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Aversive Factors in Alcohol Drinking in Humans and Animals

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MYERS, R. D. AND J. A. EWING. *Aversive factors in alcohol drinking in humans and animals.* PHARMAC. BIOCHEM. BEHAV. 13: Suppl. 1, 269–277, 1980.—In human subjects there is a wide range of response to the taste of alcohol in varying concentrations. What some people find totally aversive may be accepted with relish by others. Both individual and racial responses to ingested or injected alcohol can be aversive, since markedly dysphoric experiences can occur. Human studies also suggest that the euphorigenic properties of alcohol are very variable between subjects and that this correlates with characteristics of biogenic amines present in the body. Except for relatively low concentrations, the laboratory rat, used commonly in alcohol drinking experiments, avoids the selection of alcohol. Paralleling human studies are animal investigations that manipulate the levels of chemical substances in the brain and demonstrate effects of this upon alcohol self-selection. When certain tetrahydroisoquinolines or β-carboline substances are infused into the brain of the rat or monkey, the aversive nature of orally ingested alcohol, particularly in very high concentrations, is overcome. In contrast, however, when a high dose of a tetrahydropapaveroline is infused, the animal's volitional intake of alcohol is inhibited and even weak concentrations of alcohol are rejected. The possible mechanisms for this phenomenon and the recent investigations of agents acting on CNS opiate receptors in reinstating alcohol aversion are considered.

Acetaldehyde Alcohol and Aversion Drinking Dopamine β-hydroxylase Metabolites of alcohol Naloxone Naltrexone Oriental flush Tetrahydroisoquinolines Taste factors

THIS paper describes some of the known factors which underlie and contribute to the aversiveness of alcohol. In terms of behavioral, biochemical and psychological mechanisms underlying the rejection of alcohol-containing fluids, there is a notable parallelism between human and animal cases. A clear scientific understanding of these mechanisms is vitally important for two main reasons. An aversion to alcohol, if it can be pharmacologically induced, would be of therapeutic value to those individuals who are unable to use the drug in moderation. Second, a much needed animal model of alcoholism, based on immoderate volitional intake, cannot be derived unless the typical laboratory animal's innate aversion to alcohol can be reversed and then fully overcome.

CLINICAL OBSERVATIONS

At a purely clinical and nonexperimental level, a wide variation in human response to alcoholic beverages can be observed. Age seems to be a factor, in that beverages such as beer that are relatively aversive to children and even adolescents may be perceived as extremely desirable and flavorful to adults, although in many such instances this has to be considered an acquired taste. However, associated with the aging process is a loss, in some instances, of the gustatory attractiveness of alcoholic beverages.

Apart from the taste of alcoholic beverages, the principal impingement upon the human is its effects upon the central nervous system (CNS). Again, a very wide variation in sensitivity to these effects has been observed in the clinical setting. Thus, one of us (J.A.E.) has seen people reporting feeling very sleepy and dizzy with blood alcohol concentrations of no more than 10 mg/100 ml. Such responses appear not to be purely due to suggestion, since they can occur even in the absence of subjective knowledge of having ingested alcohol. At the other end of the scale, heavy drinkers and patients suffering from alcoholism may rate themselves as "not drunk" or "only slightly so" when blood levels of between 200 and 300 mg/100 ml are present.

Those who regularly use alcoholic beverages appear to experience in general a sense of euphoria and well-being, at least when low levels of blood alcohol concentration are present. With higher levels, many people begin to perceive the intoxication as somewhat threatening. Some seem to fear a loss of control and may even report a sense of anxiety. Although high sensitivty to low levels of alcohol and the experiencing of a dysphoric response can occur in both sexes, the symptoms have been seen more frequently in females than in males.

Human Experimental Studies Involving Taste

Wilson [57] has determined taste responses to ethanol by an adaptation of the method used by Harris and Kalmus [22] for measurement of the taste threshold of phenylthiocarbamate. Subjects are presented solutions of alcohol in water varying from 0.25 to 64% of absolute alcohol. Wilson reports that at a percentage of 4.2 ± 0.24 (SE) alcohol, the solution is perceived as sweet. A burning sensation, which Wilson

believes to be an all-or-none phenomenon, is perceived at a higher concentration of 21.2 ± 1.22 (SE) percent of absolute alcohol. Wilson finds also a circadian rhythm with the threshold being at its lowest at 18:00 hours and at its highest at 06:00 hours. Finally the subject refuses to accept higher concentrations. Introducing 30% alcohol directly into the stomach to give 0.8 g of alcohol per kg of body weight raises both thresholds. This phenomenon may also apply to the rat since Deutsch and Koopmans [9] report a large and lasting enhancement of alcohol consumption over control levels after direct infusion of 10% alcohol into the stomachs of rats for six days. On the other hand, in that experiment it is possible that the increased consumption represents a response to subjective sensations of alcohol withdrawal.

Wilson also notes that one can alter the threshold to sweet taste and burning sensation in humans, for example, by giving metronidazole at a dosage of 800 mg in 24 hours. In our laboratory we have confirmed some of Wilson's findings and carried them further with special reference to the issue of aversion. Twenty-four healthy males aged 20 to 30 years were taste-tested three times weekly during a total of four weeks. Subjects received either metronidazole or a placebo on a crossover double blind design involving ten days' administration of either the drug or the placebo. The presence of the metronidazole was associated with a significant increase in the threshold to burning sensation but not to that of sweet or aversion. For the remainder of this report we will simply discuss responses while subjects were taking placebo.

