) at 9 weeks or to benzyl

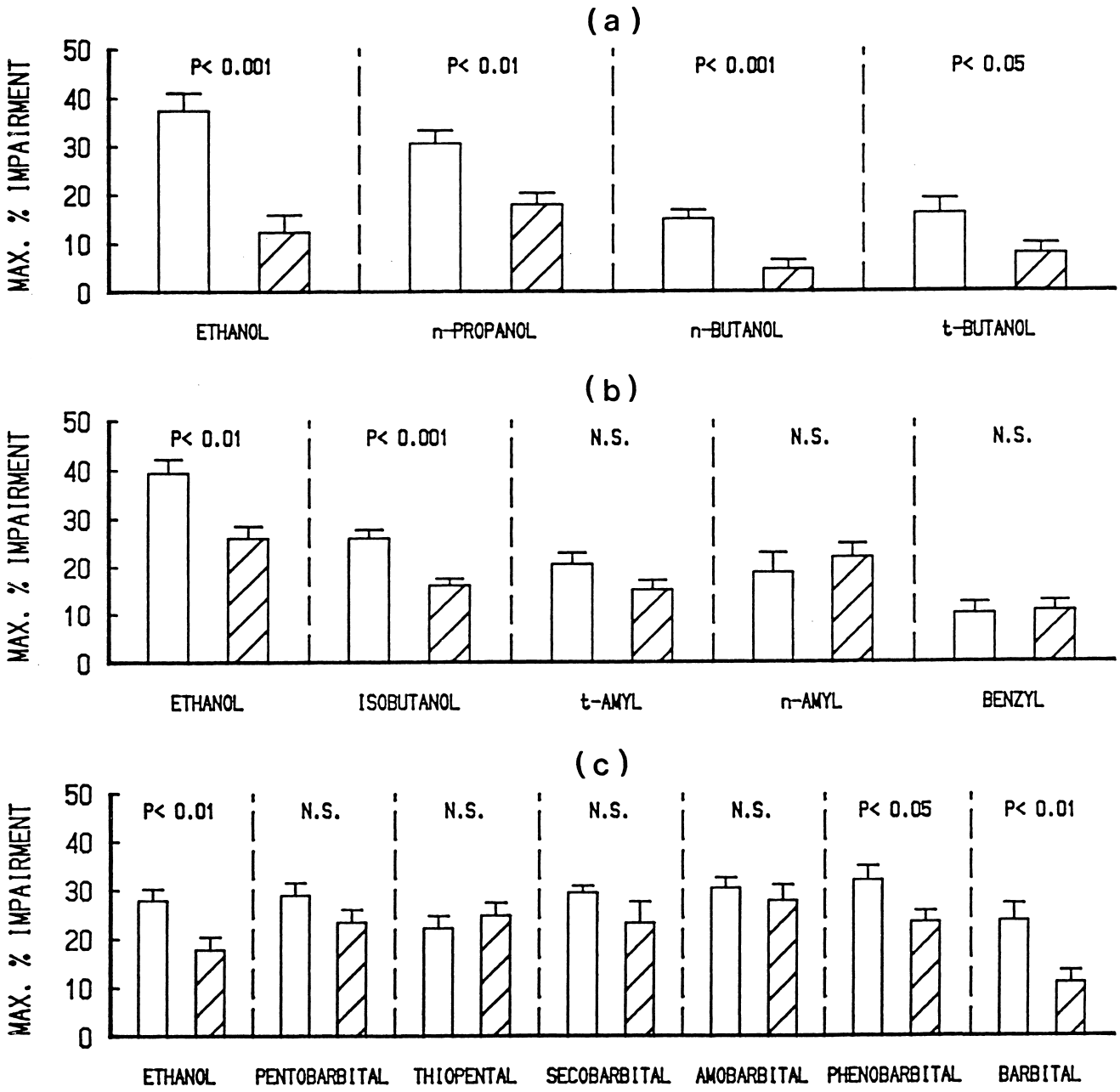

FIG. 5. Effect of chronic treatment by gastric intubation with ethanol (hatched bars) or sucrose (open bars) on impairment of tilt-plane test performance by: (a) ethanol (1.0 g/kg), *n*-propanol (0.35 g/kg), *n*-butanol (0.12 g/kg) and *t*-butanol (0.3 g/kg); (b) ethanol (1 g/kg), isobutanol (0.45 g/kg), *t*-amyl alcohol (0.32 g/kg), *n*-amyl alcohol (0.38 g/kg) and benzyl alcohol (0.45 g/kg) and (c) ethanol (1 g/kg), pentobarbital (25 mg/kg), phenobarbital (75 mg/kg), thiopental (30 mg/kg), secobarbital (23 mg/kg), amobarbital (38 mg/kg) and barbital (105 mg/kg). Vertical lines indicate positive half of the standard error, with $n = 12$–13 animals per group. The significance of difference between groups was estimated by Student's *t*-test for unpaired data. NS = not significant, $p > 0.05$.

alcohol (0.45 g/kg; $t = 0.156$, $df = 19$, $p > 0.90$) after 11 weeks of chronic ethanol treatment.

