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Neurochemical changes of the extracellular concentrations of glutamate and aspartate in the nucleus accumbens of rats after chronic administration of morphine

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Abstract

The effects of discontinuing a chronic morphine treatment on the concentrations of glutamate and aspartate were analyzed in the nucleus accumbens of unrestrained unanesthetized rats. The administration of naloxone or the cessation of morphine administration resulted in increased concentrations of glutamate and aspartate in this central nervous system area. These increased amino acid concentrations were observed a few minutes after naloxone administration and persisted in the controls 48 h after the last dose of the opiate. Morphine withdrawal was also studied in rats not injected with naloxone. In these latter animals, increased concentrations of glutamate and aspartate persisted in controls 96 h after the last dose of the opiate. Single doses of morphine, acamprosate or riluzole administered to rats previously withdrawn from chronic morphine treatment restored the amino acid concentrations to normal levels. These results suggest that the maintenance of increased levels of amino acids could be the expression of new adjustments in central nervous system neurotransmission after discontinuation of the chronic morphine treatment.

Keywords: Morphine dependence; Glutamate; Excitatory amino acid; Nucleus accumbens; Neurotransmitter; Acamprosate; Riluzole

1. Introduction

It has been established that excitatory amino acid transmission systems play a role in the development of tolerance to opiate actions as well as in the induction of dependence. These effects have been demonstrated using the administration of glutamate receptor antagonists (Trujillo and Akil, 1991; Marek et al., 1991; González et al., 1997), and of drugs that reduce glutamatergic responses, such as riluzole (Sepúlveda et al., 1999), acamprosate (Sepúlveda et al., 2002), and a glutamate transporter activator (Nakagawa et al., 2001), all of which reduce the induction of physical dependence on, and tolerance to, morphine, and decrease the signs of the naloxone-precipitated withdrawal syndrome in mice. Direct evidence for the role of glutamate in the abstinence behavior was reported by Tokuyama et al. (1996), who showed that i.c.v. administration of the amino acid to morphine tolerant rats elicited withdrawal signs similar to those evoked by naloxone. In these animals, the expression of the withdrawal syndrome induced by glutamate was suppressed by pretreatment with MK-801, a NMDA receptor antagonist.

The nucleus accumbens, an interface in the limbic motor system, is considered a pivotal brain structure for behaviorally rewarding effects of μ-opioid receptor agonists (Koob et al., 1989). Pharmacological evidence for the involvement of the nucleus accumbens in opiate reward is consistent with the anatomical localization of μ-opioid receptors in this region (Zastawny et al., 1994). In this regard, Vaccarino et al. (1985a) demonstrated that the administration of an opiate antagonist into the nucleus accumbens reduced the rewarding properties of heroin in rats.

Neurochemical changes in the nucleus accumbens following systemic morphine administration in rats have been reported by Rada et al. (1991, 1996), who showed an increase in dopamine and a simultaneous decrease in acetylcholine after a single dose of the drug. In contrast, naloxone administration to rats rendered tolerant by

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chronic morphine resulted in decreased dopamine and increased acetylcholine levels (Pothos et al., 1991; Rada et al., 1991). Furthermore, Sepúlveda et al. (1998) demonstrated an increased release of glutamate in the nucleus accumbens of morphine-tolerant rats following naloxone administration.

Martin et al. (1997) suggested that μ-opioids can regulate the level of activation of NMDA receptors in neurons of the nucleus by a balanced control at pre- and postsynaptic sites, whereas the chronic administration of opiates induced a disruption of this balance.

Nevertheless, the exact mechanisms underlying opiate dependence are still unknown. Very few studies have addressed the neurochemical interactions between opiate and excitatory amino acid transmission systems in the nucleus accumbens in animals subjected to morphine treatment and to pharmacological manipulations affecting excitatory amino acid transmission. In this study, we determined the extracellular concentrations of glutamate and aspartate in the brain nucleus of rats acutely and chronically treated with morphine, as well as during the abstinence behavior evoked by naloxone administration or by the discontinuation of the chronic morphine treatment. Our interest focussed on the analysis of the extracellular concentrations of the amino acids in rats in which the spontaneous withdrawal signs had disappeared. High levels of the amino acid concentrations could suggest the persistent presence of neurochemical events associated with a delayed abstinence syndrome. In addition, the effects of two antiglutamatergic agents, riluzole and acamprosate, were also assessed in chronically treated rats after the termination of the morphine treatment or before the precipitation of withdrawal with naloxone.

2. Materials and methods

2.1. General

Male albino rats of the Sprague–Dawley strain (weighing 250–300 g) were individually housed and maintained on a 12/12-h light–dark cycle at constant room temperature (22 ± 2 °C) with food and water ad libitum. Determinations of neurochemical responses were done in the period between 9:30 and 12:00 h. The animals were used for only one

