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Characterization of the decrease of extracellular striatal dopamine induced by intrastriatal morphine administration

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1 The effect of intrastriatally-administered morphine on striatal dopamine (DA) release was studied in freely moving rats. Morphine (1, 10 or 100 μM) was given into the striatum by reversed microdialysis, and concentrations of DA and its metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) were simultaneously measured from the striatal dialysates.

2 Intrastriatally-administered morphine significantly and dose-dependently decreased the extracellular concentration of DA, the concentrations of the acidic DA metabolites were only slightly decreased. The effect of morphine was antagonized by naltrexone (2.25 mg kg^{-1}, s.c.). Pretreatment with a preferential κ-opioid receptor antagonist, MR2266 [(−)-5,9 alpha-diethyl-2-(3-furylmethyl)-2′-hydroxy-6,7-benzomorphane; 1 mg kg^{-1}, s.c.], had no effect on the decrease of extracellular DA evoked by intrastriatal morphine (100 μM).

3 Intrastriatal administration of the selective μ-opioid receptor agonist [D-Ala^2,MePhe^4,Gly-ol^5] enkephalin (DAMGO; 1 μM), significantly decreased the extracellular concentration of DA in the striatum.

4 When the rats were given morphine repeatedly in increasing doses (10–25 mg kg^{-1}, s.c.) twice daily for 7 days and withdrawn for 48 h, the decrease of extracellular DA induced by morphine (100 μM) was significantly less than that seen in saline-treated controls.

5 Our results show that besides the well-known stimulatory effect there is a local inhibitory component in the action of morphine on striatal DA release in the terminal regions of nigrostriatal DA neurones. Tolerance develops to this inhibitory effect during repeated morphine treatment. Furthermore, our results suggest that the effect of intrastriatally-administered morphine is mediated by the μ-opioid receptors.

Keywords: Morphine; opioids; dopamine release; striatum; microdialysis; naltrexone; tolerance; MR2266; DAMGO; sensitization

Abbreviations: DA, dopamine; DAMGO, [D-Ala^2,MePhe^4,Gly-ol^5]enkephalin; DOPAC, 3,4-dihydroxyphenylacetic acid; HVA, homovanillic acid; MR2266, [(−)-5,9 alpha-diethyl-2-(3-furylmethyl)-2′-hydroxy-6,7-benzomorphane]; 3-MT, 3-methoxytyramine; MO, morphine; NTX, naltrexone; SAL, saline

Introduction

It is well known that systemic administration of opioids increases the synthesis, metabolism and release of dopamine (DA) in the striatum thereby increasing dopaminergic transmission. However, contrary to the increase of the acidic DA metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) we have repeatedly found that the *post-mortem* concentration of 3-methoxytyramine (3-MT), a metabolite considered to reflect the release of DA (for a review see Wood & Altar, 1988) tends to decrease in the striatum of rats or mice given relatively large doses of morphine (Ahtee *et al*., 1989; 1990; Airio & Ahtee, 1997). Morphine (Celsen & Kuschinsky, 1974; Kuschinsky *et al*., 1975; Marien *et al*., 1983; Westfall *et al*., 1983) and the selective μ-receptor agonist [D-Ala^2, MePhe^4, Gly-ol^5]enkephalin (DAMGO) (Widdowson & Holman, 1992) have been found to decrease the stimulated release of DA from striatal slices as well. Furthermore, there is preliminary evidence that intrastriatally-administered morphine decreases the release of striatal DA in freely moving rats (Rossetti *et al*., 1990). Thus, it appears that morphine has a dual effect on striatal DA release: an inhibitory effect in the terminal regions and a stimulatory effect in the somatodendritic regions of the nigrostriatal dopaminergic neurones. To investigate the inhibitory effect of morphine on DA release, we administered morphine directly into the striatum by reverse dialysis through a microdialysis probe and simultaneously measured the extracellular concentrations of DA and its metabolites DOPAC and HVA. Indeed, we found that intrastriatal morphine dose-dependently and in a naltrexone-reversible manner reduced the extracellular concentration of DA in the striatum indicating a reduced release of DA.

