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CCK_B Antagonists Protect Against Some Aspects of the Ethanol Withdrawal Syndrome

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WILSON, J. AND H. J. LITTLE. *CCK_B Antagonists protect against some aspects of the ethanol withdrawal syndrome.* PHARMACOL BIOCHEM BEHAV **59**(4) 967–973, 1998.—Effects of the CCK_B antagonists, CAM1028 and CI988, and the CCK_A antagonist, CAM1481, were studied on the ethanol withdrawal syndrome. When handling-induced behavior was measured hourly for 12 h from withdrawal of ethanol, a small, but significant, protective effect was seen with 3 mg/kg CAM1028, but not with 0.3, 1, or 10 mg/kg. CI988 (0.3 1, 3, or 10 mg/kg), or CAM1481 (0.1 or 1 mg/kg), had no effects. At 16 h from ethanol withdrawal, these ratings were significantly decreased by 3 mg/kg CAM1028 or CI988, but not by lower doses. At 16 h, CAM1481 had very small, but significant, protective effects. At 3 mg/kg, CAM1028, increased the latencies to audiogenic seizures, but had only small effects on convulsion incidence. CAM1481 did not alter the audiogenic convulsions. The decrease in convulsion thresholds to NMDLA, at 16 h from ethanol wtihdrawal, was completely prevented by CAM1028 or CI988, at 1 and at 3 mg/kg, but not by lower doses; CAM1481 had no significant effects. The results suggest change in CCK_B receptors may be involved in the later stages of the ethanol withdrawal syndrome. © 1998 Elsevier Science Inc.

Ethanol CCK Withdrawal syndrome

THE ethanol withdrawal syndrome in humans consists of a variety of different symptoms (2,19). Tremor and anxiety are seen early in the withdrawal period, followed by hallucinations. Increased susceptibility to seizures occurs within 2 days of withdrawal, with a peak incidence between 13 and 24 h. The acute confusional state, known as "delirium tremens," occurs later, about 2–5 days after cessation of alcohol intake (2, 19). A prolonged phase of sleep disturbances and anxiety is also seen, which can last up to 6 months.

Some drugs will decrease the symptoms of withdrawal, such as the benzodiazepines, but these are not selective and possess their own addiction liability, which can result in dependence on these as well as, or instead of, alcohol. The central nervous system possesses two types of high-affinity binding sites for CCK, CCK_A, and CCK_B, and there are selective antagonists for these. Recent work has demonstrated that antagonists of the CCK_B receptor possess anxiolytic activity (11). Unlike the majority of compounds used to treat anxiety, these compounds do not possess sedative activity and are not anticonvulsant. Experimental studies have shown that they do not appear to have dependence liability and do not potentiate the acute actions of alcohol.

Recent studies have suggested that CCK_B receptors may play a role in dependence on sedative/hypnotic drugs. The CCK_B antagonist, CI988, prevented the withdrawal anxiogenesis in mice that followed withdrawal from chronic benzodiazepine treatment (18). This compound also prevented the decrease in convulsion threshold for pentylenetetrazol, which was measured during the withdrawal phase, with no effect on control thresholds.

The present study investigated the effects of the CCK_B antagonists, CAM1028 and CI988 and the CCK_A antagonist, CAM1481, on the range of behaviors that are seen during withdrawal from chronic ethanol treatment. Convulsive changes were measured by ratings of handling-induced behavior, responses to an audiogenic stimulus, and convulsion thresholds by intravenous infusion.

METHOD

Male TO mice were used, obtained from Bantin and Kingman, UK. The weights of the mice ranged from 25 to 35 g, with no more than a 5 g range in any single experiment. They were housed, 10 per cage, at 21 ± 1°C, with a 12 L: 12 D cycle, with the light phase between 0900 to 2100 h.

Production of Physical Dependence on Ethanol

Ethanol was administered in a liquid diet schedule [Dyets, Pennsylvania; (13)]. All mice received the control diet for an initial 3-day period to accustom them to the diet. Ethanol-

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treated mice then received a diet containing 8% v/v ethanol for 7 days. Control groups were pair fed a control diet, balanced isocalorifically to match the ethanol-containing diet (13,14). There were no differences in the weights of the ethanol-treated and control mice at the end of the treatment periods. The amount of ethanol drunk by the groups of mice was measured on days 2 and 5 of the treatment and was as follows: day 2, 27.0 ± 2.0; day 5, 31.0 ± 2.6 g/kg/24 h.