Subjects were invited to go on taste-testing the various concentrations which were increasing in 5% steps between the levels of 30 and 100% absolute alcohol. They were told that they could stop the taste-testing at any time that the solutions became too unpleasant. The average percentage at which this aversive concentration was reported was 80.6 ± 21.1 (SE). However, some subjects were perfectly willing to go all the way to 100% concentration.

Humans typically choose alcoholic beverages that they find palatable when they wish to drink; therefore, these experiments have little relevance to actual drinking occasions. However, they do serve to demonstrate the wide variability in biological systems.

Nervous System Factors in Aversion

Over the course of some years we have conducted several human experiments to evaluate the possible role of the sympathetic nervous system in alcohol intoxication. Since our subjects are healthy adults and are not available for more than a few hours at a time, we are unable to collect 24-hour urine specimens. Therefore, as a possible index of sympathetic state we measure the peripheral blood levels of dopamine-β-hydroxylase (DBH), the enzyme responsible for the production of epinephrine from dopamine. In our first series of three separate studies, using the same subjects, we found the usual wide response of subjective reactions. However, when we studied subjects falling into the lower quartile of DBH level (before doing any drinking) and compared them with subjects in the higher DBH quartile, there were significant differences, even though similar doses of alcohol had been given orally and the blood alcohol concentrations were identical at an average of 64 mg/100 ml. Using an analog scale we found that while those with high levels felt a general sense of euphoria this was less so in those with the low levels of DBH. Moreover, those with low levels of DBH perceived themselves as significantly more drunk and sick [15].

These studies were essentially corroborated by a fourth one in which we invited subjects to spend an evening drinking under clinical conditions. The terms of the protocol permitted people to leave when they wanted and only fourteen subjects stayed on for a total of six hours of drinking. The seven subjects falling below the mean in terms of DBH level drank an average of 5.4 drinks and had a final blood alcohol level of 66 mg/100 ml. The seven subjects above the mean drank an average of 9.6 drinks and ended up with a final blood alcohol concentration of 131 mg/100 ml [18].

In yet another study our results appeared to be significantly confounded by a social factor, as far as we can determine. Briefly, we invited subjects to come with a spouse or a friend and found that under these conditions, the social factors overshadow the biological ones. We were no longer able to demonstrate the relationship between DBH level and amount drunk voluntarily [17]. In our most recent study, we screened subjects and selected people with high and low levels of DBH, then invited them to a cocktail party. This time, females with higher DBH levels drank more and achieved higher blood alcohol concentrations, than females with lower DBH levels, but the phenomenon could not be demonstrated in males (unpublished observation).

Thus, while it appears that the adrenergic system may relate in some way to the response to ethanol it is by no means a clearcut relationship, and it would be naive to assume that a solitary factor such as a catecholamine is at work. Nevertheless, there are reasons to believe that some form of relationship exists. Although we know of no other laboratory that has replicated our experiments, a study reported by Ahlenius *et al.*, involved premedicating subjects with α-methyl-p-tyrosine (αMpT) or a placebo before a drinking experiment [1]. Tyrosine is a precursor of norepinephrine and those premedicated with the inhibiting αMpT showed less euphoria; thus, this experiment substantiates our findings. In addition, there are a variety of animal studies that indicate the same possibility. For example, drugs can be given to animals to produce significant inhibition of DBH. Under these circumstances animals have been shown to self-select less alcohol whether by mouth [4] or by bar pressing for intragastric administration [8].

One well-known DBH inhibitor is disulfiram but this compound, of course, is also an aldehyde dehydrogenase inhibitor. Thus, animals premedicated with disulfiram may well find alcohol aversive because of higher levels of circulating acetaldehyde. However, there are drugs which inhibit DBH without interfering with the metabolic breakdown of alcohol and these, too, reduce alcohol intake.

Some have speculated that possibly an effect of administering disulfiram (Antabuse) to humans is to reduce the craving for alcohol by virtue of DBH inhibition. However, an experiment conducted in our laboratory has failed to support this concept [46]. In the first place, healthy subjects receiving Antabuse in the usual clinical dose showed no significant drop in the levels of circulating DBH. Secondly, although we could not allow our subjects actually to drink alcohol under the experimental conditions, they showed no significant change in craving to drink as based on subjective measures. It should be noted that the doses used in animal studies to produce DBH inhibition are many times greater than those given to humans.

To sum up the situation concerning the adrenergic system, it seems possible that there is a relationship between responses to alcohol and the neurotransmitters within that system. However, we have much to learn and, in any case,

we may not be dealing with an aversive component. Although our low-level DBH subjects reported feeling more sick and, in a long-term drinking experiment, did select less drinks, they may have been experiencing less euphoria rather than more aversion.

