

INDUCTION OF PHYSICAL DEPENDENCE BY ETHANOL INTOXICATION IN RATS

BY

A.K. Mashhour, A. Bastawi and A.H. Khalil

Abstract

The most dangerous characteristic of alcohol is the ease with which it induces dependence in the regular drinkers. The present study tested the hypothesis that a continuously sustained elevation of relatively high blood ethanol concentrations by forced intragastric administration for only a few days may prove adequate, and is an essential condition for rapid induction of physical dependence upon ethanol in rats. It also describes the signs and reactions of ethanol detoxication phase and dependence phase of the withdrawal period. Rats were treated by intragastric intubation of a 20% ethanol solution in doses of 9-15 g/kg in 3-5 fractions for 16 days. Both tolerance and physical dependence were demonstrated after this treatment with the maximum tolerable doses to only a few days. Tolerance was assessed by signs of severity of intoxication. During withdrawal, as blood ethanol concentrations approached 100mg/dl the ethanol dependence phase was characterized by the onset of signs and responses of progressive severity. A significant degree of tolerance was demonstrated for all signs of intoxication after 4 days of treatment. The severity of the withdrawal

reactions intensified progressively to a maximum intensity after 4 days of treatment. These different time courses suggest that tolerance and physical dependence are mediated through different mechanisms.

Introduction

Alcoholism, acknowledged to be a disease and recognized as a major public health problem, yields sufficient signs and symptoms to enable reasonable diagnostic precision. Several attempts have been made to assess the burden upon society of the adverse consequences of alcohol consumption by estimating the costs of alcoholism and alcohol misuse (Holtermann and Burchell, 1981).

It may be, however, that the most dangerous characteristic of alcohol is the ease with which it induces dependence in the regular drinker. The degree of dependence is important and clinicians and researchers pay efforts due to its assessment.

There are also forensic implications, with alcoholic dependence, since the restriction of choice in the dependent alcoholic may be such that his legal responsibility for crimes he may commit, may be called into question (Shaw, 1982).

As recently as 10 years ago, a model of physical dependence on ethanol in the rat was established (Majchrowicz, 1975), displaying many of the signs and responses of the ethanol withdrawal syndrome observed in man (Freund, 1975).

According to generally held views, physical dependence upon ethanol and the associated abstinence syndrome in humans and in experimental animals require prior consumption of large volumes of alcoholic beverages for extensive periods of time (Victor and Adams, 1953). However, clinical observations (Majchrowicz and Mendelson, 1971), suggest that this may not be true. Accordingly, the present study tested the hypothesis that a continuously sustained elevation of relatively high blood ethanol concentrations by forced intragastric administration for only a few days may prove adequate

and is an essential condition for rapid induction of physical dependence upon ethanol in the rat.

Consequently, this paper reports findings relative to a simple, rapid and reproducible technique for the induction of physical dependence upon ethanol in the rat. This technique uses the administration of ethanol by stomach tube, thus closely resembling the route of ethanol intake in man. Hunter et al. (1973) explored the relationship between the onset of the withdrawal syndrome and the elevation of blood ethanol concentration which accompanies overt intoxication. The study describes signs and reactions of the ethanol detoxication phase and of the ethanol dependence phase, of the withdrawal period, in the light of today's interest in helping alcoholics to stop drinking.

Materials and Methods

Male Spargue-Dawley rats weighing between 200-250g. were obtained from Numro 3 breeding laboratory, the animals were allowed to acclimate to the animal house condition for 3 days. The animals were housed in groups of 10 per cage and had access of food and water. The animals were treated with a 20% (W/V) aqueous solution of absolute ethanol A.R. (Prolabo 98%) by means of intragastric intubation. Ethanol was administered in doses of 9-15 g/kg/day in 3-5 fractions for 1-6 days. Each experiment was initiated with the administration of a priming ethanol dose of 5 mg/kg. Subsequent doses were determined individually for each animal utilizing the correlation between the blood ethanol concentration and the severity of intoxication (Majchrowicz, 1974). Accordingly, rats rated neutral received 4 g/kg, ataxia, 1 -3g/kg, ataxia, 2, -2g/kg and ataxia 3, g/kg. Animals which display loss of righting reflex or were comatosed received no ethanol. Since after a few days of treatment the animals developed considerable tolerance, the individual doses of ethanol were somewhat increased (Majchrowicz, 1974). However the total daily amount of alcohol given to the animals remained unaffected.

