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Sound-Induced Seizures during Ethanol Withdrawal in Mice

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Abstract. An ethanol withdrawal syndrome, consisting of tremors and convulsions, was induced in C57BL/6J mice by feeding them a liquid diet containing 27% of ethanol-derived calories at an environmental temperature of +12 to 13° C for 6 days. Control groups were pair-fed with liquid diets containing isocaloric amounts of sucrose or Laboratory Chow. Seven and 24 h after the beginning of withdrawal, all mice were exposed to bell ringing for 90 sec. This sound induced convulsions in nearly one-half of ethanol-consuming mice at 7 h and in a few mice at 25 h. Only one mouse of the control groups had a convulsion. These findings support the concept that the ethanol withdrawal syndrome is a partially latent state of hyperexcitability of the brain following depression by ethanol.

Key-Words: Alcohol — Withdrawal — Seizures — Audiogenic.

Introduction

Several hypotheses have been proposed to explain the mechanisms of convulsions occurring during withdrawal from addictive drugs (Mendelson, 1970; Symposium, 1970). Hyperirritability of the central nervous system following a period of functional depression is suggested by a lowering of the threshold for electroconvulsive seizures (McQuarrie and Fingl, 1958) and EEG changes following withdrawal from sedative drugs (Wikler and Essig, 1970). This view receives further support by the results of this study which demonstrates that a strain of mice genetically resistant to audiogenic seizures (Fuller and Wimer, 1966) becomes highly susceptible to seizures for a limited period of time following withdrawal from ethanol.

Methods

The methods for induction of ethanol withdrawal signs in mice have been described (Freund, 1969, 1970, 1971). Briefly, female C57BL/6J (Jackson Laboratory, Bar Harbor, Maine) 4-month-old mice were fed a liquid diet at libitum containing 27% of calories derived from 95%

ethanol (group E). In order to control for weight loss experienced by the ethanol-consuming mice, control groups were pair-fed with identical amounts of the liquid diet (10—15 ml/mouse/day in 25 ml inverted graduate cylinders through 3 inch long tubes with $^5/_{32}$ inch opening) except that 27 % of the calories were derived from sucrose (group S). A third group received isocaloric amounts of Guinea Pig Chow Pellets (Purina Lab Chow, Ralston Purina Co., St. Louis, Mo.) and water ad libitum (group C). All three groups were housed in individual cages in a refrigerator at temperatures maintained between +12° to +13°C for 6 days. A fourth group was kept at +25°C with access to Chow and water ad libitum (group D). On day 7 all mice received Chow and water ad libitum at +25°C environmental temperature. Withdrawal signs were recorded as described previously (Freund, 1969): Stage III, indicating clonic-tonic convulsions; and Stage IV, death during convulsion. Less severe stages of withdrawal including tremors and tail signs were included in Stages I and II. Audiogenic seizures were induced by a bell (No 340, Edwards, Norwalk, Conn.) in a sound-attenuated chamber for 90 sec. The sound intensity, measured approximately 2 cm above the chamber floor, was 118 decibels. The sound was presented to the mice 7 h after the beginning of withdrawal and repeated in approximately 25 h. Additional control groups were weight reduced from a mean body weight of 21.6 g by feeding 2.5 (8 mice), 2.0 (8 mice), 1.5 (16 mice), and 1.0 g (8 mice) Chow/mouse daily for 30 days. The mean final weights at the time of testing for audiogenic seizure susceptibility before feeding in the morning were 19.5, 17.7, 15.6, and 13.0 g.

Results

The results of ethanol withdrawal and sound-induced seizures are shown in the Table. It is apparent that with the exception of one seizure in the control group (D), only mice after ethanol withdrawal had clinical withdrawal signs including seizures, either spontaneous or sound-induced. Seven out of 9 mice which had one or two seizures during the 7-h withdrawal period had seizures again during the 90-sec exposure to the bell. Seven animals which had shown signs of withdrawal, but no seizures, developed convulsions when exposed to the sound of the bell. Twenty-four hours after the beginning of ethanol withdrawal, seizures could be induced by the bell in only 5 of 23 remaining mice. Only one (lethal) seizure was observed in the 40 mice whose weight was reduced from 21.6 g to 13.0—19.5 g body weight. This seizure occurred in a mouse weighing 15.8 g. Approximately 15 % of mice receiving the ethanol-containing diet in the refrigerator die during alcohol induced coma or because of obstruction of the drinking tube by a membrane of dried liquid diet.

