FORCED ETHANOL TREATMENT STIMULATES AND INHIBITS ETHANOL INTAKE
IN A RAT MODEL OF ALCOHOLISM

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Abstract — In a model of psychological dependence, a very stable ethanol intake was induced by a chronic (1-year) intermittent (once a week) exposure to intoxicating amounts of ethanol (24 h choice between ethanol and water, followed by 2.0 g/kg i.p.). After this year, the rats had continuous access to ethanol and water. Stability was shown by the ability of the rats to take the same dose of ethanol (in g/kg) when the concentration was changed from 10 to 20%. To study possible priming or inhibiting effects on ethanol intake, ethanol was injected i.p., first as 20%, 40% or 60% of the intake in the 24 h prior to the injection, then as fixed doses of 0.5, 1, and 2 g/kg, and the ethanol intake during the following 24-h period was recorded. The results showed that, following a low dose of ethanol, voluntary ethanol intake was increased in rats with a low, and decreased in rats with a high, ethanol intake, while high doses of ethanol seemed to decrease voluntary ethanol intake in all rats. The results are discussed in relation to theories about loss of control of drinking and relapse in humans.

INTRODUCTION

The results of earlier experiments in rats (Gauvin et al., 1993) indicate that ethanol in low doses can stimulate the subsequent voluntary ethanol intake, whereas high doses attenuate ethanol intake. This ability of ethanol to stimulate its own intake has been suspected to be one mechanism behind loss of control in drinking in humans, and also the ability of an exposure to small amounts of ethanol to cause a relapse in alcoholics. In their incentive-sensitization theory of addiction, Robinson and Berridge (1993, p. 267) stated that small doses of drugs of abuse, including alcohol, can maintain or even increase drug craving. Naltrexone, currently registered in several countries for relapse prevention in alcoholism, has been proposed to work by decreasing such an alcohol-induced craving for alcohol (O’Malley et al., 1996).

In our laboratory, a rat model of psychological dependence has been developed (Wahlström, 1994a). This model starts with a 1-year treatment period during which the rats are intermittently (once a week) exposed to intoxicating amounts of ethanol. The result of this treatment is that the rats take the same dose of ethanol independently of the offered ethanol concentration, indicating a need for a certain pharmacological effect of ethanol. The present experiment evaluates how this very stable ethanol intake is affected by pretreatments with different doses of ethanol.

Parts of this article have previously been presented at the 1999 Congress of the European Society for Biomedical Research on Alcoholism (Hedlund and Wahlström, 1999).

MATERIALS AND METHODS

Model of psychological dependence

The present model was designed to resemble the ethanol exposure pattern of human alcoholics. Three prerequisites were chosen for the model, although they might be specific only for certain types of human alcoholism. (1) Intoxication once a week is a drinking pattern that is seen in Nordic countries (Simpura, 1987) and in Wales (Bennett et al., 1991), where ‘Saturday night’ intoxication is a common feature, prior to the development of alcoholism. In the model, this is mainly accomplished by ethanol injections once a week during a 1-year treatment period. (2) Chronic treatment is important, since, in most cases, it takes a long period of exposure to ethanol before psychological dependence develops in humans. In the model, the rats are intoxicated once a week for a year, and then have a continuous choice between ethanol and water for 3 months before their psychological dependence is tested. (3) Voluntary oral intake was chosen, since in humans, alcohol is taken orally with a choice of other drinking fluids always available. In the present model, one of the bottles always contains tap water. Furthermore, after the 1-year treatment period, the oral choice is the only exposure to ethanol (no ethanol injections).

The effect of this treatment is that, during an evaluation period, rats treated according to these prerequisites take the same daily dose of ethanol in a continuous voluntary choice situation independently of the concentration of the offered ethanol solution in the range of 5 to 25% (Wahlström, 1987). This indicates a central nervous system mechanism that closely regulates ethanol intake, to reach a certain pharmacological effect. In our opinion, this defined need for a specific dose of the drug is an important part of both psychological dependence and craving. This stability of the ethanol intake independently of concentration is not seen in other rats, e.g. rats that have received, during the treatment period, saline injections instead of ethanol injections together with a continuous choice between 10% ethanol and water (Wahlström, 1987), or rats recently introduced to ethanol (Myers and Oblinger, 1977).