Morphine is a relatively selective agonist for the μ-opioid receptors, but it also has weak affinity for the rat κ-opioid receptors (Chen *et al*., 1993; Meng *et al*., 1993). κ-Opioid agonists have been consistently shown to decrease DA release in striatal tissue preparations (Mulder *et al*., 1984; 1991; Schoffelmeer *et al*., 1988). Thus, theoretically it is possible that the effect of morphine on striatal DA release is mediated through κ-opioid receptors, because naltrexone, which was used to show that the effect of morphine was mediated by opioid receptors, has high affinity to both μ- and κ-opioid receptors (Meng *et al*., 1993; Zastawny *et al*., 1994), cannot differentiate these receptor types. Therefore, we tested whether κ-opioid receptors are involved in this effect by using the relatively selective κ-opioid receptor antagonist, MR-2266 (Bhargava *et al*., 1989; Clark *et al*., 1989). To further verify the involvement of the μ-opioid receptors in this effect, we tested whether the selective μ-receptor agonist, DAMGO, is able to

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decrease the extracellular concentration of DA when given intrastriatally.

Initially, in rats large doses of morphine induce behavioural depression and catalepsy, which are followed by behavioural stimulation (Ahtee, 1974; Babbini & Davis, 1972; Vasko & Domino, 1978). During repeated administration, tolerance develops to the sedative and cataleptic effects of morphine and simultaneously, the stimulatory effects are potentiated and stereotypies occur with increasing frequency (Ahtee, 1974; Babbini & Davis, 1972). The stereotypies induced by morphine and other μ-opioids in rats treated repeatedly with opioids have been linked to the activation of the striatal dopaminergic system (Morelli *et al*., 1989). Indeed, we have shown that the effect of morphine on striatal DA transmission is augmented during withdrawal from chronic morphine treatment (Ahtee *et al*., 1989; Attila & Ahtee, 1984; Honkanen *et al*., 1994). Therefore, we investigated whether tolerance develops to the inhibitory component of morphine on striatal DA release, which in turn might contribute to the sensitization of DA release in the withdrawal from repeated opioid treatment.

Methods

Animals

Male Wistar rats weighing 250–350 g were used in the experiments. They were kept under a 12 h light/dark cycle (lights on at 06 00 h) at an ambient temperature of $24\pm2^{\circ}$C. Rat chow and tab water were available *ad libitum*. The animal experiments were approved by the local institutional animals 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'.

Microdialysis

An I-shaped microdialysis probe (Santiago & Westerink, 1990) was implanted into the rat striatum (A + 1.0, L + 3.0, D − 6.0; Paxinos & Watson, 1986) under pentobarbitone (60–80 mg kg^{-1}, i.p.) anaesthesia. The dialysis tube was prepared from a polyacrylonitrile/sodium metasulphonate copolymer (Filtral 20; o.d/i.d. 310 : 220 μm; Hospal, France). The exposed tip of the dialysis membrane was 4 mm. After the surgery, the rats were placed individually in test cages and allowed to recover for approximately 40 h. On the morning of the experimental day, the probe was connected *via* polyethylene tubing to a 1 ml microsyringe and Ringer solution (in mM: NaCl 147, $CaCl_2$ 1.2, KCl 2.7, $MgCl_2$ 1.0, ascorbic acid 0.04) was infused (2 μl min^{-1}) through the microdialysis probe with a microinjection pump. The samples (40 μl) were collected every 20 min. The basal output of DA and its metabolites was defined as an average of the first four stable samples after a stabilization period of 1 h, and was defined as 100%. The last baseline sample was collected at time point 0. It is the first sample used in the statistical analyses.

Study design

Immediately after the collection of the final baseline sample (time point 0), the intrastriatal perfusion medium was switched to a modified Ringer solution containing morphine (1, 10 or 100 μM) or DAMGO (0.3 or 1 μM). Control rats were perfused continuously with Ringer solution. The lag time for the Ringer solution containing morphine to reach the microdialysis probe in the brain through the inlet tubing was about 40 min. In addition, the outlet tubing introduced a lag time of about 20 min. Thus, the effects of morphine and DAMGO were first detected at time point 60 min. Indeed, the electrochemical signal induced by morphine in the microdialysis samples was detected first in the third microdialysis sample (time point 60 min).

To study the effects of opioid antagonists, the rats were administered subcutaneously either naltrexone (2.25 mg kg^{-1}) or MR2266 (1 mg kg^{-1}) immediately after the collection of the last baseline sample (time point 0). Control animals received corresponding volumes (2 ml kg^{-1}) of saline s.c. The effect of opioid antagonists are corrected for the lag time of 20 min (outlet tubing).