Fig. 5c shows the results of cross-tolerance tests to the motor-impairment effect of barbiturates after chronic ethanol treatment. Tolerance to ethanol after 11.5 weeks of chronic ethanol treatment was confirmed again ($t = 2.85$, $df = 21$, $p < 0.01$). Among the barbiturates tested, cross-tolerance was seen only with phenobarbital (75 mg/kg) at 10.5 weeks ($t = 2.36$, $df = 21$, $p < 0.05$) and with barbital (105 mg/kg) at 14.5 weeks ($t = 2.988$, $df = 24$, $p < 0.01$). Cross-tolerance did not occur to pentobarbital (25 mg/kg) at 9.5 weeks ($t = 1.49$, $df = 20$, $p > 0.10$), thiopental (30 mg/kg) at 17 weeks ($t = 0.727$, $df = 21$, $p > 0.50$), secobarbital (23 mg/kg) at 18 weeks ($t = 1.262$, $df = 20$, $p > 0.10$) or to amobarbital (38 mg/kg) at 19 weeks ($t = 0.69$, $df = 20$, $p > 0.50$). The data for pentobarbital and secobarbital were also subjected to a one-

TABLE 2

BLOOD LEVELS FOR VARIOUS ALCOHOLS (mg/dl) AND BARBITURATES (μg/ml) AT THE END OF TESTING, IN RATS TREATED CHRONICALLY WITH ETHANOL (E) OR SUCROSE (S)

Test Drug		Test Method (a) Hypothermia		Test Method (b) Tilt-Plane Test
(a) Alcohols				
Ethanol	E	164.1 ± 3.2**	E	203.7 ± 4.5**
	S	176.0 ± 2.5	S	220.1 ± 2.6
n-Propanol	E	91.6 ± 3.8	E	111.5 ± 5.2
	S	98.8 ± 4.0	S	119.9 ± 2.5
n-Butanol	E	2.8 ± 1.9*	E	4.1 ± 2.0*
	S	11.3 ± 1.3	S	13.5 ± 1.7
t-Butanol	E	97.4 ± 2.0	E	104.6 ± 1.0
	S	97.2 ± 1.6	S	107.4 ± 1.3
(b) Barbiturates				
Pentobarbital	E	1.57 ± 0.54	E	5.51 ± 0.43
	S	45.0 ± 0.77	S	5.10 ± 0.29
Phenobarbital	E	43.7 ± 1.6	E	70.3 ± 4.1
	S	45.0 ± 1.6	S	72.3 ± 3.1
Thiopental	E	11.5 ± 0.6	E	12.9 ± 0.8
	S	10.9 ± 0.7	S	13.5 ± 0.6
Secobarbital	E	2.63 ± 0.55	E	7.31 ± 0.56
	S	2.10 ± 0.50	S	7.69 ± 0.73
Amobarbital	E	8.85 ± 0.54	E	9.70 ± 0.98
	S	7.85 ± 0.58	S	9.29 ± 0.82
Barbital	E	71.7 ± 1.3	E	80.3 ± 1.2
	S	73.8 ± 1.0	S	81.7 ± 2.1

n = 11–12 animals per group.
*$p < 0.02$; **$p < 0.01$.

way ANOVA of results at all test times, and it did not show any cross-tolerance development for either pentobarbital ($F = 2.20$, $df = 1,20$, $p > 0.1540$) or secobarbital ($F = 1.83$, $df = 1,20$, $p > 0.192$).

Drug levels. The blood samples taken at the end of hypothermia and tilt-plane tests showed significantly lower alcohol levels in chronically ethanol-treated rats than in sucrose controls, for ethanol and *n*-butanol (Table 2). There was no difference in blood levels for *n*-propanol, *t*-butanol or for the various barbiturates (Table 2).

DISCUSSION

The results of this work indicate that chronic treatment with ethanol resulted in tolerance to ethanol on three different tests. The degree of tolerance to ethanol was roughly comparable in the three tests, but animals in the rotarod test took longer to develop tolerance than those in the other two tests. Since we used different batches of animals for different studies, we cannot tell whether the difference in time required to produce comparable levels of tolerance is due to differences between the various tests used, or differences in the batches of animals obtained.

It has been shown previously that tolerance to ethanol and other drugs has different characteristics, depending on whether the animals are or are not required to perform the tests repeatedly under the influence of the drug in question (12,13). The differentiation has been characterized by such terms as "behaviorally augmented" vs. "pharmacological" tolerance (20,21), "learned" vs. "non-learned" tolerance (29) and "environment-dependent" vs. "environment-independent" tolerance (28). While the question is not yet answered definitively, it is a reasonable hypothesis that these processes are brought about by different mechanisms, as reflected by the ability of NMDA receptor antagonists to prevent learned tolerance but not non-learned (15,27). The present work deals only with "non-learned" or "environment-independent" tolerance. The fact that intervals of at least 10 days were left between repeated tests in the same animals ensured that no carry-over of learning occurred from one test to another (20).

