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Modifications in the Response to Alcohol Following the Establishment of Physical Dependence

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Abstract. An alcohol withdrawal syndrome characterized by the successive appearance of hyperactivity and tremors, muscle spasticity and, in some animals, generalized convulsions was induced in rats. It was obtained by the administration to rats whose weight had been previously reduced, of a liquid diet containing 45% of alcohol for a period of 3 weeks. The establishment of a state of physical dependence increased the incidence of withdrawal symptoms in a subsequent period of alcoholization. The administration of the 45% alcohol liquid diet during a 4-day period did not induce any noticeable withdrawal symptomatology in animals not previously exposed to alcohol. On the other hand, when previously alcohol-dependent animals were subjected to the same procedure, 50% of them demonstrated a severe withdrawal symptomatology.

Key-Words: Alcohol — Physical Dependence — Withdrawal — Rats.

Introduction

It is well known that a state of dependence on alcohol can develop as a consequence of heavy drinking. According to Jellinek's (1952, 1960) classical description, one of the main features that characterize the "alcohol addict" is that he cannot control his intake once he has started to drink. Jellinek advanced the hypothesis that this loss of control was related to the early occurrence, during a drinking bout, of withdrawal symptoms such as tremor, restlessness and anxiety. The alcoholic's vain attempt to achieve permanent relief from these symptoms would then lead to continued uncontrolled drinking. The occurrence of withdrawal symptoms after a short period of exposure to alcohol was also reported by Mendelson *et al.* (1966) who compared the effect of a 4-day period of alcoholization in 4 alcoholic and 4 normal subjects. Following cessation of drinking, 2 of the alcoholic subjects showed some withdrawal symptomatology while none of the control subjects did. The authors interpreted their results as indicating that alcohol

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addicts had a prediposition to develop withdrawal symptoms. The purpose of the present study was to obtain an experimental verification of such a hypothesis. In a first step, a state of physical dependence on alcohol was induced in rats. Such a state has been described in other species, such as the mouse (Freund, 1969), the dog (Essig and Lam, 1968) and the monkey (Ellis and Pick, 1970). Subsequently, we attempted to verify the assumption that alcohol withdrawal symptoms would occur more readily in these alcohol dependent rats than in normal controls.

Methods

28 Long Evans hooded male rats, aged 3 to 6 months and weighing between 350 and 450 g were used in this study. They were divided into a control and an experimental group of 14 animals each, matched according to weight. During the entire study, the animals were housed individually. Each animal was weighed daily at a scheduled time and then given a measured quantity of food. During the first period, lasting usually two weeks, the animals were put on a restrictive solid diet, consisting in half of their usual daily intake of lab-chow. During this period, water was freely available. This diet was continued until the animals lost a third of their initial weight. During a second period, lasting usually one week, the experimental and control animals received a liquid diet, which did not contain alcohol. This diet consisted of a mixture of equal volumes of a commercially available liquid diet preparation (Metrecal), containing 0.95 cal/cc and of an isocaloric solution of sucrose in water (238 g/l). One liter of a Metrecal solution contains 68.7 g of protein, 12.7 g of fat, 140.6 g of carbohydrates and a specified amount of essential aminoacids, minerals and vitamins. A measured amount of the liquid diet, put in a bottle, was given to the animals every day. The bottle remained attached to the cage for a 24 h-period but was usually emptied in a much shorter time by the underweight and underfed rats. The animals did not have access to any other source of food or water. The amount of liquid diet given every day was adjusted by successive approximations so that the animals' body weight remained the same, i.e. two-thirds of the initial weight. The intake was considered stabilized if it did not induce any increase or decrease in body weight of more than 5 g when given on 3 consecutive days. The third and last period of treatment lasted 3 weeks. During that period, the control animals remained on the same liquid diet. The experimental animals received a liquid diet identical to the control diet except for the fact that the sucrose solution was partially replaced by an equivalent amount of isocaloric solution of ethanol (17 % v/v). On the first day of alcoholization, 35 % of the caloric content of the diet consisted in alcohol. This percentage was gradually increased to 45 % over a few days and maintained at that level for the remainder

Table 1. *Schedule of administration of the various diets in the experimental and control groups*

	Control group	Experimental group
Experiment 1	(14 animals)	(14 animals)
1st period (2 weeks)	SD	SD
2nd period (1 week)	LD	LD
3rd period (3 weeks)		
1—21st day	LD	AD (35—45% alcohol)[a]
22nd day	LD	LD
Experiment 2	(8 aminals)	(8 animals)
2 weeks	LD	LD
4 days	AD (45% alcohol)[b]	AD (45% alcohol)[c]
1 day	LD	LD

SD: Solid diet.

LD: Liquid diet without alcohol (50% Metrecal—50% Sucrose).

AD: Liquid diet with alcohol (50% Metrecal—35 to 45% alcohol—15 to 5% Sucrose).

Daily alcohol intake, expressed in milliliter of absolute alcohol per animal (Mean + S.D.):

[a] 4.31 ± 0.22.

[b] 4.26 ± 0.17.

