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Role of Cerebral Dopamine and Noradrenaline in the Morphine-induced Locomotor Sensitisation in Mice

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AIRIO, J. AND L. AHTEE. *Role of cerebral dopamine and noradrenaline in the morphine-induced locomotor sensitisation in mice.* PHARMACOL BIOCHEM BEHAV **58**(2) 379–386, 1997.—The effects of morphine on locomotor activity and cerebral dopamine (DA) and noradrenaline (NA) metabolism were studied in mice treated repeatedly with morphine for 5 days followed by 1, 3, or 5 days of withdrawal. Acute morphine treatment did not increase the locomotor activity of mice withdrawn for 1 day, after withdrawal for 3 days the increase was similar to that in controls, and after 5 days the increase was clearly larger than in controls. In mice withdrawn for 3 or 5 days, but not in control mice, acute morphine significantly elevated striatal 3,4-dihydroxyphenylacetic acid and homovanillic acid concentrations. Acute morphine challenge decreased striatal 3-methoxytyramine in control mice, but did not alter it in mice withdrawn for 3 or 5 days. In mice withdrawn for 3 days acute morphine increased the free 3-methoxy-4-hydroxyphenylethylene glycol in all brain areas studied clearly less than in controls, whereas in mice withdrawn for 5 days the tolerance was found only in the hypothalamus. Our results show that the morphine-induced locomotor hyperactivity is enhanced in mice after sufficiently long withdrawal, when mice are sensitised to the acute morphine-induced increase of DA turnover but the tolerance to morphine's effects on cerebral NA is disappearing, suggesting that in mice the cerebral NAergic systems, in addition to the DAergic ones, are major determinants of the behavioural response to morphine. © 1997 Elsevier Science Inc.

Morphine challenge Morphine withdrawal Locomotor activity Striatal dopamine release Brain noradrenaline release

IN most mouse strains the predominant behavioural effect of acute morphine treatment is locomotor hyperactivity (16,17,42), to which tolerance develops during chronic morphine treatment (38,41,43). However, there is some evidence that after sufficiently long chronic morphine treatment and withdrawal, mice are sensitised to the acute morphine-induced locomotor hyperactivity (23,37). In contrast to mice, in rats acute morphine, especially at larger doses, induces sedation and catalepsy, which, however, disappear after chronic morphine administration whereas motor stimulation becomes progressively more pronounced (3,15,24,29).

The limbic and striatal dopamine (DA) neurons are implicated in the locomotor stimulant effects of morphine and other opioids in rats (31–33,48), although it is recognized that part of the opioid-induced motor stimulation is DA-independent (34). In rats on withdrawal from opioids, acute opioid challenge accelerates striatal and limbic DA turnover and release even more than in naive controls (1,2,4,5,10,12,19). The effects of morphine on striatal DA metabolism are about similar in mice and rats, and withdrawal from repeated morphine sensitises also the mice to morphine-induced increase of striatal DA turnover (6,8,13).

However, the effects of morphine on cerebral noradrenaline (NA) metabolism differ in these two species. In rats, acute morphine elevates sulphated 3-methoxy-4-hydroxyphenylethylene glycol (MOPEG) concentrations in most brain areas and accelerates the α-methyl-*p*-tyrosine (αMT)-induced NA depletion in the lower brain stem but retards it in the cortex (5,9–11,50); tolerance develops only to the NA turnover-enhancing effects of morphine (5,10,11,44). In mice, acute

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morphine increases the free MOPEG concentrations as well as accelerates the αMT-induced NA depletion in all brain areas, and tolerance develops towards these effects (7,13,22, 36,47). Thus, differences in cerebral NAergic systems could be responsible for the different effects of morphine on locomotor activity in mice and rats. Indeed, in mice, the morphine-induced changes in locomotor activity have been ascribed also to cerebral NA (14,21,36,40). However, there are no studies in which the locomotor activity of mice treated acutely and chronically with morphine has been correlated with the changes in either cerebral DA or NA metabolism.

Thus, to elucidate the roles of DAergic and NAergic systems in the morphine-induced behavioural sensitisation in mice, we studied the temporal coupling of the effects of morphine withdrawal and challenge on cerebral DA and NA and on locomotor activity in mice. To estimate DA turnover and release, we measured the striatal concentrations of DA and its metabolites 3-methoxytyramine (3-MT), 3,4-dihydroxyphenylacetic acid (DOPAC), and homovanillic acid (HVA). 3-MT is generated extraneuronally by catecholoxymethyltransferase subsequent to DA release and is used as an index of DA release (35,53). DOPAC is formed by monoamine oxidase mainly intraneuronally and is used as an index of intraneuronal DA synthesis and metabolism. HVA is formed by both of these enzymes and is considered to indicate the sum of DA synthesis, metabolism, and release (51,52). We also measured the concentrations of NA and free MOPEG, the main metabolite of NA and thus a functional index of cerebral NA turnover and release in mice (18,30,45), in various brain areas.

