Pharmacology Biochemistry and Behavior, Vol. 63, No. 3, pp. 473–479, 1999

0091-3057/99/$–see front matter

PII S0091-3057(99)00017-9

# Genetic Determinants of Severity of Acute Withdrawal From Diazepam in Mice: Commonality With Ethanol and Pentobarbital

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Received 28 August 1998; Revised 11 December 1998; Accepted 22 December 1998

METTEN, P. AND J. C. CRABBE. *Genetic determinants of severity of acute withdrawal from diazepam in mice: Commonality with ethanol and pentobarbital.* PHARMACOL BIOCHEM BEHAV **63**(3) 473–479, 1999.—Potentially life-threatening seizures can occur following withdrawal from benzodiazepines, ethanol, or barbiturates. In animals, withdrawal severity has been shown to be partially genetically determined for each drug class. Susceptibility to these drugs is partially determined by common genetic factors, but the evidence is conflicting. We tested the hypothesis that acute benzodiazepine withdrawal convulsions are influenced by at least some genes that also affect withdrawal from ethanol and pentobarbital. Results in inbred mouse strains demonstrate that strain susceptibility is genetically correlated with susceptibility to ethanol and pentobarbital. The proportion of variance accounted for by genetic factors common to diazepam and ethanol was estimated at 69%. Results contrast with previous data obtained in mice that were serially tested for withdrawal severity from ethanol, pentobarbital, and then diazepam, because serial testing of mice significantly affected the previous results for some strains. Diazepam withdrawal severity was also genetically correlated with pentobarbital withdrawal. Together, these results suggest that some genes influence severity of withdrawal from several types of depressant drugs. © 1999 Elsevier Science Inc.

Benzodiazepines Diazepam Ethanol Flumazenil Genetic correlation Genetic determinants
Handling-induced convulsions Inbred strains Mouse Pentobarbital Pharmacogenetics Withdrawal

SEIZURES are a potentially life-threatening consequence of alcohol withdrawal that is common to all species studied, including mice and humans (27,36). Ethanol, barbiturates, and benzodiazepines produce many common signs and symptoms upon withdrawal, including convulsions, suggesting that they share many mechanisms (24,26,33–35,45,55). Crosstolerance and -dependence among benzodiazepines, ethanol, and barbiturates has been shown in rats (23,38–41,43,46,52,54) and mice (10,12–14). Furthermore, crosstolerance and -dependence among these drugs is suggested by the fact that ethanol and barbiturate withdrawal episodes are commonly treated in humans using benzodiazepines (45,53). Benzodiazepines and barbiturates can also reduce ethanol withdrawal convulsions in rodents (17,29).

In most studies, genes affecting withdrawal from ethanol appear to exert influences on withdrawal from other central nervous system depressant drugs, an effect known as genetic pleiotropism. Several studies in selectively bred mouse lines have supported this relationship for barbiturates and benzodiazepines (4,5,20,49). Selectively bred lines are developed by testing genetically heterogeneous animals on the trait of interest and then mating together extreme-scoring animals. Divergence in opposite directions of the lines high and low on the selection trait over generations of selection is conclusive evidence that the trait is genetically influenced. During selection, genes influencing the trait become homozygously fixed, but remaining genes (i.e., those not influencing the trait) continue to segregate according to Mendelian law (25). Differential sensitivity of the lines on a nonselected trait is evidence of pleiotropic influences of the genes fixed by selection (i.e., correlated response to selection) (21). Withdrawal Seizure-Prone (WSP) mice were shown to have more severe diazepam, pentobarbital, and phenobarbital withdrawal than their Withdrawal Seizure-Resistant (WSR) counterparts (4,5,20), although

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they were selectively bred only for differential withdrawal convulsion severity following chronic ethanol inhalation (19). This is good evidence of genetic correlation among these traits (21).

Another method of estimating genetic correlations among ethanol- and benzodiazepine-withdrawal severities is to test a number of inbred strains for withdrawal severity from each drug and correlate strain means (32). Inbred strains are developed by systematic inbreeding, commonly brother/sister matings, over 20 or more generations (25). Therefore, like-sex members of any particular inbred strain are genetically identical (16,47). Consequently, individual differences in responses within an inbred strain must be due to environmental influences, while differences among several inbred strains can be attributed to genetic factors, given equivalent testing procedures. The utility of inbred strains and methodological considerations regarding their use in pharmacogenetic research have been discussed in detail elsewhere (3,21,22,47). Use of relatively large panels of inbred strains ($\geqslant$12 strains) is recommended when attempting to ascertain genetic correlations between responses, because each strain represents a single genotype (i.e., the genetic sample size equals the number of strains being tested).

Acute ethanol, pentobarbital, and precipitated diazepam-withdrawal severities were previously assessed in 15 inbred strains (50). Consistent with findings in selectively bred mice (20,49), a genetic correlation was demonstrated between ethanol- and pentobarbital-withdrawal severities, and between pentobarbital and diazepam withdrawal. Surprisingly, no significant correlation between ethanol and diazepam withdrawal severity strain means was found (50). However, the mice were serially tested for withdrawal from ethanol, pentobarbital, and then diazepam at 1-week intervals, so an effect of repeated testing could not be ruled out. The purpose of the present experiment was to reexamine the hypothesis that some of the same genes confer susceptibility to diazepam, ethanol, and pentobarbital acute withdrawal convulsions (i.e., after a single injection) (50).