Behavioral Tests

In all the behavioral tests described below, the ratings were carried out by an observer who was unaware of which drug treatment the animals had received.

Measurement of Handling-Induced Convulsive Behavior

Ratings of handling-induced behavior were assessed by the same experimenter, on the same mice after withdrawal from ethanol. This method, widely used in the study of ethanol withdrawal, follows that of Goldstein and Pal (8), modified slightly in our laboratory (9). Each mouse was lifted gently by the tail for three seconds and held below an "Anglepoise lamp" with a 60-watt bulb. The animal was gently rotated, and its ensuing behavior rated on a scale of 0–5 according to the criteria in Table 1. Groups of 10 mice were used in each of the treatment groups, and the data were calculated as medians with interquartile ranges.

The ratings of handling behavior were made in two separate experiments. In the first, mice were withdrawn from the ethanol at 0900 h and provided with tap water, but not food, during the experiments. Ratings were made on the same mice, every hour for a period of 12 h from withdrawal; this covered the period over which hyperexcitability is normally measured in this test (20). CAM1028 or CI988 were injected subcutaneously at 0.3, 1, 3, and 10 mg/kg, and CAM1481 at 0.1 and 1 mg/kg, immediately after withdrawal and at 2 h 20 min from withdrawal.

In the second experiment, mice were withdrawn from the ethanol at 1700 h and provided with the water and control liquid diet until 16 h from withdrawal, when a single set of ratings of handling behavior was made. CAM1028 or CI988 were administered to ethanol-treated animals, subcutaneously, at 0.1, 0.3, 1, and 3 mg/kg, and CAM1481 at 0.1 and 1 mg/kg, immediately after withdrawal and 40 min before testing. Control animals were given CAM1028 or CI988, at 3 mg/kg, or CAM1481 at 1 mg/kg, at the corresponding times (the lower dose of CAM1481 was used because of the limited supply of this compound). Parallel groups of control and ethanol-treated mice were given saline injections at corresponding times.

Elicitation of Audiogenic Seizures

Susceptibility to sound-induced convulsions was measured in separate groups of 12 mice. All mice were withdrawn from the ethanol treatment at 1900 h and provided with tap water until use at 7 h from withdrawal of ethanol, a time at which a clear response is seen to an audiogenic stimulus (20). The animals were tested singly, and each individual was taken to a separate room for testing, out of hearing of the other mice, then an electric doorbell was rung for 60 s at a height of 30 cm above the cage top. The number of mice that responded by wild running and clonic or tonic convulsions was counted, and the latencies to the beginning of these signs measured. The mice were humanely killed as soon as the first signs of a full convulsion were seen. CAM1028 was injected subcutaneously

TABLE 1

RATINGS OF BEHAVIORAL RESPONSES TO GENTLE HANDLING FOR 3 S

1. Mild tremor on lifting and turning
2. Continuous severe tremor on lifting and turning
3. Clonic forelimb extensor spasm on lifting
4. Clonic forelimb extensor spasm on lifting, which continued after placing mouse on cage top
5. Spontaneous evidence of myoclonic activity followed by 4

