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Rapid Tolerance as an Index of Chronic Tolerance

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KHANNA, J M , H. KALANT, G SHAH AND J WEINER *Rapid tolerance as an index of chronic tolerance* PHARMACOL BIOCHEM BEHAV **38**(2) 427–432, 1991 —Hypothermia and motor impairment (tilt-plane test) were used to assess the phenomenon of rapid cross-tolerance between ethanol and pentobarbital in rats The hypothermic and motor-impairment responses were significantly reduced on day 2 in animals receiving ethanol on day 1, compared to the control group pretreated with saline Ethanol pretreatment, however, did not result in rapid cross-tolerance to pentobarbital on either test. Pentobarbital pretreatment on day 1 resulted in rapid tolerance to pentobarbital on day 2 However, in contrast to the lack of rapid cross-tolerance to pentobarbital after pretreatment with ethanol, pentobarbital pretreatment clearly conferred rapid cross-tolerance to ethanol Determination of ethanol and pentobarbital blood levels suggested that pharmacokinetic alterations did not contribute significantly to the observed rapid tolerance and cross-tolerance The asymmetry of rapid cross-tolerance seen in these studies mimics the results obtained by us in chronic tolerance and cross-tolerance studies reported recently These results suggest that rapid tolerance and cross-tolerance can be used as predictors of chronic tolerance and cross-tolerance

Tolerance Cross-tolerance Rapid Ethanol Pentobarbital Rat

MOST studies of acquired tolerance to alcohol and other drugs, and of cross-tolerance between them, have employed a paradigm that is usually referred to as chronic tolerance and cross-tolerance. The tolerance and cross-tolerance develop gradually and reach their maximum levels after several days or weeks of repeated or continuous administration of the drug. Both dispositional and functional components may contribute to the chronic tolerance and cross-tolerance (7). However, Crabbe et al. (3) described another model of tolerance that they designated as rapid tolerance. In this model, animals are given a drug on 2 consecutive days, and tolerance is inferred from the diminished response to the drug on day 2. Chan et al. (2) used a similar paradigm to study cross-tolerance, by giving ethanol on day 1 and chlordiazepoxide on day 2. This type of tolerance and cross-tolerance is primarily functional and does not include a significant dispositional component (2,3).

The nature of the relationship between rapid and chronic tolerance and cross-tolerance is not clear. In a recent study, Chan et al. (2) reported that mice exposed to a single acute dose of ethanol showed rapid cross-tolerance to chlordiazepoxide 24 hours later. Moreover, the extent of rapid cross-tolerance to chlordiazepoxide was similar to that of cross-tolerance to chlordiazepoxide in mice receiving ethanol chronically in a liquid diet for 15 days. Although this finding would suggest some similarity between chronic and rapid cross-tolerance, further experiments are necessary to explore the relationship between these two types of tolerance and cross-tolerance

In this paper, we have therefore examined rapid cross-tolerance between ethanol and pentobarbital in both directions, i.e., rapid cross-tolerance to pentobarbital after pretreatment with ethanol and rapid cross-tolerance to ethanol after pretreatment with pentobarbital. Tolerance and cross-tolerance were measured on two different tests, i.e., hypothermia and motor impairment (tilt plane). The major aim of these studies was to compare the results on rapid cross-tolerance with the results obtained recently on such investigations in a chronic model of tolerance and cross-tolerance (4,8).

METHOD

Animals

Male Sprague-Dawley rats were obtained from Charles River Canada, Ltd. (Montréal, Quebec) at initial body weights of 150–200 g. They were individually housed in a colony room maintained at 21 ± 1°C with lights on at 7 a.m. and off at 7 p.m. Water was available at all times. Purina Rat Chow was given ad lib until body weights reached 200–250 g. Thereafter, the daily ration was restricted and individually adjusted to maintain comparable body weights in the various groups