Racial Factors in Aversion

Although a particular form of sensitivity to alcohol in Oriental peoples may have been known to travelers for a long period of time, it appears that the first scientific study of this phenomenon was that of Wolff in 1972 [58]. He reported that native Japanese, Taiwanese and Koreans responded with a rapid facial flush after ingesting small amounts of alcohol that had almost no effect on matched groups of Euro-Americans. In 1973, he extended his work to report a vasomotor flushing in response to small quantities of alcohol in Chinese, American-born Japanese and Chinese, one tribe of North American Indians and the hybrid offspring of Caucasoid and Mongoloid parents [59]. He concluded that facial flushing to alcohol may reflect a genetic variation in vasomotor sensitivity. Wolff pointed out that the relevance of this phenomenon for the problems of alcoholism remains unclear. For example, although Chinese and American Indians appear to share the alcohol flushing response, the incidence of alcoholism in the two groups differs radically. Thus, he drew the obvious conclusion that the incidence is determined by more complex factors than those he was describing. However, he went on to say, "Conceivably, the prevalence of the highly visible flushing response will inhibit Mongoloid groups from drinking as long as their social structure is intact and exercises sanctions against intoxication. When the social cohesion of a culture is destroyed, as it has been in the case of the American Indians, a greater susceptibility to alcohol intoxication may act as one of several predisposing factors for alcoholism."

Although we do know more about the Oriental sensitivity to alcohol since Wolff wrote these words, his conclusion remains a valid one. No one today would have the temerity to expect to find a single factor of etiologic significance for alcoholism. Only a concept that includes both the predisposing and protecting aspects of psychosocial as well as biological influences can provide adequate understanding.

We first studied the phenomenon of Oriental sensitivity by comparing responses to alcohol by mouth in 24 Oriental and 24 non-Oriental subjects [16]. They were matched for previous drinking experience and we had some difficulty in finding non-Orientals who drank as little as the Orientals. In response to doses of alcohol of 0.3 and 0.4 g/kg body weight, 17 of the 24 Oriental subjects showed flushing of the face that also often appeared on the ears and neck and sometimes on the chest and the palms of the hands. Flushing was noted to a much lesser degree in non-Oriental subjects, i.e., only 3 of the 24. Significant increases in heart rate and in pulse pressure were seen in the Oriental but not the Occidental subjects. Most interesting was the listing of subjective complaints by Oriental subjects. They were significantly more likely to report feelings of dizziness, pounding in the head, muscle weakness and tingling sensations and less likely to report feeling relaxed, happy, confident and alert as did the non-Oriental subjects.

Our data suggest that the discomfort of the Oriental subjects is primarily physiological in origin and sufficiently dysphoric to be mildly aversive. This correlates well with the lower rates of alcoholism reported from Oriental countries as compared with North America and Europe [49]. In our early studies, there was a trend for blood acetaldehyde levels to be higher in the Orientals than the non-Oriental subjects. However, there are problems inherent in the measurement of blood acetaldehyde levels, and therefore in a later study, while alcohol was administered intravenously, we demonstrated significantly higher levels of acetaldehyde in the breath of Oriental than non-Oriental subjects [19]. On that occasion we also found that the flush phenomenon is not essential for the presence of dysphoric symptoms such as pounding in the head. Three Chinese subjects who had breath acetaldehyde levels significantly above the mean found in non-Orientals showed no flushing while complaining of significant dysphoria in one instance, slight dysphoria in two instances and pounding in the head in two instances.

A series of studies done elsewhere now confirms the presence of higher levels of acetaldehyde in Oriental subjects with sensitivity to alcohol [23, 45, 61]. Moreover, comparison of flushing and non-flushing Orientals has demonstrated a conspicuous rise in blood acetaldehyde levels after drinking in those who flush [32]. Ijiri has shown that catecholamine excretion does not change in any reliable direction after drinking in non-flushers but doubles in those who flush [23].

When acetaldehyde is circulating in the body at these apparently toxic levels it appears to lead to the release of various hormones; one group has speculated that the flush may be due to the vascular effects of histamine [45]. It has now been demonstrated that when subjects who normally flush after consuming alcohol are given H1 and H2 blockers (diphenhydramine and cimetidine, respectively) there is a blockade of the flushing phenomenon [41].

A possible explanation for the higher levels of acetaldehyde in Oriental subjects might be a relatively lower availability of aldehyde dehydrogenase. However, it is conceivable that an atypical alcohol dehydrogenase might lead to a more rapid production of acetaldehyde, at least until the rate limiting step is reached, which appears to depend upon available NAD [55]. Thus, Stamatoyannopoulos and colleagues demonstrated a high population frequency of the atypical form of liver dehydrogenase in Japanese [50]. A similar finding among Chinese has been reported by Teng and colleagues [52]. This is the "atypical" alcohol dehydrogenase that first was described by von Wartburg in 1965 [54]. Between 5 and 10% of English and no more than 20% of the Swiss population have the atypical form of alcohol dehydrogenase, whereas the percentage in Japanese and Chinese people has been found to be as high as 85 to 90% [52].

Thus, the existence of an inborn aversive response to alcohol is firmly demonstrated, and its correlation with higher levels of acetaldehyde is also shown. However, the exact nature of the mechanism remains unclear.

At a clinical level there is some additional evidence to support the possibility of histamine as a major component in the sensitivity reaction. For example, one physician who is married to a Chinese wife reports severe facial flushing, tachycardia, palpitations, dizziness and, in some instances, faintness in his wife and some members of her family after alcohol. On the other hand, two members of the family only experience flushing and one is unaffected by alcohol. Of great interest is his report that the reaction is more severe after drinking red wine than after drinking white wine (E. Norris, personal communication, 1977). Analytical studies of wine have shown that high levels of histamine are commonly found in red wine, which has been matured without wood [53].

