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Comparison of Effects of Diazepam on Barbiturate and on Ethanol Withdrawal[1]

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ABSTRACT

Barbiturates and ethanol produce physical dependence states that are similar but not identical. We have previously shown that when animals are treated with "chronically equivalent" doses of barbiturate or ethanol, the withdrawal syndrome after ethanol is more severe than after barbiturate. In addition, certain ethanol withdrawal signs (*e.g.*, tremors and convulsions) appeared qualitatively different than the corresponding barbiturate withdrawal signs. We recently reported that diazepam is only partially effective in suppressing severe ethanol withdrawal in our animal model and that certain ethanol withdrawal signs were resistant to diazepam treatment. In view of the differences between ethanol and barbiturate physical dependence, we compared the effects of diazepam in suppressing the two types of withdrawal. Ethanol or pentobarbital was administered introgastrically twice daily according to methods which produce severe ethanol or barbiturate withdrawal syndromes of approximately equal intensity and similar time course. A single dose of diazepam (0.5–40 mg/kg) was administered i.m. at the time of near-maximal withdrawal intensity. The peak response to diazepam was evaluated in terms of reduction of overall withdrawal intensity and effects on individual withdrawal signs. Diazepam suppressed convulsions and prevented death during both ethanol and pentobarbital withdrawal syndromes. Diazepam decreased overall withdrawal intensity dose-dependently in both types of withdrawal syndromes but failed to completely suppress either type: 5 mg/kg of diazepam produced maximal but partial suppression of withdrawal signs. However, the maximal reduction of overall withdrawal intensity by diazepam was significantly greater in barbiturate withdrawal. These results were related to the effects of diazepam on individual withdrawal signs. Many pentobarbital and ethanol withdrawal signs were completely and dose-dependently suppressed by diazepam, although a higher dose was needed to suppress most of these signs in the ethanol withdrawal syndrome. Certain ethanol withdrawal signs (*e.g.*, tremor and bizarre behavior) were resistant to diazepam. The most striking finding in this study was that diazepam suppressed tremor and bizarre behavior during the pentobarbital withdrawal syndrome. The results support the evidence that ethanol and barbiturate physical dependence are not equivalent and indicate that diazepam is less effective in suppressing the ethanol withdrawal syndrome.

Chronic administration of barbiturates or ethanol can produce tolerance and physical dependence. Barbiturates and ethanol produce similar withdrawal syndromes in man, indicating that physical dependence states produced by these drugs are similar (Fraser *et al.*, 1957). On the other hand, Wikler *et al.* (1956) reported that the electroencephalogram patterns and incidence of seizures in ethanol withdrawal were different from those previously reported in barbiturate withdrawal. However, differences between these studies with respect to the chronic drug administration schedules hinder direct comparison of barbiturate and ethanol physical dependence.

Our recent characterization of the tolerance and physical dependence capabilities of barbiturates and ethanol in an animal model (Okamoto *et al.*, 1981) supports the concept that these drugs produce similar but not identical physical dependence states. This study chose barbital and pentobarbital as prototypes of barbiturates, because our previous work had established that the acute effects and production of physical dependence by these drugs were nearly indistinguishable when the influence of their known difference in pharmacokinetics was obviated by experimental manipulation (Boisse and Okamoto, 1978). When these prototypical barbiturates were compared with ethanol (Okamoto *et al.*, 1981), it was found that the acute effects of barbiturates and ethanol and the withdrawal syndromes that followed their chronic administration were nearly identical. However, functional tolerance developed faster and the withdrawal syndrome was more severe in animals treated with ethanol than in animals treated with "chronically equivalent" (ataxia-producing) doses of barbital. Chronic barbiturate treatment to the level of anesthesia (with pentobarbital) was needed to produce a severity of physical dependence comparable to that produced by ataxigenic doses of ethanol. Furthermore, qualitative differences between certain signs ap-

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ABBREVIATIONS: CNS, central nervous system; WIR, Withdrawal Intensity Rating.

pearing in barbiturates (barbital and pentobarbital) and ethanol withdrawal were noted.

We recently reported that diazepam did not completely suppress severe ethanol withdrawal in our animal model (Aaronson *et al.*, 1982). Certain ethanol withdrawal signs (*e.g.*, tremor and "bizarre behavior") were resistant to diazepam treatment. In view of the clinical and experimental evidence that ethanol and barbiturate physical dependence are not identical, we compared the effectiveness of diazepam in suppressing barbiturate and ethanol withdrawal syndromes of comparable intensity, using pentobarbital as the prototype barbiturate.

Methods

Chronic barbiturate and ethanol administration. The methods for chronic pentobarbital and ethanol administration have been reported in detail previously (Okamoto *et al.*, 1981) and hence will be summarized only briefly.

Adult cats (2.0–3.0 kg) of either sex were prepared for chronic drug administration by surgical implantation of a gastric catheter (Okamoto *et al.*, 1975). The animals were allowed to recover from surgery for 1 week before chronic dosing began.

