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Acute Morphine Administration Increases Extracellular DA Levels in the Rat Lateral Septum by Decreasing the GABAergic Inhibitory Tone in the Ventral Tegmental Area

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We studied the effect of an acute systemic administration of morphine and of a local intra-ventral tegmental area (VTA) infusion of the same drug on extracellular levels of dopamine (DA) in the lateral septum (LS) by in vivo microdialysis in anesthetized rats. The extracellular levels of 5-hydroxytryptamine (5-HT) were also measured in all dialysate samples. The acute systemic administration of morphine dose-dependently increased extracellular levels of DA but not of 5-HT in the LS, in the absence or presence of fluoxetine. This morphine effect was antagonized by the previous administration of naloxone, a specific opioid antagonist. The local infusion of morphine in the VTA also induced a significant increase of the extracellular levels of DA in the LS, concomitantly with a decrease of γ-aminobutyric acid (GABA) extracellular levels in the VTA itself. Intriguingly, the LS extracellular levels of DA returned to basal values before the VTA GABA extracellular levels recovered. Our results show for the first time that an acute administration of morphine increases DA extracellular levels in the LS. The results also suggest that DA cells in the VTA and innervating the LS are under an inhibitory GABAergic tone sensitive to morphine. Taken together, our neurochemical data and previous studies involving LS DA in stress-related behavior support the hypothesis that DA in the LS plays a significant role in addictive behavior. The participation of LS DA and 5-HT systems in stress-induced relapse to drug seeking should be studied further.

Key words: microdialysis; addiction; opioids; limbic system; septal nuclei

Several studies indicate that opioid receptors are critically involved in various aspects of addiction to opioid drugs as well as to several non-opioid addictive drugs (Di Chiara and North, 1992; Koob, 1992; Van Ree et al., 1999; Magendzo and Bustos, 2003). The ventral tegmental area (VTA)-nucleus accumbens pathway is considered the center of the reward system (Wise, 1980). Electrophysiologic studies (Gysling and Wang, 1983) have shown that the activation of μ-opioid receptors in VTA increases the firing rate of dopaminergic neurons by inhibiting nondopaminergic VTA interneurons. Further electrophysiologic (Johnson and North, 1992a) and neurochemical (Klitenick et al., 1992) evidence showed the γ-aminobutyric acid (GABA)ergic nature of this inhibitory tone. The activation of these VTA dopaminergic neurons induced by acute or chronic morphine treatment increases dopamine (DA) extracellular levels in the nucleus accumbens (Pothos et al., 1991; Rada et al., 1991). Similarly, morphine indirectly activates dopaminergic neurons located in the substantia nigra, increasing DA extracellular levels in the striatum (Maisonneuve et al., 2001). The lateral septum (LS) is a forebrain limbic nucleus that also receives dense dopaminergic projections from the VTA (Assaf and Miller, 1977; Gaspar et al., 1985). Compelling evidence involves the LS in physiologic processes related to cognitive function, motivation, stress, and autonomic regulation (DeFrance et al., 1976; Gray, 1977; Swanson, 1987; Thomas, 1988, Menard and Treit, 1999; Sheehan et al., 2004). In addition, pioneer studies of Olds and Milner (reviewed in Milner, 1991) included the septum as a relevant part of the reward system. Esposito et al. (1984) showed that electrical self-stimulation of VTA projecting fibers increases metabolic activity, measured by deoxyglucose utilization, in discrete VTA neuronal terminal

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fields including the nucleus accumbens, lateral septum, hippocampus and mediodorsal thalamus. Sheehan et al. (2004) have reviewed recently the evidence supporting a role of LS in addiction. The medial septum has also been involved in stress-induced relapse to drug seeking (Highfield et al., 2000). Only one previous study analyzed DA extracellular levels in LS and showed that a mild stressful stimulus such as a tactile stimulation increases DA in rat LS, supporting its role in stress-related behavior.

The septum receives a significant serotoninergic input from dorsal and medial raphe that has been implicated in different aspects of the stress response (Azmitia and Segal, 1978; Vertes, 1991; Graeff et al., 1996; Vertes et al., 1999). The infusion of corticotropin-releasing hormone (CRH) in dorsal raphe induces a bimodal effect on extracellular levels of 5-HT and in the activity of LS neurons (Price and Lucki, 2001; Price et al., 2002; Thomas et al., 2003). It has also been shown that morphine activates 5-HT-containing neurons from dorsal but not medial raphe, diminishing an endogenous GABAergic inhibitory tone paralleling the effects in the VTA (Tao and Auerbach, 1996, 2002a,b, 2003). In accordance, morphine increases extracellular levels of 5-HT in nucleus accumbens and striatum, two nuclei innervated by the dorsal raphe, but not in the hippocampus, which is innervated by the medial raphe.