Induced Sensitivity to Alcohol

There are certain substances including drugs that are associated with alcohol flushing. A typical example would be the chlorpropamide-alcohol flushing reaction that appears to be genetically dominant [24]. It has been found that non-insulin dependent diabetics who show the response are less likely to develop diabetic retinopathy than those in whom the flush reaction is absent [25]. Thus a useful diagnostic test may be built around this particular type of alcohol sensitivity.

Some substances used in industry are also able to interfere with the metabolism of alcohol and we should recall that the development of disulfiram for the treatment of alcoholism followed the accidental discovery of the alcohol-disulfiram reaction [21]. A recent report describes facial flushing after workers consuming alcohol are exposed to dimethylformamide [28]. Sensitivity reactions have also been described in subjects who drink alcohol after eating a specific mushroom [6].

To sum up, there are many instances of aversive responses to alcohol in humans. These can represent individual biological variations, racial characteristics and specific responses to exogenous substances such as chemicals and ingested drugs.

ALCOHOL SELF-SELECTION IN ANIMALS

Interesting and direct corollaries of these clinical observations have evolved from studies with rodents and other species. Sensory factors, particularly in the rat and mouse, seem to play an important part in an animal's general aversion to alcohol [37]. In fact, the gustatory quality of alcohol limits an animal's voluntary intake in a self-selection paradigm as well as its ingestion of alcohol under the condition of forced choice.

Gilbert [20] has shown that the sweetening of a 5 or 10% alcohol solution with either saccharin or sucrose augments the rat's intake of alcohol sharply. Further, when the animal is deprived of food, the consumption of non-sweetened alcohol rises to 10 gm/kg, but to nearly 14 gm/kg when adulterated with sucrose. Eckardt [11] demonstrated that a learned taste aversion to a previously preferred flavor develops readily when a rat ingests a 5% solution of alcohol rapidly on five successive occasions (1.5 g/kg doses). This suggests that even at a low dose of 1.5 g/kg, alcohol produces aversive consequences internally. If given by the intraperitoneal route, alcohol also induces the same sort of learned taste aversion when paired with an originally preferred solution [12]. Thus, alcohol could be "perceived" by the animal as being equivalent to a toxic substance or poison, and hence is a fluid which should be avoided at all costs.

That olfactory cues are also involved in alcohol's aversive characteristics is well known. Anosmia produced in the rat or mouse by olfactory bulbectomy can eliminate, at least partially, the animal's aversion to alcohol [37]. However, removal of olfactory cues by surgical bulbectomy does not necessarily abolish the preference for the fluid exhibited by an alcohol-preferring animal such as the C57 BL mouse [39].

Genetic Determinants

The inheritance of an addictive liability to alcohol has begun to be accepted by an ever increasing number of scientists [43]. A large number of classical studies with animals dating back to those of Mardones, McClearn, Eriksson and their co-workers clearly implicate a complex of genetic factors in an animal's behavioral and physiological reactions to alcohol [43]. For example, in the animal that has been selectively bred for its preference or rejection of a 10% solution of alcohol, clear-cut genetic differences arise in the susceptibility to the acute, intoxicating effect of alcohol [43]. Moreover, the non-alcohol-preferring rat (ANA), in contrast to the alcohol-preferring animal (AA), fails to exhibit the "alcohol deprivation effect" in which a limited access to alcohol augments its voluntary consumption in the normal rat [47]. These two strains differ further in their urinary excretion of electrolytes as well as the osmolality of urine when the rats are treated with alcohol given systemically [27].

Physiologically, there are large differences in the drinking of alcohol by mice as based on measures of blood pressure. The hypertensive mouse consumes significantly greater quantities of alcohol in a range of concentrations between 6 and 18% than the hypotensive animal [60]. Whether a factor in the central nervous system is responsible for these differences is not known. However, it is interesting that there are remarkable differences between rats of the Sprague-Dawley, Long Evans, Holtzman, and Wistar strains in their preference-aversion patterns for alcohol following the central lesioning of serotonergic or catecholaminergic pathways by 5,6-dihydroxytryptamine or 6-hydroxydopamine, respectively [30]. Again, a biogenic amine in the CNS could influence and partially determine alcohol's action centrally.

The belief in the genetic transmissability of certain aspects of alcohol's effects is not universally accepted. In a carefully carried out study involving maternal pair-feeding, weight-matching and cross-fostering techniques, it has been shown that alcohol given to pregnant rats gives rise to offspring which exhibit significantly greater locomotor activity at 60 days post-partum [29]. Thus, alcohol could act directly on the fetus through its placental transfer to evoke changes in activity which are ascribed by some scientists to a genetic trait. Alternatively, the transfer of an ovum from a C57BL alcohol drinking mouse to the uterus of a non-drinking DBA mother, which in turn then raises the offspring, results in the sustaining of strain-typical, high alcohol preference in the C57BL animal [42]. However, the same uterine transplant of an ovum from the alcohol avoiding DBA mouse to a C57BL drinking mother nevertheless results in a significant increase in alcohol drinking in the DBA offspring. This rise is presumably due to some factor in the pre-natal or post-natal environment. Therefore, not only are the genetic factors for alcohol drinking present in the fertilized ovum, but it is clear also that the aversion to alcohol can be modified greatly by unknown factors in the fetal or neo-natal environment [42].

