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Short communication

Different sensitivity to the motor-stimulating effect of amphetamine in Sardinian alcohol-preferring and non-preferring rats

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Abstract

The selective breeding of rodents on the basis of ethanol intake and preference has led to the development of lines of alcohol-preferring and non-preferring animals. The divergent degree of alcohol preference and consumption displayed by these lines of animals appears to be related, among other factors, to the genetic differences in dopaminergic neurotransmission. Moreover, in genetically unselected rats, a positive correlation has been found between alcohol preference and several amphetamine effects, including the stimulation of motor hyperactivity, thus suggesting the hypothesis that a common neural pathway might underlie some aspects in both of the amphetamine-induced hypermotility and alcohol preference. In the present study, we compared the motor-stimulating effect of amphetamine, which is mediated by the release of dopamine in the nucleus accumbens and in the corpus striatum in two lines of rats selectively bred for high and low ethanol preference, the Sardinian alcohol-preferring (sP) and the Sardinian alcohol-non-preferring (sNP) rats, respectively. The results show that sP rats are less sensitive to the motor-stimulant effect of amphetamine with respect to sNP rats, thus suggesting a negative correlation between this behavioural response and alcohol preference. The present results might be explained by the previously reported reduced density of dopamine receptors in the nucleus accumbens of sP rats and are consistent with the view that alcohol preference is associated with a deficient dopaminergic transmission. Moreover, they are consistent with the hypothesis that alcohol preference and amphetamine motor effect share a common neural substrate and that hereditary factors determine individual variations in its sensitivity.

Keywords: Alcohol; Amphetamine; Dopamine; Motor activity

1. Introduction

The development through selective breeding of alcohol-preferring lines of rodents, along with their non-preferring counterparts, has provided an important research tool for the investigation of genetic factors involved in excessive alcohol drinking. Studies performed on alcohol-preferring and non-preferring lines of rats suggest the involvement in alcohol preference of a variety of neurotransmitter systems such as serotonin, glutamate, GABA, opioid and dopamine neural pathways (see Li, 2000 for review).

Relevant differences in dopaminergic mesotelencephalic pathways between alcohol-preferring and non-preferring animals have been demonstrated in virtually all the pairs of lines developed as yet although not without inconsistencies. In particular, alcohol-preferring lines appear to have innate deficiencies in dopaminergic mesotelencephalic pathways, such as the reduced levels of basal dopamine content (Gongwer et al., 1989; McBride et al., 1995; Murphy et al., 1982, 1987) or release (Honkanen et al., 1999 but see Smith and Weiss, 1999; Tattoli et al., 2001), reduced densities of dopamine postsynaptic receptors (De Montis et al., 1993; Stefanini et al., 1992, but see McBride et al., 1997; Syvalahti et al., 1994) and reduced density of dopamine fibers in forebrain limbic areas (Zhou et al., 1995). However, these deficiencies seem to be associated with a more pronounced increase in dopamine release after the administration of alcohol (Fadda et al., 1990; Smith and Weiss, 1999 but see Kiianmaa et al., 1995; Yoshimoto et al., 1992). Moreover, dopamine agonist administration has been found to reduce alcohol intake in alcohol-preferring animals such as UChB, P and HAD rats (Mardones and Quintanilla, 1996; Russell et al., 1996) but not in the non-preferring line UChA (Mar-

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dones and Quintanilla, 1996). The involvement of dopamine transmission in incentive motivation and drug (including alcohol)-induced reinforcement (see Spanagel and Weiss, 1999) suggests that these differences might play a crucial role in alcohol preference.

The administration of dopamine agonists such as amphetamine and apomorphine induces an increase in the motor activity which is mediated by the stimulation of dopamine receptors in the corpus striatum and nucleus accumbens (Kelly et al., 1975). It has been reported that individual differences in alcohol preference in genetically unselected rats positively correlate to the motor response to amphetamine (Fahlke et al., 1995) and apomorphine (Bisaga and Kostowski, 1993) and to the magnitude of alcohol-induced dopamine release in the nucleus accumbens (Engel et al., 1992). Moreover, sensitization to the amphetamine motor effect has been reported to develop along with the increase in alcohol preference (Fahlke et al., 1994). The correlation between the amphetamine effects and alcohol preference suggests that the same neural substrate is involved in both behavioural responses (Fahlke et al., 1995).