The method for chronic "high-dose" pentobarbital administration was used (Rosenberg and Okamoto, 1974; Okamoto *et al.*, 1975). The responses to pentobarbital were quantified on a CNS depression rating scale that ranges from 0 (no CNS depression) to 14 (near death from respiratory depression). Interobserver reliability on this scale was checked by comparing ratings made independently by the observers. For high-dose barbiturate treatment, the Na pentobarbital doses were chosen to produce a peak CNS depression rating of 11 to 12 (deep anesthesia).

Sodium pentobarbital was administered intragastrically twice a day, morning and evening, 11 to 12 hr apart. The initial dose of Na pentobarbital was 40 mg/kg. Subsequent doses were individually adjusted by 5-mg/kg increments to maintain the peak CNS response of 11 to 12. The duration of chronic treatment was 5 weeks.

The method for chronic ethanol administration was similar, the major differences being that the doses of ethanol were chosen to produce a peak CNS depression rating of 4 to 6 (gross ataxia) and that the duration of ethanol treatment was 3 weeks. Ethanol was administered intragastrically twice daily, morning and evening, 11 to 12 hr apart. The initial dose of ethanol was 2.0 g/kg. Subsequent doses were individually adjusted by 0.25-g/kg increments to achieve and maintain the peak CNS depression response of 4 to 6. All ethanol doses were mixed with 60 ml of a nutritionally complete liquid diet consisting of pureed tuna (Purina Variety Menu), Esbilac (Borden) and milk.

These barbiturate and ethanol chronic dosing methods were chosen for this study in order to produce withdrawal syndromes of comparable severity (Okamoto *et al.*, 1981).

Barbiturate and ethanol withdrawal evaluation. After the last dose of barbiturate or ethanol, the animals were placed individually in activity-monitoring cages (Okamoto *et al.*, 1976). Generalized convulsions, *i.e.*, status and isolated tonic-clonic convulsions and clonic convulsions, were continuously monitored (Okamoto *et al.*, 1976, 1981).

Evaluation of withdrawal was based on observation and subjective assessment of overt withdrawal signs as described previously (Okamoto *et al.*, 1976, 1981; Aaronson *et al.*, 1982). Twenty-one motor, autonomic and behavioral signs were rated and recorded. The intensity of most withdrawal signs was graded from 0 (no sign, normal) to 3 (severe). Several signs were simply recorded as present or absent. Interobserver reliability to withdrawal ratings was checked periodically as has been reported previously (Okamoto *et al.*, 1976). Withdrawal observations were recorded immediately before and at preset times after diazepam administration. Observations were made at 0.5, 1, 2, 3, 4, 6, 8 and 12 hr after diazepam, and subsequently at least three times daily.

Diazepam treatment. A single i.m. injection of diazepam (Hoffmann-La Roche Inc., Nutley, NJ) was administered to each animal 24 hr after the last dose of either pentobarbital or ethanol, provided that at least one convulsion had been recorded on the activity record. If no convulsion had occurred, the treatment was delayed until after the first convulsion. The maximum allowable delay in treatment was 8 hr (32 hr after the last dose of either pentobarbital or ethanol).

Diazepam, in a freshly prepared suspension in oil, was injected into the deltoid muscle. The doses were 0.5, 2.5, 10 and 20 mg/kg for the pentobarbital groups and 1, 5, 10, 20 and 40 mg/kg for the ethanol groups. The dose was delivered on 0.1 ml of suspension per kg b.wt. The injection site was massaged to enhance the absorption of the drug. The highest dose of diazepam, 40 mg/kg, was delivered as 0.2 ml/kg and divided between two injection sites. The same volume of oil was injected i.m. into several control animals.

Preliminary studies of blood levels of diazepam and desmethyldiazepam in several normal animals showed that intramuscularly administered diazepam was well absorbed: the time to the peak and the peak levels of diazepam and desmethyldiazepam were comparable to those measured after intragastric administration of the same dose (M. Okamoto, unpublished observations).

Calculations and statistical methods. The WIR is a composite score for estimation of withdrawal intensity (Okamoto *et al.*, 1981; Aaronson *et al.*, 1982). Individual withdrawal signs are given numerical values as previously described (Boisse and Okamoto, 1978). WIR is the sum of the values of the individual withdrawal signs observed at a particular time. WIR ranges from 0 (no withdrawal) to 54 (maximum).

The effectiveness of diazepam in reducing overall withdrawal intensity was measured as the percentage of reduction of WIR. Differences in response between treatment groups were tested by analysis of variance and Student-Newman-Keuls multiple range testing. The arcsine (angular) transformation was used to normalize the percentage data (Zar, 1974).