On the day of ethanol withdrawal, the animals were allowed to detoxify and the degree of intoxication

assessed at hourly intervals. Blood sample for ethanol determination were drawn immediately after each behavioral evaluation. The degree of intoxication was assessed using the signs and responses described by Majchrowicz (1974) and stated below,

- * Neutrality - no over signs of intoxication.
- * Sedation - reduced muscle tone and relaxed slow locomotor activity, no overt gait impairment or motor incoordination.
- * Ataxia 1-lowest degree of gait impairment and motor incoordination, abdomen and pelvis still markedly elevated.
- * Ataxia 2-responses falling between Ataxia 1 and Ataxia 3, accentuated staggering gait.
- * Ataxia 3 - slowed righting reflex, heavily impaired motor coordination, absence of pelvic and abdominal elevation.
- * Loss of righting reflex - inability of animal to right himself, other reflexes are still present, little or no spontaneous motor activity.
- * Coma - no signs of movements and completely unresponsive absence of eyeblink reflex.

The severity of the withdrawal syndrome was assessed on the basis of the appearance of at least three behavioral signs (Majchrowicz, 1973, 1974). The signs used in this study are listed as follows:

- * General hyperactivity - increased locomotor activity, accentuated startle response, agitation.
- * Tremors - can involve the tail, caudal region, trunk, head or the whole body.
- * Rigidity - expressed as curling of the tail around a stiff rod or increased muscle tone in the body.
- * Convulsive Seizures - spontaneous and induced tonic-clonic.

Blood Ethanol Determination

During the detoxication phase of ethanol withdrawal period when ethanol was still present, the blood samples were taken from the tail vein and analysed for ethanol using ultra violet - spectrophotometer and Boehringer Mannheim Kit catalogue number 125822 for ethanol detection.

Results

Table (1) showed the correlation between the blood ethanol concentrations and the degrees of severity of intoxication and the amount of ethanol administered with each degree of intoxication. The doses of ethanol administered for the same degree of intoxication were lower during the first 2 days of the experiment than during the last 4 days after the animals develop significant degree of tolerance to ethanol. The tolerance can be seen by the increase in blood ethanol concentrations as the duration of treatment increased. Statistically significant differences using student's t-test ($P < 0.05$) were found for sedation, ataxia 1 and ataxia 3 after as little as one day of treatment. Tolerance for all signs of intoxication occurred after 5 days.

The last dose of ethanol marked the withdrawal of the animals from ethanol and the initiation of the withdrawal period. Initially, the animals were usually intoxicated to a variable degree depending upon the blood ethanol concentration. Since the animals which were highly intoxicated at the time of the last intubation received only a small dose or no ethanol; the onset of withdrawal syndrome in these animals evolved promptly after the initiation of the observations. In contrast the animals with slight degree of intoxication received relatively large doses of ethanol.

The severity of withdrawal syndrome intensified progressively as the period of treatment with ethanol increased (Table 2). After one day of treatment a majority of the animals (53%) displayed withdrawal signs (40%) showed mild signs, while (2%) developed convulsions. There was a significant increase in withdrawal scores with each additional day of treatment. After 4 days of treatment maximum intensity of the withdrawal syndrome was observed in 69% of the rats exhibiting severe withdrawal reactions including convulsions (Table 2).

Discussion

The results of this study demonstrate that when

Table (1) Intoxication-dose relationship in rats (n=75) received an initial dose of alcohol (5g/kg body weight).

Degree of intoxication (behavioral evaluation)	Blood ethanol concentration (mg/dl) mean $\pm$ S.D.	Subsequent dose of ethanol (g/kg)		
		First 2 days	Last 4 days	
Neutrality (developed in 60 rats of the 75)	205 $\pm$ 70*	4	5	
Sedation (developed in 60 rats of the 75)	287 $\pm$ 54*	3	4	
Ataxia 1 (developed in 42 rats of the 75)	343 $\pm$ 59*	2	3	
Ataxia 2 (developed in 22 rats of the 75)	399 $\pm$ 77	1	2	
Ataxia 3 (developed in 24 rats of the 75)	468 $\pm$ 85*	0	1	
Loss of righting reflex (developed in 18 rats of the 75)	536 $\pm$ 53	0	0	
Coma (developed in 12 rats of the 75)	708 $\pm$ 125	0	0	

No. of total animals 75 * P < 0.05

rats are treated with the maximum tolerable doses of ethanol, tolerance and physical dependence can be induced only in few days. In fact it is possible to observe significant signs of both phenomena after as little as one day of treatment. This is in contrast to the generally held view that physical dependence in alcoholic persons can be induced only after prolonged consumptions of large doses of ethanol (Jellinek, 1960). However, with the use of animal models, physical dependence can be induced in a matter of days (Mello, 1973).

