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Tolerance to Ethanol and Cross-Tolerance to Pentobarbital and Barbital

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GOUGOS, A , J M KHANNA, A D LÊ AND H KALANT *Tolerance to ethanol and cross-tolerance to pentobarbital and barbital* PHARMACOL BIOCHEM BEHAV 24(4) 801–807, 1986 —A chronic regimen of ethanol by intubation, which produced clear tolerance to ethanol-induced hypothermia, ataxia and narcosis, produced only a marginal degree of cross-tolerance to these effects of pentobarbital The lack of appreciable cross-tolerance to pentobarbital-induced hypothermia and ataxia was also observed over a wide range of test doses However, cross-tolerance to barbital was observed after chronic treatment with ethanol Increased rate of drug biotransformation did not contribute significantly to the observed tolerance and cross-tolerance The difference in the extent of cross-tolerance between ethanol and the two barbiturates is consistent with the hypothesis that there is a degree of specificity in the sites of action of ethanol and other sedative-hypnotic drugs

Ethanol Pentobarbital Barbital Tolerance Cross-tolerance

ON the basis of existing theories of tolerance, one would predict the development of cross-tolerance between ethanol and barbiturates However, a review of the clinical and experimental literature on cross-tolerance [12] reveals data which are not entirely consistent with this prediction Although there is some supporting evidence, various investigators have reported a lack of cross-tolerance to pentobarbital in ethanol-tolerant subjects [5, 18, 24].

The diversity in treatment regimen, time of testing, test system, strain, species and sex, or shortcomings in experimental design, do not permit firm conclusions about the possible existence of cross-tolerance Moreover, in some studies it was difficult to determine or dismiss the contribution of dispositional changes to the reported cross-tolerance development [1, 4, 9, 10, 14], and in others it was not established that tolerance to ethanol was present prior to, or concomitantly with, cross-tolerance to the barbiturate [19, 22, 23]. In most studies, tests were done at a single time point, implying the unproven assumption that tolerance and cross-tolerance develop at a similar rate and to the same extent The single test may have been done after a period of treatment which was not long enough for the full development of tolerance to occur [5]. Further complicating the literature is the inconsistency of the duration of chronic treatment from study to study, and the diversity of methods of testing for cross-tolerance. In all of the work reviewed, there was a notable absence of appropriate dose-response experiments

The present experiments were designed to address these various problems. The development of tolerance to, and cross-tolerance between, ethanol and pentobarbital was examined by testing repeatedly over an extended period of high-dose treatment Cross-tolerance to a second barbiturate, barbital, was also assessed following chronic treatment with ethanol. Three different tests were used to measure tolerance and cross-tolerance development, and the concentrations of the test drugs in the blood were determined during each test session. Furthermore, the effect of varying the test dose on cross-tolerance development was also assessed.

METHOD

Animals

Male Sprague-Dawley rats were obtained from Taconic Farms at initial body weights of 150–200 g. They were individually housed in a colony room maintained at 21 ± 1°C with a cycle of 12 hr light, 12 hr darkness. 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, sodium pentobarbital

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(BDH) and sodium barbital (BDH). All drugs were prepared in isotonic saline on the day they were used

Chronic Ethanol Treatment

An initial dose of 3 g/kg/day was administered each morning by intubation and increased by 0 5 g/kg every 5 days to a maximum of 5 g/kg/day At this point an afternoon dose of 2 g/kg was added, and increased by 0.5 g/kg every 8 days until the total 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

Test Procedures

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

Hypothermia A 4 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 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 or barbital and 90 min after injection of pentobarbital The hypothermic effect was quantified as the maximal drop in temperature over this time period.

