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Effect of Precipitated Withdrawal on Extracellular Glutamate and Aspartate in the Nucleus Accumbens of Chronically Morphine-Treated Rats: An In Vivo Microdialysis Study

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SEPULVEDA, M. J., L. HERNANDEZ, P. RADA, S. TUCCI AND E. CONTRERAS. *Effect of precipitated withdrawal on extracellular glutamate and aspartate in the nucleus accumbens of chronically morphine-treated rats: An in vivo microdialysis study.* PHARMACOL BIOCHEM BEHAV **60**(1) 255–262, 1998.—Excitatory amino acids release during morphine or naloxone administration was studied in rats. Microdialysis in freely moving animals and capillary electrophoresis with laser-induced fluorescence detection were used to measure several amino acids including glutamate and aspartate in the extracellular fluid at the nucleus accumbens. Perfusion with a calcium-free Ringer's solution decreased glutamate and aspartate in nucleus accumbens dialysates to 35% of its baseline levels, suggesting partial synaptic origin of these amino acids. The first morphine injection decreased glutamate and aspartate to 50% of its baseline level. After repeated morphine injections this effect disappeared, suggesting tolerance. Naloxone injections to morphine-dependent rats increased 300% glutamate and aspartate release; these experiments suggest that excitatory amino acid release in the nucleus accumbens might play a role in morphine withdrawal. © 1998 Elsevier Science Inc.

Excitatory amino acids Nucleus accumbens Morphine Naloxone Tolerance Capillary electrophoresis Microdialysis

NALOXONE injections increase glutamate and aspartate release in the locus coeruleus in morphine-dependent rats (1,9, 34). Systemic and intracerebroventricular injections of NMDA and non-NMDA glutamate receptor blockers prevent some features of the morphine withdrawal syndrome (4,24,25, 27,28,32) and intracerebroventricular injections of glutamate dose dependently induce withdrawal symptoms in morphine dependent rats (26); therefore, it has been proposed that excitatory amino acids give rise to the morphine withdrawal syndrome. However, most of this research has pointed out the pivotal role of the locus coeruleus in morphine dependence, although some evidences suggest that other brain areas might be involved as well (1). For instance, while NMDA receptor antagonists suppress several symptoms of the morphine withdrawal syndrome, the glutamate receptors of locus coeruleus neurons are not of the NMDA type (3). This incongruity explains why the naloxone-induced increase in norepinephrine turnover and locus coeruleus neuronal firing is not reversed

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by NMDA receptor antagonists (21). Therefore, it has been proposed that excitatory amino acid release in the locus coeruleus as well as elsewhere in the brain might mediate the morphine withdrawal syndrome (1).

Besides the locus coeruleus, the nucleus accumbens might be another substrate for the aversiveness of opiate withdrawal (11,23). Neurochemical changes in the nucleus accumbens following morphine or naloxone administration have been reported. Acute injections of morphine increase dopamine but decrease acetylcholine in nucleus accumbens dialysates (19, 20,32). Naloxone injections in morphine-dependent rats decrease dopamine and increase acetylcholine (17,19). Morphine injections increase the expression of the immediate early genes c-*fos* and *jun*-B in several brain areas including the nucleus accumbens (5,12) and chronic morphine administration augmented chronic Fos-related antigens and decreased Cyclic AMP responsive element binding protein in the nucleus accumbens in rats (18,29). The mRNA for mu opioid receptor expresses in the nucleus accumbens, indicating the presence of morphine-sensitive receptors in the nucleus accumbens (33). All these experiments suggest that the nucleus accumbens might play a role in the neurochemical events triggered by morphine addiction. To our knowledge, the release of excitatory amino acids in the nucleus accumbens has not been studied in morphine-dependent animals. In the present series of experiments we combined microdialysis in freely moving animals and capillary zone electrophoresis with laser-induced fluorescence detection to study the effect of morphine and naloxone injections on excitatory amino acids (glutamate and aspartate) in the nucleus accumbens.

METHOD

Subjects

Male albino rats of the Wistar strain weighing between 250 and 310 g were individually housed with food and water ad lib and a 12 D:12 L cycle.

Surgery

Under ketamine (Ketalar, Parke Davis, Venezuela) anesthesia (intraperitoneal injection at the dose of 100 mg/kg) bilateral guide cannula (made of 10-mm long pieces of 21 gauge stainless steel tubing) were stereotaxically implanted at the following coordinates: A: 1.0 mm rostral to bregma, L: 1.2 mm lateral to the midsagital suture, and V: 4.0 mm ventral to the surface of the leveled skull. These coordinates place the tip of the guide cannula 2 mm above the nucleus accumbens (16). After surgery, 200,000 units of penicillin were administered intramuscularly and at least 7 postsurgical days were allowed before starting experiments. This surgical procedure has been approved by the Bioethic Committee of Los Andes University.

Microdialysis Probes

The probes were made of cellulose tubing with 12,000 molecular weight cutoff, 200 μm outside diameter and 10 μm wall thickness (6). A piece of this tube was sealed at one end with an epoxy plug. The open end was attached to the tip of a piece of 26 gauge stainless steel tube. A piece of fused silica capillary tube with polyimide cover (150 μm outside diameter and 75 μm inside diameter) was inserted into the stainless steel and cellulose tube.