## METHOD

### *Subjects*

Adult male mice (52–67-days-old at the time of testing) from 14 of the 15 inbred strains tested in our previous study (50) were used in this experiment. The following inbred strains were available in sufficient numbers for testing: 129/J, A/HeJ, AKR/J, BALB/cJ, C3H/HeJ, C57BL/6J, C57BR/cdJ, C57L/J, CBA/J, DBA/1J, DBA/2J, PL/J, SJL/J, and SWR/J. Mice were purchased from the Jackson Laboratory, Bar Harbor, ME, and housed by strain, three to four animals per polycarbonate cage ($28 \times 17 \times 11.5$ cm) and allowed at least 1 week to acclimate. Cages were lined with corn cob bedding and cleaned twice weekly. The colony was maintained on a 12 L:12 D cycle (lights on at 0600 h), and food and water were available ad lib. Colony and testing room temperatures were maintained at 22 $\pm$ 1.5°C. During the experiment, food and water were available ad lib and lights remained on.

From the previous diazepam withdrawal data in these same inbred strains, we predicted that a minimum sample size of four to six per drug group would be adequate to produce reliable means (split-half reliability = 0.93) (50). Therefore, sample sizes for each drug group were five to six animals.

### *Drugs*

Drugs were freshly mixed the morning of the experiment. Diazepam and flumazenil (Ro 15-1788) were a gift of Dr. Edward Gallaher. Doses employed were 20 mg/kg diazepam and 10 mg/kg flumazenil, injected in a 10-ml/kg volume. The vehicle for both diazepam and flumazenil contained 0.125 g/ml 2-hydroxypropyl-β-cyclodextrin (Research Biochemicals Incorporated) in 0.9% physiological saline.

### *Handling-Induced Convulsion Scoring*

The HIC scale used in the present studies has been published (15), and was modified from that of Goldstein (28). Convulsions are rated on a scale from 0 (absent) to 7 (violent tonic–clonic convulsion resulting from cage disturbance). Each mouse was picked up by the tail and observed for convulsive signs. If no signs were present within 2 s, the mouse was spun gently by the tail through a 180–360° arc and again observed. A score was assigned based on the specific convulsive sign and whether spinning was required to elicit a convulsion. For example, a tonic convulsion when lifted by the tail only was assigned a score of 4, while the same convulsion elicited by a spin was assigned a score of 2. Most scores in the current study were in the moderate range (0–4).

### *Experimental Procedures*

Experimental procedures were identical to those employed to study diazepam withdrawal in our previous experiment (50), except that animals were naive at the time of testing. The experiment commenced at 0730 h, with starting times staggered for each pair of cages (eight animals). Animals were assessed first for baseline HIC and weighed. After approximately 20 min, a second baseline HIC was scored. Immediately thereafter, a group of animals was injected within 1 min; half of the animals of each strain were injected with diazepam and half with vehicle. HICs were scored at 30 and 55 min following injection with diazepam or vehicle to establish that HICs were depressed in the diazepam groups. Sixty minutes after the first injection, all animals were injected with flumazenil. Withdrawal HICs were scored 1, 3, 5, 8, and 12 min later.

This cycle of testing was repeated for the next eight mice, and so on, with the injection times staggered. Strain order of testing was randomized so that some animals of each strain were tested at several times across a 4-h period, thus minimizing any gross circadian effects on testing. Because the maximum number of mice that could be tested on 1 day was about 60, approximately four naive mice per strain were tested on each of 3 days.

### *Data Analyses*

Withdrawal severity scores were calculated as the peak score [average of the three highest consecutive scores; (28)] minus the average vehicle group score over the same time points. Peak, rather than area, scores were employed to index withdrawal since the time course for diazepam is in minutes rather than hours, and is in unequal intervals between HIC assessments. Correction for vehicle group scores is necessary because flumazenil exerts a small, strain-dependent anticonvulsant effect on HIC (50). These scores were subjected to ANOVA (strain). The proportion of total phenotypic variance in peak drug withdrawal accounted for by genetic factors was estimated as the sum of squares for the between-groups factor (strain) divided by the total sum of squares (37).

Correlational analyses using Pearson's $r$ were performed to determine whether inbred strain mean acute withdrawal severities for diazepam in naive mice were genetically correlated with the previously collected scores (50). A reanalysis of the genetic correlations among ethanol, pentobarbital, and di-

azepam withdrawal severities was also performed including means from the present diazepam data set. Statistical significance for the tests of genetic correlation was based on a two-tailed test, with $\alpha = 0.05$. The percentage of common phenotypic variance accounted for by genetic factors was estimated as the square of the correlation coefficient (25). Split-half reliabilities of withdrawal scores and interdrug genetic correlations were calculated using the Spearman–Brown correction and the correction for attenuation, respectively (48).

## RESULTS

Results are shown in Fig. 1A and B. As we had seen previously, the inbred strains differed significantly in diazepam