Drugs

Drugs used were 95% (w/v) ethanol and sodium pentobarbital

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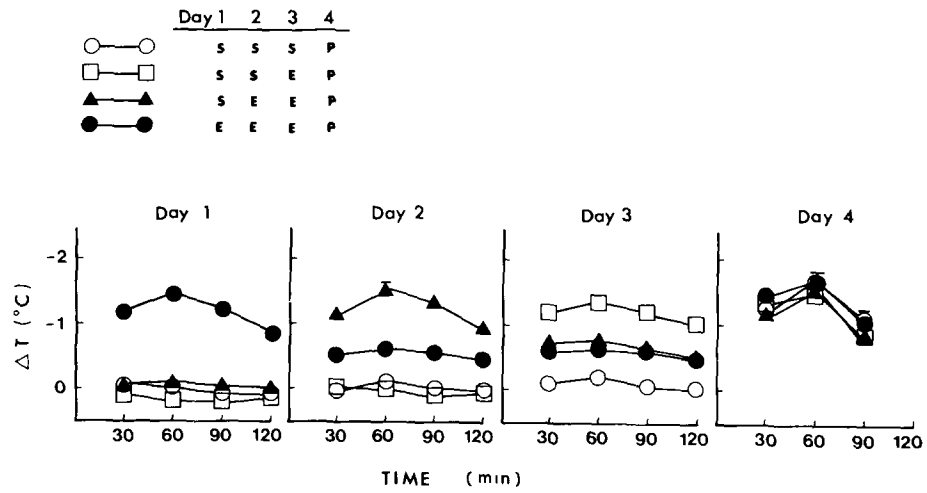

FIG 1 Hypothermic response (ΔT °C) to ethanol or pentobarbital assessed every 30 min in rats pretreated with ethanol or saline for 1, 2 and 3 days Group SSSP (○) received saline on days 1, 2 and 3 and pentobarbital on day 4 Group SSEP (□) received saline on days 1 and 2, ethanol on day 3 and pentobarbital on day 4 Group SEEP (▲) received saline on day 1, ethanol on days 2 and 3 and pentobarbital on day 4 Group EEEP (●) received ethanol on days 1, 2 and 3 and pentobarbital on day 4 Values shown are means ± SEM Only the largest SEM is shown Where no value is seen, it is within the symbol N = 18 animals per group

(BDH). All drug solutions were prepared in isotonic saline on the day they were used

Test Procedure

Hypothermia. A 5-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 prior to and at successive 30-min intervals after the intraperitoneal test injection until the temperature began to return to normal. This generally occurred about 120 min after injection of ethanol and 90 min after injection of pentobarbital.

Motor impairment. The tilting-plane test was used as a measure of motor impairment (1,4). The apparatus consists of a plane which can be inclined 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 and 90 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 for the same animal.

Drug Analysis

Blood samples (50 μl for ethanol measurement, 100 μl for pentobarbital) were taken in some experiments from the rat's tail tip immediately after the last measurement of temperature or motor impairment. This occurred about 120 min after injection of ethanol and 90 min after injection of pentobarbital. Blood ethanol was analyzed by the enzymatic method described previously (5) Pentobarbital was analyzed by gas-liquid chromatography, by an on-column methylation procedure (9)

Experimental Procedure

Rapid tolerance to ethanol and cross-tolerance to pentobarbital (hypothermia test). Rats were randomly divided into three separate groups of 24 rats. Each group of 24 rats was further subdivided into 4 subgroups of 6 animals each. The rats in each of the 4 subgroups received the appropriate saline or drug treatment according to the schedule outline in Fig. 1, in which S refers to saline, E to ethanol and P to pentobarbital. Thus on day 1 the first 3 groups received saline and the fourth group received ethanol. On day 2, the first two groups received saline and the remaining two received ethanol. This allowed us to compare tolerance to ethanol on day 2 as a result of prior administration of ethanol or saline, 24 h earlier On day 3, the first group received saline and the other 3 groups received ethanol. Measurement of responses on this day allowed us to replicate the results found on day 2, and to determine the effect of one additional injection of ethanol on further development of tolerance On day 4, all animals received pentobarbital. Cross-tolerance to pentobarbital (20 mg/kg) was studied by comparing the effect of pentobarbital in groups that had received one or more doses of ethanol previously with that in a group which had received only saline previously.