[c] 4.33 ± 0.48.

of the experiment. Minor adjustments in the amount of the liquid diet were made in control and experimental groups so that the animals' weight remained constant. On the last day of this 3-week period, the experimental animals received again the alcohol-free liquid diet consisting only of Metrecal and the sucrose solution. After having received the alcohol-free diet, the experimental animals were left in their cages and were observed constantly for a period of 12 h. Control animals were simultaneously observed, and the behavior of the two groups of animals were compared. In addition, at hourly intervals, each experimental and each control animal was, for a brief period, let free to run in a larger enclosure so that its motor behavior could be more accurately evaluated.

The second part of the study was designed to observe differences in the effect of alcoholization on control and previously alcoholized animals. Eight control and 8 experimental subjects used in the first part of the study were selected at random for this second experiment.

For a 2-week period immediately following the end of the first experiment, these 16 animals continued to receive the alcohol-free liquid diet. As in the first experiment, the amounts of food given was controlled so that the animals kept a constant weight equal to two thirds of their initial weight. After these 2 weeks, the two groups of animals were fed for 4 days with an alcohol liquid diet in which alcohol accounted for 45% of the total caloric content. After 4 days of this diet, both groups received the alcohol-free liquid diet again. Their behavior was then constantly monitored for a 12 h-period, using the same procedure as the one used during the withdrawal episode in the first experiment.

A summary of the various diet regimens in the experimental and the control groups in the first and second experiments can be seen in Table 1.

Results

The animals maintained their weight (two-thirds of their initial weight) with a daily food caloric intake ranging between 18 and 23 cal/100 g B.W. The substitution of sucrose by alcohol did not modify the amount of caloric intake necessary to maintain the same weight. Therefore, the maximum daily intake of alcohol, when it constituted 45% of the caloric content of the diet, ranged between 1.15 and 1.50 g/100 g B.W. Symptoms of alcohol intoxication were observed in all the animals taking the alcohol diet. They appeared sedated, were less active, somewhat ataxic with a broad based gait and slow motions. More severe symptoms of intoxication, such as the disappearance of the righting reflex or a coma were not observed in any of the animals. During the last days of the alcoholization period, the various manifestations of intoxication were less severe and of lesser duration than they had been during the first days.

As mentioned above, the period of alcoholization lasted 21 days during the first experiment. On the 22nd day, at the regular feeding time, the animals were presented with the control liquid diet, made of Metrecal and the sucrose solution. The first withdrawal symptoms appeared 3 to 4 h later. The animals became hyperactive and squealed spontaneously or when they were touched. They exhibited also episodes of body tremors. At a later stage, usually a couple of hours later, symptoms of spasticity occurred. The locomotion of the animals was characterized by a broad based gait and slow, rigid motions. Frequently, during that period, there was a marked dorsal flexion of the spine, giving the appearance of a hunchback and a flexion of the tail, which took an S-shape. These symptoms were observed in all animals but with varying degrees of severity. Episodes of generalized convulsions, with the clonic component most prominent, were observed in two animals after the

Table 2. *Withdrawal signs following a 21-day period and a 4-day period of alcoholization*

	Stage 1			Stage 2			Stage 3		
	Mild	Moderate	Severe	Mild	Moderate	Severe	Mild	Moderate	Severe
Experiment 1 (21 days of alcoholization)									
14 experimental rats (1st alcoholization)	0	1	13	1	6	7	0	2	0
Experiment 2 (4 days of alcoholization)									
8 control rats (1st alcoholization)	4	4	0	0	0	0	0	0	0
8 experimental rats (2nd alcoholization)	0	3	5	1	3	0	0	0	0

Stage 1: hyperactivity and tremor; Stage 2: spasticity and hypertonia; Stage 3: generalized convulsions. The numbers represent those rats, in each group, that exhibited the symptom.

development of symptoms of muscle spasticity. (The intensity of the various withdrawal symptoms observed in this first experiment can be seen in Table 2.) No death occured during the course of withdrawal. The severity of the symptoms started to decrease 4 to 8 h after the occurrence of the first symptoms and the animals appeared to have completely recovered on the following day.

In the second experiment, we compared the intensity of the withdrawal symptoms following 4 days of alcoholization in the control animals, for which it was the first exposure to alcohol and in the experimental animals, which had undergone an alcoholic withdrawal episode 2 weeks earlier. (See Table 2.) The 8 control animals showed only a mild or a moderate degree of hyperactivity and tremor. None of them showed any evidence of muscle spasticity. The withdrawal symptomatology was more fully developed in the 8 experimental animals. They all showed a degree of hyperactivity and tremor more severe than the control animals. Four of them suffered, in addition, from muscle spasticity severe enough to induce slow and rigid ambulatory motions.