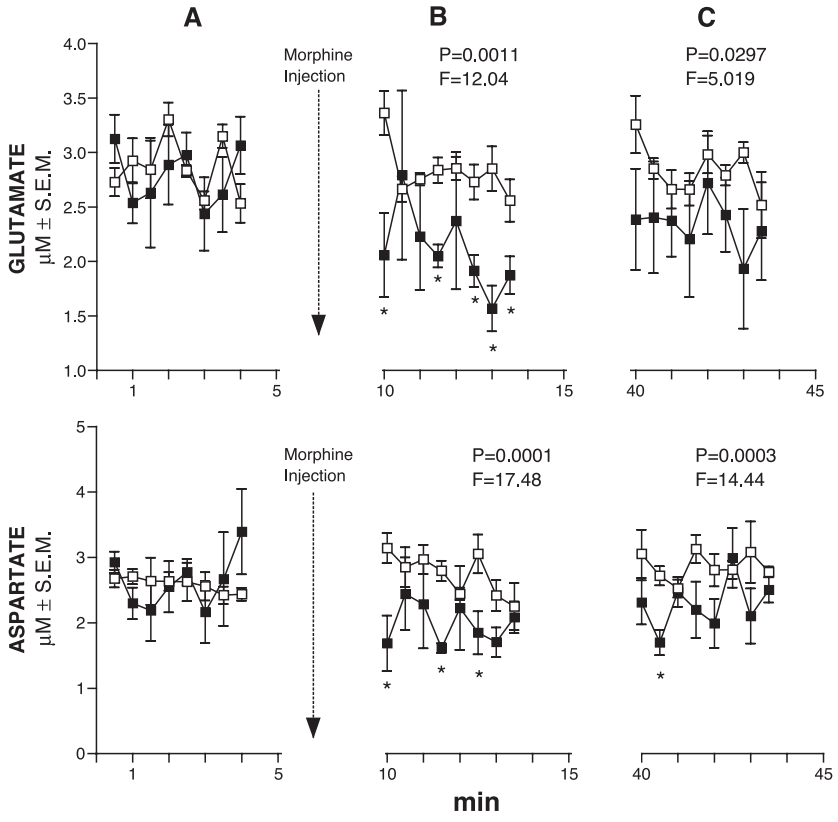

Fig. 1. Effects of morphine (10 mg/kg, i.p.) on glutamate (top) and aspartate (bottom) concentrations in nucleus accumbens dialysates of rats. The results are expressed as μM concentrations ± S.E.M. Samples obtained 5 min before treatment are shown in section A. The effects of morphine on amino acid concentrations are shown in B and C and correspond to dialysates collected during 5-min periods, 10 and 40 min after morphine. The arrows indicate the moment of drug administration. The asterisks indicate significant differences from saline-injected rats. Values of *P* and *F* are shown in the corresponding sections of the figure. $n = 4$. Values for saline-injected rats are represented by open squares (□) and those for drug-injected rats by closed squares (■). (Two-way ANOVA with drug condition as between-subjects factor and time as within-subjects factor.)

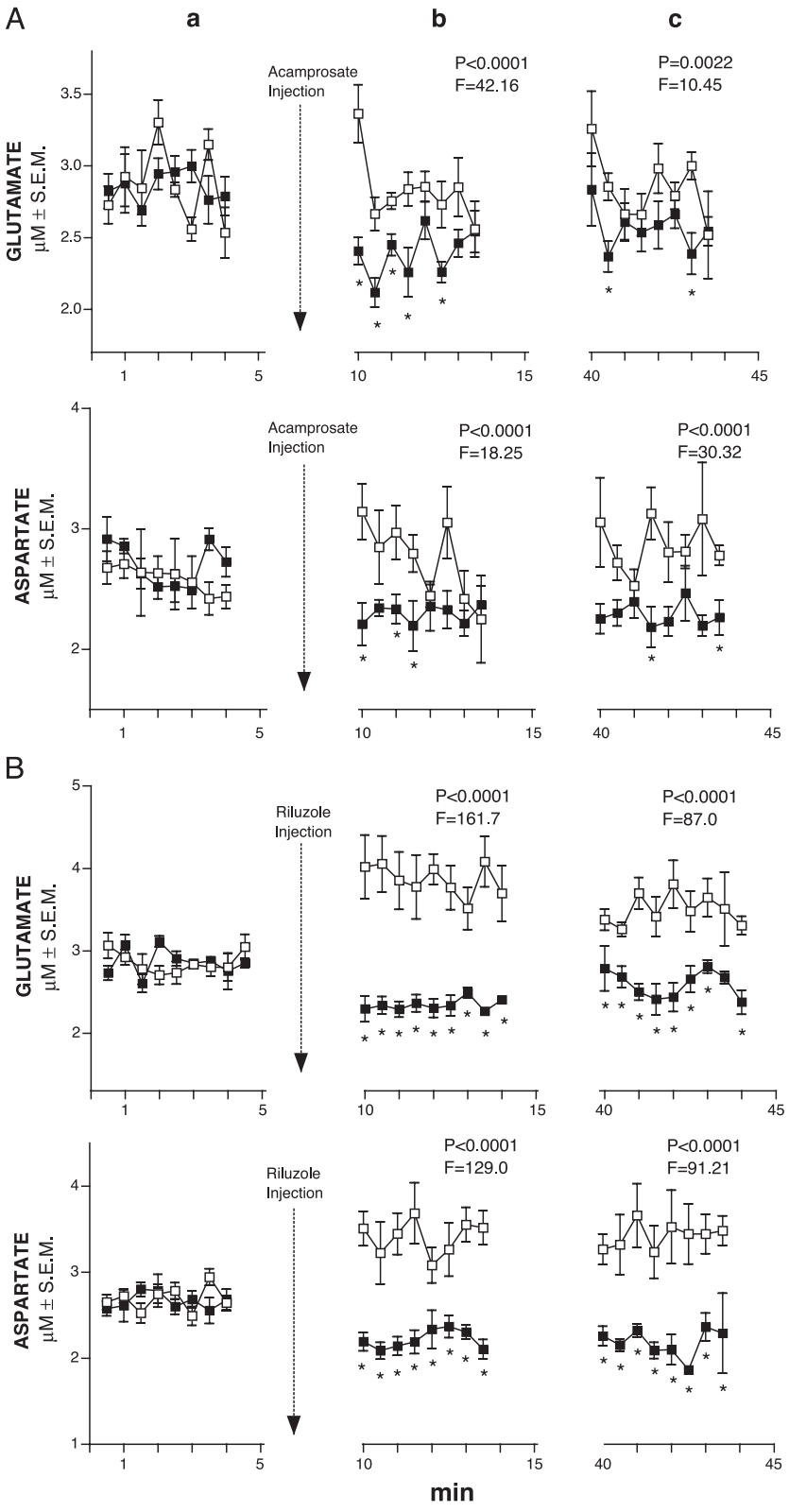

Fig. 2. Effects of acamprosate (200 mg/kg, i.p, Panel A) or riluzole (4 mg/kg, i.p., Panel B) administration on glutamate and aspartate concentrations in the nucleus accumbens dialysates of rats. The results are expressed as μM concentrations ± S.E.M. Control samples, taken over a 5-min period, are shown in section a. The effects of acamprosate and riluzole on glutamate and aspartate are shown in b and c and correspond to dialysates collected during 5-min periods, 10 and 40 min after their administration. The arrows indicate the moment of acamprosate or riluzole administration. The asterisks indicate significant differences from values for control rats. Values of *P* and *F* are shown in the corresponding sections of the figure. $n = 4$. Values for control rats are represented by open squares (□) and those for drug-injected rats by closed squares (■). (Two-way ANOVA with drug condition as between-subjects factor and time as within-subjects factor.)