On each day, the group of 24 animals was brought upstairs to the laboratory from the animal colony. They were weighed, and approximately fifteen minutes later their body temperature was recorded. This time interval was allowed to minimize the effects of any excitement or arousal due to transportation. They then received their appropriate treatment, i.e., saline or ethanol (2 g/kg IP), and their body temperature was again measured as described above A second IP injection of ethanol (2 g/kg) or saline was given at 120 min, immediately after the last temperature measurement. This procedure of giving ethanol in 2 doses of 2 g/kg each, rather than as one single dose of 4 g/kg, was employed because preliminary experiments had shown that (a) a total dose of 3–4 g/kg on day 1 is needed to produce reliably the rapid tolerance on

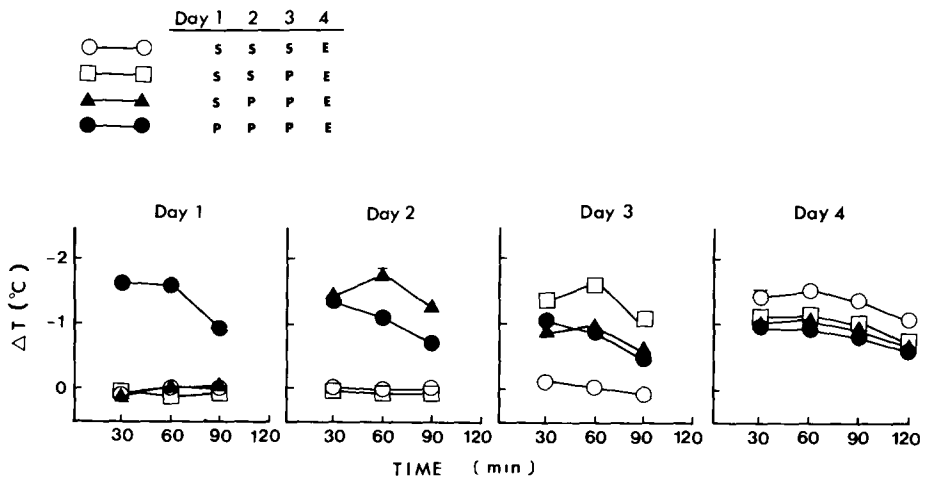

FIG 2 Hypothermic response to pentobarbital or ethanol assessed every 30 min in rats pretreated with pentobarbital or saline for 1, 2 and 3 days Group SSSE (○) received saline on days 1, 2 and 3 and ethanol on day 4 Group SSPE (□) received saline on days 1 and 2, pentobarbital on day 3 and ethanol on day 4 Group SPPE (▲) received saline on day 1, pentobarbital on days 2 and 3 and ethanol on day 4 Group PPPE (●) received pentobarbital on days 1, 2 and 3 and ethanol on day 4 N = 17 to 18 animals per group Values shown are means ± SEM Only the largest SEM is shown Where no value is seen, it is within the symbol

day 2, and (b) doses of ethanol greater than 2 g/kg may not always fall in the linear part of the dose-response curve Therefore the initial test was done with 2 g/kg and the supplementary dose at the end of the test was given to bring the total dose up to the level required to produce rapid tolerance Since only 24 animals could be tested in one day, this experiment was repeated under identical conditions with each of the two remaining groups of 24 animals. The results from the three separate groups were pooled for a two-way repeated measures analysis of variance.

Rapid tolerance to pentobarbital and cross-tolerance to ethanol (hypothermia test). The design of these studies was very similar to the one described above except that pentobarbital was used in place of ethanol on days 1, 2 and 3 (see Fig. 2). The testing dose of pentobarbital for the hypothermia study was 20 mg/kg IP, whereas the extra dose given at the end of the test was 40 mg/kg IP. Therefore, the total dose of pentobarbital on days 1, 2 or 3 was 60 mg/kg. On day 4, the whole group was tested with ethanol (2 g/kg IP). The rectal temperature was recorded before and at 30′, 60′, 90′ and 120′ after the IP injection.

Rapid tolerance to ethanol and cross-tolerance to pentobarbital (tilt-plane test). The experimental procedure for these studies was similar to that described above, except that measurements were made on only two consecutive days, because the results of the hypothermia study indicated that the degree of rapid tolerance did not change after additional treatment days.

On day 1, half of the group received IP ethanol (2.3 g/kg) and the other half was injected IP with saline. Before and at 30, 60, 90 and 120 min after ethanol or saline injections, the tilt-plane performance was measured. Immediately after the last tilt-plane test, the rats received a supplementary dose of ethanol (1.7 g/kg) or saline respectively Rats were then returned to their home cages. On day 2, an identical procedure was followed except that all animals received ethanol (2 3 g/kg). For testing cross-tolerance, pentobarbital (23 mg/kg) was administered on day 2 instead of ethanol.