These probes protruded 5 mm off the guide cannula and the efficient dialysis length (length of the dialysis section of the probe) was 2.0 mm. The in vitro recovery of these probes was assessed by dialysing a 10^{-6} M glutamate and 10^{-6} M aspartate solution. Six probes were calibrated for recovery as follows. Each probe was connected to a perfusion system and the dialysis section was immersed in a beaker containing the standard solution. The probes were perfused at 1 μl/min and two 20-min samples were collected for each probe (12 samples total) into 400 μl Eppendorf tubes. Ten aliquots of 20 μl of standard solutions were pipetted into Eppendorf tubes.

Drugs and Reagents

Fluorescein Isothiocyanate Isomer I, sodium chloride, potassium chloride, magnesium chloride, sodium bicarbonate, sodium carbonate, and naloxone were obtained from Sigma Chemical Co. (Sigma Chemical Co., St. Louis, MO) Acetone was obtained from J. T. Baker (J. T. Baker Inc., Phillipsburg, NJ) and morphine hydrochloride was obtained from E. Merck (E. Merck AG, Darmstadt, Germany).

Microdialysis Sessions

The probes were connected to a syringe pump and perfused at a flow rate of 1 μl/min. The perfusion fluid was a Ringer solution made of 135 mM NaCl, 3.7 mM KCl, 1 mM $MgCl_2$, 1.2 mM $CaCl_2$, and 10 mM $NaHCO_3$ at pH 7.4 (adjusted with 0.1 N HCl).

Neuronal Origin of Glutamate and Aspartate

Twelve hours before the experiment, microdialysis probes were inserted in the nucleus accumbens of four rats. After collecting two baseline samples the perfusion Ringer solution was disconnected from the microdialysis probe and the perfusion system thoroughly washed with calcium-free Ringer solution. Then the microdialysis probe was connected to a syringe pump loaded with calcium-free Ringer, and seven more samples were collected.

Acute Morphine Hydrochloride Administration

The animals were not habituated to the injection procedure prior to this test. Twelve hours before the experiment the microdialysis probe was inserted in one of the guide shafts (ipsilateral guide shaft). In this experiment the rats received either 10 mg/kg of morphine hydrochloride ($n = 8$) or vehicle ($n = 7$) subcutaneously. Microdialysis samples were collected every 30 s (0.5 μl total sample volume) in hematocrit tubes sealed at one end on a flame. Five baseline samples (2.5 min) were collected before injection and 10 more samples (5 min) were collected after injection. Then sample collection stopped and was resumed 25 min later, i.e., 30 min after the injection. Seven more samples were collected (3.5 min) the microdialysis probes were withdrawn and the animals returned to their home cage.

Chronic Morphine Administration

The same animals (except one of the morphine hydrochloride group that was excluded because rejected the head assembly) received 20 mg/kg of morphine twice a day for 2 days, after which the dose was increased to 30 mg/kg twice a day for days 3 and 4, and to 40 mg/kg twice a day for days 5 and 6. On day 6 the microdialysis probe was inserted in the contralateral guide shaft to avoid probe reinsertion in the same tissue. The next day, i.e., on day 7, the injection of morphine (this time 20 mg/kg) or saline was repeated and sample collection was done following the same protocol as in the first day. Six hours later the animals received an intraperitoneal injection of 5 mg/kg of

naloxone or saline. Sample collection was carried out as in the first experiment (5 samples before injection, 10 samples immediately after injection, and 7 samples starting 30 min after the injection). Each animal was used only once, either for morphine and naloxone treatment or as a control. This sampling procedure was adopted after pilot experiments showed that the main changes of glutamate and aspartate were observed in the first 5 min after the injection.

Derivatization Procedure

The samples of the in vitro recovery experiment were reacted with 4 μl of a solution made of equal volumes of 20 mM carbonate buffer at pH 9.6 and 4×10^{-4} M fluorescein isothiocyanate in acetone. The samples were reacted with 0.2 μl of this buffer–FITC solution. After 16 h of reaction in the dark the mixtures were diluted 10 times with carbonate buffer and injected into a home-made capillary electrophoresis laser-induced fluorescence detection instrument. As a standard solution a glutamate and an aspartate 1 mM solutions were prepared and 1 ml of these solutions was reacted with 20 μl of 4×10^{-6} M Fluorescein isothiocyanate in acetone solution. After 16 h of reaction in the dark, this mixture was used for spiking the samples. For this purpose 9 μl of the amino acid solution were mixed with the sample and analyzed with the capillary electrophoresis laser-induced fluorescence detection instrument.

Capillary Electrophoresis

The instrument is made of a fused silica capillary (150 μm outside diameter and 25 μm inside diameter) filled with carbonate buffer. The ends of the capillary are immersed in buffer reservoirs. Each reservoir has an electrode made of Pt-Ir wire connected to a high-voltage power supply. A 5 mm window is opened in the capillary by burning the polyimide cover. The detector is a collinear one described elsewhere (7). Briefly, the 488 nm line of a tunable Argon-ion laser is reflected by a dichroic mirror and focused on the window of the capillary through a 0.85 NA microscope objective. The same objective collects the fluorescence and after proper filtering and with the aid of an eyepiece the fluorescence is focused on the light sensitive window of a model 928, Multialkali, Hamamatsu photomultiplier tube operated at 700 V. The signal generated by the PMT was sent to a 386 PC for data acquisition with Maxima software from Waters Co. This instrument had a mass sensitivity for FITC-amino acids in the zeptomole range (10^{-21} mol) and a concentration sensitivity of 10^{-12} M. The sample was hydrodinamically injected into the anodic end of the capillary by applying a −19 psi pulse of 0.3 s duration at the cathodic end. Electrophoresis was carried out at 20 KV. The peaks were identified by migration time and by spiking of the sample with standard solutions.

