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Alcohol Exposure Pattern and Physical Dependence

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LE BOURHIS, B. AND G. AUFRERE. *Alcohol exposure pattern and physical dependence.* PHARMACOL BIOCHEM BEHAV **18:** Suppl. 1, 511–514, 1983.—Intoxicating animals by inhalation of ethanol vapor allows no possibility of determining accurate daily absorbed doses of ethanol. The daily BAC versus time curve may be considered as a picture of animal intoxication during the experimental period, and the area under the BAC curve (A) indicates the intoxication level of animal. On the other hand, to compensate the development of alcohol tolerance, it is necessary to gradually increase alcohol concentration in the atmosphere (ACA). A protocol was developped to study the withdrawal syndrome intensity (WSI) in rats, based on observations of behavioral alterations. Five successive grades were described and noted in relation to more and more serious WSI. Male rats were intoxicated in various protocols in which the length of alcohol exposure (7–35 days) and the rate of increase in ACA (0.22–0.70 mg/l/day) were changed. In the condition of constant increases of ACA (0.33 mg/l/day in average), WSI varied in relation to the length to alcohol exposure. When A was kept constant, the higher the ACA increase rate was, the higher the WSI was. These results are discussed with regard to patterns of consumption of alcoholic beverages by man.

Pattern of alcohol intoxication Physical dependence Withdrawal syndrome

THE well-known withdrawal syndrome induced following chronic alcohol administration in laboratory animals is currently described as the expression of an adaptive phenomenon [9]. However, the mechanism underlying this phenomenon has not as yet been clearly established. The withdrawal syndrome is characterized by seizures and other behavioral signs, as well as alterations in body temperature and body weight. Some of these characteristics have been individually studied by various authors in order to quantitate the intensity of the withdrawal phenomenon. However, the diversity of the methods employed indicate that no single measure is fully satisfactory.

In spite of this methodological difficulty, it has been clearly demonstrated that the intensity of the withdrawal syndrome is positively correlated with the total quantity of ethanol taken in by the animals [4, 6, 11, 18]. However, to our knowledge, no study has been undertaken to examine the influence of the pattern of alcohol administration on withdrawal syndrome intensity.

Recently, Littleton has hypothesised that the withdrawal syndrome represents an adaptive phenomenon associated with alcohol tolerance [17]. If this is the case, then the conditions of alcohol administration are important to consider. For example, it would be particularly interesting to know whether the withdrawal syndrome reaches the same intensity under different conditions of ethanol dose and duration of administration, provided that the product of dose × duration remains constant. In other words, it would be of interest to compare the effects of ethanol administered for a long duration in low doses, to those of ethanol administered for a short time in high doses.

Furthermore, under conditions where ethanol is administered in progressively increasing doses, it is of importance to determine the relative contributions of the pattern of dose increases and the dose × duration product on subsequent withdrawal syndrome intensity.

The present paper reports two experiments employing rats intoxicated by inhalation of ethanol vapor. The ethanol concentration in the atmosphere was increased throughout the intoxication period in such a way that the blood alcohol concentration (BAC) increased as a linear function of time. With alcohol administration by the pulmonary route, it is not possible to accurately determine the ethanol dose absorbed by individual animals. However, the standard of intoxication may be estimated by individual BACs, and the area under the BAC curve may be considered as an index of intoxication.

In the first experiment, area under the BAC curves was varied by changing the duration of alcohol administration but keeping the slope of BAC evolution constant. In the second experiment, the slopes of the BAC curves and the duration of alcohol administration were covaried in such a manner that the area under the BAC curves would be kept constant. In both experiments, the severity of the withdrawal syndrome was related to the area under the BAC curves.

METHOD

Male Wistar rats AF/Han provided by EVIC-CEBA Co., weighing 180–190 g at the start of experiments, were used. They were individually caged. Alcohol exposure was conducted in chambers previously described [13,14]. BACs were determined in blood samples obtained from the retro-orbital sinus, using an ADH method (Kit; Sigma).

TABLE 1

Duration of alcohol exposure (days)	Protocols of alcohol atmosphere increase: initial concentration (mg/l)	final concentration (mg/l)	steps (mg/l)	duration of steps days	Number of animals
32	15	22	1	4/5	12
27	15	22	1	3/4	20
21	15	22	1	2/3	12
9	15	22	1	1/2	20

Alcohol Administration Protocols

During the alcohol administration, food and water were provided to animals ad lib. They were taken out of the alcohol inhalation chambers twice or three times a week for 10 minutes, for taking blood samples and determining food and water consumption.

First Experiment

Seventy-six animals were divided into 5 groups of 12–19 animals each. They were simultaneously exposed to ethanol for 7, 14, 21, 29 or 35 days respectively.

The alcohol concentration in the atmosphere was increased from 15 to 27 mg/l by successive 1 mg/l steps of 1–5 days duration. At the time of removal of the rats from the chambers, the alcohol concentration in the atmosphere was 18, 22, 24, 26 or 27 mg/l, respectively.

Second Experiment

The alcohol concentration in the atmosphere increased from 15 to 22 mg/l, according to 4 different protocols (Table 1).

