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Extracellular glutamate and GABA in the ventral tegmental area of alcohol-preferring AA and alcohol-avoiding ANA rats treated repeatedly with morphine

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Abstract

Glutamate and γ-amino-butyric acid (GABA) have been implicated in neuronal plasticity related to behavioral sensitization. In the present study, we examined morphine-induced changes in the extracellular concentrations of glutamate and GABA in the ventral tegmental area in alcohol-preferring Alko Alcohol (AA) and alcohol-avoiding Alko Non-Alcohol (ANA) rats that have previously been shown to differ in morphine-induced sensitization. The rats were given escalating doses (5–20 mg/kg) of morphine every other day for five days. This treatment produced behavioral sensitization to locomotor effects of morphine in AA, but not in ANA rats, when challenged with an additional injection of morphine (10 mg/kg) 10 days later. Morphine also increased the levels of glutamate in the ventral tegmental area only in AA rats, while no significant changes were found in the extracellular concentrations of GABA between the lines. Challenging the morphine-treated AA rats with ethanol (1.5 g/kg) did not modify the levels of glutamate or GABA. No changes in the concentrations of glutamate or GABA were seen in saline-treated AA and ANA rats after morphine challenge. These results render increased glutamate transmission in the ventral tegmental area a potential contributor to the higher susceptibility of AA rats to morphine-induced behavioral and neurochemical effects relative to ANA rats.

Keywords: Behavioral sensitization; Ethanol; Excitatory amino acids; Microdialysis; Selected lines

1. Introduction

Drugs of abuse induce persistent adaptations, such as tolerance and sensitization, in the central nervous system. Behavioral sensitization, a progressive and long-lasting increase in behavioral and neurochemical effects of the drugs, can be seen as a model of neuroplastic changes associated with addiction (cf. Morgan and Roberts, 2004; Pierce and Kalivas, 1997). It has been shown to develop in experimental animals after repeated administration of various substances including morphine, ethanol and psychostimulants, and it is commonly demonstrated as an increase in their motor stimulant effects (Phillips et al., 1997; Vanderschuren and Kalivas, 2000).

The mesolimbic dopaminergic system seems to have a role in the mediation of reinforcing effects of abused drugs, and to be a substrate for neuroadaptations associated with repeated exposure to them (Anderson and Pierce, 2005; Kalivas and Duffy, 1987; Kerns et al., 2005). The activity of dopaminergic neurons in the ventral tegmental area is modified by excitatory glutamatergic afferents from the prefrontal cortex, amygdala and tegmental nuclei and inhibitory γ-amino-butyric acid (GABA) interneurons and descending afferents from the nucleus accumbens and ventral pallidum (Johnson and North, 1992b; Karreman et al., 1996; Steffensen et al., 1998; Xi and Stein, 1998). Drugs of abuse share an ability to intervene with this system and alter the function of the dopaminergic cells either directly or indirectly through other neurotransmitter systems (cf. Elliott and Beveridge, 2005; Kahlig and Galli, 2003). Morphine, for instance, binds to μ-opioid receptors on the GABAergic interneurons or on the descending GABAergic afferents in the ventral tegmental area, and by inhibiting their activity disinhibits the dopaminergic neurons (Johnson and North, 1992a; Klitenick et al., 1992).

Besides mediating the acute effects of drugs of abuse, glutamatergic and GABAergic neurotransmission have also

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been shown to have an important role in the adaptations induced by their repeated administration. Glutamatergic innervations to the dopaminergic cells in the ventral tegmental area are probably needed for the development of behavioral sensitization to psychostimulants and morphine (Vanderschuren and Kalivas, 2000; Vezina and Kim, 1999; Wolf, 1998). GABAergic mechanisms are likewise suggested to have a role in the neuroadaptations related to opioid-induced motor sensitization and reward (Laviolette et al., 2004; Leite-Morris et al., 2004).

