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Effects of Dizocilpine in Withdrawal Seizure-Prone (WSP) and Withdrawal Seizure-Resistant (WSR) Mice

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CRABBE, J., E. R. YOUNG AND J. DOROW. *Effects of dizocilpine in Withdrawal Seizure-Prone (WSP) and Withdrawal Seizure-Resistant (WSR) mice.* PHARMACOL BIOCHEM BEHAV 47(3) 443–450, 1994.—Mice selectively bred to be Withdrawal Seizure-Prone (WSP) or Seizure-Resistant (WSR) after chronic ethanol administration have been reported to be differentially sensitive to the anticonvulsant and proconvulsant effects on alcohol withdrawal of drugs interacting with glutamate receptors. Several behavioral effects of the noncompetitive glutamate receptor antagonist, dizocilpine, were determined in WSP and WSR mice to see whether their differential sensitivity generalized to effects unrelated to seizures, and to see whether it was only apparent during ethanol withdrawal. Dizocilpine potentiated the loss of righting reflex induced by ethanol, and dose-dependently stimulated habituated and nonhabituated open field activity. WSP and WSR mice were equally sensitive to these effects of dizocilpine. Pretreatment with dizocilpine increased the transcorneal amperage necessary to produce maximal electroshock seizures: WSR mice were more sensitive than WSP to this effect. Ethanol withdrawal (i.e., testing 6 h after a 24-h exposure to ethanol vapor) and dizocilpine had several effects on mice tested in the hole-in-wall apparatus. Several differences between WSP and WSR mice were also found, but in no case did dizocilpine differentially affect ethanol-withdrawing WSP and WSR mice. Across these experiments, differences between WSP and WSR mice in response to dizocilpine were rather specific. For some responses, WSP and WSR mice were equally sensitive, but only in the seizure-related measure assessed were naive WSR mice more sensitive than WSP. Since naive WSR mice are also more sensitive to NMDA-induced convulsions than WSP, these data are consistent with the hypothesis that alterations in the function of excitatory amino acid-gated ion channels are important for the convulsions accompanying withdrawal from ethanol dependence.

Selected mouse lines MK-801 Excitatory amino acids Ethanol withdrawal Pharmacogenetics
WSP WSR Seizures Anticonvulsant Dizocilpine

IT has recently been suggested that a combination of decreased function in inhibitory γ-aminobutyric acid (GABA) pathways and increased activity at *N*-methyl-D-aspartate (NMDA) receptors may underly the hyperexcitability seen during ethanol withdrawal (20). Freed and Michaelis (19) administered ethanol to mice by vapor inhalation for 72 h to induce physical dependence. Dependent mice were more sensitive than controls to seizures induced by kainic acid, but not pentylenetetrazole (PTZ), and a glutamate antagonist reduced withdrawal seizures. We recently reported similar results in mice of the Withdrawal Seizure-Prone selected line acutely withdrawing from a single 4 g/kg ethanol injection (12). C57BL/6 mice showed increased numbers of hippocampal dizocilpine (MK-801) binding sites after chronic administration of ethanol in a liquid diet (22,23). Upregulation has also been reported in cerebellar granule cells in vitro (27). Some signs of ethanol withdrawal can be inhibited by dizocilpine or the noncompetitive inhibitor, (±)-5-aminocarbonyl-10,11-dihydro-5H-dibenzo[*a*,*d*]cyclohepten-5,10-imine (ADCI), or exacerbated by NMDA (21,22,37).

Genetic animal models are rather extensively used in studies to elucidate ethanol's mechanism of action (5). One such model has recently been employed in studies with the NMDA complex (25). We have selectively bred lines of mice to be

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Withdrawal Seizure-Prone (WSP) or Withdrawal Seizure-Resistant (WSR) after chronic ethanol vapor inhalation (9). Other neuropharmacological and behavioral traits found to differ in both replicated WSP and WSR lines can be assumed to be controlled by some or all of the genes underlying withdrawal severity (13). Naive WSP mice were reported to have significantly more dizocilpine binding sites than WSR mice in hippocampus, while there was no difference between naive WSP and WSR mice in cortical binding (40). However, a recent study has been unable to replicate this finding (1). Chronic treatment with an ethanol liquid diet increased dizocilpine binding in both WSP and WSR mice to an equivalent extent (40). When WSP and WSR mice were treated for 24 h by ethanol vapor inhalation, no difference between lines, and no increase in dizocilpine binding, was seen, although this treatment produced a significant withdrawal reaction in WSP mice (1). The severity of handling-induced convulsions in WSP and WSR mice after doses of drugs too small to elicit other seizure types can be used to index small differences in seizure susceptibility. WSP mice were more sensitive than WSR mice to the effects on handling-induced convulsions of all compounds from a wide range tested (11), and WSP mice were equally or slightly more sensitive than WSR mice to seizures induced by several convulsants given intraperitoneally or by tail vein infusion (7).