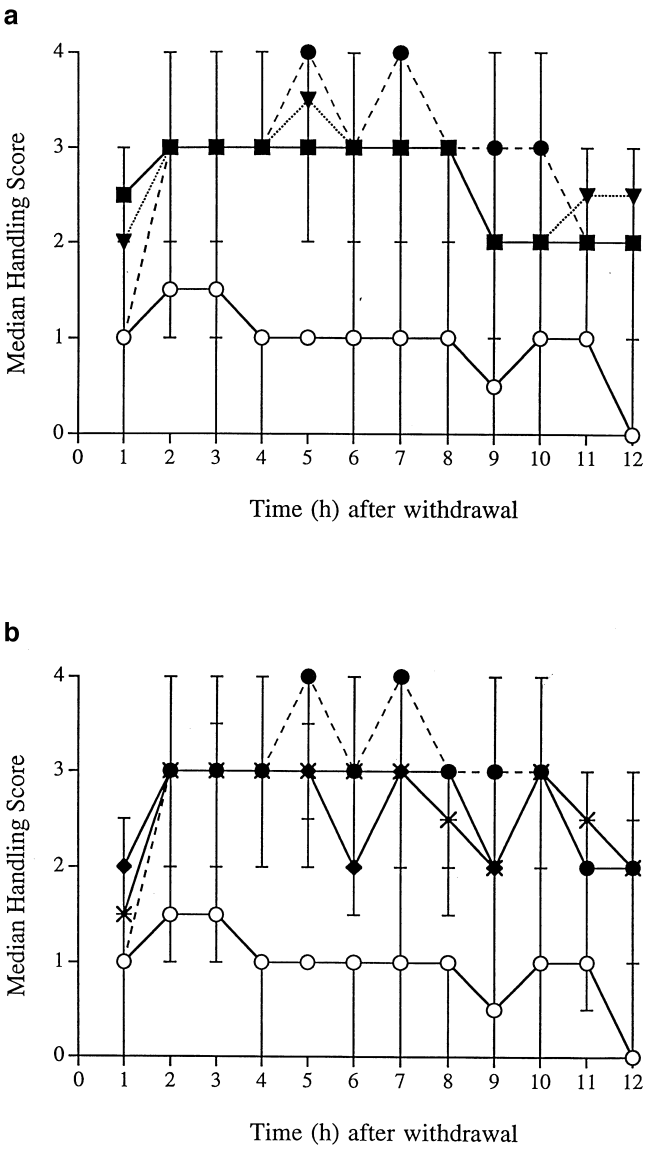

FIG. 1. Scores of behavioral responses to handling measured over a 12-h period following withdrawal from chronic ethanol treatment. Values are medians with interquartile ranges. For clarity, the effects of the two lower doses of CAM1028 are illustrated in a and those of the two higher doses in b. ○—Control-treated mice; ●—ethanol-treated mice given saline; ■—ethanol-treated mice given CAM1028, 0.3 mg/kg; ▼—ethanol-treated mice given CAM1028, 1 mg/kg; ◆—ethanol-treated mice given CAM1028, 3 mg/kg; ★—ethanol-treated mice given CAM1028, 10 mg/kg.

at 0.1, 0.3, 1, and 3 mg/kg, immediately after withdrawal and at 40 min before testing.

Intravenous Infusions of NMDLA

The method used was that of tail vein infusion (17). The convulsant drug was infused into a tail vein at a rate of 1 ml/min. The *N*-methyl-D,L-aspartate (NMDLA) concentration 70 mg/ml was chosen so that control mice convulsed within 20 to 30 s. The threshold for a full convulsion was measured as the time taken to the first sign of convulsive movement; the animals

