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Effects of repeated morphine on cerebral dopamine release and metabolism in AA and ANA rats

Janne A.V. Mikkola[a], Aapo Honkanen[a], T. Petteri Piepponen[a], Kalervo Kiianmaa[b], Liisa Ahtee[a,*]

[a]*Department of Pharmacy, Division of Pharmacology and Toxicology, University of Helsinki, P.O. Box 56, FIN-00014 University of Helsinki, Helsinki, Finland*
[b]*Department of Mental Health and Alcohol Research, National Public Health Institute, P.O. Box 719, FIN-00300, Helsinki, Finland*

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Abstract

Cerebral dopaminergic mechanisms were studied in the nucleus accumbens and caudate–putamen of alcohol-preferring AA (Alko Alcohol) and alcohol-avoiding ANA (Alko Non-Alcohol) rats after 4-day repeated morphine treatment. This treatment has been shown to enhance the locomotor activity stimulating effect of morphine in the AA but not in the ANA rats. Morphine (1 or 3 mg/kg) or saline was administered subcutaneously once daily and the extracellular concentrations of dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) were measured, in freely moving rats by in vivo microdialysis on days 1 and 4. Morphine increased accumbal DA, DOPAC and HVA similarly in rats of both lines, and no sensitization of DA release or metabolism was seen in rats of either line given morphine repeatedly. In the caudate–putamen, morphine increased DA, DOPAC and HVA significantly only in the AA rats. During repeated treatment, the morphine-induced elevation of DA metabolites, but not that of DA, was enhanced similarly in rats of both lines. These results suggest that the effects of acute morphine administration on nigrostriatal dopaminergic mechanisms are stronger in the AA than in the ANA rats, whereas the effects of morphine on mesolimbic dopaminergic systems do not differ. Furthermore, in rats of both lines, repeated morphine treatment enhanced the responses of the nigrostriatal dopaminergic systems similarly, but no enhancement occurred in the mesolimbic systems of rats of either line. These findings do not support the critical role of accumbal dopaminergic systems in morphine-induced behavioural sensitization.

Keywords: Alcohol preference; Morphine; Microdialysis; Dopamine; Selected rat lines; Sensitization

1. Introduction

Different drugs of abuse, e.g. alcohol, opioids and psychostimulants, share a common ability to increase extracellular concentration of dopamine (DA) in the nucleus accumbens and in the caudate–putamen, the terminal fields of the mesolimbic and nigrostriatal DA pathways, respectively [10]. Mesolimbic dopaminergic mechanisms are thought to mediate, at least partly, the reinforcing and locomotor activity-stimulating effects of drugs of abuse [32]. On the other hand, the nigrostriatal dopaminergic pathway plays an important role in the regulation of extrapyramidal motor functions. Repeated intermittent administration of psychostimulants, such as cocaine and amphetamine, or of μ-opioid receptor agonists, results in behavioural sensitization, i.e. the effects of these compounds on motor activity of animals are strongly potentiated following repeated treatment. This sensitization is associated with enhanced DA release in the nucleus accumbens, as well as with enhancement of reinforcing effects of these drugs [26]. Furthermore, after repeated administration opioids induce stereotypies and gnawing in rodents, which can be interpreted as overactivity of the nigrostriatal dopaminergic mechanisms [2].

Inherited characteristics seem to be involved in the susceptibility to becoming drug-dependent or an alcoholic [3,7]. Selected rat lines can be a useful tool in studies on the mechanisms of ethanol consumption, because rats of different lines should differ from each other only in the trait or related traits upon which selection has been applied. The alcohol-preferring AA (Alko Alcohol) and alcohol-avoiding ANA (Alko Non-Alcohol) rat lines have been developed by selective outbreeding for differences in voluntary alcohol consumption [11].

* Corresponding author. Tel.: +358-9-191-59472; fax: +358-9-191-59471.
E-mail address: liisa.ahtee@helsinki.fi (L. Ahtee).

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Interestingly, in addition to alcohol, the AA rats consume more etonitazene, an μ-opioid receptor agonist, in drinking fluid than the ANA rats [17]. Given that alcohol drinking activates brain dopaminergic pathways in AA rats [14], this finding may indicate that in the AA rats, these brain mechanisms are also sensitive to other abused drugs. This idea is supported also by recent findings, that acute morphine administration increases locomotor activity more in the AA than in the ANA rats, and that during repeated treatment, this locomotor activity-stimulating effect is enhanced in the AA rats but not in the ANA rats [16]. These findings suggest that the reinforcing effects of opioids may be stronger in AA than in ANA rats. This suggestion is also in line with the evidence that the endogenous opioid system seems to play an important role in the reinforcing properties of ethanol (for a review see Ref. [13]). Acute administration of moderate-to-high doses of opiates prior to drinking episodes seem to decrease alcohol consumption, while low doses of opiates seem to increase it [30]. Alcohol also alters opioid receptor signalling and biosynthesis of opioid peptides [6,12]. Several differences in opioid receptors and endogenous opioid systems have been found between AA and ANA rats [8,9,22,28,31], which may contribute to the differential alcohol intake between the rat lines.

