INTRODUCTION

Cerebral dopaminergic mechanisms seem to be involved in the locomotor activity-stimulating and reinforcing effects of drugs of abuse. It is well known that different kinds of abused drugs, e.g. opioids, psychostimulants and alcohol, increase dopamine (DA) release in the nucleus accumbens and in the caudate-putamen (Di Chiara and Imperato, 1988). Repeated administration of μ-opioids or psychostimulants results in behavioural sensitization, which is associated with enhanced DA release in the nucleus accumbens (Kalivas and Stewart, 1991), as well as with enhancement of the reinforcing effects of these drugs (Robinson and Berridge, 1993). Furthermore, after repeated administration to rats, opioids simultaneously enhance striatal DA turnover and induce stereotypic behaviours and gnawing, which can thus be interpreted as an overactivity of the nigrostriatal dopaminergic mechanisms (Ahtee and Attila, 1987).

It is generally accepted that inherited characteristics are involved in the development of alcoholism. This has been shown both in experimental animals and humans (for reviews see Cloninger, 1987; Ball and Murray, 1994; Crabbé et al., 1994). The alcohol-prefering AA (Alko Alcohol) and alcohol-avoiding ANA (Alko Non-Alcohol) rat lines have been developed by selective outbreeding for high and low voluntary alcohol intakes, respectively (Eriksson, 1968). These rat lines are a useful tool in studies of the mechanisms of ethanol consumption, because rats of these lines should differ from each other only in the traits related to voluntary alcohol consumption. In addition to alcohol, the AA rats consume more aqueous solutions of cocaine and etonitazene, a μ-opioid receptor agonist, than ANA rats or non-selected Wistar rats (Hyttä and Sinclair, 1993). Morphine increases horizontal locomotor activity of AA rats, more than that of ANA or non-selected Wistar rats, and, furthermore, AA, but not ANA or Wistar rats, show sensitization to this locomotor-activating effect of morphine by the fourth day of repeated treatment with a 1 mg/kg dose of morphine (Honkanen et al., 1999b).

Given that psychomotor stimulation seems to be associated with the reinforcing effects of drugs of abuse (for a review, see Wise and Bozarth, 1987), these results suggest that the reinforcing effects of opioids may be stronger in AA than ANA rats and raises the possibility that properties of the endogenous opioid system may play a role in the differential alcohol consumption behaviours of these rats. This is in line with the suggestion that the endogenous opioid system and regulation of brain dopaminergic mechanisms by opioids play a role in alcohol addiction (Herz, 1997). Thus, the differential effects of opioids on brain dopaminergic systems in AA and ANA rats might be involved in the difference between the alcohol consumptions of these rats.

We have previously found that the effect of acute morphine administration on mesolimbic DA release does not differ between AA and ANA rats, whereas the nigrostriatal DA system seems to be more sensitive to morphine in AA rats than in ANA rats (Honkanen et al., 1999a). Thus, in the present study, we wanted to clarify whether synaptic DA release or metabolism is involved in the differential behavioural sensitization induced by morphine in AA and ANA rats. We studied whether repeated morphine pretreatment induces sensitization of DA systems to acute morphine in AA and ANA rats. We measured concentrations of DA and its metabolites, 3-methoxytyramine (3-MT) and homovanillic acid (HVA), in the nucleus accumbens, caudate-putamen and olfactory tubercle of AA and ANA rats after acute or repeated morphine treatment. To prevent the post-mortem changes in the concentration of DA and its metabolites, the rats were killed by head-focused microwave irradiation.

*Author to whom correspondence should be addressed.
In addition to brain opioidergic and dopaminergic systems, cerebral serotonergic mechanisms have been suggested to be involved in alcohol-drinking behaviour both in humans (Linnoila et al., 1994; Virkkunen and Linnoila, 1997) and animals (McBride et al., 1993). Thus, the effects of repeated morphine treatment on concentrations of serotonin (5-hydroxytryptamine, 5-HT) and its metabolite, 5-hydroxyindol-3-ylacetic acid (5-HIAA), were also assessed.