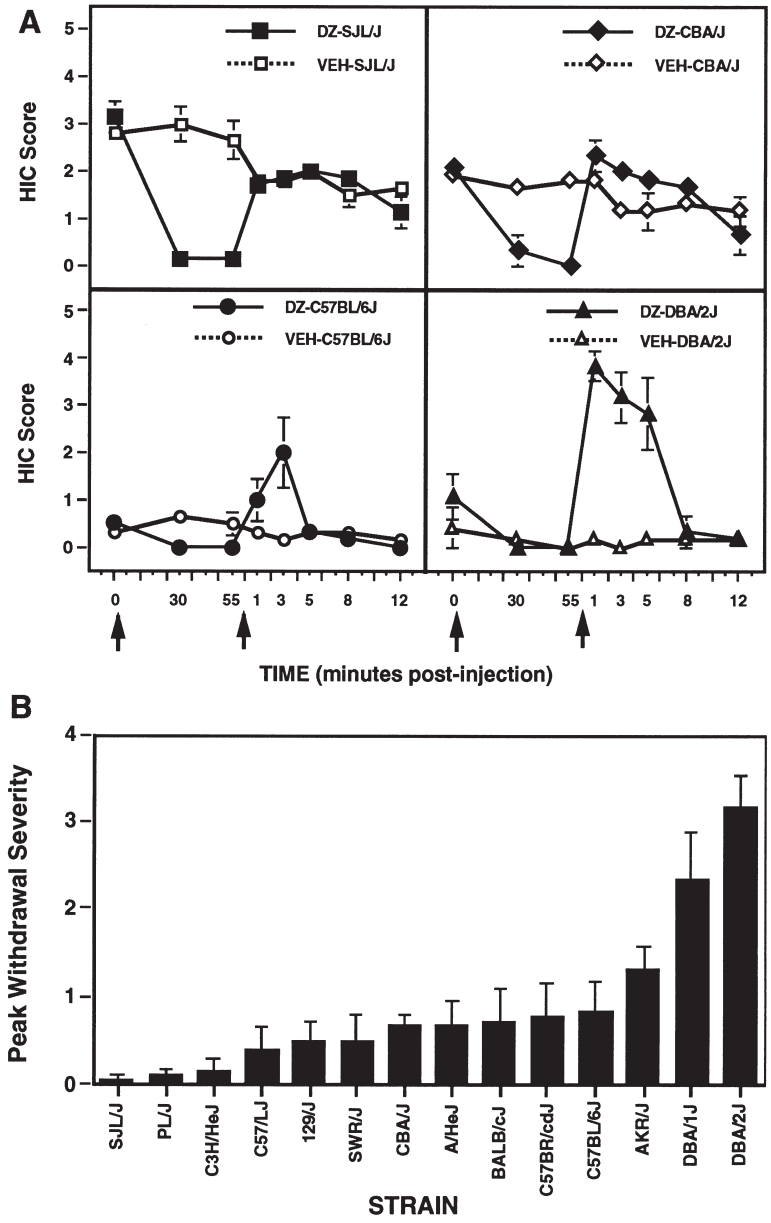


FIG. 1. (A) Time courses of precipitated diazepam withdrawal in 4 of 14 inbred strains. Symbols represent mean ± SEM for each strain. Standard error bars not shown are smaller than the symbol. Y-axes: handling-induced convulsion (HIC) score. X-axes: time, in minutes, following injection. Diazepam (DZ) or vehicle (VEH) injection occurred at the first (left) arrows, immediately following predrug baseline HIC assessments. Flumazenil was injected at the second (right) arrows (at 60 min). Closed symbols represent the diazepam-treated animals. (B) Rank-ordered diazepam withdrawal severity in 14 inbred strains. X-axis: inbred strains, rank ordered by withdrawal severity. Y-axis: strain mean peak withdrawal severity, calculated as discussed in the text. Error bars represent SEM. Strains differed significantly in withdrawal severity, $F(13, 71) = 8.91$, $p < 0.01$.

withdrawal severity (50). The time courses of withdrawal for four representative strains are depicted in Fig. 1A. The inbred strains are known to differ considerably in basal HIC severity (18,50), and a significant main effect of strain on baseline HIC was detected, $F(13, 140) = 31.05$, $p < 0.01$; range of strain mean values: 0–4. As expected, treatment groups within strain did not differ with respect to baseline HICs. In all strains having baseline HICs greater than 0, diazepam depressed HIC scores at 30 and 55 min following injection. Flumazenil injection restored HIC severity in the diazepam-treated animals to near baseline or higher levels in all strains. The modal peak withdrawal time for all strains was either 1 or 3 min; therefore, the average of the first three time points was used as the index of peak HIC severity.

Analysis of the vehicle group data revealed significant differences among strains for the sum of the postflumazenil HIC scores, $F(13, 69) = 24.17$, $p < 0.01$. This finding confirmed the need to control for strain differences in response to flumazenil. Therefore, for each vehicle-treated animal, the mean of the first three postflumazenil HIC scores was determined, and the strain mean vehicle scores were calculated. To index diazepam withdrawal, the appropriate vehicle group strain mean was subtracted from the peak HIC score for each individual animal in the diazepam/flumazenil group. Strains differed significantly, $F(13, 71) = 8.91$, $p < 0.01$, in diazepam withdrawal severity (Fig. 1B). As shown in Fig. 1A and B, some strains (e.g., SJL/6J—solid squares, and CBA/J—solid diamonds) had insignificant or slight withdrawal from diazepam. Other strains (e.g., C57BL/6J—solid circles, and DBA/2J—solid triangles) had moderate to severe withdrawal from diazepam. The proportion of variance accounted for by genetic factors (broad-sense heritability) was 0.62 in this experiment.