Animals

Male Sprague-Dawley rats (Mo:SPRD Han) were purchased from Mølkegaards Breeding Centre Ltd, Li Skensved, Denmark. They were kept in individual cages for 10 days prior to the start of experiments. Each cage was equipped with two drinking bottles. One of the bottles always contained tap water and the other contained either water or an ethanol solution (10 or 20% w/v). Food [commercial rat pellets, R34,
Lactamin, Stockholm, Sweden, consisting of 16.5% raw protein, 4.0% raw fat and 58.0% NFE (carbohydrates), 1255 KJ/100 g] was available ad libitum. The room where the rats were housed had a reversed light/dark schedule (lights on 19:00–07:00) and a room temperature around 23°C. The age of the animals was determined from the age/weight curve (supplied by the breeder) and was approximately 5 weeks at the start of the experiment. The experiment was approved by the regional ethical committee for animal research (Umeå djurförskötselsäteck).

Drugs

Ethanol (AB Svensk Sprit, Sweden) as a drinking fluid was mixed with tap water at concentrations of 10 and 20% (w/v). Ethanol for injection was dissolved in a 0.9% (w/v) NaCl solution and administered i.p. Control animals were given saline injections at a corresponding volume.

Treatment of psychologically dependent rats

Forty-eight rats were assigned randomly to four groups. Group 1 was a control group that received saline injections. Groups 2, 3, and 4 received ethanol injections to study the effect on voluntary ethanol intake. The groups are described in more detail below. As seen in the Results section, a few rats in each group died during the experiment, but no deaths occurred during the two tests with ethanol. The experimental design used in this experiment is illustrated in Fig. 1. Details of the model and the basic design have been described in earlier publications (Wahlström, 1987, 1994a,b). The present experiment consisted of two periods. The first was a 1-year treatment period during which psychological dependence was induced. In the following evaluation period, the psychological dependence of the rats was tested according to our empirical criterion, and the pretreatments with alcohol injections were given.

During the treatment period (weeks 1–54), the weekly ethanol exposure consisted of a voluntary choice between 10% (v/v) ethanol and water for 24 h, with ethanol in the bottle from which the rat had consumed most water during the previous 6 days. This 24-h period ended each week by an ethanol injection (2.0 g/kg, i.p.) to make certain of intoxication once a week. This intermittent exposure to intoxicating amounts of ethanol is a key feature of the present model.

During the evaluation period (weeks 55–74), the rats had a voluntary and continuous oral choice between ethanol and water, with random placement of the ethanol bottle each week. No ethanol injections were given, except for the two ethanol treatments (see Fig. 1). The basic ethanol concentration was 10% (v/v). The ethanol concentration was changed prior to the treatments to 20% for 3 weeks (weeks 60, 61, and 62) to test the psychological dependence of the rats. In this test, the average individual intake on 10% in the 2 weeks before and the 2 weeks after the period with 20% ethanol was compared by regression analysis to the average individual intake on 20% ethanol. Our main criterion of psychological dependence was that the individual rats must have the same ethanol intake in g/kg/day for both concentrations. The statistical criteria consisted of: (1) there had to be a significant correlation between the intakes of the two concentrations; (2) the slope of the regression line did not deviate from 1.0; (3) the regression line did not deviate from origin.

The first ethanol treatment was given on the first day of week 69. Group 2 was injected with 20%, group 3 was injected with 40% and group 4 was injected with 60% of the mean daily intake on the first 6 days of the previous week. The control group (group 1) was injected with saline. The ethanol was mixed with saline at concentrations of 4%, 8%, and 12% respectively. This procedure gave all rats a volume in ml determined by: (ethanol intake in g/kg/day) × 5. No rat was injected with more than 12 ml.

A second ethanol treatment was given on the first day of week 71. This time, the rats received predetermined doses. Group 2 was injected with 0.5 g/kg, group 3 with 1.0 g/kg, group 4 with 2.0 g/kg, and group 1 with saline. The volume was 2.0 ml/kg body wt.

