SUCROSE SELF-ADMINISTRATION PREDICTS ONLY INITIAL PHASE OF ETHANOL-REINFORCED BEHAVIOUR IN WISTAR RATS

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Abstract — Aims: To characterize the relationship between the sucrose- and ethanol-reinforced behaviour in Wistar rats. Methods: Subjects (n = 31) were trained to lever press for 8% (v/v) ethanol in an operant procedure where increasing concentrations of ethanol were introduced in the presence of 8% (w/v) sucrose. The sucrose concentration was subsequently decreased from 8 to 0%. Subjects were allowed to stabilize their intake of 8% ethanol over the next 20 days. Results: Self-administration of 8% sucrose (ml/kg) significantly correlated with ethanol consumption (g/kg) on days 1–5 of the 8% ethanol self-administration period. This relationship completely disappeared during the subsequent weeks of ethanol self-administration (days 6–20). Conclusions: The results of the present study, combined with our previous findings, may indicate that self-administration of sucrose predicts only the initial phase of ethanol-taking behaviour in Wistar rats.

INTRODUCTION

Both laboratory animals and humans demonstrate marked individual differences in their propensity to a drug- and alcohol-taking behaviour (Cloninger et al., 1988; Piazza et al., 1989; Bisaga and Kostowski, 1993; Koros et al., 1998; Kampov-Polevoy et al., 1999). From a clinical perspective, it would be particularly important to define biological markers (‘predictors’) of vulnerability towards excessive drug or alcohol consumption. An excessive intake of psychoactive drugs may be a risk factor for the development of abuse and dependence (Moore et al., 1990; Sussman et al., 2000).

Several authors have reported that rodents with a high preference for sweet substances consume more ethanol and acquire self-administration of psychostimulants more quickly than those with a low preference for these substances (Gosnell and Krahn, 1992; Kampov-Polevoy et al., 1995; Bachmanov et al., 1996; DeSousa et al., 2000; Gosnell, 2000). The positive relationship between consumption of sweet substances and non-operative free-choice alcohol intake has been reported for both genetically selected alcohol-preferring (Sinclair et al., 1992; Kampov-Polevoy et al., 1996) and outbred strains of rats (Gosnell and Krahn, 1992; Overstreet et al., 1993). However, it has been repeatedly shown that the magnitude of the correlation between the intake of sweet solutions and ethanol in rodents is critically dependent on the length of alcohol exposure and/or its concentration (Belknap et al., 1993; Kampov-Polevoy et al., 1996; Koros et al., 1998, 1999b). Moreover, no correlation has been reported between preference for sweet solutions and voluntary ethanol consumption by several authors (Overstreet et al., 1996, 1999; Salimov, 1999; Agabio et al., 2000). In line with these latter studies, no relationship has been found between the preference for sweet substances and alcohol dependence in recent studies on adult male alcoholics (Bogucka-Bonikowska et al., 2001) or children of alcohol-dependent fathers (Kranzler et al., 2001; Scinska et al., 2001; but see also Kampov-Polevoy et al., 1997).

Several studies have examined the relationship between saccharin and alcohol drinking in non-operative procedures (Sinclair et al., 1992; Overstreet et al., 1993; Kampov-Polevoy et al., 1995, 1996; Koros et al., 1998, 1999b; Agabio et al., 2000). In a single previous study, where an operant oral ethanol self-administration procedure was employed, Bell et al. (1994) used groups of male Wistar rats showing a high, intermediate, or low saccharin preference. All rats were subsequently initiated to lever press for ethanol in a food-induced drinking acquisition procedure. In 29 of 32 experimental conditions (a different fixed ratio size combined with different alcohol concentrations), the mean number of responses for ethanol tended to be higher in the high-saccharin-prefering, than in low-saccharin-prefering, subjects. However, the between-group differences did not reach any significance (Bell et al., 1994). Considering these latter findings, one should be aware of the fact that no consistent relationship between free-choice ethanol drinking and operant ethanol self-administration has been detected across a number of rodent genotypes including genetically selected alcohol-prefering and non-prefering rats (Samson et al., 1989; George and Ritz, 1993; Ritz et al., 1994; Koros et al., 1999a).

