RELATIONSHIP BETWEEN Dopamine D2 RECEPTOR-ASSOCIATED RESPONSES AND OPERANT ETHANOL SELF-ADMINISTRATION IN THE RAT: A FACTOR ANALYSIS

ARTUR ROGOWSKI1, DARIUSZ ROKICKI2, WOJCIECH KOSTOWSKI1,3 and PRZEMYSLAW BIENKOWSKI1*

1Department of Pharmacology, Institute of Psychiatry and Neurology, Sobieskiego 9 St., PL-02957 Warszawa, 2Medagro International, Warszawa and 3Department of Experimental and Clinical Pharmacology, Warsaw Medical Academy, Warszawa, Poland

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Abstract — Aims: To characterize the relationship between dopamine D2 receptor-associated responses and operant ethanol self-administration in Wistar rats. Methods: Thirty-two rats were first tested for apomorphine-induced sniffing and raclopride-induced catalepsy. Subsequently, the same subjects were initiated to lever press for ethanol in the sucrose-fading procedure. The subjects were allowed to respond for 8% v/v ethanol for 20 days. A factor analysis was used to characterize the relationship between D2-associated responses and parameters of sucrose and ethanol self-administration. Results: The analysis revealed three factors accounting for 88.3% of the total variability. The first factor comprised only parameters of ethanol-reinforced behaviour. Parameters of sucrose self-administration and cataleptic responses to raclopride loaded heavily on the second and third factors, respectively. None of the factors comprised apomorphine-induced stereotypy. Conclusions: It appears that there is no relationship between apomorphine-induced sniffing, raclopride-induced catalepsy and operant responding for ethanol in Wistar rats. Our results, combined with previous reports, suggest that D2 receptors are not primarily involved in the regulation of ethanol reinforcement.

INTRODUCTION

Brain dopamine receptors are thought to play an important role in the regulation of drug self-administration. It is widely accepted that the dopamine D1- and D2-like receptor families both mediate the positive reinforcing properties of psycho-stimulants (for a review see Self and Nestler, 1995; Wise, 1998; Tzschentke, 2001). However, in the case of alcohol (ethanol) the situation is less clear. In most studies, the effects of dopamine D2 receptor ligands on ethanol self-administration were either small in magnitude or non-selective (Lyness and Smith, 1992; Goodwin et al., 1996; Silvestre et al., 1996; Samson and Chappell, 1999).

Both animals and humans demonstrate marked individual differences in their propensity to ethanol self-administration (e.g. Cloninger et al., 1988; Piazza et al., 1989; Kampov-Polevoy et al., 1999). Several preclinical and clinical studies aimed to identify trait markers for excessive alcohol intake (Bisaga and Kostowski, 1993; Salimov, 1999; Scinska et al., 2000). However, in the case of alcohol (ethanol) the situation is less clear. In most studies, the effects of dopamine D2 receptor ligands on ethanol self-administration were either small in magnitude or non-selective (Lyness and Smith, 1992; Goodwin et al., 1996; Silvestre et al., 1996; Samson and Chappell, 1999).

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Subjects

Thirty-two male Wistar rats weighing 300–400 g at the beginning of the study were housed two per cage. The subjects were kept in a room under standard environmental conditions (22 ± 1°C, relative humidity of 60%, 12 h–12 h light–dark cycle with lights on at 07:00 h). The animals were supplied by a breeder (Medical Research Centre, Polish Academy of Sciences, Warszawa, Poland) 2 weeks before the onset of the experiments. During this time, the subjects were weighed and handled several times. Standard laboratory chow (Labofeed H, WPIK, Kcynia, Poland) was available ad libitum. Tap water was always available, except as noted below.
Treatment of the rats in the present study was in full accordance with the ethical standards laid down in respective Polish and European (directive No. 86/609/EEC) regulations. All procedures were reviewed and approved by an ethics committee on animal studies.

**Raclopride-induced catalepsy**

Before the evaluation of the D₂ receptor-associated responses, the rats were repeatedly familiarized with a horizontal wooden bar and an observational cage used for measurement of raclopride-induced catalepsy and apomorphine-induced sniffing, respectively. Raclopride-induced catalepsy was assessed first. Four days later, apomorphine-induced sniffing was evaluated in the same rats.