METHODS

Animals

Male NMRI mice weighing 21–30 g at the beginning of the experiments were housed 9 or 10 to a cage at an ambient temperature of 21–23°C under a 12 L:12 D cycle (lights on at 0600 h). The mice had free access to fresh tap water and standard pellet food. They were weighed daily at 0800–0900 h. At the beginning of repeated treatment, the mean weight ± SEM of the control mice was 25.8 ± 0.2 g (n = 120) and that of the morphine-treated mice was 26.3 ± 0.2 g (n = 124).

Drugs and Drug Treatment

Isotonic morphine HCl (*Ph. Eur.*, 2nd ed.) solutions were given subcutaneously (SC) in a volume of 0.1 ml/g. All doses refer to the free base. During the repeated treatment (6), the mice were given morphine three times daily (at 0800–0900 h, 1400–1500 h, and 2200–2300 h) for 5 days. The dose of morphine was increased from 100 mg/kg × 3 on day 1 to 150 mg/kg × 3 on day 2 to 200 mg/kg × 3 on days 3–5. On day 5, the third daily morphine dose was omitted. The control mice were given similar volumes of 0.9% NaCl solution SC. After withdrawal periods of 1, 3, or 5 days, the acute treatments (morphine, saline) were given in both the behavioural and biochemical experiments between 1100 and 1400 h. The dose of acute morphine was 10 mg/kg. In the biochemical experiments, the mice were killed by decapitation between 1200 and 1500 h.

Dissection of the Brain

After decapitation, the brains were rapidly excised and dissected on an ice-cooled glass plate into four parts: a) striatum, b) hypothalamus, c) lower brain stem, and d) area designated "rest of forebrain + midbrain," consisting mainly of cortical areas, hippocampus, and thalamus, as described earlier (13). The brain parts were frozen on dry ice immediately after dissection, weighed, and stored at −80°C until the concentrations of DA, NA, or their metabolites were estimated.

After decapitation, DA is released and metabolised rapidly. In rats, postmortem 3-MT content is increased particularly quickly, and therefore microwave irradiation is the preferred method of sacrifice (51). However, in mouse striatum, the steady-state content of 3-MT is several times larger and the turnover as well as the postmortem formation of 3-MT clearly slower than in rats (27,54). Thus, microwave irradiation is not necessary in studies of mouse striatal 3-MT content, provided that the dissection time is constant and short enough. In the present experiments, the striata were dissected and frozen within 1 min 30 s to 2 min after decapitation, when the postmortem elevation of 3-MT concentration is about 20% (27,54).

Assays

Concentrations of DA and its metabolites DOPAC, 3-MT, and HVA, as well as NA and its metabolite free MOPEG were estimated as described by Haikala (28). Briefly, the proteins from the tissue samples were precipitated by perchloric acid. The samples were then purified and isolated using Sephadex G-10 gel chromatography. The fractions of the Sephadex G-10 eluates were injected onto C18 reversed-phase chromatographic columns. The detector used consisted of an amperometric rotating disc working electrode (28); in some of the experiments, an ESA Coulochem model 5100 or model II detector was used.

Locomotor Activity

After 1, 3, or 5 days of withdrawal, the mice were given a challenge dose of morphine (10 mg/kg) or saline SC and were placed in groups of three mice (n = 4–9 groups) in cages 18 × 33 × 15 cm. Groups of three mice, taken from the same home cage, were used in order to not affect the social behaviour of the animals. Interruptions of photocell beams (40 photocells per cage) were registered by a computerized counter. Locomotor activity was measured immediately after morphine or saline administration at 5-min intervals for 2 h between 1100 and 1600 h. Each mouse was used only once. The magnitude and duration of the exploration phase, when compared with the onset and magnitude of the effect of acute morphine, was found to be small in our previous experiments (7). Therefore, mice were not habituated before measurement.

Statistics

A two-tailed Student's t-test was used when comparing the means of body weight changes. DA, NA, and their metabolite data were analysed by two-way analysis of variance (ANOVA) to estimate the effects of acute and repeated treatment (2 × 2 groups) and acute × repeated treatment interaction. The statistical tests were performed separately for 3- or 5-day withdrawal. When significant ($p < 0.05$) main effects (acute or repeated treatment) were found, the comparisons of the group means were performed using Student's t-test with pooled variance. In the locomotor activity experiments, ANOVA with repeated measures was used both for the overall effect and for the effects of acute and repeated treatments. A significant ($p < 0.001$) overall effect was found at each withdrawal time (1, 3, or 5 days). In addition, two-way ANOVA was used to estimate the effects of acute and re-

peated treatment (2 × 2 groups) and acute × repeated treatment interaction at each 15-min time point separately. When significant main effects (acute or repeated treatment) were found, comparisons of the group means at each time point were performed using Student's *t*-test with pooled variance. All ANOVAs were calculated using SuperAnova or BMDP Statistical Software.