Rapid tolerance to pentobarbital and cross-tolerance to ethanol (tilt-plane test). For these studies, pentobarbital (23 mg/kg) or saline was given by IP injection on day 1. After the last tilt-plane test on day 1, a second IP dose of pentobarbital (37 mg/kg) or saline, respectively, was given. On day 2, pentobarbital (23 mg/kg) was administered again to all animals for studies on tolerance testing. In studies on cross-tolerance, ethanol (2 3 g/kg IP) was given to all animals on day 2.

RESULTS

Rapid Tolerance to Ethanol and Cross-Tolerance to Pentobarbital (Hypothermia)

The results from 3 separate experiments were pooled as there was no significant effect of replications. Analysis of variance showed no significant change in baseline temperatures across days in any of the treatment groups. On day 1, rats injected with ethanol showed the expected hypothermia. On day 2, these animals (EE group) showed a significantly, $F(1,34) = 22.58$, $p < 0.001$, smaller hypothermic response to ethanol (Fig. 1) when compared to rats which had received saline on day 1 (SE group). On day 3, the extent of tolerance did not increase further with one additional ethanol injection, i.e., group EEE did not differ significantly from group SEE, $F(1,34) = 0.53$, $p > 0.47$. However, this latter group was significantly different from SSE, $F(1,34) = 16.53$, $p < 0.001$, corroborating the difference between SE and EE groups on day 2. Comparison between groups SSE and EEE showed significant group effect, $F(1,34) = 26.26$, $p < 0.001$. The absence of a significant interaction between time × group suggests that the time course of hypothermia was similar for both groups, $F(3,102) = 0.62$, $p > 0.603$. These results indicate a rapid development of tolerance to ethanol on the day following the two initial ethanol injections, and further administration of ethanol on day 3 did not increase the tolerance seen after the second day's injections.

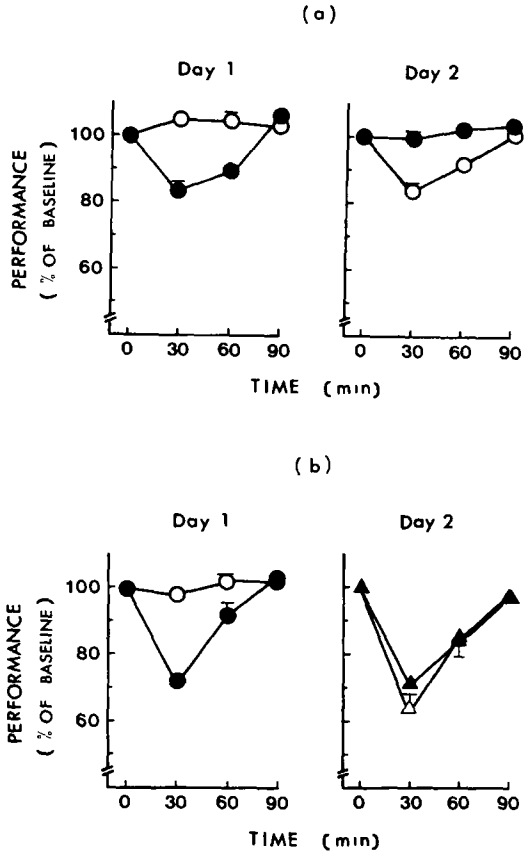

FIG 3. (a) Percentage performance to ethanol on the tilt-plane test assessed every 30 min in EE rats treated with ethanol on both days (●), compared to the SE group (○) which received saline on day 1 and ethanol on day 2 N = 10 to 13 animals per group Values shown are means ± SEM Only the largest SEM is shown Where no value is seen, it is within the symbol (b) Percentage performance to pentobarbital on the tilt-plane test assessed every 30 min in EP rats (▲) treated with ethanol on day 1 and pentobarbital on day 2 compared to the SP group (△) which received saline on day 1 and pentobarbital on day 2. N = 10 to 13 animals per group Values shown are means ± SEM. Only the largest SEM is shown Where no value is seen, it is within the symbol