Reversability of Aversion to Alcohol

In keeping with the clinical reports described earlier in this paper, it is apparent that acetaldehyde given to an animal also exerts profound effects on the ingestion of alcohol as well as on other behavior. Oritz and colleagues [40] have found that chronic exposure to either alcohol or acetaldehyde induces similar signs of withdrawal and a subsequent depression of locomotor activity. Further, in the C57BL alcohol-preferring mouse, the blood level of acetaldehyde is higher following an anesthetic dose of alcohol [26]. Studies with disulfiram and other evidence obtained with the enzyme aldehyde dehydrogenase also point to the fact that a systemically elevated level of acetaldehyde tends to reduce an animal's alcohol consumption [13]. Again, as with the human patient, acetaldehyde clearly induces an aversion to alcohol

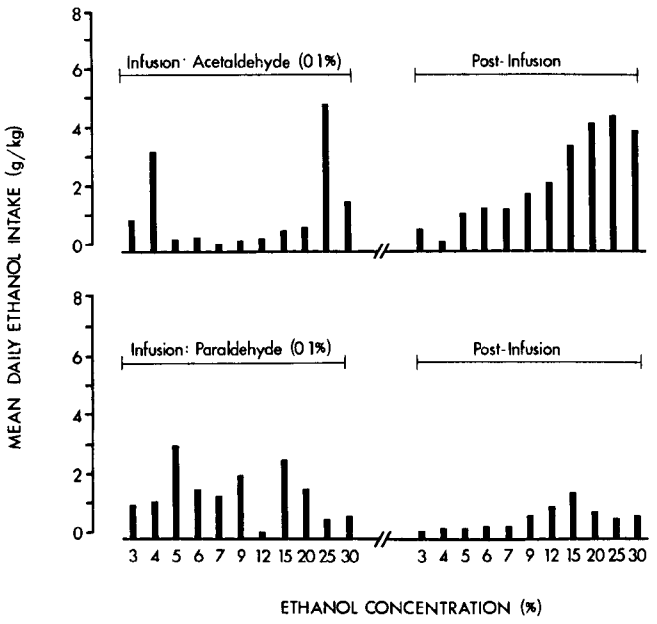

FIG. 1. Daily intake of alcohol (g/kg) in two rhesus monkeys. 0.1% acetaldehyde (TOP) or 0.1% paraldehyde (BOTTOM) was infused into the respective monkey's lateral cerebral ventricle in a volume of 100 μl every 15 min around the clock during the first 12-day (3–30% alcohol) preference sequence (abscissa). One week later, after infusions had been discontinued, the 3–30% alcohol preference sequence was repeated. From Myers *et al.*, 1972 [38].

possibly by a mechanism involving biogenic amine metabolism in the brain [13,36]. Interestingly, Amir [2] has found a significant positive relationship between alcohol intake and the level of aldehyde dehydrogenase in the brains of rats not only from different strains but of either sex.

In experiments done in our laboratory in the late 1960's, we found that acetaldehyde, when infused chronically and directly into the cerebral ventricle of the rat, causes a significant enhancement in alcohol preference in the range of concentrations between 3 and 30% [36]. Similarly, Amit and co-workers [5] have found that a rat depresses a lever to deliver a minute amount of acetaldehyde into its cerebral ventricle. Thus, in both instances it would appear that acetaldehyde acting within the central nervous system exerts a rewarding effect which is associated with the imbibition of alcohol. However, a direct causal relationship between levels of acetaldehyde in the brain and either the preference for or aversion to alcohol does not necessarily exist. Eriksson and colleagues [14] have demonstrated that the reduction of alcohol intake caused by a high dose of thiamine incorporated in the diet of the rat is not correlated in any way with a change in blood acetaldehyde concentration.

A persistent question remains concerning the possible role of acetaldehyde, if present in the brain of a human, and the characteristics of a person's consequent drinking behavior. In an attempt to simulate the human situation neurochemically, we have used the unanesthetized rhesus monkey as a model for studying the role of cerebral acetaldehyde in the induction of an immoderate intake of alcohol. In these experiments, each monkey was injected with 100 μl of the test solution into its lateral cerebral ventricle every 15 min around the clock [38]. Whereas 8% alcohol given intraventricularly induced alcohol drinking in three of four monkeys, during the course of a 12 day infusion regimen, paraldehyde and acetaldehyde were essentially without effect on the voluntary self-selection of the fluid [38]. However, as illustrated in Fig. 1, the intake of alcohol increased substantially following the termination of acetaldehyde infusion. As the concentrations of alcohol offered to the primate were increased daily in the 9 to 30% range, the alcohol consumption reached 4.0 g per kg levels. On the other hand, paraldehyde, which was used as a hydrocarbon control, was essentially without effect when infused identically into the cerebral ventricle of a matched-pair monkey.

Why acetaldehyde should produce a delayed effect on an animal's consumption of alcohol is not known. In view of the accumulated evidence that aldehyde-amine condensation products not only form endogenously [34], but also exert profound pharmacological effects, including the induction of alcohol drinking, it is conceivable that a metabolic by-product of acetaldehyde metabolism is responsible for the post-infusion selection of alcohol offered in the normally aversive range of concentrations [34]. Acetaldehyde does penetrate the blood-brain barrier and readily enters the brain. Following the intragastric administration of alcohol, it is detectable by means of perfusion techniques in the interstitial fluid of the brain [56]. Since the concentration of acetaldehyde in the interstitial fluid can be quantitated, whereas that in whole brain can not, it is probable that acetaldehyde is rapidly catalysed cellularly by aldehyde dehydrogenase present in the brain's nerve and neuroglial elements [56].