Alcoholization Index

The alcohol concentration in the atmosphere was periodically increased in such a manner that the mean of BACs would be a simple linear regression with time. For alcoholization index calculation, each animal was individually considered, independently from the alcoholization protocol to which it was submitted. Individual normal equations were determined and corresponding correlation coefficients (r) were calculated. Only animals having $r \geq 0.7$ were considered. This value corresponds to a statistically significant regression, $p < 0.05$, on the condition that the degrees of freedom would be ≥ 6. In the present experiments this was always the case. Then individual A values were calculated (Fig. 1):

$$A = \frac{T \times B}{2}\ ;\ B = \text{BAC calculated at day T (g/l)}$$

Withdrawal Syndrome

The severity of the withdrawal syndrome was evaluated by using a method described by Aufrere [1]. Animals were periodically individually observed over 12 hours after removal from the alcohol inhalation chamber, and scored for the occurrence of 4 well-defined behavioral disorders. These disorders were related to an increasing stage of severity of the withdrawal syndrome (Aufrere [1]: Stage A: normal; stage B: stereotyped head and limb movements; stage C: tremors; stage D: squealing; stage E: ventro medio-distal flexion (pudicity). Finally each animal was classified according to the stage of maximum severity reached.

RESULTS AND DISCUSSION

In the first experiment it was observed (Table 2) that due to differences in individual sensitivity, the slopes of BAC curves (not shown) were not identical for the various groups. However, the differences between groups were not statistically significant. On the other hand, it was observed that the higher A was, and therefore the longer the period of alcohol administration was, the more severe was the withdrawal syndrome. These results are in agreement with those obtained by other researchers using different methods [4, 6, 11, 18], and our results support the view that the severity of the ethanol withdrawal syndrome is closely related to the total dose of ethanol administered.

In the second experiment, values of A were quite close to each other, except for the 9-day ethanol-administration group (Table 3). Nevertheless, the differences between groups, compared 2 by 2, were never statistically significant. On the other hand, it was observed that the steeper the slope of the evolution of the BAC curves, the more severe was the withdrawal syndrome (Table 3). In other words, even if A was kept constant, the withdrawal syndrome intensity rose in accordance with the speed of increase of BAC.

Thus, when the same total amount of ethanol was absorbed by the animals, the more rapidly the ethanol dose level increased, the more dramatic were the chronic effects on the central nervous system. These results are paralleled by those obtained in man by Eggleton [3], who showed that in acute alcohol intoxication, the CNS effects of ethanol were related to the slope of the increasing phase of the BAC curve.

Hill and Bangham [8] have argued that ethanol physical dependence characterized by the withdrawal syndrome, represents the state of functional tolerance and dependence de-

TABLE 2

Duration of alcohol exposure (days)	Number of animals	A (mg/l/day / 2 ±SEM)	Frequency of withdrawal syndrome stages (%) A	B	C	D	E
7	12	1.4 ± 0.4	100	0	0	0	0
14	12	9.5 ± 1.4	41.5	41.5	17	0	0
21	12	16.5 ± 2.1	25	25	33	17	0
29	14	30.9 ± 2.3	7	7	43	43	0
35	19	43.2 ± 2.0	0	0	26	42	33

TABLE 3

Duration of alcohol exposure (days)	Slope of BACs curves (g/l ± SEM)	A (g/l/day / 2 ±SEM)	Frequency of withdrawal syndrome stages (%) A	B	C	D	E
32	0.03 ± 0.005	15.6 ± 2.6	50	33	17	0	0
27	0.042 ± 0.003	15.0 ± 1.3	47	32	16	5	0
21	0.075 ± 0.008	16.5 ± 2.1	25	25	33	17	0
9	0.306 ± 0.048	12.4 ± 0.9	11	17	22	22	28

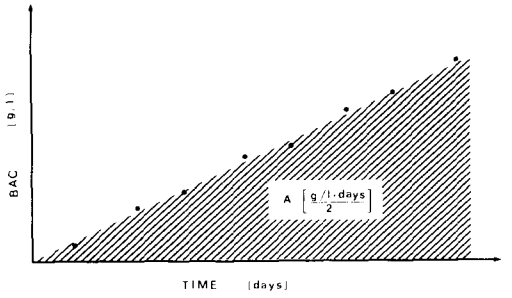

FIG. 1. Shaded area="Alcohol Exposure Index" = area under curve (g/l × days/2).

veloped by neurones during chronic alcohol intoxication. In other words, they assume that functional tolerance and dependence are two distinct aspects of a single phenomenon. However, it has been recently demonstrated [16,19] that tolerance to and dependence on alcohol can be dissociated. It seems now well demonstrated that tolerance is the consequence of a rapid adaptation of the synaptic membrane to the effects of ethanol [2, 7, 9, 10, 12, 16]. Furthermore, Littleton [17] has suggested as a mechanism of dependence on ethanol the following hypothesis: In chronic ethanol administration, a stage exists where tolerance is progressively increasing to a maximum, but where animals are not dependent on ethanol. It is assumed that at this stage the central nervous system is still able to adapt quickly and the behaviour of the animals can rapidly return to its initial state after alcohol removal. However, if alcohol intoxication is prolonged, this possibility of adaptation is increasingly reduced and eventually removal of ethanol would induce the so-called withdrawal syndrome. In other words, physical dependence will occur only when tolerance has reached a stage of irreversibility.

The present results are not in opposition to this hypothesis. Indeed, they demonstrate that inependently of the total amount of alcohol absorbed by animals during chronic treatment, the time course of the appearance of the withdrawal syndrome is positively correlated with the time course of the increase in ethanol dose. This suggests that the physicochemical changes in synaptic membranes related to dependence [17] may occur more rapidly, and be more significant, under conditions where ethanol intake is increasing at a rapid rate.

The results presented in this paper are limited to the effects of ethanol on nervous cells. It would be of interest to consider other types of cells or organs. For instance, it would be interesting to determine if the same alcohol exposure protocols, including progressively increasing administered doses, would induce the same variation of effects on liver as on brain. The present results suggest it would be possible to explain in part the diversity of disorders produced by excessive alcohol ingestion in relation to customs of alcoholic beverage consumption [5,15].

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