Ethanol induces changes in central nervous system excitability even several hours after a single high-dose treatment (36). WSP mice show a rebound elevation in handling-induced convulsions 6–10 h after an acute injection of 4 g/kg ethanol (32). Acutely withdrawing WSP mice are more sensitive than naive WSP mice to the effect of NMDA, but not PTZ, to elevate handling-induced convulsions (4,12). Furthermore, acutely withdrawing WSP mice are more sensitive than naive WSP to the anticonvulsant effects of dizocilpine (12), but not diazepam (4,12).

The noncompetitive glutamate receptor antagonist, dizocilpine, exerts a spectrum of sedative behavioral effects in rodents (18,31). Dizocilpine potentiates ethanol-induced loss of righting reflex (15). It stimulates locomotor activity in mice (35) and rats (38), and produces ataxia and stereoptypies at higher doses (24,35). It has been reported to attenuate the development of rapid tolerance to ethanol-induced hypothermia (28–30). As discussed above, it inhibits alcohol withdrawal convulsions (12,16,21,22,37), in addition to its other anticonvulsant effects (2,39). These effects are presumed to depend upon interactions with an active NMDA-gated ion channel, where the binding site for dizocilpine is thought to reside.

The evidence reviewed above suggests that alterations in glutamatergic function may be an important part of the neuropharmacological adaptation to chronic ethanol treatment, and/or accompany subsequent withdrawal hyperexcitability. Since WSP and WSR mice have been specifically bred to express high and low withdrawal convulsions after chronic ethanol, it was reasonable to see whether WSP mice were more sensitive than WSR mice to dizocilpine, using several behavioral responses known to be sensitive to dizocilpine. WSR mice do not show measurable handling-induced convulsions during acute ethanol withdrawal (32). Therefore, the lines could not easily be compared for sensitivity of the handling-induced convulsion to agents affecting glutamatergic systems. In the current studies, we tested WSP and WSR mice for dizocilpine sensitivity, using as end points the ethanol-induced loss of righting reflex; open-field activity; susceptibility to maximal electroshock seizures; and several behaviors affected by withdrawal from acute or short-term chronic ethanol.

METHOD

Subjects

WSP and WSR mice were bred in our laboratory in Portland, OR. Drug-naive adult male or female male mice (age range 50 to 100 days) from selected generation 26 (filial generations G_{39}–G_{41}) were used. The WSP/WSR selective breeding experiment is replicated (9); thus, there are two independently derived WSP lines and two WSR lines. Roughly equal numbers (dependent upon availability) of mice of each WSP line and each WSR line were tested unless otherwise noted. In a given experiment, mice of a given genotype were randomly assigned to experimental groups. Mice were maintained under a 12 L : 12 D cycle of 0600–1800 light; testing was performed starting at 0900–1400 h.

Drug Source and Preparation

Dizocilpine was a gift of Dr. Aaron Janowsky. It was dissolved in saline. Ethanol was prepared as a 20% (v/v) solution in saline. Both drugs were administered IP.

Blood Ethanol Determinations

A 20 μl blood sample was drawn from the tail. Each sample was processed and assayed using previously published methods (3,6).

Statistical Analyses

In initial analyses, selected line, replicate (i.e., WSP-1 and WSR-1 vs. WSP-2 and WSR-2), and appropriate factors related to drug dosing were included. To simplify analyses, and to avoid having to interpret four-way interactions, if no interpretable effects of replicate were found, data were collapsed across this factor; if there were important differences between replicates, data were analyzed separately for each replicate. Post hoc tests were performed using simple main effects, or Newman–Keuls analysis, as appropriate (41).

Experiment 1

Female mice were weighed and injected with saline or dizocilpine (1 mg/kg). Thirty minutes later, all mice were given ethanol (3.5 g/kg). As soon as possible, each mouse was placed on its back in a V-shaped trough, and latency to lose righting reflex was recorded to the nearest second. Any mouse failing to lose righting reflex within 3 min was deleted from the experiment. When a mouse righted itself twice within 30 s, time to regain righting reflex was recorded, and a 20 μl blood sample was taken from the tip of the tail.