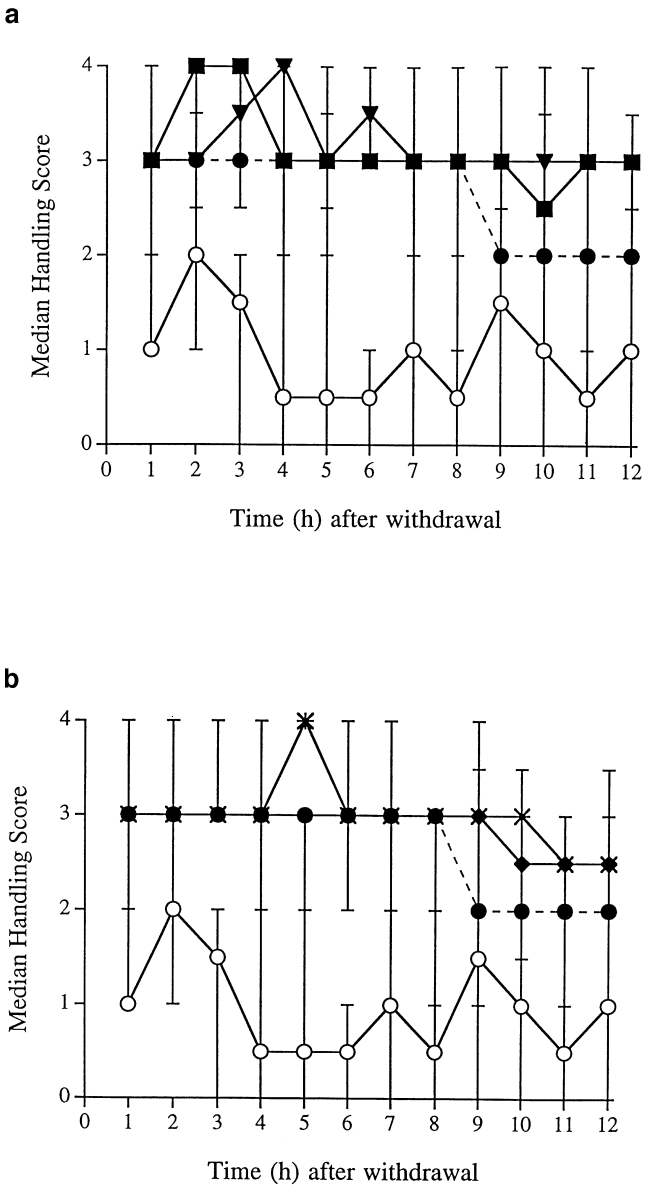

FIG. 2. Scores of behavioral responses to handling measured over a 12-h period following withdrawal from chronic ethanol treatment. Values are medians with interquartile ranges. For clarity, the effects of the two lower doses of CI988 are illustrated in a and those of the two higher doses in b. ○—Control-treated mice; ●—ethanol-treated mice given saline; ■—ethanol-treated mice given CI988, 0.3 mg/kg; ▼—ethanol-treated mice given CI988, 1 mg/kg; ◆—ethanol-treated mice given CI988, 3 mg/kg; ★—ethanol-treated mice given CI988, 10 mg/kg.

