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Dopamine Release and Metabolism in Nucleus Accumbens and Striatum of Morphine-Tolerant and Nontolerant Rats

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JOHNSON, D. W. AND S. D. GLICK. *Dopamine release and metabolism in nucleus accumbens and striatum of morphine-tolerant and nontolerant rats.* PHARMACOL BIOCHEM BEHAV 46(2) 341-347, 1993.—Morphine administered at high doses produces a biphasic locomotor effect, characterized by an initial locomotor depression, followed a short time later by hyperlocomotion. Prior exposure to morphine produces tolerance to the motor-depressive effects and sensitization to the motor-activating effects of morphine. Little is known of the neurochemical changes that occur to produce tolerance and sensitization to morphine. In the present study, we developed a morphine pretreatment regimen in rats that produced both tolerance and sensitization to a high (30 mg/kg) dose of morphine. Using in vivo microdialysis, we then measured dopamine (DA), dihydroxyphenylacetic acid (DOPAC), and homovanillic acid (HVA) release in the nucleus accumbens (NAC) and striatum (STR) in morphine- and saline-pretreated rats after acute morphine administration. In morphine-tolerant/sensitized rats, basal DA concentrations in the NAC were higher and levels of DOPAC and HVA in the NAC after acute morphine injection were greater compared to controls. These results suggest that the NAC, but not the STR, may be important in mediating tolerance and sensitization to opiates.

Locomotor activity Morphine Nucleus accumbens Striatum Microdialysis Tolerance Sensitization

IT is well known that low doses of systemically administered morphine in the rat produce an increase in locomotor activity, while high doses produce a biphasic locomotor effect, characterized by an initial decrease in locomotor activity, followed by hyperactivity later. Tolerance to the initial locomotor-depressant effect and sensitization to the later locomotor-excitatory effect occurs if high doses of morphine are administered repeatedly (2,4,25).

The mesolimbic and nigrostriatal dopamine (DA) systems, with cell bodies in the midbrain and axon projections to the nucleus accumbens (NAC) and striatum (STR), play an important role in mediating the locomotor effects of morphine (20,28). The firing frequency of mesolimbic neurons is generally enhanced in response to morphine (18,23), and morphine-induced locomotor activity is blocked by DA receptor antagonists (10); morphine also increases DA turnover in mesolimbic and nigrostriatal DA terminal fields, specifically the NAC and STR (19,27,29).

Little is known concerning the neural mechanisms underlying the phenomenon of tolerance. The development of the technique of in vivo brain microdialysis has made it possible to measure catecholamines and their metabolites in the neuronal extracellular environment of specific brain regions of awake and freely moving rats. Our laboratory previously reported the effects of low (5 mg/kg) and high (30 mg/kg) doses of morphine on the concentration of DA and DA metabolites in the STR and NAC of naive rats (16). The objectives of the present study were to develop a pretreatment regimen of morphine injections that would produce tolerance to a locomotor-inhibiting dose of morphine and, using in vivo microdialysis, subsequently measure changes in the concentrations of DA and DA metabolites in the extracellular environment of the NAC and STR of nontolerant and tolerant/sensitized rats.

METHOD

Animals and Pretreatment Regimens

Female Sprague-Dawley rats (250-275 g) were obtained from Charles River Laboratories (Wilmington, MA) and used in all experiments. Animals were housed in metal cages at 21-23°C and maintained on Purina rodent chow and tapwater ad lib. Lights were on between 8:00 a.m. and 8:00 p.m. Animals

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used for activity studies were housed together in treatment groups and animals used for microdialysis studies were housed individually after cannula implantation.