RESULTS

Body Weight during Treatment and Withdrawal

During the 5-day repeated treatment, the control mice given three daily saline injections gained 2.9 ± 0.3 g (mean ± SEM, $n = 120$), whereas the mice repeatedly given morphine lost 3.2 ± 0.1 g ($n = 123$, $p < 0.001$). During the first withdrawal day, the control mice continued to gain weight, gaining 0.5 ± 0.1 g ($n = 120$), whereas the morphine-withdrawn mice lost 0.3 ± 0.1 g ($n = 122$, $p < 0.001$), indicating physical dependence (49). After longer withdrawal periods (2–5 days), no further loss of weight occurred and the morphine-withdrawn mice gained more weight than the corresponding control mice ($p < 0.05$–0.001; data not shown).

Locomotor Activity

Withdrawal. The locomotor activity of mice withdrawn for 1 day from the 5-day repeated morphine treatment was slightly decreased. Thus, during the first hour of measurement, the cumulative locomotor activity counts of mice treated repeatedly with morphine and acutely with saline were smaller than those of control mice treated repeatedly and acutely with saline (Fig. 1; $p < 0.01$ at 15, 30, and 45 min; $p < 0.05$ at 60 min). Repeated-measures ANOVA also showed a significant difference [$F(1, 16) = 11.22$, $p < 0.001$]. Locomotor activity of the mice was not altered after 3 or 5 days of withdrawal from the repeated morphine treatment (Fig. 1).

Morphine challenge. In control mice, the acute morphine challenge dose of 10 mg/kg increased locomotor activity [Fig. 1; 1-day: $F(1, 16) = 13.95$, $p < 0.01$; 3-day: $F = 16.49$, $p < 0.01$; 5-day: $F(1, 27) = 15.80$, $p < 0.001$; repeated-measures ANOVA]. At 120 min, the mean ± SEM cumulative locomotor activity counts in morphine-challenged control mice were 3347 ± 585 (1-day withdrawal), 3426 ± 131 (3-day withdrawal), and 4070 ± 646 (5-day withdrawal), being larger ($p < 0.001$) than the corresponding counts (1199 ± 41, 788 ± 108, and 755 ± 106, respectively) for control mice given saline acutely and repeatedly.

In mice withdrawn for 1 day, the morphine challenge dose did not increase locomotor activity at all. Thus, the locomotor activity of these mice was significantly smaller than that of control mice given the morphine challenge [Fig. 1; $F(1, 16) = 11.14$, $p < 0.001$; repeated-measures ANOVA]. Furthermore, in two-way ANOVA, a significant acute × repeated treatment interaction was found at 75 min [$F(1, 16) = 5.61$, $p < 0.05$], 90 min [$F(1, 16) = 7.90$, $p < 0.05$], 105 min [$F(1, 16) = 9.74$, $p < 0.01$], and 120 min [$F(1, 16) = 11.14$, $p < 0.01$] after acute morphine administration, indicating different responses to morphine challenge in control and morphine-withdrawn mice.

In mice withdrawn for 3 days, the morphine challenge dose increased the locomotor activity to a degree similar to that seen in corresponding controls treated repeatedly with saline (Fig. 1). Furthermore, no acute × repeated treatment interaction by two-way ANOVA was found at any time point, indicating similar responses to morphine challenge in control and morphine-withdrawn mice.