Histological Studies

After the experiments the animals were sacrificed with an overdose of chloroform and their brain perfused through the heart. The brains were then dissected out and fixed in formaline for 5 days. The brains then were frozen and sectioned in 40 μm thick slices. Then the slices were set on a macrophotography stage and transilluminated. The brain structures were distinguished by the contrast due to the low refringency of the lipids and the high transparency of the cell bodies (birefringency method). The probe tracts were easily localized by this method.

Statistical Analysis

The data were analyzed by two-way ANOVA with drug condition as between subjects factor and time as within-subjects factor.

RESULTS

At least 34 peaks were present in the electropherogram (see Fig. 1). Glutamate and aspartate were among the last peaks because of their negative charge at pH 9.6. Glutamate and aspartate were easily identified by spiking the sample (see Fig. 2). The in vitro percent recovery was 20 and 15% per mm of dialysis hollow fiber for glutamate and aspartate, respectively. The RSD of the assay was 7.9% and the confidence limits ±18.17%. The concentration of glutamate was 0.48 ± 0.1 μM and aspartate 0.32 ± 0.1 μM. The perfusion with calcium-free Ringer solution significantly decreased glutamate and aspartate, $F(1, 8) = 15.035$, $p < 0.001$) (see Fig. 3). The result of the injection of 10 mg/kg of morphine hydrochloride is shown in Fig. 4. Glutamate and aspartate decreased in the first 5 min after the morphine injection as compared to the vehicle injection [glutamate: $F(1, 15) = 6.51$, $p < 0.02$; aspartate: $F(1, 15) = 9.98$, $p < 0.006$]. This decrease did not last for the seven samples collected between 30 and 33.5 min after the injection because the controls returned to the baseline levels. Figure 5 shows that the last morphine injection (i.e., the seventh day after the beginning of the chronic treatment) had no effect on glutamate or aspartate [glutamate: $F(1, 15) = 0.13$, NS; aspartate: $F(1, 15) = 0.37$, NS]. Naloxone injection induced teeth chattering, hyperactivity, wet dog shakes, rearing, grooming, sometimes vocalization, jumping, and diarrhea. Of all these symptoms, hyperactivity was observed from the first minute after the injection of naloxone. In addition, naloxone injection significantly increased the two amino acids [glutamate: $F(1, 15) = 5.41$, $p < 0.05$; aspartate: $F(1, 15) = 11.53$, $p < 0.01$] (see Fig. 6). The increase of excitatory amino acids lasted only 5 min. Thirty minutes after the naloxone injection the levels

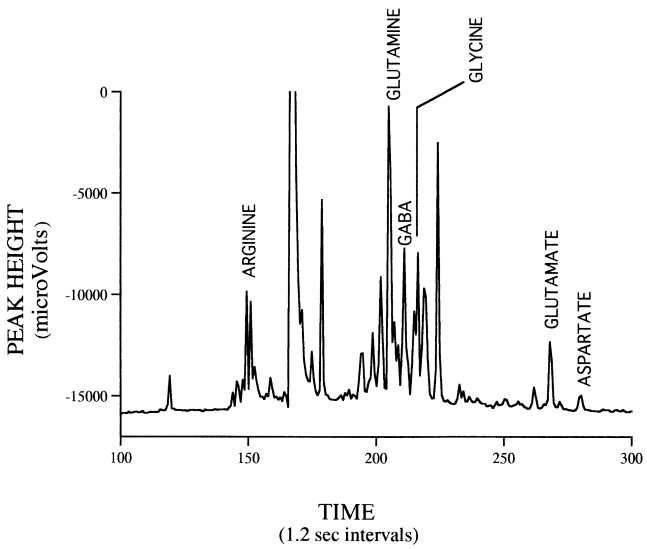

FIG. 1. Electropherogram of a dialysate. The sample shows 34 peaks. The ones corresponding to glutamate and aspartate migrate among the last peaks.

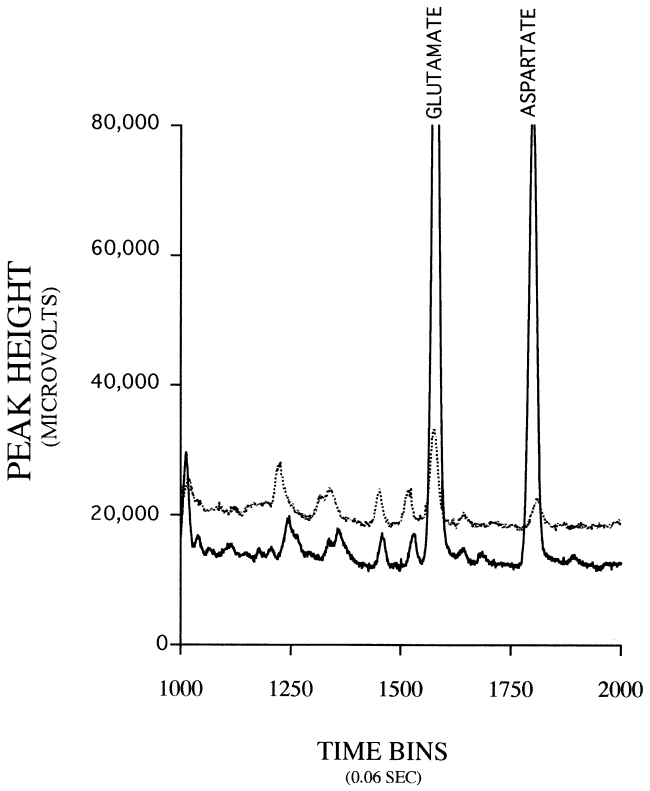

FIG. 2. Spiking the sample with glutamate and aspartate increases the putative glutamate and aspartate peaks. The electropherogram of the sample is shown by a dotted line. The electropherogram of the spiked sample is shown as a continuous line.