Probit analysis was used to analyze the dose-response relationship for suppression of individual withdrawal signs by diazepam. The ratings for each sign before and 2 hr after diazepam were used to determine the proportion of animals showing complete suppression of the sign (*i.e.*, rated 0 or normal). Two hours after diazepam treatment was selected because this was the time when diazepam produced maximal reduction of overall withdrawal intensity. Animals not displaying the sign before treatment were excluded from the analysis of that sign. Log dose vs. percent effect curves were fitted for each of 13 withdrawal signs by computer (IBM 370/168) using a modified version of the "Bliss 20" program (Finney and Craigie, 1979) for analysis of quantal response data. This program estimates the ED_{50} (the dose which is required to suppress completely the sign in 50% of animals) and confidence limits by fitting a normal sigmoid curve to the data, using the principle of maximum likelihood estimation (Finney, 1971).

The nonparametric Kruskal-Wallis analysis of variance by ranks (Zar, 1974) was used when parametric methods were inappropriate. The significance of observed differences in proportions was assessed using Fisher's exact test (Zar, 1974).

The incidence of abnormal behavior after diazepam treatment was compared with that during the corresponding period in untreated or ethanol withdrawal. Five abnormal behaviors were evaluated: "apprehensive"; "passive"; "aggressive"; "overly affectionate"; and "bizarre." These behaviors have been described previously (Okamoto *et al.*, 1976). Bizarre behavior consisted of visual tracking in the absence of visual stimulation observable by the experimenter and was usually accompanied by apprehensive, aggressive, or "mouse-hunting" behavior. For untreated controls, the total incidence of each abnormal behavior during the period 24 to 48 hr after the last dose of ethanol was estimated as the percentage of animals displaying these behaviors at 24, 36 or 48 hr after the last dose of pentobarbital or ethanol. For diazepam-treated groups, the incidence was estimated as the percentage of animals displaying each behavior at 1, 12 or 24 hr after diazepam (approximately 25, 36 and 48 hr after the last dose of barbiturate or ethanol). The χ^2 test with Yates correction was used to compare the incidence of abnormal behaviors in these groups.

Results

Barbiturate and ethanol withdrawal controls. The time course and the intensity of untreated barbiturate and ethanol withdrawal are represented in figure 1. As previously reported (Okamoto *et al.*, 1981), 3 weeks of "low-dose" (ataxic level) treatment with ethanol produced withdrawal syndromes of intensity comparable to that produced by 5 weeks of high-dose (anesthetic level) treatment with barbiturate.

The number of tonic-clonic convulsions per animal during pentobarbital withdrawal was, however, consistently higher than the number during ethanol withdrawal (fig. 1). During the period of peak overall withdrawal intensity, 24 to 36 hr after the last dose of pentobarbital or ethanol, the average number of convulsions per animal was 4.0 ± 0.3 in barbiturate withdrawal and 2.38 ± 0.7 in ethanol withdrawal groups (mean ± S.E.).

Effect of diazepam on barbiturate and ethanol withdrawal. Diazepam suppressed withdrawal convulsions during barbiturate and ethanol withdrawal (fig. 2). Low doses of diazepam temporarily suppressed convulsions, whereas single high doses completely suppressed convulsions throughout withdrawal. The threshold dose of diazepam needed to suppress convulsions appeared higher in pentobarbital withdrawal than in ethanol withdrawal: 2 mg/kg did not significantly reduce the total incidence of barbiturate convulsions compared to untreated barbiturate controls (P = .252, Fisher's exact test), whereas 1 mg/kg significantly reduced the total incidence of ethanol withdrawal convulsions compared to untreated ethanol controls (P = .0186, Fisher's exact test).

Diazepam also reduced the total number of convulsions per animal during both types of withdrawal in a dose-dependent manner (Kruskal-Wallis analysis of variance by ranks, P < .001 for both ethanol and pentobarbital withdrawal).

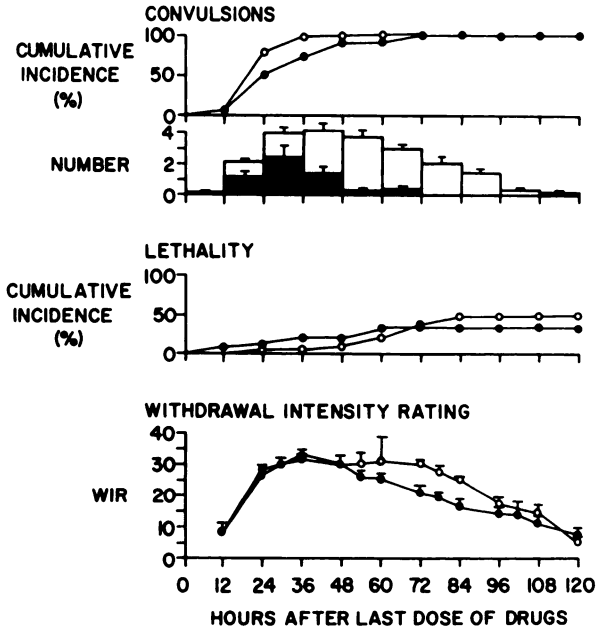

Fig. 1. Time course of untreated barbiturate and ethanol withdrawal. Abscissa: hours after last dose of Na pentobarbital or ethanol. Ordinates (from top): cumulative incidence of convulsion; number of convulsions per animal (mean ± S.E.): cumulative incidence of lethality; and, WIRs (mean ± S.E.). Barbiturate withdrawal, open circle or open bar; ethanol withdrawal, solid circle or solid bar. Number of animals per group (n) = 40 to 80 for barbiturate (includes data previously published in Okamoto *et al.*, 1981) and 10 to 15 for ethanol.