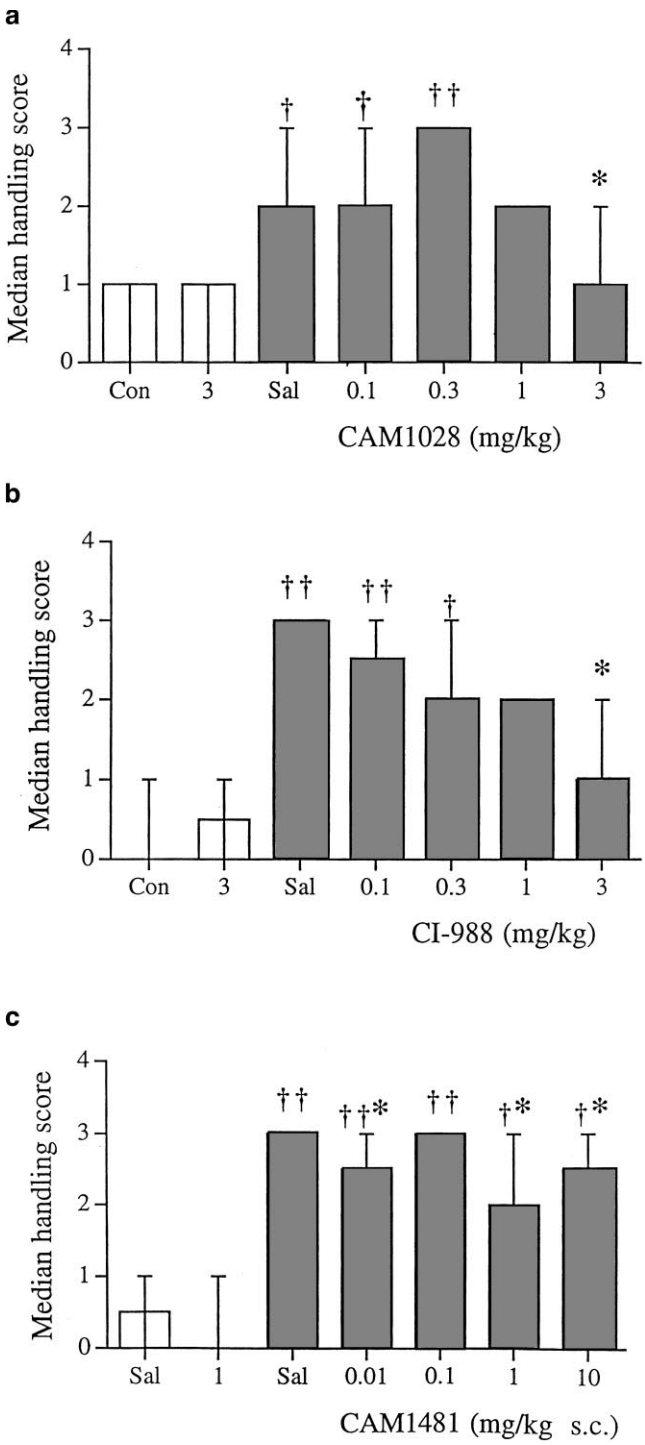

FIG. 3. Scores of behavioral responses to handling measured at 16 h after withdrawal from chronic ethanol treatment. (a) this illustrates the effects of CAM1028; (b) illustrates the effects of CI988; (c) illustrates the effects of CAM1481 (the CCK$_A$ antagonist). Values are medians with interquartile ranges. Open columns = control mice; shaded columns = ethanol-treated mice; $^{\dagger}p < 0.01$ compared with control values; $^{*}p < 0.05$ compared with values after saline administration.

TABLE 2

EFFECT OF CAM1028 ON THE INCIDENCE OF AUDIOGENIC SEIZURES 7 HOURS FOLLOWING REMOVAL OF ETHANOL

	Control diet (Saline)	Ethanol diet (Saline)	Ethanol diet (CAM1028 0.1 mg/kg)	Ethanol diet (CAM1028 0.3 mg/kg)	Ethanol diet (CAM1028 1 mg/kg)	Ethanol diet (CAM1028 3 mg/kg)
30 s cutoff	0/18	10/19†	5/18	7/18†	6/18*	4/18†
60 s cutoff	0/18	17/19†	10/18*†	14/18*	12/18*	11/18*

*$p < 0.05$ compared with control diet + saline group, †$p < 0.05$ compared with ethanol diet + saline group.

were then humanely killed. Groups of 12 mice were used for each treatment group. CAM1028 or CI988 at 0.1, 0.3, 1, and 3 mg/kg, CAM1481 at 0.1 and 1 mg/kg, or saline were injected subcutaneously to ethanol-treated animals immediately after withdrawal and at 40 min before testing. Control animals were given CAM1028 or CI988, at 3 mg/kg, or CAM1481 at 1 mg/kg, at the corresponding times.

Drugs Used

CAM1028 (Butanoic acid, 4-[[2-[[3-(1H-indol-3-yl)-2-methyl-1-oxo-2-[[[1.7.7-trimethylbicyclo [2.2.1] hept-2-yl)oxy]carbonyl]amino]propylamino] 1-phenylethyl] amino-4-oxo-.[1S-1α.2β [S(S)].4α]]-.*N*-Methyl-D-glucamine (Bicyclo system 1S-endo). CI988 (Butanoic acid, 4-[[2-[[3-(1H-indol-3-yl)-2-methyl-1-oxo-2-[[tricyclo[3.3.1.1^3.7] dec-2-yloxy) carbonyl]amino] propyl]amino]-1-phenylethyl]amino-4]oxo[R-(R.R)]-*N*-Methyl-D-glucamine. CAM 1481 b/-Alanine, *N*-[!a/-methyl-*N*-[(tricyclo [3.3.1.1>3, 7] dec-2-yloxy) carbonyl]-L-tryptophyl]-D-3-(phenylmethyl)-1-deoxy-1-(methylamino)-D-glucitol (1:1) (AdOC-(!a/Me)Ltrp-(D-3-Bzl)bAla.D-MeGluc).

All the above compounds were dissolved in saline; fresh solutions were made daily. NMDLA (Sigma) was dissolved in saline and brought to pH 7.4 with 1 M NaOH, before use.

Statistical Analysis

Differences in the ratings of handling-induced convulsive behaviour were analyzed using a nonparametric two-way analysis of variance designed for repeated measures on the same animal (8) when ratings were made every hour for 12 h, and by the Mann–Whitney *U*-test when a single set of measurements was made. The differences in convulsion thresholds for NMDLA and the latencies to audiogenic convulsions were analyzed by a two-tailed unpaired Student's *t*-test. Fisher's exact probability test was used to compare the incidence of audiogenic convulsions.

RESULTS

Measurement of Handling-Induced Convulsive Behavior

When the ratings of behavior were made hourly for 12 h (Fig. 1), CAM1028, at 3 mg/kg, caused a small decrease in the median values, a change that was significant when analyzed over the whole time period ($p < 0.05$). No significant changes were seen after any of the other doses tested (0.3, 1, and 10 mg/kg). CAM1028 had no effect on behavior in control animals when these were handled in the same way as the ethanol-treated animals (data not shown).