Experiment 2

To see whether dizocilpine differentially influenced locomotor activity in WSP and WSR mice, female mice were tested for 1 h in Omnitech open field apparatus housed in sound-attenuating chambers. Mice were injected with saline or dizocilpine (0.1, 0.2, 0.4, or 0.8 mg/kg) and placed immediately into the open field. Infrared beam interruptions were recorded automatically each 5 min. The apparatus floor was wiped clean with 70% ethanol after each mouse.

Experiment 3

The results of Experiment 2 were difficult to interpret due to differences in baseline (saline) activity between WSP and WSR mice (see the Results section). We therefore sought to

eliminate those differences by prior habituation of the mice to the open field apparatus before dizocilpine administration. Groups of female mice were given saline injections and tested for 1 h for 4 consecutive days. On the fifth day, half the mice were given saline and half 0.4 mg/kg dizocilpine, and all mice were tested for 1 h.

Experiment 4

Effects of dizocilpine on activity might only be different in mice undergoing ethanol withdrawal. In this experiment, we administered groups of male mice (female mice were unavailable) an injection of saline or ethanol (4 g/kg). Six hours after the initial injection, at a time when WSP mice are known to display increased handling-induced convulsion severity (10), mice were given either saline or dizocilpine (0.4 mg/kg), placed into the open fields, and tested for 1 h.

Experiment 5

To see whether the WSP and WSR lines differed in sensitivity to the anticonvulsant effects of dizocilpine, we administered separate groups of 16 male mice saline or dizocilpine (0.4 mg/kg, IP). Thirty minutes later, mice were administered transcorneal electroshock (ECS) with a fixed, 200-ms pulse duration, as previously reported (14). ECS of varying amperage (10.4–59.7 mA) was given to determine the CA_{50}, the amperage that would induce a tonic hindlimb extensor (THE) seizure in 50% of the mice in that group. The Up-and-Down method was used (17). If a mouse responded with a THE seizure, the next mouse in that group was given a lower amperage. If a mouse failed to respond, the next was given a higher amperage. Each mouse was euthanized by cervical dislocation immediately after testing.

Experiment 6

Groups of 15–16 female mice were given saline or ethanol (2.4 g/kg for WSR, 2.0 g/kg for WSP) and were placed in an inhalation chamber. Ethanol vapor was maintained at 15 mg/l air; control mice were placed in a chamber where only air was circulated. Details of the apparatus and general procedures for inducing dependence by inhalation have been published (8). After 24 h of inhalation, mice were removed from the chamber and a 10 μl blood sample was drawn from the tip of the tail. Samples from ethanol-treated mice were subsequently analyzed by gas chromatography; control samples were discarded. Five and one-half hours after removal, half the mice of each genotype and treatment group combination were injected with saline, and half were given dizocilpine (0.4 mg/kg). Starting 6 h after removal (i.e., 30 min after injection), mice were tested in the hole-in-wall apparatus for 5 min. The hole-in-wall test has been used in mice to demonstrate several signs of withdrawal following chronic ethanol treatment (26). This apparatus consists of a square box, 31.5 × 31.5 × 17 cm high. Interior walls running from corner to corner divide the box into four triangular-shaped compartments, alternately painted black or white. A 4-cm hole in the wall connects each pair of compartments. Each mouse is initially placed into a white compartment. During the test, several variables are scored: latency to enter a black compartment for the first time; total time spent in black compartments; number of headpokes through a hole without crossing; total number of crossings; and number of rears (lifting both forepaws off the floor). The apparatus floor was wiped clean with 70% ethanol after each mouse.

RESULTS

Experiment 1

Sensitivity was indexed using two measures, duration of loss of righting reflex and blood alcohol level upon recovery: these were analyzed with separate three-factor ANOVAs (line × replication × drug). Table 1 shows the results of Experiment 1. The main effect of dizocilpine was significant, $F(1, 86) = 314.2$, $p < 0.0001$, reflecting a potentiation of the ethanol-induced duration of loss of righting reflex. The main effect of replicate was also significant: collapsed over treatment groups, WSP-1 and WSR-1 mice were less sensitive than their counterparts in replicate 2, $F(1, 86) = 8.0$, $p < 0.01$. Both the replicate × line and replicate × drug interactions were also significant ($p < 0.01$). Dizocilpine potentiation was also seen as a reduction in the blood alcohol level at which animals regained righting reflex, $F(1, 87) = 14.3$, $p < 0.001$. Collapsed over treatment groups, replicate 1 mice were less sensitive than replicate 2, $F(1, 87) = 5.6$, $p < 0.05$, but no other effects reached significance. Since no effects involving line and drug were significant for either measure, we conclude that the WSP and WSR mice were equally sensitive to dizocilpine in this experiment.