Catalepsy was observed 30, 60 and 90 min after administration of 1 mg/kg raclopride (Ögren et al., 1986; Wadenberg and Ahlenius, 1991). Cataleptic responses were determined by a modification of the bar method (Undie and Friedman, 1988). Each rat was placed on a clean, smooth table with the wooden bar suspended 10 cm above the working surface. The animal’s front paws were gently placed over the bar. The length of time (in seconds) the animal touched the bar with both front paws was measured up to a pre-set cut-off time of 180 s. Cataleptic responses were averaged across the three observation times.

**Apomorphine-induced stereotypy**

Apomorphine-induced sniffing was assessed in the rectangular cage (height × width × length: 25 × 25 × 42 cm) with wooden chip bedding on the floor. Twenty minutes after administration of 0.5 mg/kg apomorphine, each rat was placed in the observational cage and its sniffing behaviour was recorded for 30 min using a video camera (Ögren et al., 1986; Kostowski and Krzascik, 1989).

**Operant oral ethanol self-administration**

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 et al., 1999). A white house light was centred near the top of the front of the cage. The start of test sessions was signalled by turning the house light on. The cage was also equipped with two response levers, separated by a liquid delivery system (a liquid dipper; Coulbourn Instruments, Inc.). 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 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 Instruments, Inc.) running on an IBM-compatible PC.

The self-administration procedure started 10 days after the assessment of apomorphine-induced sniffing. The rats were trained to respond for 8% v/v ethanol according to the sucrose-fading procedure (Samson, 1986) with some modifications (Bienkowski et al., 1999, 2001). All sessions were 30 min long and there was only 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 a 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% w/v sucrose (Phase 2). Then, over the next 14 sessions (sucrose fading; Phase 3), ethanol concentrations were gradually increased from 0 to 8%, and sucrose concentrations were decreased from 8 to 0%. The 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 (2 days); 8% ethanol/2% sucrose (2 days); and 8% ethanol/1% sucrose (3 days).

The subjects were then allowed to make their 8% ethanol consumption stable during the next 20 days (8% ethanol self-administration; Phase 4).

**Drugs**

Raclopride and apomorphine (both from Sigma, Poznan, Poland) were dissolved in sterile distilled water and administered intraperitoneally in a volume of 1 ml/kg. Raclopride and apomorphine solutions were prepared immediately prior to use and protected from light. Sucrose and ethanol (95% rectified spirit; Polmos, Zielona Gora, Poland) used in the self-administration procedure were dissolved in tap water. The solutions were prepared daily and stored at room temperature.

**Data analysis**

The ‘active’ lever responding (lever presses/30 min) was averaged across the 2 days of 8% sucrose availability (Phase 2) and across days 1–5, 6–10, 11–15 and 16–20 of 8% ethanol self-administration (Phase 4). Sucrose (ml/kg/30 min; Phase 2) and ethanol (g/kg/30 min; Phase 4) intakes were estimated by measuring the amount of solution remaining in the dipper after the self-administration session.

A principal components factor analysis with varimax rotation (Salimov, 1999) was run for 12 variables, i.e. raclopride-induced catalepsy, apomorphine-induced sniffing, responding for and intake of 8% sucrose, and responding for and intake of 8% ethanol in the four successive weeks of Phase 4. All variables included in the factor analysis were independent and normally distributed. The Statistica software package for Windows (StatSoft, Tulsa, OK, USA) was used to analyse all data.

**RESULTS**

Table 1 presents the basic characteristics of raclopride-induced catalepsy, apomorphine-induced sniffing, and 8% sucrose and 8% ethanol self-administration. Individual responding on the ‘active’ lever averaged across the 5 day periods of ethanol availability ranged from 0.3 to 99.4 responses/30 min. Ethanol consumption ranged from 0.01 to 1.5 g/kg/30 min. Responding on the ‘inactive’ lever was always marginal and ranged from 0 to 2.4 responses/30 min. The pattern of ethanol consumption in the present study followed that found in our previous experiments (Bienkowski et al., 1999, 2001).
The parameters are marked by one-letter codes. * indicates that the parameter was not associated with the other variables.