MATERIALS AND METHODS

Animals

Experimentally naive male AA and ANA rats, 3–4 months old, from generation F75, were used. The rats of each line were housed in groups of four or five per cage, except as described below, under 12-h/12-h light/dark cycle (lights on at 06:00) at an ambient temperature of 22°C. The animals were accustomed to handling for at least 3 days before the beginning of the experiment. Tap water and rat chow (RM1 (E) SQC pellets from SDS, Witham, Essex, UK) were available ad libitum.

The animal experiments were approved by the local institutional animal care and use committee and conducted according to the ‘European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes’.

Drug treatment

Forty AA and ANA rats were given repeatedly morphine (1 mg/kg, 1 ml/kg, s.c., supplied by the University Pharmacy, Helsinki, Finland) or saline (1 ml/kg, s.c.) for 4 days. The rats were kept overnight in groups of four or five of each line. The rats were placed individually in cages 20 min before morphine or saline injection and were kept in these cages for 2 h after the injection, after which they were returned to the original cages. The rats of both lines were divided into four experimental groups receiving different treatments. Group 1: morphine once daily for 4 days; group 2: saline once daily for 4 days; group 3: morphine once daily for 3 days and saline on day 4; group 4: saline once daily for 3 days and morphine on day 4. Each cage housed rats given different treatments.

Sixty minutes after the last administration of morphine or saline (on day 4), each rat was taken from its cage to another room, killed with head-focused microwave irradiation (7 kW for 1.4 s) using model NJE 2603-10 kW microwave instrument (New Japan Radio Inc., Japan), and decapitated. The brains were removed from the skull and placed on a brain mould (RBM-4000C; ASI Instruments, USA) cooled on ice, and sectioned coronally with razor blades at 2.7 and –0.3 mm from bregma (Paxinos and Watson, 1986). The caudate-putamen and nucleus accumbens were dissected from the second slice by using needles with inner diameters of 2 and 3 mm, respectively. Tissue ventral to the nucleus accumbens was dissected as the olfactory tubercle. The tissues were immediately frozen on dry ice and stored at –80°C until assayed for DA, serotonin and their metabolites.

Estimation of DA, 3-MT, HVA, 5-HT and 5-HIAA

Samples were homogenized in 1 ml of 0.2 M HClO₄ after which 25 μl KOH/HCOOH buffer were added to the homogenates to adjust the pH to 2.4. Samples were centrifuged at 5500 g for 45 min. The supernatants were purified using a method described earlier (Haikala, 1987) with slight modifications. In brief, a 950 μl sample of supernatant was pipetted onto Sephadex G-10 columns and washed with 3.0 ml of 0.01 M HCl. DA and 3-MT were collected by washing the columns with 1.5 ml of 0.01 M HCl and 1.0 ml of 0.02 M NH₃. HVA, 5-HT and 5-HIAA were collected by subsequent washing of the columns with 1.0 ml of 0.02 M NH₃ and 4.0 ml of 0.01 M KOH. Thirty μl of 2.6 mM sodium pyrosulphite and 5.7 mM ascorbic acid (in 0.01 M HCl) were added into the tubes containing DA/3-MT and HVA/5-HT/5-HIAA, respectively. The samples were assayed for concentrations of these parameters by using high-performance liquid chromatography with electrochemical detection as described earlier (HonKanen et al., 1994).

Statistical analysis

Differences in the basal concentrations of DA, 5-HT and their metabolites between the rat lines were tested with Student’s t-test in rats treated repeatedly with saline (= group 2, see Materials and methods section). Effects of drug treatments (saline or morphine on day 4) within each rat line and the interactions between drug pretreatments (saline or morphine for 3 days) and drug treatments within rat lines were tested with 2-way analysis of variance (ANOVA) followed by Tukey’s compromise test.