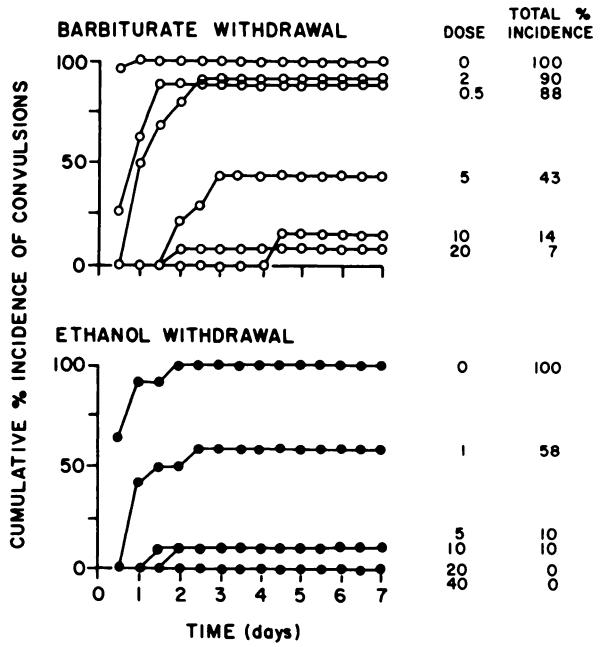

Fig. 2. Effect of diazepam on incidence of convulsions in barbiturate and ethanol withdrawal. Abscissa: time in days after diazepam treatment (treated groups), or after 24 hr after the last dose of Na pentobarbital or ethanol (untreated controls). Ordinate: cumulative percentage of incidence of convulsion. ○, barbiturate withdrawal; ●, ethanol withdrawal. On right, the dose of diazepam in milligrams per kilogram and the corresponding total percentage of incidence of convulsions. Number of animals per group (n) = 10 to 14 for barbiturate and 10 to 12 for ethanol.

Diazepam significantly reduced the incidence of death during both pentobarbital and ethanol withdrawal syndromes (Fisher's exact test, P < .05 for each diazepam-treated group compared with its respective control). Diazepam (0.5 mg/kg) reduced the incidence of lethality during pentobarbital withdrawal from 49% in untreated withdrawal to 25% after the diazepam treatment; none of the animals given diazepam in doses of 2 mg/kg or higher died. The incidence of lethality in ethanol withdrawal after diazepam (1 mg/kg and higher) was 3%, whereas 46% of the animals not treated with diazepam died.

Diazepam also produced dose-dependent reductions of overall withdrawal intensity (WIR) of both pentobarbital and ethanol withdrawal (fig. 3). Within each type of withdrawal, the difference between diazepam-treated groups was significant (pentobarbital withdrawal: F = 22.82, P < .01; ethanol withdrawal: F = 5.78, P < .01). Neither type of withdrawal was completely suppressed: increasing the dose of diazepam above 5 mg/kg did not further reduce WIR. However, the apparent "ceiling" in reduction of overall withdrawal intensity was higher in pentobarbital withdrawal than in ethanol withdrawal (P < .001 for comparison between barbiturate and ethanol withdrawal at each dose of diazepam: 5, 10 and 20 mg/kg).

Gross observation suggested that, as in ethanol withdrawal (Aaronson *et al.*, 1982), the individual withdrawal signs in pentobarbital withdrawal did not respond equally to diazepam treatment. Accordingly, the dose-response relationships for individual withdrawal signs which contribute to WIR were analyzed in order to compare quantitatively the responses of individual pentobarbital and ethanol withdrawal signs to diazepam.

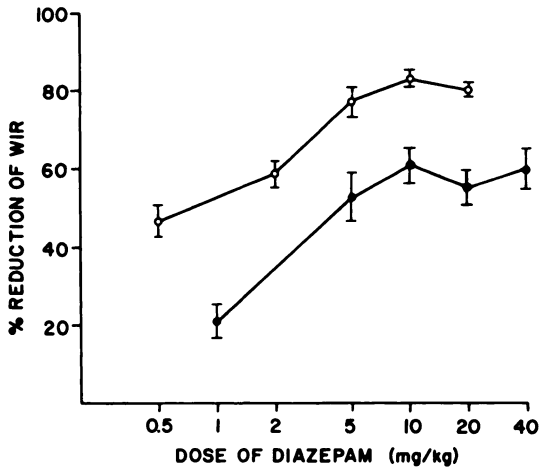

Fig. 3. Peak reduction of overall barbiturate and ethanol withdrawal intensity by diazepam. Abscissa: dose of diazepam (milligrams per kilogram) on a logarithmic scale. Ordinate: peak percentage of reduction of WIRs. ○, barbiturate withdrawal; ●, ethanol withdrawal. Points are means, and bars are 95% confidence intervals. (*n*) = 10 to 14 per group for barbiturate and 10 to 12 per group for ethanol.