Motor Activity Monitoring

Animals used in the activity studies were pretreated with IP injections of either morphine sulfate (30 mg/kg, dissolved in saline, Mallinckrodt, Inc., St. Louis, MO) with dose expressed as the salt or saline (1 ml/kg). The schedule for pretreatment injections to produce tolerance was according to one of two paradigms: a) once daily for 4 days; or b) twice daily for 3 days, then once on day 4. Twice-daily injections were administered at least 8 h apart. On day 5, all rats were injected IP with 30 mg/kg morphine and immediately placed in photocell activity cages (7), and locomotor activity was measured for 3 h. The 30-mg/kg test dose of morphine used on day 5 was selected because we (17) have previously shown this dose to produce locomotor depression in rats for 2 h after injection, followed by locomotor hyperactivity in the third hour after injection. All experiments were conducted during the light phase of the rats' light/dark cycle in a quiet room.

Microdialysis Experiments

Under pentobarbital anesthesia, rats were implanted stereotaxically with guide cannulae in the left NAC and right STR. The coordinates for the guide cannula placement in the NAC were: rostral +1.6 mm from bregma, lateral +1.5 mm, and ventral −4.6 mm from the skull surface. For the STR, coordinates were: rostral +0.5 mm, lateral −2.9 mm, and ventral −3.0 mm (24). The cannulae were fixed firmly to the skull with four screws and dental cement. A "fence" of perforated aluminum (Small Parts, Inc., Miami, FL, J-PMA-062) was cemented around the perimeter of the implant area to protect the probes and provide a site of attachment later for the swivel tether.

Two days after surgery, rats were assigned to either morphine or saline pretreatment, and the second paradigm pretreatment injection schedule was used (described above). At least 5 h after receiving the final pretreatment injection on day 4, rats were lightly anesthetized with methohexital (6 mg/kg) and placed in a cylindrical Plexiglas testing chamber (30 cm diameter). A CMA/12 3-mm microdialysis probe (Bioanalytical Systems, West Lafayette, IN. Cat. 8309563) was lowered into each guide cannula. The tips of the probes were 4 mm lower than the tips of the guides. The probe inlets were connected to a liquid swivel that was tethered to the skull pedestal. All probes were calibrated at least 24 h prior to use in artificial cerebrospinal fluid (CSF) (25°C) containing 146 mM Na^+, 2.7 mM K^+, 155 mM Cl^-, 1.2 mM Ca^{++}, 1 mM Mg^{++}, and 1 μM ascorbic acid, degassed with argon. The CSF was delivered by a Harvard syringe pump (Harvard Apparatus, South Natick, MA) at a flow rate of 1 μl/min for calibrations and experiments. Probes were reused no more than three times, and recoveries for all compounds were in the 20–35% range.

All experiments were carried out between 9:00 a.m. and 4:00 p.m. the day after rats were placed in the Plexiglas testing chamber. During each experiment, the animal's movement was inhibited only by its cable connection from the skull pedestal to the swivel. The collection tubes, containing 2 μl 5 N perchloric acid solution, were placed in a holder on the swivel tether so the samples could be removed without disturbing the animal. Six 20-min fractions for each brain area were collected

