Differential Regulation of the Endocannabinoids Anandamide and 2-Arachidonylglycerol within the Limbic Forebrain by Dopamine Receptor Activity

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ABSTRACT

Glutamatergic synaptic transmission within the striatum and prefrontal cortex regulates the neuronal synthesis of endocannabinoids. Because a primary role of dopamine is to modulate this excitatory transmission, we tested the hypothesis that dopaminergic transmission modulates endocannabinoid content in the limbic forebrain. Liquid chromatography/mass spectrometry was used to determine endogenous anandamide and 2-arachidonylglycerol (2-AG) contents within the limbic forebrain of mice after pharmacological manipulation of dopaminergic transmission. Increasing synaptic dopamine concentrations with methylphenidate significantly and dose dependently decreased both anandamide and 2-AG content. The selective dopamine reuptake inhibitor 1-[2-[bis(4-fluorophenyl)methoxy]ethyl]-4-(3-phenylpropyl)piperazine (GBR 12909) also significantly decreased anandamide and tended to decrease 2-AG content. The D1 receptor antagonist R-(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine (SCH 33390) increased and the D1 receptor agonist 2,3,4,5-tetrahydro-7,8-dihydroxy-1-phenyl-1H-3-benzazepine (SKF 33393) decreased anandamide content. 2-AG content was unaffected by SCH 33390 but was significantly increased by the D2 receptor antagonist eticlopride, which had no effect on anandamide content. The D2 agonist quinpirole had a biphasic effect on anandamide content with low, autoreceptor-prefering doses increasing anandamide and higher doses decreasing it back toward control. Quinpirole did not significantly affect 2-AG content. Together, these data indicate that endogenous dopamine exerts a differential, net suppressive effect upon anandamide and 2-AG content via activation of D1 and D2 receptors, respectively. These data are consistent with the hypothesis that modulation of endocannabinoid content by dopamine is secondary to changes in glutamatergic transmission, and they provide a pharmacological framework for the rational development of endocannabinoid-based therapeutic interventions for dopamine-related neuropsychiatric disorders.

Several endogenous neuronal cannabinoid receptor (CB1) ligands, including anandamide and 2-AG, have been identified that fulfill established criteria as neuromodulators (for review, see Hillard, 2000). Anandamide is synthesized by sequential activities of an N-acyltransferase and phospholipase D, whereas 2-AG is synthesized by phospholipase C-mediated cleavage of phosphatidylinositol bis-phosphate, generation of diacylglycerol, and the subsequent activity of a diacylglycerol lipase. The effects of anandamide and 2-AG are terminated via degradation by fatty acid amide hydrolase and monoglyceride lipase, respectively (Cravatt et al., 2001; Dinh et al., 2002). Several in vitro studies suggest that endocannabinoids serve as activity-dependent retrograde inhibitors of neurotransmitter release within the striatum and prefrontal cortex (Auclair et al., 2000; Gerdeman et al., 2002; Dinh et al., 2002). Several in vitro studies suggest that endocannabinoids serve as activity-dependent retrograde inhibitors of neurotransmitter release within the striatum and prefrontal cortex (Auclair et al., 2000; Gerdeman et al., 2002; Robbe et al., 2002). According to this hypothesis, endocannabinoids are produced by postsynaptic cells (striatal medium spiny neurons and cortical pyramidal neurons) in response to glutamate receptor activation. Endocannabinoids then activate presynaptic CB1 receptors, which function to inhibit further glutamate release (Gerdeman and Lovinger, 2001; Huang et al., 2001).