On the basis of these findings, it is possible that the effects of opioids on the brain dopaminergic mechanisms may be stronger in the AA than in the ANA rats. This difference, therefore, could play a role in the differential alcohol intake in these animals. Thus, in the present study, we investigated whether acute and repeated morphine administration differently activate mesolimbic and nigrostriatal dopaminergic mechanisms in the AA and ANA rats. Using in vivo microdialysis, we measured the extracellular concentrations of DA and its metabolites, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), in the nucleus accumbens and in the caudate–putamen in freely moving AA and ANA rats after acute and repeated treatment with morphine.

2. Method

2.1. Animals

Experimentally naïve male AA and ANA rats, 3 to 4 months of age (from generations F_{71}–F_{75}, Alcohol Research Center, National Public Health Institute, Helsinki) were used. Before surgery, the rats were housed in groups of four to five rats of each line per cage under 12/12 h light/dark cycle (lights on at 6 A.M.) at an ambient temperature of 22–23°C. Tap water and standard laboratory food were available ad libitum. The animal experiments were approved by the local institutional animal care and use committee and the chief veterinarian of the county administrative board, and were conducted according to the "European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes".

2.2. Surgery

The rats were implanted with guide cannulae (CMA/11 or BAS MD-2250) under halothane anaesthesia (3.5% during induction for 5 min and then 2.5–1% during surgery). The guide cannulae were calculated relative to bregma and were aimed at the point above the nucleus accumbens (NAC), A/P=+1.7, L/M=−1.2, D/V=−6.8, or the caudate–putamen (CPU), A/P=+1.0, L/M=+2.7, D/V=−4.0, according to the atlas by Paxinos and Watson [23]. The cannula was fastened to the skull with dental cement (Aqualox, Voco, Germany) and three stainless steel screws. After the surgery, the rats were placed into individual test cages (30 × 30 × 40 cm) and allowed to recover at least for 4 days before the experiment. The rats were weighed and handled for at least on 2 days before the beginning of the microdialysis experiments.

2.3. Microdialysis

The rats were treated with morphine-HCl (1 or 3 mg/kg as base, 1 ml/kg, sc, supplied by the University Pharmacy, Helsinki, Finland) or saline (0.9% NaCl solution, 1 ml/kg, sc) once daily on 4 consecutive days. Microdialysis experiments were performed on days 1 and 4. In the morning of the experiment days, a microdialysis probe (CMA/11, 2 mm membrane, od 0.24 mm or BAS, MD-2200, 2 mm membrane) was inserted into the guide cannula. Modified Ringer solution (147 mM NaCl, 1.2 mM $CaCl_2$, 2.7 mM KCl, 1.0 mM $MgCl_2$ and 0.04 mM ascorbic acid) was infused through the probe at a flow rate of 1.5 μl/min. The collection of microdialysis samples (every 15 min, 22.5 μl/sample) was started 2.5–3 h after the probe insertion. The samples were discarded until a stable baseline was achieved; the average concentration of the first three to four stable samples was used as basal level. Thereafter, the rats were given morphine or saline, and the samples were collected for the next 4 h. The probes were thereafter removed and inserted again on day 4. In the caudate–putamen experiments, the basal concentrations of DA remained stable throughout the experiments (days 1–4, see Fig. 3). Furthermore, it has been shown that extracellular DA concentrations in the striatum remain constant even after 10 dialysis experiments (i.e. 10 probe insertions and removals) over a 23-day period, and thus, repeated microdialysis may be used in that brain region [20]. In nucleus accumbens experiments conducted by inserting the probes on day 1, the average extracellular accumbal concentrations of DA declined during the experiments to approximately 50% of basal levels, and surprisingly, morphine was without effect on DA. Therefore, the experiments were conducted by inserting a probe into the guide cannula 4 days before the actual micro-

dialysis experiment, perfusing the probe with a Ringer solution for 6 h and then removing the probe. Four days thereafter, the actual accumbal microdialysis experiment was conducted as described above, either in control rats receiving morphine for the first time in their life or in rats, which had received morphine on 3 previous days. The rats were kept individually in the same cages throughout the experiments and received morphine or saline repeatedly in the same environment.