Pharmacological Reversal of Alcohol Aversion

Many attempts have been made by scientists in many disciplines to induce alcohol drinking pharmacologically in the rat and other animals [37]. However, other than in those investigations in which the intracerebro-ventricular approach has been used, there is little evidence to suggest that an animal will self-select alcohol over time to the point of physical dependence and withdrawal. For example, Bass and Lester [7] have shown that during the period of recovery from a severe thiamine deficiency, rats will voluntarily ingest up to 13 g/kg alcohol per day for a short time. Nevertheless, no signs of physical dependence nor withdrawal appear as a result of this high intake, even though the amounts of ingested alcohol exceed the rat's capacity to metabolize alcohol.

Amine-aldehyde condensation products have been implicated theoretically in the etiology of alcoholism for many years [33]. Until relatively recently, experimental information had not been obtained to support the idea of a possible involvement of this class of endogenously formed substances [34] in the disease. From experiments undertaken in our laboratory with both rats and monkeys, it would now seem clear that certain of the amine-aldehyde conjugates could play some part in the development of abnormal patterns of alcohol drinking.

Over the last five years, a number of studies have been published which show that the administration of certain dopamine and serotonin-aldehyde metabolites profoundly affect an animal's self-selection of alcohol [34]. For instance, Melchoir and Myers [31] have reported that the tetrahydroisoquinoline derivative, tetrahydropapaveroline (THP), sharply increases the animal's voluntary consumption of alcohol when the compound is infused in minute doses into the cerebral ventricle of the Sprague-Dawley rat every 30

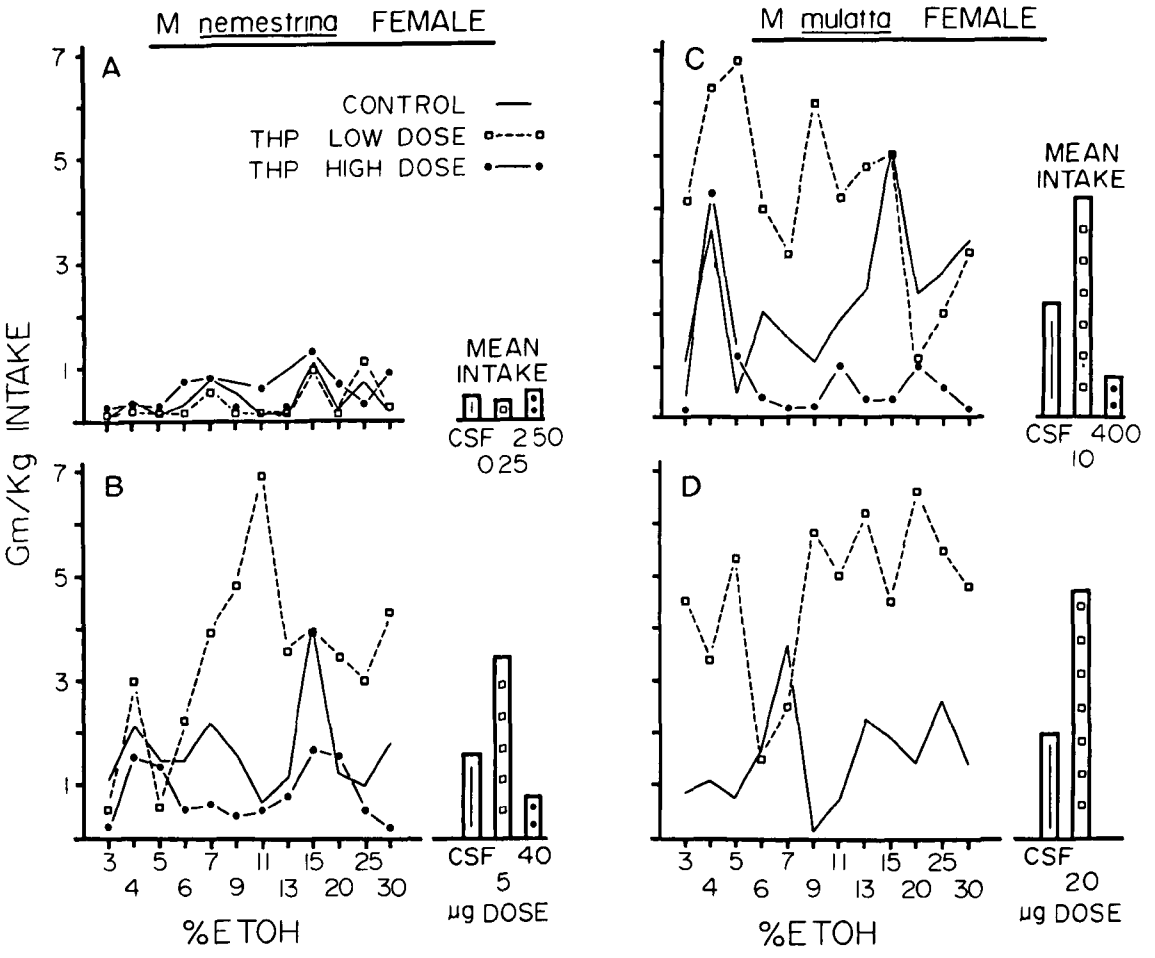

FIG. 2. Daily intake of alcohol (g/kg) in two pig-tailed (LEFT) and two rhesus (RIGHT) monkeys. Artificial CSF was infused in the lateral cerebral ventricle of each monkey, in a volume of 400 μl, during the first 12 day alcohol (3–30%) preference sequence. Next the lower dose of THP (abscissa) was infused identically during the second 12-day preference sequence. Finally, the higher dose of THP was infused during the third test sequence (except monkey D). From Myers, Ruwe and McCaleb, 1980, in preparation.