RESULTS

Basal concentrations of DA, 5-HT and their metabolites

The basal concentrations of DA, 5-HT and 5-HIAA are presented in Table 1. In the nucleus accumbens, the concentrations of 5-HT and 5-HIAA, and in the olfactory tubercle, the concentrations of DA, 5-HT and 5-HIAA were higher in AA rats than in ANA rats. In the caudate-putamen, the concentration of 3-MT was smaller in AA than in ANA rats (Fig. 1), but the HVA concentrations did not differ significantly between rats of the two lines (Figs 1–3).

Effects of acute morphine on DA metabolism in rats pretreated with saline or morphine

DA. Morphine did not affect the concentrations of DA either in saline or in morphine-pretreated rats of either line in any of the brain areas studied (Table 1).

3-MT. Morphine increased the concentrations of 3-MT in the caudate-putamen [treatment effect: F(1,33) = 28.28, P < 0.001 for AA and F(1,35) = 10.36, P = 0.003 for ANA rats, 2-way ANOVA], in the nucleus accumbens [treatment effect: F(1,34) = 59.21, P < 0.001 for AA and F(1,35) = 32.66, P < 0.001 for ANA rats, 2-way ANOVA] and in the olfactory tubercle [treatment effect: F(1,34) = 5.84, P = 0.02 for AA and F(1,33) = 11.70, P = 0.002 for ANA rats, 2-way ANOVA] in rats of both lines. Post hoc analysis revealed that morphine elevated 3-MT in the caudate-putamen significantly after both pretreatments in AA rats, whereas in ANA rats, the effect of morphine did not reach statistical significance after either pretreatment (Fig. 1). The effect of morphine on 3-MT in the nucleus accumbens was significant after both pretreatments in rats of both lines (Fig. 2). In the olfactory tubercle, the effect of morphine in ANA rats did not reach statistical significance in either pretreatment group, whereas in ANA rats pretreated...
with morphine, the effect of morphine was significant (Fig. 3). Neither pretreatment altered significantly the effects of morphine on 3-MT in rats of either line in any of the brain areas studied (pretreatment × treatment interaction $P > 0.05$ for rats of both lines, 2-way ANOVA).

$HVA$. Morphine increased the concentration of HVA in the caudate-putamen [treatment effect: $F(1,34) = 15.73, P < 0.001$ for AA and $F(1,36) = 12.97, P < 0.001$ for ANA rats, 2-way ANOVA], in the nucleus accumbens [treatment effect: $F(1,34) = 16.52, P < 0.001$ for AA and $F(1,36) = 23.22, P < 0.001$ for ANA rats, 2-way ANOVA] and in the olfactory tubercle [treatment effect: $F(1,34) = 22.96, P < 0.001$ for AA and $F(1,36) = 26.95, P < 0.001$ for ANA rats, 2-way ANOVA] in rats of both lines. Post hoc analysis revealed that the effect of morphine in the caudate-putamen was significant in saline-pretreated AA rats and morphine-pretreated ANA rats (Fig. 1). The effect of morphine in the nucleus accumbens was significant after both pretreatments in ANA rats and after morphine pretreatment in AA rats (Fig. 2). In the olfactory tubercle, morphine increased HVA significantly after both pretreatments in rats of both lines (Fig. 3). Neither pretreatment altered significantly the effects of morphine on HVA in any of the brain areas studied in rats of either line (pretreatment × treatment interaction $P > 0.05$ for rats of both lines, 2-way ANOVA).

Effects of acute morphine on serotonergic transmission in rats pretreated with saline or morphine

$5-HT$. Morphine did not significantly affect the concentrations of 5-HT in any of the brain areas studied either in saline- or in morphine-pretreated rats of either line (Table 1). 5-HIAA. Morphine increased the concentration of 5-HIAA significantly in the caudate-putamen and olfactory tubercle of AA, but not in those of ANA, rats [treatment effect: $F(1,33) = 6.61, P = 0.01$ for AA and $F(1,36) = 1.13, P = 0.30$ for ANA rats in the caudate-putamen and treatment effect: $F(1,33) = 5.96, P = 0.02$, for AA and $F(1,35) = 0.86, P = 0.36$ for ANA rats in the olfactory tubercle, 2-way ANOVA]. Post hoc analysis conducted within AA rats showed that the effect of morphine was not significantly altered in either pretreatment group in the caudate-putamen, but in the olfactory tubercle the effect of morphine was significant in saline, but not in morphine-pretreated, AA rats (Table 1). In the nucleus accumbens, acute administration of morphine did not significantly affect 5-HIAA after either pretreatment in rats of either line (Table 1). Neither pretreatment altered significantly the effects of morphine on 5-HIAA in rats of either line in any of the brain areas studied (pretreatment × treatment interaction $P > 0.05$ for rats of both lines, 2-way ANOVA).

