

## Effects of Chronic Alcohol Intake on Anesthetic Responses to Diazepam and Thiopental in Rats

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The effect of chronic alcohol intake on anesthetic responses to alcohol, thiopental, or diazepam was examined in adult male Sprague-Dawley rats. Alcohol-fed animals were maintained solely on a complete balanced liquid diet containing 6.54% ethanol (w/w) for 21 days; pair-fed control animals received equal amounts of the same diet with alcohol isocalorically replaced by sucrose or dextrin. Nine hours after diets were withdrawn on the twenty-second day, the following drug/dose combinations were administered intraperitoneally to separate groups of alcohol-fed and control rats (10-15 animals in each group): ethanol 2.4, 3.2, and 4.0 g/kg; thiopental 20, 40, and 80 mg/kg; and diazepam 10, 20, and 40 mg/kg. Three different responses were assessed in every animal: 1) loss of righting reflex (induction of anesthesia); 2) response to a painful stimulus (analgesia); and 3) sleeping time (duration of anesthesia). Alcohol-fed rats compared with controls were significantly less tolerant of pain at an acute alcohol dose of 2.4 g/kg, and loss of righting reflex and sleeping time were reduced at 4.0 g/kg. All three anesthetic responses were also attenuated in alcohol-fed rats at a diazepam dose of 20 mg/kg. In contrast, none of the three responses was reduced in alcohol-fed rats at any of the three thiopental doses. Thus, chronic alcohol intake sufficient to produce tolerance to anesthetic doses of alcohol in rats also produced cross-tolerance to diazepam but not to thiopental in equianesthetic doses. These results suggest that blanket recommendations for adjusting intravenous anesthetic dosages in alcoholic humans may be inadequate as guides to anesthetic management. (Key words: Alcohol: addiction; cross-tolerance; tolerance. Analgesics: tolerance. Anesthetics, intravenous: diazepam; thiopental. Tolerance: alcohol; diazepam.)

IT IS WIDELY ACCEPTED in anesthesia practice that chronic alcoholic patients not acutely intoxicated are resistant to the usual doses of general anesthetics. However, as pointed out by Bruce,<sup>1</sup> this phenomenon of cross-tolerance to anesthetics in alcohol-tolerant individuals has not been adequately characterized in either humans or animals. Experimental study in humans is limited to an abstract reporting increased MAC for halothane in six patients with chronic alcoholism.<sup>2</sup> Mice receiving 10% alcohol as their only source of liquid developed cross-tolerance to isoflurane that persisted for several weeks after cessation of alcohol administration,<sup>3</sup> and similar results were obtained with halothane in alcohol-fed rats.<sup>4</sup>

With regard to intravenous agents, dose requirements

for benzodiazepine anesthesia in alcohol-tolerant individuals have not been investigated at all, and studies of barbiturates have not produced a clear-cut result. Whereas pentobarbital sleep time was shortened in alcohol-tolerant rabbits<sup>5</sup> and rats,<sup>6,7</sup> a comparable study of ultrashort-acting agents in rats concluded that "established ethanol tolerance has no appreciable influence on induction or maintenance response to thiopental, methohexital or Innovar[®]."<sup>8</sup> Despite these negative findings in animals and the lack of any controlled testing in humans, the need for higher doses of thiopental to induce anesthesia in chronic alcoholics has been widely promoted.<sup>9-12</sup>

The present work has (re-)examined in rats the effect of chronic alcohol intake on anesthetic responses to two commonly used intravenous agents, thiopental and diazepam. This study has improved on those cited previously by confirming that the alcohol feeding regimen employed did produce alcohol tolerance and by monitoring responses to the test drug that more comprehensively reflected the anesthetic state.

### Materials and Methods

Male Sprague-Dawley rats, housed two per cage, were allowed *ad libitum* access to a complete balanced liquid diet containing 6.54% ethanol (w/w) as their only source of food and water for 3 weeks. The diet consisted of 315 ml of chocolate Sustacal®, 151 ml of water, and 34 ml of 95% ethanol. This level of ethanol, in a complete diet given for 21 days, has been shown to produce tolerance and at the same time permit normal growth and avoid nutritional deficiencies.<sup>13,14</sup> Pair-fed control rats were given exactly the same volume of diet consumed by their treated counterparts over the previous 24 h but with sucrose or white dextrin isocalorically substituted for ethanol. All liquid diets were withdrawn at midnight of the twenty-second day, 9 h prior to testing, and the animals given free access to water.