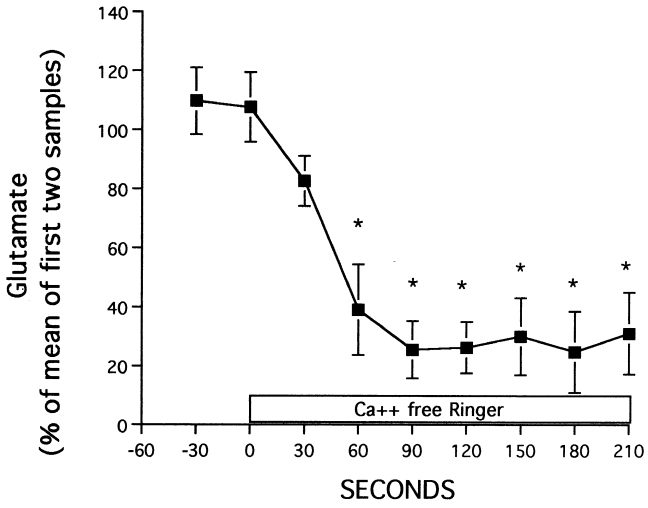

FIG. 3. Perfusion with a calcium-free Ringer's solution (horizontal bar) decreased glutamate in nucleus accumbens dialysates. Data are plotted as mean ± standard error of the mean. Asterisks indicate statistically significant difference at the 0.01 level.

of these amino acids were back to baseline, although the withdrawal symptoms had not disappeared.

The microdialysis probe tracks were located between planes 10 and 16 of the Paxinos and Watson atlas. The probes sampled from the shell and the core on the nucleus accumbens. No anatomical response pattern within the nucleus accumbens was observed.

DISCUSSION

Both glutamate and aspartate in nucleus accumbens dialysates were calcium-dependent. Perfusion with calcium-free Ringer solution significantly decreased glutamate and aspartate baseline levels. This suggests that glutamate and aspartate were at least partially of neuronal origin. Several authors have reported 20% of the glutamate in brain dialysates to be calcium dependent or no calcium dependency at all (8,15,22, 30). The larger calcium dependency reported here might be due to several technical differences between our experiment and others. We used CZE-LIFD, which have larger peak capacity than HPLC. We also used FITC as a derivatizing agent rather than Ortophtalaldehide used in other reports. FITC derivatives tend to be more stable than OPA derivatives. We carefully washed the perfusion lines to eliminate as much as possible calcium residues. In any event, comparisons of the two technical procedures need to be done but they are beyond the scope of the present report.

The present experiments suggest that morphine effects are correlated with glutamate and aspartate changes in the nucleus accumbens. The first morphine injection decreased glutamate and aspartate levels. However, this decrease seems to be a combination of no significant increase of excitatory amino acids in the control group and a significant decrease in the morphine group. Therefore, this decrease might be interpreted as less stress in the morphine-injected rats than in the vehicle-injected rats. Moreover, because the physicochemical properties of the solutions were different, it is possible that local effects of the vehicle and morphine injection could have been different. For instance, the pH of the vehicle (7,8) and the morphine hydrochloride solution (4.4) and the osmolarity as well were different. Therefore, the fast initial decrease of glutamate and aspartate might be due to sensory phenomena. If the vehicle injection caused pain and the morphine injection local anesthesia nucleus accumbens excitatory amino acids could have changed in opposite direction. In any event, this issue should be examined with 5-s time resolution microdialysis to establish the latency of excitatory amino acid variation after acute morphine administration.

Thirty minutes after the injection the glutamate and aspartate levels were similar in both the vehicle and morphine groups. By this time the controls had returned to baseline levels rendering statistically nonsignificant the difference between both groups.

The effects of morphine injection on glutamate and aspartate disappeared after repeated administration of morphine, suggesting tolerance.

Naloxone increased glutamate and aspartate levels in morphine-dependent rats, but this increase lasted shorter than the withdrawal syndrome. These findings suggest that excitatory amino acids trigger the morphine withdrawal symptoms but do not maintain them. Other neurochemical events that last longer (dopamine decrease and acetylcholine increase among other neurochemical changes) might maintain the symptoms. Because NMDA blockers suppress the withdrawal symptoms, it would be interesting to find out whether or not the injection of NMDA blockers 5 min after naloxone injection can still suppress the withdrawal syndrome.

Glutamatergic cell bodies that innervate the nucleus accumbens are located in the hippocampus and the prefrontal

cortex (2,10). Therefore, it is quite possible that the acute injection of morphine inhibits these neurons and that the chronic administration of morphine induces tolerance as shown by the lack of excitatory amino acid increase after the last morphine injection. By contrast, naloxone-induced withdrawal should activate them. Alternatively, dopamine released by morphine, or dopamine decreased by naloxone might presinaptically decrease or increase respectively