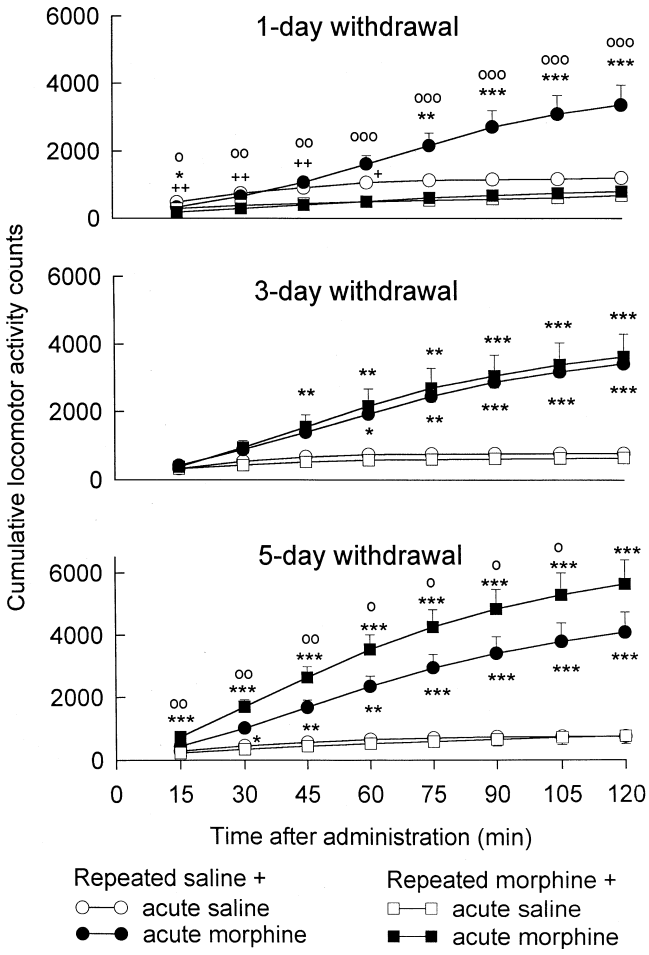

FIG. 1. Effect of acute morphine challenge (10 mg/kg SC) on locomotor activity of mice withdrawn for 1, 3, or 5 days from 5-day repeated morphine treatment. Each point gives the mean ± SEM cumulative locomotor activity counts of four to nine groups of three mice. Statistical significances for the effect of repeated morphine treatment: repeated morphine + acute saline group (□): vs. corresponding repeated saline + acute saline group (○): $^{+}p < 0.05$, $^{++}p < 0.01$, and repeated morphine + acute morphine (■) vs. repeated saline + acute morphine (●): $^{\circ}p < 0.05$, $^{\circ\circ}p < 0.01$, $^{\circ\circ\circ}p < 0.001$; and for the effect of acute morphine treatment: repeated saline + acute morphine vs. repeated saline + acute saline or repeated morphine + acute morphine vs. repeated morphine + acute saline: $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ (two-way ANOVA followed by Student's *t*-test with pooled variance at each time point).

In mice withdrawn for 5 days, the morphine challenge dose increased the locomotor activity clearly more than in control mice. Thus, the acute morphine-induced locomotor activity counts were higher in withdrawn mice than in corresponding controls [Fig. 1; $F(1, 27) = 6.03$, $p < 0.05$; repeated-measures ANOVA]. Furthermore, a significant acute × repeated treatment interaction was found by two-way ANOVA at 15 min [$F(1, 27) = 7.66$, $p < 0.01$], 30 min [$F(1, 27) = 6.48$, $p < 0.05$], and 45 min [$F(1, 27) = 5.02$, $p < 0.05$] after acute morphine administration, indicating that morphine challenge increased

the locomotor activity more in morphine withdrawn than in control mice.

Striatal DA

Withdrawal. In mice withdrawn for 3 or 5 days from repeated morphine treatment, the striatal DA, DOPAC, HVA, and 3-MT concentrations were not altered (Fig. 2, columns C vs. A).

Morphine challenge. The acute morphine challenge dose of 10 mg/kg did not alter the striatal DA concentrations in control or morphine-withdrawn mice (Fig. 2, columns B vs. A or D vs. C). In control mice, the morphine challenge tended to increase the striatal DOPAC (by 19–38%, NS, Fig. 2, columns B vs. A) and HVA (by 5–16%, NS) concentrations. Furthermore, acute morphine decreased the striatal 3-MT concentration (by 29–31%, $p < 0.01$–0.001).

In mice withdrawn from morphine for 3 or 5 days, the challenge dose significantly increased striatal DOPAC ($p < 0.05$, Fig. 2, columns D vs. C) and HVA ($p < 0.01$–0.001) concentrations. At 5-day withdrawal, a significant acute × repeated treatment interaction [$F(1, 56) = 4.40, p < 0.05$] was found by two-way ANOVA for HVA concentration, showing that morphine challenge increased striatal HVA concentration more in mice withdrawn from repeated morphine for 5 days than in corresponding control mice. In contrast to the result in control mice, morphine challenge did not significantly alter the striatal 3-MT concentration in withdrawn mice. Thus, the striatal 3-MT concentration was larger in mice withdrawn from morphine and treated acutely with morphine than in corresponding controls treated acutely with morphine ($p < 0.001$, Fig. 2, columns D vs. B). Furthermore, significant acute × repeated treatment interactions were found by two-way ANOVA for striatal 3-MT concentration at 3-day [$F(1, 52) = 6.91, p < 0.05$] and at 5-day [$F(1, 56) = 7.65, p < 0.01$] withdrawal, indicating that control and morphine-withdrawn mice responded differently to morphine challenge.

Cerebral NA

Withdrawal. Withdrawal from repeated morphine treatment for 3 or 5 days did not alter the NA or free MOPEG concentrations in the area "rest of forebrain + midbrain" or in the lower brain stem (Fig. 3, Fig. 4, columns C vs. A). Neither was the free MOPEG concentration in the hypothalamus altered at either day; however, the NA concentration was elevated at 3 days but not at 5 days after withdrawal ($p < 0.01$, Fig. 3, columns C vs. A).