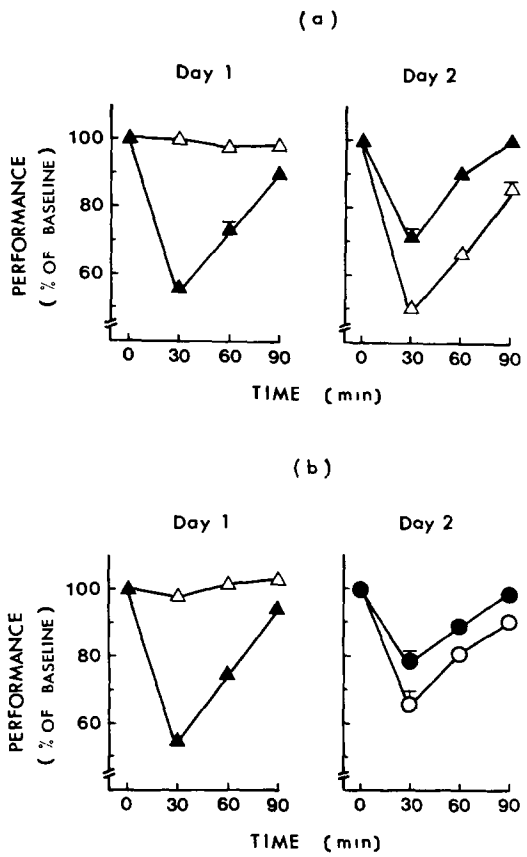

FIG 4 (a) Percentage performance to pentobarbital on the tilt-plane test assessed every 30 min in PP rats (▲) treated with pentobarbital on both days compared to the SP group (△) which received saline on day 1 and pentobarbital on day 2 N = 10 to 13 animals per group Values shown are means ± SEM Only the largest SEM is shown Where no value is seen, it is within the symbol (b) Percentage performance to ethanol on the tilt-plane test assessed every 30 min in PE rats (●) treated with pentobarbital on day 1 and ethanol on day 2 compared to the SE group (○) which received saline on day 1 and ethanol on day 2 N = 10 to 13 animals per group Values shown are means ± SEM Only the largest SEM is shown Where no value is seen, it is within the symbol

Cross-tolerance to pentobarbital, tested on day 4, was not seen after either one, two or three days with two ethanol injections per day. The hypothermic response was identical in all groups.

Rapid Tolerance to Pentobarbital and Cross-Tolerance to Ethanol (Hypothermia)

Rapid tolerance to pentobarbital following pentobarbital pretreatment 24 h earlier was also observed [Fig. 2, day 2 comparison of SP and PP groups $F(1,34) = 16.19$, $p < 0.003$]. Groups SSP and SPP were also significantly different on day 3, $F(1,34) = 16.34$, $p < 0.03$, corroborating the difference between SP and PP groups on day 2. Groups SPP and PPP were, however, not significantly different, $F(1,34) = 0.05$, $p > 0.829$, from each other, i.e., there was no further increase in tolerance with an additional pentobarbital injection. Cross-tolerance to ethanol on day 4, after 1–3 days of pentobarbital pretreatment, was clearly significant [for comparison of SSSE vs. SSPE: $F(1,33) = 5.67$, $p < 0.02$]. Moreover, the extent of cross-tolerance to ethanol after pentobarbital pretreatment was indistinguishable among the three groups receiving either one, two or three prior injections of pentobarbital.

Rapid Tolerance to Ethanol and Cross-Tolerance to Pentobarbital (Tilt-Plane Test)

The results of this experiment are shown in Fig. 3. Rats injected with ethanol on both days (EE) showed significantly, $t(24) = 3.57$, $p < 0.005$, less motor-impairing effect of ethanol on day 2 than those injected with saline 24 h earlier (SE) (Fig. 3a). Thus a single prior exposure to ethanol resulted in a rapid development of tolerance to the motor-impairment effects of ethanol [30 min. $t(24) = 4.0$, $p < 0.001$; 60 min. $t(24) = 3.63$, $p < 0.005$] Comparison of maximum percentage impairment between SP and EP groups on day 2 showed no significant difference, $t(18) = 1.53$, $p > 0.02$ (Fig. 3b) Similarly, no significant differences were obtained between SP and EP groups when the experimental data were subjected to two-way analysis of variance for repeated mea-

sures over all time points, $F(1,18)=0.41$, $p>0.53$.