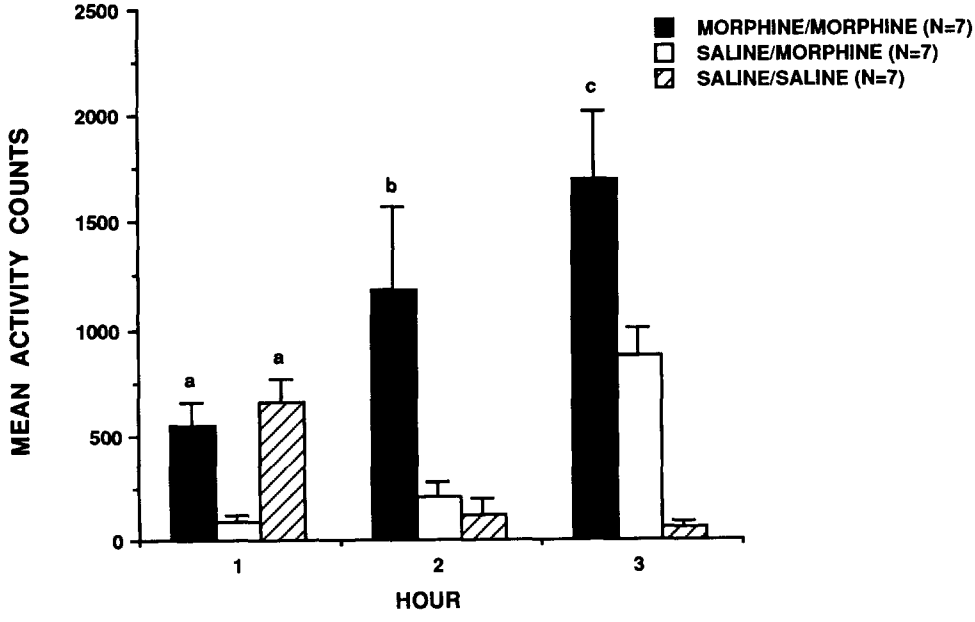

FIG. 1. Effect of morphine or saline pretreatment on mean (± SEM) activity counts at 1, 2, and 3 h after injection of morphine (30 mg/kg) or saline. Morphine/morphine, morphine pretreatment, 30 mg/kg, twice daily for 3 days, then once on day 4; morphine, 30 mg/kg, immediately prior to entering activity boxes on day 5; saline/morphine, saline pretreatment, 1 ml/kg, as above; saline/saline, saline pretreatment, 1 ml/kg, as above; saline, 1 ml/kg, immediately prior to entering activity boxes on day 5. All injections were administered IP. [a]Differs from saline/morphine ($p < 0.01$). [b]Different from other two groups ($p < 0.05$). [c]Different from other two groups ($p < 0.05$–0.001).

as baseline samples. At the end of the sixth baseline sample collection, all rats received an IP injection of 30 mg/kg morphine and collection of 20-min fractions continued for an additional 3 h. Cannula placements were confirmed at the end of each experiment by standard histological procedures.

Catecholamine Assay

Perfusate samples were measured using a Waters high-performance liquid chromatography (HPLC) system consisting of a refrigerated 712 WISP autosampler, 510 pump, and a 464 electrochemical detector. The column was a 3-μM reverse-phase column (Phase Separation Spherisorb Column S3 ODS2; 10 cm × 4.6 mm). The mobile phase consisted of 6.9 g/l sodium monobasic phosphate, 250 mg/l heptane sulfonic acid, 80 mg/l disodium EDTA, and 50 ml/l methanol, was adjusted to pH 3.6 with HCL, and pumped at a rate of 1.2 ml/min. A flow cell from the Waters 460 electrochemical detector was used in the 464 detector; it was set at a potential of +0.80 V vs. Ag/AgCl. Chromatograms were integrated, compared to standards, and analyzed using Waters Maxima Software. The approximate sensitivity limits of the assay with these detector settings and this chromatographic separation were 2–3 pg for DA, 5 pg for dihydroxyphenylacetic acid (DOPAC), and 10 pg for homovanillic acid (HVA).

Statistical Analysis

For the neurochemical data, a three-factor repeated-measures analysis of variance (ANOVA), with pretreatment as the major variable and time and brain region as repeated measures, was conducted to evaluate pretreatment and time effects across brain regions for each amine. A two-factor repeated-measures ANOVA was carried out on activity data and, where appropriate, paired or unpaired *t*-tests were calculated to test individual mean differences.