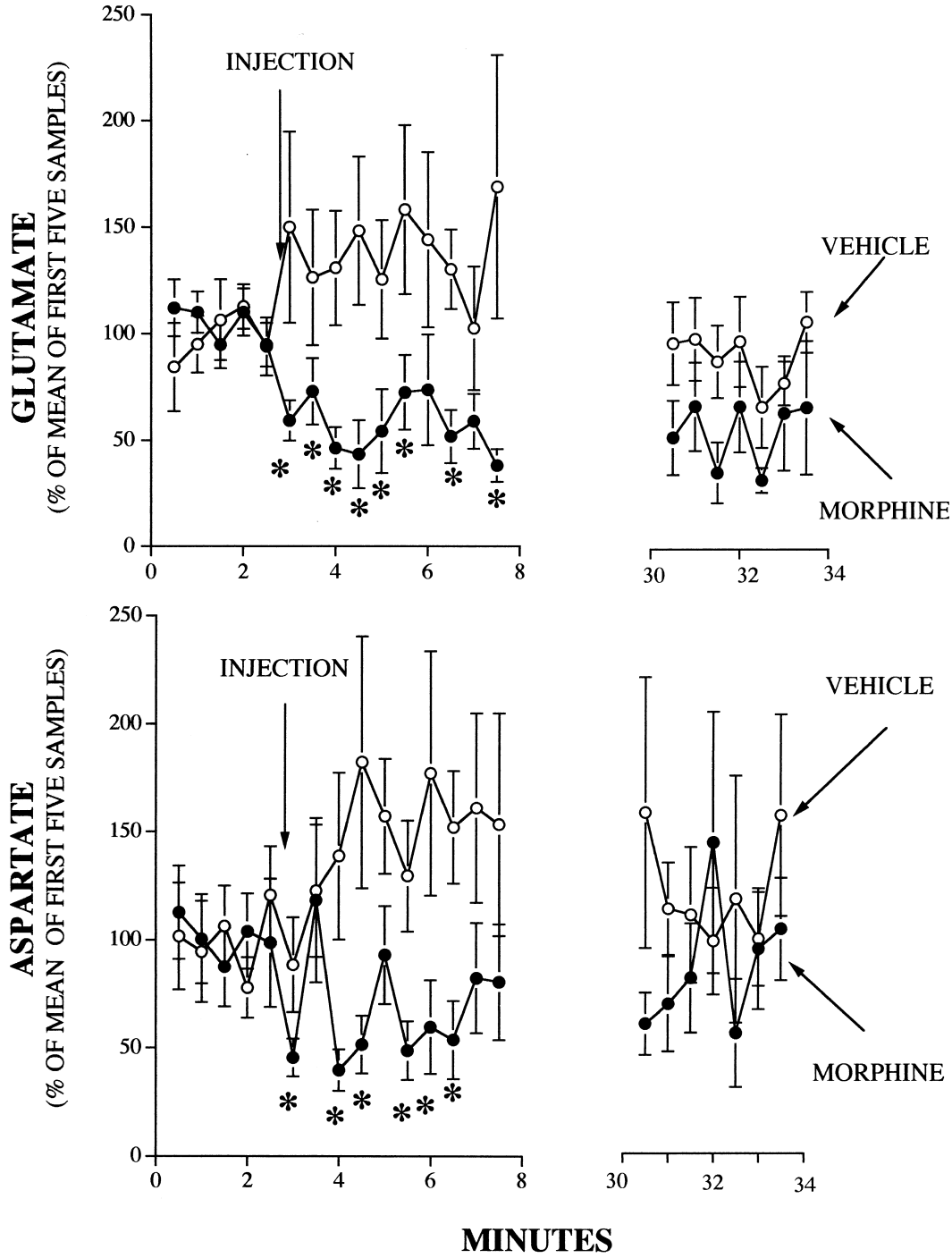

FIG. 4. Effect of the first morphine HCl injection (10 mg/kg SC) on glutamate (top) and aspartate (bottom) levels in nucleus accumbens dialysates. Glutamate and aspartate levels decreased in morphine HCl-injected rats (filled circles) compared to vehicle-treated rats (open circles). Data are expressed as percent of the mean of the five preinjection samples ± standard error of the mean. Asterisks mark the samples of morphine HCl-injected rats significantly different from the baseline at the $p < 0.05$ level.

glutamate and aspartate. An inhibitory action of dopamine on glutamate and aspartate release in the basal ganglia has been shown (13,14,31). However, the increase of dopamine after repeated morphine injections did not show tolerance. In other words, the last injection of morphine still increased dopamine. By contrast, in the present study the last morphine injection did not change glutamate or aspartate release. One possibility could be that chronic morphine might downregulate dopamine receptors, decreasing dopamine inhibition of glutamate. In any event, the role of dopamine on excitatory amino acid release in the nucleus accumbens warrants further research.

In conclusion, we have successfully used CZE-LIFD to improve the time resolution of brain microdialysis. The method allows simultaneous monitoring of glutamate and aspartate in