Motor impairment The tilting plane test was used as a measure of motor impairment [2] 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 prior to and at 30, 60 and 90 min after the injection of the drug The degree of post-drug ataxia was expressed as the percentage change in the sliding angle, compared to the pre-drug value. Maximum impairment, regardless of the time of its occurrence, was employed as the measure of the drug's effect

Sleeping time The sleep onset time was defined as the time interval between injection and loss of the righting reflex The sleeping time was defined as the time elapsed between loss and recovery of the righting reflex The criterion for recovery of the righting reflex was the ability of the rat to right itself 3 times within 30 sec

Drug Analysis

Blood ethanol was analyzed by the enzymatic method described previously [6]. Barbiturates were analyzed by gas-liquid chromatography, by an on-column methylation procedure, described recently [11]

EXPERIMENT I: THE EFFECT OF CHRONIC ETHANOL TREATMENT BY GASTRIC INTUBATION ON THE DEVELOPMENT OF TOLERANCE TO ETHANOL AND CROSS-TOLERANCE TO PENTOBARBITAL AND BARBITAL

In Experiment I, three groups of rats (n=20 each) were tested for their hypothermic and motor impairment responses to either ethanol, pentobarbital or barbital Each group was then subdivided into two subgroups matched with respect to their maximum hypothermic and motor impairment responses One subgroup was designated to serve as the chronically treated group and the other as the control Treated animals received ethanol by gastric intubation for approximately 70 days and control animals received equicaloric doses of sucrose solution At 10-day intervals for the first 50 days of the treatment period, one ethanol and one sucrose group were tested for tolerance to the hypothermic and motor-impairing effects of ethanol, while the remaining groups were tested for cross-tolerance to these same effects of either pentobarbital or barbital Equipotent test doses of ethanol (2 6 g/kg), pentobarbital (26 mg/kg) and barbital (142 mg/kg) had been determined previously for these groups by the use of dose-response curves Tail blood samples were taken immediately after the last motor impairment measurement and stored at 4°C until analysis was done The duration of sleep induced by ethanol (3.5 g/kg) and pentobarbital (40 mg/kg) was measured on day 63 of treatment, while barbital (200 mg/kg) sleep was measured on day 74 Tail blood samples were taken upon recovery of the righting reflex

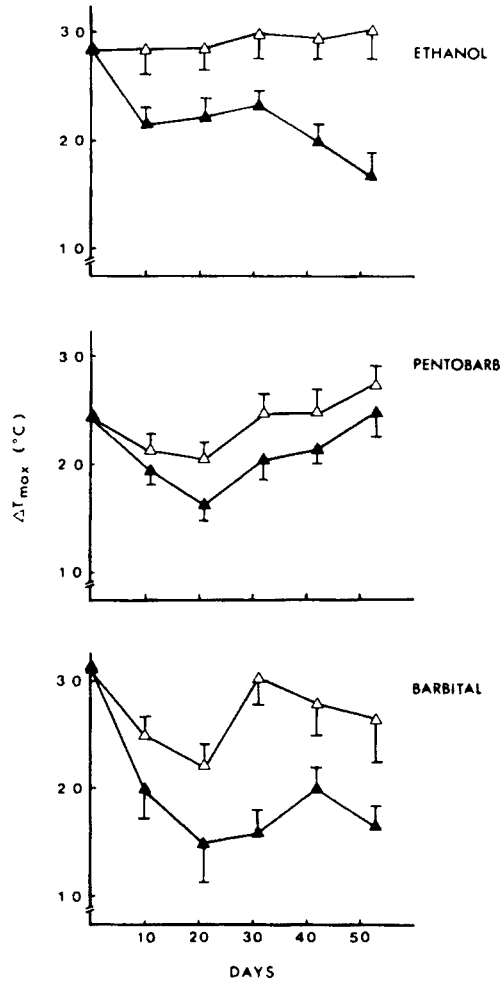

FIG 1 The effect of chronic ethanol treatment by gastric intubation, on the hypothermic response to ethanol, pentobarbital or barbital Three groups of rats were tested approximately every 10 days with either 2 6 g/kg IP of ethanol, 26 mg/kg IP of pentobarbital or 142 mg/kg IP barbital Chronic ethanol (▲) versus equicaloric sucrose (△) Results shown are means±S E M with n=8–10 animals per group

