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Endocannabinoid release from midbrain dopamine neurons: A potential substrate for cannabinoid receptor antagonist treatment of addiction

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Abstract

Substantial evidence suggests that all commonly abused drugs act upon the brain reward circuitry to ultimately increase extracellular concentrations of the neurotransmitter dopamine in the nucleus accumbens and other forebrain areas. Many drugs of abuse appear to increase dopamine levels by dramatically increase the firing and bursting rates of dopamine neurons located in the ventral mesencephalon. Recent clinical evidence in humans and behavioral evidence in animals indicate that cannabinoid receptor antagonists such as SR141716A (Rimonabant) can reduce the self-administration of, and craving for, several commonly addictive drugs. However, the mechanism of this potentially beneficial effect has not yet been identified. We propose, on the basis of recent studies in our laboratory and others, that these antagonists may act by blocking the effects of endogenously released cannabinoid molecules (endocannabinoids) that are released in an activity- and calcium-dependent manner from mesencephalic dopamine neurons. It is hypothesized that, through the antagonism of cannabinoid CB1 receptors located on inhibitory and excitatory axon terminals targeting the midbrain dopamine neurons, the effects of the endocannabinoids are occluded. The data from these studies therefore suggest that the endocannabinoid system and the CB1 receptors located in the ventral mesencephalon may play an important role in regulating drug reward processes, and that this substrate is recruited whenever dopamine neuron activity is increased.
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The neurons located in the mammalian ventral midbrain that synthesize and release dopamine (DA) are implicated in a variety of neuropsychiatric and neurodegenerative diseases, as well as in the development and maintenance of compulsive drug use in humans. Virtually every abused drug increases extracellular DA levels in the axon terminal fields of the DA neurons whose cell bodies are located in the area of the mesencephalon known as the ventral tegmental area (VTA). The axons from these neurons course rostrally to innervate, primarily, the medial prefrontal cortex (PFC) and the ventral striatum, also known as the nucleus accumbens (NAc). This VTA DAergic projection can therefore be further anatomically subdivided into both the mesocortical and mesolimbic pathways (Fig. 1A). The VTA DA neurons represent the primary source of DA in these forebrain areas, where this

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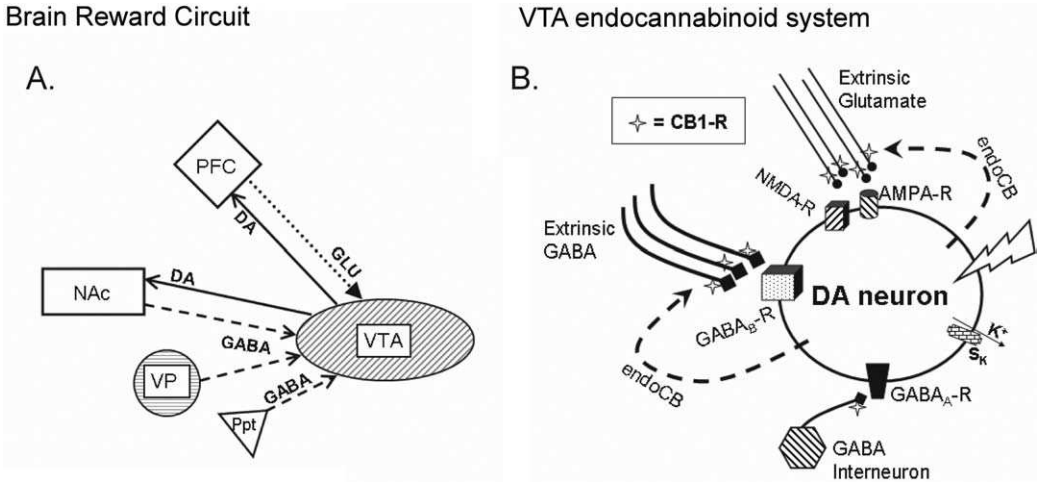

Fig. 1. Brain reward circuit and known substrates for endocannabinoid action in the VTA. (A) A simplified schematic diagram illustrating some of the important inputs and outputs of the mesencephalic midbrain (VTA). Abbreviations: Ppt, pedunculopontine nucleus; VP, ventral pallidum; NAc, nucleus accumbens; PFC, prefrontal cortex; DA, dopamine; GLU, glutamate. Note that the diagram is oversimplified for clarity. (B) Locations of cannabinoid CB1 receptors, and mechanisms of endocannabinoid (endoCB) release in the VTA. The locations of CB1 receptors were determined by electrophysiological studies cited in the text. The lightning bolt-represents the depolarization necessary to initiate endocannabinoid release from VTA DA neurons. The small conductance calcium-sensitive potassium channel (S_K) is also shown. Blockade of this channel by apamin increases DA neuron sensitivity to excitatory afferent input or to diminished inhibitory input, thereby initiating bursting and enhanced endocannabinoid release.

neurotransmitter clearly plays important roles in cognition, reward, and motivational processes (Di Chiara and Imperato, 1988; Wise, 2004).

1. Different drugs increase extracellular DA concentrations through distinct mechanisms

Although an increase in DA function is the ultimate result of the use of commonly abused drugs, the mechanisms through which this occurs can differ among distinct classes. Opiate drugs, such as heroin and morphine are thought to increase DA release in the NAc by inhibiting the release of the inhibitory neurotransmitter GABA onto VTA DA neurons (Johnson and North, 1992; Devine and Wise, 1994). This "disinhibition" of the DA neurons increases their rates of spontaneous firing, and increases the frequency with which these cells fire in bursts (Kiyatkin, 1988) (discharges separated by distinct pauses in firing). This is significant because these bursts of DA neuron activity are associated with more efficacious terminal releases of DA than simple increases in firing rates alone (Gonon, 1988). This ultimately results in increases in DA levels in the NAc and PFC that appear to be necessary to elicit the rewarding effects of these drugs (Di Chiara and Imperato, 1988).