The most striking finding (table 1) was that pentobarbital withdrawal tremor was suppressed by diazepam in a dose-dependent manner, in contrast to ethanol withdrawal tremor, which was not suppressed by diazepam even at the highest dose used. Except for ethanol withdrawal tremor, the signs shown in table 1 were diazepam sensitive in both types of withdrawal. However, a higher dose of diazepam was usually needed to suppress the diazepam-sensitive ethanol withdrawal signs than the diazepam-sensitive pentobarbital withdrawal signs (*i.e.*, the ED_{50} values for diazepam were higher in ethanol withdrawal).

Diazepam was also more effective in suppressing several types of abnormal behavior during pentobarbital withdrawal than during ethanol withdrawal (table 2). In pentobarbital withdrawal, doses of diazepam greater than 0.5 mg/kg produced a prolonged (24 hr) reduction of apprehensive behavior compared with untreated controls, whereas even 40 mg/kg of diazepam failed to reduce apprehensive behavior during the corresponding period of ethanol withdrawal. The higher doses of diazepam also effectively reduced the incidence of bizarre behavior during pentobarbital withdrawal, but did not reduce bizarre behavior during ethanol withdrawal.

Diazepam increased the incidence of several types of abnormal behavior in both pentobarbital and ethanol withdrawal (table 2). Stereotyped overly affectionate behavior, which usually emerges on the 3rd day of untreated pentobarbital or ethanol withdrawal (Okamoto *et al.*, 1976), was more prevalent in diazepam-treated groups than in untreated groups on the 2nd day of withdrawal (table 2). A similar effect of diazepam on body shakes was observed (data not shown). Diazepam also increased the incidence of passive behavior in both types of withdrawal (table 2). This indicated a direct depressant effect of diazepam (see below). After treatment with high doses of diazepam, animals undergoing pentobarbital withdrawal became passive when left alone, and overly affectionate when handled. In contrast, animals undergoing ethanol withdrawal displayed a complex mixture of passive and hyperexcitable behavior after diazepam treatment. They tended to be passive, staring blankly when they were left alone in the cage, but became apprehensive and aggressive upon handling.

Diazepam increased the intensity of certain signs in both types of withdrawal. In addition to increasing passive behavior, diazepam increased motor incoordination and weakness in both types of withdrawal; the threshold doses for these effects were 5 mg/kg for weakness and 10 mg/kg for motor incoordination, for both barbiturate and ethanol withdrawal syndromes. As reported previously (Aaronson *et al.*, 1982), diazepam has similar toxic CNS depressant effects in naive animals: 5 mg/kg (i.m.) produced ataxia in three of four naive animals and a peak CNS depression rating of 2.5 ± 0.5; 10 mg/kg produced ataxia in seven of eight naive animals and a peak CNS depression rating of 4.6 ± 0.5, representing a severe degree of motor incoordination and weakness. Therefore, the increases in motor incoordination and weakness observed after diazepam treatment of both barbiturate and ethanol withdrawal probably represent direct effects of diazepam.

In summary, the individual pentobarbital and ethanol withdrawal signs can be categorized according to their response to diazepam treatment. Group I consists of signs which were suppressed in a dose-related manner during pentobarbital withdrawal, and were not suppressed during ethanol withdrawal. This group includes head tremor, body tremor, intention tremor and bizarre behavior. Group II consists of signs which were suppressed in a dose-related manner during both types of withdrawal, but which required higher doses of diazepam for suppression during ethanol withdrawal. These signs were

TABLE 1

Suppression of individual withdrawal signs by diazepam: pentobarbital *vs.* ethanol withdrawal