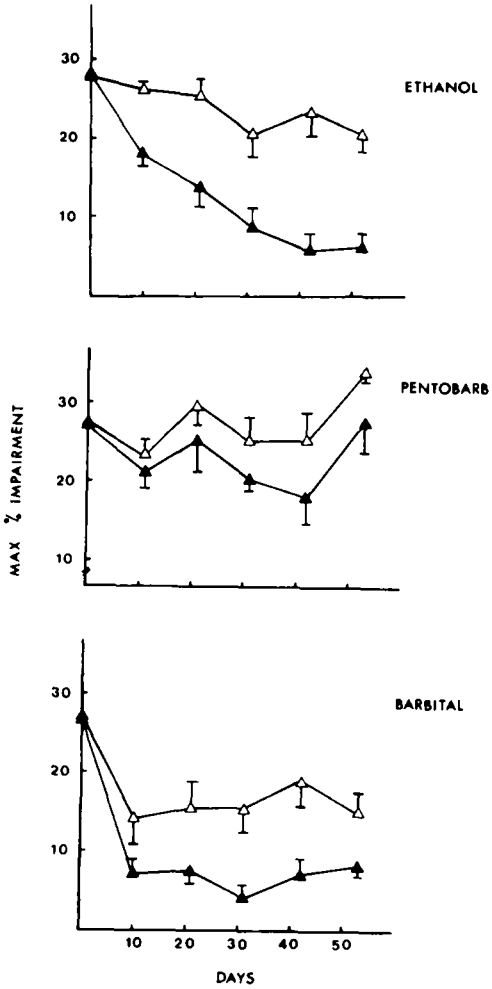

FIG 2 The effect of chronic ethanol treatment by gastric intubation, on motor impairment induced by ethanol, pentobarbital or barbital as measured by the tilting plane test Three groups of rats were tested approximately every 10 days with either 2 6 g/kg IP of ethanol, 26 mg/kg IP of pentobarbital or 142 mg/kg IP barbital Chronic ethanol (▲) versus equicaloric sucrose (△) Results shown are means±S E M with n=8–10 animals per group

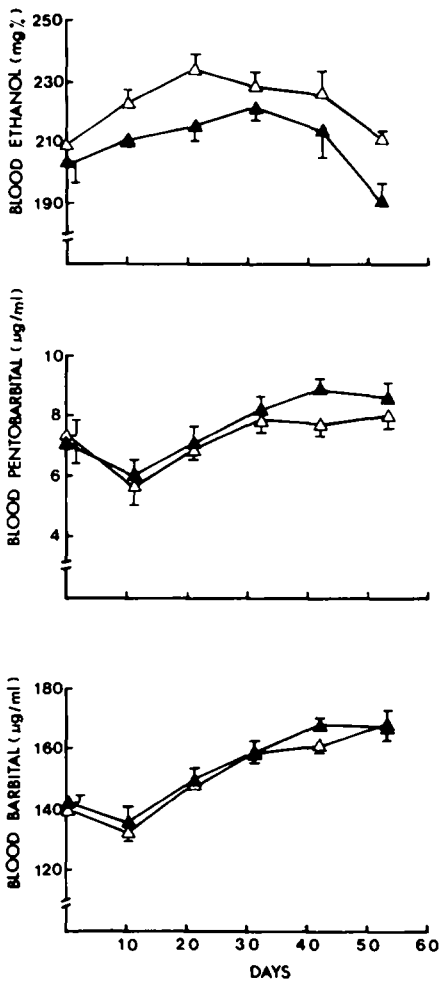

FIG 3 The concentration of ethanol, pentobarbital or barbital in the blood of rats treated chronically with ethanol or sucrose by gastric intubation Samples were taken at the end of each test session (90 min after IP injection of 26 mg/kg pentobarbital and 120 min after injection of 2 6 g/kg of ethanol or 142 mg/kg of barbital) Chronic ethanol (▲) versus equicaloric sucrose (△) Results shown are means±S E M with n=8–10 animals per group

EXPERIMENT II: DOSE-RESPONSE STUDY OF TOLERANCE TO ETHANOL AND CROSS-TOLERANCE TO PENTOBARBITAL

Two groups of rats (n=32 each) received either ethanol or isocaloric sucrose by gastric intubation for approximately 26 days. On day 18, one half of each group was randomly divided into 4 groups (n=4), each one receiving a different test dose of ethanol (1.9, 2.3, 2.7, 3 1 g/kg). Tolerance to the hypothermic and motor-impairing effect of ethanol was then measured On the next day, the other half of each treatment group was similarly tested, and the results from both days were pooled. The same procedure was carried out for pentobarbital (20, 25, 30, 35 mg/kg) on days 25 and 26 of treatment.