The aim of the present work was to study further the effect of systemic and local intra-VTA administration of morphine on extracellular DA levels in LS. GABA extracellular levels in VTA were also studied. Additionally, extracellular 5-HT levels were measured in all dialysate samples. One microdialysis probe was placed in the LS to measure DA and 5-HT extracellular levels, and another in the VTA to infuse morphine and at the same time measure extracellular GABA levels. All experiments were carried out in chloral hydrate-anesthetized rats. A preliminary account of this work has been presented elsewhere (Gysling et al., 2002).

MATERIALS AND METHODS

Reagents and Drugs

DA, 5-HT, EDTA, and 1-octanesulfonic acid were purchased from Sigma Chemical Co. (St. Louis, MO), and naloxone was obtained from RBI (Natick, MA). Fluoxetine hydrochlorate was donated by Ahumada Pharmacies Co. (Santiago, Chile). Morphine sulfate was obtained from the Public Health Institute of Chile (ISP; Santiago, Chile). All other reagents were of analytical grade.

Animal Preparation

Male Sprague-Dawley rats weighing 250–285 g were anesthetized with choral hydrate (400 mg/kg, intraperitoneally [i.p.]) and placed in a stereotaxic apparatus (Stoelting, Wood Dale, MA). Corporal temperature of the animals was maintained at 37°C with an electrical blanket controlled by a thermostat. A quarter of the initial dose of choral hydrate was given every hour to maintain the animal anesthetized during the course of the experiments. All experiments were conducted in accordance with institutional (Catholic University of Chile) and international guidelines (NIH Guide for the Care and Use of Laboratory Animals).

Administration of Morphine, Naloxone, and Fluoxetine

Systemic administrations of morphine and naloxone were made intravenously (i.v.) in one of the tail lateral veins. Morphine (2.5 and 5 mg/kg) was injected over 2 min. The higher dose of morphine (10 mg/kg) was injected through the tail vein in 10 min. To study the effect of naloxone, 0.5 mg/kg of the drug were administered 10 min before morphine injection. In some experiments, fluoxetine (1 μM) was administered by continuous perfusion through the microdialysis probe during the development of the experiment. For the local intra-VTA administration of morphine, the drug was perfused through a microdialysis probe placed in the VTA that also served to collect fluid for determining extracellular GABA levels. The presence of morphine in the perfusion solution did not interfere with the determination of GABA (data not shown).

Experimental Procedure for In Vivo Microdialysis

Rats were anesthetized deeply; the skull exposed by a skin incision and a small hole was drilled in the area overlying the LS, VTA, or both. Concentric brain microdialysis probes (2-mm membrane; model MD-2200; BAS, West Lafayette, IN) were implanted in LS, VTA, or both using the following coordinates according to the atlas of Paxinos and Watson (1986): LS, 0.20 mm anterior to bregma, 0.8 mm lateral and 5.0 mm ventral to the dura surface; VTA, 6.0 mm posterior to bregma, 0.7 mm lateral and 8.0 mm ventral. In initial pilot experiments, the following coordinates for LS were also used: 0.30 mm posterior to bregma, 0.8 mm lateral and 5.0 mm ventral. During implantation and throughout the experiment, microdialysis probes were perfused with Krebs-Ringer's phosphate buffer (KRP) at a rate of 1 μl/min with or without fluoxetine (1 μM) using a Harvard infusion pump (Model 22; Dover, MA). The composition of the KRP buffer was 120 mM NaCl, 2.4 mM KCl, 1.2 mM $CaCl_2$, 0.9 mM NaH_2PO_4, and 1.4 mM Na_2HPO_4, adjusted to pH 7.4. After a stabilization period of 90 min, nine perfusion samples of 10 min each were collected in 2 μl of an antioxidant mix (Kankaanpaa et al., 2001). Perfusion samples were maintained on ice during the experiment and stored at −80°C until analysis.

At the end of each experiment, animals were sacrificed and brains quickly removed and stored in formalin. Brain sections of 50 μm were stained with cresyl violet to verify probe location. Placement of the probe was examined microscopically.