Experiment 2

Total activity counts were analyzed with a three-factor ANOVA (selected line × replicate × dose). The main effect of replicate, its interaction with selected line, and the three-way interaction were all statistically significant. Therefore, the data were analyzed in separate two-way ANOVAs for each replicate, and results are presented in Fig. 1 (panels a and b) separately for each selected line. In replicate 1, there was no significant difference between WSP and WSR mice ($F < 1$), but the main effect of dose was highly significant, $F(4, 52) = 7.1$, $p < 0.0001$. Post hoc analyses revealed that significant stimulation of activity was produced by both the 0.2 and 0.4 mg/kg doses ($p < 0.05$ and 0.001, respectively), but not the 0.8 mg/kg dose. In replicate 2 mice, a slightly different result was obtained. The main effects of selected line (WSR > WSP), $F(1, 56) = 12.8$, $p < 0.001$, dose, $F(4, 56) = 15.7$,

TABLE 1

EFFECT OF DIZOCILPINE ON ETHANOL-INDUCED LOSS OF RIGHTING REFLEX

Selected Line	Pretreatment	Duration of Loss (min)	Blood Ethanol at Recovery (mg/ml)
WSP-1	Saline	46.9 ± 6.3	2.81 ± 0.24
	Dizocilpine	208.1 ± 26.6	2.07 ± 0.16
WSR-1	Saline	24.6 ± 4.1	2.92 ± 0.17
	Dizocilpine	143.6 ± 4.4	2.59 ± 0.06
WSP-2	Saline	37.5 ± 4.7	2.66 ± 0.16
	Dizocilpine	172.5 ± 10.0	2.52 ± 0.12
WSR-2	Saline	55.2 ± 9.2	2.97 ± 0.14
	Dizocilpine	159.0 ± 11.4	2.57 ± 0.18

Groups of 11–12 mice were given saline or 1 mg/kg dizocilpine. Thirty minutes later, all mice were given 3.5 g/kg ethanol. Dizocilpine significantly enhanced sensitivity to ethanol-induced loss of righting reflex (i.e.; increased duration of loss of righting reflex, and decreased blood ethanol levels at recovery), but the WSP and WSR mice were equally sensitive. For statistical results, see text. Means ± SE are shown.

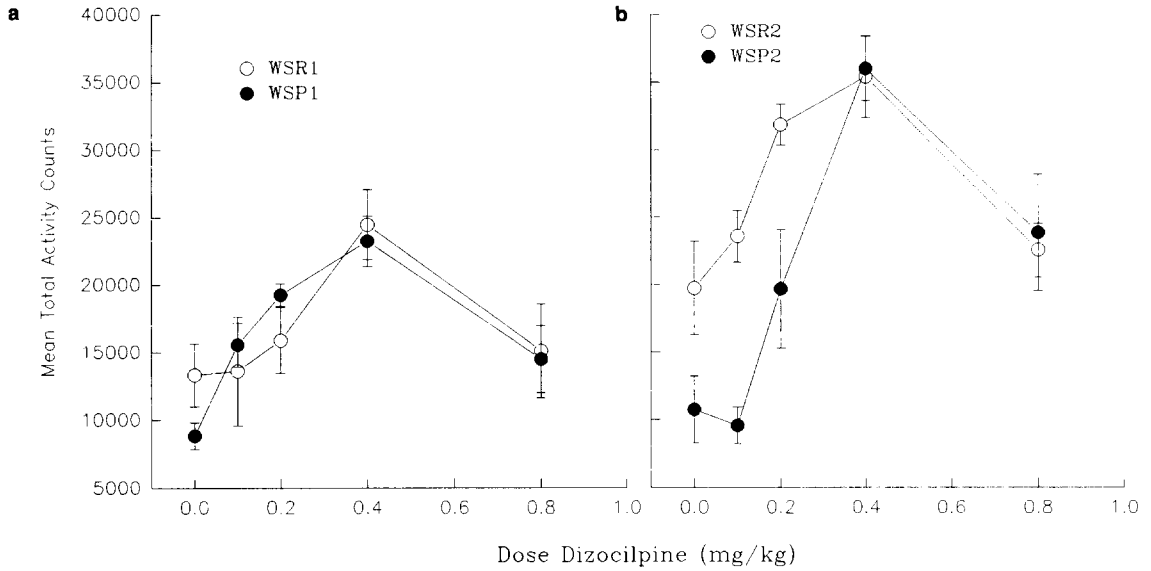

FIG. 1. Results of Experiment 2 for groups of five to seven mice at each dose are shown. WSP-1 and WSR-1 (left), WSP-2 and WSR-2 (right). Mean ± SE shown: absent SE bars are smaller than symbol size. Dizocilpine induced a biphasic stimulant response in all selected lines. For statistical results, see text.