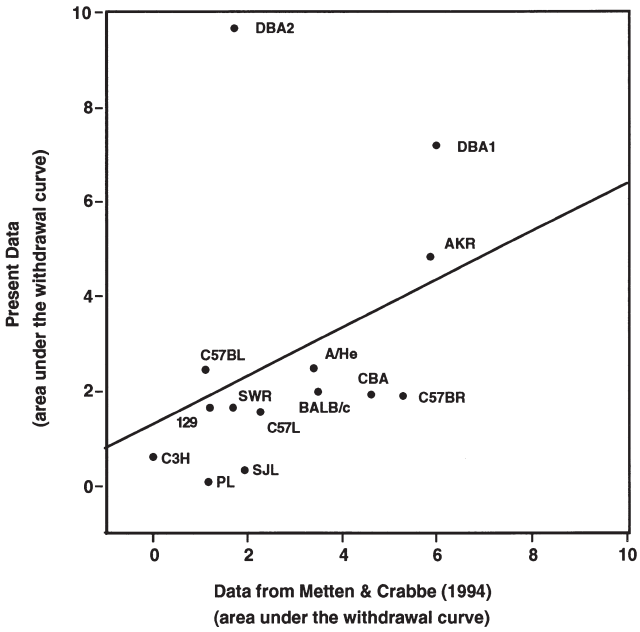


FIG. 2. Scatterplot and line of least-squares regression of diazepam withdrawal severity strain means form Metten and Crabbe [(50); X-axis] and the present data set (Y-axis). Withdrawal severities in both studies were calculated as the between-groups area under the curve, as discussed in the text. Labeled symbols represent inbred strain means. Withdrawal severities between the two studies were not significantly genetically correlated ($r_G = 0.368$). Withdrawal severity strain means were found to be significantly genetically correlated when the outlier DBA/2J strain data were removed from the analysis, $r(11) = 0.74$, $p < 0.01$.

### *Comparison of Present With Previous Data*

As discussed above, the previous diazepam withdrawal data were collected in the same inbred strains, plus one additional strain, CE/J, which was not available for testing at this time; however, those animals were tested serially for withdrawal from ethanol, pentobarbital, and diazepam (50). Withdrawal from ethanol and pentobarbital was assessed over a 12-h period (not minutes, as with precipitated diazepam withdrawal). In the earlier study, diazepam withdrawal was calculated as the area under the curve of the diazepam-treated animals minus the strain mean area of the vehicle treated animals (i.e., between groups). Diazepam withdrawal was also calculated the same way as for ethanol and pentobarbital withdrawal (i.e., within subjects). These two measures of diazepam withdrawal were significantly genetically correlated ($r = 0.79$) (50).

The present and previous data were first compared by recalculating the present withdrawal severity means using the between-groups area measure. Peak and area withdrawal severities were significantly genetically correlated, $r(12) = 0.99$, $p < 0.01$, and strains differed significantly in withdrawal severity using the area measure, $F(13, 71) = 8.76$, $p < 0.01$. The scatterplot and least-squares regression line representing the genetic correlation between the present and previous withdrawal area data are shown in Fig. 2. The apparent lack of correlation between the two data sets (Fig. 2), $r(12) = 0.37$, $p = 0.20$, seemed to be due to one outlier strain, DBA/2J. To address the issue of whether the withdrawal severity scores of any strains were significantly different between the two experiments, the data were subjected to an experiment × strain ANOVA. As expected, there was a significant main effect of Strain, $F(13, 146) = 7.66$, $p < 0.01$, and a significant experiment × strain interaction, $F(13, 146) = 3.98$, $p < 0.01$. The main effect of the experiment was not significant, $F(1, 146) = 0.08$, $p = 0.78$. The significant interaction was pursued by simple main effects analyses (37). DBA/2J had significantly higher scores in the present experiment, $F(1, 146) = 39.42$, $p < 0.01$. Two other strains, C57BR/cdJ and CBA/J, had significantly lower scores in the present experiment [both $F$s(1, 146) $> 7.66$, $p$s $< 0.01$]. Despite a reduction in power, the correlation between the previous and present data sets without these strains was nearly significant, $r(9) = 0.58$, $p = 0.06$. The correlation was significant when omitting the most obviously affected DBA/2J strain, $r(11) = 0.74$, $p < 0.01$.

### *Genetic Correlations Among Diazepam, Ethanol, and Pentobarbital*

Figures 3A and B show the scatterplots and lines of least-squares regression of the genetic correlations of the present diazepam withdrawal severity mean area scores with those previously collected for ethanol and pentobarbital, respectively (50). In contrast to previous findings, diazepam- and ethanol-withdrawal severity scores were significantly genetically correlated, indicating that there is substantial overlap in genes influencing acute withdrawal from these two drugs, $r(12) = 0.83$, $p < 0.01$ [see (50)]. Additionally, diazepam-withdrawal severity scores correlated significantly with corresponding strain mean pentobarbital-withdrawal severities, in agreement with previous findings, $r(12) = 0.75$, $p < 0.01$. The