Analysis of Dialysate Samples

Quantification of DA and 5-HT. Using a Rheodyne injector valve, 5 μl of dialysate were injected into a high performance liquid chromatography (HPLC) system (BAS, West Lafayette, IN) with the following configuration: a pump (model PM-80), a SepStick microbore column, and an amperometric detector (model LC-4C). The HPLC mobile phase, containing 0.1 M NaH_2PO_4, 1.8 mM 1-octanesulfonic acid, and 1 mM EDTA (pH adjusted to 2.3) was pumped at a

TABLE I. Effect of Perfusion With Fluoxetine (1 μM) on Extracellular Levels of DA and 5-HT in Lateral Septum[+]

Treatment	DA (fmol/μl)	5-HT (fmol/μl)
Control	0.99 ± 0.02	2.38 ± 0.04
Fluoxetine (1 μM)	1.62 ± 0.04[*]	4.16 ± 0.04[*]

[+]Fluoxetine (1 μM) was perfused through the microdialysis probe throughout the experiment. At 90 min after the insertion of the probe, nine basal samples of 10 min each were collected. Extracellular levels of dopamine (DA) and 5-HT were quantified in each sample. Results are the mean ± standard error of the mean of four independent experiments. [*]$P < 0.0001$ compared to the respective control value.

flow rate of 80 μl/min. The potential of the amperometric detector was set at 0.650 V. Under these experimental conditions, retention times were 6.80 min for DA and 19.10 min for 5-HT. Dialysate samples were analyzed by comparing their peak area and elution times with reference standards. The detection limit for DA and 5-HT was 0.1 fmol/μl, allowing measurement of baseline levels of DA and 5-HT at both rostrocaudal levels of the LS studied.

Quantification of GABA. GABA in the perfusates was assayed as described previously (Forray et al., 1999) using the method of Lindroth and Mopper (1979), i.e., precolumn derivatization and HPLC coupled with fluorometric detection. Briefly, 5 μl of dialysis perfusate were mixed with 5 μl of KRP, 2 μl of borate buffer (pH 10.8), and then the mixture was derivatized by adding 2 μl of fluorogenic reagent (20 mg of orthophthaldehyde and 10 μl of β-mercaptoethanol in 5 ml of ethanol). At 90 sec after derivatization, samples were injected into a HPLC system with the following configuration: a one-piston minipump (LDC Milton Roy, Riviera Beach, FL), a C-18 reverse phase column (LichroCART 125-4; Merck, Darmstadt, Germany), and a fluorometric detector (Fluoromonitor III; LDC Milton Roy). An isocratic gradient was used. A mobile phase containing 0.1 mM NaH_2PO_4, 1.0 mM EDTA, and 26% CH_3CN (pH 5.8) was pumped at a flow rate of 0.7 ml/min during 20 min. To eluate the rest of the fluorescent products, a washing solution containing 50/50 CH_3CN/H_2O (vol/vol) was pumped for a 5-min period before the next sample. Fluorometer filters were configured for an excitation wavelength of 340–380 nm and an emission wavelength of 418–700 nm. Under these experimental conditions, the retention time for GABA was 12 min.

Calculations and Statistical Analysis

Data are presented as absolute values except for Figure 2. Each value corresponds to the mean ± standard error of the mean (SEM) of three to five independent experiments. The values shown in Table I were statistically analyzed by unpaired *t*-test. Data for the different morphine doses in Figure 2 were plotted as percentage of the respective basal level. Data in Figures 3 and 4 were analyzed statistically by one-way analysis of variance (ANOVA) followed by Newman-Keuls post-hoc test. All statistical analysis was carried out with GraphPad Prism v3.0 (GraphPad Software, San Diego, CA).