RESULTS

Activity Studies

Compared to saline-pretreated/saline-injected controls, rats pretreated with morphine (30 mg/kg) or saline only once daily for 4 days showed no tolerance in the first hour to the locomotor-depressive effects of a 30-mg/kg morphine injection administered just prior to entering activity boxes on day 5 ($p < 0.01$; data not shown). Mean activity counts in the third hour after morphine injections were the same for both saline- and morphine-pretreated rats; however, mean activity counts in the third hour for both these groups were higher than that of saline-pretreated/saline-injected controls ($p < 0.01$; data not shown). When the pretreatment paradigm was expanded to twice-daily injections of 30 mg/kg morphine or saline for 3 days, with a single injection on day 4, tolerance to the locomotor-depressive effects of a 30-mg/kg injection of morphine was evident in morphine-pretreated rats as compared to saline-pretreated rats in hour 1 ($p < 0.01$; Fig. 1); in addition, rats receiving morphine on day 5 that were pretreated with morphine were sensitized to the locomotor-activating effects of morphine in hours 2 ($p < 0.01$) and 3 ($p < 0.05$) compared to rats pretreated with saline and injected with morphine on day 5 (Fig. 1).

Effect of Morphine or Saline Pretreatment on Baseline DA, DOPAC, and HVA Levels in the Striatum and Nucleus Accumbens

Pretreatment with 30 mg/kg morphine using the tolerance/sensitization-producing paradigm above did not change baseline levels of DOPAC or HVA in either the STR or NAC on day 5 compared to saline-pretreated rats (Tables 1 and 2). Basal DA concentrations did not differ between groups in the STR, but in the NAC morphine pretreatment resulted in a baseline DA concentration more than double that measured in rats pretreated with saline (28.6 vs.12.8 nM; $p < 0.01$; Fig. 2). This did not occur in rats pretreated with 30 mg/kg morphine using the non-tolerance-producing regimen (saline vs. morphine; 8.1 vs.7.3 nM in STR; 7.4 vs.8.2 nM in NAC).

TABLE 1

BASAL CONCENTRATIONS OF DOPAC AND HVA IN THE STRIATUM OF RATS PRETREATED WITH EITHER MORPHINE OR SALINE

Compound	*n*	Pretreatment	Mean ± SE (μM)
DOPAC	13	Saline	5.9 ± 1.1
	12	Morphine	6.9 ± 1.2
HVA	13	Saline	5.0 ± 0.8
	12	Morphine	4.9 ± 0.8

Saline pretreatment, saline, 1 ml/kg, twice daily for 3 days, then once on day 4; morphine pretreatment, morphine pretreatment (30 mg/kg) twice daily for 3 days, then once on day 4. Basal means for both metabolites derived from three to six baseline samples from each rat.

Effect of Acute Morphine Injection on DA, DOPAC, and HVA Release in the STR and NAC in Rats Pretreated With Saline or Morphine

Rats pretreated with either saline or morphine and dialyzed on day 5 experienced a gradual increase in the % release (relative to baseline concentrations) of both DOPAC and HVA over a 2- to 3-h period in both the STR and NAC following acute injection of 30 mg/kg morphine ($p < 0.001$; Figs. 3 and 4). In the NAC, there was a significant pretreatment × time interaction ($p < 0.001$) for both DOPAC and HVA release after morphine injection (Fig. 4). There were no effects of acute morphine injection in either group on DA concentrations in either the STR or NAC (results not shown).

DISCUSSION

The biphasic locomotor response seen here after morphine (30 mg/kg) administration in rats pretreated with saline is a

TABLE 2

BASAL CONCENTRATIONS OF DOPAC AND HVA IN THE NUCLEUS ACCUMBENS OF RATS PRETREATED WITH EITHER MORPHINE OR SALINE

Compound	*n*	Pretreatment	Mean ± SE (μM)
DOPAC	12	Saline	4.0 ± 0.9
	12	Morphine	5.5 ± 1.0
HVA	13	Saline	1.8 ± 0.3
	12	Morphine	2.2 ± 0.4

Saline pretreatment, saline, 1 ml/kg, twice daily for 3 days, then once on day 4; morphine pretreatment, morphine pretreatment (30 mg/kg) twice daily for 3 days, then once on day 4. Basal means for both metabolites derived from three to six baseline samples from each rat.