Statistical Analysis

All time-course data were subjected to analysis of variance using the statistical package BMDP-2V. Group means of treated and untreated groups taken on single test days were compared using Student's *t*-test for unpaired data.

RESULTS

Experiment I

The time course of effect of chronic ethanol treatment on the hypothermic response to ethanol, pentobarbital, and barbital is shown in Fig. 1 An analysis of variance shows that ethanol treatment significantly attenuated the hypothermic effect of ethanol, $F(1,14)=12.2$, $p<0.004$, and barbital, $F(1,18)=10.0$, $p<0.005$, indicating the development of tolerance to ethanol and cross-tolerance to barbital. The extent of tolerance and cross-tolerance was dependent on the duration of ethanol treatment as shown by the significant interaction between treatment and time, $F(5,70)=4.4$, $p<0.002$; $F(5,90)=2.6$, $p<0.03$, respectively. Although the chronic treatment group had a consistently lower mean

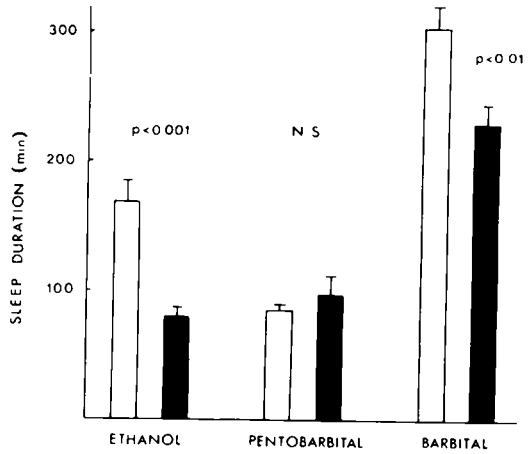

FIG 4 The effect of chronic ethanol treatment by gastric intubation, on the duration of sleep induced by ethanol, pentobarbital or barbital Three groups of rats were tested with IP injections of either 3 5 g/kg of ethanol or 40 mg/kg of pentobarbital on day 63 of treatment, or 200 mg/kg of barbital on day 74 Chronic ethanol (■) versus equicaloric sucrose (□) Results shown are means±S E M with n=9–14 animals per group

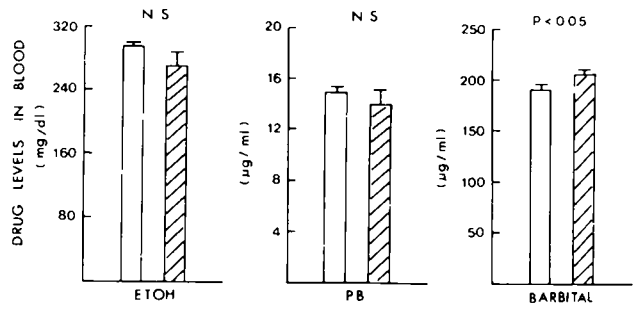

FIG 5 The concentration of ethanol, pentobarbital or barbital at awakening in the blood of rats treated chronically with ethanol or sucrose by gastric intubation for 60–70 days The doses administered were 3 5 g/kg IP of ethanol, 40 mg/kg IP of pentobarbital and 200 mg/kg IP of barbital Chronic ethanol (striped area) versus equicaloric sucrose (□) Results shown are means±S E M with n=10–14 animals per group

hypothermic response to pentobarbital than did the control group, analysis of variance showed no significant difference between them, $F(1,18)=2\ 3$, $p>0\ 2$ This indicates that chronic ethanol treatment failed to produce any significant cross-tolerance to the hypothermic effect of pentobarbital

The effect of chronic ethanol treatment on ethanol-, pentobarbital-, and barbital-induced motor impairment is shown in Fig 2 Ethanol treatment significantly decreased the motor impairment induced by ethanol, $F(1,15)=68\ 5$, $p<0\ 001$, and barbital, $F(1,18)=12\ 0$, $p<0.003$, indicating the development of tolerance to ethanol and cross-tolerance to barbital The degree of tolerance or cross-tolerance was dependent on the duration of ethanol treatment since a significant interaction between treatment and time was observed for both drugs, $F(5,75)=3\ 6$, $p<0\ 006$, $F(5,90)=2\ 4$, $p<0\ 05$, respectively. In contrast to pentobarbital-induced hypothermia, chronic ethanol treatment produced a marginally significant decrease in pentobarbital-induced ataxia, $F(1,17)=5\ 0$, $p<0\ 04$, indicating the acquisition of cross-tolerance to pentobarbital However, there was no significant interaction between treatment and time, $F(5,85)=0\ 4$, $p>0\ 84$

