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Effects of Acute and Chronic Morphine on DOPAC and Glutamate at Subcortical DA Terminals in Awake Rats

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HUANG, N. K., C. J. TSENG, C. S. WONG AND C. S. TUNG. *Effects of acute and chronic morphine on DOPAC and glutamate at subcortical DA terminals in awake rats.* PHARMACOL BIOCHEM BEHAV **56**(3) 363–371, 1997.—Effects of morphine and naloxone on the levels of 3,4-dihydroxy-phenylacetic acid (DOPAC) and glutamate in the striatum and nucleus accumbens of awake rats were studied with *in vivo* microdialysis. Acute morphine (50 mg/kg, IP) treatment increased the levels of DOPAC and glutamate in the striatum and nucleus accumbens, but both decreased from the elevated levels when naloxone (10 mg/kg, IP) was given 2 h later. Chronic morphine treatment, twice daily for 5 days in incremental doses (5, 10, 20, 40 and 50 mg/kg, IP), increased the level of DOPAC but decreased that of glutamate in the striatum and nucleus accumbens. When naloxone was given 2 h later, the reverse of the above phenomena are found. After given repeated morphine treatment and experiencing naloxone-precipitated withdrawal, the rats with an intact cortex and the rats with ibotenic acid (5 μg/0.5 μl/2.5 min) lesions on the medial prefrontal cortex and sulcal cortex have similar alternations in the levels of DOPAC and glutamate in the striatum. However, in the nucleus accumbens, the level of DOPAC dropped more and the level of glutamate increased more in the intact rats than the lesioned rats during the withdrawal stage. These data suggested that the intact cortex ordinarily exerted an inhibitory role to influence the level of DOPAC in the striatum and nucleus accumbens during chronic morphine treatment. In conclusion, morphine seems to activate different pathways in dependent and non-dependent rats.

DOPAC Glutamate Morphine Striatum Nucleus accumbens Cortex

THE mesotelencephalic dopamine (DA) system is commonly divided into two parts, the nigrostriatal DA pathway originating from DA cells in zona compacta of the substantia nigra, and the mesocorticolimbic DA pathway originating from DA cells in the area of the ventral tegmentum. The latter has been suggested as a main component of the reward circuit (28), which relates to various reinforcing behaviors including those induced and maintained by drugs such as morphine and amphetamine. In the reward circuit, the nucleus accumbens nearby the ventral striatum is an area receiving most of the DAergic input from the ventral tegmentum neurons. This area and its output, ventral pallidus, are recognized to be very essential for the signals from the mesocorticolimbic pathway, basal ganglia and corticofugal system to be integrated and transformed to a variety of motivated behaviors (20). Although there are some substances such as opioids and other DA-unrelated neurochemicals taking part in the reward mechanisms, DA, especially in the nucleus accumbens, is still the most crucial.

On the other hand, it is known that morphine can enhance the synthesis/turnover rate of DA in the mesotelencephalic DA system and then induces different patterns of psychomotor behavior in rats. Nevertheless, recent reports also indicated that DA in the nucleus accumbens not only is important in the rewarding effects of abused drugs, but also plays a pivotal role in opiate withdrawal (21). One explanation for the findings of rising DAergic transmission in this system is that when morphine acts on the μ opioid receptors, the hyperpolarization of the interneurons and afferents leads to the reduction of spontaneous GABA-mediated influence on cellular firing in the substantia nigra or ventral segmental area (25). This influence finally thus affects the synthesis/turnover rate of DA in

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the striatum and nucleus accumbens (13,35), where the major subcortical DA neuron terminals innervating areas are located.

The corticofugal system has aroused our concern because it is found to control mental as well as motor activities (4,7). The cortico-striatal and cortico-accumben projections utilize excitatory amino acids (EAA), such as glutamate/asparate, as their neurotransmitters. These projections functionally and anatomically interact with the nigrostriatal and mesocorticolimbic DA pathways (10). They are similar to the projection from the nucleus paragigantocellularis to the locus coeruleus, which is involved in behavioral arousal and vigilance control (2,3). There is strong evidence to prove the fact that glutamate is as important as DA in the mammalian central nervous system, involved in the expression of proper conditional, emotional and motivational responses. Besides, glutamate receptors such as NMDA receptor are currently regarded to play an important role in the formation of synaptic plasticity, which seems to be essential for the generation of some animal compulsive behaviors (12). We know that different concentrations of glutamate induce a variety of changes in the level of the endogenous DA (26). This phenomenon is possibly caused by two types of glutamate/asparate-DA interactions at the subcortical DA terminals (18,41). The first type may be an action of EAA on the activity of DAergic neurons or DA release. Extra-synaptic receptors on nerve terminals are activated by diffusion of glutamate that is not from the synapse, which may contribute to this effect. The second type may be a direct and post-synaptic interaction. Base on the above understanding, our hypothesis is that the glutamate released from the corticofugal neuron terminals primarily activates the post synaptic element, and this element may be a dendritic spine of an intrinsic neuron connecting EAA and DA terminals.

In addition, the studies of drug addiction currently emphasizes the role of the EAA system. In vivo microdialysis study demonstrated that the efflux of EAA (glutamate/aspartate) dramatically increased in the locus coeruleus during naloxone (or naltrexone)-precipitated morphine withdrawal (2,43). Glutamate receptor antagonists, kynurenate, AP5 and CNQX, have been shown to (a) block morphine withdrawal responses of locus coeruleus neurons (3,40); (b) inhibit the development of morphine tolerance and dependence (39); and (c) attenuate the addictive effects of cocaine and ethanol (25,27). Thus, the neuroadaptive changes of glutamate receptors are hypothesized to mediate various drug abuse and withdrawal symptoms (15). All the above information has given us a lot of help to manage the symptoms of drug abuse, particularly to deal with the negative reinforcement caused by physical dependence (aversion). However, the role of intrinsic EAA transmission in mediating the positive reinforcement caused by psychological dependence (reward) has not been studied throughout.