Table 1. Raclopride-induced catalepsy, apomorphine-induced sniffing, 8% sucrose (Phase 2) and 8% ethanol self-administration (Phase 4) in Wistar rats (n = 32 rats)

<table>
<thead>
<tr>
<th>Behavioural parameters</th>
<th>Mean ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raclopride catalepsy (s)</td>
<td>92.2 ± 10.3</td>
</tr>
<tr>
<td>Apomorphine sniffing (c)</td>
<td>638.6 ± 59.1</td>
</tr>
<tr>
<td>8% sucrose self-administration</td>
<td></td>
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<tr>
<td>Responses/30 min</td>
<td>110.1 ± 7.9</td>
</tr>
<tr>
<td>ml/kg/30 min</td>
<td>28.0 ± 2.5</td>
</tr>
<tr>
<td>8% ethanol self-administration</td>
<td></td>
</tr>
<tr>
<td>Responses/30 min</td>
<td></td>
</tr>
<tr>
<td>Days 1–5</td>
<td>35.1 ± 3.9</td>
</tr>
<tr>
<td>Days 6–10</td>
<td>34.6 ± 3.8</td>
</tr>
<tr>
<td>Days 11–15</td>
<td>29.9 ± 3.7</td>
</tr>
<tr>
<td>Days 16–20</td>
<td>26.9 ± 3.1</td>
</tr>
<tr>
<td>g/kg/30 min</td>
<td></td>
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<tr>
<td>Days 1–5</td>
<td>0.53 ± 0.06</td>
</tr>
<tr>
<td>Days 6–10</td>
<td>0.52 ± 0.05</td>
</tr>
<tr>
<td>Days 11–15</td>
<td>0.44 ± 0.05</td>
</tr>
<tr>
<td>Days 16–20</td>
<td>0.38 ± 0.04</td>
</tr>
</tbody>
</table>

Parameters of sucrose and ethanol self-administration were averaged across respective periods of reinforcer availability (see Materials and methods for details).

Table 2 presents a full matrix of correlations between the behavioural parameters included in the factor analysis. The factor analysis revealed three factors accounting for 88.3% of the total variability (Table 3). The first factor (‘ethanol self-administration’) comprised the parameters of ethanol-reinforced behaviour. The second factor (‘sucrose self-administration’) comprised the parameters of sucrose-reinforced behaviour. Cataleptic responses to raclopride loaded on the third factor (‘raclopride catalepsy’). Apomorphine-induced stereotypy was not associated with the other variables.

**DISCUSSION**

The present study examined the relationship between the D2-associated behavioural responses and ethanol-reinforced behaviour initiated in the sucrose-fading procedure. The factor analysis indicated no consistent relationship between apomorphine-induced sniffing, raclopride-induced catalepsy and operant ethanol self-administration. The results of the present study are in agreement with our previous report (Bisaga and Kostowski, 1993) that apomorphine-induced stereotypy did not correlate with non-operant two-bottle choice alcohol drinking. In this respect, our findings support several previous reports indicating marginal, if any, role of D2 receptors in the reinforcing properties of ethanol in rodents (Risinger et al., 1992; Goodwin et al., 1996; Silvestre et al., 1996; Samson and Chappell, 1999; Nowak et al., 2000; but see also Samson et al., 1993; Salimov et al., 2000).

The results of factor analysis were at variance with the reports of Cools and co-workers (Cools and Gingras, 1998; Sluyter et al., 2000; see Introduction); however, one should bear in mind that a non-operant ethanol drinking procedure was used in these latter studies. In another study on this topic, Behnert et al. (1987) analysed the relationship between ethanol drinking and subsequent behavioural reactivity to apomorphine. The authors have reported that apomorphine-induced stereotypy did not differ between alcohol-prefering and alcohol-naive rats. More recently, Samson and Chappell (1995) have reported no correlation between amphetamine-induced locomotion and non-operant ethanol intake. As mentioned in the Introduction, clinical studies have suggested some association between the A1 allele of DRD2 gene and alcohol dependence (e.g. Blum et al., 1991). However, the most recent large family-based study (the COGA project) was negative (Edenberg et al., 1998). Taken together, the present and previous studies suggest that D2-associated responses may not serve as predictors of ethanol self-administration.

It can be argued that the behavioural responses assessed in the present study were associated with D2 receptors located in the dorsal striatum (Arnt et al., 1985; Klockgether et al., 1988), while it is dopamine transmission in the nucleus accumbens that plays a specific role in drug reinforcement (Wise, 1998). However, D2 receptors in the nucleus accumbens may also contribute to cataleptic responses induced by dopamine antagonists (Al-Khatib et al., 1989; Ossowska et al., 1990). Similarly, various stereotypical responses, including apomorphine-induced sniffing, may depend on both striatal and accumbal dopamine transmission (Arnt, 1985; Bradberry et al., 1991).