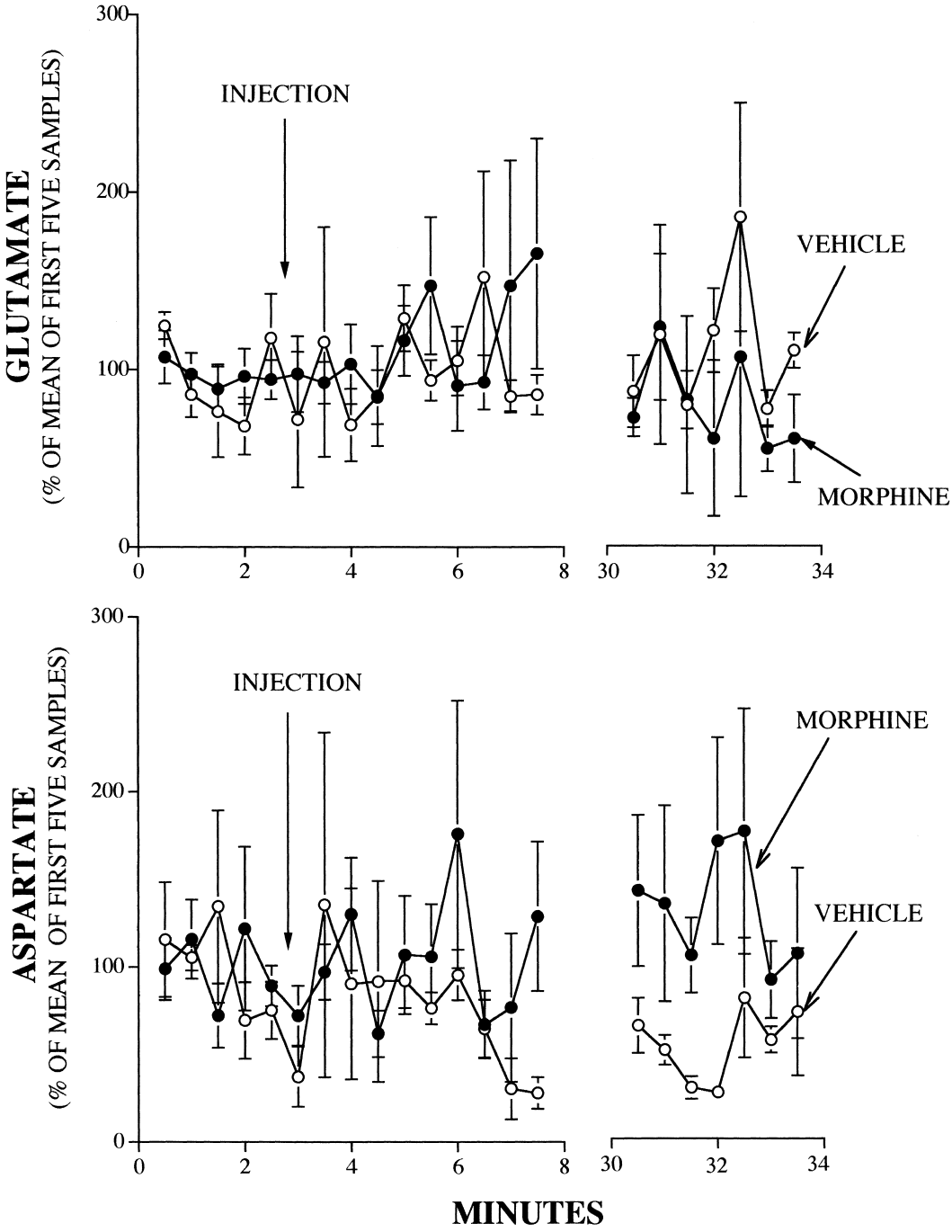

FIG. 5. Effect of the last morphine (HCl injection (20 mg/kg SC) on glutamate (top) and aspartate (bottom) levels in nucleus accumbens dialysates in morphine-dependent rats (filled circles). After chronic administration of morphine, no statistically significant difference in the concentration of either glutamate or aspartate between control (open circles) and morphine-dependent rats. Data are plotted as mean ± standard error of the mean.

a 30-s frame. The results showed that glutamate and aspartate decrease for the first 5 min after an acute injection of morphine. This effect showed tolerance. Withdrawal induced by naloxone increase extracellular glutamate and aspartate in the nucleus accumbens.

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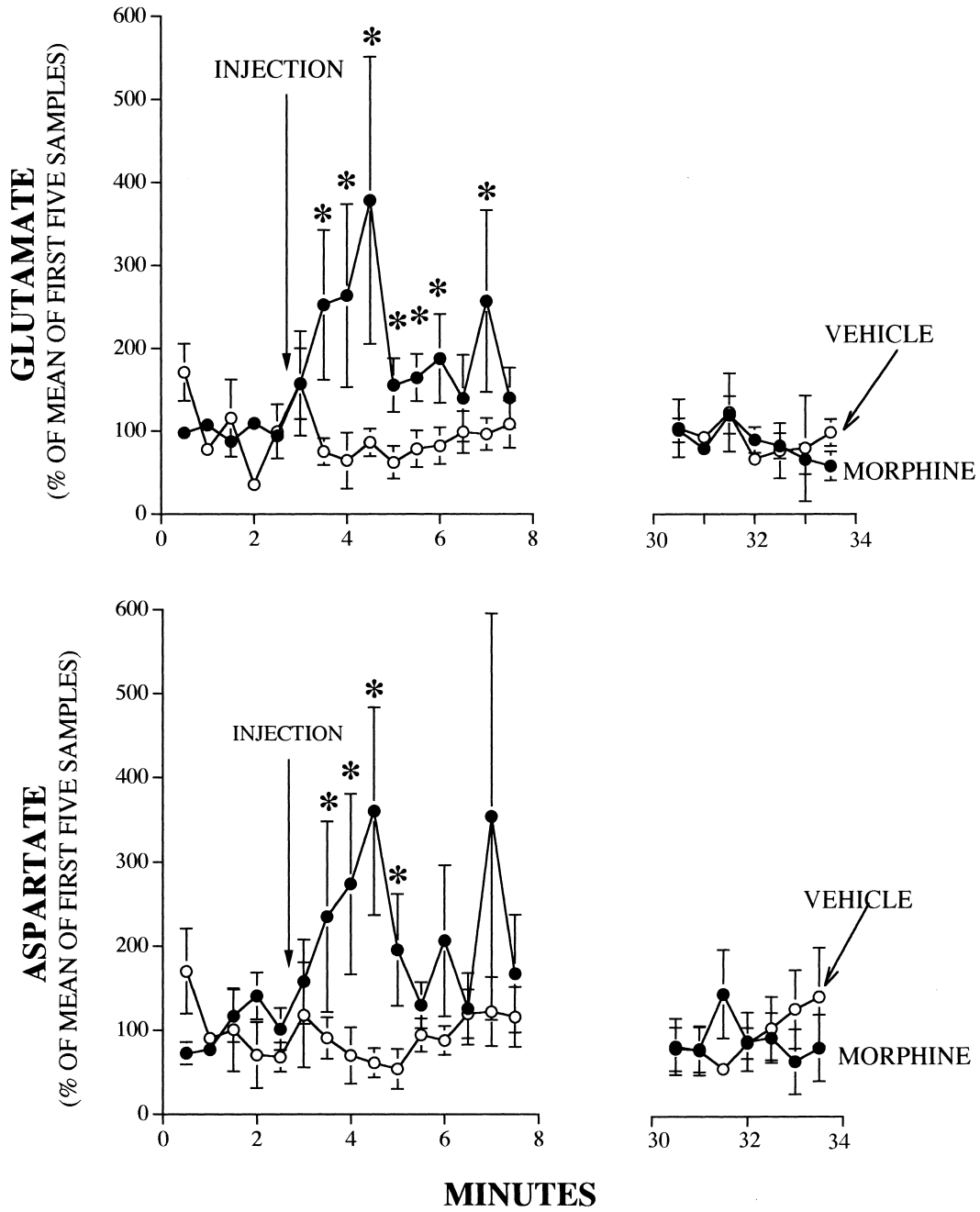