Morphine challenge. The acute morphine challenge dose of 10 mg/kg did not alter the NA concentrations in any of the

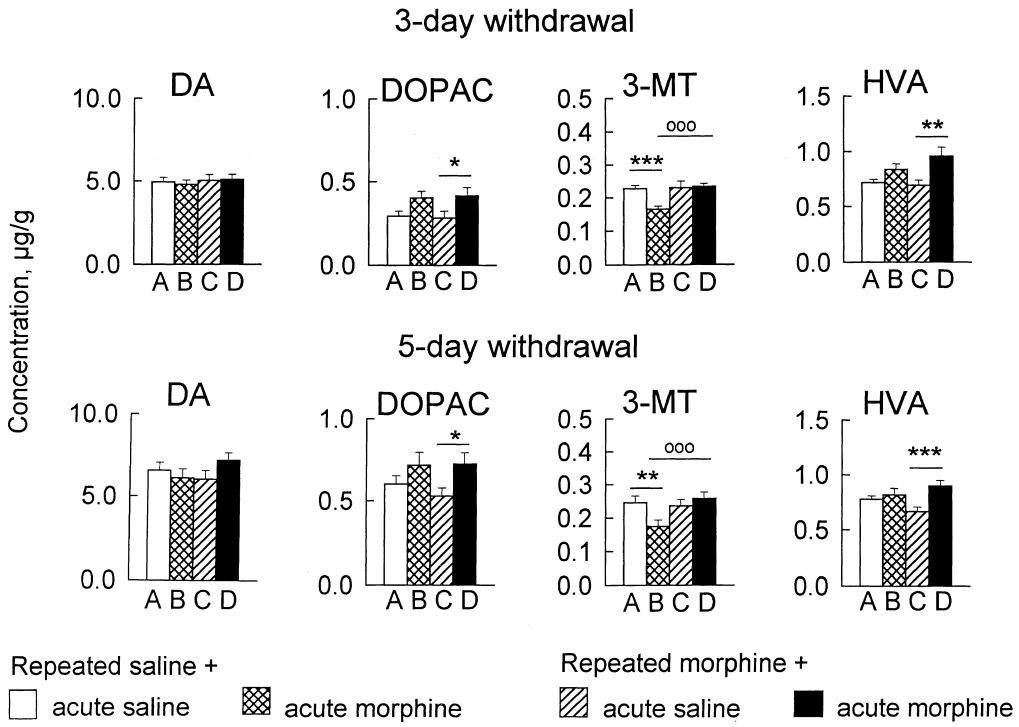

FIG. 2. Effect of acute morphine challenge dose (10 mg/kg SC, 1 h before decapitation) on striatal dopamine (DA), 3,4-dihydroxy-phenylacetic acid (DOPAC), 3-methoxytyramine (3-MT), and homo-vanillic acid (HVA) concentrations in mice withdrawn for 3 or 5 days after 5-day repeated morphine treatment. The columns give the means ± SEM (µg/g) and represent the following treatments: columns A (open), repeated saline + acute saline, n = 12–15; columns B (cross-hatched), repeated saline + acute morphine, n = 14–17; columns C (hatched), repeated morphine + acute saline, n = 12–15; and columns D (solid): repeated morphine + acute morphine, n = 14–16. Statistical significances for the effect of repeated morphine treatment: repeated morphine group vs. corresponding repeated saline group (columns D vs. B): $^{ooo}p < 0.001$; and for the effect of acute morphine treatment: acute morphine group vs. corresponding acute saline group (columns B vs. A or columns D vs. C): $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ (two-way ANOVA followed by Student's t-test with pooled variance).