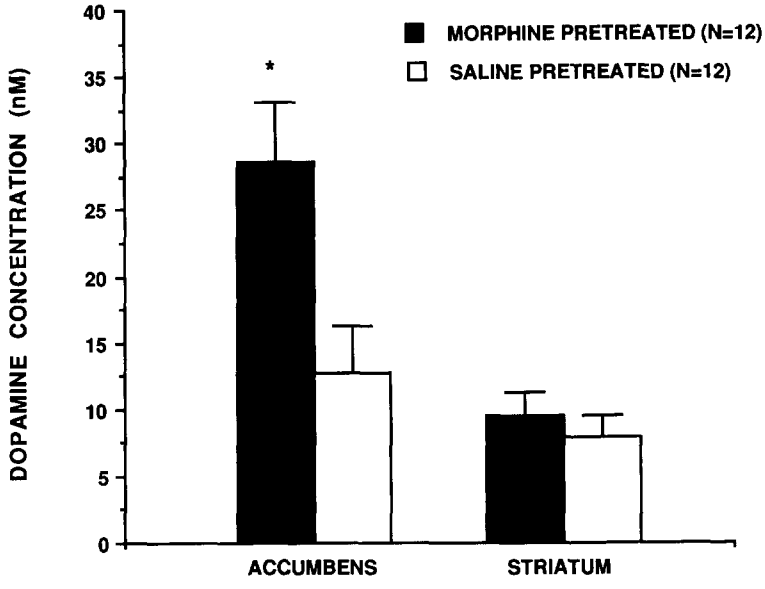

FIG. 2. Effect of morphine (30 mg/kg) or saline (1 ml/kg) pretreatment on mean (± SEM) basal dopamine concentrations in the nucleus accumbens and striatum of dialyzed rats. Morphine and saline were administered IP twice daily for 3 days, then once on day 4. Rats were dialyzed on day 5. Each mean represents the average of 3-6 baseline samples taken from 12 rats. *Different from saline-pretreated, nucleus accumbens (repeated-measures analysis of variance followed by unpaired t-test; $p < 0.01$).

well-established occurrence after injection of a high dose of morphine (2,3,17,25). The activity data in the present study demonstrates that, under these conditions, pretreatment of rats once daily for 4 days with a high dose (30 mg/kg) of morphine is inadequate to produce either tolerance to the initial locomotor-depressing effect of this dose or sensitization to the later locomotor-activating component. However, both tolerance and sensitization to the hypo- and hyperlocomotor effects, respectively, of a 30-mg/kg dose of morphine can be produced by increasing the number of morphine pretreatment injections while keeping the dose and number of days over which the pretreatments are administered constant.

The most robust neurochemical finding of this study was that in dialyzed rats pretreated with the tolerance/sensitization-producing regimen of morphine basal levels of DA in the NAC were more than twice that of controls, while basal DA levels in the STR were the same in the two groups. Basal extracellular DA concentrations as measured by microdialysis likely reflect an exocytotic process that is dependent upon the physiological activity of dopaminergic neurons (9). In fact, extracellular recordings demonstrate that morphine and other opiates stimulate dopaminergic neurons (8,22), including the A10 dopaminergic neurons (18), which originate in the ventral tegmental area (VTA) and provide the major dopaminergic input to the NAC (15). Therefore, the elevated levels of basal DA in the NAC of tolerant/sensitized rats in the present study likely reflect an increase in the physiological activity of the mesolimbic DA pathway.

The elevated levels of basal DA found in the NAC of tolerant/sensitized rats in this study suggests that hyperactivation of the mesolimbic pathway may, at least in part, mediate some aspects of the tolerance/sensitization to acute morphine seen here. Sensitization to the motor-stimulatory effects of an acute dose of morphine can be produced by prior activation of opiate receptors on the cell bodies of the A10 neurons in the VTA (12,26), and this in turn is associated with an increased capacity of opioids to increase DA metabolism in the NAC (11). Thus, the increased basal DA concentrations found in the NAC of tolerant/sensitized rats in this study seems consistent with the above model, at least with regard to the sensitization component. Although sensitization is a long-lasting phenomenon, we are unaware if the sensitization produced here to acute morphine persists as long-term studies were not conducted.