Blood ethanol levels in animals chronically treated with ethanol were slightly but significantly lower than those of controls. While this is consistent with reports of a metabolic component in ethanol tolerance (18) it is unlikely that metabolic changes contributed significantly to the tolerance observed in the present work. This is based on various studies which indicate that blood ethanol levels in ethanol-treated and control rats are not significantly different at 30–60 min, the time of peak effect for motor impairment and hypothermia. Moreover, we have previously argued that because tolerance can be demonstrated after intracerebroventricular injection of ethanol, tolerance on these tests was largely functional rather than metabolic in nature (8).

In a previous study (16) we observed that such tolerance to ethanol was accompanied by functional cross-tolerance to *n*-propanol and *t*-butanol, but not to *n*-butanol or pentobarbital. These results raised the possibility that lipid/water partition coefficient might be an important determinant of the production of cross-tolerance, but the range of compounds studied was small, and only a single test, the loss of righting reflex (LRR), was used. The present work confirms and extends the earlier findings, with drugs having a considerably wider range of lipid/water partition coefficients, and with several tests differing substantially from each other and from LRR. The results indicate that on all three tests there was cross-tolerance between ethanol and the relatively polar alcohols and barbiturates, but not between ethanol and the more non-polar compounds in both series. Since approximately equieffective doses of the various drugs were used, the difference can not reasonably be attributed to the choice of relative doses selected for the tests.

The one ambiguous result was that obtained with *n*-butanol, which showed cross-tolerance from ethanol on all three tests despite having a relatively high lipid/water partition coefficient. This may not reflect true functional cross-tolerance. Rather, it appears to be metabolic in origin, because the *n*-butanol blood levels in ethanol-treated animals are about one-fourth of those in their controls. In the case of other alcohols and barbiturates tested, the issue of pharmacodynamic vs dispositional tolerance is less important either because the treated and control groups showed the same drug concentrations, or because there was no difference in response in ethanol-treated and control groups.

Since the longer acting barbiturates (barbital and phenobarbital) were usually tested near the end of the study in order to avoid complications due to their very long half-life, it could be argued that cross-tolerance was seen with these drugs because it had more time to develop than with the short-acting ones. However, this is not true since no cross-tolerance was seen when some short-acting barbiturates were tested on the tilt-plane test between 17–19 weeks, yet phenobarbital and barbital showed clear cross-tolerance at 11 and 14.5 weeks respectively.

Previous studies from this laboratory have shown that when

tolerance to ethanol was produced by treatment procedures which involved learning, such as intoxicated practice and environmental conditioning (5,17), cross-tolerance to pentobarbital could be demonstrated. However, no or minimal cross-tolerance to pentobarbital occurred when animals were gavaged with ethanol daily in the animal quarters, even when they were tested repeatedly without drug at frequent intervals in the laboratory. Apparently, little or no tolerance-related learning takes place in the latter condition. Therefore, it is unlikely that the observed differences in cross-tolerance among various drugs are related to learning factors.

The mechanism by which lipid/water partition coefficient might influence the development of unlearned cross-tolerance is not yet clear. A plausible hypothesis is that the degree of polarity of the individual compounds produces relatively specific patterns of interaction with different lipid and protein constituents of cell membranes. If tolerance arises from adaptive changes in these constituents, cross-tolerance would be more probable between compounds with fairly similar degrees of polarity and hence with similar patterns of membrane interaction. This is consistent with the finding that tolerance to ethanol, a polar compound, is accompanied by cross-tolerance to other polar alcohols (up to *t*-butanol) and to the relatively polar barbiturates (barbital and phenobarbital), but not to the less polar compounds in either series. To test this hypothesis, it would be desirable to produce unlearned tolerance to a non-polar alcohol, and look for cross-tolerance to other non-polar compounds in both series, and lack of such cross-tolerance to the polar ones.

In this reasoning, the relevant expression of lipid/water partition coefficient would not appear to be the absolute value, but the relative one within each homologous series. Thus, functional cross-tolerance was seen from ethanol to phenobarbital (coefficient 1.42) but not to *t*-amyl alcohol (coefficient 0.89). This should not be regarded as a contradiction of the hypothesis, because a drug interacts with a wide range of different membrane constituents, for each of which there may be a different partition coefficient, but only a few of these may be involved in the adaptive changes giving rise to tolerance. For the same reason, there is no inherent contradiction between the finding of a single regression line for partition coefficient vs acute impairment by both alcohols and barbiturates (Fig. 2) and the apparent existence of separate regression lines for tolerance. The spectrum of membrane interactions for barbiturates is probably not identical to that for alcohols, but they may share a limited number related to cross-tolerance. If that is so, the relative value of the partition coefficient within each homologous series would be expected to be more important than the absolute value.

Two additional points must be emphasized. The first is that this hypothesis refers only to unlearned tolerance; learned tolerance may well have a different molecular basis. The second is that the effect of degree of polarity does not yet help to identify the site(s), such as $GABA_A$-linked chloride channel, NMDA-linked cation channel, adenosine-linked cAMP formation, or other specific molecular mechanisms that have been proposed as targets for ethanol action and tolerance. The exploration of those possibilities requires a quite different approach from that used in the present work.

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