2.4. Determination of DA, DOPAC and HVA

The samples were analyzed with HPLC immediately after collection to estimate the concentrations of DA, DOPAC and HVA. The system used for determination of the extracellular concentrations of DA, DOPAC and HVA consisted of an ESA Coulochem II detector (ESA, MA, USA) equipped with a model 5014A microdialysis cell, a Pharmacia LKB model 2248 HPLC pump (Pharmacia LKB, Sweden) and a SSI model LP-21 pulse damper (Scientific Systems, PA, USA). The column (Spherisorb ODS2, 3 μm, 4.6 × 100 mm or Spherisorb ODS 2, 3 μm, 2.0 × 100 mm) was kept at 40°C with a column heater (Croco-Cil, France). The mobile phase used consisted of 0.1 M NaH_2PO_4 buffer, pH 4.0 (adjusted with 1.0 mM citric acid), 0.1–0.2 mM octane sulfonic acid, 16% methanol and 1.2 mM EDTA. Twenty microliters of the dialysate sample was injected with a CMA/200 autoinjector (CMA, Stockholm, Sweden). DA was reduced with an amperometric detector (potential −80 mV) and DOPAC and HVA were oxidized with a coulometric detector (+300 mV). The flow rate of the HPLC pump was set at 0.3 ml/min (with 2.0 × 100-mm column) or 1.0 ml/min (with 4.6 × 100-mm column) and the chromatogram was processed with a Hitachi D-2000 chromato-integrator.

2.5. Histology

After completion of the experiment, the positions of the probes were verified by fixing the brain in formalin and then making frozen 100-μm coronal sections stained with thionine. The locations of probes were verified from both nucleus accumbens and caudate–putamen experiments. Since the nucleus accumbens is significantly smaller in size than the caudate–putamen, and it can be further divided to two subdivisions, the core and the shell, the locations of probes implanted in the nucleus accumbens of rats that received morphine are shown in Fig. 1.

2.6. Statistical analysis

Statistical analysis was carried out on data normalized to percentage of the preinjection baseline values for each rat using three-way analysis of variance (ANOVA) for repeated measures. Between-factors were rat line (AA, ANA), treatment (saline, morphine 1 or 3 mg/kg) and day (Day 1 or 4), and within-factor time (0–180 min). When appropriate, two-way ANOVA was conducted within the rat lines or days. When three-way ANOVA showed a significant treatment effect, the effect of different doses of morphine was compared with corresponding saline group with Student–Newman–Keuls post hoc test. Possible differences in the histologically verified coordinates of probes implanted in the nucleus accumbens between the rat lines were investigated with Mann–Whitney *U* test in rats that received morphine.

3. Results

3.1. Locations of the accumbal dialysis probes

No significant differences were found in the histologically verified coordinates (A/V, L/M, D/V) of dialysis probes between AA and ANA rats (Fig. 1), indicating that there were no systematic differences in the placements of the probes between the AA and ANA rats, which might affect the responses measured.

3.2. Nucleus accumbens

3.2.1. Dopamine

Morphine significantly elevated the extracellular concentration of DA in the nucleus accumbens [treatment effect: $F(2,62) = 35.20$, $P < .0001$, three-way ANOVA, Fig. 2]. There were no differences in the effects of morphine between the rat lines or days [rat line effect: $F(1,62) = 1.47$, $P = .23$; day effect: $F(1,62) = 0.86$, $P = .36$, three-way ANOVA]. Further analysis showed that the effect of morphine on day 4 was statistically significant only with the higher (3 mg/kg) dose of morphine (morphine 1 mg/kg: AA, day 1, $P < .05$ and day 4, $P > .05$; ANA, day 1, $P < .05$ and day 4, $P > .05$ and morphine 3 mg/kg: AA, day 1 and day 4, $P < .01$; ANA, day 1, $P < .01$ and day 4, $P < .05$, Student–Newman–Keuls test).

3.2.2. 3,4-Dihydroxyphenylacetic acid

Morphine significantly elevated the extracellular DOPAC concentrations [treatment effect: $F(2,62) = 84.10$, $P < .0001$, three-way ANOVA]. The effects of morphine did not differ significantly between days or rat lines [day effect: $F(1,62) = 0.001$, $P = .98$; rat line effect: $F(1,62) = 0.82$, $P = .37$, three-way ANOVA]. Further analysis showed that the effect of morphine reached statistical significance with both doses (1 and 3 mg/kg) and on both days in rats of both lines (Fig. 4).

3.2.3. Homovanillic acid

Morphine elevated the extracellular HVA concentrations in rats of both lines as well [treatment effect: $F(2,62) = 111$, $P < .0001$, three-way ANOVA]. The effect