Figure 2 shows that CI988 had no effect on the handling response when this behavior was measured hourly for 12 h from withdrawal ($p > 0.05$). CAM1481 also had no effect (data not shown).

A single set of behavioral ratings on the handling response was made at 16 h into the withdrawal period. Both CAM1028 and CI988 were clearly effective at 3 mg/kg in decreasing the

TABLE 3

THE LATENCIES (s) TO THE ONSET OF WILD RUNNING AND TO SEIZURES IN RESPONSE TO AN AUDIOGENIC STIMULUS, MEASURED 7 HOURS AFTER CESSATION OF CHRONIC ETHANOL TREATMENT

	Saline	CAM1028 0.1 mg/kg	CAM1028 0.3 mg/kg	CAM1028 1 mg/kg	CAM1028 3 mg/kg
Wild running	27 ± 5	39 ± 6	35 ± 5	38 ± 5	45 ± 4*
Clonic seizure	30 ± 5	41 ± 5	38 ± 5	40 ± 5	46 ± 4*
Tonic seizure	33 ± 6	43 ± 5	39 ± 5	42 ± 5	49 ± 4*

*$p < 0.05$ compared with saline values.

TABLE 4

EFFECT OF CAM1481 ON THE INCIDENCE OF AUDIOGENIC SEIZURES 7 HOURS FOLLOWING REMOVAL OF ETHANOL

	Control diet (Saline)	Ethanol diet (Saline)	Ethanol diet (CAM1481 0.1 mg/kg)	Ethanol diet (CAM1481 0.3 mg/kg)	Ethanol diet (CAM1481 1 mg/kg)	Ethanol diet (CAM1481 3 mg/kg)
30 s cutoff	0/15	8/16*	8/15*	6/16*	7/14*	10/15*
60 s cutoff	0/15	15/16*	13/15*	14/16*	12/14*	14/15*

*$p < 0.05$ compared with control diet + saline group.

rise in behavioral ratings seen during ethanol withdrawal (Fig. 3a and b). This effect was significant for both drugs ($p < 0.05$). For both drugs, the effects of 1 mg/kg in ethanol-treated animals just failed to reach significance ($p = 0.06$ for CAM1028 and $p = 0.07$ for CI988). Very small, but significant, protective effects of CAM1481 were seen at doses of 0.01, 1, and 10 mg/kg (Fig. 3c), although the ratings remained significantly above the saline values in each case. None of the drugs had any effects in control animals, when tested at 3 mg/kg for CAM1028 and CI988 and at 1 mg/kg for CAM1481 (Fig. 3).

Elicitation of Audiogenic Seizures

A significant protective effect of the 3 mg/kg dose of CAM1028 was found on both seizure incidence at 30 s in response to the audiogenic stimulus (Table 2), and on the latencies to the onset of wild running, clonic, and to tonic convulsions (Table 3). These effects were significant ($p < 0.05$). No changes were seen in the incidence of seizures (Table 2) or the latencies to the above behaviors (Table 3) after any of the lower doses of CAM1028, with the exception of a small but significant decrease in seizure incidence at 60 s after administration of the 0.1 mg/kg dose. Wild running, clonic, and tonic convulsions were seen in all animals that had previously been treated with ethanol, and the CAM1028 did not affect the progressive pattern of behavior. CAM1481 did not alter the seizure incidence (Table 4) or latencies (data not shown) at any of the doses tested. Control animals did not show any signs of this behavior in response to the stimulus.

Intravenous Infusions of NMDLA

The convulsion thresholds to NMDLA were lowered at 16 h from withdrawal of ethanol (Fig. 4), as reported previously (17). At 1 and 3 mg/kg, CAM1028 completely prevented this fall in NMDLA thresholds (Fig. 4a). Comparison of the results after either dose, compared with saline, gave p-values of less than 0.001. The lower doses, 0.1 and 0.3 mg/kg, did not have any significant effects.

CI988, at either 1 or 3 mg/kg (Fig. 4b), also prevented the decrease in NMDLA thresholds ($p < 0.05$ for 1 mg/kg, $p < 0.0001$ for 3 mg/kg). CAM1481 did not alter the effects of ethanol withdrawal on NMDLA seizure thresholds at any of the doses tested (Fig. 4c). None of the compounds had any effects on NMDLA thresholds in control animals (Fig. 4).