Therefore, it may be meaningful to study the functions of the cortex and the relationships between the cortex and the striatum or nucleus accumbens during the development of morphine dependence and withdrawal. Through in vivo microdialysis and electrochemical detection, we examined the extracellular levels of glutamate and DOPAC (an index of the new synthesis rate of DA) in the rats with intact cortex and the rats with limbic cortex lesions during morphine dependence and naloxone-precipitated withdrawal. Comparing acute and chronic morphine treatments, we found that the increasing profiles of DOPAC are consistent but the efflux profiles of glutamate are incompatible, no matter if located in the striatum or nucleus accumbens.

METHODS

Animals

Morphine-naive male Sprague–Dawley rats (270–350 g) were used for these experiments. The rats were housed in wiremesh cages and allowed free access to food and water in a room with a temperature control (21–24°C) and a 12:12 h light/dark cycle.

Procedure

Rats were divided into 3 groups. Group 1 was given acute (single dose) morphine treatment. Group 2 was given chronic (repeated doses) morphine treatment. Group 3 was ibotenic acid lesion of limbic cortex and then given chronic morphine treatment. Naloxone was given 2 h later after the single injection of morphine in Group 1 or the last injection of morphine in Groups 2 and 3.

Acute Morphine Treatment. In order to make the comparisons among different groups meaningful, the dose must be consistent with the last dose of the chronic morphine treatment even though such a high dose will cause pathological morphine actions. Rats were injected with single morphine HCl (50 mg/kg, IP) or saline once and then with saline or naloxone (50 mg/kg, IP). Based on the first (morphine or saline) and the last (nalaxone or saline) drug treatment, this group of rats were divided into three subgroups, morphine/saline, morphine/naloxone and saline/naloxone. This classification was also applied in the following experiments.

Chronic Morphine Treatment. Rats were injected with morphine HCl twice daily for 5 days in increasing doses of 5, 10, 20, 40, and 50 mg/kg IP, which is a schedule of producing physical dependence (37). Naloxone (10 mg/kg, IP) or saline was given 2 h later after the last injection of morphine.

Chronic Morphine Treatment with Lesions of Medial Prefrontal Cortex and Sulcal Cortex. The lesions were induced 7 to 10 days before the chronic morphine treatment. The lesion method is detailed below (23,30).

After anesthesia with chloral hydrate (400 mg/kg, IP), rats were immobilized in the stereotaxic frame (Kopf Instruments) with the incisor set at 2.5 mm below the interaural line. Ibotenic acid (5 μg/0.5 μl) dissolved in 0.1 M phosphate buffered saline (pH 7.4) was stereotaxically administered bilaterally through a 30-gauge cannula with an infusion pump (CMA 100, Carnegie Medicine AB, Sweden) over a period of 2.5 min. The coordinates for the medial prefrontal cortex were AP +3.5 mm, ML ± 0.7 mm and VD −3.5 mm; the sulcal cortex coordinates were AP +3.2 mm, ML ± 2.0 mm and VD −6.1 mm at an angle of 16° from the bregma point and the aura. The cannula were in place for 5 min after the end of infusion.

Drugs

DOPAC, *o*-phthalaldehyde, *t*-butylthiol and iodoacetamide were purchased from BAS (West Lafayette, IN, USA). Glutamate and ibotenic acid came from RBI (MA, USA) and morphine HCl from the NIH of the ROC. Other chemicals used in this study were from Sigma (St. Louis, USA).

Brain Dialysis

Eighteen to twenty hours before the acute morphine or the last morphine treatment, microdialysis probes were implanted in the striatum and nucleus accumbens. Concentric dialysis probes (26) were used to monitor the extracellular DOPAC and glutamate in the striatum and nucleus accum-

bens. The dialysis membranes were prepared from polyacrylonitrile/sodium methalyl sulfonate copolymer (ID: 0.22 mm; OD: 0.31 mm; AN 69, Hospal, Bologna, Italy) and its exposed tips were 4 mm (striatum) and 2 mm (nucleus accumbens). Coordinates for implantation in the striatum were AP +1.0 mm, LM +2.8 mm, and VD −6.5 mm; the coordinates of nucleus accumbens were AP +2.2 mm, LM −1.4 mm, and VD −7.5 mm (30). Rats were kept awake with free movements. The Ringer's solution (NaC1 140 mM, $CaC1_2$ 1.2 mM, KCI 3.0 mM, $MgC1_2$ 1.0 mM, and ascorbic acid 0.04 mM) was continuously perfused (1.0 μl/min) via probes throughout the experiments.

Chemical Analysis

One hour before the acute or the last dose of the chronic morphine treatment (either in the intact or cortex lesions group), 30 μl of dialysate was collected every 30 min for 5 h at a flow rate of 1 μl/min. Dialysates were immediately assayed, 5 μl for detecting DOPAC and 20 μl for detecting glutamate by independent high performance liquid chromatography with electrochemical detection. A BAS PM 60 pump was used in conjunction with a reverse-phase C18 microbore column (100 × 1 mm; particle size, 3 μm). Then DOPAC was detected by glassy carbon working electrode set at 650 mV (with respect to an Ag/AgCl reference electrode) and magnified by a detector (LC-4B, BAS, USA). The mobile phase consisted of a mixture of monochloroacetic acid 0.1 M, EDTA 0.5 mM, sodium octyl sulfate 0.15 g/L, acetonitrile 50 ml/L, and tetrahydrofuran 7 ml/L at pH 3.1. The retention peak for DOPAC appears at 5 min ($\kappa = 2.5$). Glutamate in dialysate was assayed by precolumn derivatization with an *o*-phthaladehyde/*t*-butylthiol reagent and iodoacetamide/methanol scavenger (5). The derivatized sample was then injected into an amino acid-II cartridge column (100 × 3.2 mm; particle size, 3 μm) and detected by a glassy carbon working electrode set at 700 mV. A gradient of two eluents was used to separate the amino acids. Eluent A was 0.1 M sodium acetate buffer (pH 6.8)/acetonitrile (84:16). Eluent B was 0.1 M sodium acetate buffer (pH 6.8)/acetonitrile (15:85). The gradient program was as follows: at 0 min, 0% B; at 6 min, 5.6% B; at 8 min, 5.6% B; at 9 min, 80.6% B; at 9.9 min, 80.6% B; at 10 min, 0% B. Mobile phase flow rate was 0.9 ml/min. The retention peak for glutamate appears at 2 min ($\kappa = 2.0$) (Fig. 1).