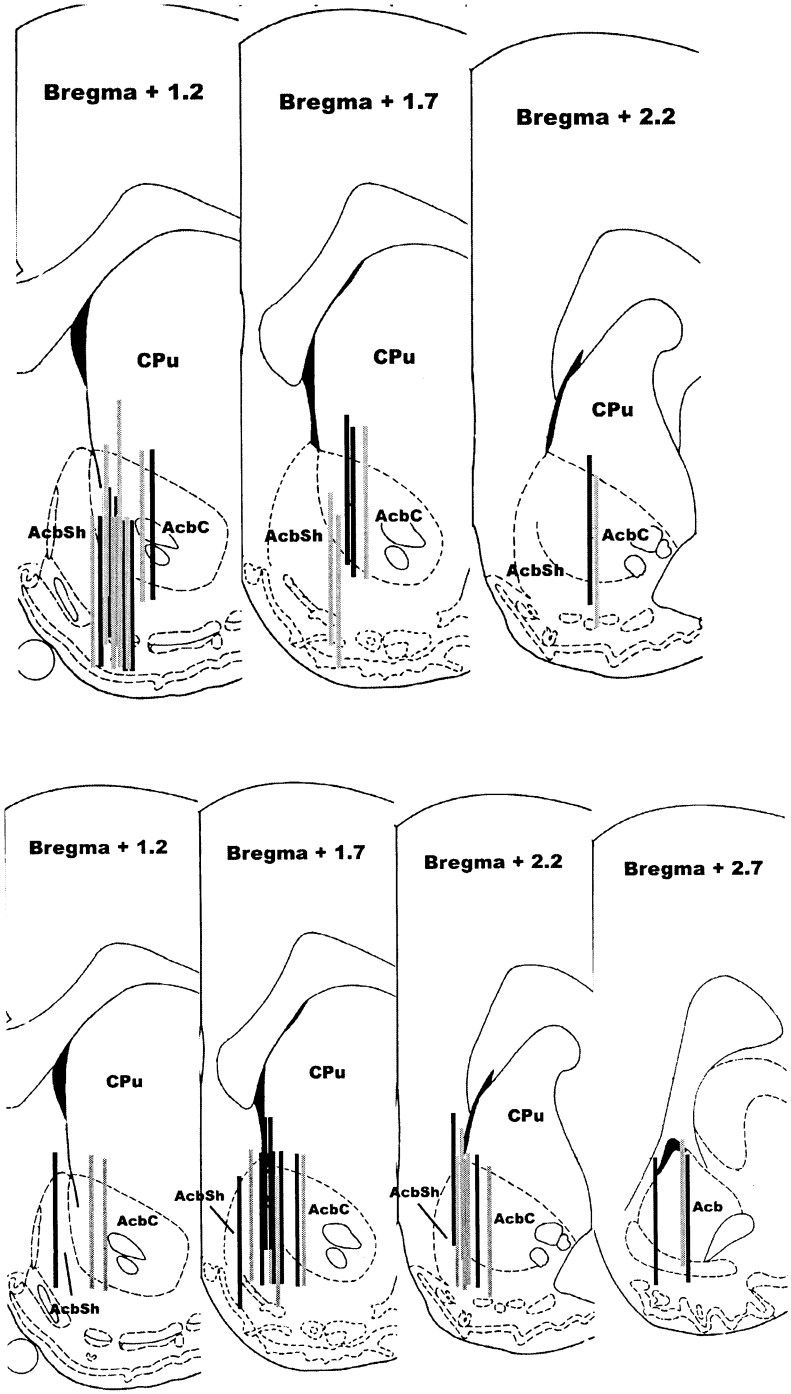

Fig. 1. The placements of probes implanted in the nucleus accumbens of AA (grey bars) and ANA (black bars) rats included in statistical analyses. For clarity, the locations of probes of rats that received saline are not included. The locations are drawn to the nearest corresponding slice. No significant differences were found in the coordinates of the probes between the rat lines (Mann–Whitney *U* test). Upper panel: experiments on day 1, lower panel: experiments on day 4. Figure modified from the atlas of Paxinos and Watson [23]. CPU, caudate–putamen; Acb, nucleus accumbens; AcbC, nucleus accumbens, core; AcbSh, nucleus accumbens, shell.

again did not differ between days or rat lines [day effect: $F(1,62)=2.59$, $P=.11$; rat line effect: $F(1,64)=0.96$, $P=.33$, three-way ANOVA]. Further analysis showed that the effect of morphine was statistically significant with both doses of morphine (1 and 3 mg/kg) and on both days (Fig. 4).

3.3. Caudate–putamen

3.3.1. Dopamine

Morphine elevated the extracellular concentrations of DA in the caudate–putamen [treatment effect: $F(2,64)=24.45$, $P<.001$, three-way ANOVA, Fig. 3]. The effect of morphine