Blood ethanol levels taken at the end of each test period are shown in Fig 3 Analysis of variance showed this difference between the treated and control group to be significant, $F(1,15)=16\ 7$, $p<0\ 001$, but there was no interaction between time and treatment, $F(5,75)=0\ 6$, $p>0\ 7$ The blood barbital concentrations of the treated animals were not significantly different from control, $F(1,18)=0\ 8$, $p>0\ 4$ There was no difference between treated and control groups with respect to blood pentobarbital levels, although the means were consistently slightly higher in the ethanol treatment group, $F(1,18)=0\ 8$, $p>0.4$

The effect of chronic ethanol treatment on the duration of sleep induced by ethanol, barbital and pentobarbital is shown in Fig 4 Ethanol treatment significantly reduced the duration of ethanol- and barbital-induced sleep ($p<0\ 001$ and $p<0\ 01$ respectively), but not of pentobarbital-induced sleep This is indicative of the development of tolerance to ethanol and cross-tolerance to barbital but not to pentobarbital Drug concentrations in blood taken at the time of awakening are shown in Fig 5 Ethanol and pentobarbital blood levels were not significantly different in the treated group compared with control A marginally higher blood barbital level, however, was observed in the treatment group ($p<0\ 05$)

Experiment II

The effect of chronic ethanol treatment on the hypothermic and motor-impairing effects of various doses of ethanol and pentobarbital is shown in Figs 6 and 7, respectively Ethanol-treated animals showed significantly diminished hypothermic and ataxic responses to ethanol compared to controls over the given dose range, $F(1,53)=35\ 5$, $p<0\ 0001$ for hypothermia, $F(1,55)=15\ 1$, $p<0\ 0003$ for motor impairment This indicates the development of tolerance to these effects of ethanol. This treatment, however, did not confer cross-tolerance to pentobarbital as there were no significant differences in the degree of hypothermia or ataxia produced by various doses of pentobarbital between the ethanol- or sucrose-treated groups, $F(1,55)=1\ 0$, $p>0\ 34$ for hypothermia, $F(1,55)=1\ 4$, $p>0\ 26$ for motor impairment

DISCUSSION

The results of this study indicate that a chronic regimen of ethanol which conferred clear-cut tolerance to ethanol-induced hypothermia, ataxia and hypnosis produced only a minimal degree of cross-tolerance to these effects of pentobarbital The lack of appreciable cross-tolerance to pentobarbital-induced hypothermia and ataxia was observed over a wide range of test doses It is worthy of note, however, that although a statistically significant level of cross-tolerance to pentobarbital was not always detected, the trend towards cross-tolerance was present in every case, especially if one takes into consideration the fact that pentobarbital concentration in the ethanol treated group was somewhat higher than in the control group In any event, the fact remains that the extent of cross-tolerance to pentobarbi-