To study the effect of repeated morphine administration, the rats were given morphine s.c. twice daily (at 08.00 and 18.00 h) for 7 days according to the following schedule: day 1: 10 and 10 mg kg^{-1}; day 2: 15 and 10 mg kg^{-1}; day 3: 15 and 15 mg kg^{-1}; day 4: 20 and 15 mg kg^{-1}; day 5: 20 and 20 mg kg^{-1}; day 6: 25 and 20 mg kg^{-1}; day 7: 25 and 25 mg kg^{-1}. The control animals received saline s.c. using the same schedule. On the morning of day 8, a further dose of morphine (15 mg kg^{-1} to avoid respiratory depression during the surgery) was given. The rats were operated upon about 4 h after the last dose of morphine. The rats were thereafter withdrawn from repeated morphine or saline treatment for approximately 48 h, and then microdialysis samples were taken from the striatum and morphine (100 μM) was administered intrastriatally as described above.

Analytical procedure

Extracellular concentrations of DA and its metabolites DOPAC and HVA were measured by h.p.l.c. with electrochemical detection. Slightly different analytical procedures were used in the experiments with morphine and DAMGO, because relatively large concentrations of morphine in the perfusion fluid induce interferences with the analysis of DA when ordinary reverse phase columns are used.

Experiments with morphine Thirty μl and 5 μl of the dialysate sample were injected for the analysis of DA and DOPAC/

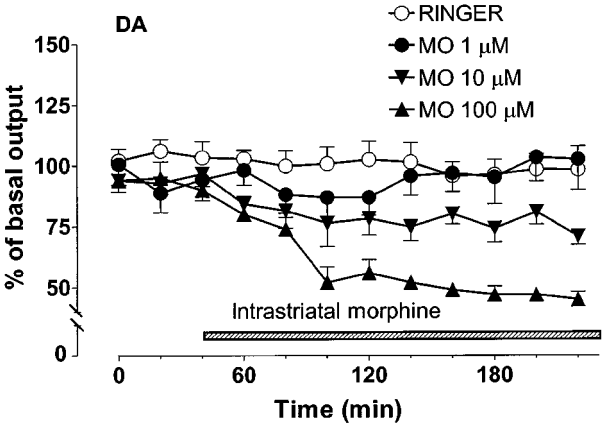

Figure 1 Effect of intrastriatal morphine (1, 10 or 100 μM) on the extracellular concentration of dopamine (DA) in the rat striatum. Morphine (MO) was adminstered intrastriatally *via* microdialysis probe instead of Ringer solution as shown by the shaded horizontal bar. All values are presented as percentages of the basal level ± s.e.mean ($n = 5$). Contrast analysis after analysis of variance for repeated measurements; 0–220 min: Morphine 100 μM vs Ringer $P < 0.001$.

HVA, respectively, with a CMA/200 autoinjector (CMA, Stockholm, Sweden). The system for determining DA consisted of an ESA Coulochem 5100A detector (ESA Inc., MA, U.S.A.) equipped with a model 5014A analytical cell and a Pharmacia LKB model 2150 h.p.l.c. pump (Pharmacia LKB, Sweden) with a SSI model 20-0225 pulse damper (Scientific Systems Inc., PA, U.S.A.). The separation and quantification of DA was performed as described by Lagerqvist (1991). The column (Nucleosil SA 5 μm, 20 cm, i.d. 4 mm) was kept at 45°C with a Bio-Rad column heater. The mobile phase was 15/85 (v v^{-1}) mixture of solutions A (citric acid 300 mM, NaOH 700 mM) and B (citric acid 75 mM, NaOH 175 mM, 30 v v^{-1} methanol) and contained 0.004% (w v^{-1}) EDTA. The flow rate of mobile phase was 0.6 ml min^{-1}.

The system for determining DOPAC and HVA consisted of a Waters 464 amperometric detector, a Beckman model 110B pump (Beckman Instruments Inc., U.S.A.) with a SSI model LP-21 pulse damper and a Spherisorb 5 μm 25 cm column. The mobile phase was prepared by mixing 0.1 M citric acid and 0.2 M Na_2HPO_4 in order to set the pH to 4.3. The mobile phase also contained 10% (v v^{-1}) methanol and EDTA (0.2 mM) and the flow rate was 1.2 ml min^{-1}.