In contrast to the opiates, the psychostimulants cocaine and amphetamine appear to increase DA function in the NAc and PFC through inhibition of the DA transporter (DAT) that is located on axon terminals, and that normally clears DA from the extracellular space following its release (Wise and Bozarth, 1985). In addition, amphetamine can also stimulate the release of DA from axon terminals by inhibiting the storage of monoamines in synaptic vesicles. Thus, unlike the opiates, these psychostimulants can increase the duration of the DA signal in the NAc and PFC through the inhibition of the mechanism that limits its duration of action in the extracellular space. The inhibition of DAT activity has also been observed at the cell bodies and dendrites of the DA neurons located in the VTA. However, when extracellular DA levels increase in the VTA this can lead to a well-described *decrease* in DA neuron firing rates through the activation of inhibitory DA D2 receptors located on these cells (Bonci et al., 2003; Brodie and Dunwiddie, 1990; Einhorn et al., 1988). Therefore, one might assume that the net effect of cocaine and amphetamine might be to actually *dampen* DA neuron activity and output to the NAc and PFC. However, several plausible mechanisms have also been identified that may account for the ability of psychostimulants to *increase* the firing and bursting rates of these VTA DA neurons under certain conditions (Shi et al., 2000; Trent et al., 1991). One of the ways in which amphetamine or cocaine might increase the activity of VTA DA neurons is through the enhancement of extracellular DA concentrations, following DAT inhibition, and the subsequent activation of α_1-adrenergic receptors by DA (Paladini et al., 2001). These receptors are known to stimulate the release

of calcium, and deplete it from phosphoinositide-sensitive intracellular stores. By depleting this source of intracellular calcium, metabotropic glutamate type-1 receptors (mGluR-1) are prevented from synaptically activating calcium-sensitive potassium channels (S_K channels) (Kohler et al., 1996) that normally act to limit DA neuron excitability by generating an IPSP. This can lead to increased excitation of VTA DA neurons through disinhibition (Fiorillo and Williams, 1998; Paladini et al., 2001). Other plausible mechanisms through which psychostimulants might increase VTA DA activity include the blockade of the serotonin (5-HT) transporter by low concentrations of cocaine and the subsequent presynaptic inhibition of GABA release onto VTA DA neurons via 5-HT1D receptors (Cameron and Williams, 1994), or through the activation of DAT-associated excitatory membrane currents that are elicited by DA or amphetamine in midbrain DA neurons (Ingram et al., 2002). It is also interesting to note in this latter study that the concentrations of DA that caused the DAT-dependent increase in neuronal excitability were lower than those necessary to activate inhibitory D2 receptors (Ingram et al., 2002). Thus, although the most frequently described effect of the psychostimulants is the inhibition of DA neuron firing, several mechanisms and conditions have been identified that could account for increases in the activity of these cells by cocaine and amphetamine, under certain physiological conditions.

The neurobiological mechanisms underlying the rewarding effects of nicotine also appear to involve increased DA function in the axon terminal fields of the VTA neurons (Nisell et al., 1994). Both direct and indirect mechanisms accounting for an increase in VTA DA neuron activity have been identified (Pidoplichko et al., 1997, 2004), and data are available to support the idea that nicotine can induce DA release in the NAc via an effect on DA axon terminals (Ferrari et al., 2002). Furthermore, whereas the direct excitatory action of nicotine on VTA DA neurons appears to desensitize rapidly, the ability of nicotine to increase DA neuron excitation through the facilitation of glutamate release onto these cells is longer-lived, and may therefore represent the primary rewarding effect of nicotine consumption (Pidoplichko et al., 2004).

Although the neurobiological mechanisms through which ethanol influences brain reward circuitry are more poorly understood than the preceding abused drugs, it is well established that it can substantially increase the firing rates of VTA DA neurons (Brodie and Appel, 1998; Brodie et al., 1990), resulting in elevated extracellular DA levels in the NAc (Di Chiara and Imperato, 1988). This suggests that at least part of the rewarding and euphoric actions of ethanol may be mediated by large increases in VTA DA neuron firing rates.

2. Cannabinoid receptor substrates for marijuana effects on reward circuits

In contrast to the relatively well-understood neurobiological effects of the commonly abused drugs described above, the primary sites of interaction of the primary psychoactive constituent in marijuana, Δ^9-THC, are only now being elucidated (Lupica et al., 2004). Our relatively primitive state of understanding of the effects of this drug on central reward pathways is primarily attributable to two factors. First, the Δ^9-THC molecule and all experimentally employed cannabinoid drugs are extremely hydrophobic, making them very difficult to suspend in aqueous solution, and limiting their bioavailability in behavioral assays, such as self-administration (Tanda and Goldberg, 2003). Second, up until several years ago, there were few pharmacological agents available to probe for cannabinoid receptor function in the brain. However, with better understanding of the physical properties of these molecules, the cloning of a cannabinoid receptor binding site located in the CNS, the CB1 receptor (Matsuda et al., 1990), and the development of agonists and antagonists of this receptor (Pertwee, 1997), the pace of research into the neurobiological activity of Δ^9-THC, and other cannabinoid drugs has been greatly accelerated. Armed with newly developed tools such as the antagonist SR141617A (Rimonabant, Rinaldi-Carmona et al., 1994), researchers quickly defined roles for a new class of brain-derived lipids that act as agonists at CB1 receptors in the CNS (Petrocellis et al., 2004; Freund et al., 2003). The endogenous cannabinoids (i.e. endocannabinoids) that have received the most experimental attention are the arachidonic acid-containing lipids known as anandamide and 2-arachidonylglycerol (Mechoulam et al., 1998). Thus far, the most prominent and best studied physiological role for these molecules is as synaptic *retrograde messengers*, where they are released from postsynaptic neurons upon depolarization, activate CB1 receptors located on axon terminals, and thereby inhibit neurotransmitter release. This endocannabinoid action thus exploits the ubiquitous distribution of CB1 receptors on axon terminals throughout the brain, permitting postsynaptic neurons to regulate their own synaptic inputs as a function of their relative level of activity (Alger, 2002; Wilson and Nicoll, 2002). This retrograde signaling function of endocannabinoid molecules appears to be widely distributed throughout the CNS (Alger, 2002), although it appears to be limited to only certain neuronal phenotypes within particular brain regions (Hoffman et al., 2003b).