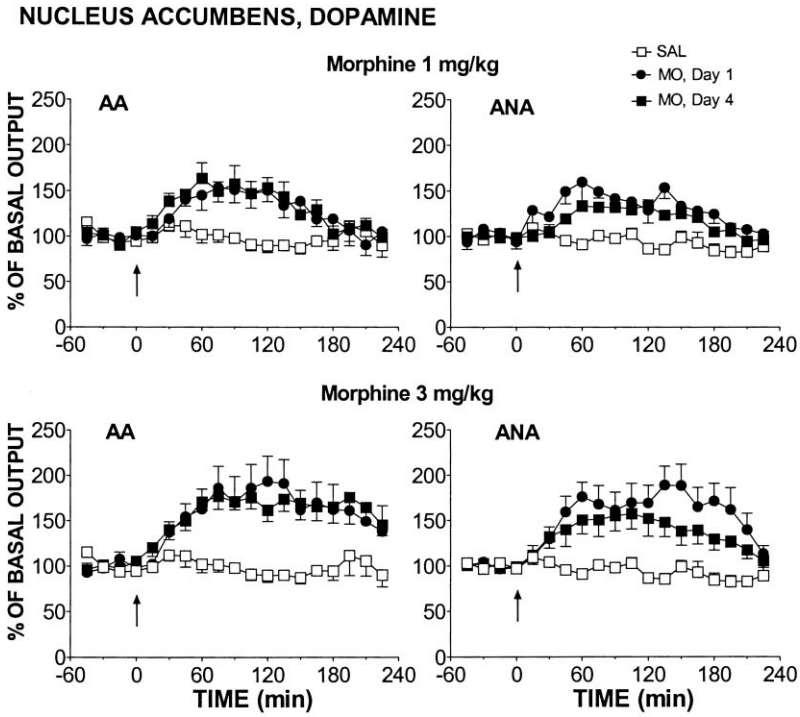

Fig. 2. Time course of extracellular DA concentrations in the nucleus accumbens in alcohol-preferring AA and alcohol-avoiding ANA rats after acute (day 1) or repeated 4-day treatment (day 4) with morphine (MO, 1 and 3 mg/kg, sc, once daily) or saline (SAL, sc, once daily). Morphine or saline was given at the time point indicated by the arrow. All results are shown as means ± S.E.M. ($n = 6–8$). The basal levels of DA were (fmol/20 μl, $n = 32$) AA 14.6 ± 2.2, ANA 17.2 ± 3.1.

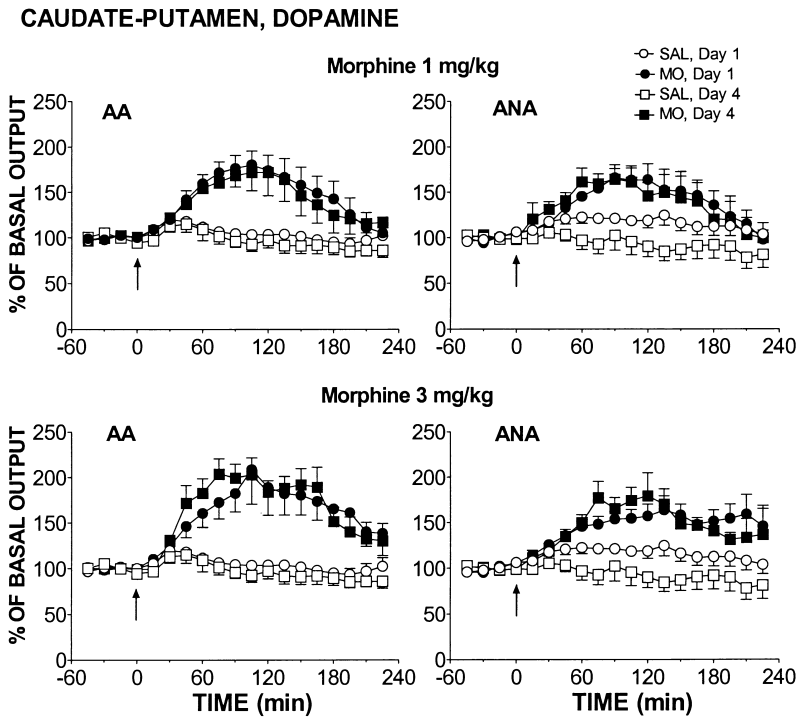

Fig. 3. Time course of extracellular DA concentrations in the caudate–putamen in alcohol-preferring AA and alcohol-avoiding ANA rats after repeated 4-day treatment with morphine (MO, 1 and 3 mg/kg, sc, once daily) or saline (SAL, sc, once daily). Microdialysis experiments were conducted on days 1 and 4. Morphine or saline was given at the time point indicated by the arrow. All results are shown as means ± S.E.M. ($n = 6–8$). The basal levels of DA were (fmol/20 μl) on day 1: AA 69.3 ± 5.3, ANA 75.9 ± 5.7 and on day 4: AA 79.3 ± 9.5, ANA 63.7 ± 7.9, respectively.

did not differ significantly between the days or rat lines [day effect: $F(1,64)=0.54$, $P=.46$; rat line effect: $F(1,64)=1.62$, $P=.21$, three-way ANOVA]. However, further analysis showed that effect of morphine did not reach statistical significance in the ANA rats on day 1 ($P>.05$ and $P<.05$, AA rats, morphine 1 and 3 mg/kg, respectively, and $P>.05$, ANA rats, both doses of morphine, Student–Newman–Keuls test), whereas on day 4, the effect of morphine reached statistical significance in rats of both lines ($P>.05$ and $P<.01$, AA rats, morphine 1 and 3 mg/kg, respectively, and $P<.05$, ANA rats, both doses of morphine, Student–Newman–Keuls test).