Histology

After the experiments, rats were given an overdose of chloral hydrate and their brains were fixed with 4% paraformaldehyde through intracardiac perfusion. Coronal sections (50 μm thick) were made and stained with crystal violet, in order to verify the location of dialysis probes or the lesion sites. The data from the animals with incorrect probe placement or lesion sites were deleted from analysis.

Statistics

All data were analyzed by an IBM-compatable statistical software package (SPSS for Windows, Ver. 6.0). The significance of the drug effects (Figs. 2–4) and treatments (Fig. 5) was determined by two-way analysis of variance (ANOVA) with repeated measures. If there were significant interactions, the simple main effect of each factor would be analysed by separate analysis of variance. Post hoc comparisons were carried out either between means or within means, according to the suitability. The area under the curve (AUC) in Fig. 5 was calculated by means of the differences between the AUC in the morphine/naloxone group and the saline/naloxone group from 0–120 and 120–240 min. Statistical significance was set at $p < 0.05$.

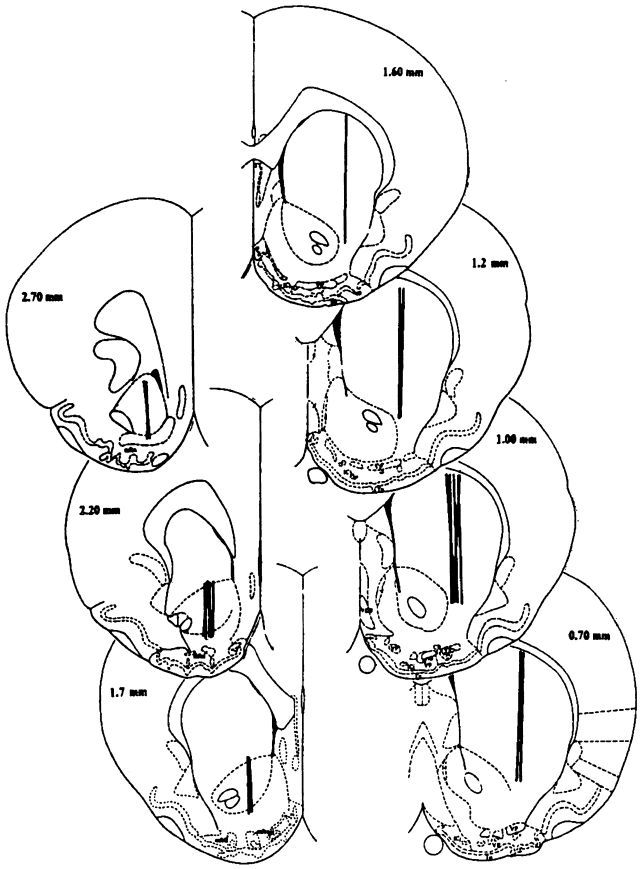

FIG. 1. Location of the partial microdialysis probes in the left nucleus accumbens and right striatum shown by the lines. The numbers indicate millimeters rostral to bregma according to the atlas of Paxinos and Watson (1986).

RESULTS

Behavior and Histology Examination

The awake rat is put in a container for microdialysis and this system inevitably limits its mobility. Under the circumstances, merely partial morphine-induced or naloxone-induced behaviors can be observed. We found that the chronic morphine-treated rats always underwent akinesia and catalepsia throughout the dialysis process after given the last dose of morphine. On the other hand, several withdrawal symptoms such as diarrhea, chewing, burrowing, jumping, and teeth chattering occurred in all rats right after the naloxone injection. The intensity of these responses maximized within twenty minutes and then gradually declined. At last, the rats showed flappy, hypokinesia and some nonspecific weaknesses.

Figure 1 indicates the locations of the active dialyzing probes used in the experiment. When ibotenic acid lesions of medial prefrontal cortex occurred, necrosis was found not only in the cingulate cortex area 3, but also in three other regions, part of the most anterior aspect of the cingulate cortex (cingu-

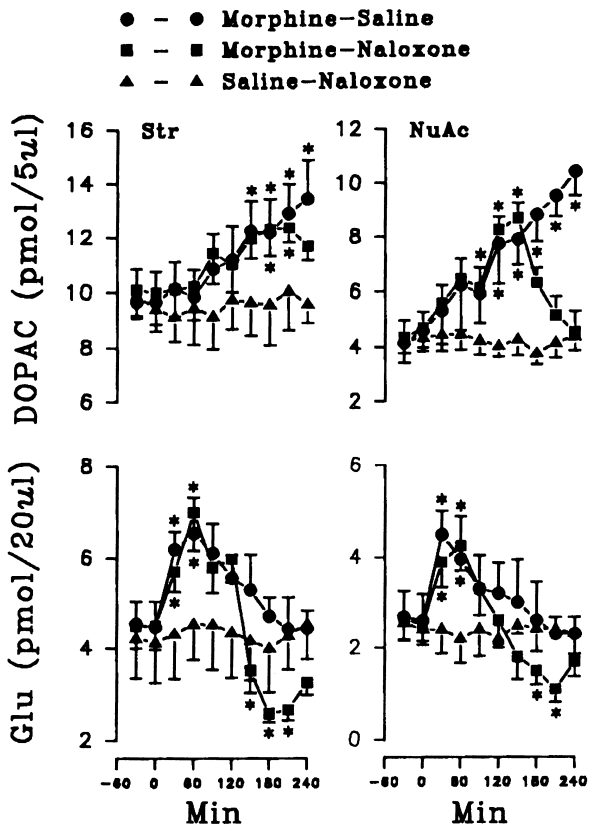

FIG. 2. Changes of DOPAC and glutamate from the perfusate in the striatum (Str) and nucleus accumbens (NuAc) of morphine (Mor) naive rats after the acute morphine treatment (50 mg/kg, IP) followed by the challenge of naloxone (Nal; 10 mg/kg, IP; ■-■; $n = 5$) or saline (Sal; ●-●; $n = 5$) 2 h later. Control group (▲-▲; $n = 4$) was first treated with saline instead of morphine and then given naloxone 2 h later. Values are expressed as means ± SEM, $^*P < 0.05$.

late cortex areas 1 and 2), the medial and ventral orbital cortices. When proceeding with sulcal cortex lesions, the agranular insular cortex and the anterior lateral orbital cortex are also influenced. Nevertheless, the most important point is that the subcortical regions, striatum and nucleus accumbens are never damaged by these lesions.