In addition to this short-term regulation of synaptic strength, endocannabinoids appear to be necessary to observe certain forms of long-lasting synaptic plasticity, such as long-term depression (LTD) of glutamate release

in areas of the brain such as the NAc, and the dorsal striatum (Lupica et al., 2004; Hoffman et al., 2003a; Robbe et al., 2002; Gerdeman and Lovinger, 2003; Gerdeman et al., 2002). Furthermore, because of the prominent role that these brain structures play in mediating the rewarding and motivational effects of abused drugs, it has been proposed that the reliance upon endocannabinoids for LTD in the NAc may represent a cellular adaptation that may be involved in the progression from casual drug use to drug addiction (Gerdeman et al., 2003; Hoffman et al., 2003a).

In an effort to understand the properties of marijuana that sustain its continued use in humans, and to compare the effects of marijuana with the effects of other abused drugs, several studies have examined the effects of Δ^9-THC and other cannabinoid agonists on the activity of VTA DA neurons and on extracellular DA levels in the axon terminal fields of these cells (Lupica et al., 2004; Gardner and Vorel, 1998). These studies have demonstrated that cannabinoid agonists, when administered i.v. in either anesthetized or freely moving rodents, can increase the level of activity of VTA DA neurons (Wu and French, 2000; French et al., 1997; Gessa et al., 1998). This augmentation of VTA DA neuron activity included both an increase in baseline firing rates and an increase in the frequency of action potential bursting. Moreover, this effect was also observed in an in vitro brain slice preparation containing the VTA, suggesting that cannabinoid agonists may act via local neuronal circuitry in the VTA to produce this same effect (Cheer et al., 2000). This cannabinoid-induced increase in DA neuron activity was apparently responsible for the increased extracellular levels of DA observed in the NAc (Ng Cheong Ton et al., 1988; Chen et al., 1990; Tanda et al., 1997), since no effect of cannabinoid agonists on extracellular DA concentrations were observed when they were infused directly into the NAc (Szabo et al., 1999). This latter study is also consistent with data demonstrating that DA neurons do not appear to express CB1 receptors (Herkenham et al., 1990, 1991; Matsuda et al., 1993, but see Wenger et al., 2003). Collectively, the above studies suggest that, like other drugs of abuse, marijuana appears to increase the extracellular concentrations of DA in the NAc, and increases the activity of DA neurons in the VTA. Furthermore, the studies demonstrating that locally applied cannabinoids in the NAc did not affect extracellular DA concentrations (Szabo et al., 1999), but did increase the firing and bursting rates of VTA DA neurons in a brain slice preparation (Cheer et al., 2000), implied that the effects of Δ^9-THC and other cannabinoids were likely on the local circuitry of the VTA (Fig. 1B). Since the primary mechanism through which cannabinoids appeared to affect this drug reward pathway was by increasing VTA DA neuron activity, subsequent studies sought to define the cellular mechanisms involved in this action. As mentioned above, since CB1 receptor protein or mRNA appears to be absent in VTA DA neurons (Herkenham et al., 1991; Matsuda et al., 1993), this argues against a more direct role for these receptors in regulating DA neuron activity. Therefore, indirect mechanisms involving the well-known presynaptic inhibition by these receptors were investigated in the VTA.

There are at least two other neuronal phenotypes to consider in addition to DA neurons in the VTA (Cameron et al., 1997). A substantial number of these non-DA neurons are GABAergic and their output forms a projection to the NAc (Van Bockstaele and Pickel, 1995), and the PFC (Carr and Sesack, 2000). These neurons have also been hypothesized to provide substantial synaptic inhibitory input to $GABA_A$ receptors located on DA neurons via axon collaterals (Johnson and North, 1992; Steffensen et al., 1998). Since opiates increase DA neuron activity through a disinhibitory mechanism involving the inhibition of GABA release from local circuit neurons (Johnson and North, 1992), and CB1 receptors are known to mediate this effect throughout the brain (Hoffman and Lupica, 2000, 2001), this seemed a likely possibility in the VTA. Consistent with this hypothesized mechanism, application of the non-selective cannabinoid receptor agonist WIN55,212-2 to brain slices containing the VTA reduced electrically evoked IPSCs mediated by the activation of $GABA_A$ receptors (Szabo et al., 2002). This effect appeared to be mediated by CB1 receptors located on the axon terminals of intrinsic VTA neurons (Fig. 1B), since it was blocked by the antagonist SR141716A and was not observed when IPSCs were evoked by direct application of the $GABA_A$ agonist muscimol (Szabo et al., 2002). Furthermore, miniature spontaneously occurring tetrodotoxin-resistant IPSCs were unaffected by WIN55,212-2, further suggesting that the cannabinoid agonist acted presynaptically (Szabo et al., 2002). The results of this study suggested that CB1 receptors were located on GABAergic axon terminals targeting $GABA_A$ receptors in the VTA, and identified a potential substrate through which cannabinoids might act to increase the activity DA neurons in the VTA, and augment extracellular DA concentrations in the NAc (Fig. 1). However, in deference to this possibility, there is at least one additional potential mechanism for the cannabinoid-dependent disinhibition of VTA DA neurons that is known to exist. VTA DA neurons also receive substantial innervation from GABAergic neurons extrinsic to this brain structure, originating in the NAc (Sugita et al., 1992; Walaas and Fonnum, 1980), the ventral pallidum (Kalivas, 1993), and the pedunculopontine nucleus (Fig. 1) (Charara et al., 1996). Furthermore, these GABAergic axons are thought to primarily target $GABA_B$ receptors, rather than $GABA_A$ receptors located on VTA DA neurons,