The aim of the present study was to test this hypothesis by comparing the sensitivity to the amphetamine motor-stimulating effect between two lines of rats selectively bred for high and low ethanol preference and intake: Sardinian preferring (sP) and Sardinian non-preferring (sNP) rats, respectively (see Colombo, 1997).

2. Materials and methods

The present study was carried out in accordance with the Italian law, which allows experiments on laboratory animals only after the submission of a research project to the competent authorities, and in accordance with the "Principles of Laboratory Animal Care" (NIH Publication No. 85-23, revised 1985).

2.1. Subjects

The experiments were performed on alcohol-naive male Sardinian ethanol-preferring (sP) and Sardinian ethanol-non-preferring (sNP) rats weighing 250–300 g (Charles River, Como, Italy). They were housed in groups of two to three per cage in air-conditioned rooms. The rooms were lit between 0800 and 2000 h and maintained at a temperature of 22 °C (±2) and humidity of 50–60%. The animals had water and standard laboratory diet ad libitum.

2.2. Drugs and treatments

The animals from each line (sP and sNP) were divided into two groups so that four experimental groups were formed: (1) sP rats treated with vehicle ($n=12$), (2) sP rats treated with amphetamine ($n=13$), (3) sNP rats treated with vehicle ($n=12$) and (4) sNP rats treated with amphetamine ($n=13$). Amphetamine sulphate dissolved in distilled water was administered intraperitoneally at the dose of 1 mg/kg in a volume of 1 ml/kg.

2.3. Motor activity

Motor activity was measured by an apparatus consisting of a mobile rack (height: 180 cm, width: 100 cm and depth: 60 cm) with eight compartments ($h=40$ cm, $w=45$ cm and $d=50$ cm) into which a transparent perspex cage (height: 19 cm, floor area: 23×33 cm) was placed (Imetronic, Pessac, France). The motor activity is detected by a system of photocell infrared beams, dividing the cage area into two sectors, rear and front sectors. In particular, the interruption of two photocell beams belonging to two different sectors is recorded as the "long movement" motility count. The interruption of two photocell beams belonging to the same sector is recorded as the "short movement" motility count. A barrier of infrared photocell beams placed at the height of 15 cm detects the rearing activity. The apparatus was connected to a personal computer by an electronic interface.

Experiments were performed between 0900 and 1900 h. After 1 h of habituation to the motility cages, all the rats were intraperitoneally injected with 1 mg/kg amphetamine sulphate and the motor response was recorded for the following 2 h. Data have been collected in 5-min time bins.

2.4. Statistics

The results were analysed by the analysis of variance (ANOVA) supplemented by the *F*-tests for contrast using the appropriate error term (Winer, 1971).

Habituation and amphetamine challenge data have been analysed separately. The analysis involved two between-group factors, *strain* (with two levels: *sNP* and *sP*) and *amphetamine* (with two levels: *vehicle* and *amphetamine*).

3. Results

3.1. Long movements

3.1.1. Habituation

No statistically significant differences between the groups were revealed (Fig. 1A).

3.1.2. Challenge

ANOVA showed a significant main effect of amphetamine [$F(1,46)=53.42$; $P<10^{-6}$]. The effect of the factor amphetamine reflects the strong potentiation of the locomotor activity observed in both groups of rats (Fig. 2A). The interaction between the factors amphetamine and strain was

on the verge of statistical significance [$F(1,46) = 3.14$; $P = 0.083$] due to the lower level of activation achieved in the sP group with respect to sNP.

3.2. Rearing

3.2.1. Habituation

ANOVA showed a significant main effect of strain [$F(1,48) = 4.41$; $P = 0.04$] due to higher level of activity of sP rats (Fig. 1B).