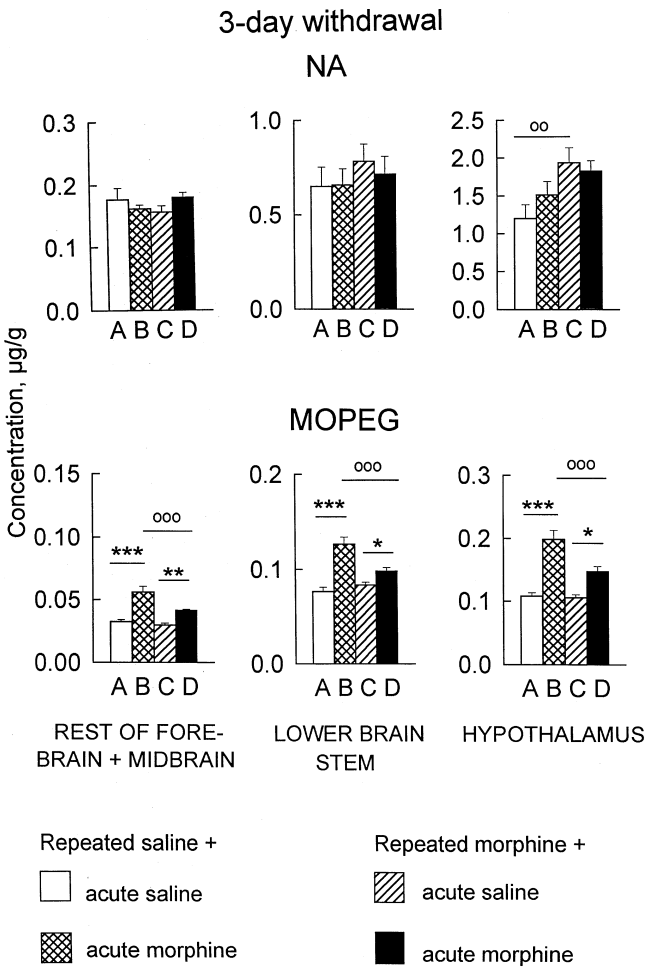

FIG. 3. Effect of acute morphine challenge dose (10 mg/kg SC, 1 h before decapitation) on noradrenaline (NA) and free 3-methoxy-4-hydroxyphenylethylene glycol (MOPEG) concentrations in the area "rest of forebrain + midbrain," lower brain stem, and hypothalamus in mice withdrawn for 3 days after 5-day repeated morphine treatment. The columns give the means ± SEM (n = 5–8) of NA and free MOPEG concentrations (µg/g) and represent the following treatments: columns A (open), repeated saline + acute saline; columns B (cross-hatched), repeated saline + acute morphine; col-umns C (hatched), repeated morphine + acute saline; and columns D (solid), repeated morphine + acute morphine. Statistical signif-icances for the effect of repeated treatment: repeated morphine group vs. corresponding repeated saline group (columns C vs. A or D vs. B): $^{oo}p < 0.01$, $^{ooo}p < 0.001$; and for the effect of acute treatment: acute morphine group vs. corresponding acute saline group (columns B vs. A or D vs. C): $*p < 0.05$, $**p < 0.01$, $***p < 0.001$ (two-way ANOVA followed by Student's t-test with pooled variance).

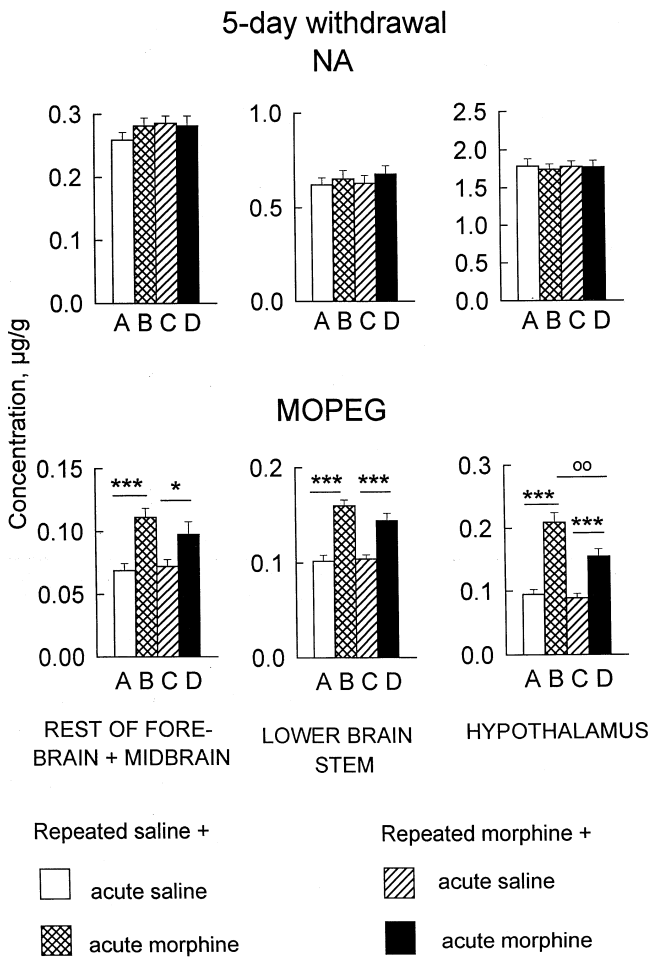

FIG. 4. Effect of acute morphine challenge dose (10 mg/kg SC, 1 h before decapitation) on noradrenaline (NA) and free 3-methoxy-4-hydroxyphenylethylene glycol (MOPEG) concentrations in the area "rest of forebrain + midbrain," hypothalamus, and lower brain stem in mice withdrawn for 5 days after 5-day repeated morphine treatment. The number of mice was 14 or 15. For other explanations, see legend for Fig. 3.

brain areas studied in either the control or the morphine-withdrawn mice (Fig. 3, Fig. 4, columns B vs. A or D vs. C).