On the other hand, selective lesions of mesolimbic dopaminergic neurones with 6-hydroxydopamine (6-OHDA) failed to alter either free-choice ethanol drinking or operant responding for ethanol (Lyness and Smith, 1992; Rassnick et al., 1993; Ikemoto et al., 1997; Koistinen et al., 2001). Notably, Ikemoto et al. (1997) have shown that although the 6-OHDA lesion did

Table 2. A matrix of correlations (Pearson’s r values) between parameters used in a factor analysis (n = 32 rats)

<table>
<thead>
<tr>
<th>Behavioural parameters</th>
<th>a</th>
<th>b</th>
<th>c</th>
<th>d</th>
<th>e</th>
<th>f</th>
<th>g</th>
<th>h</th>
<th>i</th>
<th>j</th>
<th>k</th>
<th>l</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raclopride-induced catalepsy (a)</td>
<td>1.00</td>
<td>0.17</td>
<td>–0.08</td>
<td>–0.12</td>
<td>0.00</td>
<td>–0.02</td>
<td>–0.11</td>
<td>–0.13</td>
<td>0.08</td>
<td>0.05</td>
<td>0.10</td>
<td>0.08</td>
</tr>
<tr>
<td>Apomorphine-induced sniffing (b)</td>
<td>1.00</td>
<td>0.17</td>
<td>0.12</td>
<td>0.19</td>
<td>0.15</td>
<td>0.38*</td>
<td>0.34</td>
<td>0.32</td>
<td>0.32</td>
<td>0.29</td>
<td>0.26</td>
<td></td>
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<tr>
<td>Responses for 8% sucrose (c)</td>
<td>1.00</td>
<td>0.97**</td>
<td>0.14</td>
<td>0.18</td>
<td>0.35</td>
<td>0.41*</td>
<td>0.25</td>
<td>0.30</td>
<td>0.24</td>
<td>0.28</td>
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<tr>
<td>8% sucrose consumption (d)</td>
<td>1.00</td>
<td>0.12</td>
<td>0.20</td>
<td>0.34</td>
<td>0.43*</td>
<td>0.21</td>
<td>0.29</td>
<td>0.22</td>
<td>0.29</td>
<td></td>
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<tr>
<td>Responses for 8% ethanol, days 1–5 (e)</td>
<td>1.00</td>
<td>0.99**</td>
<td>0.89**</td>
<td>0.87**</td>
<td>0.84**</td>
<td>0.83**</td>
<td>0.89**</td>
<td>0.87**</td>
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<tr>
<td>Ethanol consumption, days 1–5 (f)</td>
<td>1.00</td>
<td>0.88**</td>
<td>0.89**</td>
<td>0.82**</td>
<td>0.83**</td>
<td>0.87**</td>
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<td>Responses for 8% ethanol, days 6–10 (g)</td>
<td>1.00</td>
<td>0.98**</td>
<td>0.91**</td>
<td>0.91**</td>
<td>0.86**</td>
<td>0.84**</td>
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<tr>
<td>Ethanol consumption, days 6–10 (h)</td>
<td>1.00</td>
<td>0.88**</td>
<td>0.91**</td>
<td>0.83**</td>
<td>0.85**</td>
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<td>Responses for 8% ethanol, days 11–15 (i)</td>
<td>1.00</td>
<td>0.99**</td>
<td>0.86**</td>
<td>0.83**</td>
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<tr>
<td>Ethanol consumption, days 11–15 (j)</td>
<td>1.00</td>
<td>0.85**</td>
<td>0.84**</td>
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<tr>
<td>Responses for 8% ethanol, days 16–20 (k)</td>
<td>1.00</td>
<td>0.98**</td>
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<tr>
<td>Ethanol consumption, days 16–20 (l)</td>
<td>1.00</td>
<td></td>
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</table>

The parameters are marked by one-letter codes. *P < 0.05; **P < 0.001.
not alter ethanol consumption in rats that had prior experience with ethanol, it slowed the acquisition of ethanol preference in subjects with no ethanol-drinking history. In two of the above studies, genetically selected alcohol-preferring rats served as subjects (Iketomo et al., 1997; Koistinen et al., 2001).

The results of the present study and the data cited above do not exclude the possibility that other dopamine receptor subtypes play a more prominent role in the regulation of alcohol reinforcement. For example, D1 receptors seem to be related to drug-taking motivation, while D2 receptors may be primarily involved in the regulation of ethanol reinforcement.

In conclusion, there is no consistent relationship between apomorphine-induced sniffing, raclopride-induced catalepsy and operant responding for ethanol in Wistar rats. Our results, combined with previous reports, suggest that D3 receptors are not primarily involved in the regulation of ethanol reinforcement.

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REFERENCES


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