3.2.2. Amphetamine challenge

ANOVA showed a significant main effect of amphetamine [$F(1,46) = 83.77$; $P < 10^{-6}$] due to the strong increase in the activity observed in both groups of rats. As shown in Fig. 2B, the effect of amphetamine on rearing on the two strains of rats did not show any difference.

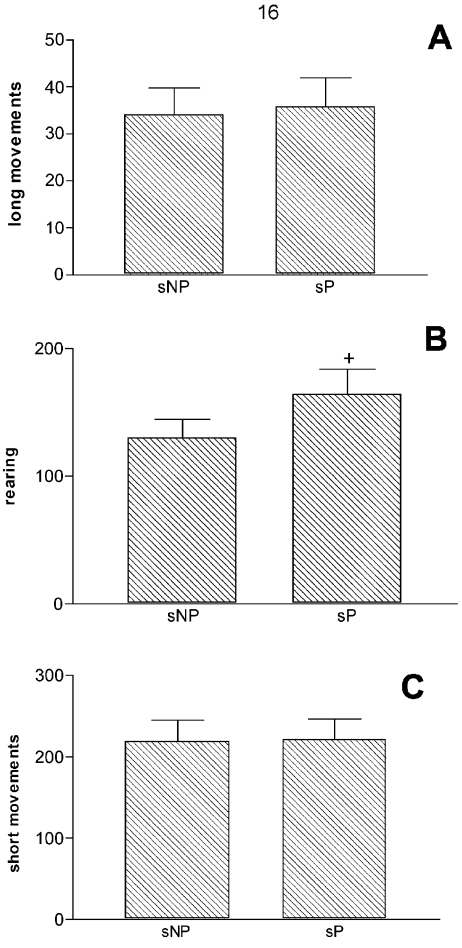

Fig. 1. Habituation. sNP: Sardinian alcohol-non-preferring; sP: Sardinian alcohol-preferring. Each value represents the means ± S.E.M. from 25 rats. Long movements (A), rearing (B) and short movements (C) were recorded for 1 h after placing the animals into the motility cages. $+P < 0.05$, effect of strain (ANOVA followed by F-tests for contrasts).