Dopaminergic transmission within the limbic forebrain contributes to optimal cognitive function, psychomotor control, and the expression of motivated behavior (Braver and...
An essential function of dopaminergic transmission is to enhance the signal-to-noise ratio of information processing via modulation of cortical and striatal glutamatergic transmission and the responsibility of postsynaptic neurons to glutamatergic inputs (Horvitz, 2002; O'Donnell, 2003). Although the precise molecular mechanisms by which this function is expressed remain controversial, studies suggest that activation of D1 family (D1 and D5) receptors decreases the responsibility of striatal GABAergic and cortical pyramidal neurons to non-N-methyl d-aspartate (NMDA) receptor activation, while enhancing signaling through NMDA receptors (Cepeda et al., 1993; Seamans et al., 2001; Wang and O'Donnell, 2001). Such a mechanism has been suggested to facilitate strong, sustained, and highly efficacious synaptic activity that results from NMDA receptor activation, while decreasing the effects of weak and transient non-NMDA-mediated depolarization (Seamans et al., 2001). Activation of D2 family (D2, D3, and D4) receptors located postsynaptically is associated with either a decrease or no effect on NMDA and non-NMDA receptor responsivity (Cepeda et al., 1993; Cepeda and Levine, 1998). However, evidence suggests that DA inhibits glutamate release via activation of presynaptic D2 heteroreceptors on cortico-striatal glutamatergic afferents (Hsu et al., 1995); consequently, D2 receptor knockout mice exhibit increased striatal glutamatergic transmission (Cepeda et al., 2001).

Therefore, because dopaminergic activity seems to negatively modulate the overall response of striatal and prefrontal cortical neurons to glutamatergic input (Kiyatkin and Rebec, 1999; Seamans et al., 2001; Horvitz, 2002; O'Donnell, 2003), and glutamatergic input is likely an important activator of endocannabinoid synthesis in the limbic forebrain, we have tested the hypothesis that increased dopaminergic tone results in decreased endocannabinoid content in mouse limbic forebrain. There is support for this hypothesis from other laboratories. In one study, lesioning of dopaminergic neurons with 6-hydroxy dopamine was found to produce a 3-fold increase in anandamide, but not 2-AG, content within the striatum (Gubellini et al., 2002). This increase was reversed when dopamine was restored by chronic L-DOPA treatment (Maccarrone et al., 2003). It has also been reported that monoamine depletion by reserpine results in a 2.5-fold increase in both anandamide and 2-AG content within the striatum (Di Marzo et al., 2000b). In addition, Giuffrida et al. (1999) demonstrated that local infusion of the D2 receptor agonist quinpirole increased anandamide outflow within the dorsal striatum, whereas no 2-AG was detected in dialysate samples (Giuffrida et al., 1999). Because activation of D2 autoreceptors inhibits release of dopamine (Mottola et al., 2002), these data are consistent with the hypothesis that decreased dopaminergic transmission increases endocannabinoid content within forebrain regions that receive dopaminergic innervation.

We have investigated the effects of acute regulation of dopamine tone on limbic forebrain anandamide and 2-AG content. We report that basal dopaminergic transmission exerts a net inhibition of both anandamide and 2-AG content via activation of D1 and D2 receptors, respectively. In addition, augmentation of dopaminergic transmission decreases both anandamide and 2-AG content. These data shed new light on the dopaminergic modulation of endocannabinoid signaling and suggest the possibility of functionally separate anandamide and 2-AG endocannabinoid systems within the limbic forebrain.

### Materials and Methods

**Drugs and Animals.** ICR male albino mice (25–35 g) from Harlan (Madison, WI) were used in all experiments and were housed on a 12:12-h light cycle (lights on at 6:00 AM) with ad libitum access to

![Fig. 1. A representative chromatograph depicting the presence of deuterated standards, [1H6]anandamide (AEA) and [1H6]2-AG, and endogenous endocannabinoids, AEA and 2-AG, within lipid extracts from the limbic forebrain of control animals.](image-url)
were resuspended in 500 μl of methanol to recapture any lipids adhering to the glass tube, and dried again. Finally, lipid extracts were suspended in 20 μl of methanol, 5 μl of which was used for analysis by liquid chromatography/mass spectrometry.