$p < 0.0001$, and their interaction, $F(4, 56) = 3.1$, $p < 0.05$, were all significant. Analyses of simple main effects showed that there were significant effects of dose in both selected lines (WSR-2, $p < 0.01$; WSP-2, $p < 0.0001$). WSR-2 mice had higher activity than WSP-2 mice at the zero, 0.1, and 0.2 mg/kg doses ($p < 0.05$, 0.01, and 0.01, respectively), but at the higher doses, the selected lines did not differ ($F < 1$).

Since the WSP-2 and WSR-2 mice differed significantly when given saline, we also examined these data as a percent increase over saline-treated groups. The increase over saline was 19%, 61%, 79%, and 14% for WSR-2 mice given 0.1, 0.2, 0.4, and 0.8 mg/kg dizocilpine, respectively. The equivalent increases for WSP-2 mice were −11%, 83%, 235% and 122%, respectively. Although WSP mice appeared to be more sensitive to dizocilpine than WSR mice in terms of percent increase in activity, the pattern of activity over time (data not shown) suggested another interpretation. WSP mice treated with saline or the ineffective (0.1 mg/kg) dose of dizocilpine showed a steadily declining level of activity over the hour-long test, while their WSR counterparts did not. Thus, the apparent difference between WSP-2 and WSR-2 in stimulation, which was most pronounced late during the activity test (data not shown), could have been due to differences in habituation in the saline groups, rather than in sensitivity to dizocilpine.

Experiment 3

Analyses revealed significant effects of replicate, but since they did not affect interpretation, results are presented collapsed on this factor. On the first daily test, WSP mice displayed significantly less activity than WSR mice, $F(1, 62) = 9.3$, $p < 0.01$. Groups that were eventually to be treated differentially with dizocilpine or saline did not differ significantly, or interact with selected line ($F < 1$). During the 4 days of habituation to saline, WSP mice displayed a steady decrease in activity, suggestive of habituation, while WSR mice remained highly active (data not shown). On the test day (day 5: see Fig. 2), saline-treated WSP and WSR mice showed greatly attenuated activity in the open field compared to their day 1 scores. Dizocilpine stimulated activity significantly, $F(1, 62) = 84.9$, $p < 0.0001$; neither the main effect of selected line or its interaction with drug treatment were significant ($F < 1.4$).

Experiment 4

Initial analyses of total activity during the 1-h test period revealed only a significant effect of replicate, $F(1, 73) = 4.0$, $p = 0.05$, and a significant stimulant effect of dizocilpine treatment, $F(1, 85) = 100.7$, $p < 0.0001$. Examination of Fig. 3 suggests that any effects due to ethanol were rapidly overcome by the strong stimulant effect of dizocilpine. To explore these data further, we analyzed the 5-, 10-, and 15-min

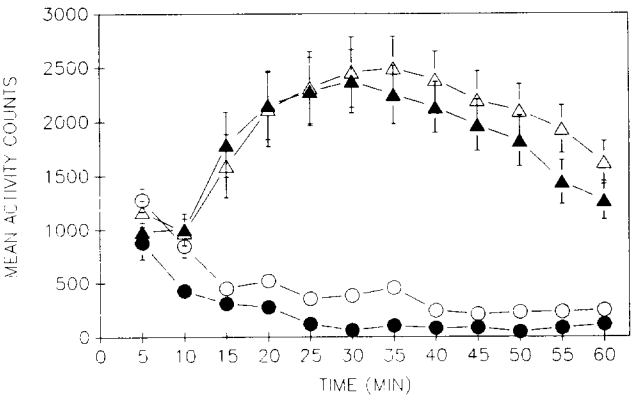

FIG. 2. Mean ± SE activity on test day 5 for groups of 16–18 mice, previously habituated for 4 days, given saline (circles) or 0.4 mg/kg dizocilpine (triangles) and tested in the open field. WSP: dark symbols. WSR: open symbols. For statistical analyses, see text.