Susceptibility to and intensity of the drug-induced behavioral and neurochemical changes differs among selectively bred or inbred rodent lines (Grahame et al., 2000; Lessov et al., 2001). Consequently, selectively bred alcohol-preferring AA (Alko Alcohol) and alcohol-avoiding ANA (Alko Non-Alcohol) rat lines (Eriksson, 1968) have been shown to differ in their susceptibility to morphine-induced sensitization (Honkanen et al., 1999; Mikkola et al., 2002; Ojanen et al., 2003). Furthermore, neurochemical and behavioral studies have revealed that there are specific differences in the opioidergic systems between the two rat lines. In addition to showing higher preference for ethanol, the AA rats also consume more aqueous solutions of μ-opioid receptor agonist, etonitazene, than the ANA rats (Hyytiä and Sinclair, 1993). Moreover, studies on the distribution of opioid receptors, receptor density, opioid propeptide mRNA levels, as well as G-protein coupled receptor function in various nuclei of the mesolimbic system suggest higher opioidergic tone in the AA than ANA rats (de Waele et al., 1995; Gianoulakis et al., 1992; Marinelli et al., 2000; Nylander et al., 1994; Soini et al., 2002). Therefore, we have speculated that increased susceptibility to morphine-induced sensitization in AA rats compared to ANA rats may be a reflection of functional differences in their opioidergic systems and may further contribute to their differential ethanol self-administration behavior (Hyytiä and Sinclair, 1993; Ojanen et al., 2005, 2006). In theory, selection for high and low ethanol drinking should have created differences in other traits than the selected ones only when they are genetically correlated to the selected trait (Sinclair et al., 1989).

Considering the possible role tegmental glutamatergic and GABAergic innervations have in the mediation of the effects of acute and repeated administration of morphine, one can speculate that the differential sensitivity of AA and ANA rats to the locomotor and neurochemical effects of morphine may be based on morphine-induced changes in the glutamatergic or GABAergic input of the mesolimbic dopaminergic neurons on the level of ventral tegmental area. The mechanisms involved may also contribute to the differences in ethanol intake between the lines. The present study sought to determine with the help of *in vivo* microdialysis, if repeated administration of morphine produces differential changes in the extracellular levels of glutamate or GABA in the ventral tegmental area of AA and ANA rats after a challenge dose of morphine. Since cross-sensitization between the locomotor stimulant effects of morphine and those of ethanol has been reported (Lessov and Phillips, 2003; Nestby et al., 1997), we also investigated, if an ethanol challenge modifies the extracellular levels of glutamate or GABA in AA rats sensitized to morphine.

2. Materials and methods

2.1. Animals

Male alcohol-preferring AA and alcohol-avoiding ANA rats (Alcohol Research Centre, National Public Health Institute, Helsinki) from generation F_{84} were used. They were about three months old and weighed from 250 to 300 g at the beginning of the experiments. The rats were housed in groups of four in plastic cages (Macrolon IV, 31 cm × 54 cm × 19 cm) with free access to food (SDS RM1 (E) SQC, Witham, Essex, England) and water. In the microdialysis experiments, the animals were housed individually in Plexiglas cages (24 cm × 24 cm × 30 cm) after surgical implantation of the guide cannula. Ambient temperature was 22 ± 1 °C, humidity 55 ± 10% and the light/dark cycle 12/12 h (lights on at 0600 h). A group of 24 rats was used to test locomotor activity and 71 rats were used in the microdialysis studies. The experiments were conducted in accordance with the European Communities Council Directive (86/609/EEC), and were approved by the Institutional Animal Care and Use Committee at the National Public Health Institute and the Chief Veterinarian of the County Administrative Board.

2.2. Morphine treatment

The rats were injected repeatedly with morphine or saline in home cages every other day for five days, and challenged with an additional dose of morphine or saline ten days after discontinuation of the repeated injections. The injections were given subcutaneously in a volume of 1 ml/kg. Groups AA morphine-morphine (AA-MM) and ANA morphine-morphine (ANA-MM) received escalating doses of morphine (day 1: 5 mg/kg, days 3 and 5: 10 mg/kg, days 7 and 9: 20 mg/kg), while groups AA saline–morphine (AA-SM), ANA saline–morphine (ANA-SM), AA saline–saline (AA-SS) and ANA saline–saline (ANA-SS) received an equal number of vehicle (0.9% sodium chloride) injections. Groups AA-MM, ANA-MM. AA-SM and ANA-SM were challenged with morphine (10 mg/kg) and groups AA-SS and ANA-SS with saline. In the ethanol experiment, all animals received repeated injections of morphine, but animals in group AA-ME were challenged with ethanol (1.5 g/kg, 12% w/v, i.p.) and animals in group AA-MS with vehicle (i.p.).