Less is known of the neurochemistry concerning tolerance development to the effects, for example, catalepsy, of high doses of acute morphine, however. Tolerance to the antinociceptive properties of morphine in rats can occur after just one prior injection of the opiate (13), but we are unaware of any studies showing tolerance to an acute cataleptic dose of morphine with only one prior exposure to the opiate. It appears that to produce tolerance to opiates binding to the opiate receptor for at least a short period of time must occur and intracellular protein synthesis is required because prior administration of naloxone or protein synthesis inhibitors blocks tolerance development (5,21). The locomotor-depressing effects of high doses of morphine may be due to reduction of DA release in motor pathways (14). This could involve κ-receptor activation, as high doses of morphine can activate κ-opiate receptors and subsequently decrease DA release from mesolimbic terminal fields in the NAC, which reduces motor activity (6). Such effects of κ-agonists are not blocked by specific μ-receptor antagonists and are blocked only by relatively high doses of naloxone, suggesting little or no involvement of μ-opiate receptors (6). It should be noted, however, that we did not find a change in extracellular DA concentrations in

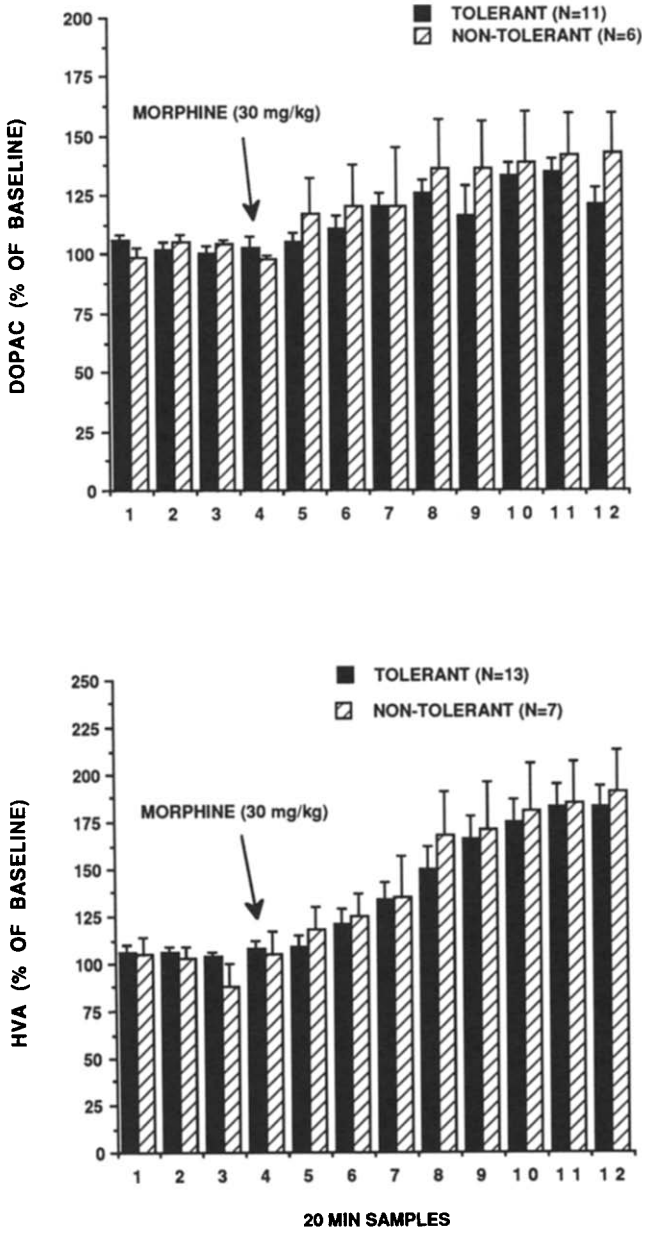

FIG. 3. Effects of acute morphine injection (30 mg/kg, IP) on dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) release (mean ± SEM) in the striatum of tolerant (pretreated with 30 mg/kg morphine, IP, twice daily for 3 days, then once on day 4) and nontolerant (saline pretreated, same protocol as tolerant) rats. All rats were dialyzed on day 5. Morphine was administered immediately after sample 3 in the figure. DOPAC and HVA in the dialysate gradually increased in both groups over 2–3 h after morphine injection (repeated-measures analysis of variance, $p < 0.001$). Levels of DOPAC and HVA were not different between tolerant and nontolerant groups.

the nontolerant/sensitized group after injection of 30 mg/kg morphine in our dialysis study. Therefore, in the present study this may not be the mechanism responsible for the decreased locomotor activity seen in these animals after acute morphine injection.

The protocol and design of this study provides little neurochemical information regarding the development of tolerance or sensitization as measurements of mesolimbic and nigrostriatal DA and DA metabolites in morphine-pretreated rats were taken after both phenomena were established. However, we previously reported (16) that naive rats of the same gender,