In the light of our work, it appears as suggested earlier by Goldstein, (1974) and later by Tarter (1980), that the length of time necessary for physical dependence to be expressed as a withdrawal syndrome depends on the total amount of ethanol consumed per day, specially when the dose is distributed over the 24 hours. In alcoholic men under laboratory conditions a withdrawal syndrome has been demonstrated after a week of continuous drinking (Majchrowicz and Mendelson, 1971).

One reason why it might take longer for alcoholic people in society to induce a state of physical dependence is their inability to sustain severe degrees of intoxication for longer periods of time without rendering themselves unconscious. In our experiment the rats were severely intoxicated and were still given ethanol doses at a time when alcoholic subjects might not be able to drink. The conclusion to be drawn is that in order to attain a certain level of physical dependence, a given blood concentration of ethanol must be maintained throughout the 24 hour period and for a requisite number of days (Seevers and Deneau, 1963).

The biochemical basis underlying the altered functioning of the central nervous system in dependence is not yet established and probably more than one mechanism are involved. Alcohol interferes with the function of the nerve-cell membrane by increasing the "fluidity" or disorder of the lipoprotein membrane, thus altering neurotransmitter release, the function of membrane-bound enzyme, ion conductance channels and Ca^{++} binding sites. Animal studies showed that, in a matter of minutes or hours, nerve cells adapt to the presence of alcohol by reducing the proportion of polyunsaturated fatty

Table (2) Percentage of animals in 5 separate categories of ethanol withdrawal reactions.

Days of Treatment	Total No. of rats	Percentage of animals in each designated withdrawal category				
		0 No withdrawal signs	1 mild	2 Moderate	3 Severe; Severe excluding convulsions only	3 Severe excluding convulsions
1	30	47%	40%	11%	2%	0%
2	27	27%	23%	25%	9%	16%
3	25	11%	19%	17%	34%	19%
4	27	1%	14%	16%	27%	42%
5	23	2%	11%	19%	30%	42%
6	24	0%	9%	35%	24%	32%

acids in the membrane phospholipids and increasing the cholesterol content of the membrane. In this way the membrane becomes less fluids and less prone to perturbation by alcohol (Littleton, 1980). Initially this adaptive state is readily reversible but, with chronic exposure to alcohol, reversibility becomes less rapid and is accompanied by withdrawal symptoms (Shaw, 1982).

Our data suggested that maximal physical dependence, in our studied animals, was observed after 3-4 days of intragastric administration of ethanol, and appears to be responsible for the maintenance of uninterrupted elevation of blood ethanol concentrations above the threshold value. Therefore, the role of other variables, such as individual susceptibility, genetic predisposition, and previous induction of tolerance to ethanol under these conditions had little effect on the development of dependence.

References

- Freund, G. (1975): Induction of physical dependence on alcohol in rodents. In *Biochemical Pharmacology of ethanol*, E. Majchrowicz, ed., *Advac. Exp. Biol. Med.*, Vol. 56, pp. 311-326, New York, London: Plenum Press.
- Goldstein, D.B. (1974): Rates of onset and decay of alcohol physical dependence in mice. *J. Pharmacol. Exp. Ther.* 190, 377.
- Holtermann, S. & Burchell, A. (1981): The costs of alcohol misuse (Government Economic Service Working Paper No.37). Department of Health and Social Security, London.
- Hunter, B.E., Boast, C.A., Walker, D.W., Zornetzer, S.F. (1973): Alcohol withdrawal syndrome in rats: neural and behavioral correlates. *Pharmacol. Biochem. Behav.* 1, 719.
- Jellinek, E.M. (1960): The disease concept of alcoholism. Highland Park. N.J.: Hillhouse Press.
- Littleton, J.M., (1980): In: Richter D. Ed. *Addiction and Brain damage*. pp. 46-74. Croom Helm, London.
- Majchrowicz, E. (1973): Induction of physical dependence on alcohol and the associated metabolic and behavioral changes in rats. *Pharmacologist* 15. 159, abs.