Effects of Acute Morphine Treatment

Figure 2 indicates the changes of DOPAC and glutamate in the striatum and nucleus accumbens during acute morphine treatment. The concentrations of basal dialysate in the striatum were DOPAC 9.6 ± 1.2 pmol/5 μl and glutamate 4.1 ± 0.4 pmol/20 μl. In the nucleus accumbens, the basal dialysate concentrations were DOPAC 4.2 ± 1.4 pmol/5 μl and glutamate 2.6 ± 0.9 pmol/20 μl.

In the striatum, there was a significant drug × time interaction in the changes of DOPAC and glutamate [$F(18, 99) = 2.19$ and 6.50, respectively, $p < 0.01$]. About the changes of DOPAC, its simple main effect of time within drug indicated that the drug effects were significant in the morphine/saline and morphine/naloxone groups [$F(9, 99) = 9.01$ and 4.67, respectively, $p < 0.001$] but not in the saline/naloxone group. Post hoc comparisons within means revealed that the changes of DOPAC significantly elevated from 150 ($t = 2.31$, $p < 0.05$) to 240 min ($t = 2.06$, $p < 0.05$) in the morphine/saline group and from 180 ($t = 2.14$, $p < 0.05$) to 210 min ($t = 2.22$, $p < 0.05$) in the morphine/naloxone group. Regarding the change of glutamate, its simple main effect of time within drug indicated that the drug effects were significant in the morphine/saline and morphine/naloxone groups [$F(9, 99) = 8.27$ and 28.30, respectively, $p < 0.001$] but not in the saline/naloxone group. Post hoc comparisons within means revealed that the change of glutamate significantly increased from 30 ($t = 3.0$, $p < 0.05$) to 60 min ($t = 2.03$, $p < 0.05$) in the morphine/saline group. In the morphine/naloxone group, the glutamate was also found to have increased, but it began going decreasing at 150 min ($t = 2.08$, $p < 0.05$) following the naloxone treatment.

In the nucleus accumbens, there was a significant drug × time interaction in the changes of DOPAC and glutamate [$F(18, 99) = 16.21$ and 6.42, respectively, $p < 0.001$]. About the change of DOPAC, its simple main effect of time within drug indicated that the drug effects were significant in the morphine/saline and morphine/naloxone groups [$F(9, 99) = 43.09$ and 21.78, respectively, $p < 0.001$], but not in the saline/naloxone group. Post hoc comparisons within means revealed that the change of DOPAC significantly elevated from 120 ($t = 2.11$, $p < 0.05$) to 240 min ($t = 5.50$, $p < 0.05$) in the morphine/saline group. However, the comparison between means indicated that the change of DOPAC significantly elevated from 60 ($t = 2.05$, $p < 0.05$) to 240 min ($t = 4.48$, $p < 0.05$). In the morphine/naloxone group, the change of DOPAC significantly elevated from 90 ($t = 3.24$, $p < 0.05$) to 150 min ($t = 2.08$, $p < 0.05$). However, the comparison between means indicated that the change of DOPAC significantly elevated from 60 ($t = 2.62$, $p < 0.05$) to 210 min ($t = 2.58$, $p < 0.05$). Regarding the change of glutamate, its simple main effect of time within drug indicated that the drug effects were significant in the morphine/saline and morphine/naloxone groups [$F(9, 99) = 7.77$ and 24.73, respectively, $p < 0.001$], but not in the saline/naloxone group. Post hoc comparisons within means revealed that the change of glutamate significantly increased from 30 ($t = 2.08$, $p < 0.05$) to 60 min ($t = 2.10$, $p < 0.05$) in the morphine/saline group. In the morphine/naloxone group, the increase of glutamate was also found from 30 ($t = 2.06$, $p < 0.05$) to 60 min ($t = 2.5$, $p < 0.05$), but it reversed and even decreased significantly at 180 min ($t = 2.54$, $p < 0.05$) following the naloxone treatment.

Effects of Chronic Morphine Treatment

Figure 3 indicates the changes of DOPAC and glutamate in the striatum and nucleus accumbens during chronic morphine treatment in rats with an intact cortex. By the last morphine injection on the 5th day, the concentrations of basal dialysate in the striatum were DOPAC 8.8 ± 2.3 pmol/5 μl and glutamate 3.3 ± 0.3 pmol/20 μl. In the nucleus accumbens, the basal dialysate concentrations were DOPAC 3.8 ± 1.9 pmol/5 μl and glutamate 1.9 ± 0.1 pmol/20 μl.

In the striatum, there was a significant drug × time interaction in the changes of DOPAC and glutamate [$F(18, 117) = 3.90$ and 12.26, respectively, $p < 0.001$]. About the change of DOPAC, its simple main effect of time within drug indicated that the drug effects were significant in the morphine/saline and morphine/naloxone groups [$F(9, 117) = 9.84$ and 8.56, respectively, $p < 0.001$] but not in the saline/naloxone group. Post hoc comparisons within means revealed that the change of DOPAC significantly elevated from 90 ($t = 2.10$, $p < 0.05$) to 210 min ($t = 2.06$, $p < 0.05$) in the morphine/saline group.