experimental condition. All experiments were performed in accordance with the institutional guidelines and with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

2.2. Drugs and reagents

Acetone, fluorescein isothiocyanate isomer I, sodium chloride, potassium chloride, magnesium chloride, sodium bicarbonate, sodium carbonate, naloxone (Sigma, St. Louis, MO, USA), morphine hydrochroride (E. Merck, Darmstadt, Germany), acamprosate (Lipha, Lyon, France), riluzole (Research Biochemicals Internationals, Natick, MO, USA) were used. Drugs were dissolved in saline (except riluzole, which was dissolved in 10% Tween 80).

2.3. Surgery

Under ketamine (2 mg/kg) plus pentobarbital (60 mg/kg) intraperitoneal (i.p.) anesthesia, a guide cannula consisting of 10 mm long pieces of 21-gauge stainless steel tubing was stereotaxically implanted at the following coordinates: A: 1.0 mm rostral to the bregma; L: 1.2 mm lateral to the midsagittal suture; 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 (Paxinos and Watson, 1986). After surgery, seven post surgical days were allowed before experiments were initiated.

2.4. Microdialysis probes

The probes were made of cellulose tubing (12.000 molecular weight cut off) of 200 μm outside diameter and 10 μm wall thickness, 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 silica capillary tube with polyimide cover (150 μm outside and 75 μm inside diameter) was inserted into a stainless steel and cellulose tube. These probes protruded 5 mm from the guide cannula and the efficient dialysis length was 2.0 mm.

2.5. Microdialysis sessions

Twelve hours before the experiment, microdialysis probes were inserted in the guide shaft, connected to a

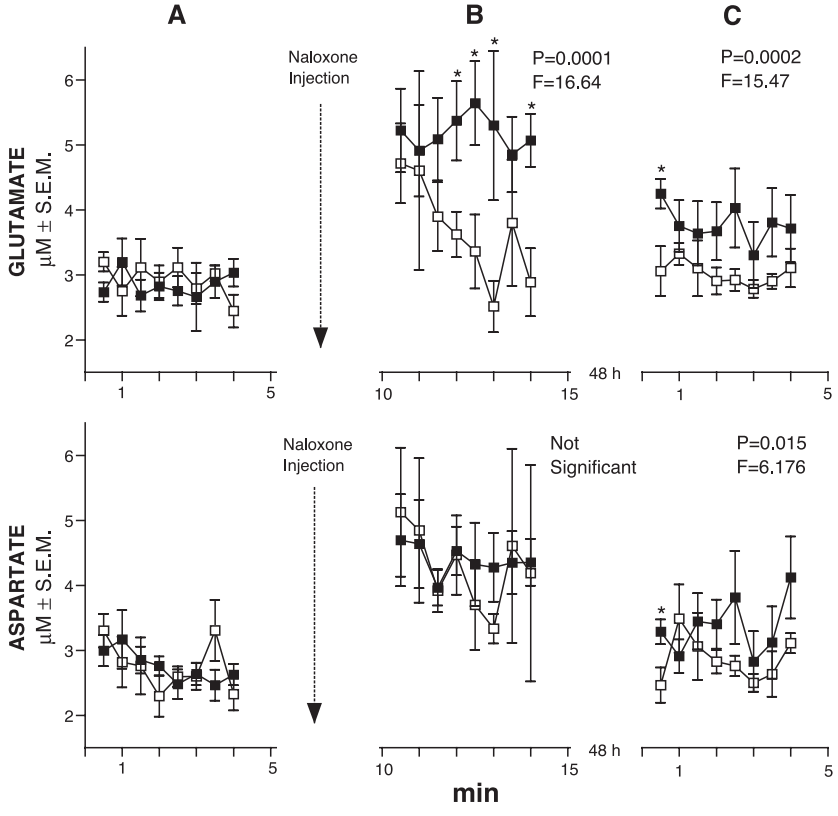

Fig. 3. Effects of acute withdrawal induced by naloxone (4 mg/kg, i.p.) in chronic morphine-treated rats (see Materials and methods) on glutamate (top) and aspartate (bottom) concentrations in the nucleus accumbens dialysates of these rats. Results are expressed as μM concentrations ± S.E.M. Samples obtained during 5-min periods before naloxone are shown in section A. The effects induced by naloxone correspond to dialysates collected during 5-min periods 10 min (B) and 48 h (C) after its administration. The asterisks indicate significant differences from saline-injected rats. Values of P and F are shown in the corresponding sections of the figure. $n = 4$. Values obtained from saline-injected rats are represented by open squares (□) and those from morphine-injected rats by closed squares (■). (Two-way ANOVA with drug condition as between-subjects factor and time as within-subjects factor.)

syringe pump and perfused at a flow rate of 1 μl/min with Ringer's solution of the following composition (mM) NaCl 135.0, KCl 3.7, $MgCl_2$ 1.0, $CaCl_2$ 1.2, and $NaHCO_3$ 10.0 (adjusted to pH 7.4 with 0.1 N HCl). Microdialysis samples (0.5 μl total sample volume) were collected every 30 s.

