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Cannabinoid Modulation of Opiate Reinforcement through the Ventral Striatopallidal Pathway

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Recent evidence indicates that cannabinoid-1 (CB_1) receptors play a role in the mediation of opiate reward, though the neural mechanisms for this process have not been characterized. The present experiments investigated the influence of CB_1 receptors in the ventral striatopallidal system on opiate-induced neurochemical events and opiate self-administration behavior in rats. Acute morphine administration (3 mg/kg) significantly reduced ventral pallidal GABA efflux in a manner similar to that produced by heroin self-administration. This neurochemical effect was reversed by doses of the selective CB_1 antagonist SR 141716A (Rimonabant; 1 and 3 mg/kg) that also significantly reduce opiate reward. Morphine-induced increases in nucleus accumbens dopamine levels were unaltered by SR 141716A. Intravenous heroin self-administration (0.02 mg/infusion) was significantly reduced by intra-accumbens, but not intraventral pallidal SR 141716A infusions (1 and 3 μg/side), implicating nucleus accumbens CB_1 receptors in the modulation of opiate reinforcement. In contrast, SR14716A did not alter cocaine self-administration (0.125 mg/inf), cocaine-induced (10 mg/kg) decrements in ventral pallidal GABA efflux or cocaine-induced increases in accumbens dopamine. This is consistent with evidence that selective inactivation of CB_1 receptors reduces opiate-, but not psychostimulant-maintained self-administration. The CB_1 receptor agonist WIN 55,212-2 (5 mg/kg) reduced pallidal GABA efflux in a manner similar to morphine, and this effect was reversed by the opiate receptor antagonist naloxone. Collectively these findings suggest that CB_1 receptors modulate opiate reward through the ventral striatopallidal projection and that the modulation of this projection system may be involved in the reciprocal behavioral effects between cannabinoids, and opioids.

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INTRODUCTION

There is accumulating evidence that cannabinoid-1 (CB_1) receptors are involved in mediating the rewarding properties of opiates. For example, genetic ablation of CB_1 receptors in mice greatly reduces both opiate self-administration (Ledent *et al*, 1999; Cossu *et al*, 2001) and opiate-induced conditioned place preference (Martin *et al*, 2000; Rice *et al*, 2002). Similarly, administration of the selective CB_1 receptor antagonist SR 141716A (Rimonabant) attenuates both morphine-induced conditioned place preference (Mas-Nieto *et al*, 2001) and heroin self-administration in rodents (Navarro *et al*, 2001; Caillé and Parsons, 2003; De Vries *et al*, 2003; Solinas *et al*, 2003). In addition, it has been shown that the reinforcing and motivational effects of heroin-paired stimuli are mediated, at least in part, by activation of cannabinoid CB_1 receptors (De Vries *et al*, 2003; Fattore *et al*, 2003). However, little is known regarding the neurobiological mechanisms through which CB_1 receptors modulate opiate reinforcement.

Numerous studies have implicated the nucleus accumbens (NAC) as a neuroanatomical substrate involved in mediating opiate reward. For example, opiate infusions directly into the NAC of rodents induce conditioned place preference (Van Der Kooy *et al*, 1982) and support operant self-administration behavior (Olds, 1982; David *et al*, 2002). Conversely, intra-NAC opioid antagonist administration decreases the reinforcing effects of intravenous heroin (Vaccarino *et al*, 1985). Intra-ventral tegmental area opiate administration has been shown to inhibit GABAergic interneuronal input to dopamine (DA) cells, thereby increasing the firing of midbrain DA cells that terminate in the NAC (Johnson and North, 1992; Wise *et al*, 1995; Xi and Stein, 2000) and increasing NAC DA release (Hemby *et al*, 1995; Pontieri *et al*, 1995; Tanda *et al*, 1997; Xi and Stein, 1999). It has been postulated that this process contributes to the positive reinforcing effects of opiates. In a similar manner, CB_1 receptor activation increases NAC DA release (French *et al*, 1997; Tanda *et al*, 1997; Gessa *et al*, 1998a) and accordingly it may be hypothesized that the

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modulation of opiate reward by CB_1 receptors involves a dopaminergic link. However, several lines of evidence suggest that opiate reward is mediated in part through DA-independent mechanisms (Pettit *et al*, 1984; Gerrits and Van Ree, 1996; Shippenberg and Elmer, 1998; Platt *et al*, 2001) and the reduction in opiate self-administration induced by the CB_1 antagonist SR 141716A does not appear to involve a dopaminergic link in the NAC (Caillé and Parsons, 2003).

An additional neural substrate in the mediation of opiate reward is the ventromedial ventral pallidum (VP). The VP receives a dense GABAergic input from the NAC (Groenewegen and Russchen, 1984; Chang and Kitai, 1985); while the NAC shell projects to the ventromedial part of the VP, the NAC core projects mainly to the dorsolateral part of the VP. Opiates strongly inhibit the spontaneous activity of the NAC GABAergic neurons (Hakan and Henriksen, 1989; Bardo, 1998; Lee *et al*, 1999; Xi and Stein, 2000, 2002) thereby reducing VP GABA efflux (Caillé and Parsons, 2004). The resultant disinhibition of the VP is thought to contribute to the positive reinforcing effects of opiates. Accordingly, it has been shown that the reinforcing properties of heroin self-administration are blocked by pharmacologically-induced increases in VP GABA levels (Bardo, 1998; Xi and Stein, 2000, 2002) and that intra-VP injections of a μ-agonist produced reward increases (Johnson *et al*, 1993). Moreover, electrical stimulation of the VP produces a rewarding effect comparable to that produced by stimulating the ventral tegmental area, lateral hypothalamus, or amygdala (Panagis *et al*, 1995). CB_1 receptors are localized in both the NAC (Herkenham *et al*, 1991; Robbe *et al*, 2001) and the VP (Herkenham *et al*, 1991), and thus it is possible that these receptors participate in the mediation of opiate reward by modulating the activity of the accumbal-pallidal GABA projection.