3.3.2. 3,4-Dihydroxyphenylacetic acid

Morphine elevated the extracellular DOPAC concentrations in the caudate–putamen [treatment effect: $F(2,64)=52.55$, $P<.001$, three-way ANOVA, Fig. 4]. However, morphine elevated the concentrations of DOPAC on day 1 only in the AA rats and the effect of morphine was stronger in the AA rats as compared with the ANA rats [treatment × rat line interaction: $F(2,64)=10.96$, $P<.001$, three-way ANOVA]. The effect of morphine was enhanced in rats of both lines on day 4 as compared with day 1 [treatment × day interaction: $F(2,64)=14.47$, $P<.001$, three-way ANOVA]. The magnitude of the enhancement did not differ between the rat lines [rat line × treatment × day interaction: $F(2,64)=0.23$, $P=.80$]. When the rat lines were analyzed separately, the effect of morphine was similarly enhanced in both rat lines on day 4 as compared with day 1 [treatment × day interaction: $F(2,34)=6.83$, $P=.003$ and $F(2,30)=9.23$, $P<.001$ for AA and ANA rats, respectively]. Further analysis showed that the enhancement was significant only with the larger 3 mg/kg dose of morphine [dose 1 mg/kg, treatment × day interaction: $F(1,23)=2.59$, $P=.12$ and $F(1,20)=1.59$, $P=.22$ for AA and ANA rats, respectively; dose 3 mg/kg, treatment × day interaction: $F(1,22)=20.63$, $P<.001$ and $F(1,20)=20.99$, $P<.001$ for AA and ANA rats, respectively]. When the effect of morphine was compared between the rat lines within days 1 and 4, the effect of morphine was significantly stronger in the AA rats on both days [treatment × rat line interaction: $F(2,34)=4.69$, $P=.016$ and $F(2,30)=6.15$, $P=.006$, days 1 and 4, respectively, two-way ANOVA].

3.3.3. Homovanillic acid

Morphine elevated the extracellular HVA concentrations in the caudate–putamen [treatment effect: $F(2,64)=43.67$, $P<.001$, three-way ANOVA, Fig. 4]. However, morphine elevated the concentrations of HVA on day 1 only in the AA rats and the effect of morphine was stronger in the AA rats as compared with the ANA rats [treatment × rat line interaction: $F(2,64)=10.34$, $P<.001$, three-way ANOVA]. The effect of morphine was enhanced in rats of both lines on day 4 as compared with day 1 [treatment × day interaction: $F(2,64)=13.08$, $P<.001$, three-way ANOVA]. The magnitude of the enhancement did not differ between the rat lines [rat line × treatment × day interaction: $F(2,64)=0.60$, $P=.55$]. When the rat lines were analyzed separately, the effect of morphine was enhanced in both rat lines on day 4 as compared with day 1 [treatment × day interaction: $F(2,34)=8.18$, $P=.001$ and $F(2,30)=5.23$, $P=.011$ for AA