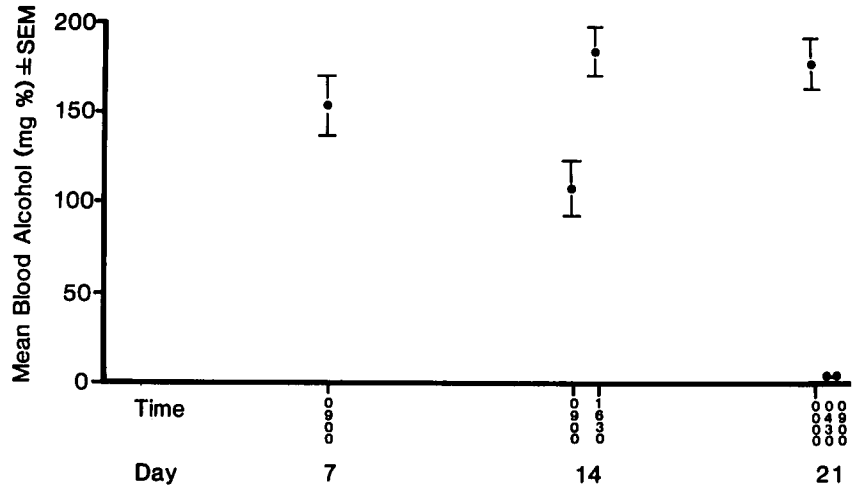
A separate group of ten rats were fed the alcohol-containing diet on exactly the same schedule and used only for monitoring blood alcohol levels. Blood samples were drawn by retrobulbar venous aspiration under light halothane anesthesia and analyzed for alcohol levels by gas chromatography.<sup>15</sup> Samples were drawn at 0900 h on the seventh day, at 0900 h and 1630 h the fourteenth day; and at midnight, 0430 h, and 0900 h on the twenty-second day.

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FIG. 1. Blood alcohol levels at selected times during chronic alcohol administration. Values given are means  $\pm$  SEM for ten animals. Alcohol was withdrawn at midnight (0000 h) of day 22 and acute drug testing was begun at 0900 h.



Acute testing of anesthetic responses was begun 9 h after withdrawal of diets, using the following drugs and doses: thiopental 20, 40, and 80 mg/kg; diazepam 10, 20, and 40 mg/kg; and ethanol (15% v/v solution) 2.4, 3.2, and 4.0 g/kg. All drugs were administered intraperitoneally. Ten or 15 rats in both the experimental and control groups were tested at each dose level of each drug.

After each animal was injected with anesthetic, it was returned to its cage and observed for loss of righting reflex (induction of anesthesia), defined as failure of the rat, when placed on its back, to roll to a lateral or upright position within 30 s. Immediately on loss of righting reflex, sleep monitoring was carried out by placing the animal at the center of an 18  $\times$  24 in rectangle outlined on the floor and noting the time required for the animal to move out of that area. When the rat did so, it was returned to its cage, and its sleeping time (duration of anesthesia) was recorded as the interval between loss of righting reflex and movement out of the rectangle. At 10 min after loss of righting reflex or 20 min after injection, if loss of righting reflex had not occurred, analgesia was measured by placing the rat on a heated surface (60° C) and timing the appearance of avoidance behavior (escape from surface or licking of paws). The animal was removed from the surface as soon as avoidance occurred or at 15 s, if there was no response by then. Rats that had still been sleeping at the time of analgesia testing were immediately returned to their rectangles for continued sleep monitoring. Animals that had not lost their righting reflex by the time of analgesia testing, and therefore had not slept, were assigned a sleeping time of 0 min.

For each measure of anesthesia, response times were considered in relation to a specific reference value. Thus, responses are reported as follows: for loss of righting reflex, the percentage of animals in the group that retained the reflex (remained upright) for at least 10 min after

injection; for analgesia, the percentage that demonstrated escape/avoidance within 15 s; and for sleeping, the percentage that had sleeping times of less than 10 min (*i.e.*, that either stayed awake throughout the observation period or that reawakened within 10 min after loss of righting reflex). The results for corresponding treated and control groups were compared using the Fisher exact probability test, with  $P < 0.05$  considered statistically significant.

## Results

Over the 3-week feeding period, both alcohol-fed and control animals showed steady weight gains that were not significantly different at the time of the drug testing (data not shown). Figure 1 shows that average blood alcohol levels were consistently elevated in the group ingesting alcohol. After withdrawal of alcohol at midnight on the twenty-second day, alcohol levels all reached zero within 4½ h, well before acute drug testing was begun.