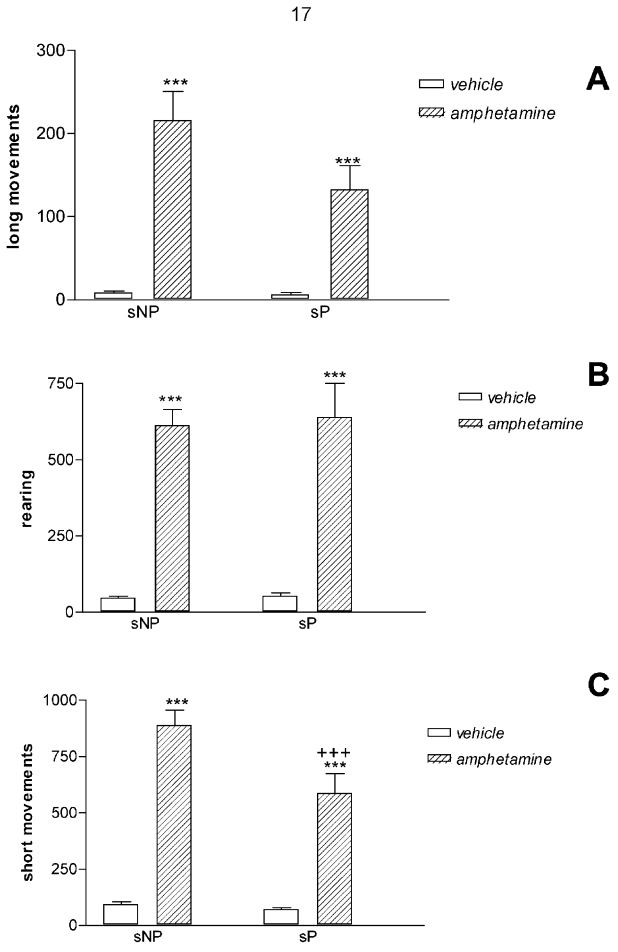

Fig. 2. Amphetamine challenge. sNP: Sardinian alcohol-non-preferring; sP: Sardinian alcohol-preferring. Each value represents the means ± S.E.M. from 12–13 rats. After 60 min of habituation to the motility cages, long movements (A), rearing (B) and short movements (C) were recorded for 2 h, following 1 mg/kg i.p. amphetamine sulphate or vehicle injection. $***P < 0.001$, effect of amphetamine (ANOVA); $+++P < 0.001$, effect of strain (ANOVA followed by F-tests for contrasts).

3.3. Short movements

3.3.1. Habituation

No statistically significant differences between the groups were revealed (Fig. 1C).

3.3.2. Amphetamine challenge

ANOVA showed a significant main effect of strain [$F(1,46) = 7.94$; $P = 0.007$] and amphetamine [$F(1,46) = 128.45$; $P < 10^{-6}$]. Moreover, a significant interaction between strain and amphetamine was present [$F(1,46) = 5.72$; $P = 0.02$]. The F-test for contrasts showed that sNP rats were more sensitive to the amphetamine motor effect with respect to sP rats (Fig. 2C).

4. Discussion

The present data show that sP rats are less sensitive to the motor-stimulating effect of amphetamine with respect to sNP

rats as indicated by the reduced "short movement" motility counts in this experimental group after the amphetamine challenge. A similar trend (i.e. a difference on the verge of statistical significance) was observed also in the "long movement" motility counts. Moreover, we observed a slightly higher level of activity in sP rats in the habituation period as shown by an increased number of rearing episodes with respect to sNP rats. This observation is in contrast with a previous study showing a decreased motor response to a novel environment in sP rats (Agabio et al., 2001). The small size of our motility cages with respect to the large (60×60 cm) open field arena used by Agabio et al. may account for this inconsistency.

These results are consistent with the data from earlier experiments performed in sP and sNP rats. Although no difference in the magnitude of the amphetamine-induced dopamine release was found between sP and sNP rats, with the former showing an increased basal level (Tattoli et al., 2001), a reduced dopamine receptor density in the nucleus accumbens of sP rats (De Montis et al., 1993; Stefanini et al., 1992) may account for their reduced sensitivity to the amphetamine-stimulating effect. Taken together, these findings are consistent with the view that increased alcohol preference is associated to a deficient mesotelencephalic dopaminergic transmission (see: Gongwer et al., 1989; Honkanen et al., 1999; McBride et al., 1995; Murphy et al., 1982, 1987; Zhou et al., 1995).

It has been reported that individual differences in alcohol preference in genetically unselected rats positively correlate to the motor response to amphetamine (Fahlke et al., 1995). Two alternative hypotheses have been proposed to explain this correlation. One possibility is that prior consumption of ethanol induced cross-sensitization to the amphetamine motor-stimulating effect (for instances of cross-sensitization between different classes of drugs, see Fahlke et al., 1994; Kalivas and Stewart, 1991; Robinson and Becker, 1986). Alternatively, hereditary and/or experiential factors may account for the individual variations in the responsiveness of the dopaminergic pathways involved both in the sensitivity to amphetamine and in controlling alcohol drinking and preference (Fahlke et al., 1995). Both explanations posit a common neural substrate for amphetamine-induced hyperactivity and alcohol preference, i.e. the dopaminergic mesotelencephalic pathways. The present results, i.e. the reduced sensitivity to the amphetamine motor-stimulating effect in the alcohol-preferring sP rats, are consistent with this hypothesis, and suggest that hereditary factors underlie individual variations in the sensitivity of this system, thus resulting in individual differences both in the amphetamine motor effect and in alcohol preference. However, in contrast to the study of Fahlke et al. in alcohol-naive sP and sNP rats, the correlation between alcohol preference and sensitivity to amphetamine results to be negative. The positive correlation founded by Fahlke et al. (1995), as they suggested as a possibility, might indeed be due to the cross-sensitization between alcohol and amphetamine.

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