min around the clock. Of particular interest is the fact that highly aversive solutions of alcohol, in concentrations ranging from 11 to 30%, are consumed by the THP-infused animal, and the g/kg intake of alcohol is steadily augmented [31]. Two other important facets of this observation are notable. First, the animal that drinks alcohol, following intracerebroventricular THP infusion, exhibits a permanent preference for alcohol even as long as six months after the last exposure to alcohol or THP infusion [31]. The permanency of the alkaloid's effect has its counterpart in the human alcoholic condition. Second, THP-injected rats show signs of withdrawal and ataxia as well as significantly elevated blood alcohol levels [31]. Thus, the THP-induced alcohol-consuming rat might eventually serve as an animal model for the syndrome of pathological drinking observed in the human.

The infra-human primate has also been used as an animal test model of the amine-aldehyde condensation product hypothesis. In investigations done in our laboratory, *Macaca mulatta* (rhesus) and *Macaca nemestrina* (pig-tailed) monkeys were surgically fitted with chronically indwelling intracerebroventricular cannulae designed for chronic or acute injections. Post-operatively, a control pre-screen with CSF injections was done to determine the preference-aversion curve for each monkey at concentrations of alcohol from 3 to 30% offered over a 12 day interval. Following an initial day of priming, in which the selected dose of THP was infused unilaterally into the monkey's cerebral ventricle, in a 400 μl volume, the animal was then given the same dose at the same time each day during the course of the alcohol preference test. After the initial CSF control infusions, each of the successive 12-day preference sequences was separated by an interval of 2 to 14 days.

Figure 2 presents the records of four adult female monkeys, two rhesus (Fig. 2, *right*) and two pig-tailed (Fig. 2, *left*). In record A, the monkey drank no alcohol during the first test sequence, while the CSF control infusions were given. Neither a dose of 0.25 μg THP given in the second 12-day preference sequence nor the higher dose of 2.5 μg THP infused during the third 12-day test sequence caused any change whatsoever in the monkey's g/kg intake of alcohol. In record B, a dose of 5.0 μg THP infused intracerebroventricularly in the second pig-tailed monkey began to induce a rise in alcohol intake at the 7% concentration. This

reached a level of intake of 7 g/kg of the 11% solution, after which the monkey's intake of alcohol stabilized at approximately 4.0 g/kg per day through the 30% concentration. During the third 12-day preference test, 40.0 μg of THP infused into the ventricle daily caused a sharp suppression of alcohol intake. At virtually every concentration offered to the monkey, the g/kg consumption fell to a level even below that of the CSF control.

The two rhesus monkeys gave nearly identical results. As shown in record C, 10.0 μg of THP infused into the monkey's ventricle produced alcohol drinking up to the 20% solution in quantities from 3.0 to 7.0 g/kg/day. Once again, when the dose of THP was elevated 10-fold to a 400 μg level during the third preference test sequence, the monkey's intake of alcohol was markedly suppressed (except for the 4% solution) to well below the initial CSF control level. The fourth monkey (D) was given what was thought to be an optimal dose, i.e., betweeen 10 μg and 40 μg. As shown in record D, a dose of 20 μg THP more than doubled the primate's intake of alcohol above that of the CSF control level. At concentrations from 9 through 30%, the animal drank between 4 and 7 g/kg alcohol per day. Of interest here is the fact that in this monkey, the intake of alcohol seemed to accelerate at the higher, more aversive concentrations of the fluid, which parallels that seen in the rat [35].

Overall, these findings with the infra-human primate demonstrate several points. First, the monkey, which displays an aversion to alcohol under normal circumstances, can be induced neurochemically to drink the fluid. Second, THP administered directly into the CNS of the primate exerts a somewhat similar action as that seen in the rat given corresponding intraventricular infusions of the condensation product. Third, the aversion to alcohol production in the rat by a high dose of a tetrahydroisoquinoline alkaloid is equally evoked in the primate. In this case, the same inhibiting effect of THP on alcohol drinking occurs as that described previously in the rat [10,35].