DISCUSSION

The main finding in the present study was that the 4-day treatment with morphine (1 mg/kg) did not induce any detectable sensitization in acute morphine’s effects on cerebral DA release or metabolism or on 5-HT metabolism in either rat line. This is not in line with our previous findings concerning locomotor activity, where the morphine-induced locomotor
stimulation of AA rats was more easily sensitized by repeated administration of 1 mg/kg of morphine than that of ANA rats (Honkanen et al., 1999b).

The AA rats have been found to have more DA in the whole brain as well as in the caudate-putamen than the ANA rats (Ahtee and Eriksson, 1975; Kiianmaa et al., 1991; Honkanen et al., 1999a). A similar difference was found in the olfactory tubercle in the present study. As found previously (Honkanen et al., 1999a), the 3-MT concentrations were smaller in the caudate-putamen of AA, than ANA, rats. In contrast to P/NP and HAD/LAD rats (McBride et al., 1993; McBride and Li, 1998), AA rats have higher cerebral concentrations of 5-HT and 5-HIAA than ANA rats (Ahtee and Eriksson, 1973; Korpi et al., 1988; Honkanen et al., 1999b), which is in line with the results of the present study. Interestingly, low concentrations of 5-HIAA in the cerebrospinal fluid have been associated with type 2 alcoholism in humans (Cloninger, 1987; Virkkunen and Linnoila, 1997). Thus, in respect to 5-HIAA, the AA rats may represent a different type of alcohol preference than the P and HAD rats.

In agreement with our earlier findings (Honkanen et al., 1999a) acute morphine administration enhanced the accumbal DA metabolism similarly in rats of both lines. This finding gives further evidence that the increase in mesolimbic DA release or metabolism induced by morphine is not involved in the differences seen between AA and ANA rats in the locomotor stimulation after acute morphine administration (Honkanen et al., 1999a,b). Instead, the effect of morphine on DA metabolism in the caudate-putamen was previously found to be stronger in the AA than in the ANA rats (Honkanen et al.,

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Fig. 1. Acute effects of morphine on concentrations of 3-methoxytyramine (3-MT) and homovanillic acid (HVA) in the caudate-putamen of AA and ANA rats pretreated repeatedly with saline (SAL) or morphine (MO).

Rats received saline or morphine (1 mg/kg) s.c. 1 h before death and were pretreated with either saline or morphine (1 mg/kg) s.c. for 3 days. Results are means ± SEM (n = 8–10). *P < 0.05 in comparison with corresponding AA group (Student’s t-test). †P < 0.05 and ††P < 0.01, in comparison with corresponding saline group (ANOVA followed by Tukey’s compromise test). □ Acute saline; ■ acute morphine.

Fig. 2. Acute effects of morphine on concentrations of 3-methoxytyramine (3-MT) and homovanillic acid (HVA) in the nucleus accumbens of AA and ANA rats pretreated repeatedly with saline (SAL) or morphine (MO).

Rats received saline or morphine (1 mg/kg) s.c. 1 h before death and were pretreated with either saline or morphine (1 mg/kg) s.c. for 3 days. Results are means ± SEM (n = 8–10). †P < 0.05 and ††P < 0.01, in comparison with corresponding saline group (ANOVA followed by Tukey’s compromise test). □ Acute saline; ■ acute morphine.