Figure 2 presents the results of testing animals with ethyl alcohol. Alcohol-fed rats showed significant attenuation in loss of righting at an alcohol dose of 4.0 g/kg, in pain tolerance at 2.4 g/kg, and in sleeping time at 4.0 g/kg. Figure 3 shows that for all three measures of anesthesia, the control rats were significantly more affected by diazepam at 20 mg/kg than were alcohol-tolerant rats. The data of figure 4 indicate that with thiopental over a four-fold dose range, there were no differences between control and alcohol-tolerant rats for any of the three anesthesia-related responses.

## Discussion

The present study has demonstrated that rats chronically treated with alcohol, thereby becoming tolerant to

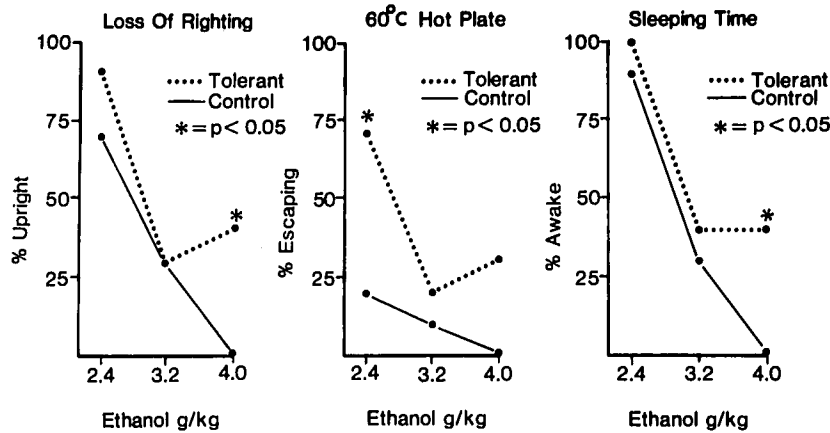


FIG. 2. Anesthetic responses of chronically alcohol-fed rats to acutely administered alcohol. Values on the ordinate are the percentage of animals tested ( $n = 10-15$ ) that gave the indicated response. For details of testing procedures, see "Materials and Methods."

its anesthetic effects, did not show cross-tolerance to anesthetic effects of thiopental. This finding agrees with that of an earlier study<sup>8</sup> in which alcohol tolerance acquisition was assumed rather than confirmed by direct testing. Subject to the limitations of extrapolating from animals to humans, the two studies taken together offer no support for increasing thiopental doses to achieve satisfactory anesthesia in chronic alcoholic patients.

In contrast to thiopental, cross-tolerance to diazepam was observed for all three anesthetic responses. This result suggests that alcoholic patients might well require more diazepam than nonalcoholic patients to achieve the same level of anesthesia.

The adequacy of the experimental protocol for the acquisition of alcohol tolerance, suggested but not proven by the duration of alcohol consumption and consistent elevation of blood alcohol levels (fig. 1), was verified by demonstrating attenuated anesthetic responses to acutely administered alcohol in alcohol-fed rats compared with pair-fed controls. Blood alcohol levels were zero at the time of testing, insuring that the observed effects were

attributable solely to previous alcohol exposure and not to the persistence of alcohol in body fluids. Differences in nutritional status that could have contributed to the observed effects of chronic alcohol treatment were minimized by the pair-feeding regimen, which insured equivalent intakes of calories and of the balanced diet formulation by both groups of rats.

For all three anesthetic responses to both alcohol and diazepam, tolerance was statistically significant at only one of the three test doses. This result reflects the fact that a broad range of each dose-response curve was chosen for study and that the separation between curves for tolerant and nontolerant animals is larger at the intermediate dose and smaller at both high and low doses, where the curves would be converging. Alcohol tolerance was significant at different doses for different responses, suggesting that the latter did not show the same dependence on alcohol dose (*i.e.*, that the dose range chosen encompassed slightly different portions of the dose-response curves for the three different measures of anesthesia).