2.3. Locomotor activity

The rats were first familiarized to the measuring apparatus in three training sessions during the interval between termination of the repeated injections and challenging them with morphine. Two sessions were carried out without injections and one with a vehicle injection. The rats were weighed, placed into transparent plastic cages (18 cm × 33 cm × 15 cm), and left undisturbed for 10 min to reduce handling-induced activity. After an injection, horizontal locomotor activity was recorded for 4 h with computer controlled photocells (Cage Rack Activity System, San Diego Instruments, CA, USA). The session with morphine challenge took place ten days after the end of the repeated

treatment with saline or morphine. Training and experiments were conducted in a quiet room adjacent to the colony room, where the animals were maintained.

2.4. Surgery for microdialysis

Three days after discontinuation of repeated morphine or saline injections the rats were anaesthetized with halothane (4% during induction for 5 min and 1.5–2% during surgery) and attached to the stereotaxic frame for the implantation of a guide cannula into the brain. The cannula was lowered above the ventral tegmental area, angled toward the midline at 11° from the vertical to avoid damage to the periaqueductal gray area. The coordinates used for the microdialysis probe were 5.2 mm posterior to bregma, 0.6 mm lateral to midline and 8.4 mm below dura (Paxinos and Watson, 1997). The cannula was fastened to the skull with three stainless steel screws and dental cement. After the surgery, the rats were administered buprenorphine (0.05 mg/kg, s.c.) once, placed in individual cages, and allowed to recover for 7 days. The rats were habituated to the experimental procedures by tethering them to a counterbalancing arm several times during this recovery period.

2.5. Microdialysis

Microdialysis was performed in the home cages. A microdialysis probe (CMA/12, membrane length 1 mm, o.d. 0.5 mm, polycarbonate membrane with a 20,000-Da cutoff, CMA Microdialysis, Stockholm, Sweden) was inserted into the guide cannula at 1600 h in the day preceding the experiment and was left there without perfusion until the next morning. In the morning (at 0700 h), the rats were tethered to the counterbalancing arm and modified Ringer solution (147 mM NaCl, 3 mM KCl, 1.3 mM $CaCl_2$, 1 mM $MgCl_2$ in 0.1 M Na_2HPO_4, pH 7.25) was perfused through the probe with a flow rate of 1.5 μl/min using a CMA 100 microinjection pump. Collection of microdialysis samples (every 15 min, 22.5 μl/sample) was started 1 h after turning the flow on with a refrigerated sample collector (Univentor 820, Zejtun, Malta). A baseline collection (60 min, four samples) was followed by an injection of morphine, ethanol, or saline, and collection of the samples was continued for 4 h. An aliquot (6 μl) was taken from the vial for the determination of glutamate content, while the remaining 16.5 μl was used for the determination of GABA content. The samples were stored at −70 °C, and were analyzed later.