A recent examination of CB_1 receptor distribution in the NAC by Pickel *et al* (2004) supports this possibility. Using electron microscopic immunocytochemistry, this study showed a major presynaptic distribution of CB_1 receptors on terminals forming excitatory-type synapses and CB_1 receptors on somata and dendrites of presumed GABAergic projection neurons in both the NAC shell and core, indicating a significantly higher number of CB_1-immunogold particles in the NAC shell than in the NAC core. Interestingly, in many instances CB_1 receptors were colocalized with μ-opioid receptors within the same or synaptically linked neurons in the NAC shell and core (Pickel *et al*, 2004). Based on these collective findings it may be hypothesized that CB_1 receptors participate in the mediation of opiate reward by modulating the effect of opiates on the activity of both NAC core and shell striatopallidal GABAergic projections.

The present experiments tested this hypothesis by investigating alterations in morphine-induced decreases in VP GABA efflux induced by pretreatment with the CB_1 receptor antagonist SR 141716A. To evaluate a potential influence of DA input to the striatopallidal GABA system, the effects of SR 141716A on morphine-induced increases in NAC DA were also characterized. Although recent investigations indicate that CB_1 receptor stimulation modulates the reinforcing properties of cocaine (Vlachou *et al*, 2003) and reinstates cocaine-seeking behavior (De Vries *et al*, 2001), a large number of studies show that CB_1 receptor blockade alters opiate-, but not psychostimulant-maintained self-administration (Fattore *et al*, 1999; Ledent *et al*, 1999; Cossu *et al*, 2001; Vlachou *et al*, 2003). Thus, the effect of SR 141716A on cocaine self-administration and cocaine-induced alterations in NAC DA and VP GABA efflux were also evaluated. Further, the influence of bilateral microinfusions of SR 141716A into the NAC or VP on heroin self-administration behavior was tested in an effort to identify the locus through which CB_1 receptors modulate opiate reinforcement. Finally, the reciprocity of the interaction was studied by examining the ability of the opioid receptor antagonist naloxone to block the effects of the CB_1 receptor agonist WIN55,212 on VP GABA efflux. Our findings indicate that CB_1 receptors participate in opiate-, but not cocaine-induced decreases in VP GABA efflux through a DA-independent mechanism, and that intra-NAC SR 141716A administration attenuates the reinforcing effects of heroin self-administration.

MATERIALS AND METHODS

Subjects

A total of 114 male Wistar rats (300 g, Charles River, Wilmington, MA) were used. All animals were housed in groups of three in a temperature-controlled vivarium (22°C) with a 12 h light/dark cycle (lights on at 0800), and given *ad libitum* access to food and water. The studies were conducted in accordance with the *Guide for Care and Use of Laboratory Animals* provided by the National Institutes of Health.

Drugs and Reagents

Morphine sulfate, heroin hydrochloride, and cocaine hydrochloride were obtained from the National Institute on Drug Abuse (Washington, DC, USA). SR 141716A was generously provided by the National Institute of Mental Health Chemical Synthesis and Drug Supply Program (Washington, DC, USA). Naloxone, WIN 55,212-2 and all other reagents and neurotransmitter standards were obtained from Sigma (St Louis, MO, USA). Morphine, heroin, cocaine, and naloxone were dissolved in a vehicle of sterile 0.9% saline. SR 141716A and WIN 55,212-2 were dissolved in a vehicle of ethanol : emulphor : saline (1 : 1 : 18).

Surgery

Intracerebral microdialysis. Anesthetized animals (isoflurane 1.5–2.0% vapor) were implanted with a microdialysis guide cannula (21 gauge, Plastics One, Roanoke, VA, USA) aimed at either the NAC shell (from bregma: AP, +1.6 mm, ML, ±0.8 mm, and DV, −6.0 mm) or the VP (from bregma: AP, −0.6 mm, ML, ±2.2 mm, and DV, −6.7 mm) (Paxinos and Watson, 1998) and cemented to the skull. All animals were permitted a minimum of 5 days of postsurgical recovery prior to experimentation. In Figure 1, the corresponding probe placements for both structures are presented (see Histology for more detailed description of the range of placements in these experiments).

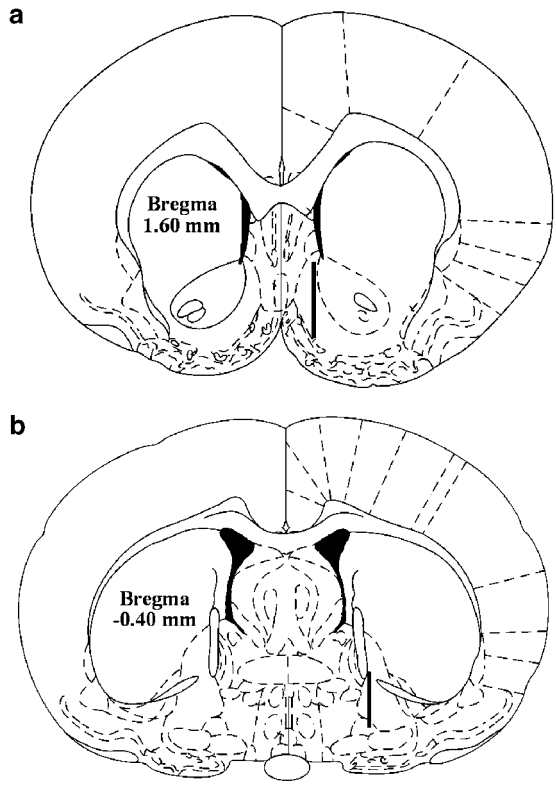

Figure 1 Schematic representation of the standard location of the 2-mm and the 1.5-mm dialysis probe membranes within the nucleus accumbens shell ($n=41$) and the ventral pallidum ($n=52$) respectively. Distances shown are in mm from bregma (adapted from Paxinos and Watson, 1998).