The morphine challenge dose increased the free MOPEG concentration in the "rest of forebrain + midbrain" of the control mice ($p < 0.001$, Fig. 3, Fig. 4, columns B vs. A). Also, in mice withdrawn for 3 days, the morphine challenge dose increased the free MOPEG concentration ($p < 0.01$, Fig. 3, columns D vs. C) but clearly less than in corresponding control mice ($p < 0.001$, columns D vs. B). In mice withdrawn for 5 days, the morphine-induced increase of the free MOPEG concentration ($p < 0.05$, Fig. 4, columns D vs. C) did not significantly differ from that in the control mice (Fig. 4, columns D vs. B). Furthermore, a significant acute × repeated treatment interaction was found by two-way ANOVA for free MOPEG concentration at 3-day [$F(1, 24) = 4.92$, $p < 0.05$] but not at 5-day withdrawal, indicating that the free MOPEG concentration-increasing effect of acute morphine was reduced at 3 days but not at 5 days after withdrawal.

The morphine challenge dose increased the free MOPEG concentration in the lower brain stem of control mice ($p < 0.001$, Fig. 3, Fig. 4, columns B vs. A). In mice withdrawn for 3 days, the morphine challenge slightly increased the free MOPEG concentration ($p < 0.05$, Fig. 3, columns D vs. C), but clearly less than in corresponding control mice ($p < 0.001$, Fig. 3, columns D vs. B). In mice withdrawn for 5 days, the morphine challenge increased the free MOPEG concentration ($p < 0.001$, Fig. 4, columns Ds vs. C) to the same extent as in the corresponding control mice (Fig. 4, columns D vs. B). Furthermore, a significant acute × repeated treatment inter-

action was found by two-way ANOVA for free MOPEG concentration at 3-day [$F(1, 24) = 14.15$, $p < 0.01$] but not at 5-day withdrawal, indicating that the free MOPEG concentration-increasing effect of acute morphine was no longer reduced at 5 days after withdrawal.

The morphine challenge dose increased the free MOPEG concentration in the hypothalamus of control mice ($p < 0.001$, Fig. 3, Fig. 4, columns B vs. A). In mice withdrawn for 3 days or 5 days, the morphine challenge dose increased the free MOPEG concentration (3 days: $p < 0.05$, Fig. 3, columns D vs. C; 5 days: $p < 0.001$, Fig. 4, columns D vs. C), but less than in the corresponding control mice (3 days: $p < 0.001$, Fig. 3, columns D vs. B; 5 days: $p < 0.01$, Fig. 4, columns D vs. B). Furthermore, a significant acute × repeated treatment interaction was found by two-way ANOVA for free MOPEG concentration both at 3-day [$F(1, 22) = 8.21$, $p < 0.01$] and at 5-day [$F(1, 55) = 4.95$, $p < 0.05$] withdrawal, indicating that the hypothalamic free MOPEG concentration-increasing effect of acute morphine was reduced for at least 5 days after withdrawal of morphine treatment.

DISCUSSION

The present experiments show that mice are similarly sensitised to the acute morphine-induced increase of striatal DA release after 3 and 5 days of withdrawal. However, the mice were dissimilarly tolerant towards the cerebral NA turnover and release-increasing effect of acute morphine at 3-day and 5-day withdrawal, so we found tolerance in all brain areas studied at 3-day withdrawal but only in the hypothalamus at 5-day withdrawal. At 1-day withdrawal mice were tolerant to the acute morphine-induced locomotor hyperactivity, at 3-day withdrawal morphine increased their locomotor activity similarly to that of the control mice, but at 5-day withdrawal mice were sensitised to this effect.

In the present experiment, the acute 10-mg/kg challenge dose of morphine clearly increased the DOPAC and HVA concentrations in mice withdrawn for either 3 or 5 days from repeated morphine treatment but did not alter the 3-MT concentration, whereas in the control mice this dose tended to elevate DOPAC or HVA and even decreased 3-MT. As stated in the introduction, changes in 3-MT concentration reflect changes in DA release, and changes in HVA concentration reflect changes in DA turnover and release. Thus, the changes of 3-MT and HVA in the striata of morphine-withdrawn mice can be interpreted as enhanced turnover and release of DA. Furthermore, the present results agree with our earlier finding that after long enough repeated morphine treatment and withdrawal, mice (8), like rats (4,5,10,12), are sensitised to the acute morphine-induced increase of striatal DA turnover and release (as measured by estimating DA metabolites) as well as the αMT-induced DA depletion.

Previously it has been shown that the striatal DOPAC and HVA concentrations fall and the αMT-induced DA depletion is retarded in mice at 1-day but no longer at 3-day withdrawal from repeated morphine treatment (6,8,22). In agreement with these findings, in the present experiment neither 3- nor 5-day withdrawal from repeated morphine treatment altered the concentrations of striatal DA or its metabolites.