2.6. Derivatization procedure

The samples were treated with 0.2 μl of a solution composed of equal volumes of 20 mM carbonate buffer at pH 9.6 and 4×10^{-4} M fluorescein isothiocyanate (FITC) in acetone. After a 16-h reaction time in the dark, the samples were diluted 110-fold with carbonate buffer and injected into a homemade capillary electrophoresis laser-induced fluorescence detection instrument (CE-LIFD). Glutamate and aspartate solutions (1 mM) were prepared as standards and 1 ml of these solutions was reacted with 20 μl of 4×10^{-4} M FITC in acetone solution. After 16-h reaction in the dark, this mixture was used for spiking the samples. For this purpose 9 μl of the amino acid standard solution was mixed with the sample and analyzed by CE-LIFT.

2.7. Capillary electrophoresis

The instrument consisted of a fused silica capillary (150 μm outside diameter and 25 μm inside diameter) filled with carbonate buffer. The ends of the capillary were immersed in buffer reservoirs. Each reservoir contained a Pt–Ir wire electrode connected to a high voltage power supply. A 5-mm window was opened in the capillary by burning the polyimide cover. The collinear detector used has been described elsewhere (Hernández et al., 1991). The samples were hydrodynamically injected into the anodic end of the capillary by applying a −19 psi pulse of 0.2 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 samples with standard solutions.

2.8. Histological studies

After the experiments, the animals were killed with an overdose of ethylether, their brains were perfused through the heart, then dissected out, fixed in formalin for 5 days and subsequently frozen The brain structures and the probe tracts were localized by birefringence.

2.9. Morphine dependence

The rats were treated with increasing s.c. doses of morphine according to the following scheme: 20 mg/kg on the first and second day, the doses were increased to 30 mg/kg on the third and fourth days, and to 40 mg/kg on the fifth to seventh day. Naloxone (4 mg/kg) was administered 6 h after the last chronic morphine dose. Doses of morphine and naloxone were similar to those used in a previous study (Sepúlveda et al., 1998).

In separate experiments, three groups of rats were treated as described in the preceding paragraph, then subjected to drug deprivation starting on the seventh day. Amino acid concentrations in the nucleus accumbens of one group of animals were determined 96 h after the last dose of morphine. After sample collection, the animals received an additional dose of morphine (20 mg/kg, i.p.) and further samples were obtained 15 min later.

The two additional groups of animals were injected with acamprosate (200 mg/kg) or riluzole (4 mg/kg) 96 h after discontinuation of the chronic treatment and the amino acid concentrations in the nuclei were analyzed 15 and 60 min later. Acamprosate or riluzole were given in doses that decrease the abstinence behaviour in mice (Sepúlveda

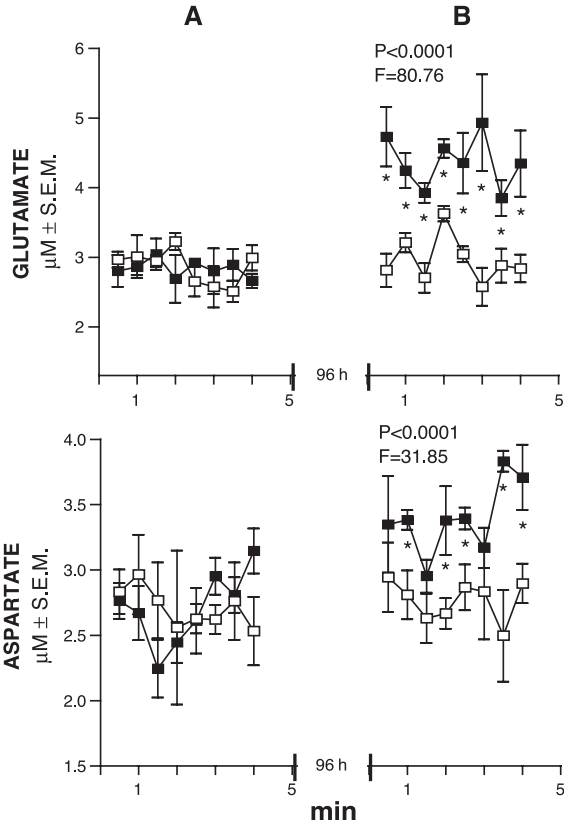

Fig. 4. Effects of morphine withdrawal on glutamate and aspartate concentrations in the nucleus accumbens dialysates of rats. The results are expressed as μM concentrations ± S.E.M. Values obtained from saline-injected rats are represented by open squares (□) and those from chronic morphine-treated rats by closed squares (■). Values shown in A correspond to amino acid concentrations observed 6 h after the last dose of saline or morphine. The effects of treatment withdrawal are shown in B and correspond to glutamate and aspartate concentrations in the nucleus accumbens dialysates of rats, collected during a 5-min period 96 h after the last dose of saline or morphine. Values of P and F are shown in the corresponding sections of the figure. $n = 4$. The asterisks indicate significant differences from the values obtained in saline-injected rats. (Two-way ANOVA with drug condition as between-subjects factor and time as within-subjects factor.)

et al., 1999, 2002) and tested in rats for the same purpose (unpublished results).

2.10. Neuronal origin of amino acids

Microdialysis probes were inserted in the nucleus accumbens 12 h before the experiment. After three baseline samples had been collected, the perfusion Ringer's solution was disconnected from the microdialysis probe and the perfusion system was thoroughly washed with calcium-free Ringer's solution. The microdialysis probe was connected to a syringe pump loaded with calcium-free Ringer, and seven more samples were collected.

2.11. Statistical analysis

The data were analyzed by two-way analysis of variance (ANOVA) with drug condition as between-subjects factor and time as within-subjects factor. Differences with P values < 0.05 or less were considered statistically significant.

3. Results

3.1. Acute morphine

The effects of acute morphine administration (10 mg/kg, i.p.) on amino acid levels in the nucleus accumbens, compared with those of saline administration, are illustrated in Fig. 1. Ten measurements were performed before treatment was started. These results are referred to as basal values and are presented as micromolar concentration of the amino acids ± S.E.M. Ten new samples were obtained 10 min after morphine administration and again after 40 min.