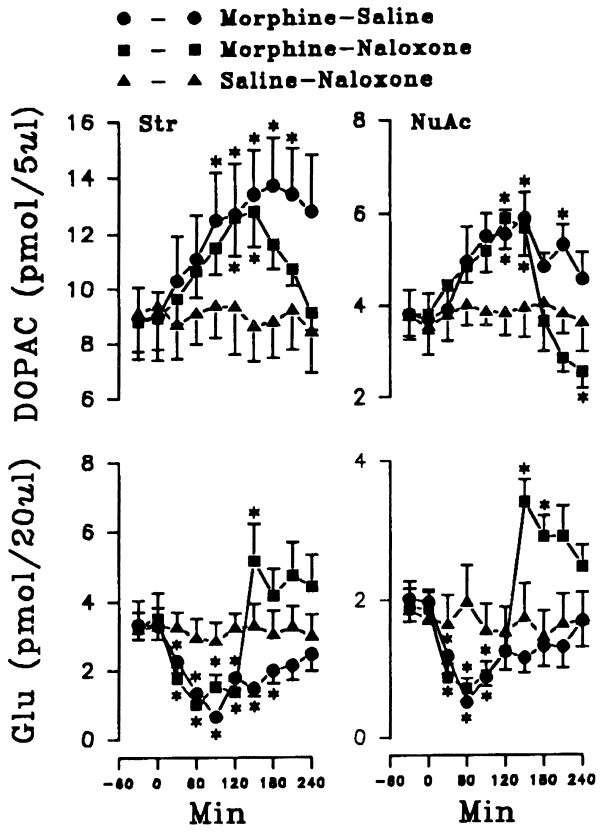

FIG. 3. Changes of DOPAC and glutamate from the perfusate in the striatum and nucleus accumbens of the cortex-intact rats after the last dose (50 mg/kg, IP) of chronic morphine treatment and after given naloxone (■-■; $n = 6$) or saline (●-●; $n = 6$). The control group (▲-▲; $n = 4$) was also chronically treated with saline and then given naloxone 2 h later at the last day. Values are expressed as means ± SEM, $^*P < 0.05$.

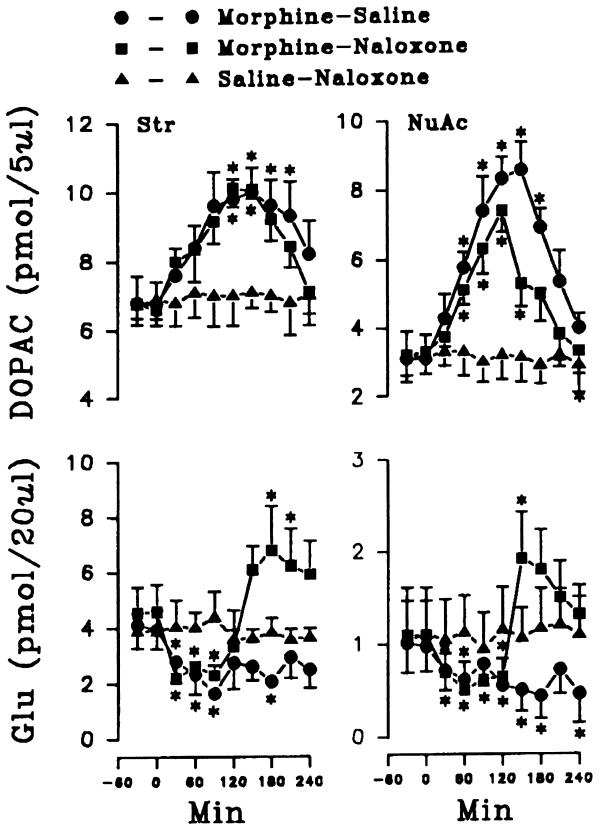

FIG. 4. Changes of DOPAC and glutamate from the perfusate in the striatum and nucleus accumbens of the cortex-lesioned rats after the last dose (50 mg/kg, IP) of chronic morphine treatment and after given naloxone (■-■; $n = 4$) or saline (●-●; $n = 5$). The control group (▲-▲; $n = 4$) was also chronically treated with saline and then given naloxone 2 h later at the last day. Values are expressed as means ± SEM, $^*P < 0.05$.

In the morphine/naloxone group, the DOPAC had also significant increased at 120 min ($t = 2.20, p < 0.05$) and 150 min ($t = 2.34$, $p < 0.05$). Regarding the change of glutamate, its simple main effect of time within drug indicated that the drug effects were significant in the morphine/saline and morphine/naloxone groups [$F(9, 117) = 11.73$ and 38.78, respectively, $p < 0.001$], but not in the saline/naloxone group. Post hoc comparisons within means revealed that the changes of glutamate significantly decreased from 30 ($t = 2.94$, $p < 0.05$) to 180 min ($t = 3.42$, $p < 0.05$) in the morphine/saline group. In the morphine/naloxone group, the decrease of glutamate was also found but the phenomenon reversed at 150 min ($t = 2.72$, $p < 0.05$) following the naloxone treatment.

In the nucleus accumbens, there was a significant drug × time interaction in the changes of DOPAC and glutamate [$F(18, 117) = 5.53$ and 13.10, respectively, $p < 0.001$]. Regarding the change of DOPAC, its simple main effect of time within drug indicated that the drug effects were significant in the morphine/saline and morphine/naloxone groups [$F(9, 117) = 9.37$ and 19.83, respectively, $p < 0.001$] but not in the saline/naloxone group. Post hoc comparisons within means revealed that the change of DOPAC significantly elevated at 120 ($t = 2.23$, $p < 0.05$), 150 and 210 ($t = 2.23$, $p < 0.05$) min in the morphine/saline group. In the morphine/naloxone group, the change of DOPAC significantly elevated at 120 ($t = 3.51$, $p < 0.05$) and 150 min ($t = 2.09$, $p < 0.05$), but the elevation reversed after the injection of naloxone and even decreased significantly below its basal level at 240 min ($t = 2.02$, $p < 0.05$). Regarding the change of glutamate, its simple main effect of time within drug indicated that the drug effects were significant in the morphine/saline and morphine/naloxone groups [$F(9, 117) = 8.81$ and 36.70, respectively, $p < 0.001$] but not in the saline/naloxone group. Post hoc comparisons within means revealed that the change of glutamate significantly decreased from 30 ($t = 2.30$, $p < 0.05$) to 90 min ($t = 4.31$, $p < 0.05$). In the morphine/naloxone group, the decrease of glutamate was also found from 30 ($t = 2.20$, $p < 0.05$) to 90 min ($t = 3.94$, $p < 0.05$), but it reversed and was even significantly elevated at 150 min ($t = 2.60$, $p < 0.05$) following the naloxone treatment.