In the present study, we decided to further evaluate the relationship between the consumption of sweet substances and alcohol in the operant oral self-administration procedure. For this purpose, rats were trained to lever press for ethanol in the sucrose-fading procedure (Samson, 1986; Koros et al., 1999a). The sucrose-fading procedure is most frequently applied among many operant procedures used to initiate responding for ethanol (e.g. Samson et al., 1988, 1989; Sławecki et al., 1997; Piasecki et al., 1998; Bienkowski et al., 1999a,b; Samson and Chappel, 1999). Therefore, it would be useful to define behavioural parameters (e.g. sucrose intake) which predict ethanol self-administration in this procedure.

MATERIALS AND METHODS

Subjects

Thirty-one male Wistar rats weighing 320–420 g at the beginning of the study were kept (two or three in a cage) in a...
room under controlled environmental conditions (22 ± 1°C, relative humidity 60%, a 12-h light:12-h dark cycle with lights on at 07:00). The animals were supplied by a breeder (HZL, Warsaw, Poland) 10 days before the start of the experiment. Standard lab chow (Labofeed H; WPIK, Kcynia, Poland) was available ad libitum. Tap water was always available except as noted below.

The treatment of the rats in the present study was in full accordance with the ethical standards laid down in respective European and Polish regulations. All procedures were reviewed and approved by a local Ethics Committee.

**Apparatus**

Ethanol-reinforced behaviour was studied in eight standard operant chambers (Coulbourn Instruments, Inc., Allentown, PA, USA). The chambers consisted of modular test cages placed inside sound-attenuating cubicles with ventilation fans and background white noise (for details, see Bienkowski and Kostowski, 1998). A white house light was centred near the top of the front of the cage. The start of test sessions was signalled by the turning on of the house light. The cage was also equipped with two response levers, separated by a liquid delivery system (a liquid dipper, Coulbourn). Only one lever (an ‘active’ lever) activated the liquid dipper. Presses on the other lever (an ‘inactive’ lever) were recorded but not reinforced. The liquid delivery system presented a respective fluid in a 0.1-ml portion for 5 s. The availability of reinforcer was signalled by a brief audible click and a small white light (4 W) located inside the liquid dipper hole. The programming of each session, as well as data recording, were based on the L2T2 Software package (Coulbourn) running on an IBM-compatible PC.

**Operant oral ethanol self-administration**

The rats were trained to respond for 8% (v/v) ethanol according to the sucrose-fading procedure introduced by Samson (1986) with some modifications (Bienkowski et al., 1999a,b). All sessions were 30-min long and there was one session daily (Monday to Friday). The whole procedure consisted of four phases. During the first 4 days of training, the animals were deprived of water for 22 h/day and shaped to lever press for water according to a fixed ratio 1 (FR1) schedule of reinforcement (Phase 1). As soon as the lever pressing was established (≥100 presses on the ‘active’ lever/30 min), tap water became freely available in the home cages.

During days 5–6, the animals received 8% (v/v) sucrose (the sucrose self-administration; Phase 2). Subsequently, over the next 15 sessions (sucrose-fading; Phase 3), ethanol concentrations were gradually increased from 0 to 8%, and sucrose concentrations were decreased from 8 to 0%. Rats were given the following combinations of ethanol and sucrose solutions: 2.5% ethanol/8% sucrose (1 day); 5% ethanol/8% sucrose (2 days); 6.5% ethanol/8% sucrose (2 days); 8% ethanol/6% sucrose (2 days); 8% ethanol/4% sucrose (3 days); 8% ethanol/2% sucrose (2 days); 8% ethanol/1% sucrose (3 days).

Subjects were allowed to stabilize their 8% ethanol consumption over the next 20 days (a period of an 8% ethanol self-administration; Phase 4).

**Data analysis**

Sucrose and alcohol intakes were estimated by measuring the amount of solution remaining in the dipper after the sucrose or ethanol self-administration session, respectively. Alcohol intakes for the successive weeks of ethanol self-administration (days 1–5, 6–10, 11–15, and 16–20 of Phase 4) were compared by a one-way analysis of variance (ANOVA) with repeated measures. The Pearson product-moment test was used to study correlations between an 8% sucrose self-administration (in ml/kg/30 min) and an 8% ethanol consumption (in g/kg/30 min). P < 0.05 was considered significant.