- Majchrowicz, E. (1974): Spectrum and continuum of ethanol intoxication and withdrawal in rats. *Pharmacologist* 16, 304.
- Majchrowicz, E. (1975): Induction of physical dependence upon ethanol and the associated behavioral changes in rats. *Psychopharmacologia (Berl.)* 43, 245.
- Majchrowicz, E., Mendelson, J.H. (1971): Blood methanol concentrations during experimentally induced ethanol intoxication in alcoholics. *J. Pharmacol. Exp. Ther.* 179, 293.
- Mello, N.K. (1973): A review of methods to induce alcohol addiction in animals. *Pharmacol. Biochem. Behav.* 1, 89.
- Seevers, M.H., Deneau, G.A. (1963): Physiological aspects of tolerance and physical dependence. In *physiological pharmacology*, Vol. 1, W.S. Root and E.G. Hofmann, eds. pp. 565-640. London, Academic Press.
- Shaw, G.K. (1982): Alcohol Dependence and Withdrawal. *Br. Med. Bulletin.* Vol. 38, No. I, p. 99.
- Tarter, R.E. (1980): In: Richter D., Ed. *Addiction and brain damage* pp. 267-297. Croom Helm, London.
- Victor, M., Adams, R.D. (1953): The effect of alcohol on the nervous system, *Res. Publ. Ass. Nerv. Ment. Dis.* 32, 526.

Acknowledgement

We are grateful to Prof. Dr. Refki Faris, Head of Preventive Medicine Department. Ain Shams University for his statistical assistance.

Authors

A.K. Mashhour. Ass. Prof. of Forensic Medicine
 A. Bastawi. Prof. of Pharmacology.
 Afaf Hamed Khalil Lecturer in Psychiatry. Ain Shams University.

إحداث اعتماد عضوى باستخدام التسمم الأيثانولى فى فئران التجارب موجز

إن أخطر صفة للكحوليات هى فى سهولة تحول شاربها إلى الإدمان وهذه الدراسة هى لفحص نظرية تفترض أن الزيادة المستمرة فى تركيز مستوى الكحول فى الدم عن طريق إدخاله لمعدة الفئران لفترة عدة أيام قد تكون كافية — وهى شرط ضرورى — لإيجاد إدمان بالفئران . وهى تصف أيضاً علامات وآثار فترة التطهير من الكحول.

أعطى للفئران عن طريقة أنبوبة بالمعدة سائل كحولى بتركيز ٢٠٪ فى جرعات مقدارها يتراوح بين ٩ جم ، ١٥ جم لكل كيلو جرام من وزن الجسم مقسمة على ثلاثة أو خمسة أجزاء لمدة ١٦ يوم .

وقد أمكن إظهار علامات التحمل وعلامات الاعتماد على الكحول بعد هذه المعالجة بأقصى جرعات محتملة لعدة أيام فقط . وقد قيس درجة التحمل بعلامات شدة التسمم . وفى أثناء فترة السحب وعندما اقتربت مستويات تركيز الكحول فى الدم من ١٠٠ مجم لكل ديسيلتر ، تميزت فترة الاعتماد على الكحول بعلامات ، واستجابات بشدة متزايدة وقد أمكن إظهار درجة عالية من التحمل لكل علامات التسمم بعد أربعة أيام من العلاج . وقد ازدادت شدة أعراض السحب تدريجياً لتصل لأعلى درجة بعد ٤ أيام وإن هذه البرامج توحي بأن التحمل والإدمان تتم عن طريق عمليات مختلفة .

ABSTRAIT

LA PRODUCTION DE LA DÉPENDENCE PHYSIQUE PAR L'INTOXICATION DE L'ÉTHANOL CHEZ LES RATS

La facilité avec laquelle l'alcool produit la dépendance, chez ceux qui le boivent régulièrement, est son caractère le plus dangereux. L'étude examine l'hypothèse qu'une élévation, continuellement prolongée, des concentrations relativement hautes de l'éthanol dans le sang, par une administration intragastrique forcée, pour quelques jours, peut être suffisante pour le développement rapide d'une dépendance physique sur l'éthanol chez les rats. L'étude décrit aussi les signes et les réactions de la phase de détoxification de l'éthanol et la phase de dépendance dans la période de sevrage. Les rats sont traités par une intubation intragastrique avec une solution de 20% éthanol dans des doses de 9 à 15 g/kg pour 1 à 6 jours. La tolérance et la dépendance physique ont été montrées avec les maximales doses tolérables par ce traitement. La tolérance a été estimée par les signes de sévérité de l'intoxication. Durant le sevrage, quand les concentrations de l'éthanol dans le sang aboutissent à 100 mg/dl, la période de dépendance de l'éthanol est caractérisée par le commencement des signes et des réponses d'une sévérité progressive. Dans 4 jours du traitement un degré de tolérance significatif a été démontré par tous les signes de l'intoxication.