2.6. Analysis of glutamate and GABA

The concentrations of glutamate and GABA in the microdialysis samples were determined with a high performance liquid chromatography (HPLC) system using fluorescent detection. The system consisted of an isocratic pump with a degasser unit and a refrigerated autoinjector (Hewlett Packard 1100 series, Palo Alto, CA, USA), and a multi wavelength fluorescence detector (Waters 2475, Milford, MA, USA). The detector was equipped with an 8 μl flow-cell and was operated at maximal excitation wavelength of 354 nm and emission at 489 nm. The column was a Discovery RP Amide C16, 150 × 3 mm i.d. with a particle size of 5 μm (Supelco, Bellefonte, PA, USA). The concentrations of GABA and glutamate were determined in separate runs. The mobile phase for glutamate was a 0.3 M acetic acid buffer containing 17% (v/v) acetonitrile and 0.1 mM ethylenediaminetetra acetic acid (EDTA) at pH 5.80, and for GABA a 0.1 M acetic acid buffer containing 37% (v/v) methanol and 0.1 mM EDTA at pH 5.36. The flow rate of the mobile phase was set to 0.3 ml/min. The microdialysis samples for glutamate assay were mixed with 1 μl (reaction time 90 s), and those for GABA assay with 2 μl (60 s) of *O*-phthalaldehyde-β-mercaptoethanol for pre-column derivatization. The injection volumes were 4.5 μl and 15 μl for glutamate and GABA, respectively. The chromatograms were acquired and processed with Class VP software (v 6.12, Schimadzu Corporation, Kyoto, Japan). The raw microdialysis data (μM or nM) were converted into percentages of the baseline consisting of the mean of four baseline samples. The limit of detection for glutamate was 20 nM and for GABA 5 nM.

2.7. Histology

The brains fixed in 10% formalin solution were frozen and cut into 100 μm thick coronal sections. The positions of the probes were verified by microscopic examination of the sections stained with thionine. Only the rats where more than 50% of the probe membrane was verified to be in the ventral tegmental area were included into the results.

2.8. Chemicals and reagents

Morphine-hydrochloride was purchased from University Pharmacy (Helsinki, Finland) and was dissolved in sterile saline to reach the final concentration of 5–20 mg/ml. *O*-phthalaldehyde-β-mercaptoethanol was obtained from Pickering Laboratories (Mountain View, CA, USA). Other reagents and solvents were HPLC-quality, and were obtained from Sigma-Aldrich Finland Oy (Helsinki, Finland) or Merck GmbH (Darmstadt, Germany).

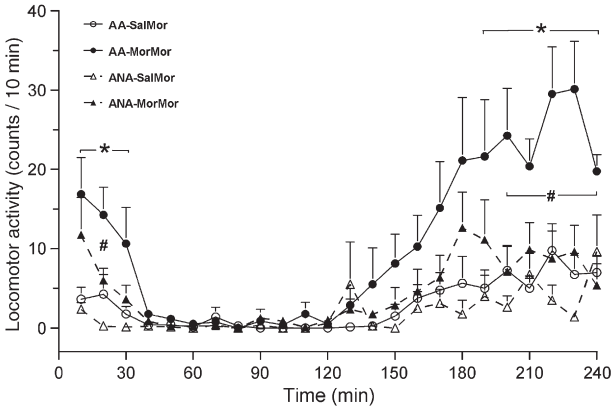

Fig. 1. Effect of a challenge dose of morphine (10 mg/kg sc) on locomotor activity in AA and ANA rats treated repeatedly with morphine (5–20 mg/kg, five injections in total). *$P<0.05$, represents difference from the AA-SM group, # $P<0.05$, relative to the corresponding group of the ANA line. Mean photocell counts per 10 min ± S.E.M. are given, $n=8$.