where they act to attenuate their activity in vivo (Erhardt et al., 2002).

Based upon this anatomical information, we hypothesized that CB1 receptor activation might modulate these GABAergic inputs impinging upon $GABA_B$ receptors found on VTA DA neurons, and that this might represent a substrate for the disinhibitory effects of Δ^9-THC and other cannabinoids on these cells (Riegel and Lupica, 2004). When GABAergic axons were activated using trains of electrical stimulation consisting of 6 pulses at 50 Hz in brain slices containing the VTA, $GABA_B$ receptor-mediated IPSCs were recorded in the DA neurons (Fig. 2A). The cannabinoid receptor agonist WIN55,212-2 dose-dependently inhibited these $GABA_B$ IPSCs (Fig. 2C), and this effect could be reversed by the CB1 receptor antagonist, AM251 (Riegel and Lupica, 2004). In addition, we found that $GABA_B$ receptor-mediated ion currents activated by the direct pressure application of GABA to VTA DA neuron dendrites were insensitive to WIN55,212-2 (Fig. 2D). This suggested that CB1 receptors are located on GABAergic afferents that target $GABA_B$ receptors located on VTA DA neurons (Riegel and Lupica, 2004). Because $GABA_B$ receptor antagonists can disinhibit VTA DA neuron activity, increasing their rates of firing and bursting in vivo (Erhardt et al., 2002), and because Δ^9-THC is know to also initiate this effect (French et al., 1997), we hypothesize that this may represent a substrate to explain both the increase in forebrain extracellular DA concentrations, and the rewarding effects of Δ^9-THC in humans and animals.

3. The retrograde signaling capacity of endocannabinoids

One objective of the preceding studies has been to discover the substrates upon which Δ^9-THC acts upon in the drug reward circuitry that may account for its ability to support marijuana use in humans (Lupica et al., 2004; Riegel and Lupica, 2004). However, the physiological effects of endocannabinoids in other brain areas (Alger, 2002) suggest that this system may