Discussion

The present study indicates that a withdrawal syndrome can be induced in rats by the administration to animals whose weight had been previously reduced of a liquid diet containing 45 % of alcohol for a period of 3 weeks. Freund (1969) has recently reported that the administration

to mice which had previously lost a third of their weight of a similar liquid diet for a period of 4 days induced a state of alcohol dependence. According to this author, the prior reduction in weight decreased the rate of ethanol metabolism and as a consequence, facilitated the establishment of alcohol dependence. Our study differs from Freund's in that the food intake of our experimental animals was controlled. In Freund's study, as the liquid diets were freely available, the animals on the alcohol-free diet gained weight while those on the alcohol diet lost some. In our study, however, the caloric intake of the experimental and control animals were regulated so that there were no changes in body weight. As the amount of proteins and other essential nutrients were equivalent in both groups, it is unlikely that behavioral differences observed during the period of alcoholization and at the time of withdrawal can be explained by dietary differences.

During the withdrawal syndrome which took place at the end of the 3-week period of alcoholization, we observed the successive emergence of 3 clusters of symptoms. Hyperactivity and tremulousness appeared first. They were followed by symptoms resulting from muscle spasticity, such as slow and rigid walking motions. Finally, episodes of generalized convulsions occurred in some animals. This symptom pattern is similar to the one previously described in other species. In the dog (Essig and Lam, 1968), the earliest and longest lasting symptom was tremulousness of the extremities. Episodes of tonic extension of the limbs were later followed by generalized convulsions in 5 out of 8 animals. Freund (1969) described 3 successive stages in the mice. Overactivity and tremor were seen in Stage I. A slow, broad-based gait and various symptoms of hypertonia were observed in Stage II. Stage III was characterized by episodes of generalized convulsions in some animals. In the monkey (Ellis and Pick, 1970) there was a successive occurrence of a tremulous stage, a spastic stage and later, a convulsive stage. The complex clinical picture observed during the alcohol withdrawal syndrome in humans has been described in detail by a number of authors (Victor and Adams, 1953; Isbell *et al.*, 1955; Mendelson and La Dou, 1964). As in other species, this syndrome is characterized by the early occurrence of tremors. Somewhat later, a certain degree of hyperreflexia develops, which could be considered as the equivalent of the spastic stage, noted in the various species mentioned above. Convulsions occur later and only in a relatively small number of cases.

In the second experiment, we compared the effects of a 4-day period of alcoholization in the control and the experimental animals. There was virtually no withdrawal symptomatology in animals which had not been previously exposed to alcohol. However, 50% of the 8 experimental animals, which had experienced an alcohol withdrawal syndrome 2 weeks

earlier, showed clear-cut evidence of withdrawal symptoms at the end of this short period of alcoholization. In a study, comparing 4 normal and 4 alcoholic subjects, Mendelson *et al.* (1969) had also reported that after a 4-day period of alcoholization, withdrawal symptoms could be observed in some of the alcoholics but in none of the control subjects. It appears thus that, in animals or humans having previously experienced an alcohol withdrawal syndrome, the time of exposure to alcohol necessary to induce another withdrawal syndrome is decreased. This corroborates Jellinek's clinical observations (1952, 1960) that, in alcohol addicts, the ingestion of a limited amount of alcohol results in what is "felt by the drinker as a physical demand for alcohol" that leads to further ingestion of this drug. Jellinek emphasized that this modified reactivity to alcohol persists even after a long period of abstinence and that it is likely to play a role in the maintenance of alcohol addiction.

There are a number of observations indicating that, in morphine addiction too, the prolonged exposure of the organism to the drug leads to the occurrence of physiological modifications that are observable for a long period of time after the disappearance of the acute withdrawal symptoms. Himmelsbach (1942) found that, in human subjects, previously addicted to morphine, changes in various physiological parameters persisted for at least 6 months after cessation of drug intake. More recently, Martin and Jasinski (1969) have distinguished two successive phases in the morphine abstinence syndrome in humans. An early phase or primary abstinence is characterised by mydriasis and by an increase in blood pressure, respiratory rate, cardiac rate and body temperature. A second phase or protracted abstinence, beginning 6 to 9 weeks after morphine withdrawal and lasting 6 months or more, manifests itself by myosis and by a decrease in blood pressure, respiratory rate, cardiac rate and body temperature. Martin *et al.* (1963) have observed in rats, experimentally addicted to morphine over a period of 42 days, the successive occurrence of a "primary abstinence syndrome" lasting about 72 h and of a "secondary abstinence syndrome" that could be observed for as long as 6 months after morphine withdrawal. The various physiological changes noted during the secondary abstinence syndrome were an elevated body temperature, an increase in metabolic rate and an increase in water consumption. These long lasting physiological modifications may reflect changes in the central nervous system that facilitate the occurrence of drug seeking behavior. This interpretation is suggested by reports from a number of authors (Nichols and Davis, 1959; Wikler *et al.*, 1963; Wikler and Pescor, 1967; Stolerman and Kumar, 1970) who observed that the consumption of morphine and other opioids is greater in rats previously addicted to morphine than in control animals.

In view of the foregoing, it is of interest to examine further the evolution of changes occurring in rats made physically dependent on alcohol. One can wonder, for example, if the modifications found in the present experiment persist indefinitely or disappear progressively. Further investigation of the biochemical and neurophysiological changes occurring during the period following withdrawal and of their eventual contribution to a modified reactivity to alcohol should aid in the elucidation of the mechanisms involved in alcohol addiction.

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