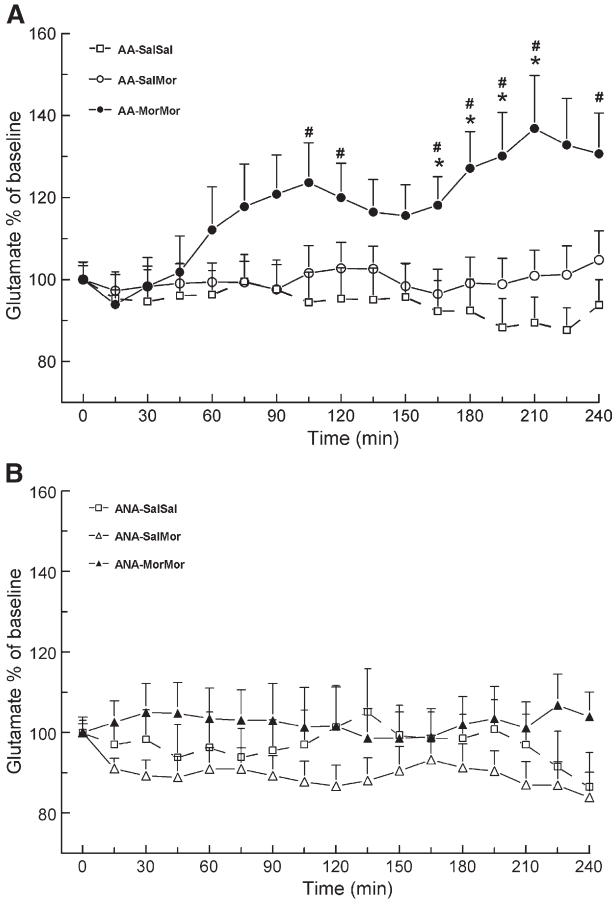

Fig. 2. Effect of a challenge dose of morphine (10 mg/kg sc) on the extracellular concentrations of glutamate in the ventral tegmental area of AA (A) and ANA (B) rats treated repeatedly with morphine (5–20 mg/kg). The raw microdialysis data were converted into percentages of the baseline consisting of the mean of the four baseline samples. The data are given as a moving average of three consecutive time points±S.E.M., n=8–11/group. *P<0.05, relative to SM group; # P<0.05, relative to the corresponding group in the ANA line, Student–Newman–Keuls post hoc comparison.

2.9. Statistical analysis

The locomotor activity scores and the microdialysis data were analyzed with a mixed-design three-way analysis of variance (ANOVA) with treatment (saline, morphine) and rat line (AA, ANA) as the between-subjects factors and measuring interval (time) as the within-subjects repeated measure. After significant main effects, differences within the rat lines were examined with a subsequent repeated measures two-way ANOVA. Post hoc comparisons between the group means (AA-SS, AA-SM, AA-MM, ANA-SS, ANA-SM and ANA-MM) were conducted using a Student–Newman–Keuls test when appropriate. The statistical analysis on the data from the animals challenged with ethanol was performed using a two-way ANOVA with repeated measures on time and the challenge treatment (saline, ethanol) as the between-subjects independent variable. The placement of the probes was compared between the groups with a nonparametric Kruskal–Wallis test. The criterion for significance was set at 0.05.

3. Results

3.1. Locomotor activity

Following repeated treatment with saline or morphine, a challenge with morphine induced more locomotor activity in morphine-treated than in saline-treated rats [F(23,644)=4.68, P<0.001, for time×treatment], and there was a significant difference in the effect of morphine between AA and ANA lines [F(23,644)=3.16, P<0.001, for time×rat line] (Fig. 1). The separate ANOVAs conducted on the treatments showed that the AA rats treated with morphine were sensitized to the locomotor-stimulating effects of morphine [F(1,14)=11.25, P=0.005], while only a tendency for stimulation was seen in the ANA rats [F(1,14)=4.30, P=0.057]. Post hoc comparisons between the group means showed that the AA and ANA rats injected with saline did not differ in morphine-induced locomotor activity.

3.2. Extracellular glutamate

Fig. 2 shows the effect of morphine challenge on the extracellular concentrations of glutamate in the ventral tegmental