Effects of Chronic Morphine Treatment in Cortex-Lesioned Rats

Figure 4 indicates the changes of DOPAC and glutamate in the striatum and nucleus accumbens during chronic morphine treatment in rats with a lesioned cortex. The concentrations of basal dialysate in the striatum were DOPAC 6.8 ± 1.3 pmol/5 μl and glutamate 4.1 ± 0.7 pmol/20 μl. In the nucleus

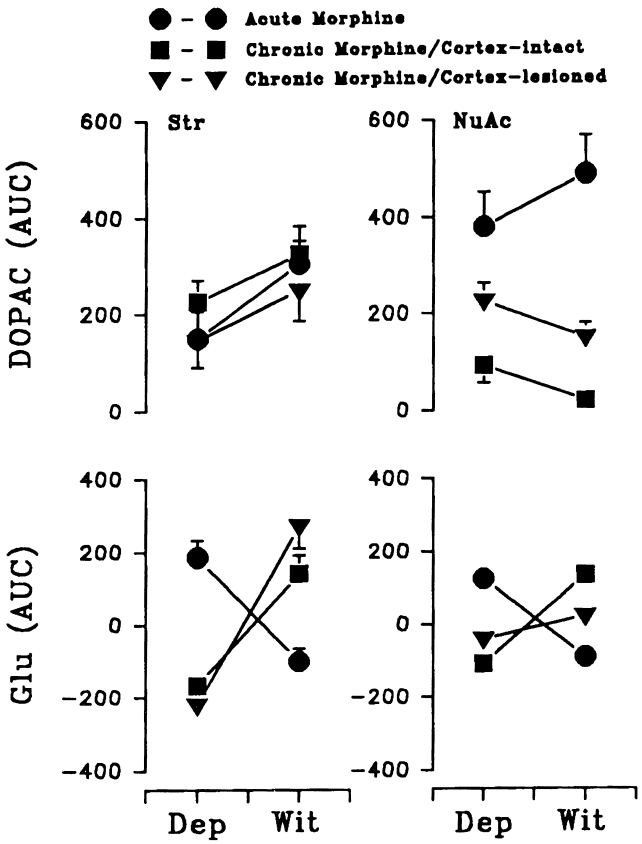

FIG. 5. The AUC of DOPAC and glutamate in the striatum and nucleus accumbens during the drug treatments in the morphine/naloxone groups from 0 to 120 and 120 to 240 min. Values are expressed as means ± SEM.

accumbens the basal dialysate were DOPAC 3.1 ± 0.8 pmol/5 μl and glutamate 1.0 ± 0.2 pmol/20 μl.

In the striatum, there was a significant drug × time interaction in the changes of DOPAC and glutamate [$F(18, 90)$ = 3.73 and 5.42, respectively, $p < 0.001$]. About the change of DOPAC, its simple main effect of time within drug indicated that the drug effects were significant in the morphine/saline and morphine/naloxone groups [$F(9, 90)$ = 9.03 and 7.98, respectively, $p < 0.001$] but not in the saline/naloxone group. Post hoc comparisons within means revealed that the change of DOPAC significantly elevated from 120 ($t = 2.13, p < 0.05$) to 210 min ($t = 3.3$, $p < 0.05$) in the morphine/saline group and from 120 ($t = 2.12$, $p < 0.05$) to 150 min ($t = 2.71$, $p < 0.05$) in the morphine/naloxone group. Regarding the change of glutamate, its simple main effect of time within drug indicated that the drug effects were significant, in the morphine/saline and morphine/naloxone groups [$F(9, 90)$ = 3.41 and 13.95, respectively, $p < 0.001$] but not in the saline/naloxone group. Post hoc comparisons within means revealed that the change of glutamate significantly decreased from 30 ($t = 2.9$, $p < 0.05$) to 90 min ($t = 3.25$, $p < 0.05$) and at 180 min ($t = 2.91, p < 0.05$) in the morphine/saline group. In the morphine/saline group, the decrease of glutamate was also found but the phenomenon reversed and even elevated significantly at time 180 min ($t = 2.30$, $p < 0.05$) following the injection of naloxone. Although the elevation of glutamate at 150 min was not significant within means, it was significantly elevated between means when comparing with the saline/naloxone group ($t = 2.21, p < 0.05$).

In the nucleus accumbens, there was a significant drug × time interaction in the changes of DOPAC and glutamate [$F(18, 90)$ = 7.72 and 4.51, respectively, $p < 0.001$]. About the change of DOPAC, its simple main effect of time within drug indicated that the drug effects were significant in the morphine/saline and morphine/naloxone groups [$F(9, 90)$ = 33.28 and 12.79, respectively, $p < 0.001$] but not in the saline/naloxone group. Post hoc comparisons within means revealed that the changes of DOPAC significantly elevated from 60 ($t = 4.94$, $p < 0.05$) to 180 min ($t = 2.66$, $p < 0.05$) in the morphine/saline group. In the morphine/naloxone group, the change of DOPAC significantly elevated from 60 ($t = 2.28$, $p < 0.05$) to 150 min ($t = 3.52$, $p < 0.05$). Regarding the change of glutamate, its simple main effect of time within drug indicated that the drug effects were significant in the morphine/saline and morphine/naloxone groups [$F(9, 90)$ = 2.52 and 10.20, respectively, $p < 0.001$] but not in the saline/naloxone group. Post hoc comparisons within means revealed that the change of glutamate in the morphine/saline group significantly decreased from 30 ($t = 2.71, p < 0.05$) to 180 min ($t = 2\ 38$, $p < 0.05$) and at 240 min ($t = 2.25$, $p < 0.05$). In the morphine/naloxone group, the decrease of glutamate was also found, but the decrease reversed and was even significantly elevated at 150 min ($t = 2.22$, $p < 0.05$) following the naloxone treatment.