In the present experiment, the free MOPEG concentrations were not altered by morphine withdrawal in any brain area, whereas the hypothalamic NA concentration was slightly increased after 3-day withdrawal. In addition, we previously found that in mice withdrawn for 1 day from 5-day repeated morphine treatment, when the withdrawal-induced weight loss is at its peak, the changes in cerebral NA turnover were relatively modest (7). Furthermore, withdrawal from morphine does not alter the αMT-induced depletion of cerebral NA or the cortical MOPEG concentration in mice and even retards the synthesis of cerebral NA (22,25,47). Thus, we suggest that in mice the cerebral NAergic mechanisms play only a minor role in stressful situations like repeated morphine treatment and withdrawal. Furthermore, it is evident that morphine withdrawal-induced effects on cerebral NA in mice differ from those in rats, because it has been repeatedly shown that morphine withdrawal clearly increases NA turnover and release in several areas of the rat brain, including cortex, lower brain stem, and hypothalamus (5,10,11,20).

Previously we reported that in mice withdrawn for 1 day from 5 days of repeated morphine treatment, acute morphine did not elevate free MOPEG at all in any brain area studied (7). In the present experiments, acute morphine somewhat elevated free MOPEG concentration in mice withdrawn for 3 days, although there was a clear tolerance to the acute morphine-induced increase of free MOPEG concentration in all brain areas. In mice withdrawn for 5 days, the acute morphine-induced elevation of the free MOPEG concentration in the cortical areas and in the lower brain stem did not differ from that in controls, although in the hypothalamus of these mice there was still tolerance to the morphine-induced MOPEG elevation. These findings suggest that, in mice, tolerance to the cerebral NA turnover-increasing effect of acute morphine disappears within a few days, even after such a substantial treatment as was used in our study.

Acute morphine challenge did not induce locomotor hyperactivity in mice withdrawn for 1 day from repeated morphine treatment, increased it in mice withdrawn for 3 days to about same extent as in the controls, and increased the motility of mice clearly more than in the controls after 5-day withdrawal. Thus, a sensitisation of the locomotor activity-increasing effect of acute morphine could be seen at 5-day withdrawal. As in rats (see introduction), the cerebral DAergic neurons are considered to be important in the mediation of the morphine-induced locomotor activity in mice (17, 26,39,42), but in mice the morphine-induced increase of locomotor activity is also attributed to the stimulation of cerebral NAergic systems (see introduction). In the present experiments we found that mice were sensitised to the morphine-induced enhancement of striatal DA turnover and release to about similar degrees after withdrawal for 3 or 5 days. However, whereas after 3-day withdrawal a clear tolerance was found to the NA turnover-increasing effect of acute morphine, after 5-day withdrawal this tolerance was greatly reduced. Thus, it seems that when the tolerance to morphine's effects on cerebral NA has disappeared and when mice are sensitised to the striatal DA turnover- and release-increasing effects of acute morphine, acute treatment with morphine increases locomotor activity more in the withdrawn mice than in the control mice. The temporal coupling between morphine-induced changes in locomotor activity and in cerebral DA and NA suggests that the morphine-induced sensitisation of locomotor activity is expressed in mice first when they are no longer tolerant to the NA turnover-increasing effects of morphine.

As stated in the introduction, there are differences in the behavioural effects of morphine in mice and rats. Further, the effects of morphine on cerebral NAergic systems differ fundamentally between mice and rats. In contrast to mice, in the rat cortex, acute morphine does not elevate the MOPEG concentration and even retards the αMT-induced NA depletion (see introduction). Furthermore, as discussed above, it is evident

that morphine withdrawal-induced effects on cerebral NA turnover in mice differ from those in rats. On the other hand, the present results, together with earlier ones (6,8,13), suggest that the morphine-induced changes in striatal DA metabolism are about similar in mice and rats. Thus, we suggest that, as in rats (33), the sensitisation of morphine-induced hyperactivity in withdrawn mice is mediated by DAergic systems. However, in morphine-withdrawn mice this effect can be masked by tolerance of their cerebral NAergic systems to the actions of morphine. Only when the DAergic regulation of locomotor activity (basically similar in mice and rats) becomes unmasked do morphine-withdrawn mice (like rats) react to morphine challenge with locomotor hyperactivity. Thus, the differences in the NAergic systems may be responsible for the different effects of morphine on locomotor activity in these two species.

In conclusion, we found that mice are sensitised to morphine's effects on locomotor activity when the tolerance to its effects on NA turnover has disappeared and when, moreover, mice are sensitised to the striatal DA turnover- and release-increasing effects of acute morphine. Our findings support the view that in mice the cerebral NAergic systems, in addition to the DAergic ones, are major determinants of the locomotor response to morphine. Taken together, our results suggest that, in addition to the DAergic systems, the expression of the behavioural sensitisation in mice is determined by morphine-induced changes in NAergic systems. The morphine-induced behavioural sensitisation has been implicated in its reinforcing effects (46). Therefore, it cannot be excluded that the NAergic systems are also of importance in morphine-seeking behaviour.

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