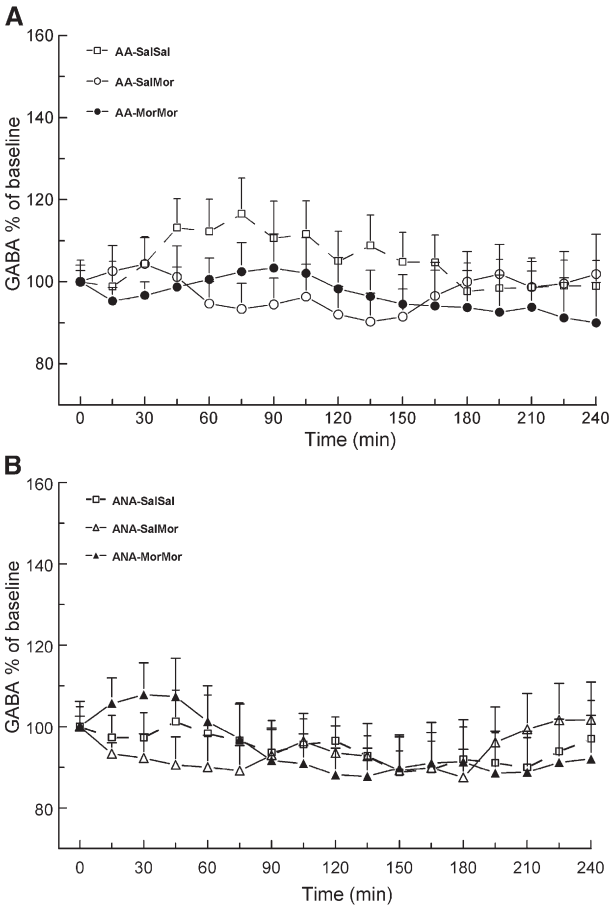

Fig. 3. Effect of a challenge dose of morphine (10 mg/kg sc) on the extracellular concentrations of GABA in the ventral tegmental area of AA (A) and ANA (B) rats treated repeatedly with morphine (5–20 mg/kg). The raw microdialysis data were converted into percentages of the baseline consisting of the mean of the four baseline samples. The data are given as a moving average of three consecutive time points±S.E.M., n=7–11/group.

area of AA and ANA rats treated repeatedly with morphine or saline. As seen in the figure, the glutamate levels in the saline-treated animals were not affected by the saline or morphine challenge. In the morphine-treated groups the effect of the morphine challenge on the concentrations of glutamate was significant [$F(1,58)=16.85$, $P<0.001$, for treatment]. The rat lines also showed a differential response to the morphine challenge [$F(15,810)=2.55$, $P=0.001$, for time × rat line × treatment]. Post hoc analyses revealed that the concentration of glutamate was increased in the AA-MM rats after the morphine challenge in comparison to the both AA-SM rats ($P<0.01$) and ANA-MM rats ($P<0.05$). Basal levels of glutamate in the ventral tegmental area were 0.71 μM in AA and 0.72 μM in ANA rats with no significant differences between the experiments, rat lines or treatments.

3.3. Extracellular GABA

Challenging the morphine- or saline-treated AA and ANA rats with morphine did not modify the extracellular levels of GABA in the ventral tegmental area (Fig. 3). The basal concentrations of GABA were 13.5 nM and 15.5 nM for AA and ANA rats, respectively ($P=0.36$).

3.4. Ethanol challenge

As shown in Fig. 4, the challenge with ethanol could not significantly modify the levels of glutamate or GABA in the AA rats treated repeatedly with morphine.

3.5. Histology

A total of 71 rats (58 rats in the morphine experiment and 13 in the ethanol experiment) was included in the final data analysis. There were no significant differences in the coordinates (AP = 5.3 ± 0.2, L = 0.6 ± 0.3, DV = 8.5 ± 0.4, mean of all rats ± S.D.) among the groups ($P>0.05$, for AP, L and DV, Kruskal–Wallis test).

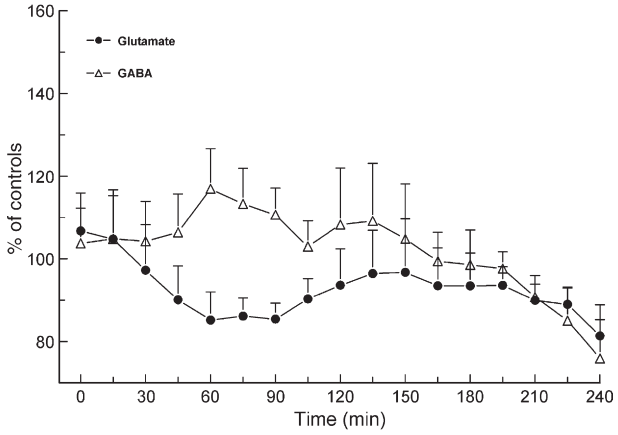

Fig. 4. Extracellular concentrations of glutamate and GABA in the ventral tegmental area of AA rats treated repeatedly with morphine and challenged with ethanol (group AA-ME). The data are shown as a difference from the saline injected controls (group AA-MS). The data are given as a moving average of three consecutive time points ± S.E.M., n = 6–7/group.