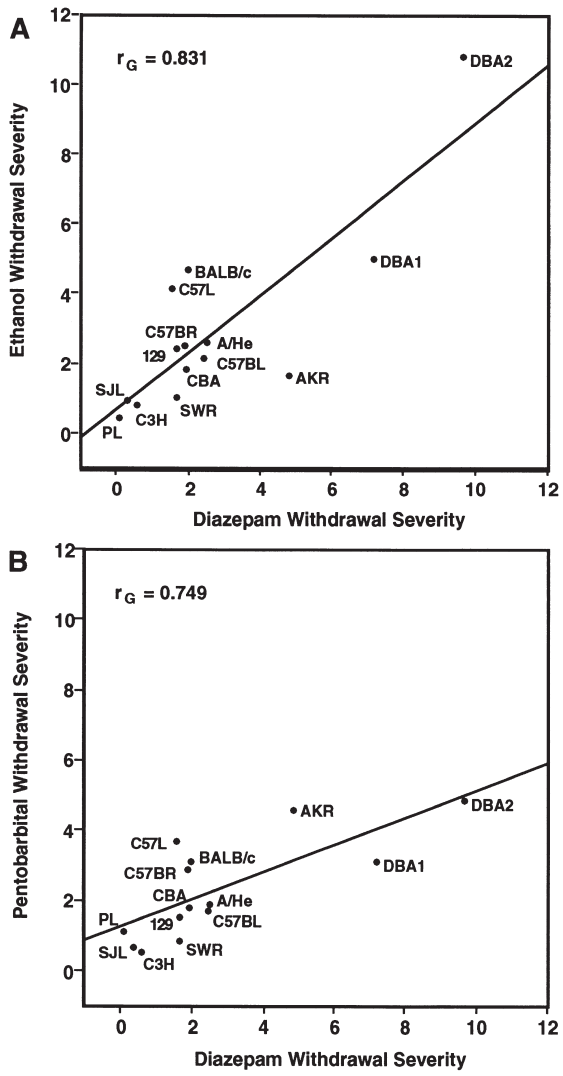


FIG. 3. Scatterplots and lines of least squares regression of strain means showing genetic correlation of withdrawal severity scores of diazepam (present data; Y-axes) with (A) ethanol [(50); Y-axis] and (B) pentobarbital [(50); Y-axis]. Labeled symbols represent inbred strain means. $r_G$, the Pearson's correlation coefficient, is shown for each relationship. (A) Ethanol and diazepam withdrawal severities were significantly genetically correlated ($p < 0.01$). (B) Pentobarbital and diazepam withdrawal severities were significantly genetically correlated ($p < 0.01$).

proportions of phenotypic variance accounted for by common genetic factors were 0.69 and 0.56, respectively.

Correlations were also calculated after excluding the DBA/2J strain, because it had an extremely high score for ethanol withdrawal (50), and because the data for this strain were not reliable between the two experiments (Fig. 2). Removal of the DBA/2J strain from the current experiment did not affect the conclusions regarding the genetic correlations of diazepam with ethanol and pentobarbital withdrawal severities. Diazepam and ethanol withdrawal severities remained significantly genetically correlated, $r(11) = 0.58$, $p < 0.05$, as did diazepam- and pentobarbital-withdrawal severity scores, $r(11) = 0.64$, $p < 0.05$. Without DBA/2J, the proportions of variance accounted for by common genetic factors were 0.33 and 0.40, respectively.

*Data Reliability Assessment*

The current diazepam withdrawal severity scores, calculated as the peak corrected for vehicle treatment, were examined for split-half reliability. Animals were pseudorandomly assigned to one of two groups, A or B, for data reanalysis. This yielded sample sizes of three animals per strain per drug group. Strain means within each half were calculated for the average vehicle score and split-half correlations were performed using Pearson's $r$ (i.e., the strain means for half A were correlated with the strain means for half B). Vehicle group ($r = 0.96$, reliability = 0.98, $p < 0.01$) and diazepam scores ($r = 0.88$, reliability = 0.93, $p < 0.01$) were significantly reliable. The "true" genetic correlations of diazepam with ethanol and pentobarbital withdrawal severities were estimated according to McNemar [(48), p. 153]. Using the correction for attenuation, the diazepam/ethanol correlation ($r = 0.91$) and the diazepam/pentobarbital correlation ($r = 0.88$) were significant (both $p$s $< 0.01$).

## DISCUSSION

The results of this study provide strong evidence that there are common genetic determinants of diazepam, ethanol, and pentobarbital withdrawal convulsions. There is substantial evidence to suggest that there should be a common neural mechanism underlying withdrawal from these three types of drugs. One receptor system known to mediate some of the effects of all three of these drugs is the GABA/benzodiazepine receptor/chloride ionophore complex (GRC). Besides distinct binding sites for GABA and benzodiazepines, the GRC has binding sites for many compounds, including neuroactive steroids, barbiturates, competitive and noncompetitive antagonists, and zinc ions (56). Functional similarity among these drugs is shown by the fact that in vitro GABA-stimulated chloride flux is enhanced by ethanol, benzodiazepines, and barbiturates (31,51). Brain region-specific changes in GRC subunit mRNA levels have been shown by chronic ethanol or pentobarbital treatment in vivo (9,44), implying that these drugs may regulate neural function by affecting GRC constituency.

Comparison of the present results with those collected in our earlier study suggested that there may have been effects of previous testing (i.e., for ethanol and pentobarbital withdrawal) on diazepam withdrawal in some strains (50). Particularly, the DBA/2J strain seemed to have lower withdrawal scores with repeated testing. The reason for this is not clear. Others have shown that C3H/HeJ inbred and outbred Swiss–Webster mice have significantly greater ethanol withdrawal scores with subsequent ethanol treatment/withdrawal episodes when fewer than 12 h separate the exposure periods (1,2,30). However, Goldstein (30) noticed that Swiss–Webster mice had withdrawal seizures that were no more, or less, severe after four cycles of ethanol exposure when 24 h separated the exposure periods. Belknap et al. (7) found that DBA/2J mice had fewer seizures if 24-h periods separated three 3-day cycles of phenobarbital exposure admixed in the diet. All of these studies only examined withdrawal seizures after all of the exposure/rest cycles were completed, used independent groups as controls, and used chronic drug-exposure regimens. In contrast, our study tested the mice for acute withdrawal

seizure severity at 1-week intervals, once after each drug (50). Regardless of the reason, the present results strongly suggest that repeated testing of the same animals for CNS depressant drug withdrawal should be avoided in studies examining genetic susceptibility to withdrawal from a single drug.