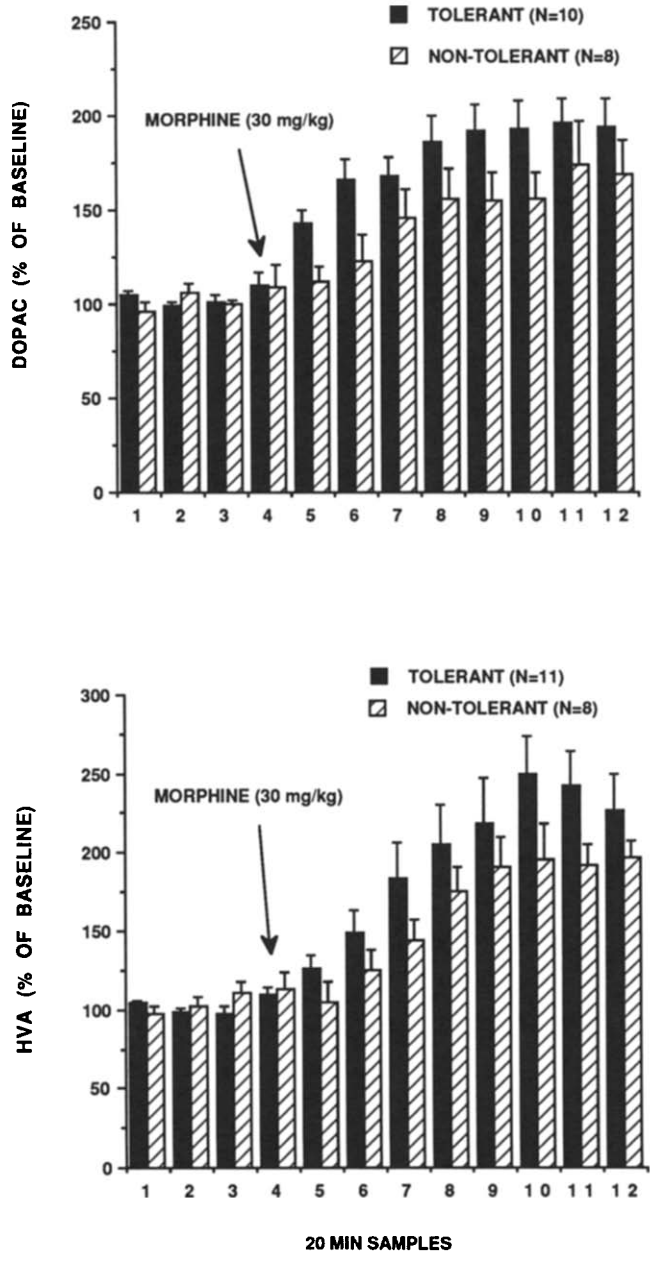

FIG. 4. Effects of acute morphine injection (30 mg/kg, IP) on dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) release (mean ± SEM) in the nucleus accumbens of tolerant (pretreated with 30 mg/kg morphine, IP, twice daily for 3 days, then once on day 4) and nontolerant (saline pretreated, same protocol as tolerant) rats. All rats were dialzyed on day 5. Morphine was administered immediately after sample 3 in the figure. DOPAC and HVA in the dialysate increased in both groups over 2–3 h after morphine injection (repeated-measures analysis of variance, $p < 0.001$). Release of DOPAC and HVA was greater over time in tolerant rats (pretreatment × time, $p < 0.001$) compared to nontolerant rats.

weight, and strain used here had basal DA concentrations approximately 60% higher in the STR than in the NAC (24.0 vs. 14.3 nM, respectively). In morphine-tolerant/sensitized rats used in this microdialysis study, the situation was reversed, with basal DA levels in the NAC averaging some 320% higher than levels in the STR (28.6 vs. 9.4 nM, respectively). Therefore, if the initial locomotor-inhibiting effects observed here after injection of a high dose of morphine were indeed due to reduction of DA release in motor pathways, in particular the mesolimbic pathway, then the apparent hyperactivity of the mesolimbic pathway in tolerant/sensitized rats, as reflected by the higher basal DA concentrations in the NAC, may have been at least partially responsible for the failure of a high dose of morphine to produce the initial locomotor depression in these rats in the activity studies as compared to the nontolerant/sensitized-pretreated rats.

We reported previously (16) that IP injection of naive rats with 30 mg/kg morphine resulted in no measurable changes in extracellular DA concentrations over a 3-h period in either the NAC or STR but extracellular DOPAC and HVA concentrations increased in both brain regions within 60–80 min after morphine injection compared to saline-injected rats. In both tolerant/sensitized and nontolerant/sensitized rats in this