Experiments with DAMGO Twenty μl of the dialysate sample were injected into the chromatographic system. The column (Spherisorb ODS2, 3 μm, 4.6 × 100 mm) was kept at 40°C with a column heater (Croco-Cil, France). The mobile phase consisted of 0.5 M NaH_2PO_4 buffer, pH 4.0 (adjusted with 1.0 mM citric acid), octane sulphonic acid (1–2 mM), 16% (v v^{-1}) methanol and 0.004% (w v^{-1}) EDTA. The flow rate of the mobile phase was 1.0 ml min^{-1}. DA was reduced with the amperometric detector (potential −80 mV) and DOPAC and HVA were oxidized with the coulometric detector (+300 mV).

In vitro *recovery of the probes*

In vitro recovery rates of DA, DOPAC and HVA through the membrane of the probe were determined by placing probes ($n=3$) in Ringer solution (22°C) containing 8 nM of DA and 400 nM of DOPAC and HVA. The probes were perfused with Ringer solution at a flow rate of 2 μl min^{-1}. After a stabilization period of 2 h, six consecutive 20 min samples were collected and assayed as described above (experiments with morphine), and their mean concentrations of DA, DOPAC and HVA were determined (concentrations inside the dialysis membrane). To estimate the concentrations of DA, DOPAC and HVA outside the dialysis membrane, the same Ringer solution to which the probes had been added was also assayed, and the recoveries were calculated as the ratio of concentrations inside and outside the dialysis membrane.

The recovery rate of morphine was not analysed in detail. However, the peaks in the chromatograms identified to be morphine were approximately 12% smaller in the dialysates from rats receiving 100 μM of morphine intrastriatally than in the Ringer solution containing 100 μM of morphine, suggesting that the *in vivo* recovery of morphine was about 12%. The recovery rate of DAMGO was not analysed.

Drugs

Intrastriatally-administered morphine hydrochloride (supplied by the University Pharmacy, Helsinki, Finland) or DAMGO (Bachem, Switzerland) were dissolved in the Ringer solution. Subcutaneously-administered morphine hydrochloride, naltrexone hydrochloride (Sigma, MO, U.S.A.) and MR2266 [(−)-5,9 alpha-diethyl-2-(3-furymethyl)-2′-hydroxy-6,7-benzomorphane, generously supplied by Boehringer Ingelheim KG, Germany] were dissolved in 0.9% NaCl solution (saline). Doses of morphine, naltrexone and MR2266 refer to the base form.

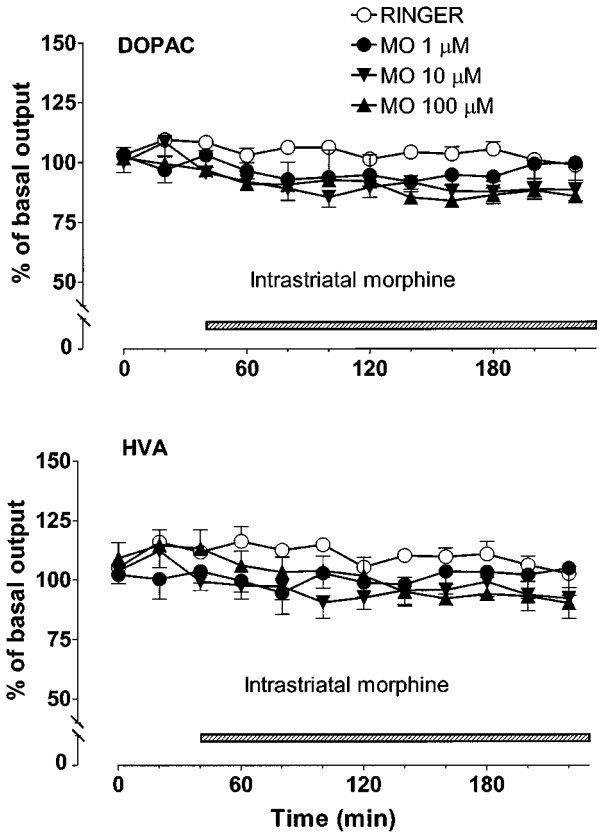

Figure 2 Effect of intrastriatal morphine (1, 10 or 100 μM) on the extracellular concentrations of DOPAC and HVA in the rat striatum. Morphine (MO) was administered intrastriatally *via* microdialysis probe instead of Ringer solution as shown by the shaded horizontal bar. All values are presented as percentages of the basal level ± s.e.mean ($n=5$).