To test the neuronal source of glutamate and aspartate, the nucleus was perfused with calcium-free Ringer's solution. Perfusion with the calcium-free solution decreased the basal concentrations of glutamate from 2.86 ± 0.08 to

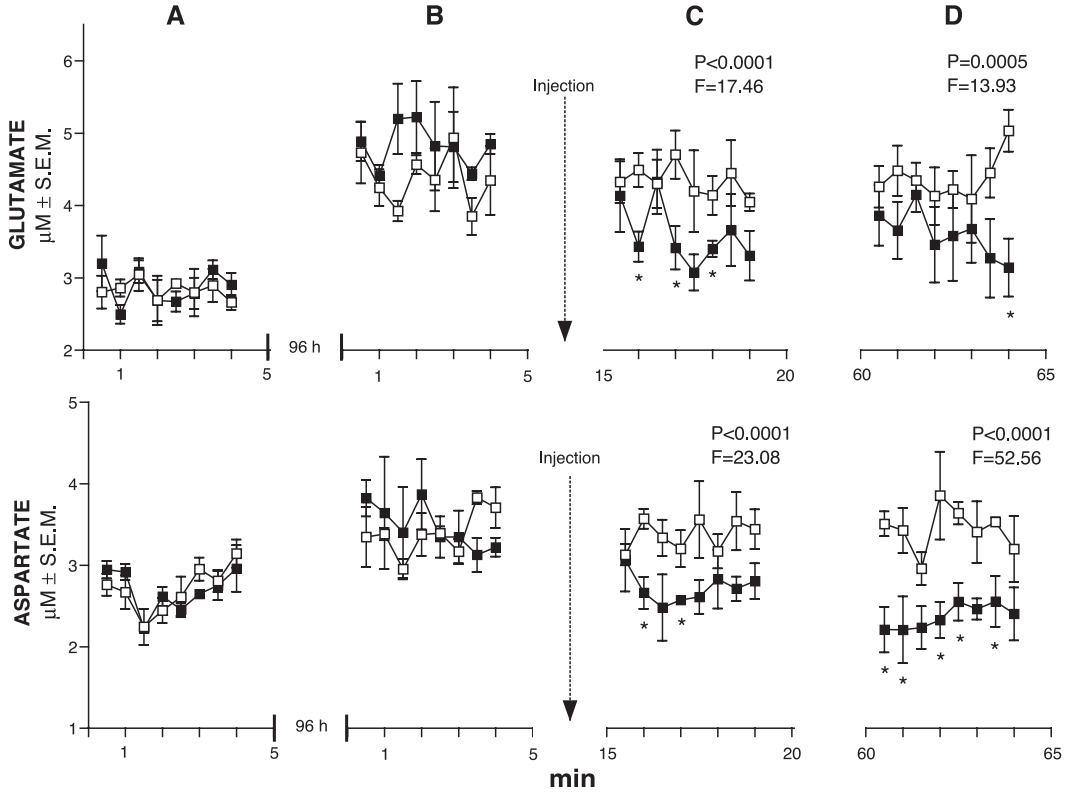

Fig. 5. Effects of the cessation of chronic morphine treatment (see Materials and methods) on glutamate and aspartate concentrations in the nucleus accumbens dialysates of rats, and their reversal induced by morphine. The results are expressed as µM concentrations ± S.E.M. Both groups of rats were chronically treated with morphine; 96 h after the last dose of the chronic treatment, the group of rats represented by closed squares received an additional dose of morphine (20 mg/kg, i.p). The group represented by open squares was injected with saline. Values shown in A correspond to amino acid concentrations observed 6 h after the last dose of morphine. Amino acid concentrations 96 h after the last dose of morphine are presented in B. Values shown in C and D are those for dialysates obtained during 5-min periods starting 15 and 60 min after the additional dose of morphine (closed squares) or saline (open squares). The results are expressed as µM concentrations ± S.E.M. The arrows indicate the moment of morphine or saline administration. The asterisks indicate significant differences from the values for saline-injected rats. Values of P and F are shown in the corresponding sections of the figure. $n = 4$. (Two-way ANOVA with drug condition as between-subjects factor and time as within-subjects factor.)

1.75 ± 0.08 μM ($F = 0.405$ and $P \leq 0.001$) and aspartate from 2.69 ± 0.15 to 1.73 ± 0.09 μM ($F = 1.53$ and $P \leq 0.001$). In both cases $n = 3$.

3.2. Effects of acamprosate (200 mg/kg, i.p.) and riluzole (4 mg/kg, i.p.) on excitatory amino acid concentrations in the nucleus accumbens

As shown in Fig. 2, both drugs reduced glutamate and aspartate levels. The effects of riluzole were greater than those of acamprosate and the concentrations of the amino acids continued to be below basal values at the end of the 40 min control period.

3.3. Effect of acute withdrawal elicited by naloxone administration on excitatory amino acid levels in the nucleus accumbens

As seen in Fig. 3, at the end of the chronic morphine treatment, and in contrast to the effects induced by an acute dose of morphine, there were no significant differences in glutamate and aspartate levels in the nucleus accumbens. Naloxone was administered 6 h after the last dose of morphine; the opiate antagonist increased the levels of these amino acids in the nuclei of these rats. The abstinence behavior observed during the first 15 min following naloxone administration consisted of piloerection, micturation, diarrhea, tremors and hypersensitivity to tactile and acoustic stimulations. Thereafter, the animals exhibited reduced motor activity, which lasted for the next 2 h. Twenty-four hours after naloxone, only increased reactions to tactile and acoustic stimulations were observed.

The concentrations of excitatory amino acids were measured 48 h after naloxone. As shown in the figure, the concentrations of glutamate and aspartate remained high when compared to the basal values at the end of the chronic morphine treatment.