Withdrawal Sign	Type of Withdrawal	ED_{50}[a]	95% Confidence Limits		P
			Lower	Upper	
		mg/kg	*mg/kg*	*mg/kg*	
Auditory startle response	Pentobarbital	0.04		0.7	<.05
	Ethanol	6.5	3.4	10.7	
Apprehensive behavior	Pentobarbital	0.2		1.1	<.05
	Ethanol	8.6	13.2	4.9	
Panting	Pentobarbital	0.9	0.02	2.3	N.S.
	Ethanol	2.1	0.4	4.2	
Pupillary light reflex	Pentobarbital	1.0	0.1	2.2	N.S.
	Ethanol	0.3		1.7	
Tactile startle response	Pentobarbital	1.0	0.1	2.2	<.05
	Ethanol	10.1	4.3	27.7	
Myoclonic jerks	Pentobarbital	1.0	0.3	1.9	<.05
	Ethanol	8.7	4.6	15.9	
Twitches	Pentobarbital	2.4	0.9	4.3	<.05
	Ethanol	8.7	4.6	15.9	
Pupillary dilatation	Pentobarbital	2.7	1.2	4.5	N.S.
	Ethanol	2.3		6.3	
Muscle spasticity	Pentobarbital	2.8	1.4	4.3	<.05
	Ethanol	10.2	4.5	26.0	
Abnormal movement qualitative	Pentobarbital	3.5	0.3	10.6	N.S.
	Ethanol	15.2	6.3	91.4	
Intention tremor	Pentobarbital	4.7	3.0	6.4	
	Ethanol[b]				
Body and limb tremor	Pentobarbital	7.8	4.8	12.3	
	Ethanol[b]				
Head tremor	Pentobarbital	14.4	4.9		
	Ethanol[b]				

[a] Dose of diazepam to produce complete suppression of withdrawal sign in 50% of animals.

[b] This withdrawal sign was never or rarely completely suppressed by diazepam (up to 40 mg/kg).

TABLE 2

Effect of diazepam on abnormal behaviors during barbiturate and ethanol withdrawal

Values are percentages of animals displaying abnormal behavior: see "Methods."

Diazepam Dose	Apprehensive	Aggressive	Bizarre	Overly Affectionate	Passive
mg/kg					
			Barbiturate Withdrawal		
0	94.1	20.6	35.3	47.1	38.2
0.5	75.0	25.0	37.5	75.0	62.5
2	50.5*	20.0	60.0	100.0*	70.0
5	27.3*	0.0	0.0*	66.7	46.2
10	23.1*	0.0	0.0*	71.4	71.4
20	7.6*	0.0	0.0*	46.2	76.9*
			Ethanol Withdrawal		
0	100.0	50.0	41.7	33.3	0.0
1	100.0	58.3	41.7	66.6	16.7
5	80.0	50.0	30.0	70.0	70.0*
10	100.0	30.0	30.0	90.0*	60.0*
20	80.0	20.0	30.0	100.0*	80.0*
40	66.7	0.0*	22.2	77.8	88.9*

* $P < .05$, 2×2 χ^2 test with Yates correction, compared with control (diazepam, 0 mg/kg dose) withdrawal.

twitches, muscle spasticity, myoclonic jerks, startle responses, apprehensiveness, pupillary signs and panting. Group III signs were increased by diazepam in both types of withdrawal. These signs were motor incoordination, weakness, overly affectionate and passive behaviors and body shakes. Withdrawal signs in group IV (abnormal movements, abnormal posture, piloerection) were not consistently affected by diazepam in either type of withdrawal. Figure 4 illustrates the relationship between these categories of withdrawal signs and the effects of diazepam on overall withdrawal intensity (WIR).

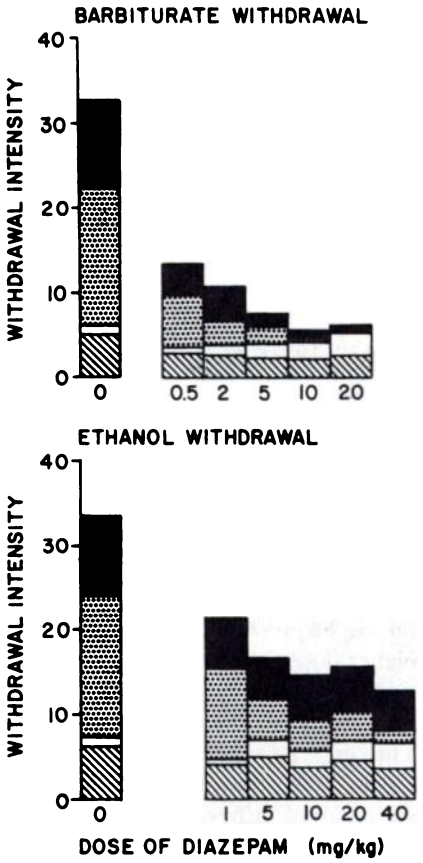

Fig. 4. Effect of diazepam on barbiturate and ethanol withdrawal signs. Abscissa: dose of diazepam (milligrams per kilogram). Diazepam 0 indicates the withdrawal intensity before diazepam (all treatment groups, pooled). Ordinate: sum of average intensity of the withdrawal signs. Groups I (solid area), II (dotted area), III (white area) and IV (hatched area). See text for description of groups of signs.