Preliminary analyses revealed that the sucrose intake correlated with ethanol consumption in days 1–5 but not in the subsequent weeks of Phase 4. As ethanol intakes in the first (days 1–5) and in the last (days 16–20) week of Phase 4 were strongly correlated (r = 0.81, P < 0.0001), it was surprising that sucrose intake did not correlate with the ethanol self-administration in all weeks of Phase 4. Thus, we assumed that an early ethanol acceptance (days 1–5) might share some regulating factor(s) with both sucrose intake and long-term ethanol self-administration. To test the above hypothesis, a principal components factor analysis with varimax rotation (Salimov, 1999) was run for 10 variables (the lever pressing for, and the intake of, 8% sucrose; the lever pressing for, and the intake of, 8% ethanol in the 4 successive weeks of Phase 4).

The ‘Statistica’ software package for Windows was used to analyse all data.

**RESULTS**

The parameters of sucrose and ethanol self-administration are summarized in Table 1. One-way ANOVA revealed that the self-administration of 8% ethanol (in g/kg/30 min) remained stable over the whole Phase 4 [F(4,150) = 0.94, P = 0.44; Table 1]. As mentioned above, ethanol consumption in days 1–5 and 16–20 of Phase 4 were significantly and positively correlated.

Sucrose self-administration was significantly and positively correlated with ethanol self-administration in days 1–5 of the 8% ethanol availability period (Table 2). Notably, those correlations diminished over days 6–20 of Phase 4. As shown in Table 2, r values gradually decreased and P values increased.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean ± SEM</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>8% sucrose Responses/30 min</td>
<td>210.3 ± 11.3</td>
<td>63.5</td>
<td>415.5</td>
</tr>
<tr>
<td>ml/kg/30 min</td>
<td>59.1 ± 3.6</td>
<td>19.8</td>
<td>127.8</td>
</tr>
<tr>
<td>8% ethanol Responses/30 min</td>
<td>Days 1–5</td>
<td>37.9 ± 4.1</td>
<td>2.6</td>
</tr>
<tr>
<td>Days 6–10</td>
<td>34.2 ± 4.1</td>
<td>3.0</td>
<td>85.0</td>
</tr>
<tr>
<td>Days 11–15</td>
<td>32.9 ± 3.8</td>
<td>1.0</td>
<td>83.8</td>
</tr>
<tr>
<td>Days 16–20</td>
<td>36.2 ± 4.1</td>
<td>1.6</td>
<td>113.0</td>
</tr>
<tr>
<td>g/kg/30 min</td>
<td>Days 1–5</td>
<td>0.55 ± 0.06</td>
<td>0.04</td>
</tr>
<tr>
<td>Days 6–10</td>
<td>0.48 ± 0.06</td>
<td>0.04</td>
<td>1.22</td>
</tr>
<tr>
<td>Days 11–15</td>
<td>0.44 ± 0.05</td>
<td>0.01</td>
<td>1.07</td>
</tr>
<tr>
<td>Days 16–20</td>
<td>0.47 ± 0.05</td>
<td>0.02</td>
<td>1.45</td>
</tr>
</tbody>
</table>

n = 31 rats. For experimental details, see Materials and methods.
Table 2. Correlations between 8% sucrose consumption (ml/kg/30 min) and subsequent 8% ethanol self-administration (g/kg/30 min)

<table>
<thead>
<tr>
<th>Phase of 8% ethanol self-administration</th>
<th>Correlation coefficient ($r$)</th>
<th>Significance ($P$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Days 1–5</td>
<td>0.45</td>
<td>0.01</td>
</tr>
<tr>
<td>Days 6–10</td>
<td>0.35</td>
<td>0.12</td>
</tr>
<tr>
<td>Days 11–15</td>
<td>0.17</td>
<td>0.36</td>
</tr>
<tr>
<td>Days 16–20</td>
<td>0.14</td>
<td>0.43</td>
</tr>
</tbody>
</table>

$n = 31$ rats. For experimental details, see Materials and methods.

when the absolute alcohol intake in the 4 successive weeks of Phase 4 was correlated with the 8% sucrose consumption.