Intravenous self-administration. In all, 21 rats were prepared with chronic silastic jugular catheters under isoflurane anesthesia (1.5–2.0%) as described elsewhere (Caine *et al*, 1993). At the time of surgery, 14 of the animals were also stereotaxically implanted with bilateral 22-gauge, 12 mm stainless-steel guide cannulae that terminated 2 mm above the NAC 'shore' (from bregma: AP, $+1.7$ mm, ML, ± 2.0 mm and DV, -5.0; $n=7$) or the VP (from bregma: AP, -0.6 mm, ML, ± 2.2 mm and DV, -5.6 mm; $n=7$) and cemented to the skull. Catheters were flushed daily with sterile heparinized saline (30 USP U/ml) and the animals were allowed a minimum of seven postoperative recovery days prior to the initiation of self-administration training.

In Vivo Microdialysis

Approximately 3 h prior to the start of microdialysis, animals were lightly anesthetized (1.5–2.0% isoflurane), and microdialysis probes were inserted through the guide-cannulae. Microdialysis probes were constructed as described previously (Frantz *et al*, 2002) and employed an active membrane length that extended either 1.5 mm (for the VP) or 2 mm (for the NAC) beyond the end of the guide cannula. After probe implantation, artificial cerebral spinal fluid (aCSF, see Frantz *et al*, 2002 for details) was perfused at a flow-rate of either 0.3 μl/min for NAC DA analysis or 0.6 μl/min for VP GABA analysis for the duration of the experiment. Dialysate samples were collected at 10-min intervals during the experiments, and frozen at -70°C until assayed for DA content by high-performance liquid chromatography (HPLC) with electrochemical detection (Frantz *et al*, 2002) or for GABA content by capillary electrophoresis-coupled laser-induced fluorescence (CE-LIF; Roberto *et al*, 2004).

Operant Self-Administration

The self-administration chambers consisted of operant boxes enclosed in sound-attenuating, ventilated environmental cubicles (Frantz *et al*, 2002). Drug self-administration training was conducted in 3 h sessions 6 days per week. At the start of each session the operant lever was extended into the chamber, and lever pressing was reinforced by the intravenous delivery of 0.1 ml of a drug solution on a fixed-ratio (FR) time out 20-s (TO20) schedule of reinforcement. Heroin self-administration training and testing was conducted under an FR1 schedule of reinforcement with a unit dose of 0.02 mg/infusion. Cocaine self-administration training and testing were conducted under an FR5 schedule of reinforcement with a unit dose of 0.125 mg/infusion. An FR5 schedule of reinforcement was employed for cocaine self-administration as this higher response requirement allows for the evaluation of the temporal proximity of lever-pressing activity with reinforcer delivery, with higher response rates immediately prior to reinforcer delivery being indicative of goal-directed behavior. This is particularly important with psychomotor-stimulant reinforcers as motor activation and stereotypy can compromise controlled operant performance. However, the use of high-ratio requirements typically reduces opiate self-administration. As such, a continuous reinforcement schedule was employed for heroin self-administration to avoid a confounding 'floor effect' while evaluating SR 141716A that was anticipated to reduced heroin intake. For each drug, training continued until the total number of infusions per session stabilized to within $\pm 10\%$ of the mean for 3 consecutive days (baseline criterion).

Intracerebral Injection Procedure

Stylets were removed from the cannulae and bilateral injectors (33 gauge) were inserted, extending 2 mm beyond the tip of the guide cannulae. Infusions (1 μl) were made over a 2 min period, followed by an additional 1 min to allow for drug diffusion prior to injector removal. Subsequently, the stylets were replaced and the rats were allowed immediate access to intravenous heroin self-administration.

Experimental Design

Experiment 1: Effect of SR 141716A on morphine-induced decreases in VP GABA efflux, and morphine-induced increases in NAC shell DA efflux. All animals in this experiment were implanted with a single microdialysis probe in either the ventromedial VP or NAC shell. Following the collection of three baseline samples, each animal received an intraperitoneal drug pretreatment followed 30 min later by a morphine challenge injection (3 mg/kg, s.c.) and an additional 180 min of dialysate collection. The drug pretreatments were vehicle (SR0;

$n=6$ (VP) and $n=8$ (NAC)); 1 mg/kg SR 141716A (SR1; $n=6$ (VP) and $n=8$ (NAC)) or 3 mg/kg SR 141716A (SR3; $n=7$ (VP) and $n=10$ (NAC)). Control groups received SR3 ($n=5$ (VP) and $n=5$ (NAC)) followed by a saline injection.

Experiment 2: Effect of SR 141716A on cocaine-induced decreases in VP GABA efflux, and cocaine-induced increases in NAC shell DA efflux. As in Experiment 1, all animals in this experiment were implanted with a single microdialysis probe in either the ventromedial VP or NAC shell. Following the collection of three baseline samples, each animal received an intraperitoneal drug pretreatment followed 30 min later by a cocaine challenge injection (10 mg/kg, i.p.) and an additional 180 min of dialysate collection. The drug pretreatments were vehicle (SR0; $n=5$ (VP) and $n=5$ (NAC)) or 3 mg/kg SR 141716A (SR3; $n=5$ (VP) and $n=5$ (NAC)).

Experiment 3: Effect of intra-VP and intra-NAC SR 141716A administration on intravenous heroin self-administration. To establish stable patterns of operant heroin intake animals were allowed daily access to heroin self-administration for 3 h/day for 21 days as described above. Subsequently, alterations in heroin self-administration induced by SR 141716A microinfusions (veh, 1 and 3 μg/side) into either the NAC ($n=7$; injector placement in the 'shore') or VP ($n=7$) were tested using a within-subjects repeated measures design, with the order of drug presentation randomized between subjects. The baseline criterion for stable self-administration behavior was established between each pretreatment test.