Discussion

The similarities between barbiturate and ethanol withdrawal have long been recognized (Kalinowsky, 1942; Isbell *et al.*, 1955; Victor and Adams, 1953; Wikler *et al.*, 1955, 1956; Eddy *et al.*, 1965; Kalant, 1977). However, clinical differences between barbiturate and ethanol withdrawal have been noted (Wikler *et al.*, 1955, 1956), and "cross dependence" studies suggest that physical dependence on barbiturate and ethanol are not identical (Fraser *et al.*, 1957; Victor, 1966; Norton, 1970; Yanagita and Takahashi, 1973). By controlling and matching conditions of chronic drug administration in our animal model, we have characterized the similarities and differences between barbiturate and ethanol withdrawal (Okamoto *et al.*, 1981). Ethanol produced a more severe withdrawal syndrome than barbiturates when the level of intoxication during chronic drug adminstration was comparable. Furthermore, differences in individual barbiturate and ethanol withdrawal signs (*e.g.*, convulsions and tremor) were observed. We previously reported that diazepam, another CNS depressant, was only partially effective in suppressing severe ethanol withdrawal, and that the failure to completely suppress ethanol withdrawal was due to the resistance of certain ethanol withdrawal signs to diazepam (Aaronson *et al.*, 1982). The present study extends the comparison of barbiturate and ethanol withdrawal by comparing their response to diazepam.

In this study, the chronic pentobarbital and ethanol dosing regimens were selected to produce withdrawal syndromes of comparable intensity. Both regimens resulted in 100% incidence of convulsions in animals not treated with diazepam, and comparable peak overall withdrawal intensity. In addition, the time course of the untreated withdrawal was very similar in pentobarbital and ethanol withdrawal. Diazepam was administered at the time of near maximal withdrawal intensity in both types of withdrawal.

Diazepam was more effective in suppressing pentobarbital withdrawal than ethanol withdrawal. The effective suppression of barbiturate withdrawal was consistent with the limited experimental evidence that benzodiazepines (*e.g.*, diazepam, chlordiazepoxide and oxazepam) can completely suppress barbiturate withdrawal, but in a relatively high dose range (Yanagita and Takahashi, 1973). Our results also support the limited clinical evidence that benzodiazepines cannot completely suppress severe ethanol withdrawal once it develops (Thompson *et al.*, 1975; Kramp and Rafaelson, 1978; Woo and Greenblatt, 1979).

The difference in the effectiveness of diazepam in the two types of withdrawal was related to its effects on individual withdrawal signs. Firstly, certain withdrawal signs which were not suppressed by diazepam during ethanol withdrawal were suppressed by diazepam in a dose-dependent manner during barbiturate withdrawal. These signs include tremor and bizarre behavior. We previously reported that ethanol and barbiturate tremor appear to be qualitatively different; ethanol withdrawal tremor is usually more "coarse," with a higher and more irregular amplitude (Okamoto *et al.*, 1981). Our present findings

that diazepam suppresses barbiturate withdrawal tremor but not ethanol withdrawal tremor suggest that the qualitative differences in the appearance of this sign may reflect fundamental differences in neuropathophysiological mechanism of tremor in the two types of withdrawal.

Secondly, in general, a higher dose of diazepam was needed to suppress diazepam-sensitive signs in ethanol withdrawal than in pentobarbital withdrawal. Also, the ED_{50} values for the ability of diazepam to suppress both withdrawal syndromes were higher than the effective dose range in most animal screening tests for diazepam effects (0.1–1.0 mg/kg k.m.; *cf.*, Randall and Kappell, 1973). These findings suggest that cross-tolerance to diazepam developed during the chronic administration of ethanol and pentobarbital, but that greater cross-tolerance (functional or dispositional) to diazepam developed during chronic ethanol administration. Considering that the level and duration of chronic ethanol administration was much less than that of chronic barbiturate administration, this implies a remarkable ability of ethanol to induce cross-tolerance to diazepam. This issue is of considerable potential clinical importance and will be pursued in future studies.

A slightly higher dose of diazepam was needed to suppress pentobarbital withdrawal convulsions than ethanol withdrawal convulsions, in contrast to the effects of diazepam on most other signs. This may have been due to another underlying difference between barbiturate and ethanol withdrawal. We have previously reported differences between the patterns of ethanol and barbiturate withdrawal convulsions (Okamoto *et al.*, 1981): barbiturate withdrawal convulsions usually consist of isolated or status tonic-clonic convulsions, whereas animals undergoing ethanol withdrawal also display clonic convulsions without tonic components. In the present study, the most apparent quantitative difference between untreated pentobarbital and ethanol withdrawal was that the number of pentobarbital withdrawal convulsions was greater. These qualitative and quantitative differences may account for the finding that slightly higher doses of diazepam were needed to suppress pentobarbital withdrawal convulsions.

The present study and our previous study (Okamoto *et al.*, 1981) show that although there are many similarities in the physical dependency states produced by barbiturates and ethanol, qualitative and quantitative differences do exist. Further studies are needed to determine whether these differences extend to structurally dissimilar general CNS depressants.

Acknowledgments

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References

AARONSON, L. A., HINMAN, D. J. AND OKAMOTO, M.: Effects of diazepam on ethanol withdrawal. J. Pharmacol. Exp. Ther. **221:** 319–325, 1982.