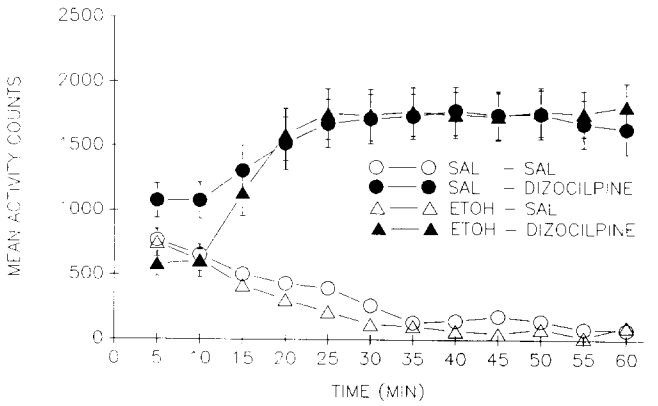

FIG. 3. Mean ± SE activity for groups of 20-24 mice given saline or 0.4 mg/kg dizocilpine. Absent SE were smaller than symbol size. Half the groups were tested during withdrawal from an acute 4 g/kg injection of ethanol; the other half were given saline. For statistical analyses, see text.

time points in separate ANOVAs. Although replicate differences were significant in some analyses, this factor did not interact significantly with other factors. Selected lines did not differ for any analysis. Therefore, results shown in Fig. 3, and reported below, are for data collapsed over the factors selected line and replicate. During the first 5 min, there was a significant reduction in activity of approximately 30% due to ethanol withdrawal, $F(1, 85) = 6.0$, $p < 0.05$, and this interacted significantly with the effect of dizocilpine, $F(1, 85) = 5.1$, $p < 0.05$. Although dizocilpine significantly stimulated saline-treated mice ($F = 4.4$, $p < 0.05$), it had no effect in mice previously given ethanol ($F = 1.2$). The reduced activity in ethanol-withdrawing mice was significant in mice also given dizocilpine ($F = 11.4$, $p < 0.001$), but not in mice given saline ($F < 1$). Results were similar at 10 min. By 15 min, only the stimulant effect of dizocilpine was significant ($F = 287.3$, $p < 0.0001$).

Experiment 5

Results of Experiment 5 are given in Fig. 4. Replicates did not differ, so they were pooled for analysis. Saline-treated WSP and WSR mice had equivalent seizure thresholds. Dizocilpine significantly ($p < 0.05$) elevated ECS thresholds in both selected lines. The increase was 75% for the WSP mice. WSR mice were much more sensitive: their ECS threshold was elevated over 450% by dizocilpine treatment.

Experiment 6

Results of Experiment 6 are summarized in Table 2: only significant main effects and interactions are tabled. The individual variables scored in the hole-in-wall apparatus were subjected to separate ANOVAs. Preliminary analyses including the factor replicate showed some significant effects, but the pattern of results was unaffected by replicate lines. Consequently, to simplify presentation and analysis, all results are collapsed over replicate. Exposure to ethanol vapor for 24 h led to similar, relatively low, blood ethanol levels in the selected lines ($F < 1$). Latency to enter a dark compartment was unaffected by selected line and did not appear as a sign of ethanol withdrawal. However, the main effect of dizocilpine treatment was to significantly increase latency, $F(1, 119) = 5.0$, $p < 0.05$. Animals spent about 60% of the time in dark compartments, but no main effects or interactions were significant (all $F < 2$), so these data are not reported. The number of head pokes was significantly reduced in animals withdrawing from ethanol, $F(1, 119) = 4.9$, $p < 0.05$. WSP and WSR mice responded differentially to dizocilpine on this variable, $F(1, 119) = 9.3$, $p < 0.01$. Saline-treated WSP mice had more head pokes than WSR. WSP mice responded to dizocilpine with decreased pokes, while WSR mice showed increases. However, the line differences in response to dizocilpine were not significantly different in naive and ethanol-withdrawing groups.

All three main effects were significant for total compartment crossings. WSR crossed more than WSP, $F(1, 119) = 17.2$, $p < 0.001$, and withdrawing mice crossed less than ethanol-naive mice, $F(1, 119) = 112.7$, $p < 0.0001$. Dizocilpine reduced crossing to 25% of control levels, $F(1, 119) = 4.3$, $p < 0.05$. None of the interactions achieved significance. The three main effects for rears were also significant. However, the effects of selected line (WSR > WSP) and ethanol condition (EtOH > air) were more pronounced ($p < 0.001$) than that of dizocilpine condition (dizocilpine < saline; $p < 0.05$). The interaction of selected line and ethanol treatment was marginally significant, $F(1, 119) = 3.7$, $p = 0.05$: ethanol withdrawal increased rearing more in WSR than in WSP mice.