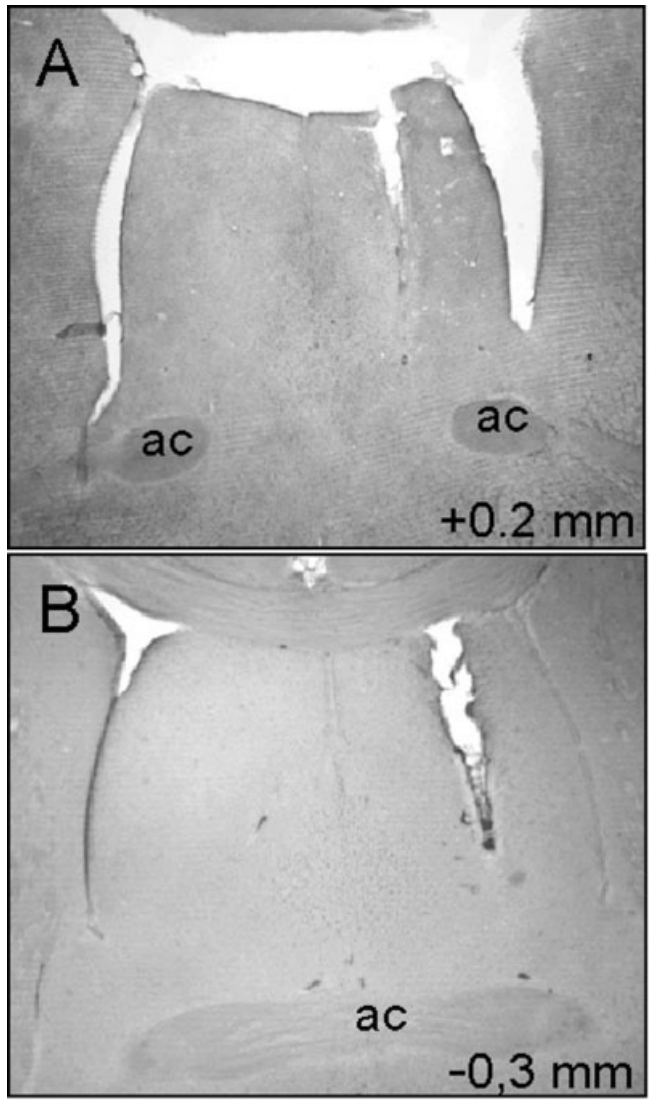

Fig. 1. Typical placement of microdialysis probe in lateral septum at each rostrocaudal level from bregma studied; ac, anterior commissure. **A:** +0.2 mm. **B:** −0.3 mm.

RESULTS

Basal Extracellular DA and 5-HT Levels in the LS at Two Rostrocaudal Levels

KRP in the presence or absence of 1 μM fluoxetine was perfused through microdialysis probes located in the posterior LS at two rostrocaudal levels from bregma (+0.2 and −0.3 mm; Fig. 1). After a stabilization period of 90 min, five samples of 10 min each were collected and DA and 5-HT were measured in each sample. Basal DA levels in dialysates collected from LS at +0.2 mm and −0.3 mm from bregma were 0.97 ± 0.05 and 0.42 ± 0.08 fmol/μl, respectively ($n = 5$; $P < 0.05$). Basal levels of 5-HT in dialysates collected from LS at +0.2 mm and −0.3 mm from bregma were 2.38 ± 0.04 and 0.28 ± 0.16 fmol/μl, respectively ($n = 5$;

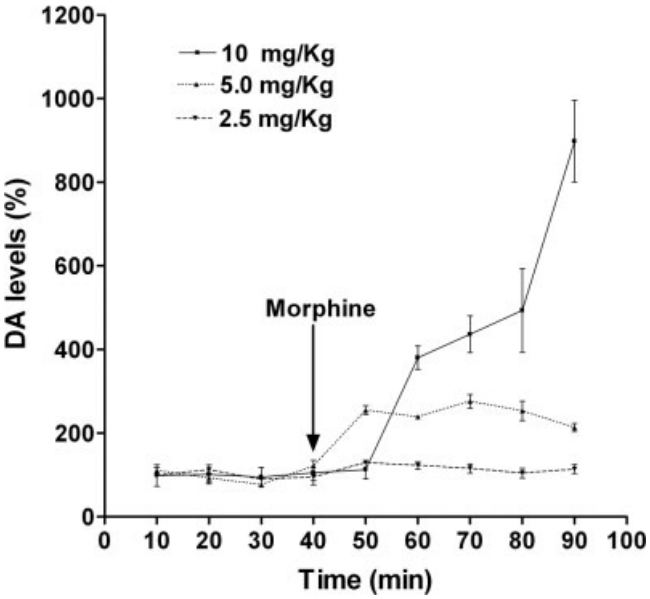

Fig. 2. Acute intravenous administration of morphine induced a dose-dependent increase in extracellular dopamine (DA) levels in lateral septum (LS). A microdialysis probe was placed in the LS at +0.2 mm from bregma (shown in Fig. 1A). After a stabilization period of 90 min, samples were collected every 10 min and DA and 5-HT were measured. Morphine (2.5 and 5 mg/kg) was injected through a lateral tail vein over a period of 2 min, at the time indicated by the arrow. In the case of the higher dose of morphine (10 mg/kg), the drug was infused slowly through the tail vein, during 10 min to avoid lethal high concentrations of the drug. Values are expressed as percentage of the respective basal levels of three to five independent experiments.

$P < 0.05$). Both DA and 5-HT extracellular levels were thus significantly higher at the more rostral level of the posterior LS studied. Subsequently, all the experiments were carried out at 0.20 mm anterior to bregma.