microdialysis study, no changes were measured in DA concentrations in the perfusate samples collected over a 3-h period from either the STR or NAC after a 30-mg/kg morphine challenge. We (16) and others (1,6) have shown that lower doses of morphine do increase DA concentrations in the STR and NAC. The absence of an increase in DA concentrations after high doses of morphine is possibly due to high concentrations of the opiate in the brain interacting with receptors other than the μ-receptor, perhaps κ-receptors, which, as described previously, might interfere with mesolimbic release of DA (6).

Interestingly, both tolerant/sensitized rats and nontolerant/sensitized rats here responded to a 30-mg/kg morphine challenge with similar increases in DOPAC and HVA levels over a 3-h period in the STR. Tolerant/sensitized rats, however, had higher DOPAC and HVA levels in the NAC over a 3-h period after acute morphine administration than did nontolerant/sensitized rats. This suggests that rats tolerant/sensitized to a locomotor-depressive dose of morphine, in addition to having higher basal DA levels in the NAC as a probable result of a physiologically more active mesolimbic pathway, may also metabolize and/or have a greater DA turnover in the NAC after acute morphine challenge compared to nontolerant/sensitized rats.

In conclusion, these findings indicate that, under our conditions, once-daily injections of a locomotor-depressive dose of morphine for 4 days are inadequate to produce either tolerance or sensitization. However, both tolerance and sensitization to this dose of morphine can be produced over the same time period by increasing the number of morphine pretreatment injections while keeping the dose constant. The results also indicate that DA released in the NAC and DA metabolism and/or turnover in the NAC are different in tolerant/sensitized rats compared to nontolerant/sensitized rats, suggesting that the NAC, but not the STR, may play an important role in mediating tolerance and/or sensitization to high doses of morphine.

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