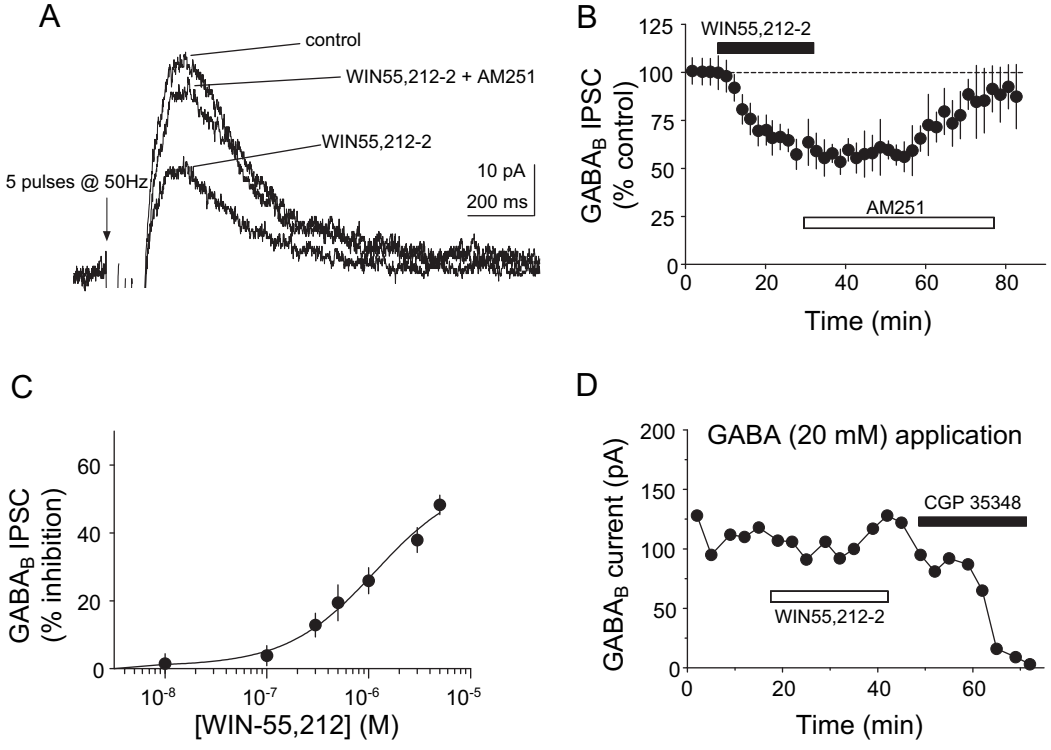

Fig. 2. CB1 receptors modulate GABAergic inputs to VTA DA neurons in mesencephalic brain slices. (A) Whole-cell electrophysiological recordings of $GABA_B$ receptor-mediated IPSC from a single VTA DA neuron voltage clamped at −60 mV. These currents were evoked by electrically stimulating the VTA brain slice with a high frequency (50 Hz) train of 6 pulses (downward arrow) during each of the indicated conditions. These recordings were performed in the presence of antagonists for $GABA_A$ receptors (picrotoxin, 100 μM), DA D2 receptors (eticlopride, 30 nM), and NMDA receptors (MK-801, 100 μM). Note the pronounced reduction of the IPSC in WIN55,212-2 (1 μM), and the antagonism of this effect by the CB1 receptor antagonist AM251 (2 μM). Concentrations of the cannabinoid drugs are the same in this, and all subsequent experiments, unless otherwise noted. (B) Mean time course ($n = 7$ DA neurons) for the effect of WIN55,212-2 and AM251 on $GABA_B$ IPSCs. (C) Concentration-dependent effects of WIN55,212-2 on $GABA_B$ IPSCs ($n = 6$–7 DA neurons). (D) WIN55,212-2 did not affect $GABA_B$ currents evoked by direct application of GABA to the DA neuron dendrites via a pressure application pipette. However, the selective $GABA_B$ receptor antagonist CGP35348 (200 μM) completely blocked these currents.

regulate ongoing brain function, and, if present in reward pathways, may also be involved in gating changes in synaptic efficacy that may play a role in addiction. Because of this possibility, another objective of more recent work has been to identify and define potential roles of endocannabinoids in the brain reward circuitry. The retrograde signaling capacity of endocannabinoids was first described by Wilson and Nicoll (2001) in the hippocampus. This system has yielded a wealth of information as to the requirements for, and the function of endocannabinoid release in functional circuits, and serves as an introduction to this process for purposes of the present discussion. Wilson and Nicoll (2001) demonstrated that the prolonged (5 s) depolarization (from −60 mV to 0 mV) of hippocampal pyramidal neurons could result in a transient reduction of $GABA_A$ receptor-mediated IPSCs recorded in these cells. This effect, known as the depolarization-induced suppression of inhibition (DSI) (Pitler and Alger, 1992), was determined to be mediated by an endocannabinoid released from the hippocampal pyramidal neurons because the presynaptic inhibition of GABA release following the depolarization of the postsynaptic pyramidal neuron was blocked by the cannabinoid receptor antagonists SR141716A or AM251. Also, this DSI effect was occluded by WIN55,212-2, or the endocannabinoid uptake inhibitor, AM404 (Wilson and Nicoll, 2001). This study also demonstrated that the release of the endocannabinoid depended upon an increase in intracellular calcium, since DSI was also initiated by the rapid release of calcium in the hippocampal pyramidal neuron via photolysis of caged calcium. This endocannabinoid-dependent DSI in the hippocampus relied upon the previously identified substrate of large numbers of CB1 receptors on the axon terminals of cholecystokinin-co-localizing GABAergic interneurons that provide strong inhibitory input to $GABA_A$ receptors located on the pyramidal neuron somatic membrane (Katona et al., 1999; Hoffman and Lupica, 2000; Wilson et al., 2001). Therefore, these endocannabinoid-sensitive receptors appeared to be identical to those activated by Δ^9-THC, and other cannabinoids, such as WIN55,212-2.

4. VTA DA neurons release endocannabinoids

In an attempt to determine whether endocannabinoids might play a role in the VTA, a similar approach to that of Wilson and Nicoll (2001) was utilized during patch clamp recordings from DA neurons (Melis et al., 2004b). However, in this case, rather than measuring endocannabinoid effects on GABAergic synaptic currents, glutamatergic, NMDA receptor-dependent synaptic currents (EPSCs) were measured (Melis et al., 2004b). It was found, similar to previous reports in other brain areas that the prolonged (5–10 s) depolarization (from −70 mV to +40 mV) of VTA DA neurons could transiently suppress NMDA EPSCs in these cells, by activating CB1 receptors located on glutamatergic afferents (Melis et al., 2004b). This endocannabinoid-mediated depolarization-induced suppression of excitation (DSE) effect in the VTA was also blocked by cannabinoid receptor antagonists, and by intracellular perfusion of a calcium chelator, confirming a role for calcium in endocannabinoid release. A similar DSE phenomenon has been described in several brain areas, where it may represent a mechanism to permit neurons to regulate their own level of excitatory input, based on the relative degree of activation (Alger, 2002).

Although the release of endocannabinoids following prolonged depolarization has been instrumental in establishing their roles as retrograde messengers, the physiological relevance of DSE and DSI has been questioned based upon the assertion that neurons rarely encounter such prolonged and pronounced levels of depolarization under non-pathological conditions. Furthermore, an in vivo study investigating this issue was unable to demonstrate DSI-like effects using stimulation protocols similar to those employed in vitro (Hampson et al., 2003). Because of these issues more recent studies have focused on the regulation of *intrinsic* neuronal mechanisms and afferent synaptic pathways that regulate neuronal excitability, and their roles in modulating physiologically relevant endocannabinoid release in the VTA.