Experiment 4: Effect of SR 141716A pretreatment on intravenous cocaine self-administration. To establish stable patterns of operant cocaine (0.125 mg/infusion) intake, animals were allowed daily access to drug self-administration on an FR5 schedule of reinforcement for 3 h/day for 21–33 days as described elsewhere (Frantz *et al*, 2002). Once stable patterns of self-administration were established, the effect of pretreatment with SR 141716A (veh, 1 and 3 mg/kg, i.p.) on cocaine self-administration was tested using a within-subjects repeated measures design ($n=7$), with the order of dose presentation randomized between subjects. The baseline criterion for stable self-administration behavior was established between each pretreatment test.

Experiment 5: Effect of WIN 55,212-2 on VP GABA efflux, and reversal of WIN 55,212-2 effect by naloxone. All animals in this experiment were implanted with a single microdialysis probe in the ventromedial VP. Following the collection of three baseline samples, each animal received an intraperitoneal drug pretreatment followed 10 min later by a WIN 55,212-2 challenge injection (5 mg/kg, i.p., dose selected based on the previously published reports Gessa *et al*, 1998b; Tzavara *et al*, 2003) and an additional 180 min of dialysate collection. The drug pretreatments were vehicle (NAL0; $n=8$) or 1 mg/kg s.c. naloxone (NAL1; $n=6$). An additional control group received NAL1 ($n=4$) followed by a saline injection.

Histology

After each experiment, the rats were euthanized by isoflurane overdose and the brain was removed and immediately frozen on dry ice. Subsequently, the brain was mounted on a cryostat and sliced into 50 μm sections. Both probe and injector tip placements were then verified; all active probe membranes were located within the following stereotaxic boundaries as described by Paxinos and Watson (1998) from bregma: VP, AP −0.4 to 0.7, ML ±1.8 to 2.2, DV −8.2 to 9.0; NAC shell, AP +1.6 to 1.9, ML ±0.6 to 1.2, DV −7.6 to −8.2. Only those subjects with accurate placement were included in the final data analyses.

Data Analysis

Drug self-administration behavior was expressed as the percent change in the total number of reinforcers earned in each drug pretreatment test relative to the average number of reinforcers earned over three baseline sessions that preceded the pretreatment test day. These percent change values for drug self-administration behavior were analyzed by repeated measures analysis of variance (ANOVA), with SR 141716A dose as the within-subjects factor.

Between-group differences in baseline microdialysate DA or GABA concentrations were first compared by ANOVA. Following confirmation of no group differences in baseline DA and GABA concentration, the data for each animal were converted to the percent change from the average baseline concentration obtained prior to the pretreatment injection. The temporal effect of drug pretreatment on morphine-, cocaine- or WIN 55,212-2-induced changes in dialysate GABA or DA levels was then evaluated using ANOVA with repeated measures over time and pretreatment dose as the between-subjects factor. Area under the curve (AUC) measures were used for comparison of overall pretreatment effects on morphine-, cocaine- or WIN 55,212-2-induced neurochemical events. The AUC was calculated for each animal by subtracting 100 from the percent of baseline value for each data point, and subsequently summing all data points collected during the post-morphine, -cocaine or -WIN 55,212-2 period of dialysate sampling. Significant differences in AUC measures were determined using ANOVA with pretreatment dose as the between-subjects factor. In each case, *post hoc* comparisons were made using Fisher's PLSD.

RESULTS

Experiment 1: Effect of SR 141716A on Morphine-Induced Decreases in VP GABA Efflux, and Morphine-Induced Increases in NAC Shell DA Efflux

VP GABA. Baseline GABA concentrations in VP dialysates did not differ between pretreatment groups ($F(2,16)=1.69$, NS) and were as follows: SR0, 38.95 ± 7.28 nM; SR1, 55.00 ± 4.13 nM; SR3, 39.25 ± 8.15 nM. In vehicle-pretreated animals morphine administration induced a significant decrease ($F(18,90)=1.88$, $P<0.02$) in dialysate GABA levels that began within the first 20 min after the injection and persisted for approximately 150 min (Figure 2a). Pretreatment with the CB_1 antagonist SR 141716A dose-dependently