**Liquid Chromatography/Mass Spectrometry.** The amounts of anandamide and 2-AG were determined by liquid chromatography-atmospheric pressure chemical ionization-mass spectrometry (1100 LC-MSD, SL model; Agilent Technologies Inc., Wilmington, DE). Samples (5 μl) were separated on a reverse-phase C18 column (Kromasil, 250 × 2 mm, 5-μm diameter) using mobile phase A (deionized water, 1 mM ammonium acetate, and 0.005% acetic acid) and mobile phase B (methanol, 1 mM ammonium acetate, and 0.005% acetic acid). Samples were eluted at a flow rate of 300 μl/min by a linear gradient. The percentage of solvent B increased linearly from 85% solvent B to 100% solvent B in 25 min then held at 100% solvent B for 10 min. Over the next 10 min, solvent B decreased linearly from 100% to 85%, and was held at 85% for an additional 10 min. Detection was made in a positive ion mode. Selective ion monitoring was used to detect [3H]anandamide (m/z 356; retention time = 13.7 min), anandamide (m/z 348; retention time = 13.9 min), [3H]2-AG, and 1(3)-AG (m/z 387; retention times = 14.3 and 15.1 min, respectively), and 2-AG and 1(3)-AG (m/z 379; retention times = 14.5 and 15.3 min, respectively). 2-AG is usually observed as a doublet because it isomerizes to 1(3)-AG during extraction (Stella et al., 1997), the area of both peaks were combined to yield total 2-AG. Endocannabinoid contents were normalized to wet tissue weight.

**Statistical Analysis.** Differences in the mean endocannabinoid content between treatment groups were determined by one-way analysis of variance followed by post hoc Dunnett’s or Bonferroni’s test as indicated. Data are presented as percentage of control endo-

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**Fig. 2.** Effects of the psychostimulant methylphenidate (MP) on anandamide (AEA) (a) and 2-AG (b) content within the limbic forebrain. *, p < 0.05; ***, p < 0.001, statistically different from vehicle treatment; Dunnett’s multiple comparisons analysis. Vehicle content; AEA 28.0 ± 1.8 ng/g; 2-AG 2.5 ± 0.2 μg/g.

**Fig. 3.** Effects of the dopamine uptake inhibitor GBR 12909 (GBR) on anandamide (AEA) (a) and 2-AG (b) content within the limbic forebrain. ***, p < 0.001, statistically different from vehicle treatment; Dunnett’s multiple comparisons analysis. Vehicle content; AEA 15.6 ± 0.77 ng/g; 2-AG 4.0 ± 0.6 μg/g.
cannabinoid content (n = 5 animals/group throughout). A p < 0.05 was considered significant throughout.

Results

Detection of Endocannabinoids within the Limbic Forebrain. We have studied the effects of changes in dopamine tone on anandamide and 2-AG content within the limbic forebrain (dorsal and ventral striatum and frontal cortex) because this brain region receives the majority of the mesotelencephalic dopaminergic projection. In addition, an analogous region in both rats and mice has been used extensively for endocannabinoid determination in other laboratories (Di Marzo et al., 2000a; Wang et al., 2003). In vehicle-treated mice, anandamide and 2-AG were both easily detected within the limbic forebrain; a representative chromatograph depicting the presence of deuterated standards and endogenous anandamide and 2-AG from this region is shown (Fig. 1). The mean values (± S.E.M., n = 23) obtained were 18.3 ± 2.2 ng/g tissue weight for anandamide and 3.6 ± 0.2 μg/g tissue weight for 2-AG. These values agree with those reported by other laboratories (Di Marzo et al., 2000b; Wang et al., 2003). The limbic forebrain endocannabinoid content of control animals varied among individual experiments due to small changes in the sensitivity of analytical hardware; therefore, each experiment included a control (vehicle-treated) group, and statistical analyses were confined to comparisons among samples subjected to the same experimental and analytical procedures.