DISCUSSION

The results in this article showed that the CCK_B antagonists, CAM1028 and CI988, had effects in protecting mice against some, but not all, of the effects of withdrawal from chronic ethanol treatment. The CCK_A antagonist, CAM1481, did not share these actions, except for a small effect in the 16-h handling study. We have previously shown (20) that the ethanol withdrawal syndrome in this species is composed of several different phases, which may have different underlying mechanisms, and which appear to parallel the effects of ethanol withdrawal in humans (2,19). The hyperexcitable responses to gentle handling and the convulsive responses to an audiogenic stimulus are seen early in the withdrawal phase, with decrease in the seizure threshold to NMDLA appearing later. The latter change was significant only at 16 h from cessation of ethanol treatment. These differences in time course of the phases of withdrawal were utilized in the present study and the effects of the CCK antagonists differed according to the behavior tests and the time at which the tests were carried out.

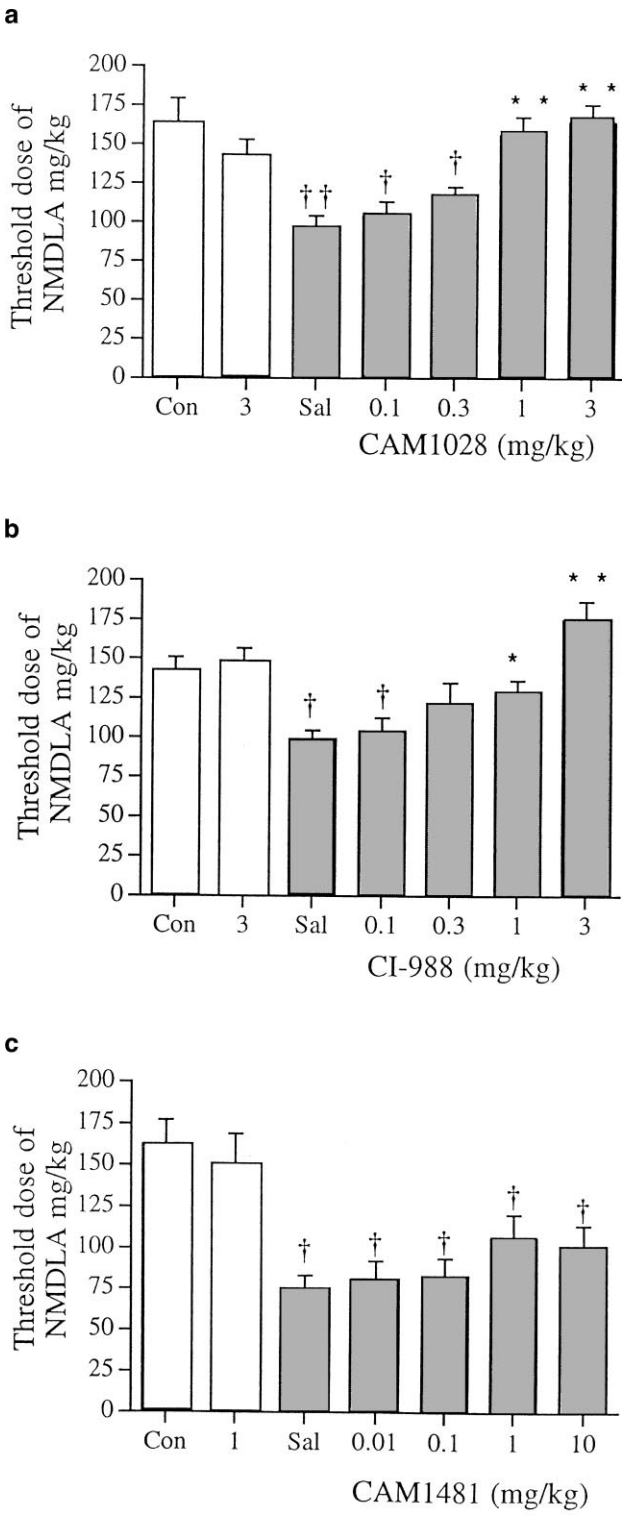

FIG. 4. Thresholds for convulsive responses to NMDLA, measured by intravenous infusion, 16 h from withdrawal from chronic ethanol treatment. (a) Illustrates the effects of CAM1028; (b) illustrates the effects of CI988; (c) illustrates the effects of CAM1481 (the CCK_A antagonist). Values are means ± SEM Con = control diet; other results obtained after ethanol diet. Open columns = control mice; shaded columns = ethanol-treated mice. $^{\dagger}p < 0.01$ compared with control values; $^{*}p < 0.05$; $^{**}p < 0.0001$, compared with values after saline administration.