Statistical methods

Conventional parametric statistical methods were used. Differences between two groups were tested with Student’s t-test. A P < 0.05 was used as the basic level of significance. No significance was denoted by NS. n denotes number of observations. Error bars denote 1 standard error of the mean (SEM). Correlation (r) and regression (b) coefficients were tested against 0. If the regression coefficient differed from 0, it was also tested against 1.0. In this case, NSD1 denotes that the slope of the regression line was not significantly different from 1.0.

RESULTS

Before the ethanol injections all groups fulfilled our criterion of psychological dependence (Fig. 2), except for group 1 (Fig. 2A) that had a cut-off of the regression line with the ordinate that significantly differed from origin, and where the slope of the regression line significantly differed from 1.0. Thus, the intermittent ethanol treatment had established a need for a defined dose of ethanol in groups 2, 3, and 4, but the presence of dependence in group 1 was more uncertain.

The effect on mean ethanol intake of both ethanol treatments is given in Table 1. The intake of the control group was unaffected on both occasions. On the first treatment day, only the group injected with 40% of their pretreatment intake significantly decreased their intake. On the second treatment day, all ethanol-injected groups significantly decreased their intake.
If the ethanol that was injected had been a pure substitute for orally consumed ethanol, the change in/kg/day would have been equal to the mean dose administered, but this was not the case.

A more detailed analysis of the results of the first ethanol injections is given in Fig. 3. The control group, injected with 0% ethanol (Fig. 3A) had a significant correlation between the pretreatment intake and the intake on the treatment day. The slope of the regression line did not deviate from 1.0, indicating that the individual rats had the same intake of ethanol. When the ethanol pretreatments were given, this correlation was first changed (20%, Fig. 3B) and then disappeared. When the results of the three ethanol-treated groups were added (Fig. 3E), a pattern emerged, which showed that below a certain pretreatment intake, almost all rats were above the 1.0 regression line. These rats had increased their intake of ethanol.

Furthermore, all rats except one with a pretreatment intake above this cut-off value decreased their intake. As stated in the text below, this cut-off value was approximately 1.67 g/kg/day.

A more detailed analysis of the results of the second ethanol treatment is given in Fig. 4. This time, the control rats had an ethanol intake on the treatment day that was not significantly related to the pretreatment intake. For all ethanol treatments, there were significant correlations between the pretreatment intake and intake on the treatment day. However, the slope of the regression line dropped with increasing doses of ethanol (Fig. 4B–D). The fact that no regression line had a cut-off with the corresponding ordinate that significantly differed from origin shows that increasing doses of ethanol affected the rats with a high pretreatment intake more than those with a low pretreatment intake. When the results of the ethanol treatments were added (Fig. 4E), there was no distinct pretreatment

### Table 1. Effects of ethanol injections on voluntary ethanol intake

<table>
<thead>
<tr>
<th>Treatment</th>
<th>0%</th>
<th>20%</th>
<th>40%</th>
<th>60%</th>
<th>0 g/kg</th>
<th>0.5 g/kg</th>
<th>1.0 g/kg</th>
<th>2.0 g/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pretreatment intake (g/kg/day)</td>
<td>1.75 ± 0.28</td>
<td>2.62 ± 0.31</td>
<td>2.25 ± 0.25</td>
<td>2.10 ± 0.39</td>
<td>1.55 ± 0.30</td>
<td>2.67 ± 0.29</td>
<td>2.02 ± 0.26</td>
<td>2.17 ± 0.47</td>
</tr>
<tr>
<td>Intake after injection (g/kg/day)</td>
<td>1.60 ± 0.23</td>
<td>2.22 ± 0.22</td>
<td>1.48 ± 0.22</td>
<td>1.62 ± 0.21</td>
<td>1.73 ± 0.21</td>
<td>2.04 ± 0.22</td>
<td>1.48 ± 0.15</td>
<td>0.84 ± 0.15</td>
</tr>
<tr>
<td>Change (g/kg/day)</td>
<td>-0.15 ± 0.22</td>
<td>-0.40 ± 0.24</td>
<td>-0.77 ± 0.31*</td>
<td>-0.48 ± 0.49</td>
<td>0.18 ± 0.33</td>
<td>-0.64 ± 0.24**</td>
<td>-0.54 ± 0.20*</td>
<td>-1.33 ± 0.39**</td>
</tr>
<tr>
<td>Mean dose administered (g/kg)</td>
<td>0.53 ± 0.06</td>
<td>0.90 ± 0.10</td>
<td>1.26 ± 0.24</td>
<td>0</td>
<td>0.5</td>
<td>1.0</td>
<td>2.0</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± 1 SEM. *P < 0.05, **P < 0.01, Student’s t-test, one sample.
ethanol intake level above which a change from an increase to a decrease could be recorded. This result clearly differs from the changes seen after the first injection (Fig. 3E). This difference could be due to the fact that, on the occasion of the second injection, some rats with a low pretreatment intake were injected with high doses of ethanol (see Fig. 4A, e.g., Fig. 4D).