4. Discussion

In parallel with our earlier findings, alcohol-preferring AA rats showed more morphine-induced locomotor activity than morphine-treated ANA rats after repeated treatment with morphine (Ojanen et al., 2003). Since the regimen of morphine administration was different from the one used in our earlier study and context dependent cues of the testing environment were missing in the present study, the difference between the lines is not specific to the treatment protocol. In contrast to the earlier study, where the rats were tested for locomotion after every saline injection, the difference between the saline-treated AA and ANA rats in locomotor activity did not reach significance here after an acute dose of morphine.

The AA rats that had been treated repeatedly with morphine showed elevated extracellular concentrations of glutamate after the challenge injection of morphine, while similarly treated ANA rats did not. This shows that besides differing in their sensitivity to morphine-induced locomotor sensitization and dopamine release after repeated treatment with morphine (Ojanen et al., 2003), AA and ANA rat lines also differ in their sensitivity to the effects of repeated administration of morphine on glutamatergic transmission, and suggests that glutamatergic mechanisms are involved in the higher susceptibility to morphine-induced behavioral sensitization in AA rats relative to ANA rats.

Morphine-induced increase in the level of glutamate in AA rats seen in the present study is consistent with earlier findings showing enhanced drug-induced glutamate levels in the ventral tegmental area after intermittent administration of amphetamine or cocaine (Kalivas and Duffy, 1998; Xue et al., 1996). A possible explanation for this effect is given by Carlezon and Nestler (2002). Drugs of abuse have been shown to enhance the extracellular levels of dopamine in both the nucleus accumbens and ventral tegmental area. In the ventral tegmental area, this could lead to stimulation of dopamine D_1 receptors on excitatory glutamatergic neurons and enhanced glutamate release, which in turn result in activation of α-amino-3-hydroxy-5-methyl-isoxazole propionate (AMPA) and *N*-methyl- D-aspartate (NMDA) receptors on dopaminergic or other neurons. Repeated exposure to drugs leads then to specific alterations within the ventral tegmental area, including changes in glutamate receptor subunits. These alterations coincide with potentiation of drug-induced increases in dopamine and glutamate levels, and contribute to behavioral sensitization (Carlezon and Nestler, 2002). Supporting this hypothesis, blocking the dopamine D_1 receptors attenuates cocaine- or amphetamine-induced increase in the glutamate levels and the development of behavioral sensitization (Giorgetti et al., 2002; Kalivas and Duffy, 1998; Vezina and Stewart, 1989; Wolf and Xue, 1998). The findings on the role of dopamine D_1 receptors in sensitization to morphine are not, however, equally consistent. While the dopamine D_1 receptor antagonist SCH-23390 diminishes the expression of sensitization to morphine, it does not block the development of sensitization induced by morphine administered systemically or directly into the ventral tegmental area (Jeziorski and White, 1995; Vezina and Stewart, 1989).

The mechanisms underlying the insensitivity of ANA rats to the effect of repeated administration of morphine on morphine-induced increase in locomotion and in the levels of glutamate are not clear. Opioids enhance the extracellular levels of dopamine in both the nucleus accumbens and ventral tegmental area probably by binding to μ-opioid receptors on GABAergic interneurons or afferents thereby disinhibiting mesolimbic dopaminergic neurons (Johnson and North, 1992a; Klitenick et al., 1992). Taken the higher density of μ opioid receptors and opioid propeptide mRNA levels in several brain areas in AA rats relative to ANA rats (Marinelli et al., 2000), one may speculate that higher opioidergic tone in AA rats might account for the difference in the effects of morphine. Administration of morphine to AA rats would possibly result in enhanced morphine-induced suppression in the levels of GABA in the ventral tegmental area relative to ANA rats, which in turn might trigger differential adaptation to repeated exposure to morphine between AAs and ANAs. In line of this view, we previously found that morphine, if given 7 days after discontinuation of repeated treatment with morphine, also induces enhanced release of dopamine in the nucleus accumbens of AA but not in that of ANA rats (Ojanen et al., 2003). The augmented increase in the levels of dopamine was not seen, however, in the AA rats challenged with morphine five weeks after discontinuation of the repeated treatment with morphine, although similarly treated animals still showed behavioral sensitization. Whether this is true also for the levels of glutamate in the ventral tegmental area is not known, since in the present study the animals were challenged with morphine only at 10 days after discontinuation of the morphine treatment.