There was very little effect of the CCK antagonists on handling-induced behavior during the first 12 h of the withdrawal period. The only significant change was after administration of 3 mg/kg CAM1028, the protective effect of this dose was small, and higher dose, 10 mg/kg, did not show any action. A similar pattern was seen when the responses to an audiogenic stimulus was measured at 7 h into the withdrawal period, as again 3 mg/kg of CAM1028 was the only dose to have a significant protective effect.

In contrast, both CAM1028 and CI988 at 3 mg/kg clearly prevented the increase in the ratings produced by ethanol withdrawal when the handling behavior was measured at 16 h from cessation of ethanol administration. At this time, CAM1481 had significant effects at a range of doses, but the changes were very small. The difference between these and the results when handling was measured over 12 h from cessation of the ethanol treatment may indicate different mechanisms involved in the responses at these times (see below). Our previous work has suggested that repeated measurements of the handling response at hourly intervals do not affect the ratings.

When the decrease in the seizure threshold to NMDLA was measured, also at 16 h from cessation of ethanol treatment, both the 1 and 3 mg/kg doses of CAM1028 and of CI988, prevented the change, and the resultant thresholds were not significantly different from control values. CAM1481 had no effects, even at a dose as high as 10 mg/kg. The time after ethanol withdrawal at which the effects of the CCK_B antagonists was, therefore, seen similar to that at which the changes in the effects of NMDLA were evident; both were maximal later in the withdrawal period. Electrophysiological studies have shown that the effects of NMDA on hippocampal pyramidal cells was increased later, but not early, in the withdrawal period (21). A direct comparison of the time course of the changes during withdrawal in vivo and in vitro, however, is not possible because of the different mechanisms of removal of ethanol from the whole brain and from isolated hippocampal slices. The effects of CI988 have been studied on withdrawal hyperexcitability in isolated hippocampal slices. This compound, at 1 μM, had a small but significant effect on the lowering of thresholds for elicitation of population spikes, which is seen when hippocampal slices are prepared after chronic ethanol treatment in vivo (1).

The results on the handling response and the NMDLA infusion parallel those obtained when these CCK_B antagonists were tested in the elevated plus-maze, in both rats and mice, during withdrawal from chronic ethanol treatment (23). In this study, both compounds showed significant action against the anxiety-related behavior recorded in this test 16 h after the end of the ethanol treatment. The doses that were effective on the plus-maze, however, were slightly lower, at 0.1 and 1 mg/kg for both compounds.

In some experiments the dose–response relationship was not straightforward. The effect of CAM1028, 3 m/kg, on handling-induced convulsions was small but significant, and this was not seen with lower or higher doses. The effect of CAM1028 on audiogenic seizures was significant only after the 0.1 mg/kg dose. "Bell-shaped" dose–response curves have often been reported with CCK antagonists, for example, in anxiety models (4,15) and in their effects on analgesia (5,12). Possible explanations for these include the existence of receptor subtypes (3,6,7,16,22), that may mediate different effects or the activation of additional mechanism(s), which oppose the functional effects of CCK receptor blockade.

The results at the different time intervals from withdrawal of ethanol suggest that changes might be produced in CCKB receptors later, but not earlier, in the withdrawal period. Harro et al. (10) reported that binding of [^{3}H]-CCK8, thought to be to CCKB receptors, was increased in frontal cortex when measured 24 h after cessation of prolonged ethanol treatment. These authors did not carry out a time course study, but the method and duration of ethanol treatment was different from that of the present.

The phase of "delirium tremens," which appears clinically later than the other symptoms of the ethanol withdrawal syndrome, consists of confusional states and delusions (19). It is possible that NMDA receptors are involved in the generation of this aspect, as some NMDA antagonists produce psychotropic effects and changes in NMDA receptor-mediated transmission appear to occur late in the withdrawal. The present results suggest that CCK_B antagonists may be of some benefit in the treatment of these later stages of the ethanol withdrawal syndrome.

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