BOISSE, N. R. AND OKAMOTO, M.: Physical dependence to barbital compared to pentobarbital. III. Withdrawal characteristics. J. Pharmacol. Exp. Ther. **204:** 514–525, 1978.

EDDY, N. B., HALBACKH, H., ISBELL, H. AND SEEVERS, M. H.: Drug dependence: Its significance and characteristics. Bull. W. H. O. **32:** 721–733, 1965.

FINNEY, D. J.: Probit Analysis, Cambridge University Press, Cambridge, England, 1971.

FINNEY, D. J. AND CRAIGIE, I. R.: Bliss 20. Department of Statistics and ARCUS, University of Edinburgh, 1979.

FRASER, H. F., WIKLER, A., ISBELL, H. AND JOHNSON, N. K.: Partial equivalence of chronic alcohol and barbiturate intoxication. Q. J. Stud. Alcohol **18:** 541–551, 1957.

ISBELL, H., FRASER, H. F., WIKLER, A., BELLEVILLE, R. E. AND EISEMAN, A. J.: An experimental study of the etiology of "rum fits" and delirium tremens. Q. J. Stud. Alcohol **16:** 1–33, 1955.

KALANT, H.: Comparative aspects of tolerance to and dependence on alcohol, barbiturates and opiates. Adv. Exp. Med. Biol. **858:** 169–186, 1977.

KALINOWSKY, L. B.: Convulsions in non-epileptic patients on withdrawal of barbiturates, alcohol and other drugs. Arch. Neurol. Psychiatry **48:** 946–956, 1942.

KRAMP, P. AND RAFAELSON, O. J.: Delirium tremens: A double-blind comparison of diazepam and barbital treatment. Acta Psychiatr. Scand. **58:** 174–190, 1978.

NORTON, P. R. E.: The effects of drugs on barbiturate withdrawal convulsions in the rat. J. Pharm. Pharmacol. **22:** 763–766, 1970.

OKAMOTO, M., HINMAN, D. J. AND AARONSON, L. M.: Comparison of ethanol and barbiturate physical dependence. J. Pharmacol. Exp. Ther. **218:** 701–708, 1981.

OKAMOTO, M., ROSENBERG, H. C. AND BOISSE, N. R.: Tolerance characteristics producing during the maximally tolerable chronic pentobarbital dosing in the cat. J. Pharmacol. Exp. Ther. **192:** 555–564, 1975.

OKAMOTO, M., ROSENBERG, H. C. AND BOISSE, N. R.: Withdrawal characteristics following chronic pentobarbital dosing method. Eur. J. Pharmacol. **40:** 107–119, 1976.

RANDALL, L. O. AND KAPPELL, B.: Pharmacological activity of some benzodiazepines and their metabolites. *In* The Benzodiazepines, ed. by S. Garrattini, E. Mussini and L. O. Randall, pp. 27–51, Raven Press, New York, 1973.

ROSENBERG, H. C. AND OKAMOTO, M.: A method for producing maximal pentobarbital dependence in cats: Dependency characteristics. *In* Drug Addiction: Neurobiology and Influences on Behavior, ed. by J. M. Singh and H. Lal, vol. 3, pp. 89–103, Miami Symposia Specialists, Miami, 1974.

THOMPSON, W. L., JOHNSON, A. D. AND MADDREY, W. L.: Diazepam and paraldehyde for treatment of severe delirium tremens: A controlled trial. Ann. Intern. Med. **82:** 175–180, 1975.

VICTOR, M.: Treatment of alcoholic intoxication and the withdrawal syndrome. Psychosom. Med. **28:** 636–650, 1966.

VICTOR, M. AND ADAMS, R. D.: The effects of alcohol on the nervous system. Res. Publ. Assoc. Res. Nerv. Ment. Dis. **32:** 526–593, 1953.

WIKLER, A., FRASER, H. F., ISBELL, H. AND PESCOR, F. T.: Electroencephalograms during cycles of addiction to barbiturates in man. Electroencephalogr. Clin. Neurophysiol. **7:** 1–13, 1955.

WIKLER, A., PESCOR, F. T., FRASER, H. F. AND ISBELL, H.: Electroencephalographic changes associated with chronic alcoholic intoxication and the alcohol abstinence syndrome. Am. J. Psychiatry **113:** 106–114, 1956.

WOO, E. AND GREENBLATT, D. J.: Massive benzodiazepine requirements during acute alcohol withdrawal. Am. J. Psychiatry **136:** 821–823, 1979.

YANAGITA, T. AND TAKAHASHI, S.: Dependence liability of several sedative-hypnotic agents evaluated in monkeys. J. Pharmacol. Exp. Ther. **185:** 307–316, 1973.

ZAR, J. H.: Biostatistical Analysis, Prentice-Hall, Englewood Cliffs, N J., 1975.

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