3.4. Effects of cessation of morphine treatment on excitatory amino acid concentrations in the nucleus accumbens of rats

After morphine administration, in doses similar to those given to rats receiving naloxone, the animals were observed for the presence of withdrawal signs after 12, 24, 36, 48, 72

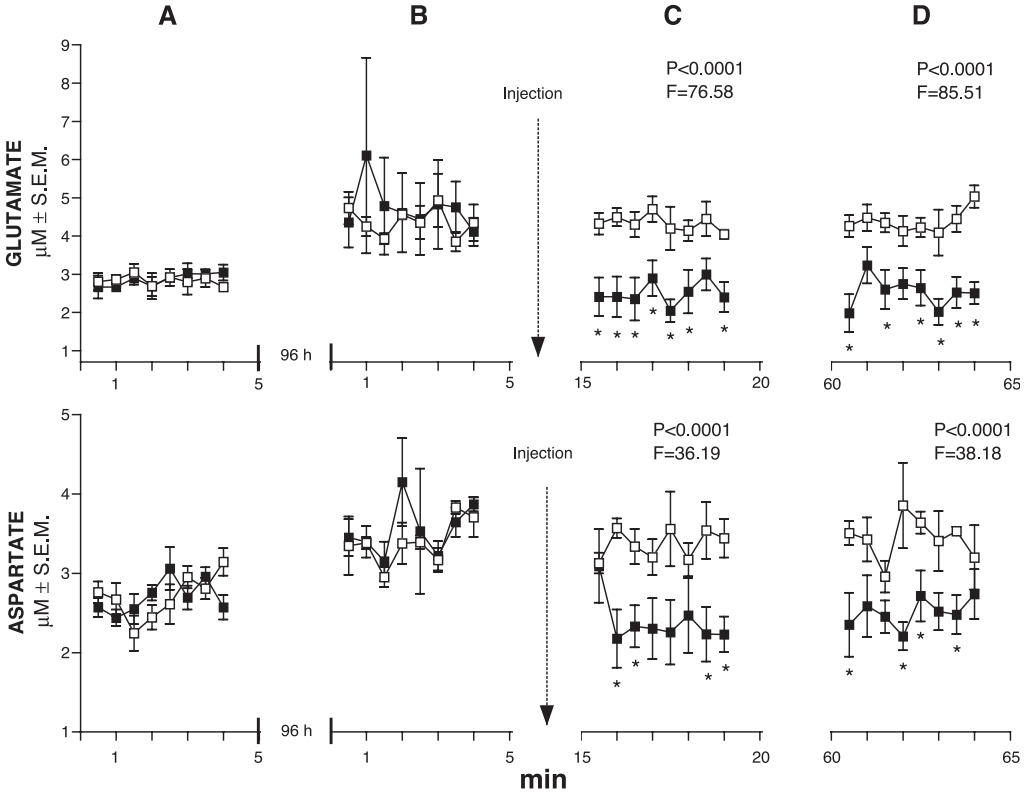

Fig. 6. Effects of acamprosate (200 mg/kg, i.p.) on glutamate (above) and aspartate (below) levels in the nucleus accumbens dialysates of rats after the cessation of chronic morphine treatment (see Materials and methods). Acamprosate or saline were administered i.p., 96 h after the last dose of morphine. Amino acid levels observed 96 h after the last dose of morphine are presented in B. Values shown in C and D are those for dialysates obtained during 5-min periods starting at 15 and 60 min after the injection of acamprosate. The results are expressed as μM concentrations ± S.E.M. The arrows indicate the moment of acamprosate or saline administration. The asterisks indicate significant differences from the values for saline-injected rats. Values of P and F are shown in the corresponding sections of the figure. $n = 4$. The symbols ■ and □ represent values for acamprosate-injected rats and for saline-injected rats, respectively. (Two-way ANOVA with drug condition as between-subjects factor and time as within-subjects factor.)