Fig. 3. Acute effects of morphine on concentrations of 3-methoxytyramine (3-MT) and homovanillic acid (HVA) in the olfactory tubercle of AA and ANA rats pretreated repeatedly with saline (SAL) or morphine (MO).

Rats received saline or morphine (1 mg/kg) s.c. 1 h before death and were pretreated with either saline or morphine (1 mg/kg) s.c. for 3 days. Results are means ± SEM (n = 8–10). †P < 0.05 and ††P < 0.01, in comparison with corresponding saline group (ANOVA followed by Tukey’s compromise test). □ Acute saline; ■ acute morphine.
Also in the present study, acute morphine elevated the concentrations of 3-MT by 43% and 21% and the concentrations of HVA by 49% and 19% in the caudate-putamen of AA and ANA rats, respectively; these effects being significant only in AA rats. Thus, it seems that morphine’s effects on DA metabolism differ in the caudate-putamen but not in the limbic areas between the two rat lines.

We have previously found that, in contrast to ANA rats, AA rats become sensitized to the horizontal locomotor stimulatory effect of morphine after a 4-day treatment at the dose used in the present study (Honkanen et al., 1999b). In the present study, no significant sensitization of the response of DA release or metabolism to morphine in any of the brain areas studied was seen in rats of either line, despite the fact that the drug administration was paired to a distinct context which should increase the probability of sensitization (Di Chiara, 1995). Thus, the difference in the morphine-induced behavioural sensitization between rats of these lines could not be linked to changes in the mesolimbic or nigrostriatal DA systems. Therefore, these results do not support the proposal that dopaminergic mechanisms are involved in the sensitization of the locomotor stimulation after repeated morphine treatment, at least as far as DA release or metabolism is concerned. However, it was recently shown that the effect of repeated morphine treatment on accumbal DA release may differ between the two subdivisions of the nucleus accumbens, the core and the shell (Cadoni and Di Chiara, 1999). Thus, DA release was context-independently sensitized only in the core of the nucleus accumbens after repeated morphine treatment, whereas, in the shell, tolerance developed to the morphine-induced DA release. In our study, the subdivisions of the nucleus accumbens could not be differentiated due to dissection technique limitations. It is thus possible that the sensitization of DA release in the core could be masked by a tolerance in the shell. Therefore, our results concerning overall sensitization in the nucleus accumbens should be interpreted with caution. On the other hand, sensitization of mesolimbic DA release appears to require higher doses and longer withdrawal periods from repeated morphine administration than those used in the present study (Kalivas and Stewart, 1991; Acquas and Di Chiara, 1992; Spanagel et al., 1993; Honkanen et al., 1994; Cadoni and Di Chiara, 1999).

Cerebral serotonergic mechanisms have been suggested to be involved in the regulation of alcohol consumption, locomotor activity and mesolimbic DA release (Benloucif et al., 1993; Geyer, 1996; Gillies et al., 1996; Mylecharane, 1996; Koob et al., 1998). In our study, acute administration of morphine increased 5-HT metabolism in the AA rats by ~20%, but clearly less so in the ANA rats. The elevation occurred especially in the olfactory tubercle, and may contribute to the stronger locomotor activity of the AA rats, as compared with ANA rats, after acute morphine administration. However, repeated morphine administration caused tolerance to, rather than sensitization in, the effects of morphine on tissue concentrations of 5-HT and 5-HIAA, and, thus, it seems that serotonergic mechanisms are not involved in the differences of the morphine-induced locomotor stimulation between AA and ANA rats after repeated administration.

In conclusion, with the methods used, we did not find any significant differences in the opioid regulation of the mesolimbic DA system that could contribute to differences in alcohol preference between AA and ANA rats or to differences in morphine-induced sensitization of locomotor activity found previously between rats of these lines. However, acute morphine administration tends to increase the metabolism of DA in the caudate-putamen, as well as that of 5-HT in the olfactory tubercle and caudate-putamen, more in the AA than in the ANA rats, which effects may contribute to the acute morphine’s larger stimulatory effect on locomotor activity in the AA, compared with ANA, rats.

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REFERENCES