Acute or Chronic Morphine/Naloxone Treatment in Alternation of DOPAC and Glutamate Levels

Figure 5 indicated the net responses (represented by the AUC of DOPAC and glutamate) of the striatum and nucleus accumbens at the dependent stage (0–120 min) and withdrawal stage (120 to 240 min) in all morphine/naloxone groups.

In the striatum, there was no interaction between treatment and stage in the AUC of DOPAC but the interaction existed in the AUC of glutamate [$F(2, 12) = 130.28$, $p < 0.001$]. In the AUC of glutamate, the simple main effect of treatments within stages indicated that treatments were significant in the dependent and withdrawal stages [$F(2, 24)$ = 26.97 and 20.72, respectively, $p < 0.001$]. Post hoc comparison revealed that, in the dependence stage, the chronic morphine treatment with an intact cortex was different from the acute morphine treatment ($t = 6.95$, $p < 0.001$) but there was no difference with the chronic morphine treatment in those with a lesioned cortex. The comparison was also significant in the withdrawal stage. On the other hand, the simple main effect of the stages within treatments indicated that the dependence and withdrawal stages were significant in all three morphine/naloxone groups [$F(1, 12)$ = 76.82, 105.99, and 132.39, respectively, $p < 0.001$]. Post hoc comparison revealed that the AUC of dependent and withdrawal stages were very different in these three groups (t = 4.84, 4.70, and 7.06, respectively, $p < 0.01$).

In the nucleus accumbens, there were interactions in the AUC of DOPAC and glutamate [$F(2, 12)$ = 17.23 and 707.60, respectively, $p < 0.001$]. In the AUC of DOPAC, the simple main effect of treatments within stages indicated that treatments were significant in the dependent and withdrawal stages [$F(2, 24)$ = 11.68 and 32.07, respectively, $p < 0.001$]. Post hoc comparison revealed that, in the dependent stage, the chronic morphine treatment with an intact cortex was different from the acute morphine treatment and the chronic morphine treatment with a lesioned cortex. (t = 4.83 and 2.07, respectively,

$p < 0.05$). The comparison was also significant in the withdrawal stage. Besides, the chronic morphine treatment with a lesioned cortex was also different from the acute morphine treatment in the dependent and withdrawal stages ($t = 2.66$ and 4.86, respectively, $p < 0.05$). On the other hand, the simple main effect of the stages within treatments indicated that the stage of dependence and withdrawal were significant in all three morphine/naloxone groups [$F(1, 12) = 18.59$, 9.86, and 7.11, respectively, $p < 0.05$]. However, post hoc comparison revealed that the stages of dependence and withdrawal were not different in any of them. In the AUC of glutamate, the simple main effect of treatments within stages indicated that treatments were significant in the dependent and withdrawal stages [$F(2, 24) = 54.13$ and 52.98, respectively, $p < 0.001$]. Post hoc comparison revealed that the chronic morphine treatment with an intact cortex was different from the acute morphine treatment and the chronic morphine treatment with a lesioned cortex ($t = 10.25$ and 2.71, respectively, $p < 0.05$). The comparison was also significant in the withdrawal stage. On the other hand, the simple main effect of the stages within treatments indicated that the dependent and withdrawal stages were significant in all three morphine/naloxone groups [$F(1, 12) = 594.96$, 835.87, and 40.0, respectively, $p < 0.001$]. Post hoc comparison revealed that the AUC of dependent and withdrawal stages were very different in these three groups ($t = 11.44$, 13.42, and 3.38, respectively, $p < 0.01$).

DISCUSSION

The present experiments examine the changes of glutamate and DOPAC in the nucleus accumbens and striatum at the morphine dependent and withdrawal stages. Comparing the acute and chronic morphine/naloxone treatments, the trends of the change in the extracellular glutamate are different but they are similar in DOPAC. These findings indicate that there seems to be a synaptic adaptation for the transmission between the corticofugal EAA system and the mesotelencephalic DA system. On the other hand, we find that the striatum and nucleus accumbens respond differently to naloxone-precipitated morphine withdrawal when the limbic cortex is damaged. Therefore, we believe that these two areas have different self-control mechanisms to monitor the presynaptic modulations. All our findings are discussed below.

Effects of Morphine on Neuronal Activities of the Mesotelencephalic DA System

Since the DOPAC is suggested to be derived from an intraneuronal pool of the new synthesized DA, we use the change of extracellular DOPAC level as an index for the biosynthetic activity of the DA neurons (42). Our data support the previous findings that morphine increases the activities of the mesotelencephalic DA neurons no matter if the administration is acute or chronic (13,35). This activation is believed to be due to a reduction of the spontaneous GABA-mediated input to the DA cells (24). In addition, the rising profiles of DOPAC in the striatum and nucleus accumbens are distinct and this result is consistent with the findings of others (13,14,24,29), proposed that the difference might be caused by the variation of the density of μ opioid receptors, the quantity of DA autoreceptors or the potency of negative-feedback-controlled circuits within these two DA cell targeting areas. The above mechanisms possibly make the increase of DOPAC in the striatum caused by the acute morphine treatment not reversible even after given naloxone.

During naloxone-precipitated morphine withdrawal, the rising profiles of DOPAC caused by the chronic treatment drop to the basal level in the striatum and even lower in the nucleus accumbens. These findings support the studies of Acquas et al. because they also found a profound depression of the mesolimbic DA transmission after morphine withdrawal in the dependent rats (1).