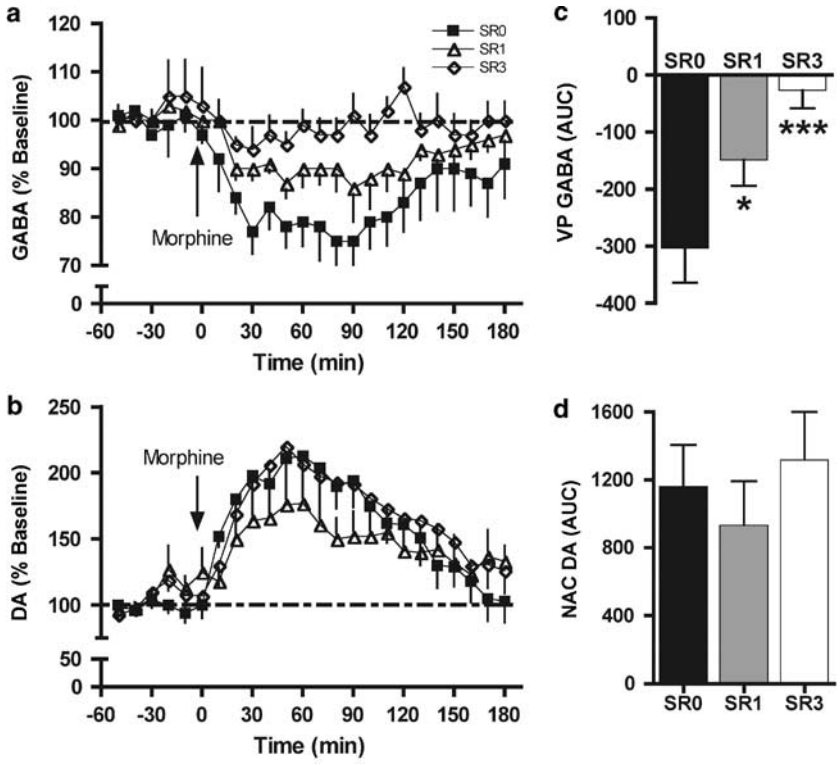

Figure 2 Effect of SR 141716A pretreatment on (a) the decreased GABA efflux in the VP and (b) increased interstitial DA in the NAC shell associated with an acute morphine injection (3 mg/kg; injection indicated by arrow). Data are expressed as the percentage change from baseline levels (mean ± SEM). The AUC data show that although SR 141716A pretreatment dose-dependently blocked morphine-induced decreases in VP GABA efflux (c), there was no significant effect of SR 141716A in morphine-induced increase in NAC shell DA levels (d). The pretreatment doses of SR 141716A are as follows: vehicle (SR0), 1 mg/kg (SR1) and 3 mg/kg (SR3). Asterisks denote significant differences from vehicle control as determined by Fisher's PLSD *post hoc* test (*$P<0.05$, ***$P<0.001$).

blocked the effect of morphine on VP GABA efflux ($F(2,16)=9.15$, $P<0.005$) (Figure 2c). When administered in the absence of morphine there was no significant effect of SR 141716A on VP GABA efflux ($F(18,72)=0.36$, NS; data not shown).

NAC DA. Baseline DA concentrations in NAC shell dialysates did not differ between pretreatment groups ($F(2,23)=0.69$, NS) and were as follows: SR0, 1.41 ± 0.23 nM; SR1, 1.68 ± 0.31 nM; SR3, 1.86 ± 0.28 nM. In vehicle-pretreated animals morphine administration induced a significant increase ($F(18,126)=14.68$, $P<0.0001$) in dialysate DA levels that began within the first 20 min after the injection and persisted for approximately 150 min (Figure 2b). There was no significant effect of SR 141716A pretreatment on morphine-induced increases in NAC DA efflux ($F(2,23)=0.53$, NS) (Figure 2d). When administered in the absence of morphine there was no significant effect of SR 141716A on NAC DA efflux ($F(18,72)=1.25$, NS; data not shown).

Experiment 2: Effect of SR 141716A on Cocaine-Induced Decreases in VP GABA Efflux, and Cocaine-Induced Increases in NAC Shell DA Efflux

VP GABA. Baseline GABA concentrations in VP dialysates did not differ between pretreatment groups ($F(1,8)=0.06$, NS) and were 39.73 ± 2.78 and 40.74 ± 3.82 nM for the SR0 and SR3 pretreatment groups, respectively. Similar to the effect produced by morphine, cocaine induced a significant decrease ($F(4,18)=4.42$, $P<0.0001$) in dialysate GABA levels in vehicle-pretreated animals that began within the first 10 min after the injection and persisted for approximately 90 min (Figure 3a). However, in contrast to the effect of SR 141716A on morphine-induced decrements in VP GABA, there was no significant effect of this CB_1 antagonist on cocaine-induced reductions in VP GABA ($F(1,8)=0.02$, NS) (See Figure 3c).

NAC DA. Baseline DA concentrations in NAC shell dialysates did not differ between pretreatment groups ($F(1,8)=0.80$, NS) and were 2.7 ± 0.2 and 2.4 ± 0.3 nM for the SR0 and SR3 pretreatment groups, respectively. Cocaine administration significantly increased dialysate DA concentrations in vehicle-pretreated animals ($F(12,48)=75.32$, $P<0.0001$) and this effect was unaltered by SR 141716A pretreatment ($F(1,8)=0.03$, NS) (see Figure 3b and d).

Experiment 3: Effect of Intra-VP and Intra-NAC SR 141716A Administration on Intravenous Heroin Self-Administration

During the final three self-administration sessions prior to the pretreatment tests animals in the VP group obtained an average of 15 ± 1 heroin reinforcers per session, and animals in the NAC group obtained an average of 19 ± 2 heroin

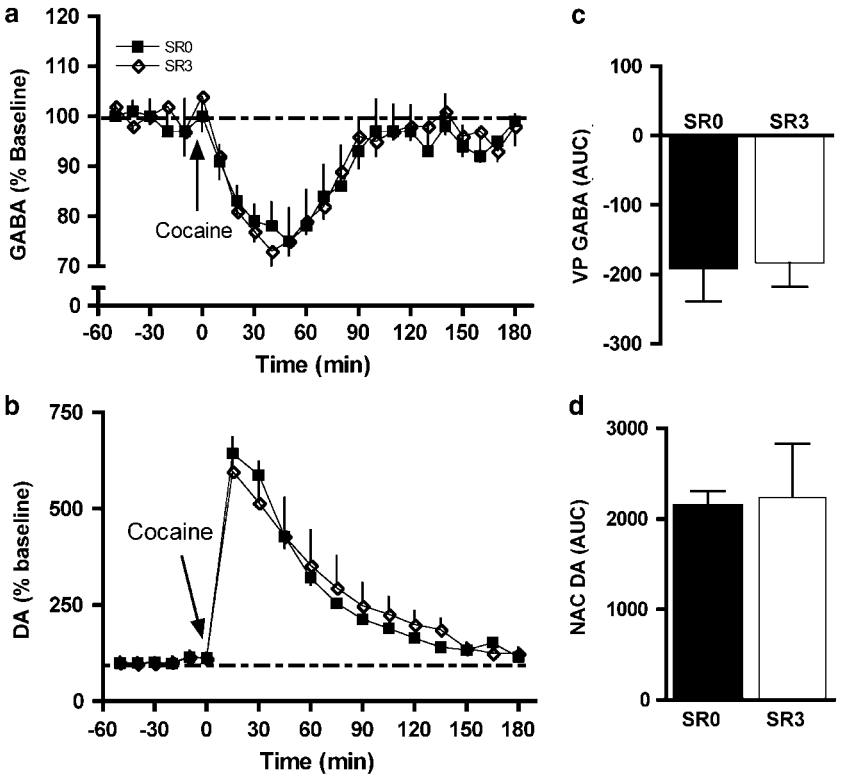

Figure 3 Effect of SR 141716A pretreatment on (a) the decreased GABA efflux in the VP and (b) increased interstitial DA in the NAC shell that are associated with an acute cocaine injection (10 mg/kg; injection indicated by arrow). Data are expressed as the percentage change from baseline levels (mean ± SEM). The AUC data show that SR 141716A did not alter either VP GABA efflux (c) or DA levels in the NAC shell (d). The pretreatment doses of SR 141716A are as follow: vehicle (SR0) and 3 mg/kg (SR3).