The activity of VTA DA neurons in vivo is dominated by pacemaker-like regular firing interrupted by phasic bursts (Cooper, 2002). The latter pattern results in a transient increase in DA release in the NAc, particularly in relation to environmental stimuli possessing strong reward salience (Schultz, 1998). The shift from tonic pacemaker firing to bursting is strongly controlled by the patterns of synaptic input from glutamatergic and GABAergic afferents to the DA neurons in vivo (Grace and Bunney, 1985; Chergui et al., 1994; Tepper et al., 1995). Burst activity is absent under control conditions in vitro brain slice preparations. However, it can be restored by manipulations that augment glutamate function in the presence of the polypeptide bee venom toxin, apamin (Johnson et al., 1992; Seutin et al., 1993; Kitai et al., 1999). Apamin increases DA neuron excitability by blocking calcium-sensitive potassium channels (S_K channels, Fig. 1B) that mediate an inhibitory afterhyperpolarization that normally limits sustained action potential activity. Blockade of S_K thus causes these cells to be more sensitive to excitatory afferent input, or to diminished inhibition (Cooper, 2002; Overton and Clark, 1997). In an effort to determine whether increased VTA DA neuron activity and bursting were associated with endocannabinoid release, we examined the effects of apamin on both

$GABA_B$ receptor-mediated IPSCs, and AMPA receptor-mediated EPSCs in these cells in vitro. Similar to prior studies, we found that apamin induced an increased sensitivity of VTA DA neurons to glutamatergic input, resulting in increased firing rates and episodic membrane oscillations during electrical stimulation (Riegel and Lupica, 2004). This augmented activity resulted in an increase in endocannabinoid release that was revealed when the CB1 receptor antagonist AM251 was applied (Fig. 3A). Thus, AM251 caused large increases in both the $GABA_B$ IPSCs (Fig. 3A) and the AMPA EPSCs (Fig. 4) during apamin application, but had no effect on these synaptic responses when applied in the absence of apamin (Riegel and Lupica, 2004).

Another potent regulator of excitability and the state of action potential firing of VTA DA neurons is the strong glutamatergic input from areas of PFC and the pedunculopontine nucleus. However, this input is

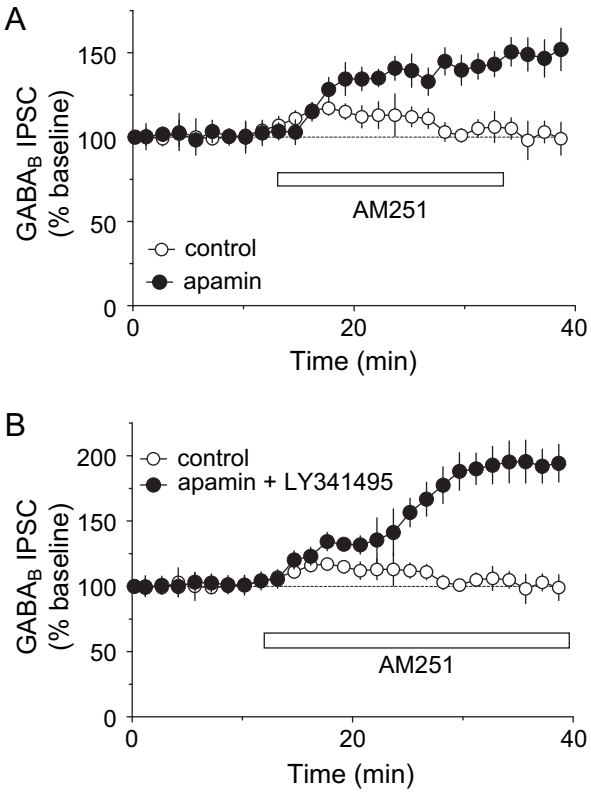

Fig. 3. The S_K channel blocker apamin (100 nM) and the mGluR antagonist LY341495 (200 μM) increase endocannabinoid release, and cause the inhibition of $GABA_B$ IPSCs in VTA DA neurons. (A) Mean time courses of the effect of the CB1 antagonist during control conditions, and after pre-incubation with apamin. Note the relatively large increase in $GABA_B$ IPSC amplitude by AM251 following apamin treatment. This indicates that apamin stimulated the release of an endocannabinoid that then activated CB1 receptors located on GABAergic terminals to inhibit the IPSCs. (B) Combined pre-incubation with apamin and LY341495 increased endocannabinoid release, and augmented the effect of AM251 on $GABA_B$ IPSCs ($n = 7$ VTA DA neurons).