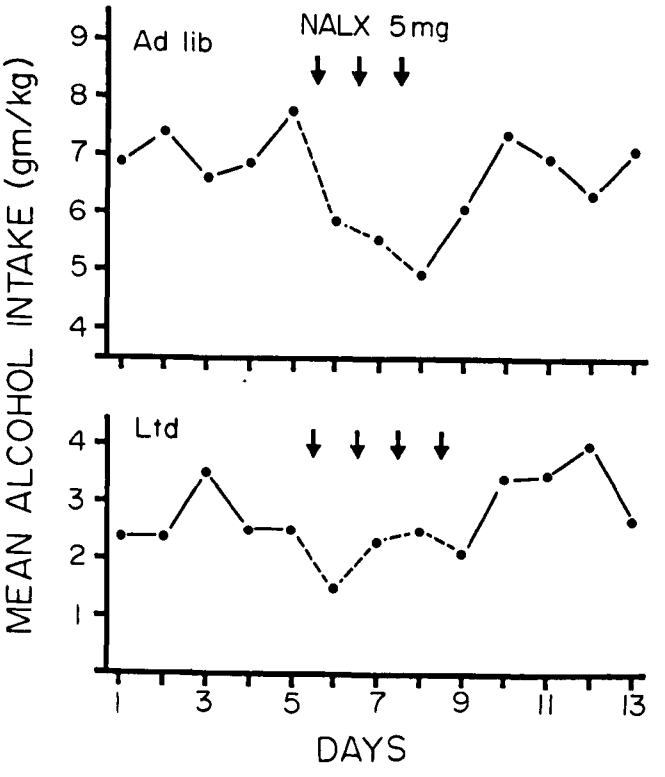

FIG. 3. Mean alcohol intake (g/kg) of rats infused earlier with THP or a protoberberine given intraventricularly. Each animal was given the choice of water and its most preferred alcohol solution (range 9–20%) which was held constant over the 13 test days. TOP: 5.0 mg/kg naltrexone injected subcutaneously (N=4) at 1700 and 2100 hr on days indicated by arrows. BOTTOM: 5.0 mg/kg naltrexone injected subcutaneously at 900 hr on days indicated by arrows to rats (N=6) just prior to a 6 hr period of access (Ltd) to alcohol, water and food. From Myers and Patel, 1980, in preparation.

Relationship of Opiate Receptors to Condensation Product Action

The potent pharmacological activity of certain tetrahydroisoquinolines could be related to the activity of opiate receptors in the brain. Naloxone, the stereospecific opiate antagonist, has been the chief tool for the investigation of this relationship. To illustrate, naloxone antagonizes the analgesic state produced by certain condensation products and can alter alcohol preference; its binding to brain tissue homogenate also is inhibited by certain alkaloid conjugates including protoberberines [33,51].

In experiments done in our laboratory since 1977, we have attempted to manipulate pharmacologically the level of alcohol intake in rats following their infusion of a given tetrahydroisoquinoline derivative in the cerebral ventricle. In confirmation of the finding of Sinclair *et al.* [48], morphine administered to the THP-treated, high drinking rat does suppress alcohol preference significantly. Concomitant with this is an equal reduction in food and overall fluid intake, suggesting that this narcotic analgesic could affect alcohol drinking by virtue of a non-specific sedative action. Following the administration of naloxone in small doses at intervals throughout the day, the intake of the high alcohol preference rat is again suppressed, but in this instance without notable changes in food or water intake (Lin and Myers, 1973, unpublished observations).

More recently we have examined the effects of a longer lasting opiate antagonist, naltrexone [3]. First, alcohol intake was determined in animals treated either with 1.0 μg/20 μl THP or 800 ng/20 μl of a protoberberine given once a day into the cerebral ventricles during the alcohol preference test sequence. Then naltrexone was administered subcutaneously twice a day at 1700 and 2100 hr, each time in a dose of 5 mg/kg, on three successive days in the middle of the preference test. Each of the four animals (two treated with THP, two with protoberberine) was offered its own preferred concentration of alcohol, which ranged from 9 to 20%, together with water ad lib. Figure 3 (top) illustrates the suppressant effect of naltrexone given to these animals, which were drinking approximately 7.0 to 8.0 g/kg alcohol per day. The opiate antagonist reduced the rats' consumption to below 5.0 g/kg/day following the drug's third successive injection ($t=3.06$; $p<0.01$). Thereafter, alcohol intake returned to its previous baseline level on the second day following the cessation of naltrexone administration.

As presented in Fig. 3 (bottom), a second group of rats was provided limited (Ltd) access to food, water and alcohol for only 6 hr per day. Under this condition, the six rats, which had been treated similarly with the same doses of THP or a protoberberine, averaged between 2 and 3 g/kg intake of their preferred solution during the 6 hr test period. Naltrexone injected again in the 5.0 mg/kg dose on four succes-

sive days, 15 min prior to the presentation of the food, water and respective alcohol solution, exerted no effect on alcohol drinking. Thus, a single administration of naltrexone had no effect on the animal which has been fasted and restricted to its intake of alcohol. Alternatively, as seen in Fig. 3 (top), repeated doses of naltrexone given at an optimal time at the first part of the rat's day-night cycle does cause a significant reduction below the basal levels of alcohol intake. One could envisage the possibility that the opiate antagonist reduces alcohol drinking, since naloxone occupies brain-stem opioid receptors which would be occupied ordinarily by a product of alcohol's metabolism to acetaldehyde. Alternatively it could act as an opiate agonist [44] and produce a morphine-like action at receptor sites or in precipitating a withdrawal-like condition, the effect would not only be aversive to the animal but perhaps be associated with the ingestion of alcohol.

CONCLUSION

The ingestion of alcohol is aversive to many humans and to numerous species of animals. If ingested in concentrations which would cause physical symptoms such as tolerance or dependence, alcohol would not be preferred in a free-choice situation. Many known biological factors contribute to the perceived qualities of alcohol and/or the deleterious consequences which can follow its consumption. These include, on the one hand, the sensory modalities of taste and smell, and on the other, internal factors of a biochemical nature. Acetaldehyde, the enzyme for acetaldehyde's degradation, and the by-products of its own metabolism could be equally important not only in terms of the aversive consequences of alcohol drinking but also in relation to the mechanisms in the brain which underlie the abnormal intake of alcohol. Further research into these complex neurochemical systems and pathways will perhaps reveal the factors responsible for uncontrolled drinking.

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