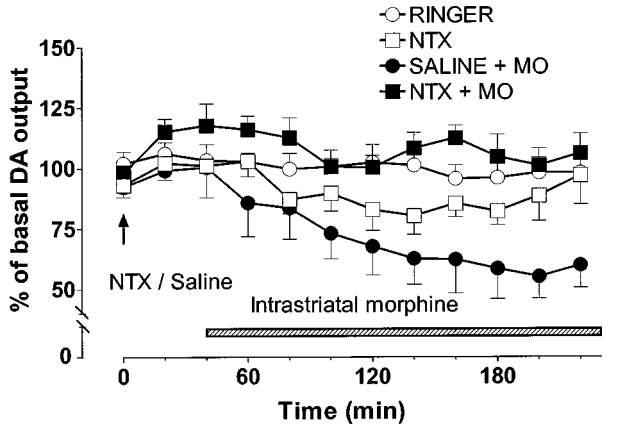

Figure 3 Effect of naltrexone (NTX, 2.25 mg kg^{-1}) alone or in combination with intrastriatal morphine (100 μM) on the extracellular concentration of dopamine (DA) in the rat striatum. Morphine (MO) was administered intrastriatally *via* microdialysis probe instead of Ringer solution as shown by the shaded horizontal bar. Naltrexone or saline was given at the time indicated by the arrow. All values are presented as percentages of the basal level ± s.e.mean ($n=5-6$). Contrast analysis after analysis of variance for repeated measurements; 0–220 min: Saline + morphine vs naltrexone + morphine $P<0.01$.

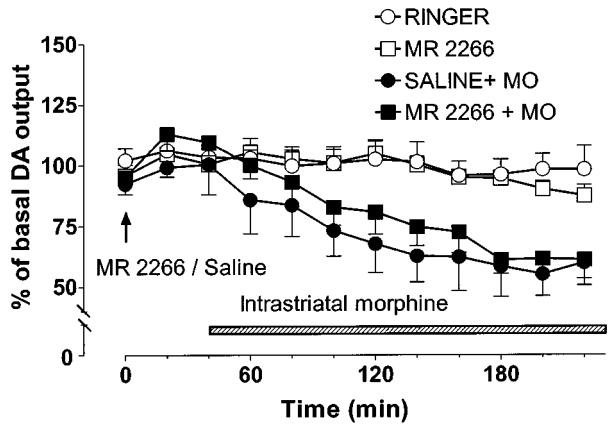

Figure 4 Effect of MR2266 (1 mg kg^{-1}) alone or in combination with intrastriatal morphine (100 μM) on the extracellular concentration of dopamine (DA) in the rat striatum. Morphine (MO) was administered intrastriatally via microdialysis probe instead of Ringer solution as shown by the shaded horizontal bar. MR2266 or saline was given at the time indicated by the arrow. All values are presented as percentages of the basal level $\pm$ s.e.mean ($n = 5-7$).

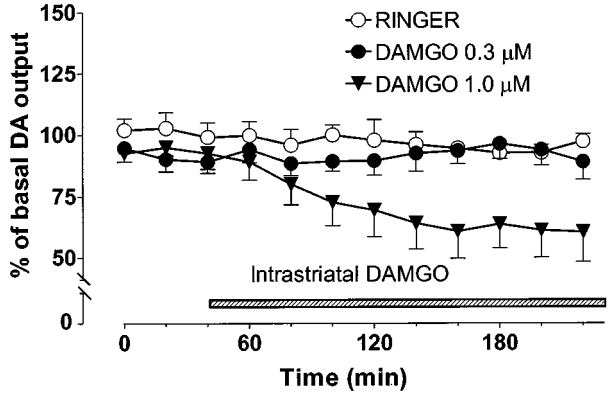

Figure 5 Effect of intrastriatal DAMGO (0.3 or 1 μM) on the extracellular concentration of dopamine (DA) in the rat striatum. DAMGO was administered intrastriatally *via* microdialysis probe instead of Ringer solution as shown by the shaded horizontal bar. All values are presented as percentages of the basal level $\pm$ s.e.mean ($n = 5-6$). Contrast analysis after analysis of variance for repeated measurements; 0–220 min: Ringer vs DAMGO 1 μM $P < 0.05$.