Effects of Indirect Dopaminergic Agonists on Endocannabinoid Content. We determined the effects of indirect dopaminergic agonists on anandamide and 2-AG within the limbic forebrain. The effects of methylphenidate and cocaine on anandamide and 2-AG content were determined 40 min after drug administration, a time point at which maximal elevations in dopamine levels have been reported (Gerasimov et al., 2000; Carboni et al., 2001). The effects of GBR 12909 were determined 60 min after administration due to its relatively slow onset of action (Carboni et al., 2001). Methylphenidate (Ritalin; 1, 10, and 20 mg/kg), a potent dopamine- and norepinephrine-releasing compound (Kuczenski and Segal, 1997; Gerasimov et al., 2000), produced significant and dose-related decreases in both anandamide and 2-AG content, reaching maximal inhibition at 10 mg/kg (Fig. 2). GBR 12909, a compound known to selectively inhibit uptake of dopamine and increase extracellular dopamine within the striatum (Carboni et al., 2001), produced a dose-dependent decrease in anandamide, reaching statistical significance at 10 mg/kg (Fig. 3a). GBR 12909 also tended to decrease 2-AG, but the reduction was not significant (Fig. 3b). Interestingly, cocaine, a nonselective monoamine uptake inhibitor, reduced anandamide content slightly at 1 mg/kg but did not reach statistical significance at any dose tested (Fig. 4a). Conversely, cocaine significantly increased 2-AG content at a dose of 20 mg/kg (Fig. 4b).

Effects of Dopamine Receptor Subtype-Specific Antagonists on Endocannabinoid Content. To determine whether endogenous dopamine modulates anandamide or

Fig. 4. Effects of the psychostimulant cocaine (coc) on anandamide (AEA) (a) and 2-AG (b) content within the limbic forebrain. *, p < 0.05, significantly different from vehicle treatment; Dunnett’s multiple comparisons analysis. Vehicle content; AEA 20.2 ± 2.2 ng/g; 2-AG 3.1 ± 0.3 μg/g.

Fig. 5. Effects of the D1 receptor antagonist SCH 23390 (SCH) on anandamide (AEA) (a) and 2-AG (b) content within the limbic forebrain. *, p < 0.05; **, p < 0.001, significantly different from vehicle treatment; Dunnett’s multiple comparisons test. Vehicle content; AEA 8.6 ± 0.5 ng/g; 2-AG 2.8 ± 0.1 μg/g.
2-AG content within the limbic forebrain via activation of D1 or D2 receptors, we administered the D1 receptor antagonist SCH 23390 (0.2 and 2 mg/kg) or the D2 receptor agonist eticlopride (0.2 and 2 mg/kg). Amounts of anandamide and 2-AG were determined 40 min after drug administration. SCH 23390 dose dependently increased anandamide content but did not affect 2-AG content (Fig. 5). Eticlopride significantly increased 2-AG content but did not affect anandamide content at any dose (Fig. 6).

Effects of Dopamine Receptor Subtype-Specific Agonists on Endocannabinoid Content. To further evaluate the contribution of D1 and D2 receptor activation to the suppression of endocannabinoid content by dopamine, we administered the D1 agonist SKF 33393 (1, 10, and 30 mg/kg) or the D2 agonist quinpirole (0.1, 1, and 10 mg/kg). The effects of SKF 33393 on the amount of anandamide and 2-AG were determined 40 min after drug administration. SKF 33393 dose dependently decreased anandamide (Fig. 7a) but exhibited a biphasic effect on 2-AG (Fig. 7b); a significant increase in 2-AG was observed at 10 mg/kg but not 30 mg/kg. When administered systemically, quinpirole exhibits a biphasic dose- and time-dependent effect on locomotion, with early locomotor suppression seen at all doses, and locomotor stimulation seen at longer time points at high doses only (Horvitz et al., 2001). To more accurately correlate the behavioral state with endocannabinoid content, the effects of quinpirole on anandamide and 2-AG content were determined 2 h after drug administration, a time point at which a 0.1-mg/kg dose produces locomotor inhibition, whereas 1- and 10-mg/kg doses produce locomotor stimulation (S. Patel and C. J. Hillard, unpublished observations; Horvitz et al., 2001). Quinpirole administration resulted in a biphasic effect on anandamide, with 0.1 and 1 mg/kg significantly increasing, and 10 mg/kg demonstrating no difference from control, but a significant decrease from the 0.1-mg/kg dose (Fig. 8a). Quinpirole also tended increase 2-AG at 0.1 and 1 mg/kg; however, these effects did not reach statistical significance (Fig. 8b).