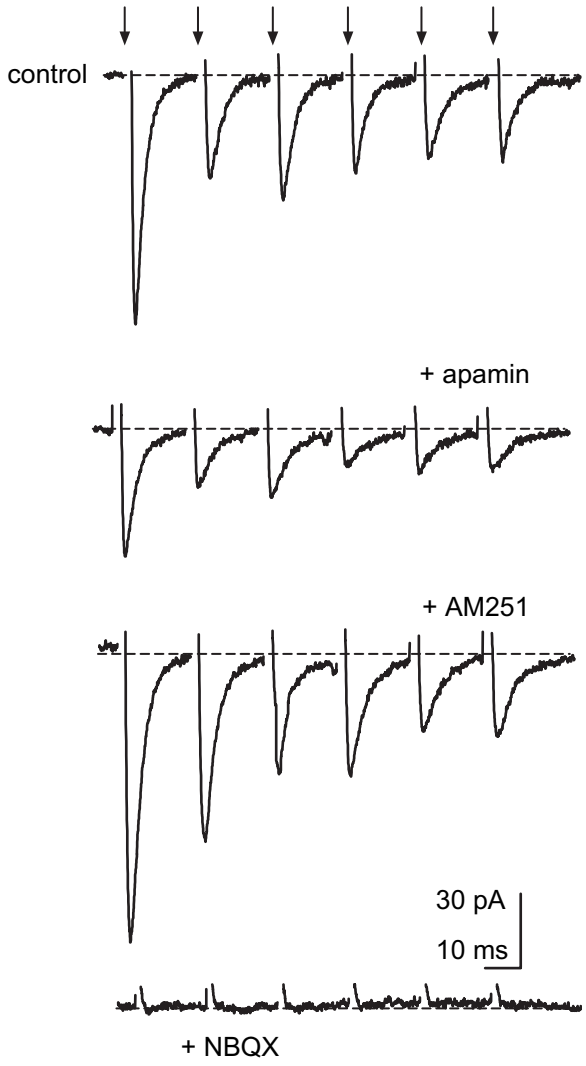

Fig. 4. Apamin application stimulates the release of endocannabinoids that inhibit AMPA-kainate EPSCs in VTA DA neurons. Trains of electrical stimuli (6 pulses at 50 Hz, arrows) were used to evoke multiple EPSCs from VTA DA neurons. Apamin application caused a pronounced inhibition of the EPSCs that was reversed by AM251, indicating that blockade of S_K channels also caused the release of endocannabinoids that then inhibited glutamate release onto VTA DA neurons. These EPSCs were recorded in the presence of the $GABA_B$ receptor antagonist CGP35348 (200 μM) used to block IPSCs. The selective AMPA-kainate antagonist NBQX completely blocked the EPSCs evoked by this stimulation.

under tight control by presynaptic metabotropic glutamate autoreceptors, predominantly of the type II and type III classes, that act to diminish the strength of the glutamatergic signal through a negative feedback mechanism (Bonci et al., 1997). In our study, antagonism of these presynaptic metabotropic autoreceptors (mGluRs) resulted in large increases in EPSCs evoked by high frequency (50 Hz) electrical stimulation in VTA DA neurons suggesting an augmentation of excitatory drive to the VTA DA

neurons. In addition, antagonism of these presynaptic mGluR-II/IIIs increased endocannabinoid release from the DA neurons, as demonstrated by an increase in $GABA_B$ IPSCs when AM251 was applied (Riegel and Lupica, 2004). Thus, the increased strength of synaptic glutamatergic inputs to the VTA DA neurons, and the enhanced postsynaptic sensitivity to this input during the blockade of postsynaptic S_K channels stimulated the release of an endocannabinoid in the VTA. As would be expected if these mechanisms were independent, combined treatment with both mGluR-III antagonists and apamin resulted in an additive increase in endocannabinoid release, and a larger effect of AM251 on $GABA_B$ IPSCs (Fig. 3B). The endocannabinoid release was also blocked by the intracellular application of the rapid calcium chelator, BAPTA-AM, demonstrating the calcium-dependency of endocannabinoid production, and further that the source of the endocannabinoid was the DA neurons themselves (Riegel and Lupica, 2004).

Collectively, these data suggest that increased DA neuron activity results in endocannabinoid release from these cells that then acts to simultaneously inhibit glutamatergic and GABAergic synaptic inputs that are derived from sources extrinsic to the VTA. Perhaps more importantly, since the combined administration of apamin and the facilitation of glutamate receptor transmission facilitates DA neuron bursting in vivo and in vitro (Overton and Clark, 1997; Seutin et al., 1993; Kitai et al., 1999), it is likely that endocannabinoid release is a consequence of bursting activity in vivo, and that endocannabinoids will be released in the presence of abused drugs that augment VTA DA neuron activity. This suggests that endocannabinoid release from VTA DA neurons and the activation of presynaptic CB1 receptors by these lipid molecules may represent a common consequence of the actions of a wide variety of abused drugs.

In further support for a potential physiological role for endocannabinoid release from VTA DA neurons in vivo, another recent study has shown that the selective activation of PFC glutamatergic afferents to the VTA appears to cause the release of an endocannabinoid that then acts to presynaptically limit glutamate release from this projection (Melis et al., 2004a). This was demonstrated by electrically stimulating the PFC while measuring the extracellular firing rates of VTA DA neurons in anesthetized rodents. When the PFC was stimulated, the firing rates of the VTA DA neurons were transiently increased, followed by a pronounced depression of spontaneous DA neuron activity that was reversed by i.v. administration of the cannabinoid receptor antagonist SR141716A (Melis et al., 2004a). This suggested that stimulation of this afferent projection at naturally occurring frequencies of activation (i.e. 5–10 Hz) provided sufficient excitation of VTA DA neurons to initiate endocannabinoid release that then acted in a retrograde manner to activate CB1 receptors on these glutamate terminals, limiting further glutamate release (Melis et al., 2004a). Therefore, our study (Riegel and Lupica, 2004) and those of Melis et al. (2004a, b) all demonstrate the dependence of endocannabinoid release from VTA DA neurons on the presence of intact excitatory glutamatergic inputs to these cells.