Statistics

Statistical analysis was carried out using one-way analysis of variance (ANOVA) for repeated measures (0–220 min). When appropriate, multiple comparisons were conducted using the contrast analysis with Bonferroni levels. P values < 0.05 were considered to be statistically significant. All results are presented as mean $\pm$ s.e.mean.

Results

The basal levels of DA, DOPAC and HVA in the striatal dialysates were 132.6 $\pm$ 7.2 fmol, 14.1 $\pm$ 0.8 pmol and 10.0 $\pm$ 0.5 pmol per 40 μl sample (in 20 min), respectively. *In vitro* experiments showed that the recovery rates of the probes for DA, DOPAC and HVA were 47.9 $\pm$ 2.5, 35.5 $\pm$ 2.3 and 28.5 $\pm$ 1.8%, respectively.

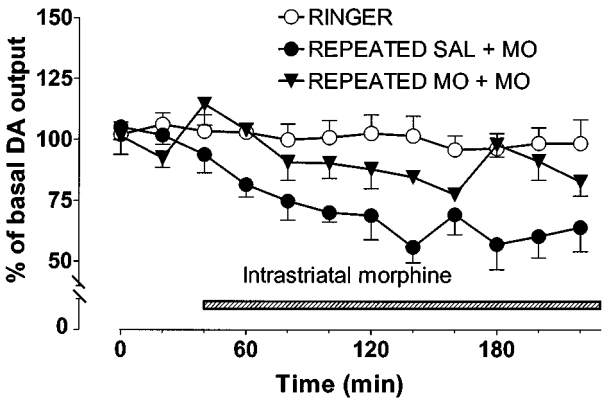

Figure 6 Effect of intrastriatal morphine (100 μM) on the extracellular concentration of dopamine (DA) in the striatum of rats withdrawn for 48 h from repeated saline (SAL) or morphine (10 to 25 mg kg^{-1}, s.c. twice daily for 7 days) treatment. Morphine (MO) was administered intrastriatally *via* microdialysis probe instead of Ringer solution as shown by the shaded horizontal bar. All values are presented as percentages of the basal level $\pm$ s.e.mean ($n = 5$). Contrast analysis after analysis of variance for repeated measurements; 0–220 min: Repeated saline + morphine vs repeated morphine + morphine $P < 0.05$.

Morphine dose-response

Intrastriatally-administered morphine dose-dependently decreased the extracellular concentration of DA in the striatum [Figure 1, Dose effect $F(3, 16) = 7.02$, $P < 0.0032$; Dose $\times$ Time interaction $F(3, 33) = 5.687$, $P < 0.0001$]. Multiple comparisons showed that only the largest dose (100 μM) of morphine significantly decreased ($P < 0.001$) the concentration of DA in the dialysates (maximally by about 50%) as compared with the control. The decrease induced by 10 μM of morphine (maximally by about 30%) was not statistically significant ($P < 0.1$) and 1 μM of morphine had no effect on the concentration of DA in the dialysates. The concentrations of DOPAC and HVA in the dialysates were not significantly decreased by morphine [Figure 2, Dose effect $F(3, 16) = 2.57$, $P < 0.1$; $F(3, 16) = 1.313$, $P > 0.3$ for DOPAC and HVA, respectively].

Effects of opioid antagonists

In rats pretreated with saline, intrastriatal morphine (100 μM) decreased the concentration of DA in the dialysate by about 40%. Pretreatment with naltrexone (2.25 mg kg^{-1}, s.c., 40 min before morphine) completely prevented the effect of morphine on extracellular DA [saline + morphine vs naltrexone + morphine $P < 0.01$, Figure 3]. The κ-opioid receptor antagonist, MR2266 (1 mg kg^{-1}, s.c. 40 min before morphine), had no significant effect on the morphine-induced decrease of extracellular DA (Figure 4). Administration of naltrexone or MR2266 alone had no significant effect on striatal extracellular DA concentration.

Effects of DAMGO

Intrastriatally-administered DAMGO decreased the extracellular concentration of DA in the striatum [Figure 5, Dose effect $F(2, 14) = 4.25$, $P < 0.0362$; Dose $\times$ Time interaction $F(2, 22) = 3.581$, $P < 0.0006$]. The larger dose (1 μM) of DAMGO significantly decreased ($P < 0.05$) the concentration of DA in the dialysates (maximally by about 40%) as compared with the control. The smaller dose (0.3 μM) of DAMGO had no effect

on the concentration of DA in the dialysates. The larger dose of DAMGO slightly (by about 15%), but not significantly decreased the output of DOPAC, the output of HVA was not altered (data not shown).