Extracellular DA and 5-HT Levels in the LS After Acute Systemic Administration of Morphine

As can be seen in Figure 2, acute systemic administration of morphine induced a dose-dependent increase in extracellular DA levels in the LS. Morphine (2.5 mg/kg, intravenously) significantly increased extracellular DA levels in LS from 1.39 ± 0.07 to 1.82 ± 0.01 fmol/μl ($P < 0.005$). Extracellular 5-HT levels in the same samples did not change significantly (data not shown). Morphine (5 mg/kg, i.v.) further increased DA extracellular levels in LS. This increase was significantly higher than was the increase induced by morphine (2.5 mg/kg). Again, extracellular 5-HT levels in the same samples did not change significantly (see Fig. 3B). The effect of morphine (10 mg/kg, i.v.) was also studied. In this case, morphine was infused over a period of 10 min to avoid animal death due to a rapid increase in blood morphine levels. In this condition, a further significant increase again was observed in extracellular DA but not 5-HT levels in the LS (Fig. 2).

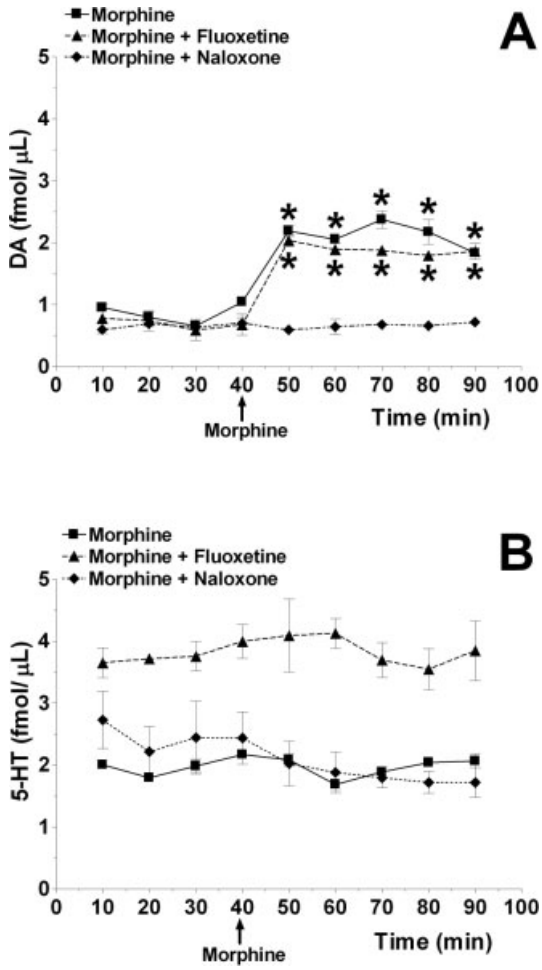

Fig. 3. Effect of acute intravenous administration of morphine on extracellular dopamine (DA; **A**) and 5-HT (**B**) in the lateral septum (LS). A microdialysis probe was placed in the LS at +0.2 mm from bregma. After a stabilization period of 90 min, samples were collected every 10 min and DA and 5-HT were measured. Morphine (5 mg/kg) was administered at the time indicated by the arrow. Naloxone (0.5 mg/kg, intravenously) was administered 10 min before morphine. In some cases, fluoxetine (1 μM) was perfused through the dialysis probe during the entire experiment. Values are the mean ± standard error of the mean of three to five independent experiments. *$P < 0.001$ compared to respective basal levels.

Effect of Fluoxetine and Naloxone on Basal and Morphine-Induced Extracellular DA and 5-HT Levels in the LS

As shown in Table I, perfusion through the probe with fluoxetine (1 μM) significantly increased basal extracellular levels of DA and 5-HT in the LS compared to that in control rats. As shown in Figure 3A, the acute administration of morphine (5 mg/kg, i.v.) in the absence and presence of fluoxetine (1 μM) in the dialysate significantly increased extracellular DA levels in the LS. Even in the presence of fluoxetine (1 μM) that significantly increased basal LS 5-HT levels, however, the administration of morphine (5 mg/kg, i.v.) did not