5. Interpreting VTA endocannabinoid effects

The true consequences of this activity-dependent modulation of synaptic inputs to the VTA DA neurons in the behaving organism are at present unknown. However, we can now make predictions based upon our knowledge of the substrates for endocannabinoid action in the VTA. Part of the difficulty in interpreting these data comes from the observations that both inhibitory GABAergic and excitatory glutamatergic synaptic inputs to VTA DA neurons can simultaneously be inhibited by endocannabinoids acting on axon terminal CB1 receptors (Riegel and Lupica, 2004) (Fig. 1B). Since these two opposing actions might be expected to result in a minimal net alteration in DA neuron activity, then how can this mechanism be invoked to explain a modulatory role for endocannabinoids in these cells? We speculate that the contribution of this endocannabinoid-dependent regulation of synaptic inputs to VTA DA neurons will depend upon the relative level of activation of these afferent pathways under distinct behavioral circumstances (Riegel and Lupica, 2004; Lupica et al., 2004). For example, the presynaptic inhibition of GABA release by endocannabinoids from afferent axon terminals might be critical to increased DA neuron activity during increased activation of the NAc output to the VTA. Under these circumstances the release of endocannabinoids from the VTA DA neurons might be expected to disinhibit this inhibitory input, thereby resulting in enhanced DA neuron activity. Similarly, the endocannabinoid action on glutamatergic afferents to the VTA DA neurons might be involved during heightened activation of cortical afferents to the VTA (Melis et al., 2004a), such as during the process of psychostimulant sensitization (Wolf et al., 2004). Clearly, there are multiple scenarios that can be envisioned in which the activity-dependent release of endocannabinoids from VTA DA neurons might play a role in retrogradely modulating inhibitory and excitatory afferent pathways to the VTA; perhaps regulating the balance of these influences and burst firing itself. In addition to this implication, the preceding data strongly suggest that endocannabinoids likely play an important role in the ongoing regulation of brain function,

including those involved in mediating reward, pleasure, motivation, and perhaps addiction.

6. Implications for cannabinoid receptor antagonists in the treatment of addiction

The cannabinoid receptor antagonist SR141716A (Rimonabant) can block the subjective rewarding effects of smoked marijuana, and therefore Δ^9-THC, in humans (Huestis et al., 2001), as well as eliminate the self-administration of Δ^9-THC in monkeys (Tanda et al., 2000). Furthermore, SR141716A appears to show promise as a useful medication for the treatment of cigarette smoking in humans (Le Foll and Goldberg, 2005), and it has been shown to be effective at blocking the self-administration of nicotine in rodents, as well as the nicotine-induced increase in extracellular DA concentrations in the NAc in rats (Cohen et al., 2002). Laboratory studies have also shown that either the genetic deletion of CB1 receptors (Cossu et al., 2001; Martin et al., 2000), or their antagonism by SR141716A (Chaperon et al., 1998) can reduce or eliminate the rewarding properties of opiates. In addition, SR141716A can also block relapse to heroin self-administration caused by a variety of priming stimuli known to initiate heroin seeking in behaviorally extinguished animals (De Vries et al., 2003). Among the most promising uses of cannabinoid receptor antagonists for the treatment of addiction may be seen in their ability to dramatically reduce ethanol seeking and consumption (Le Foll and Goldberg, 2005). Thus, SR141716A pretreatment or genetic deletion of the CB1 receptor has been shown to dramatically decrease the amount of ethanol consumed by rodents (Arnone et al., 1997; Colombo et al., 1998; Poncelet et al., 2003). Clearly, this could be an effective treatment for alcoholic patients.

In contrast to the implied roles of CB1 receptors in the effects of the abused drugs described above, it has been more difficult to provide evidence for the involvement of these receptors in the acute rewarding properties of psychostimulants, such as cocaine and amphetamine (Le Foll and Goldberg, 2005; De Vries et al., 2001). However, involvement of cannabinoid receptors in the relapse to cocaine seeking after its extinction has been demonstrated, suggesting that they may play an important role in psychostimulant addiction (De Vries et al., 2003). In these studies, SR141716A diminished relapse to cocaine self-administration that was induced by environmental stimuli associated with the psychostimulant, as well as the relapse caused by a priming injection of this drug (De Vries et al., 2003). A similar finding was obtained for the effects of SR141716A on methamphetamine seeking that was reinstated by a priming dose of the drug (Anggadiredja et al., 2004). Collectively, these studies demonstrate that cannabinoid receptor antagonists may prove useful in the treatment of drug addiction, or in the prevention of relapse to compulsive drug seeking (Le Foll and Goldberg, 2005).

7. The anti-addictive properties of cannabinoid receptor antagonists may reflect their interaction with endocannabinoids in the VTA

Although the neurobiological mechanisms for the potentially beneficial effects of cannabinoid antagonists on drug use and addiction are at present unknown, we propose that the antagonism of CB1 receptors located within the VTA, and the blockade of endocannabinoid action on these receptors represents a critical substrate (Fig. 1). This idea is supported by recent data described above that endocannabinoids are released in an activity-dependent fashion from mesencephalic DA neurons (Melis et al., 2004b; Riegel and Lupica, 2004), and by the ability of these drugs to obtund the acute rewarding effects of these drugs in behavioral assays. In further support of this idea, there also appears to be a correlation between the ability of many abused drugs to act by increasing DA neuron activity, and the ability of drugs like SR141716A and AM251 to diminish their rewarding capacity. For example, as described above, these CB1 antagonists clearly diminish the tendency of animals to self administer opiates, ethanol, Δ^9-THC, and nicotine; all drugs that have been shown to increase NAc DA concentrations by increasing VTA DA neuron firing and burst rates. However, as several studies have demonstrated, the acute rewarding effects of psychostimulants such as cocaine and amphetamine seem somewhat refractory to treatment with cannabinoid antagonists (De Vries et al., 2001). We suggest that this differential effect may be due to the ability of psychostimulants to increase extracellular DA concentrations through actions at DA axon terminals, rather than by increasing DA neuron firing rates, and thus endocannabinoid release. Since the critical endocannabinoid substrate described here may be shared by many abused drugs, it is possible that cannabinoid receptor antagonists will be useful for the treatment of many forms of addiction. However, before this can be evaluated, it will be necessary to more firmly understand the role that the endocannabinoids play in regulating ongoing normal neurobiological function in these reward pathways.

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