Table. *Spontaneous and sound-induced seizures in mice following withdrawal from ethanol-containing and control diets*

Diet	Group			
	Ethanol (E)	Sucrose (S)	Chow (C)	Chow (D)
Environmental temperature (°C)	+12	+12	+12	+25
Total number of mice	32	24	24	24
Number of mice in withdrawal before sound-induction				
Withdrawal Stage 0	0	24	24	24
Withdrawal Stage I and II	22	0	0	0
(Seizures) III	9	0	0	0
(Lethal seizures) IV	1	0	0	0
Number of Audiogenic Seizures 7 h after withdrawal				
Non-lethal	6	0	0	1
Lethal	8	0	0	0
Number of Audiogenic Seizures 25 h after withdrawal				
Non-lethal	4	0	0	0
Lethal	1	0	0	0
Mean weight loss (g) at beginning of withdrawal	4.2	3.9	4.4	0

Discussion

C57BL/6J mice are genetically resistant to the induction of audiogenic seizures. This has been attributed to higher endogenous brain concentrations of serotonin and norepinephrine than in seizure susceptible genotypes (Schlesinger, Boggan and Freedman, 1965). Other investigators, however, have not confirmed this biochemical correlation (McGeer, Ikeda, Asakara and Wada, 1969). The effects of induced changes of brain biogenic amines upon audiogenic seizures is also controversial (Platnikoff, 1960; Schlesinger, Boggan and Freedman, 1968; Schlesinger, Boggan and Griek, 1968). It appears well-established, however, that susceptibility to audiogenic seizures depends not only upon genetic predisposition but also upon environmentally induced changes of the brain. It has been possible to sensitize genetically resistant strains of mice to audiogenic seizures by exposure to loud noise during the second and third week after birth (Fuller and Collins, 1968). It was further demonstrated that this induced susceptibility can be localized to one side of the brain (Collins, 1970).

This investigation was designed to further test the hypothesis that withdrawal seizures are a manifestation of increased susceptibility of

the brain to seizure activity following a period of depression by the sedative drug alcohol. As ethanol is aversive to rodents, it is difficult to induce them to drink amounts of alcohol which lead to physiological dependence and withdrawal signs upon discontinuation of alcohol (Freund, 1969). In this experiment, mice were forced to increase their ethanol intake by incorporating it into liquid diets and raising the caloric requirement of the mice by exposure to low environmental temperatures. After discontinuation of the alcohol in the diet and return to room temperature, the mice developed a withdrawal syndrome (Freund, 1969, 1971). Control animals subjected to the identical procedure, except that ethanol was omitted from the diet, developed no signs of withdrawal. During a 7-h withdrawal period, seizures occurred in approximately one-third of the ethanol-consuming mice (Table). It appears that susceptibility to seizures was latent and made overt by the sound. This proneness to seizures was maximal 7 h after withdrawal and less after 25 h. The possibility that weight loss, dietary factors, or exposure to cold temperatures by themselves were responsible for increased seizure susceptibility were excluded by appropriate controls: weight reduction at room temperature, weight reduction with Chow in the refrigerator, and pair-feeding in the refrigerator with a liquid diet which contained isocaloric amounts of sucrose instead of ethanol. Under conditions of reduced environmental temperatures, the rate of decline of blood ethanol concentrations is not appreciably accelerated (Platonow *et al.*, 1963). It is assumed that the induction of intoxication and ethanol withdrawal seizures by reduced environmental temperatures is the result of increased consumption of ethanol-containing diets in order to satisfy increased energy requirements. The principles of this method may be applicable to other animal species.

The results of this study indicate that the alcohol withdrawal state renders the brain highly susceptible to the induction of seizures by sound. This supports the concept that withdrawal phenomena are related to a partially latent rebound hyperexcitability following depression of the nervous system.

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