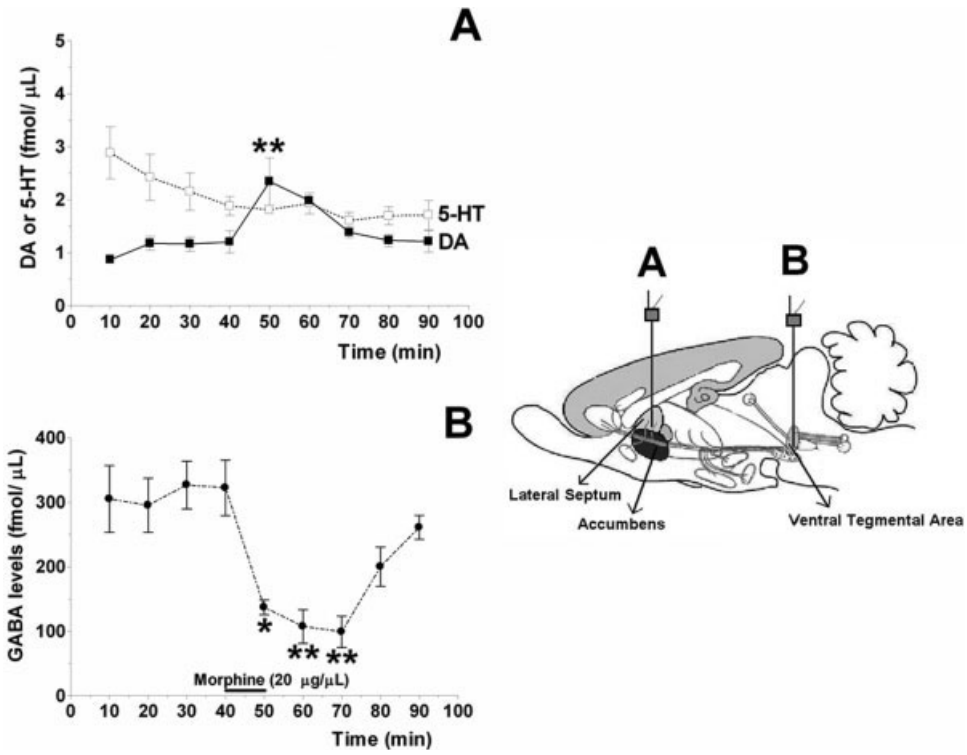

Fig. 4. Effect of intra-ventral tegmental area (VTA) infusion of morphine through a microdialysis probe on the extracellular dopamine (DA) and 5-HT levels in lateral septum (LS), and extracellular of γ-aminobutyric acid (GABA) levels in VTA. A microdialysis probe was placed in VTA and another in LS of the same animal, as depicted in the sagittal schema. **A:** Extracellular levels of DA and 5-HT in LS. **B:** Extracellular levels of GABA in VTA. Morphine (20 µg/µl) was perfused through the probe placed in VTA during 10 min as shown. Values are the mean ± standard error of the mean of five independent experiments. $*P < 0.05$ compared to basal levels. $**P < 0.001$ compared to respective basal levels.

significantly change extracellular 5-HT levels in the LS (Fig. 3B). The administration of naloxone (0.5 mg/kg, i.v.) 10 min before morphine administration completely antagonized the increase in extracellular DA levels induced by morphine (5 mg/kg, i.v.; Fig. 3A). The presence of naloxone did not induce significant changes in basal extracellular DA and 5-HT levels (Fig. 3A,B).

Extracellular DA and 5-HT Levels in the LS After Local Infusion of Morphine in the VTA

A dual microdialysis probe approach (see diagram in Fig. 4) was used to study the effect of intra-VTA morphine infusion on extracellular DA levels in LS as well as GABA levels in the VTA. The probe located in the LS (Fig. 4A) was used to collect extracellular fluid to measure DA and 5-HT levels. The second probe located in the VTA (Fig. 4B) was used to infuse morphine (20 µg/µl during 10 min) and at the same time to collect samples of extracellular fluid from the VTA to measure GABA levels. As shown in Figure 4A, the local infusion of morphine (20 µg/µl) intra-VTA through the microdialysis probe placed in the area significantly increased DA extracellular levels in LS. As expected, 5-HT extracellular levels in the same dialysate samples were not significantly changed (Fig. 4A). As shown in Figure 4B, the local infusion of morphine (20 µg/µl) intra-VTA induced a significant decrease in extracellular GABA levels in the VTA, concomitantly with the observed increase in DA extracellular levels in the LS (Fig. 4A). Notably, the extracellular DA level in the LS returned to basal value before the VTA extracellular GABA level was recovered (Fig. 4A,B).