It should be emphasized that the purpose of this study

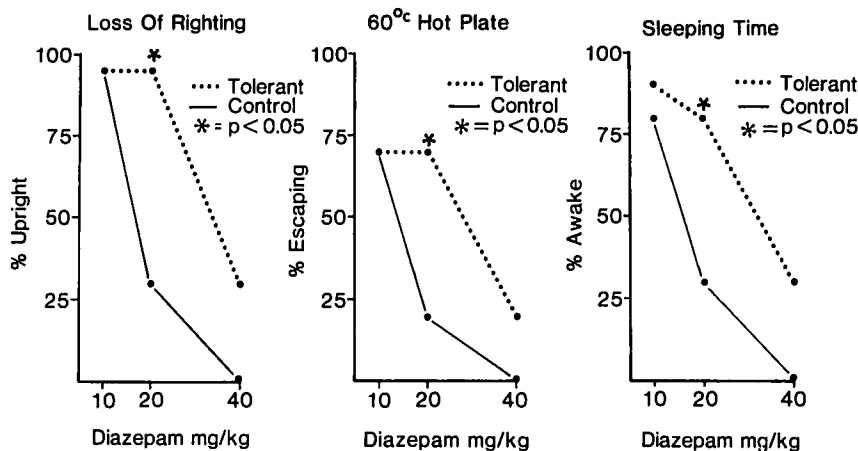


FIG. 3. Anesthetic responses of chronically alcohol-fed rats to acutely administered diazepam. For other details, see legend to figure 2 and "Materials and Methods."

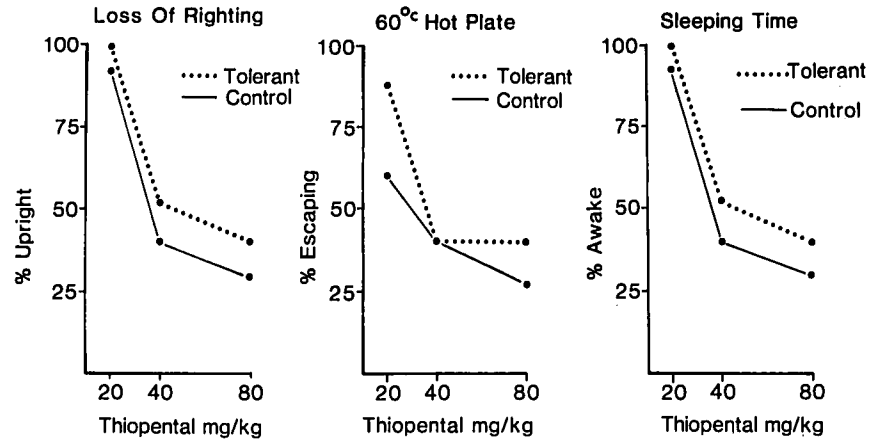


FIG. 4. Anesthetic responses of chronically alcohol-fed rats to acutely administered thiopental. For other details, see legend to figure 2 and "Materials and Methods."

was only to determine whether cross-tolerance to other anesthetics could be demonstrated in rats tolerant to alcohol. Further work will be necessary to elucidate the pharmacologic mechanism(s) responsible for the patterns of tolerance that were observed. For example, direct measurements of blood or brain levels of anesthetic would be required to ascertain whether blunted anesthetic responses to alcohol or diazepam were due to a lowering of effective dose levels by enhanced metabolism or by other alteration in drug disposition (pharmacokinetic mechanisms) or to an actual diminution in CNS responsiveness (a pharmacodynamic mechanism). Measurement of drug levels actually attained would be particularly important with drugs administered intraperitoneally, as they were in this study, because of the greater uncertainties concerning kinetics of drug uptake and distribution. Previous work has established that chronic alcohol treatment enhances the activity of hepatic drug-oxidizing systems by which both barbiturates<sup>15</sup> and benzodiazepines<sup>16</sup> are metabolized. However, the possible impact of such enhanced metabolism may be questioned in light of the rapid onset and short duration of two of the three measured responses. Over relatively short time intervals corresponding to periods of anesthetic induction, prevailing levels of injected anesthetic are thought to be principally regulated not by metabolism but by redistribution of the agent from blood into other fluid and tissue compartments.<sup>17-19</sup> On the other hand, the latter process may also be subject to alteration by chronic alcohol treatment.

Another possible contributor to the effects measured in this study may have been alcohol-induced alterations in glucose availability and ensuing effects on CNS function. The hypoglycemic effect of alcohol metabolism, which inhibits gluconeogenesis, combined with food deprivation, which depletes hepatic glycogen stores, is well documented in a variety of species, including rats.<sup>20,21</sup> In

the present study, 9 h of fasting accompanied by metabolism of residual alcohol elapsed prior to administration of drugs and monitoring of anesthetic responses. Measurements of blood glucose at the time of testing would be necessary to determine whether hypoglycemia may have been present. Although at least 24 h of fasting is required to deplete liver glycogen in normal rats, chronically intoxicated animals may have had reduced glycogen stores due to suboptimal caloric intake and a decreased proportion of dietary nutrients capable of being converted to glycogen.

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