DISCUSSION

These results provide clear evidence that WSP and WSR mice do not differ in sensitivity to all effects of dizocilpine. Potentiation of the loss of righting reflex induced by ethanol was equivalent in the lines, and could not be explained by differences in ethanol pharmacokinetics. Taken together, the results of Experiments 2 and 3 also showed no differences between WSP and WSR mice. It is instructive to note that

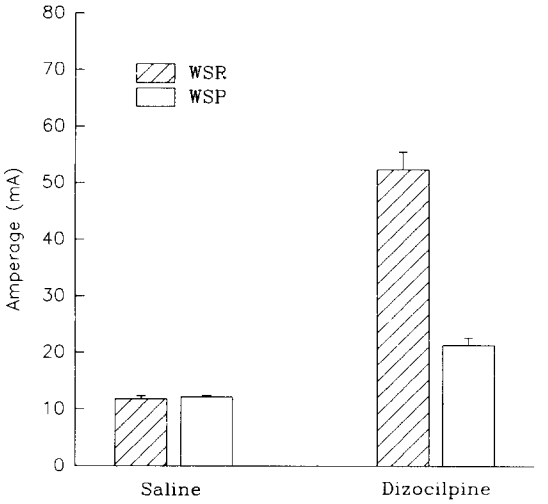

FIG. 4. Mean ± 95% confidence interval for the CA_{50} (convulsant amperage in mA causing a tonic hindlimb extensor seizure in 50% of the mice in a group). Saline: mice pretreated 30 min earlier with saline. Dizocilpine: mice pretreated with 0.4 mg/kg dizocilpine. Where the mean for a group lies outside the confidence interval for another, those groups differ significantly ($p < 0.05$). Confidence intervals are slightly asymmetric about the mean due to the log scale employed to adjust mA administered to the mice (17).

TABLE 2

EFFECT OF DIZOCILPINE ON HOLE-IN-WALL BEHAVIOR IN WSP AND WSR MICE

Selected Line	Treatment	Drug	Response Variable (mean ± SE for significant effects only, except for blood level)
			Blood ethanol level (mg/ml)
WSP			0.62 ± 0.13
WSR			0.45 ± 0.15
			Latency to enter dark (sec)
		Saline	18 ± 3
		Dizocilpine	34 ± 6
			Head pokes
	Air		32 ± 2
	EtOH		26 ± 2
WSP		Saline	32 ± 3
WSP		Dizocilpine	26 ± 2
WSR		Saline	24 ± 3
WSR		Dizocilpine	35 ± 3
			Crossings
WSP			21 ± 3
WSR			35 ± 3
	Air		31 ± 3
	EtOH		25 ± 3
		Saline	45 ± 3
		Dizocilpine	11 ± 2
			Rears
WSP			5 ± 1
WSR			15 ± 3
	Air		5 ± 1
	EtOH		15 ± 2
		Saline	13 ± 2
		Dizocilpine	7 ± 2
WSP	Air		3 ± 1
WSP	EtOH		8 ± 2
WSR	Air		7 ± 3
WSR	EtOH		22 ± 4

Experiment 2 could easily have been misinterpreted if one had simply adopted the usual strategy of examining the magnitude of differences between groups. However, Experiment 3 suggests strongly that a ceiling effect was operative in the second experiment, because against the low basal activity levels in WSP and WSR after 4 habituation days, dizocilpine stimulated the lines to an equivalent degree. Attempts to quantify the magnitude of genotypic differences in sensitivity in psychopharmacological experiments should be undertaken with care. Alternatively, it is possible that the different results seen in Experiments 2 and 3 reflect a difference between the sensitivity of habituated and nonhabituated locomotor activity to dizocilpine treatment.

Using essentially the paradigm employed in Experiment 5, we have tested WSP and WSR mice for sensitivity to the anti-ECS effects of C_1–C_5 straight-chain alcohols, ethchlorvynol, methyprylon, barbital, phenobarbital, pentobarbital, diazepam, valproic acid, and phenytoin (14), as well as carbamazepine (7). For each drug, WSR mice showed at least twofold, and as much as fourfold, greater elevations in ECS seizure thresholds than WSP mice. Dizocilpine had a similar profile. It should be noted that these were male mice, so a sex difference between Experiments 5 (and 4) vs. Experiments 1-3 and 6 must be considered.

Other evidence for differential sensitivity of glutamatergic systems of WSP and WSR mice includes the susceptibility to seizures induced by timed tail vein infusion of convulsants. WSP mice are slightly (10–15%) more susceptible than WSR to seizures induced by picrotoxin, CHEB, and 4-aminopyridine (14). They are also equally or slightly more susceptible to pentylenetetrazole, strychnine, TBPS, bicuculline, DMCM, and kainic acid (Kosobud and Crabbe, unpublished). However, we have recently reported that WSR mice are more sensitive to seizures induced by NMDA (34). Thus, the NMDA sensitivity difference, and Experiment 5 reported here, are consistent with a specific difference in WSP and WSR mice in glutamatergic function related to seizures.