For both treatments, the effects of dose or pretreatment intake of ethanol were compared by regression analysis to the change (treatment–pretreatment) in ethanol intake (Fig. 5). For both treatments, more of the variability was explained by the pretreatment intake than by the administered dose of ethanol. When the ethanol dose was determined as a defined part of the pretreatment intake the regression line intersected the line of no change at the pretreatment intake of 1.67 g/kg/day (Fig. 5C). When the ethanol dose was fixed at 0.5, 1 or 2 g/kg, the regression line intersected the line of no change at the pretreatment intake of 1.27 g/kg/day (Fig. 5D).

DISCUSSION

An important consideration in relation to the present results is the dose of ethanol administered. An i.p. injection of ethanol at a 1.0 g/kg dose to Mol:SPRD Han rats using the same technique gives a blood-alcohol concentration (BAC) just above 1.0 mg/ml, while the dose of 0.5 g/kg (that we consider low) gives a BAC around 0.5 mg/ml (L. Hedlund, unpublished data). High consuming rats included in previous experiments through voluntary intake could achieve BACs just below 1.0 mg/ml (Wahlström, 1987; Hedlund and Wahlström, 1998). This means that after both ethanol injections most rats had a BAC that they had not experienced since the treatment period, while the lower doses (0.5 g/kg and below) resulted in BACs the rats were used to. However, an injection probably leads to a more rapid rise in BAC, which could lead to pharmacodynamic differences. These could partly explain why the injected ethanol did not substitute for a corresponding amount of ethanol taken voluntarily as a drinking fluid (Table 1).

Another important consideration is the pharmacokinetic situation after the ethanol injections compared to the ensuing voluntary intake. The intoxicating effect of the ethanol injection subsides within a few hours, while other effects might be more long lasting. During which part of the 24-h period the ethanol intake is most influenced cannot be ascertained from the present experiment. In an earlier experiment, Gauvin et al. (1993) administered ethanol 15 min before a 30-min ethanol choice period. In that experiment, the intake of ethanol was increased after the dose of 0.25 g/kg, and decreased after 1.0, 1.5 and 2.0 g/kg. The 2.0 g/kg dose in that experiment reduced ethanol intake to 5–10% of the pretreatment intake, whereas in the present experiment only 2.0 g/kg reduced ethanol intake to 39% of the pretreatment intake (Table 1). One explanation of this difference could be that most of the voluntary intake of ethanol in the present experiment occurred late during the 24-h period.

The method of ethanol administration is of course important in relation to the results. In this experiment, the effect on voluntary intake was studied, whereas other investigators have...
found differences in the effects of intragastric vs i.p. injections on conditioned place preference (CPP; Ciccolioppo et al., 1999a) and conditioned taste aversion (CTA; Ciccolioppo et al., 1999b), where CTA developed with lower doses of ethanol when administered i.p. Furthermore, it is possible that different behavioural effects of ethanol are seen at different doses. For example, the discriminative stimulus effects of ethanol can be learned easily at doses above 1 g/kg (Kostowski and Bienkowski, 1999), whereas the increase in ethanol intake mentioned above was seen at 0.25 g/kg, and CPP as well as CTA are seen with doses between 0.25 g/kg and 1 g/kg. However, we believe that the behavioural measure of most interest in relation to alcoholism in humans is the voluntary oral intake of ethanol.