FIG. 6. Effect of naloxones injection (5 mg/kg IP) on glutamate (top) and aspartate (bottom) levels in morphine-dependent (filled circles) and control (open circles) rats. Glutamate and aspartate concentration in the dialysates increased significantly (asterisks indicate statistically significant differences at the $p < 0.05$ level) in the first 5-min after injection in morphine-dependent rats. Thirty minutes later glutamate and asparte levels were back to baseline. After naloxone injection no effect was observed in control rats. Data are plotted as mean ± standard error of the mean.

REFERENCES

1. Aghajanian, G. K.; Kogan, J. H.; Moghaddam, B.: Opiate withdrawal increases glutamate and aspartate efflux in the locus coeruleus: An in vivo microdialysis study. Brain Res. 636:126–130; 1994.
2. Beckstead, R. M. J.: An autoradiographic examination of cortical and subcortical projections of the medio-dorsal projection (prefrontal) cortex in the rat. Comp. Neurol. 184:43–62; 1979.
3. Ennis, M.; Aston-Jones, G. J.: Activation of locus coeruleus from nucleus paragigantocellularis: A new excitatory amino acid pathway in brain. Neuroscience 8:3644–3657; 1988.
4. Fundytus, M. E.; Coderre, T. J.: Effect of activity at metabotropic, as well as ionotropic (NMDA) glutamate receptors on morphine dependence. Br. J. Pharmacol. 113:1215–1220; 1994.
5. Garcia, M. M.; Brown, H. E.; Harlan, R. E.: Alterations in immediate early gene proteins in the rat forebrain induced by acute morphine injection. Brain Res. 692:23–40; 1995.
6. Hernandez, L.; Stanley, B. G.; Hoebel, B. G.: A small removable microdialysis probe. Life Sci. 39:2629–2637; 1986.
7. Hernandez, L.; Escalona, J.; Joshi, N.; Guzman, N. A.: Laser-induced fluorescence and fluorescence microscopy for capillary electrophoresis zone detection. J. Chromatogr. 559:183–196; 1991.
8. Herrera-Marchitz, M.; You, Z. B.; Goiny, M.; Meana, J. J.; Silveira, R.; Godukhin, O. V.; Chen, Y.; Espinoza, S.; Pettersson, E.; Loidl, C. F.; Lubec, G.; Andersson, K.; Nylander, I.; Terenius, L.; Ungerstedt, U.: On the origin of extracellular glutamate levels monitored in basal ganglia of the rat by in vivo microdialysis. J. Neurochem. 66:1726–1735; 1996.
9. Hong, M.; Milne, B.; Jhamandas, E.: Evidence of the involvement of excitatory amino acid pathways in the development of precipitated withdrawal from acute and chronic morphine: An in vivo voltammetric study in the rat locus coeruleus. Brain Res. 623:131–141; 1993.
10. Kelley, A. E.; Domesick, V. P.: The distribution of the projection from the hippocampal formation to the nucleus accumbens in the rat: An anterograde and retrograde-horseradish peroxidase study. Neuroscience 7:2321–2325; 1982.
11. Koob, G. F.; Wall, T. L.; Bloom, F. E.: Nucleus accumbens as a substrate for the aversive stimulus effects of opiate withdrawal. Psychopharmacology (Berlin) 98:530–534; 1989.
12. Liu, J.; Nickolenko, J.; Sharp, F. R.: Morphine induces c-*fos* and junB in striatum and nucleus accumbens via D1 and *N*-methyl-D-aspartate receptors. Proc. Natl. Acad. Sci. USA 91:8537–8541; 1994.
13. Maura, G.; Giardi, A.; Raiteri, M.: Release-regulating D-2 dopamine receptors are located on striatal glutamatergic nerve terminals. J. Pharmacol. Exp. Ther. 247:680–684; 1988.
14. Maura, G.; Carbone, R.; Raiteri, M.: Aspartate releasing nerve terminals in the rat striatum possess D-2 dopamine receptors mediating inhibition of release. J. Pharmacol. Exp. Ther. 251:1142–1146; 1989.
15. Morari, M.; O'Connor, W. T.; Darvelid, M.; Ungerstedt, U.; Bianchi, C.; Fuxe, K.: Functional neuroanatomy of the nigrostriatal and striatonigral pathways as studied with dual probe microdialysis in the awake rat—I. Effects of perfusion with tetrodotoxin and low-calcium medium. Neuroscience 72:79–87; 1996.