reinforcers. This baseline level of drug intake was stable within each group between pretreatment tests. Heroin self-administration was significantly reduced by intra-NAC SR 141716A administration ($F(2,12) = 4.52$, $P < 0.05$). Pretreatment with 1 or 3 μg SR 141716A/side significantly decreased heroin intake to 72 ± 10% (1 μg/side) and 71 ± 11% (3 μg/side) of baseline self-administration levels (both doses $P < 0.05$) (Figure 4a). In contrast, there was no significant effect of intra-VP SR 141716A administration on heroin self-administration behavior ($F(2,12) = 0.33$, NS). Location of injector tips are presented in Figure 4b.

Experiment 4: Effect of SR 141716A Pretreatment on Intravenous Cocaine Self-Administration

Baseline cocaine intake prior to SR 141716A testing was 70 ± 8.6 infusions per 3 h session. Consistent with recent findings by others (De Vries *et al*, 2001) pretreatment with SR 141716A produced no significant alteration in cocaine self-administration ($F(2,12) = 0.224$; NS). Cocaine self-administration was 106 ± 3, 111 ± 7, and 109 ± 8% of baseline levels following pretreatment with vehicle, 1 and 3 mg/kg SR 141716A, respectively (data not presented in graphic or tabular form). The inability of SR 141716A to alter cocaine self-administration is in contrast to the significant reduction in operant responding for heroin under both FR and progressive-ratio schedules of reinforcement induced by these same SR 141716A doses previously observed both in our laboratory (Caillé and Parsons, 2003) and by others (De Vries *et al*, 2003; Solinas *et al*, 2003).

Experiment 5: Effect of WIN 55,212-2 on VP GABA Efflux, and Reversal of WIN 55,212-2 Effect by Naloxone

Baseline GABA concentrations in VP dialysates were 40.14 ± 1.64, 49.72 ± 2.95 and 50.55 ± 1.54nM for the VEH–WIN, NAL–WIN, and NAL–VEH groups, respectively, and there were no significant differences between groups in baseline levels ($F(2,15) = 1.53$, NS). In vehicle-pretreated animals WIN 55,212-2 administration induced a significant decrease ($F(18,126) = 8.57$, $P < 0.001$) in dialysate GABA levels that began within the first 20 min after the injection and persisted for approximately 150 min (Figure 5a). Pretreatment with the nonselective opioid receptor antagonist naloxone (1 mg/kg) significantly blocked the effect of WIN 55,212-2 on VP GABA efflux ($F(1,12) = 7.17$, $P < 0.02$) (Figure 5b). There was no significant effect of naloxone alone on VP GABA efflux ($F(18,54) = 1.26$, NS; data not shown).

DISCUSSION

A growing body of evidence suggests that CB_1 receptors modulate opiate but not psychostimulant reward. CB_1 receptor knockout mice display significantly attenuated heroin-induced conditioned place-preference and heroin

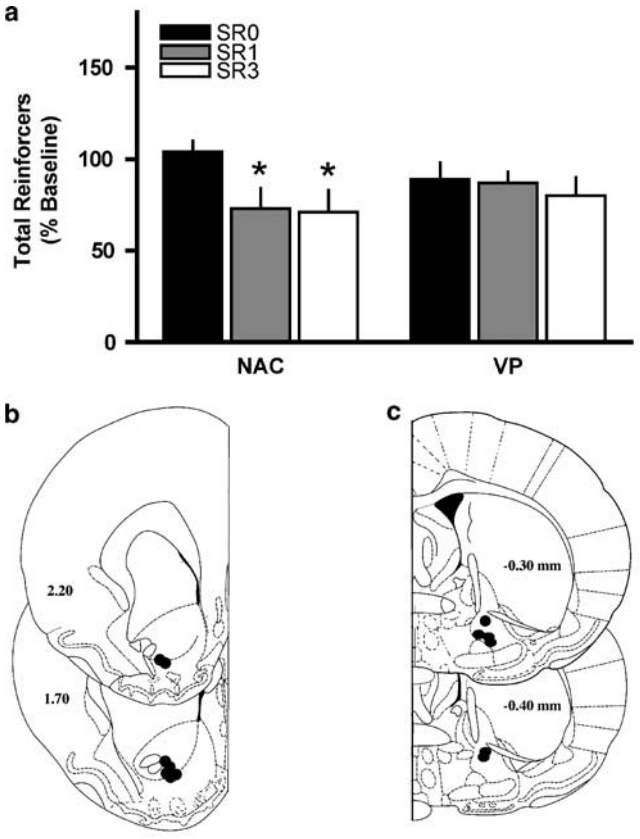

Figure 4 Effect of bilateral intra-VP and intra-NAC SR 141716A infusions on heroin self-administration (0.02 mg/infusion, FR1 schedule) (a). SR 141716A administered into the nucleus accumbens (NAC), but not the ventral pallidum (VP), reduced heroin self-administration behavior. The SR 141716A doses were: vehicle (SR0), 1 μg/μl/side (SR1) and 3 μg/μl/side (SR3). Asterisks denote significant differences from vehicle control as determined by Fisher's PLSD *post hoc* test (*$P<0.05$). Schematic representation of the location of the injector tips within the NAC (b) and the VP (c). Distances are in mm anterior to bregma (adapted from Paxinos and Watson, 1998).