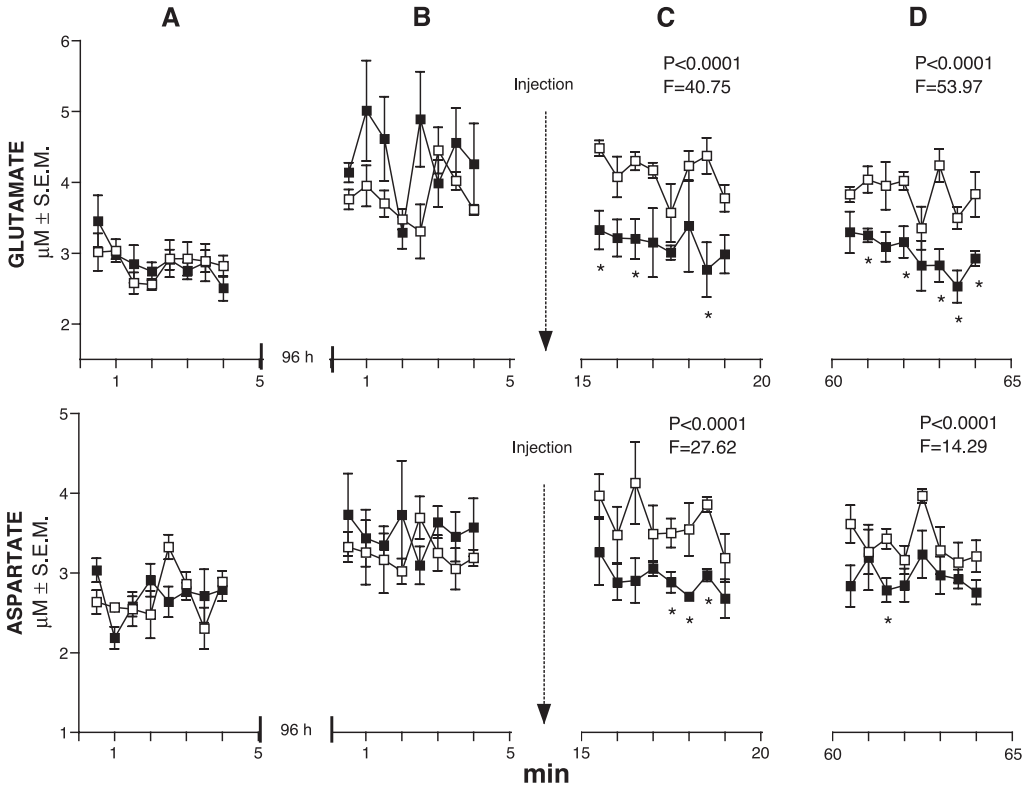

Fig. 7. Effects of riluzole (4 mg/kg, i.p.) on glutamate (above) and aspartate (below) levels in the nucleus accumbens dialysates of rats after the cessation of chronic morphine treatment (see Materials and methods). The experiments are similar to those shown in Fig. 5. The arrows indicate the moment of riluzole or vehicle administration. The asterisks indicate significant differences from the values observed in vehicle-injected rats. Values of P and F are shown in the corresponding sections of the figure. $n=4$. The symbols ■ and □ represent the values obtained in riluzole-injected rats and in vehicle-injected rats, respectively. (Two-way ANOVA with drug condition as between-subjects factor and time as within-subjects factor.)

and 96 h. After 24 h of withdrawal, the animals exhibited tremors and hypersensitivity to tactile and acoustic stimulations which persisted for 48 h.

Fig. 4 shows the concentrations of amino acids in the nucleus accumbens 96 h after discontinuation of the morphine treatment. The withdrawal of morphine treatment induced significant increases in the concentrations of both amino acids as compared to the levels observed in control saline-treated rats.

Fig. 5 shows the results observed in two groups of rats chronically treated with morphine. Amino acid concentrations in the nucleus accumbens 6 and 96 h after the last dose of the opiate are shown in Fig. 5 (panels A and B). Morphine re-administration (20 mg/kg, i.p.) to one group of rats, reduced the levels of glutamate and aspartate (panels C and D) to well below the levels in the saline-injected animals.

3.5. Effects of acamprosate and riluzole on amino acid concentrations in the nucleus accumbens of morphine-treated rats

As in the case of acute morphine administration, 96 h after the chronic morphine pretreatment, acamprosate (Fig. 6) or riluzole (Fig. 7) reduced the concentrations of the amino acids in the nucleus accumbens dialysates of rats.

4. Discussion

In this study, we showed that both discontinuation of chronic morphine treatment and naloxone-precipitated withdrawal from the opiate are accompanied by increased levels of excitatory amino acids in the nucleus accumbens. These results confirm the involvement of these neurotransmitters in the expression of the abstinence signs. The results of several studies have suggested a role for glutamate in the adaptive processes evoked by morphine administration. The nucleus accumbens has been proposed as a central nervous system area responsible for the reinforcing effects of drug abuse (Vaccarino et al., 1985a,b) and is known to have a large population of opiopeptidergic neurons and opioid receptors (Minami and Satoh, 1995; Zastawny et al., 1994).

Although the acute withdrawal syndrome is characterized by a short excitatory period, i.e. approximately 15 to 20 min, the increased excitability is followed by a longer period in which the prominent sign is a decrease in the animal's motor activity. All studies have investigated the neurochemical events during the acute excitatory phase of the with-

drawal syndrome (Mackler and Eberwine, 1994; Nestler and Aghajanian, 1997) and no reports are available concerning the delayed phase of the withdrawal process. The main interest of our study was focused on the duration of the neurochemical effects in the nucleus accumbens. Our results showed that, in contrast to the short duration of the hyperexcitability observed in rats after naloxone, the increase in amino acid levels persisted for a longer period as observed in the controls 48 h after precipitating withdrawal (Fig. 3). These effects cannot be attributed to the drug, but rather to the discontinuation of morphine administration. As observed in another group of rats, the high amino acid concentrations were still present 96 h after the last dose of morphine (Fig. 4). During the first 2 days of discontinuation of morphine injections, the presence of a mild withdrawal syndrome was observed, i.e. the animals exhibited increased responses to tactile and acoustic stimulation, whereas the other withdrawal signs, evoked by naloxone, were absent. The persistence of high concentrations of glutamate and aspartate observed 4 days after the withdrawal of morphine could be the expression of a delayed withdrawal. It can be assumed that these neurochemical events represent the development of new adjustments in neurotransmission in the nucleus accumbens and may contribute to the disphoria resulting from discontinuation of drug administration. These neurochemical effects were completely reversed by the administration of an additional dose of morphine.

The administration of the antiglutamatergic drugs, acamprosate or riluzole, reduced the release of glutamate and aspartate induced in the nucleus accumbens by morphine deprivation. The ability of these agents to restore the increased concentrations of excitatory amino acids shows that they interfere with the neurochemical processes initiated by morphine withdrawal. The mechanisms responsible for the effects of riluzole and acamprosate on glutamatergic responses are not fully understood. It is accepted that riluzole interferes with glutamate neurotransmission without affecting the binding sites of the amino acid (Debono et al., 1993), whilst acamprosate interferes with NMDA receptor-mediated glutamatergic responses (Spanagel and Zieglgänsberger, 1997). The present results showed that both agents reduce glutamate and aspartate levels observed after morphine withdrawal.

The observed effects of riluzole and acamprosate on the extracellular concentrations of glutamate and aspartate agree and support the notion that the antiglutamatergic effects are responsible for the attenuation of the withdrawal syndrome induced by these drugs in morphine-dependent animals (Sepúlveda et al., 1999, 2002). It is important to note, however, that riluzole reduces the withdrawal syndrome if co-administered with the priming doses of morphine or when given a few times before a dose of naloxone to morphine-treated mice to precipitate a withdrawal syndrome, whereas acamprosate is only effective if injected before naloxone in morphine-dependent animals. These differences can be tentatively explained (Sepúlveda et al., 2002) considering that acamprosate, in addition to its antiglutamatergic effects, also binds to type B γ-amino butyric acid (GABA) receptors (Johnston and Brown, 1983). Although an increase in GABAergic functions may augment the processes of morphine dependence (Ho et al., 1973), the observed decrease of glutamate concentrations in the nucleus accumbens is consistent with attenuation of the abstinence behaviour.