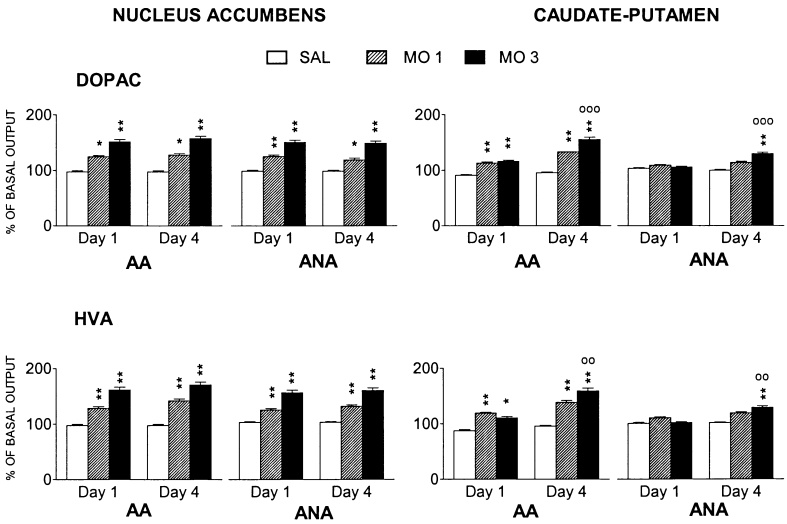

Fig. 4. Effects of acute (day 1) or repeated 4-day treatment (day 4) with morphine (MO, 1 and 3 mg/kg, sc, once daily) or saline (SAL, sc, once daily) on extracellular concentrations DOPAC and HVA in the nucleus accumbens (left panel) or in the caudate–putamen (right panel) in alcohol-preferring AA and alcohol-avoiding ANA rats. The columns represent means of the 13 samples collected during 180 min after morphine or saline injection. All results are shown as means ± S.E.M. ($n=6–8$). *$P<.05$ and **$P<.01$, when compared with the corresponding saline group (Student–Newman–Keuls post hoc test). Interaction between treatment (SAL vs. MO) × day within the rat line (two-way ANOVA): °°$P<.01$ and °°°$P<.001$. The basal levels of DOPAC and HVA in the nucleus accumbens were (pmol/20 μl, $n=32$) DOPAC: AA 4.7 ± 0.6, ANA 4.6 ± 0.6, HVA: AA 2.9 ± 0.3, ANA 2.9 ± 0.3, respectively. The basal levels of DOPAC and HVA in the caudate–putamen were (pmol/20 μl, $n=18–24$) on day 1: DOPAC, AA 24.7 ± 1.2, ANA 18.5 ± 1.0; HVA, AA 14.4 ± 0.7, ANA 11.2 ± 0.7 and on day 4: DOPAC, AA 13.2 ± 0.7, ANA 10.2 ± 0.7; HVA, AA 10.3 ± 0.5, ANA 9.1 ± 0.5, respectively.

and ANA rats, respectively]. Further analysis showed that the enhancement was significant only with the 3-mg/kg dose of morphine [dose 1 mg/kg, treatment × day interaction: $F(1,23) = 1.67$, $P = .21$ and $F(1,20) = 0.71$, $P = .41$ for AA and ANA rats, respectively; dose 3 mg/kg, treatment × day interaction: $F(1,22) = 13.88$, $P < .01$ and $F(1,20) = 14.05$, $P < .01$ for AA and ANA rats, respectively]. When the effect of morphine was compared between the rat lines within days 1 and 4 the effect of morphine was significantly stronger in the AA rats on both days [treatment × rat line interaction: $F(2,34) = 3.29$, $P = .049$ and $F(2,30) = 8.04$, $P = .002$, days 1 and 4, respectively, two-way ANOVA].

4. Discussion

The results of the present study show that there are some differences in the responses of the cerebral dopaminergic mechanisms to morphine administration among the alcohol-preferring AA and alcohol-avoiding ANA rats. The effects of acute morphine administration on accumbal DA release or metabolism did not differ between naïve AA and ANA rats, whereas the nigrostriatal DA systems appear to be more sensitive to acute morphine in the AA than in the ANA rats. The effect of morphine on accumbal concentrations of DA, DOPAC and HVA remained similar during the 4-day repeated morphine administration in rats of both lines. In the caudate–putamen, the increasing effect of morphine on the concentrations of DOPAC and HVA was enhanced in rats of both lines, whereas the effect of morphine on DA release remained similar during the 4-day repeated morphine administration.

Mesolimbic dopaminergic mechanisms are thought to be involved in the reinforcing, as well as in the locomotor activity-stimulating effects of drugs of abuse (see Section 1). We have previously found that the locomotor activities of AA and ANA rats were different after acute morphine injection, AA rats being significantly more stimulated by morphine than ANA or nonselected Wistar rats [16]. However, we now found that morphine induced a similar increase of extracellular DA levels in the nucleus accumbens in rats of both lines. The results of the present study are in agreement with our previous findings where morphine was shown to increase the accumbal DA release similarly both in the AA and ANA rats when 3-methoxytyramine (3-MT) tissue concentrations measured post-mortem were used as an indicator of DA release [15]. Morphine and opioid peptides have been shown to induce locomotor activity independently of DA when administered directly into the nucleus accumbens [19,24]. Since the effect of morphine on accumbal DA release after acute morphine administration did not differ between the rats of these lines in the present study or in our previous study [15], it seems that DA mechanisms in the nucleus accumbens are not involved in the difference seen in locomotor activity after acute morphine administration between the rat lines. On the other hand, other brain areas, e.g. the caudate–putamen (see below), or other brain transmitters, like serotonin [15], may be involved.