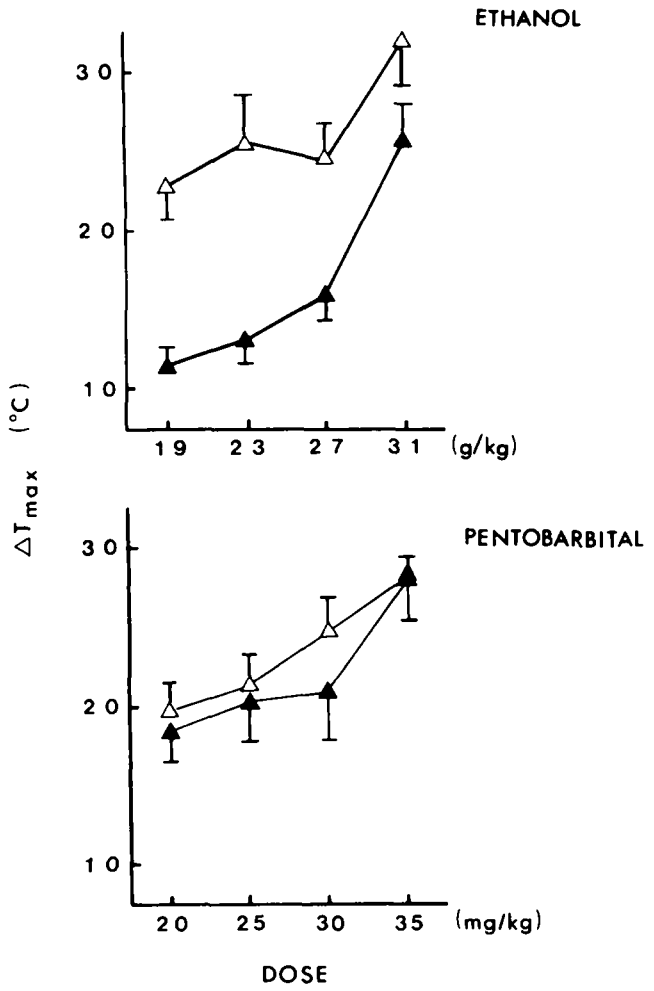

FIG 6 The hypothermic response to various doses of ethanol and pentobarbital in rats chronically intubated with ethanol Ethanol testing was done on day 18 and pentobarbital testing on day 25 Chronic ethanol (▲) versus equicaloric sucrose (△) Results shown are means±S E M with n=8 animals per group at each dose

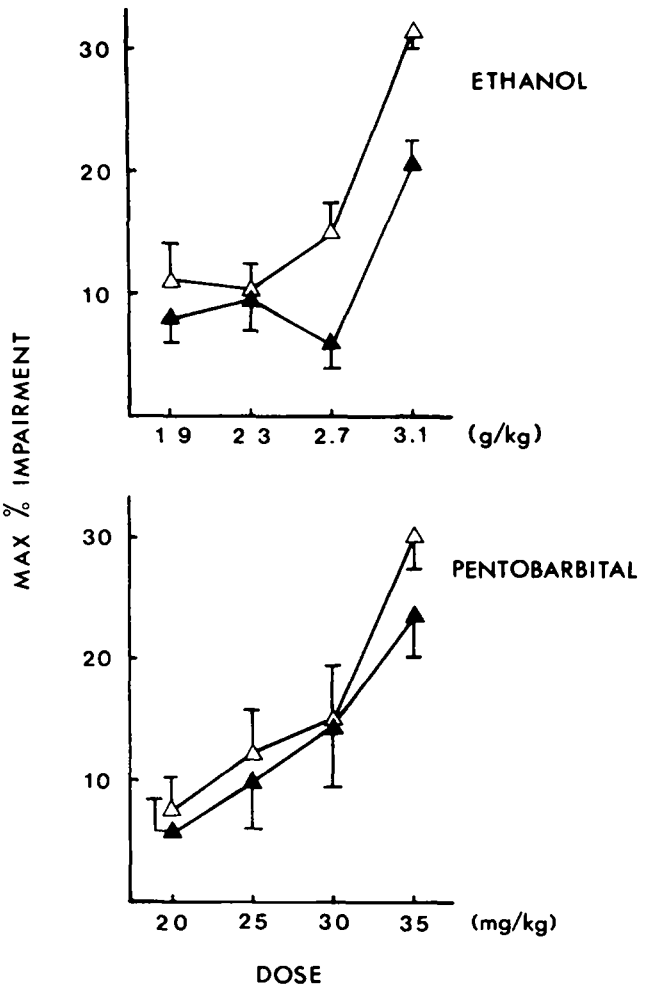

Fig. 7 The degree of motor impairment following administration of various doses of ethanol and pentobarbital in rats receiving ethanol intubation chronically Ethanol testing was done on day 18 and pentobarbital testing on day 25 of treatment Chronic ethanol (▲) versus equicaloric sucrose (△) Results shown are means±S E M with n=8 animals per group at each dose

tal was smaller than that of tolerance to ethanol itself after a similar regimen of chronic ethanol treatment