Scatterplots presenting the significant (A) and non-significant (B) correlation between the sucrose intake and ethanol self-administration in days 1–5 and 16–20, respectively, are shown in Fig. 1.

Factor analysis revealed two factors accounting for 91.8% of the total variability. The first factor had a significant positive loading (>0.95) on the parameters of the 8% sucrose consumption. In general, this factor had a negligible loading (<0.2) on the parameters of the 8% ethanol drinking, except for ethanol intake in days 1–5 of Phase 4 (factor loading = 0.37). The second factor had a positive loading (>0.85) on all ethanol consumption parameters, but not on the sucrose-reinforced behaviour parameters (factor loadings <0.1).

The mean number of ‘inactive’ lever presses in the present study did not exceed 1.1 ± 0.2 responses/session (data not shown).

**DISCUSSION**

In the present study, sucrose self-administration predicted only the initial phase of ethanol-reinforced behaviour in the sucrose-fading procedure. Our findings may support previous reports that preference of sweet solutions may be correlated with the ethanol intake in rodents (Sinclair et al., 1992; Bachmanov et al., 1996; Kampov-Polevoy et al., 1999; but see also Overstreet et al., 1999; Salimov, 1999; Agabio et al., 2000 for negative findings).

However, the correlation observed in the present study completely disappeared during the subsequent weeks of ethanol self-administration. In this respect, our results are in line with recent reports of Koros et al. (1998, 1999b). These latter authors have shown that, in Wistar rats, saccharin preference may only predict an initial acceptance of relatively low (2–6%) ethanol concentrations. Similarly, Belknap et al. (1993) have reported that saccharin consumption correlated only with 3% ethanol intakes in mice. The positive relationship between saccharin and ethanol intakes was strongest for the early stages of ethanol drinking in the study of Kampov-Polevoy et al. (1996). Our findings are also in agreement with the results of Bell et al. (1994). The latter authors found that saccharin preference did not correlate with ethanol self-administration in Wistar rats initiated to work for ethanol reinforcement in the food-induced drinking acquisition procedure.

In general, the results of the present study seem to be consistent with clinical reports showing no difference in taste responses to sucrose solutions between controls and alcoholics (Bogucka-Bonikowska et al., 2001) or between controls and children of alcoholic fathers (Kranzler et al., 2001; Scinska et al., 2001; but see also Kampov-Polevoy et al., 2001). Interestingly, no overlapping genetic control for the consumption of sweet substances and ethanol intake has been found in mice (Phillips et al., 1994; Melo et al., 1996).

Bearing in mind the data cited above and the results of the present study, one may conclude that the procedure used for the initiation of ethanol drinking, the stage of ethanol-taking behaviour as well as the ethanol concentration might all be critical points in studies on correlation between the preference for sweet substances and alcohol intake in rodents. Recently, Gosnell (2000) and DeSousa et al. (2000) have tested the relationship between individual sucrose intakes and the propensity to self-administer cocaine and amphetamine, respectively. The drug dose, the self-administration phase, and the schedule of reinforcement have been identified as factors altering the magnitude of correlation between the sucrose and drug self-administration behaviours.
Notably, in the present study, there was a strong correlation between an 8% ethanol self-administration in days 1–5 and 16–20 of Phase 4. Thus, the time factor does not seem to be a major factor limiting the relationship between the self-administration parameters. Therefore, it is rather unlikely that a 15-day interval between Phase 2 and 4 might have eliminated the correlation between the sucrose- and ethanol-reinforced behaviour. Bearing in mind the results of the factor analysis, one may rather suggest that sucrose and ethanol reinforcement were regulated by two different factors with some overlapping control for sucrose self-administration and the early phase of ethanol-reinforced behaviour.

In conclusion, the results of the present study may indicate that self-administration of sucrose predicts only the initial phase of alcohol-reinforced behaviour in Wistar rats trained in the sucrose-fading procedure. Combined with several previous reports, our results suggest that the preference for sweet substances may not predict long-term alcohol-taking behaviour.

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REFERENCES