Sensitization of locomotor activity after repeated morphine treatment has been found to be associated with enhanced DA release in the nucleus accumbens (see Refs. [18,29]). We have recently found that the stimulatory effects of morphine (daily 1 mg/kg) on locomotor activity are more enhanced in AA than in ANA rats after a 4-day morphine treatment [16]. In the present study, we did not find any enhancement of accumbal DA release in rats of either line, which finding does not support the DA hypothesis of behavioural sensitization, at least when enhanced DA release is concerned. However, sensitization of mesolimbic DA release was usually seen after 3 or more days of withdrawal after repeated morphine administration and with higher doses of morphine than those used in the present study [1,5,18,29]. Acquas and Di Chiara [1] reported a tolerance rather than sensitization of DA release after 1 day withdrawal of repeated high-dose morphine treatment and sensitization of DA release after 3 days of withdrawal. Thus, as the results of the present and our previous locomotor activity study [16] suggest, the sensitization of locomotor activity after repeated a 4-day treatment with relatively low dose of morphine may not be associated with increased DA release in the nucleus accumbens, at least after only 1 day of withdrawal. It should be noted that in the present study, the animals received repeated drug treatment in their home environment, and the experimental set up used in the behavioural experiments (drug treatment in locomotor activity testing cages) was not exactly reproduced. However, our preliminary results from experiments where 3-MT was used as an indicator of DA release suggest that the mesolimbic DA release is not significantly sensitized either in the AA or in the ANA rats after repeated 4-day treatment with 1 mg/kg of morphine even if the drug administration is paired to a distinct context as in the previous behavioural study [25]. Furthermore, repeated morphine treatment may influence differently different DA neurons. Thus, the finding of sensitization or lack of sensitization of DA release may also depend on the placement of the microdialysis probe, e.g. between the core and shell parts of the nucleus accumbens [5]. However, the placements of dialysis probes cannot explain the lack of difference in DA release between AA and ANA rats in the present experiment, since there were no differences in the placements of dialysis probes between these rats. The results of the present study suggest that differential opioid regulation of accumbal DA mechanisms is not critically involved in the differences in opioid-induced locomotor activity between these rat lines.

The AA rats seem to be more sensitive to acute morphine-induced DA release in the caudate–putamen than the ANA rats, since significant elevation in extracellular DA concentration was seen only with the higher (3

mg/kg) dose of morphine and only in the AA rats but not in the ANA rats. The first morphine injection elevated the striatal concentrations of DA metabolites (DOPAC and HVA) significantly more in the AA than in the ANA rats. The same difference was also found previously when concentrations of DOPAC, HVA and 3-MT, were measured from post-mortem tissue samples [15]. Together, these findings suggest that morphine activates the nigrostriatal DA pathway more easily in the AA than in the ANA rats. The AA rats have higher density of μ-opioid receptors in the substantia nigra, especially in the pars reticulata, and the striatal patches containing μ-opioid receptors are larger than in the ANA rats [27,28]. This may contribute to the greater DA turnover after morphine seen in the caudate–putamen of AA rats as compared to that of ANA rats. It is unclear whether this greater sensitivity of the nigrostriatal DA system in AA rats contributes to the increase in the locomotor activity after acute morphine treatment, since increased DA release in the caudate–putamen is usually linked to stereotyped behaviour, not to horizontal locomotor activity [4]. On the other hand, there is some evidence that μ-opioids may induce locomotor activity also by acting in the substantia nigra [21]. We have also recently found, that a selective μ-opioid receptor agonist, [D-Ala^2, MePhe^4, Gly-ol^5]enkephalin (DAMGO; 0.05 μg), when administered bilaterally into substantia nigra, enhances locomotor activity in addition to stereotypic gnawing in Wistar rats (unpublished results). Therefore, it is possible that the nigrostriatal DA pathway plays a role in the morphine-induced enhancement or modulation of locomotor activity in the AA rats. After repeated treatment, the effect of morphine on striatal DA metabolite concentrations was enhanced similarly in rats of both lines, and no enhancement of morphine-induced elevation of extracellular DA concentration was seen in either rat line. Morphine elevated the concentrations of DOPAC and HVA more in the AA than in the ANA rats on both days 1 and 4, but the ratio of the enhancement of metabolite concentrations between days 1 and 4 did not differ between the rats of these lines. Therefore, these results, together with previous ones [15], strongly suggest that the effects of morphine on nigrostriatal DA function are stronger in the AA than in the ANA rats, but there is no difference in the sensitization of nigrostriatal DA metabolism between these rats.

In conclusion, in the nucleus accumbens, acute morphine increased DA release and metabolism similarly in AA and ANA rats, and no sensitization of accumbal DA release or metabolism was seen in rats of either line. In contrast, the effects of acute morphine on the nigrostriatal DA mechanisms were stronger in the AA than in the ANA rats. After repeated morphine treatment, the effects of morphine on striatal DA metabolites were enhanced in rats of both lines, but the ratio of the enhancement did not differ between the rat lines. Thus, the nigrostriatal DA pathway might play a role in differential acute effects of morphine on locomotor activity between the AA and ANA rats, but neither the nigrostriatal nor the mesolimbic DA systems seem to be critical in the differential morphine-induced behavioural sensitization between these rats. Furthermore, the present study did not reveal any significant differences in the opioid regulation of brain mesolimbic dopaminergic systems between the AA and ANA rats, which might contribute to their differential alcohol preference.

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