The concept of loss of control drinking is included in the third criterion of alcohol dependence according to the diagnostic criteria (DSM-IV) of the American Psychiatric Association (1994), which is fulfilled if the patient drinks alcohol in larger amounts or over a longer time period than was initially intended. In the present experiment, an increased intake of ethanol after ethanol pretreatment was only seen in rats with a low pretreatment intake. This suggests that ethanol exposure does not increase the craving for more ethanol in all individuals. Thus, if the present model involves a system regulating ethanol intake, also present in humans, ethanol exposure does not immediately trigger the system that increases the craving for ethanol. This suggests that a direct effect of ethanol on a system regulating ethanol intake could not be the mechanism behind the concept of loss of control drinking in humans.

The theory of loss of control drinking has also been tested in humans. Marlatt et al. (1973) gave alcoholics either tonic water with or without vodka, and told half of each group that they were drinking tonic and the other half that they were drinking tonic and vodka. The consumption of alcohol in this experiment was independent of the actual alcohol content, but strongly dependent of what the subjects were told they were drinking. Those who were told that they were drinking alcohol consumed more than twice the amount of those who were told they were drinking just tonic. Paredes et al. (1973) gave abstinent alcoholics access to alcohol on two consecutive days between 13:30 and 22:00, with the size of the drinks adjusted to keep BAC below 140 mg/dl. The authors observed the behaviour and concluded that alcohol did not trigger alcohol-seeking behaviour or make the patients lose control of their behaviour. In a study by Engle and Williams (1972) alcoholics received a strongly flavoured vitamin drink, half with and half without vodka. Half of each group were told they were drinking alcohol and the other half that they were not. The alcoholics then rated their desire for alcohol. The conclusion of the authors was that the desire for alcohol was related to what the alcoholics were told they were drinking and not what they actually had drunk. Thus, also in humans ethanol exposure does not directly trigger craving for more alcohol.

Robinson and Berridge (1993), in their incentive-sensitization theory of addiction, stated that the fact that drug craving remains high or is even elevated after drug administration is not consistent with either a negative reinforcement view of craving or a pleasure-seeking view. However, these facts are consistent with their incentive-sensitization theory of addiction where ‘craving is the subjective experience associated with incentive salience attribution’. When ethanol intake increases dopamine activity, which in this sensitized system produces incentive salience, drug ‘wanting’ increases. The main foundation for their theories are reports of drug-induced drug craving for cocaine, heroin, and hydromorphone. However, the results of the present experiment and the clinical studies mentioned above indicate that small doses of alcohol do not result in increased craving or loss-of-control drinking in alcoholics. Thus, alcohol dependence does not seem to fit into this theory. Indeed, Robinson and Berridge (1993, p. 276) caution that ‘whether the addictive potential of alcohol can be accounted for by incentive-sensitization remains to be seen’.

However, other theories exist of why a relapse occurs and what role the first drink has in causing a relapse. Tiffany (1990) proposed that drug use in the addict is controlled by automatized action schemata, and that there exist in the abstinent addict non-automatic cognitive processes that counteract the urge to use the drug. These non-automatic processes (working against alcohol use) have always to be stronger than the automatic processes (working for alcohol use) if the alcoholic is to stay abstinent. The relapse then occurs ‘if their nonautomatic processing is devoted to some other task (e.g. they are distracted) and the current environmental circumstances are completely supportive of the drug-use action plan’. Furthermore, apart from the fact that alcohol reduces the efficiency of the non-automatic processes that counteract the drug-use plans, the presence of a first drink shows that stimuli that can trigger the drug-use plan are present.

The effect on ethanol intake of the ethanol injections in the rats participating in this experiment, that have had continuous access to ethanol, indicate that rats with a low intake of ethanol increase their intake when treated with low doses of ethanol, whereas rats with a high voluntary intake of ethanol decrease their ethanol intake regardless of dose. This could be consistent with the situation in humans (Engle and Williams, 1972; Marlatt et al., 1973; Paredes et al., 1973). It could also be consistent with the theories of Tiffany (1990) if it is assumed that the rats participating in this model do not have any non-automatic processes that work against ethanol intake. However, it is not certain if it is consistent with the incentive-sensitization theory of addiction, since independently of the administered dose, only rats with a low ethanol intake increased their intake.

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