The involvement of GABAergic mechanisms in the differential susceptibility of AA and ANA rats to the effects of repeated morphine remains an open question. In the present study, a challenge dose of morphine did not affect the extracellular levels of GABA in the ventral tegmental area. In contrast, a study by Klitenick et al. (1992) showed a reduction in the levels of GABA when morphine was administered through a dialysis probe directly into the ventral tegmental area. Nevertheless, we administered here morphine systemically and it is possible that morphine-induced effects in other neurotransmitter systems or brain areas could have masked the effect of morphine on GABA levels in the ventral tegmental area. Alternatively, as has been pointed out by others, there may be problems in detecting subtle changes in amino acid levels with *in vivo* microdialysis technique (Wolf and Xue, 1998), possibly because a portion of amino acids in the extracellular space is derived from sources that are not directly involved in neurotransmission (Frantz et al., 2002). This, however, does not necessarily argue against the functional significance of changes in the extracellular amino acid levels in the brain (Nyitrai et al., 2006).

Challenging the morphine-treated AA rats with ethanol produced no changes in the levels of glutamate or GABA in the ventral tegmental area. This experiment was conducted, because activation of μ-opioid receptors may have a role in ethanol-induced locomotor activity and behavioral sensitization (Pastor and Aragon, 2005; Pastor et al., 2005; Sanchis-Segura et al., 2004), and cross-sensitization between the locomotor stimulant effects of ethanol and those of morphine has been reported (Lessov and Phillips, 2003; Nestby et al., 1997; Ojanen et al., 2006). Furthermore, morphine-treated AA rats show enhanced morphine-induced ethanol intake (Ojanen et al., 2006).

Augmented morphine-induced increase in the tegmental levels of glutamate found here in AA rats relative to ANA rats may help to understand the differential susceptibility to behavioral sensitization between the lines. This can be speculated to produce more stimulation of mesolimbic dopaminergic neurons and locomotor activity in AA rats than in ANA rats. The two findings may, however, not be causally related. Since our earlier findings suggested that the role of mesolimbic dopaminergic mechanisms are probably only transient in the expression of opioid-induced behavioral sensitization, the effect of enhanced glutamate release on locomotion may be mediated through dopamine independent mechanisms (Ojanen et al., 2003). A divergent, multi-structural origin of opioid effects is suggested by studies showing that the reinforcing and stimulatory properties of opiates are not mediated exclusively by the mesolimbic dopaminergic system (Piepponen et al., 2002; Sotomayor et al., 2005). Direct non-dopaminergic connections between ventral tegmental area, nucleus accumbens and areas like ventral pallidum are also potentially important in the adaptations induced by repeated administration of morphine (Caille and Parsons, 2004; Hubner and Koob, 1990; Kalivas et al., 1983). Antagonist of μ opioid-receptors injected directly into the ventral pallidum can block the development of sensitization to morphine suggesting an important role for the ventral pallidum in psychomotor stimulant and reinforcing effects of opioids (Bardo, 1998; Pierce and Kalivas, 1997; Xi and Stein, 2000), and that self-administration of heroin by rats decreases GABA efflux and onsets a late glutamate efflux in the ventral pallidum (Johnson and Napier, 2000). Since the levels of glutamate and GABA were measured here neither in the nucleus accumbens nor ventral pallidum, the effects of morphine administration on the concentrations of amino acids in these areas are not known, and a divergent explanation for the present results cannot be provided.

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