Experiments 4 and 6 confirmed that WSP and WSR mice do not differ in all responses assessed during ethanol withdrawal (32,33). Although withdrawal from acute ethanol in Experiment 4 reduced locomotor activity in an open field during the first few minutes of testing, withdrawing mice appeared to be less sensitive than their naive counterparts to the stimulant effect of dizocilpine on ambulation. Selected lines did not differ, either in withdrawal-induced activity reduction or in sensitivity to dizocilpine.

Experiment 6 used a longer exposure to ethanol (24 h) to induce greater withdrawal than that seen after the single, acute

injection used for Experiment 4. Handling-induced convulsion scores in WSP mice after the acute withdrawal treatment employed in Experiment 4 typically peak at a score of 4 on a scale ranging from 0–7 (10), while 24-h exposure to vapor produces withdrawal scores as high as 6 for a much longer time (unpublished). Ethanol-withdrawing mice displayed fewer headpokes and crossings, but more rears, than mice exposed only to air. WSR mice were more sensitive to ethanol withdrawal-induced rearing than were WSP mice. Dizocilpine generally reduced behaviors in this task. WSP and WSR mice responded differentially to dizocilpine only in the headpoke measure, where the drug reduced pokes in WSP and increased them in WSR. Dizocilpine never interacted with ethanol treatment condition, so this experiment did not show enhanced or reduced sensitivity to dizocilpine during ethanol withdrawal, as was seen for ambulation in Experiment 4. In a separate study of dizocilpine effects on hole-in-wall behaviors during acute ethanol withdrawal (data not reported), we saw almost exactly the same pattern of results reported for Experiment 6. Although we did not directly ascertain withdrawal severity in Experiment 6 by assessing handling-induced convulsions, results of this experiment demonstrate that even a substantial degree of dependence on ethanol was unable to produce a meaningful difference between WSP and WSR mice in sensitivity to dizocilpine on the measures tested, which were unrelated to seizures.

In summary, the WSP and WSR selected lines were equally sensitive to several effects of dizocilpine, even when some of those were assessed during ethanol withdrawal. Naive WSR mice were more sensitive than WSP to the anticonvulsant effects of dizocilpine against maximal electroshock seizures. In this test, WSR mice are more sensitive to all anticonvulsants tested, so the result reported here is not specific to glutamate antagonists. However, in these and other studies, the selected lines have been reported to differ with respect to seizure-related effects of NMDA (4,34), dizocilpine (Experiment 5), and kainic acid (Kosobud and Crabbe, unpublished). Furthermore, WSP mice show enhanced sensitivity of the handling-induced convulsion to NMDA, dizocilpine, and kainic acid during acute ethanol withdrawal, compared to naive WSP mice (4,12). Since the WSP and WSR mice have been genetically selected only on the basis of their differential severity of alcohol withdrawal convulsions, these data taken as a whole lend further support to the hypothesis that alterations in the function of excitatory amino acid-gated ion channels are important for the convulsions accompanying withdrawal from ethanol dependence.

The neurochemical basis for this difference is not clear. While a week of chronic ethanol treatment by liquid diet is clearly accompanied by increases in numbers of dizocilpine receptors in some brain areas (22,23) and in vitro (27), whether naive WSP and WSR mice differ in brain receptor number is not clear (1,40). The greater sensitivity of naive WSR mice to NMDA-induced seizures after tail vein infusion (34) and their greater sensitivity to the anti-ECS effect of dizocilpine (Experiment 5) is consistent with more receptors, or greater receptor sensitivity, in WSR than WSP mice. However, the reported difference in receptor number found WSP to have more receptors in hippocampal tissue than WSR (40). The current studies found no evidence for differential sensitivity of WSP and WSR mice to effects of dizocilpine on responses unrelated to seizures, and WSP mice were not more sensitive to those effects during ethanol withdrawal. The reported upregulation of dizocilpine receptors after chronic ethanol treatment was equivalent in WSP and WSR mice (40), but WSP mice displayed significant withdrawal, while WSR did not, after 24 h of ethanol vapor inhalation, and the lines did not differ in dizocilpine binding (1). It seems likely that the neurochemical basis for the glutamatergic sensitivity differences between WSP and WSR mice must be sought at the level of glutamate release, transporter function, receptor-effector coupling, allosteric interactions with other receptors, or perhaps in the molecular diversity of NMDA receptor subunits. Such studies are in progress in other laboratories.

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