Effects of repeated morphine administration

In rats treated repeatedly with saline (twice daily for 7 days), intrastriatal morphine (100 μM) decreased the output of DA by about 40% (Figure 6). In the rats withdrawn for 2 days from repeated morphine treatment (20–50 mg kg^{-1} daily for 7 days, see Methods), the reduction of DA levels evoked by intrastriatal morphine (100 μM) was significantly smaller (maximally by about 20%) than in the saline treated controls given morphine intrastriatally (repeated saline + morphine vs repeated morphine + morphine $P<0.05$).

Discussion

Although the main effect of opioids is to enhance nigrostriatal dopaminergic transmission (Ahtee, 1974; Ahtee & Attila, 1987; Di Chiara & Imperato, 1988; Kuschinsky & Hornykiewicz, 1974), there are indications (see Introduction) that opioids may also have inhibitory effects on this system. Some previous studies showed that acute systemic administration of opioids did not increase the release of nigrostriatal DA (Ahtee *et al*., 1989; 1990; Wood & Rao, 1991; Wood & Richard, 1982; Wood *et al*., 1980; Yonehara & Clouet, 1984). Also, in studies in which DA release was measured from striatal slices, it has been shown that μ-opioids somewhat retard the release of DA (Celsen & Kuschinsky, 1974; Kuschinsky *et al*., 1975; Loh *et al*., 1976; Schlosser *et al*., 1995; Widdowson & Holman, 1992). The results of the present study confirm that there is an inhibitory, naltrexone-sensitive component in the effect of opioids on striatal DA release. Furthermore, we found that tolerance develops to this inhibitory effect by repeated morphine administration.

Electrophysiological studies have shown that morphine increases the firing activity of nigrostriatal dopaminergic neurones (Gysling & Wang, 1983; Iwatsubo & Clouet, 1977; Matthews & German, 1984; Ostrowski & Caggiula, 1991). However, as discussed above, μ-opioids when administered locally seem to decrease striatal DA release in spite of the increased activity of dopaminergic neurones. When morphine is given systemically the predominant effect is enhanced release of DA, as the increased firing activity induced by a μ-opioid receptor mediated input at the dopaminergic cell somas can overcome this presynaptic 'clamp'. However, when morphine is given directly into the striatum, the inhibitory effects predominate and the net effect on DA release is inhibition, as seen in our study. Indeed, Rossetti *et al*., (1990) reported that intrastriatally-administered opioid antagonist naloxone enhanced the effect of systemically administered morphine on striatal DA release.

In our study the output of the acidic DA metabolites, DOPAC and HVA, was not decreased by intrastriatal morphine or DAMGO. This could simply result from the fact that the decreased activity of dopaminergic neurones evoked by intrastriatal morphine increases the rate of DA synthesis (Roth *et al*., 1976; Walters & Roth, 1974). This increase in synthesis could lead to elevation of DA metabolites, DOPAC and HVA, in the dialysate. Indeed, local striatal administration of morphine increases the tissue concentration of DOPAC in the striatum (Moroni *et al*., 1979; Wood & Richards, 1982).

It is well documented that repeated administration of opioids results in development of both neurochemical and behavioural sensitization. This sensitization is more apparent in the mesolimbic dopaminergic system (for a review see Kalivas & Stewart, 1991), but the nigrostriatal system also sensitizes during repeated morphine treatment. Thus, during withdrawal from repeated morphine administration, acute challenge with morphine increases the release of DA considerably more than in naïve animals (Ahtee, 1974; Attila & Ahtee, 1984; Honkanen *et al*., 1994). Also behavioural effects of opioids linked to the activation of the nigrostriatal dopaminergic system undergo sensitization during repeated morphine administration (Ahtee, 1974; Babbini & Davis, 1972). However, the sensitization in these two systems seems to develop differently; at a molecular level, the alterations induced by repeated opioid administration are strikingly different in the somatodendritic regions of nigrostriatal and mesolimbic dopaminergic systems (Beitner-Johnson *et al*., 1992; Beitner-Johnson & Nestler, 1991). In addition, mesolimbic dopaminergic transmission can be sensitized by repeated administration of opioids in the somatodendritic region of this system (Vezina *et al*., 1987), whereas no such effect has been shown to occur in the nigrostriatal system. While we have now shown that tolerance develops to the inhibitory effect of morphine on nigrostriatal DA release, it is possible that the sensitization of this system results from the disappearance of the inhibitory component of morphine on DA release.