The blood ethanol concentration of the ethanol-treated group measured 120 min after the test injection was consistently lower than in the control over the course of the study. While this is consistent with reports of a metabolic component in ethanol tolerance, it is unlikely that metabolic changes contributed significantly to the observed tolerance to ethanol-induced hypothermia and ataxia This is because the blood ethanol levels in ethanol-treated animals and controls are not significantly different at 60 min, the time of peak hypothermia and ataxic effect It was also noted that ethanol-treated animals did not recover more rapidly from the hypothermic and ataxic effects of ethanol on successive test days This suggests that metabolic changes probably did not play a significant role in the development of tolerance to these effects of ethanol. Functional tolerance to ethanol-induced hypothermia has also been shown clearly following intracerebroventricular injection of small doses of ethanol [20]

The observed tolerance to ethanol-induced hypnosis may be metabolic to some extent since there was no difference in blood ethanol concentration between treated and control groups at awakening. However, a failure to observe this difference is not conclusive evidence that functional tolerance does not exist. First, the increased rate of metabolism (approximately 21%) due to chronic ethanol treatment (data from another study done in this laboratory for a different purpose) cannot account entirely for the 90 min difference in the duration of ethanol-induced sleep. Secondly, functional tolerance can occur if the ethanol-treated animals awakened during the rising portion of the blood ethanol curve while the control animals woke at a similar concentration during the descending portion. In the case of pentobarbital-induced hypnosis following chronic ethanol treatment, however, this explanation cannot apply because the treated and control groups showed the same blood pentobarbital concentration on awakening at the same time

In contrast to the minimal cross-tolerance to pentobarbital, cross-tolerance to barbital was observed after chronic

intubation with ethanol Since metabolism of barbital is negligible, and changes in its distribution and elimination do not play a significant role in tolerance development, cross-tolerance to the hypothermic, ataxic and hypnotic effects must be due to a decrease in CNS sensitivity

The marginal extent of cross-tolerance to pentobarbital and the differences in the extent of cross-tolerance to pentobarbital and barbital following chronic ethanol intubation are consistent with the hypothesis that there is some type of specificity in the site and/or mechanism of CNS action of sedative-hypnotic drugs, even though they are thought to exert their effects through non-specific actions on cell membranes In support of this view is the observation that some animal strains which have been selectively bred for lower sensitivity to the hypnotic and motor-impairing effects of ethanol or differ inherently in their sensitivity to ethanol are not always less sensitive to other depressants [3, 11–13, 15, 16, 21] In earlier work with the guinea pig ileum longitudinal muscle/myenteric plexus (LM/MP) preparation, Mayer *et al* [17] also showed that ethanol acts at a different site than pentobarbital and barbital Other in vivo studies on long-sleep (LS) and short-sleep (SS) mice have demonstrated that LS mice are more sensitive to ethanol but less sensitive to pentobarbital than are SS mice [3, 13, 21] The large variation in lipid solubility of ethanol and barbiturates may account for the observed differences This possibility is supported by the work of Howerton *et al* [7], who showed that mice of the SS line were more responsive to the lipid-soluble depressants than were LS mice, while LS mice were more responsive to water-soluble depressants Increasing the lipid solubility of alcohols decreased the differences between murine lines, with respect to hypothermic and hypnotic responses [8]

The demonstration of cross-tolerance to barbital and the lack of it between ethanol and pentobarbital may be accounted for in a similar way It is not inconceivable that among drugs which act on membranes in general, this specificity could involve selective actions on certain parts of the lipid bilayer depending, for example, on the lipid water partition coefficient of the drug Presumably, if the lipid solubility of a drug determines its site or mechanism of action, and if an hypothesis of tolerance based on the site of action is valid, cross-tolerance could occur between those drugs which have similar lipid solubilities It would be worthwhile to test this hypothesis by examining cross-tolerance between ethanol and different sedative/hypnotics displaying a wide range of lipid solubilities

It would also be useful to compare cross-tolerance in both directions (i e , cross-tolerance to barbiturates following chronic ethanol treatment and cross-tolerance to ethanol after chronic barbiturate treatment), using behavioral and non-behavioral paradigms These studies may help us to understand the basis for the different extent of cross-tolerance when sites of action of two drugs overlap, as well as the role of behavioral factors in the extent of cross-tolerance

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