DISCUSSION

Since the early work of Olds and Milner (reviewed in Milner, 1991), the LS was recognized as a relevant anatomic site for the sustaining of motivated behavior. Additionally, this brain region receives a significant dopaminergic innervation from the VTA, another key anatomic site for reward and addictive behavior. Few studies, however, have been focused in the LS dopaminergic system. Our results show for the first time that an acute systemic administration of morphine induces a significant and dose-dependent increase in DA extracellular levels in the LS at the rostrocaudal level studied. The results also show that the local infusion of morphine in the VTA induces a rapid and significant increase in LS extracellular DA levels, concomitantly with a decrease in VTA extracellular GABA levels.

It has been known for several years that acute morphine increases the firing rate of VTA dopaminergic neurons by decreasing a normal inhibitory tone (Gysling and Wang, 1983) of GABAergic nature (Kalivas et al., 1990; Johnson and North, 1992a,b; Klitenick et al., 1992; Henry et al., 1992). These VTA dopaminergic neurons projects to the nucleus accumbens and the prefrontal cortex, limbic areas studied extensively for their participation in the rewarding properties of substance abuse and in natural rewards (reviewed in Mathon et al., 2003). These VTA dopaminergic neurons also project to the LS (Assaf and Miller, 1977); however, there is only

one previous report studying extracellular DA levels in the LS. In this study, it was shown that tactile stimulation, a mild stressful stimulus, increases extracellular DA levels in a posterior level of the LS (Adams and Moghaddam, 2000). Extracellular DA levels thus would be regulated by stress and by morphine. Consistent evidence supports the hypothesis that the LS plays a relevant role in the reinforcing properties of addictive drugs as well as of natural reinforcers (carefully reviewed by Sheehan et al., 2004). Furthermore, the three limbic nuclei innervated by VTA dopaminergic neurons are interconnected, giving another level of complexity for the fine regulation of their activity. Altogether, our results and available information strengthen the need for further neurochemical studies to understand better the mechanisms involved in the regulation of LS DA levels and its role in addictive behavior.

Extracellular levels of DA and 5-HT were significantly higher at the rostral compared to the more caudal level of the posterior LS studied, suggesting denser dopaminergic and serotoninergic innervation at this level of the LS studied. These results are consistent with the topographic distribution of dopamine innervation to the LS reported by Assaf and Miller (1977). These authors also reported that the activation of VTA neurons in turn activates a significant number of septal neurons restricted mainly to the medial aspects of lateral septum (Assaf and Miller, 1977). Our results show that systemic or intra-VTA morphine significantly increases DA extracellular levels in the same medial aspects of the LS. It is therefore inferred that acute morphine increases LS neuronal activity.

The dual probe approach used herein to study the effect of intra-VTA infusion of morphine showed some intriguing results regarding the temporal course of the morphine effects. Extracellular DA levels in the LS were increased significantly immediately after infusing morphine through the probe located in the VTA, concomitantly with a significant decrease of GABA extracellular levels in VTA. LS DA levels returned to basal values, however, even though VTA extracellular GABA levels remained significantly decreased for the next 20 min. Several possible explanations should be considered. First, technical considerations should be tested further. The infusion of morphine through the probe located in the VTA would certainly generate a concentration gradient through the VTA parenchyma. The infusion of morphine into the VTA increased the LS DA levels, indicating that enough morphine reached the vicinity of VTA neurons projecting to the septal area being analyzed with the second probe. It could be argued, however, that the VTA extracellular GABA levels measured with the VTA probe corresponded not only to GABA in the vicinity of VTA dopaminergic neurons projecting to the LS. The different temporal courses of the LS DA increase and VTA GABA decrease were observed consistently in four independent experiments, however, weakening this argument. Second, as discussed previously, the decrease in VTA extracellular GABA levels will simultaneously increase extracellular DA levels in the nucleus accumbens, prefrontal cortex, and as shown in this work, in the LS. The high anatomic interconnection (reviewed by Sheehan et al., 2004) between these three limbic nuclei gives anatomic support to the possibility that indirect effects due to the increase in DA in nucleus accumbens and prefrontal cortex could be offsetting the increase in LS DA induced by the initial decrease in VTA GABA exerted by morphine. Third, it has been shown that the intra-VTA infusion of morphine decreases VTA GABA extracellular levels and also induces a delayed dendritic release of DA (Klitenick et al., 1992). This increase in VTA DA could in turn diminish the firing rate of dopaminergic neurons acting upon DA D2 autoreceptors and consequently facilitating the recovery of basal extracellular DA levels in the LS before VTA GABA levels regain their basal level.