As relatively high concentrations of morphine were needed to decrease the release of DA in the striatum, it is possible that opioid receptor types other than μ are involved in this effect. δ-Opioid receptors are not likely to be involved since they have been shown to either increase striatal DA release *in vivo* (Dourmap & Costentin, 1994; Dourmap *et al*., 1992; Pentney & Gratton, 1991) and *in vitro* (Chesselet *et al*., 1982; Lubetzki *et al*., 1982; Widdowson & Holman, 1992), or to have no effect on striatal DA release (Mulder *et al*., 1984; Schoffelmeer *et al*., 1988). To our knowledge, there is only one study where δ-opioids were found to inhibit the release of striatal DA *in vitro* (Schlosser *et al*., 1995). Furthermore, the effect of intrastriatal morphine on striatal DA release was readily antagonized by naltrexone, the affinity of which for the cloned δ-opioid receptors is very poor (Yasuda *et al*., 1993). In contrast, the activation of κ-opioid receptors has consistently been shown to inhibit the release of striatal DA both *in vivo* (Di Chiara & Imperato, 1988) and *in vitro* (Mulder *et al*., 1984; 1991; Schoffelmeer *et al*., 1988). However, the fact that the inhibitory effect of morphine on DA release was not antagonized by the relatively selective κ-opioid antagonist, MR2266, does not support the involvement of κ-opioid receptors in this effect. Finally, we have now shown that similarly to morphine the selective μ-receptor agonist, DAMGO, when given intrastriatally decreases the release of DA in the striatum. This finding combined with the fact that the affinity of morphine for the cloned δ- and κ-opioid receptors is poor as compared with that for the μ-opioid receptors (Fukuda *et al*., 1993; Minami & Satoh, 1995; Raynor *et al*., 1994) seems to rule out the involvement of δ- and κ-opioid receptors in this effect of morphine.

The mechanism by which morphine locally inhibits the release of DA in the striatum remains obscure. The most straightforward explanation for this effect is that there are μ-opioid receptors on the nerve terminals of nigrostriatal dopaminergic neurones that mediate the inhibition of DA release. However, the inhibitory effect of μ-opioids on nigrostriatal DA release seems to be indirect involving other neurotransmitters locally in the striatum. Thus, recent studies

have indicated that μ-opioid receptors are not located in the terminals of nigrostriatal dopaminergic neurones (Smith *et al*., 1993; Trovero *et al*., 1990; Waksman *et al*., 1987), although selective lesions of nigrostriatal dopaminergic neurones induce considerable loss (about 30%) of μ-opioid binding sites in the striatum (Bodnar *et al*., 1988; Eghbali *et al*., 1987; Smith *et al*., 1993). Striatal dopamine release is under the regulatory control of multiple excitatory and inhibitory neurotransmitters including glutamate, acetylcholine, dynorphins, and GABA (Cheramy *et al*., 1990; Ronken *et al*., 1993), and morphine might either activate the inhibitory mechanisms or inhibit excitatory mechanisms controlling DA release within the striatum. μ-Opioids have, indeed, been shown to presynaptically inhibit excitatory postsynaptic potentials, identified to be glutamatergic, in the striatum (Jiang & North, 1992). The involvement of indirect mechanisms in this effect is also supported by the fact that in none of the studies using striatal synaptosomes, which do not contain interneurones, was any effect of μ-opioids on DA release reported (Bosse & Kuschinsky, 1978; Clouet & Williams, 1974; Das *et al*., 1994; Kruk & Zarrindast, 1978; Ronken *et al*., 1993).

In conclusion, it appears that systemically-administered morphine simultaneously increases the release of striatal DA by activating dopaminergic neurones in the substantia nigra and presynaptically inhibits the release of DA in the striatum. When opioids are given systemically, their stimulatory effects on DA release predominate, but when μ-opioids are given directly to terminal areas of these neurones, it is the inhibitory effects that are predominant. Tolerance develops to the inhibitory effect of morphine on DA release, which might contribute to the sensitization of nigrostriatal dopaminergic transmission during repeated morphine administration.

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