It is also possible that previous testing for ethanol withdrawal severity affected the pentobarbital withdrawal results, but we do not believe ethanol had an important effect (50). We examined the data for effects of previous testing by analyzing the predrug baseline scores within animal for differences across drugs. Significant effects of previous testing on baseline scores were found for six strains. Of these, three strains had significantly different (i.e., lower) baseline scores prior to pentobarbital withdrawal compared to their preethanol scores. There was no effect on the ethanol/pentobarbital withdrawal correlation ($r = 0.69$, $p < 0.02$) when these three strains were removed from the analysis, arguing that the pentobarbital withdrawal scores obtained were not appreciably different than would have been obtained had the animals been tested when naive. Thus, the data now consistently support significant genetic correlations among these three drugs.

As noted, Withdrawal Seizure Prone (WSP) mice show severe withdrawal convulsions following acute (i.e., single administration) and/or chronic administration of several alcohols, barbiturates, nitrous oxide, and several benzodiazepines, including diazepam (4–6,20,42,49). WSR mice, selectively bred in parallel for minimal ethanol withdrawal severity, are generally resistant to withdrawal convulsions from these drugs. These data also support genetic commonality of ethanol, pentobarbital, and diazepam withdrawal.

Recently, another set of lines was selectively bred starting from an $F_2$ intercross of C57BL/6J and DBA/2J inbred strains. High (HAW) and Low Alcohol Withdrawal (LAW) were tested following a single hypnotic dose of ethanol (4 g/kg). Results in these lines provide independent confirmation of the data obtained in WSP and WSR mice and those reported here in inbred strains; namely, ethanol, pentobarbital, and diazepam withdrawal convulsion severities are genetically correlated (49). In addition to providing confirmation of the correlation of diazepam and ethanol withdrawal, these lines also differed in precipitated and spontaneous withdrawal from zolpidem, an imidazopyridine having agonist properties at the benzodiazepine receptor (49).

Similarly bred lines selected for High (HPW) and Low Pentobarbital Withdrawal (LPW) following a single hypnotic dose of pentobarbital (60 mg/kg) have been tested for acute ethanol and diazepam withdrawal. HPW mice had significantly greater ethanol withdrawal than LPW mice after only two generations of selective breeding (Belknap et al., in preparation). HPW mice also had greater diazepam withdrawal than LPW mice by the fourth selected generation.

Finally, no lines of mice have been selectively bred for benzodiazepine withdrawal severity to date, making the final symmetrical test of the hypothesis impossible at present. However, taken together, these studies strongly imply that the induction of convulsions by withdrawal from acute or chronic ethanol, benzodiazepines, and barbiturates occurs, at least in part, via a common genetic mechanism.

We are currently testing the set of RI strains derived from the cross of C57BL/6J and DBA/2J mice for diazepam withdrawal severity as part of our efforts to map genes involved in CNS-depressant drug withdrawal (Gallaher et al., unpublished). We plan to correlate strain mean diazepam withdrawal scores with those collected for ethanol and pentobarbital withdrawal in these same strains (8,11). The genetic correlation between ethanol and pentobarbital withdrawal in the BXD RI strains was 0.79 ($p < 0.001$) (8), in good agreement with the results found in inbred strains ($r = 0.70$, $p < 0.004$) (50).

The RI strain gene mapping data can also be used to look for quantitative trait loci (QTLs) for each drug that fall within the same chromosome regions. Such QTLs would imply that there are genes that affect withdrawal from CNS-depressant drugs in general. In fact, there may be such genes residing in three chromosomal locations [(11); (8), in preparation]. Studies are in progress to identify the specific genes that may mediate these withdrawal-related responses.

ACKNOWLEDGEMENTS

This study was supported by National Institute on Alcohol Abuse and Alcoholism Grants AA10760, AA06243, National Institute on Drug Abuse Grant DA05228, and a grant from the Department of Veterans Affairs. Pamela Metten was supported by National Institute on Drug Abuse Training Grant T32 DA 07262. We thank Sue Burkhart-Kasch for superb technical assistance, and Dr. Edward J. Gallaher for the drugs.

## REFERENCES

1. Becker, H. C.: Positive relationship between the number of prior ethanol withdrawal episodes and the severity of subsequent withdrawal seizures. Psychopharmacology (Berlin) 116:26–32; 1994.
2. Becker, H. C.; Diaz-Granados, J. L.; Hale, R. L.: Exacerbation of ethanol withdrawal seizures in mice with a history of multiple withdrawal experience. Pharmacol. Biochem. Behav. 57:179–183; 1997.
3. Belknap, J. K.: Genetic factors in the effects of alcohol: Neurosensitivity, functional tolerance and physical dependence. In: Rigter, H.; Crabbe, J. C., Jr., eds. Alcohol tolerance and dependence. Amsterdam: Elsevier/North-Holland Biomedical Press; 1980:157–180.
4. Belknap, J. K.; Crabbe, J. C.; Laursen, S. E.: Ethanol and diazepam withdrawal convulsions are extensively codetermined in WSP and WSR mice. Life Sci. 44:2075–2080; 1989.
5. Belknap, J. K.; Danielson, P. W.; Lame, M.; Crabbe, J. C.: Ethanol and barbiturate withdrawal convulsions are extensively codetermined in mice. Alcohol 5:167–171; 1988.
6. Belknap, J. K.; Laursen, S. E.; Crabbe, J. C.: Ethanol and nitrous oxide produce withdrawal-induced convulsions by similar mechanisms in mice. Life Sci. 41:2033–2040; 1987.
7. Belknap, J. K.; Ondrusek, G.; Berg, J.; Waddingham, S.: Barbiturate dependence in mice: Effects of continuous vs. discontinuous drug administration. Psychopharmacology (Berlin) 51:195–198; 1977.
8. Buck, K. J.; Metten, P.; Belknap, J. K.; Crabbe, J. C.: Quantitative trait loci affecting risk for pentobarbital withdrawal map near alcohol withdrawal loci on mouse chromosomes 1, 4, and 11. Mammalian Genome 10: in press.
9. Buck, K. J.: Molecular genetic analysis of the role of GABAergic systems in the behavioral and cellular actions of alcohol. Behav. Genet. 26:313–323; 1996.
10. Buck, K. J.; Heim, H.; Harris, R. A.: Reversal of alcohol dependence and tolerance by a single administration of flumazenil. J. Pharmacol. Exp. Ther. 257:984–989; 1991.
11. Buck, K. J.; Metten, P.; Belknap, J. K.; Crabbe, J. C.: Quantitative trait loci involved in genetic predisposition to acute alcohol withdrawal in mice. J. Neurosci. 17:3946–3955; 1997.