It has been shown that the inhibition of GABAergic control exerted by opioids in VTA participates not only in the acute effect of morphine but also in the adaptive mechanisms observed after chronic drug intake (Bonci and Williams, 1997). The relevance of the observed increase in extracellular DA levels in LS induced by acute morphine in the drug addiction process should be explored further. Interestingly, the increase in DA will be occurring at the same time in the three limbic structures, the nucleus accumbens, prefrontal cortex, and the LS, after acute and probably chronic use of morphine and other drugs of abuse like ethanol and cocaine. Morphine and probably other addictive drugs thus increase extracellular DA levels in these three limbic structures related to different physiologic functions. DA in the nucleus accumbens is a key neurotransmitter associated with the rewarding properties of drug of abuse (reviewed by Nestler, 2004). The LS has also been involved in reward (Milner, 1991). In addition, it has been proposed that LS DA plays a role in regulating the septohippocampal pathway during conditions of arousal or stress (Adams and Moghaddam, 2000). LS DA may thus have a broad physiologic spectrum integrating rewarding and stress-related phenomena. In this regard, it has been shown that stimulation of the LS induces anorexic behavior (Wang and Kotz, 2002). It will be of interest to study whether LS DA is involved in the increase in drug reward induced by food restriction reported by Carr (2002). Another good example substantiating a more specific role of the LS in addiction is the work reported by Erdtmann-Vourliotis et al. (2001). These authors have shown that cocaine-sensitized rats express significantly higher levels of GRK-5, an isoform of the G-protein coupled-receptor kinases, the expression of which is restricted mainly to the LS.

Several reports have indicated that serotoninergic neurons in the dorsal raphe are also under a tonic GABAergic inhibitory tone sensitive to morphine (Tao and Auerbach, 1996, 2002a,b, 2003). The same studies have shown that the medial raphe serotoninergic neurons are not under this GABAergic tone. As it has been reported that the LS receives serotoninergic projections

mainly from the dorsal raphe (Vertes, 1991; Vertes et al., 1999), we expected to observe an increase in extracellular 5-HT levels after morphine administration. In the present study, acute systemic morphine administration did not induce significant changes in extracellular 5-HT levels in the LS. In this regard, it has been reported that anesthetics including choral hydrate used in this study eliminate morphine effects on extracellular 5-HT levels in different brain regions (Rivot et al., 1988; Tao and Auerbach, 1994). Further studies in awake rats thus are needed to determine whether LS 5-HT is affected by morphine. Interestingly, Varga et al. (2003) were able to study the role of GABA interneurons in the regulation of dorsal raphe serotoninergic neurons by carrying out extracellular unit recording of 5-HT and non-5-HT neurons with the characteristics of GABA interneurons in rats anesthetized with choral hydrate. Furthermore, the original electrophysiological studies showing that morphine administration diminishes the firing rate of VTA nondopaminergic neurons was also carried out in choral hydrate anesthetized animals (Gysling and Wang, 1983). A recent communication reported that the striatum and the LS are innervated by distinct subgroups of 5-HT containing neurons from the dorsal raphe (Waselus et al., 2004). The serotoninergic projection to the LS arises from neurons located in the ventromedial portion of the dorsal raphe. Further studies should investigate whether these neurons are more similar to medial raphe serotoninergic neurons that are not under the GABAergic tone, and therefore are not sensitive to morphine. Even if extracellular 5-HT levels in the LS are not sensitive to morphine, the convergence of these two systems in the LS, 5-HT sensitive to stress (reviewed by Sheehan et al., 2004) and DA sensitive to morphine (our results) and stress (Adams and Moghaddam, 2000) demands more attention to the role of the LS in addictive behavior.

Concluding Remarks

The functional role of DA and 5-HT from LS in drug abuse is presently unknown; however, two lines of evidence should be considered. First, the present results indicate that LS integrates information from VTA, the site supporting morphine and ethanol reinforcement and conditioned place preference after morphine injection (McBride et al., 1999), with serotoninergic information from the raphe, the nucleus related to stress response. The possibility that LS participates in addictive behavior and particularly in stress-induced relapse to drug seeking thus should be studied further. Second, the intermediate portion of LS contains high levels of urocortin peptide and type-2 CRH receptors (Vaughan et al., 1995) and has direct anatomic and functional connections with the lateral hypothalamus, a nucleus that has been implicated in natural reward, drug abuse, and feeding behavior (reviewed by Dileone et al., 2003). In addition, food restriction increases drug reward and it has been proposed that an increased DA receptor function underlies this effect (Carr, 2002). It has been shown, however, that the activation of LS induces an anorexic-type behavior probably mediated by urocortin (Wang and Kotz, 2002). The available evidence together with the present results thus suggests the possibility that dopaminergic neurotransmission in LS is involved in increased drug reward induced by food restriction.

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