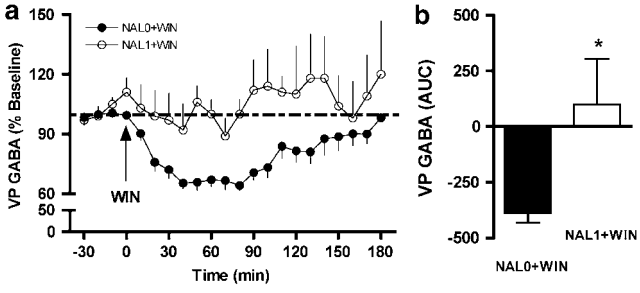

Figure 5 Effect of naloxone pretreatment on (a) decreased GABA efflux in the VP associated with an acute WIN 55.212-2 injection (5 mg/kg; injection indicate by arrow). Data are expressed as the percentage change from baseline levels (mean ± SEM). The AUC data show that naloxone pretreatment blocked WIN-induced decreases in VP GABA efflux (b). The pretreatment doses of naloxone are as follow: vehicle (NAL0) and 1 mg/kg (NAL1). Asterisks denote significant differences from vehicle control as determined by Fisher's PLSD *post hoc* test (*$P<0.05$).

self-administration but not cocaine place conditioning or self-administration (Ledent *et al*, 1999; Martin *et al*, 2000; Cossu *et al*, 2001). Similarly, the CB_1 receptor antagonist SR 141716A significantly reduces the reinforcing effects of heroin, but not cocaine, self-administration in rats (Fattore *et al*, 1999; De Vries *et al*, 2001; Mas-Nieto *et al*, 2001; Navarro *et al*, 2001; Caillé and Parsons, 2003; De Vries *et al*, 2003; Solinas *et al*, 2003). The present data demonstrate that SR 141716A dose-dependently reverses morphine- but not cocaine-induced decreases in VP GABA efflux within a dose range that significantly reduces opiate (Navarro *et al*, 2001; Caillé and Parsons, 2003; De Vries *et al*, 2003; Solinas *et al*, 2003) but not cocaine (De Vries *et al*, 2001) reward in rats. This effect is independent of alterations in morphine-induced increases in NAC DA efflux, and appears to be mediated by CB_1 receptors in the NAC rather than the VP. Moreover, the CB_1 receptor agonist WIN 55,212-2 reduced VP GABA efflux in a manner similar to morphine, and this effect was reversed by naloxone. These findings suggest that CB_1 receptors modulate opiate reward through the ventral striatopallidal GABA projection and that the modulation of this projection system may be involved in the reciprocal interactions between cannabinoids and opioids.

The VP has been implicated in mediating the rewarding effects of opiates and psychostimulants (Johnson *et al*, 1993; Robledo and Koob, 1993; Johnson and Stellar, 1994; Gong *et al*, 1996; Bardo, 1998; Xi and Stein, 2000, 2002). This region receives dense GABAergic innervation from the NAC (Groenewegen and Russchen, 1984; Chang and Kitai, 1985), and both opiate and psychostimulant drugs inhibit the activity of this projection (Hakan and Henriksen, 1989; Bourdelais and Kalivas, 1990; Bardo, 1998; Lee *et al*, 1999; Xi and Stein, 2000, 2002; Caillé and Parsons, 2004). Consistently, both morphine and cocaine were found to significantly decrease VP GABA efflux in the present study. The inhibitory effect of morphine on VP GABA was dose-dependently reversed by doses of the CB_1 antagonist SR 141716A that also significantly attenuate the breaking point of responding for heroin under a progressive-ratio schedule of reinforcement (Caillé and Parsons, 2003; De Vries *et al*, 2003; Solinas *et al*, 2003). Opiates also increase NAC DA efflux (Hemby *et al*, 1995; Pontieri *et al*, 1995) which may contribute to morphine-induced decrements in VP GABA (Yang and Mogenson, 1989; Bourdelais and Kalivas, 1992). However, SR 141716A produced no significant effect on morphine-induced increases in NAC DA, suggesting that the effects of SR 141716A on morphine-induced decrements in VP GABA occur independently of NAC DA. This is consistent with the recent observation that SR 141716A-induced reductions in heroin reward occur through a mechanism independent of NAC DA (Caillé and Parsons, 2003). There was no significant effect of SR 141716A itself on either VP GABA efflux or NAC DA efflux, suggesting that it alters opiate-induced neurochemical events rather than simply blocking tonically active processes.

In contrast to the effects of SR 141716A on opiate-induced neurochemical events and opiate-maintained behavior, the present experiments revealed no significant effect of this CB_1 antagonist on cocaine-induced decrements in VP GABA, cocaine-induced increases in NAC DA or cocaine-maintained operant behavior (also see De Vries *et al*, 2001). Since psychostimulant-induced decrements in VP GABA efflux are likely produced via increased NAC DA levels (Yang and Mogenson, 1989; Bourdelais and Kalivas, 1990, 1992), the inability of SR 141716A to alter cocaine-induced

decrements in VP GABA efflux suggests this CB_1 antagonist does not alter the inhibitory effects of NAC DA on the ventral striatopallidal GABA projection. This further supports the assertion that SR 141716A-induced reductions in opiate reward do not involve a NAC DA link.