12. Chan, A. W. K.; Langan, M. C.; Leong, F. W.; Penetrante, M. L.; Schanley, D. L.: Partial cross-dependence on ethanol in mice dependent on chlordiazepoxide. Pharmacol. Biochem. Behav. 35:379–384; 1990.
13. Chan, A. W. K.; Langan, M. C.; Leong, F. W.; Schanley, D. L.; Penetrante, M. L.: Does chronic ethanol intake confer full cross-tolerance to chlordiazepoxide? Pharmacol. Biochem. Behav. 30:385–389; 1988.
14. Chan, A. W. K.; Schanley, D. L.; Aleo, M. D.; Leong, F. W.: Cross-tolerance between ethanol and chlordiazepoxide. Alcohol 2:209–213; 1985.
15. Crabbe, J.; Kosobud, A.: Alcohol withdrawal seizures: Genetic animal models. In: Porter, R. J.; Mattson, R. H.; Cramer, J. A.; Diamond, I., eds. Alcohol and seizures. Philadelphia: F. A. Davis Company; 1990:126–139.
16. Crabbe, J. C.: Genetic animal models in the study of alcoholism. Alcohol. Clin. Exp. Res. 13:120–127; 1989.
17. Crabbe, J. C.: Antagonism of ethanol withdrawal convulsions in Withdrawal Seizure Prone mice by diazepam and abecarnil. Eur. J. Pharmacol. 221:85–90; 1992.
18. Crabbe, J. C.; Janowsky, J. S.; Young, E. R.; Rigter, H.: Handling induced convulsions in twenty inbred strains of mice. Subst. Alcohol Actions/Misuse 1:159–163; 1980.
19. Crabbe, J. C.; Kosobud, A.; Young, E. R.; Tam, B. R.; McSwigan, J. D.: Bidirectional selection for susceptibility to ethanol withdrawal seizures in *Mus musculus*. Behav. Genet. 15:521–536; 1985.
20. Crabbe, J. C.; Merrill, C.; Belknap, J. K.: Acute dependence on depressant drugs is determined by common genes in mice. J. Pharmacol. Exp. Ther. 257:663–667; 1991.
21. Crabbe, J. C.; Phillips, T. J.; Kosobud, A.; Belknap, J. K.: Estimation of genetic correlation: Interpretation of experiments using selectively bred and inbred animals. Alcohol. Clin. Exp. Res. 14:141–151; 1990.
22. Deitrich, R. A.; Spuhler, K.: Genetics of alcoholism and alcohol actions. In: Smart, R. G.; Cappell, H. D.; Glazer, R. B., Israel, Y., Kalant, H., Popham, R., Schmidt, W., Sellers, E. M., eds. Research advances in alcohol and drug problems, vol 8. New York: Plenum Press; 1984:47–98.
23. Draski, L. J.; Deitrich, R. A.; Ménez, J.-F.: Phenobarbital sensitivity in HAS and LAS rats before and after chronic administration of ethanol. Pharmacol. Biochem. Behav. 57:651–657; 1997.
24. Edwards, J. G.; Cantopher, T.; Olivieri, S.: Benzodiazepine dependence and the problems of withdrawal. Postgrad. Med. J. 66:S27–S35; 1990.
25. Falconer, D. S.; MacKay, T. F. C.: Introduction to quantitative genetics, 4th ed. London: Longman; 1996.
26. Fraser, H. F.; Wikler, A.; Isbell, H.; Johnson, N. K.: Partial equivalence of chronic alcohol and barbiturate intoxications. Q. J. Stud. Alcohol 18:541–551; 1957.
27. Friedman, H. J.: Assessment of physical dependence on and withdrawal from ethanol in animals. In: Rigter, H.; Crabbe, J. C., Jr., eds. Alcohol tolerance and dependence. Amsterdam: Elsevier/North-Holland Biomedical Press; 1980:93–121.
28. Goldstein, D. B.: Relationship of alcohol dose to intensity of withdrawal signs in mice. J. Pharmacol Exp. Ther. 180:203–215; 1972.
29. Goldstein, D. B.: An animal model for testing effects of drugs on alcohol withdrawal reactions. J. Pharmacol. Exp. Ther. 183:14–22; 1972.
30. Goldstein, D. B.: Rates of onset and decay of alcohol physical dependence in mice. J. Pharmacol. Exp. Ther. 190:377–383; 1974.
31. Grant, K. A.: Emerging neurochemical concepts in the actions of ethanol at ligand-gated ion channels. Behav. Pharmacol 5:383–404; 1994.
32. Hegmann, J.; Possidente, B.: Estimating genetic correlations from inbred strains. Behav. Genet. 11:103–114; 1981.
33. Isbell, H.; Altschul, S.; Kornetsky, C. H.; Eisenman, A. J.; Flanary, H. G.; Fraser, H. F.: Chronic barbiturate intoxication. Arch. Neurol. Psychiatry 64:1–28; 1950.
34. Isbell, H.; Fraser, H. F.; Wikler, A.; Belleville, R. E.; Eisenman, A. J.: An experimental study of the etiology of "rum fits" and delirium tremens. Q. J. Stud. Alcohol 16:1–33; 1955.