Rapid Tolerance to Pentobarbital and Cross-Tolerance to Ethanol (Tilt-Plane Test)

The results of this experiment are shown in Fig. 4. Comparison of maximum percentage impairments between SP and PP groups on day 2 indicate clearly a rapid tolerance development to the maximum motor-impairing effect of pentobarbital, $t(24)=5.85$, $p<0.001$. Rapid tolerance was also evident at all time intervals [30 min. $t(24)=5\ 85$, $p<0.001$, 60 min· $t(24)=6.88$, $p<0.001$; 90 min. $t(24)=4.94$, $p<0.001$]. Similarly, pentobarbital-treated rats (PE) injected with ethanol (2.3 g/kg) on day 2. when compared to SE rats which received ethanol injection 24 h after a saline injection, showed a significant cross-tolerance to ethanol [$t(18)=2.084$, $p<0.002$, for maximum percentage impairment]. Cross-tolerance was also evident at all time intervals [30 min· $t(18)=2.77$, $p<0.02$; 60 min $t(18)=2.18$, $p<0.05$; 90 min· $t(18)=2\ 74$, $p<0.02$].

Ethanol and Pentobarbital Blood Levels

Blood ethanol and pentobarbital levels taken at the end of temperature and motor-impairment measurements on day 2 are shown in Fig. 5. There was no significant difference in either ethanol or pentobarbital levels in animals which had received either ethanol or saline on the previous day. Similarly, pentobarbital pretreatment 24 h earlier did not affect pentobarbital or ethanol levels in pentobarbital pretreated groups compared to control groups treated with saline 24 h earlier.

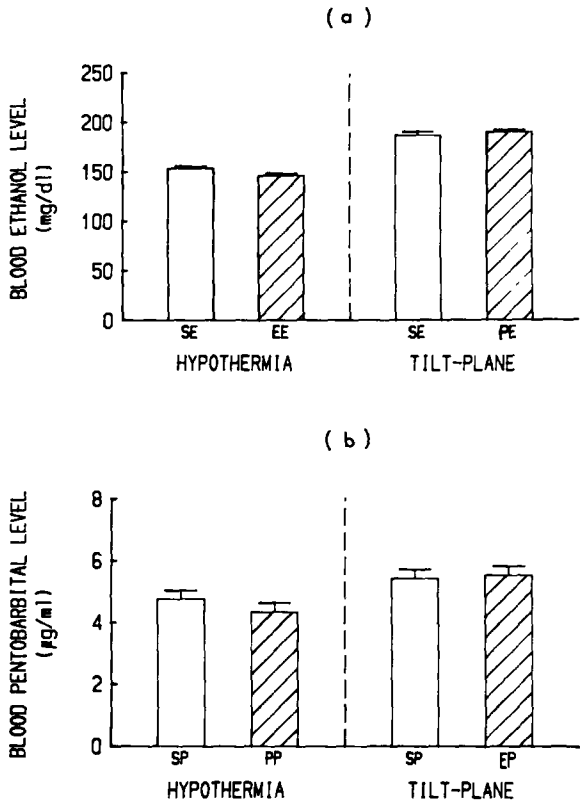

FIG 5 (a) Blood ethanol concentration in hypothermia and tilt-plane tests at the end of rapid tolerance test on day 2 in rats pretreated on day 1 with ethanol or pentobarbital and their respective saline controls Cross-hatched bars, EE animals pretreated with ethanol on day 1 and tested with ethanol on day 2, PE animals pretreated with pentobarbital on day 1 and tested with ethanol on day 2, plain bars, SE their respective control groups pretreated with saline on day 1 and tested with ethanol on day 2 Vertical lines indicate standard errors with N = 10 to 13 animals per group (b) Blood pentobarbital concentration in hypothermia and tilt-plane tests at the end of rapid tolerance test on day 2 in rats pretreated on day 1 with pentobarbital or ethanol and their respective saline controls Cross-hatched bars, PP animals pretreated with pentobarbital on day 1 and tested with pentobarbital on day 2, EP animals pretreated with ethanol on day 1 and tested with pentobarbital on day 2, plain bars, SP their respective control groups pretreated with saline on day 1 and tested with pentobarbital on day 2 Vertical lines indicate standard errors with N = 10 to 13 animals per group.