CB_1 receptors are present in both the VP and the NAC (Herkenham *et al*, 1991; Pickel *et al*, 2004) and thus the effect of SR 141716A on morphine-induced decrements in VP GABA efflux may be produced by CB_1 receptors in either of these regions. Since CB_1 receptors exert a negative influence on GABA release (Hajos *et al*, 2000) blockade of VP CB_1 receptors may reverse morphine-induced decrements in VP GABA efflux, in turn attenuating opiate reward (Bardo, 1998; Xi and Stein, 2000, 2002). CB_1 receptors in the NAC modulate the release of GABA and glutamate (Hoffman and Lupica, 2001; Pistis *et al*, 2002; Robbe *et al*, 2003) and therefore these receptors may modulate opiate reward by influencing the activity of striatopallidal projection neurons (Napier and Mitrovic, 1999). To investigate the neural locus through which CB_1 receptors modulate opiate reward, the effect of SR 141716A infusions into the VP or NAC on heroin self-administration was investigated. Previous studies have shown that peripheral SR 141716A administration dose-dependently reduces operant responding for 0.02 mg/infusion heroin under both fixed- and progressive-ratio schedules of reinforcement (Caillé and Parsons, 2003; De Vries *et al*, 2003; Solinas *et al*, 2003). These observations suggest that SR 141716A decreases the reinforcing efficacy of intravenous heroin. In the present study, intra-NAC SR 141716A infusions produced a similar and significant decrease in operant responding for 0.02 mg/infusion heroin, while intra-VP infusions of this same SR 141716A dose range did not alter heroin intake. This suggests that the NAC is an important locus for the modulation of opiate self-administration by CB_1 receptors, consistent with the proposed importance of this brain region in the mediation of opiate reward (see Introduction) and several lines of evidence which point toward convergent actions of μ-opioid- and CB_1 receptors in the NAC (Hoffman and Lupica, 2001; Manzoni and Bockaert, 2001; Pickel *et al*, 2004). Moreover, GABAergic medium spiny neurons are the predominant type of efferent neurons in the NAC core and shell (Sesack and Pickel, 1990; Smith and Bolam, 1990) and both bear CB_1 receptors (Pickel *et al*, 2004). Altogether, these findings suggest an important influence of NAC CB_1 receptors in the regulation of VP GABA transmission and the reinforcing properties of opiates. However, the relative involvement of the core and shell subregions cannot be evaluated by the present experiments as the injectors were placed in the 'shore' thereby allowing SR141716A diffusion into both the core and shell. It should also be noted that the VP receives inputs from a number of regions besides the NAC (for review see Groenewegen *et al*, 1999; Napier and Mitrovic, 1999), many of which also contain CB_1 receptors. This leaves open the likely possibility that regions other than the NAC participate in the CB_1 modulation of opiate-induced neurochemical events in the VP. Further, opioids exert direct effects in the VP (Napier and Mitrovic, 1999) and this may explain in part why blockade of NAC CB_1 receptors did not abolish completely heroin self-administration.

The neural processes through which NAC CB_1 receptors alter opiate-induced decrements in VP GABA efflux and opiate reinforcement are presently unknown, though several mechanisms may be postulated. For example, CB_1 and opiate receptors might interact at the level of their signal transduction pathways as both classes of receptors are coupled to Gi/Go-proteins and their stimulation modulates cAMP-dependent and MAP kinase pathways (Matsuda *et al*, 1990; Childers *et al*, 1992; Reisine *et al*, 1996). In addition, CB_1 receptor activation drastically reduces stimulated glutamate release in the NAC (Pistis *et al*, 2002) through the activation of presynaptic CB_1 receptors (Robbe *et al*, 2002; Robbe *et al*, 2003). Moreover, CB_1 receptors have been localized presynaptically on excitatory-type neurons that synapse with medium spiny neurons in the NAC (Pickel *et al*, 2004) whose postsynaptic activity can be suppressed by opiates (Childers *et al*, 1992). Thus, removal of the inhibitory influence of CB_1 receptors on these glutamate inputs (via SR 141716A administration) may serve to activate the VP GABA projection, thereby counteracting the morphine-induced decrease in VP GABA outflow.

The observation that naloxone, a μ-opioid receptor antagonist, blocks the CB_1 agonist-induced decrease in VP GABA, provides additional evidence of a reciprocal interaction between the cannabinoid and opioid systems in the CNS (for review see Corchero *et al*, 2004; Fattore *et al*, 2004). The interaction between the cannabinoid and opioid systems in the modulation of the accumbo-pallidal projection may be important in light of the proposed involvement of the VP in mediating drug reward, and drug-induced alterations in this interaction may contribute to neuroadaptations involved in the development of drug dependence. The mechanism(s) through which μ-receptors modulate CB_1 agonist-induced decrements in VP GABA efflux are presently unknown. Although our present and previous data suggest that the modulation of opiate-induced decreases in VP GABA efflux by CB_1 receptors occurs independent of alterations in NAC DA, it is conceivable that the μ-receptor modulation of CB_1 agonist-induced neurochemical events does involve NAC DA. For example, the CB_1 agonist WIN 55,212-2 increases mesolimbic DA cell firing through a naloxone-sensitive mechanism (Tanda *et al*, 1997) and this may influence the activity of the accumbo-pallidal GABA projection. Alternately, CB_1 agonist administration is reported to increase opioid peptide release (Mason *et al*, 1999; Welch and Eads 1999; Valverde *et al*, 2001) and it is possible that this process contributes to the μ-receptor-dependent modulation of VP GABA release by WIN 55,212-2. Regardless of the mechanism through which this interaction occurs, an important consideration for future work will be whether chronic opiate or cannabinoid exposure alters the responsivity of the VP to drug ingestion, and if so whether this alteration results from an adaptation of the cannabinoid–opioid interaction in this system.

In summary, the present results demonstrate that SR 141716A dose-dependently reverses morphine-, but not cocaine-induced decreases in VP GABA efflux within a dose range that significantly reduces opiate reward in rats. This effect does not appear to involve an alteration in morphine-induced increases in NAC DA efflux, or in the efficacy of increased NAC DA to inhibit the VP GABA projection. The blockade of WIN 55,212-2-induced decreases in VP GABA

efflux by naloxone points to a reciprocal interaction between cannabinoids and opioids in the modulation of ventral pallidal activity. These findings suggest that SR 141716A attenuates opiate reward by diminishing the inhibitory influence of opiates on NAC medium spiny neurons.

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