In summary and conclusion, this study demonstrated that the morphine withdrawal syndrome induced by naloxone, or by discontinuation of drug administration, results in increased concentrations of glutamate and aspartate in the nucleus accumbens of rats. These neurochemical events persist 96 h after the last dose of morphine and may reflect the slow development of new adjustments in the central nervous system. Administration of the antiglutamatergic drugs, riluzole or acamprosate, reduced the high levels of the excitatory amino acid observed during the withdrawal period studied.

Acknowledgements

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References

Debono, M.W., LeGuern, L., Canton, T., 1993. Inhibition by riluzole of electrophysiological responses mediated by rat kainate and NMDA receptors expressed in Xenopus oocytes. Eur. J. Pharmacol. 235, 283–289.

González, P., Cabello, P., Germany, A., Norris, B., Contreras, E., 1997. Decrease of tolerance to, and physical dependence on morphine by glutamate receptor antagonists. Eur. J. Pharmacol. 332, 257–262.

Hernández, L., Escalona, J., Joshi, N., Guzmán, N.A., 1991. A laser-induced fluorescence and fluorescence microscopy for capillary electrophoresis zone detection. J. Chromatogr. 559, 183–196.

Ho, I.K., Loh, H.H., Way, E.L., 1973. Influence of GABA on morphine analgesia, tolerance and physical dependence. Proc. West. Pharmacol. Soc. 16, 4–7.

Johnston, D., Brown, T.H., 1983. Interpretation of voltage-clamp measurements in hippocampal neurons. J. Physiol. 50, 464–486.

Koob, G.F., Wall, T.L., Bloom, F.E., 1989. Nucleus accumbens as a substrate for the aversive stimulus effects of opiate withdrawal. Psychopharmacology 98, 530–534.

Mackler, S.A., Eberwine, J.H., 1994. Cellular adaptation to opiates alters ion-channel mRNA levels. Proc. Natl. Acad. Sci. U. S. A. 91, 385–389.

Marek, P., Ben-Eliyahu, S., Gold, M., Liebeskind, J.C., 1991. Excitatory amino acid antagonists (kynurenic acid and MK-801) attenuate the development of morphine tolerance in the rat. Brain Res. 547, 77–81.

Martin, G., Nie, Z., Siggins, G.R., 1997. μ-Opioid receptors modulate NMDA receptor-mediated responses in nucleus accumbens neurons. J. Neurosci. 17, 11–22.

Minami, M., Satoh, M., 1995. Molecular biology of the opioid receptors: structures, functions and distributions. Neurosci. Res. 23, 121–145.

Nakagawa, T., Ozawa, T., Shige, K., Yamamoto, R., Minami, M., Satoh, M., 2001. Inhibition of morphine tolerance and dependence by MS-153, a glutamate transporter activator. Eur. J. Pharmacol. 419, 39–45.

Nestler, E., Aghajanian, G., 1997. Molecular and cellular basis of addiction. Science 278, 59–60.

Paxinos, G., Watson, C., 1986. The Rat Brain in Stereotaxic Coordinates Academic Press, New York.

Pothos, E., Rada, P., Mark, G., Hoebel, B.G., 1991. Dopamine microdialysis in the nucleus accumbens during acute and chronic morphine, naloxone precipitated withdrawal and clonidine treatment. Brain Res. 566, 348–350.

Rada, P., Mark, G., Pothos, E., Hoebel, B.G., 1991. Systemic morphine simultaneously decreases extracellular acetylcholine and increases dopamine in the nucleus accumbens of freely moving rats. Neuropharmacology 30, 1133–1136.

Rada, P.V., Mark, G.P., Taylor, K.M., Hoebel, B.G., 1996. Morphine and naloxone ip or locally affect extracellular acetylcholine in accumbens and prefrontal cortex. Pharmacol. Biochem. Behav. 53, 809–816.

Sepúlveda, M.J., Hernández, L., Rada, P., Tucci, S., Contreras, E., 1998. 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, 255–262.

Sepúlveda, M.J., Astorga, J.G., Contreras, E., 1999. Riluzole decreases the abstinence syndrome and physical dependence in morphine-dependent mice. Eur. J. Pharmacol. 379, 59–62.

Sepúlveda, M.J., Ortega, A., Zapata, G., Contreras, E., 2002. Acamprosate decreases the induction of tolerance and physical dependence in morphine-dependent mice. Eur. J. Pharmacol. 445, 87–89.

Spanagel, R., Zieglgänsberger, W., 1997. Anti-craving compound for ethanol: new pharmacological tools to study addictive processes. Trends Pharmacol. Sci. 18, 54–59.

Tokuyama, S., Wakabayashi, H., Ho, I.K., 1996. Direct evidence for a role of glutamate in the expression of the opioid withdrawal syndrome. Eur. J. Pharmacol. 295, 123–129.

Trujillo, K., Akil, H., 1991. Inhibition of morphine tolerance and dependence by the NMDA receptor antagonist MK-801. Science 251, 85–87.

Vaccarino, F.J., Bloom, F.E., Koob, G.F., 1985a. Blockade of nucleus accumbens opiate receptors attenuates intravenous heroin reward in the rat. Psychopharmacology 86, 37–42.

Vaccarino, F.J., Pettit, H.O., Bloom, F.E., Koob, G.F., 1985b. Effects of intraventricular administration of methylnaloxonium chloride on heroin self-administration in the rat. Pharmacol. Biochem. Behav. 23, 495–498.

Zastawny, R.L., George, S.R., Nguyen, T., Cheng, R., Tsatsos, J., Briones-Urbina, R., O'Dowd, B.D., 1994. Cloning, characterization and distribution of a mu-opioid receptor in the rat brain. J. Neurochem. 62, 2099–2105.