DISCUSSION

Tolerance to the hypothermic effect of ethanol was compared on day 2 in animals receiving either ethanol or saline 24 and 22 h earlier. The results clearly showed a lesser hypothermic response in animals pretreated with ethanol than in those pretreated with saline. Tolerance was maximal by day 2; additional administration of ethanol on day 2 did not increase the tolerance measured on day 3. These studies confirm and extend the observations on rapid tolerance first reported by Crabbe et al. (3) in mice. Similarly, we also did not find any changes in blood ethanol levels after the test on day 2. This latter finding does not entirely exclude a dispositional component in the observed tolerance, because the drug levels were measured at the end of the trials rather than at the time of maximum drug effect. Therefore it is conceivable that differences in absorption and distribution at early times after injection might have played a role.

Our experimental protocol was not exactly identical to that of Crabbe et al (3). We administered ethanol in 2 doses of 2 g/kg each on day 1, whereas Crabbe et al. (3) gave the entire dose on day 1 as a single administration. This change was made because in preliminary studies, we found that doses greater than 2 g/kg did not always give effects falling on the linear portion of the dose-response curve. Since we did not want to disregard any differences in the extent of tolerance compared across days as well as within days, we wanted to keep the same test dose on both days. However, in another study in which ethanol was given as a single dose (4 g/kg) rather than in 2 doses of 2 g/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 two doses (data not shown)

In agreement with our recent studies on lack of cross-tolerance to pentobarbital in a chronic model of alcohol tolerance (4), we also did not find any rapid cross-tolerance to pentobarbital hypothermia in animals pretreated with ethanol. However, rapid cross-tolerance to ethanol hypothermia in animals pretreated 24 and 22 h earlier with pentobarbital was seen. There was no difference in blood ethanol levels in animals pretreated with pentobarbital compared to saline-treated controls, so that the cross-tolerance did not appear to have a pharmacokinetic basis. However, the same reservation must be made as that noted above in the case of rapid tolerance to ethanol itself.

The results obtained with the tilt-plane test were essentially similar to those seen with the hypothermia test. Only a two-day design was used for motor-impairment studies because the studies with the hypothermia test had revealed that additional administration of ethanol did not further enhance the development of tolerance.

The test doses of ethanol and pentobarbital used in this study were based on previous and other ongoing studies in this labora-

tory which suggest an approximate potency ratio of ethanol to pentobarbital of 1 100 A higher ratio of treatment dose of pentobarbital (60 mg) than of ethanol (4 g/kg) was given in order to compensate for the shorter half-life of pentobarbital.

The similarity in results on rapid tolerance to those reported in models of chronic tolerance in two different tests, and in both directions, i.e., lack of tolerance to pentobarbital after ethanol pretreatment and clear evidence of tolerance to ethanol after pentobarbital pretreatment (4,8), further strengthens the earlier conclusion concerning the asymmetry of cross-tolerance. These results also suggest that rapid tolerance may be a proxy for chronic tolerance, though they do not permit any conclusion as to whether or not the two processes are identical. In other studies, Chan et al. (2) reported a similar degree of cross-tolerance to chlordiazepoxide in mice pretreated with ethanol 24 h earlier compared to mice chronically treated with ethanol on a liquid diet for 15 days. If other manipulations such as protein synthesis inhibitors, neurotransmitters modifications, etc., which are known to affect chronic tolerance, affect rapid tolerance in a similar manner, rapid tolerance may prove to be a useful, inexpensive and rapid tool to examine tolerance in general.

As in the case of chronic cross-tolerance, the asymmetry of rapid cross-tolerance between ethanol and pentobarbital cannot yet be explained. One possible explanation suggested previously (8) is that the actions of ethanol responsible for the effects measured here are a subset of a larger range of actions exerted by pentobarbital Thus pentobarbital treatment might generate a stronger stimulus to the development of cross-tolerance to ethanol than vice versa. However, this remains purely a conjecture at present.

Several investigators have indicated that tolerance to ethanol and other drugs is influenced by various behavioral factors such as practice under the influence of the drug, variation of the test system and conditional influences of environmental cues [for references, see (6)]. Practice under the influence of ethanol has already been shown to be an important factor in the production of rapid tolerance (10). Further study of the effects of behavioral, environmental and temporal factors on the expression of rapid tolerance would be a useful pursuit. Such studies are in progress.

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