16. Paxinos, G.; Watson, C.: The rat brain in stereotaxis coordinates, 2nd ed. San Diego: Academic Press Inc.; 1986.
17. Pothos, E.; Rada, P.; Mark, G.; Hoebel, B. G.: Dopamine microdialysis in the nucleus accumbens during acute and chronic morphine, naloxone-precipitated withdrawal and clonidine treatment. Brain Res. 566:348–350; 1991.
18. Nye, H. E.; Nestler, E. J.: Induction of chronic Fos-related antigens in rat brain by chronic morphine adminsitration. Mol. Pharmacol. 49:636–645; 1996.
19. Rada, P.; Mark, G.; Pothos, E.; Hoebel, B. G.: Systemic morphine simultaneously decreases extracellular acetylcholine and increases dopamine in the nucleus accumbens of freely moving rats. Neuropharmacology 30:1133–1136; 1991.
20. Rada, P. V.; Mark, G. P.; Taylor, K. M.; Hoebel, B. G.: Morphine and naloxone IP or locally affect extracellular acetylcholine in accumbens and prefrontal cortex. Pharmacol. Biochem. Behav. 53:809–816; 1996.
21. Rasmussen, K.; Fuller, R. W.; Stockton, M. E.; Perry, K. W.; Swinford, R. M.; Orstein, P. L.: NMDA receptor antagonists suppress behaviors but not norepinephrine turnover or locus coeruleus unit activity induced by opiate withdrawal. Eur. J. Pharmacol. 197:9–16; 1991.
22. Semba, J.; Kito, S.; Toru, M.: Characteristics of extracellular amino acids in striatum of freely moving rats by in vivo microdialysis. J. Neural Transm. 100:39–52; 1995.
23. Stinus, L.; Le Moal, M.; Koob, G. F.: Nucleus accumbens and amygdala are possible substrate for the aversive stimulus effects of opiate withdrawal. Neuroscience 37:767–773; 1990.
24. Tanganelli, S.; Antonelli, T.; Morari, M.; Bianchi, C.; Beami, L. G.: Glutamate antagonists prevent morphine withdrawal in mice and guinea pigs. Neurosci. Lett. 122:270–272; 1991.
25. Thorat, S. N.; Barjavel, M. J.; Matwyshyn, G. A.; Barghava, H. N.: Comparative effects of N^G-monomethyl-L-Arginine and MK-801 on the abstinence syndrome in morphine-dependent mice. Brain Res. 642:153–159; 1994.
26. Tokuyama, S.; Wakabayashi, H.; Ho, I. K.: Direct evidence for a role of glutamate in the expression of the opioid withdrawal syndrome. Eur. J. Pharmacol. 295:123–129; 1996.
27. Trujillo, K. A.; Alkil, H.: Inhibition of morphine tolerance and dependence by the NMDA receptor antagonist MK801. Science 251:85–87; 1991.
28. Tung, C. S.; Grenhof, J.; Svensson, T. H.: morphine responses of rat locus coeruleus neurons are blocked by an excitatory amino acid antagonist. Acta Physiol. Scand. 138:581–582; 1990.
29. Widnell, K. L.; Self, D. W.; Lane, S. B.; Rusell, D. S.; Vaidya, V. A.; Miserendino, M. J.; Rubin, C. S.; Duman, R .S.; Nestler, R. J.: Regulation of CREB expression: In vivo evidence for a functional role in morphine action in the nucleus accumbens. J. Pharmacol. Exp. Ther. 26:306–315; 1996.
30. Xue, C. J.; Ng, J. P.; Li, Y.; Wolf, M. E.: Acute and repeated systemic amphetamine administration: Effects on extracellular glutamate, aspartate, and serine levels in rat ventral tegmental area and nucleus accumbens. J. Neurochem. 67:352–363; 1996.
31. Yamamoto, B. K.; Davy, S.: Dopaminergic modulation of glutamate release in striatum as measured by microdialysis. J. Neurochem. 58:1736–1742; 1992.
32. Yukhananov, R. Y.; Klodt, P. M.; Il'Ina, A. D.; saitsev, S. V.; Maisky, A. I.: Opiate withdrawal intensity correlates with the presence of DSLET high-affinity binding. Pharmacol. Biochem. Behav. 49:1109–1112; 1994.
33. Zastawny, R. L.; George, S. R.; Nguyen, T.; Cheng, R.; Tsatsos, J.; Briones-urbina, R.; O'Dowd, B. F.: Cloning, characterization and distribution of a mu-opioid receptor in rat brain. J. Neurochem. 62:2099–2105; 1994.
34. Zhang, T.; Feng, Y.; Rockhold, R. W.; Ho, I. K.: Naloxone-precipitated morphine withdrawal increases pontine glutamate levels in the rat. Life Sci. 55:PL25–P31; 1994.