35. Jaffe, J. H.; Ciraulo, D. A.: Drugs used in the treatment of alcoholism. In: Mendelson, J. H.; Mello, N. K., eds. The diagnosis and treatment of alcoholism, 2nd ed. New York: McGraw-Hill Book Company; 1985:355–389.
36. Kalant, H.: Alcohol withdrawal syndromes in the human: Comparison with animal models. In: Gross, M., ed. Alcohol intoxication and withdrawal, vol. IIIb. New York: Plenum Press; 1977:57–64.
37. Keppel, G.: Design and analysis: A researcher's handbook, 3rd ed. Englewood Cliffs, NJ: Prentice Hall, Inc.; 1991.
38. Khanna, J. M.; Kalant, H.; Chau, A.; Shah, G.: Rapid tolerance and crosstolerance to motor impairment effects of benzodiazepines, barbiturates, and ethanol. Pharmacol. Biochem. Behav. 59:511–519; 1996.
39. Khanna, J. M.; Kalant, H.; Weiner, J.; Shah, G.: Rapid tolerance and cross-tolerance as predictors of chronic tolerance and cross-tolerance. Pharmacol. Biochem. Behav. 41:355–360; 1992.
40. Khanna, J. M.; Lê, A. D.; Kalant, H.; Chau, A.; Shah, G.: Effect of lipid solubility on the development of chronic cross-tolerance between ethanol and different alcohols and barbiturates. Pharmacol. Biochem. Behav. 57:101–110; 1997.
41. Kim, C. K.; Pinel, J. P. J.; Roese, N. R.: Bidirectional contingent cross tolerance between the anticonvulsant effects of pentobarbital and ethanol. Pharmacol. Biochem. Behav. 41:127–132; 1991.
42. Kosobud, A.; Crabbe, J. C.: Ethanol withdrawal in mice bred to be genetically prone or resistant to ethanol withdrawal seizures. J. Pharmacol. Exp. Ther. 238:170–177; 1986.
43. Lê, A. D.; Khanna, J. M.; Kalant, H.; Grossi, F.: Tolerance to and cross-tolerance among ethanol, pentobarbital and chlordiazepoxide. Pharmacol. Biochem. Behav. 24:93–98; 1986.
44. Lin, L.-H.; Wang, L.-H.: Region-specific changes in GABAA receptor δ subunit mRNA level by tolerance to and withdrawal from pentobarbital. Neurosci. Lett. 202:149–152; 1996.
45. Litten, R. Z.; Allen, J. P.: Pharmacotherapies for alcoholism: Promising agents and clinical issues. Alcohol. Clin. Exp. Res. 15:620–633; 1991.
46. Lytle, D. A.; Egilmez, Y.; Rocha, B. A.; Emmett-Oglesby, M. W.: Discrimination of ethanol and of diazepam: Differential cross-tolerance. Behav. Pharmacol. 5:451–460; 1994.
47. McClearn, G. E.: The tools of pharmacogenetics. In: Crabbe, J. C.; Harris, R. A., eds. The genetic basis of alcohol and drug actions. New York: Plenum Press; 1991;1–23.
48. McNemar, Q.: Psychological statistics, 3rd ed. New York: John Wiley & Sons, Inc.; 1966.
49. Metten, P.; Belknap, J. K.; Crabbe, J. C.: Drug withdrawal convulsions and susceptibility to convulsants after short-term selective breeding for acute ethanol withdrawal severity. Behav. Brain Res. 95:113–122; 1998.
50. Metten, P.; Crabbe, J. C.: Common genetic determinants of severity of acute withdrawal from ethanol, pentobarbital and diazepam in inbred mice. Behav. Pharmacol. 5:533–547; 1994.
51. Mihic, S. J.; Harris, R. A.: Alcohol actions at the GABAA receptor/chloride channel complex. In: Deitrich, R. A.; Erwin, V. G., eds. Pharmacological effects of ethanol on the nervous system. Boca Raton, FL: CRC Press; 1996:51–72.
52. Mihic, S. J.; Kalant, H.; Liu, J.-F.; Wu, P. H.: Role of the γ-aminobutyric acid receptor/chloride channel complex in tolerance to ethanol and cross-tolerance to diazepam and pentobarbital. J. Pharmacol. Exp. Ther. 261:108–113; 1992.
53. Miller, N. S.: Pharmacotherapy in alcoholism. J. Addict. Dis. 14:23–46; 1995.
54. Naruse, T.; Asami, T.: Cross-dependence on ethanol and pentobarbital in rats reinforced on diazepam. Arch. Int. Pharmacodyn. Ther. 304:147–162; 1990.
55. Sellers, E. M.: Alcohol, barbiturate and benzodiazepine withdrawal syndromes: Clinical management. Can. Med. Assoc. J. 139:113–120; 1988.
56. Sieghart, W.: $GABA_A$ receptors: Ligand-gated $Cl^-$ ion channels modulated by multiple drug-binding sites. Trends Pharmacol. Sci. 13:446–450; 1992.