Comparing the acute and chronic treatments, the DOPAC profile in morphine/saline group (Fig. 2 vs. 3) and the AUC of DOPAC in the morphine/naloxone group including dependent and withdrawal stages (Fig. 5) are different. Therefore, it is possible that the neuronal activities of DA cells undergo tolerance and it is dominant in the mesocorticolimbic pathway. Our data also indicated that the AUC of DOPAC at the dependent and withdrawal stages were obviously greater in the cortex-lesioned group than in the intact group. From the above, we support the hypothesis, raised by others, that there is an inhibitory tone exerted by the corticofugal projections on DA terminals in the nucleus accumbens (38). Thus, there is hyperactivity in the mesolimbic DA transmission if the tonic restraint is released.

Effects of Morphine on Neuronal Activities of the Corticofugal EAA System

Comparing the acute and chronic morphine treatments, the profiles of glutamate efflux are opposite not only in the nucleus accumbens but also in the striatum. These findings tell us that corticofugal glutamate transmission at the subcortical brain regions is possibly involved in addictive mechanisms. Although some researchers have successfully found that the opiate withdrawal syndrome is partly mediated by EAA systems and the mediations mainly happen in the pontine locus coeruleus (2,3,40,43), our study has not yet had sufficient evidence to explain what mechanisms make the glutamate efflux in the subcortical areas increase after the acute morphine treatment and naloxone-precipitated morphine withdrawal. Perhaps Sloviter's and other findings can partially be applied to understand the mechanisms (16,36). They suggested that a large single dose of opiate can attenuate GABA-mediated inhibition and then result in an excitation in the hippocampal pyramidal cells so that the efflux of glutamate increases.

Chronic morphine treatment has been reported to cause an adaptive desensitization of some cortical neurons or an adaptive supersensitivity in some other neurons (17,32). As we know, EAA receptors have some influences upon the synaptic plasticity and the formation of the adaptation. On the other hand, in 1942, Himmelsbach (22) proposed that morphine-induced tolerance and dependence are an adaptation of the biogenic body which passively regulates itself to reform a state of "new homeostasis." However, investigation is still needed to determine whether these adaptive changes lead to the paradox pattern in glutamate efflux. Some studies found that the glutamate efflux also increases in the pontine locus coeruleus during naloxone-precipitated morphine withdrawal. We suspect that the glutamate efflux may take part in the development of morphine tolerance and the expression of morphine withdrawal syndrome. Possibly an experiment with pretreatment of glutamate antagonists can verify this view.

In the cortex-lesioned group, the difference of glutamate efflux between the striatum and nucleus accumbens may be due to a topographically different quantity of inputs from the cortex. We suppose that the nucleus accumbens receives its input mainly from the medial refrontal cortex but the striatum may not be significantly influenced by a local cortex lesion.

The Role of Glutamate-DA Interactions in Morphine Addiction

It is known that glutamate is the main transmitter in the corticoaccumbens circuits. Therefore, limbic cortex lesion implies removing glutamate from the nucleus accumbens. Our data show that in the nucleus accumbens, the limbic cortex lesion not only can attenuate the increasing response of glutamate but also make the decreasing response of DOPAC reduce in the withdrawal stage (Fig. 3 vs. 4). These findings tell us that there must be an interaction between the glutamatergic and DAergic transmissions. If the removal of glutamate, caused by the cortex lesion, can attenuate the decreasing response of DOPAC, it is not difficult to understand that inhibitory interneurons exist between the glutamatergic and DAergic transmissions. Thus, we can find that in the nucleus accumbens, the DOPAC level of the cortex lesioned rat is higher than the DOPAC level of the intact rat (Fig. 5) because the tonic restraint has been inactivated by the cortex lesion. On the other hand, the hypothesis is also supported by the similar findings when the prefrontal cortex is inactivated by cooling, the level of DOPAC increases but the level of glutamate decreases in the subcortical brain regions (41). Moreover, a previous study found that the increase of DAergic transmission happened in the anterior striatum after given amphetamine treatment to rats with cerebral cortex lesions (23). Perhaps it is comparable to our study.

The mechanism of interaction between glutamate and DA may be a non-synaptic association (6,7) or some other models (11). We have known that an individual dendritic spine of a striatal neuron may receive the DA input at its neck and the glutamate input at its head (33) and the striatal neurons seem to be the GABAergic interneurons observed by electromicrography (8). Moreover, Freed suggested that the glutamate released from the corticostriatal axon might act on the interneurons to influence the DAergic transmission, rather than have a direct effect upon DA terminals (18). Perhaps the extrasynaptic receptors, activated by diffusion of the neurotransmitter away from the synapse, contribute to this phenomenon.

We propose that the glutamate system exerts an inhibitory tone via interneurons on regulating the DAergic transmission in the DA terminals, i.e. stratum and nucleus accumbens. This hypothesis fits to explain the opposite responses of DOPAC and glutamate between chronic and acute morphine/naloxone treatments. If morphine is administrated to the dependent rats, it will decrease the glutamate level. Consequently, the inhibitory tone via interneurons attenuates so that the DOPAC level finally increases. However, if a large single dose of morphine is given to normal rats, it will cause a large amount of glutamate release. The great increase of glutamate efflux may spill and diffuse outside the synaptic sites, so it can directly activate the DA neurons. Finally. the DOPAC level increases because the activation is stronger than the inhibition. Obviously, the acute and chronic morphine/naloxone treatments have two different kinds of regulating systems.

Apart from the interaction between the glutamatergic and DAergic transmissions, differences in the responsiveness to drugs in the EAA system and DA system can be found in this study. Comparing the intact and cortex-lesioned rats, the changing patterns of DOPAC between the dependent and withdrawal stages are the same but those of glutamate are different in the nucleus accumbens (Fig. 5). This means that the cortex lesion does not cause any change in the responsiveness of the DA system but can attenuate the responsiveness of the corticofugal EAA system to morphine and naloxone.

In conclusion, morphine can affect the glutamatergic and DAergic systems and the latter is simultaneously influenced by the two regulating systems. Perhaps these mechanisms play a role in the occurrences of morphine dependence and withdrawal.

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