Discussion

The purpose of these studies was to explore the relationship between dopaminergic transmission and endocannabinoid content within the limbic forebrain. Based upon the distribution of D1, D2, and CB1 receptors in this brain region, a working model of the relationships among these players and endocannabinoid synthesis is shown in Fig. 9a. Support for this model comes from in vitro studies demonstrating that stimulation of glutamatergic afferent fibers in the dorsal striatum and nucleus accumbens results in CB1 receptor-mediated suppression of presynaptic glutamate release (Gerden et al., 2002; Robbe et al., 2002). Similar effects have been demonstrated within the prefrontal cortex (Auclair et al., 2000). In addition, striatal and cortical neurons in culture produce anandamide in response to non-NMDA glutamate receptor activation and depolarization, whereas 2-AG is produced by cortical neurons in response to NMDA receptor
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activation (Di Marzo et al., 1994; Stella et al., 1997; Stella and Piomelli, 2001). Because dopaminergic activity modulates the response of postsynaptic neurons to glutamatergic input (see Introduction), we have tested the hypothesis that manipulation of dopaminergic tone results in alterations in endocannabinoid content of the limbic forebrain in mice. Our findings support this hypothesis and indicate that two endocannabinoids, anandamide and 2-AG, are reciprocally and differentially regulated by dopamine receptor activity in vivo.

Our results indicate that anandamide content in the limbic forebrain is under tonic inhibitory control by dopamine acting through D1 receptors. Data to support this conclusion are that the D1 receptor agonist SKF 33939 decreased, whereas the D1-selective antagonist significantly increased, anandamide content. In addition, pharmacological elevation of synaptic dopamine decreased limbic forebrain anandamide content. Overall, our findings agree with data indicating that activation of postsynaptic D1 receptors decreases non-NMDA-mediated excitatory influences on postsynaptic neurons (Cepeda and Levine, 1998; Kiyatkin and Rebec, 1999; O'Donnell, 2003) and that D1 antagonists increase the mean firing rate of striatal neurons in vivo (Kiyatkin and Rebec, 1999). In addition, dopaminergic depletion-induced elevation in cortico-striatal transmission, which is associated with increased anandamide but not 2-AG content, is dependent upon non-NMDA glutamate receptor activation (Gubellini et al., 2002). These observations strongly suggest that elevated anandamide content is correlated with increased neuronal activity in vivo. This contention is further supported by in vitro data that anandamide synthesis is increased by membrane-depolarizing agents and non-NMDA glutamate receptor agonists (Di Marzo et al., 1994).

We have also determined the effects of D2 receptor ligands on anandamide content. Whereas the D1 receptor is predominantly postsynaptic, at least three functionally distinct D2 receptor "pools" are operative in the striatum. First, D2 receptors on glutamatergic terminals inhibit glutamate release (Hsu et al., 1995; Cepeda et al., 2001); second, D2 receptors on dopaminergic neurons act as release- and synthesis-inhibiting autoreceptors (Skirboll et al., 1979); and third, D2 receptors are expressed postsynaptically on those GABAergic neurons that mediate outflow via the indirect, striatopallidal pathway (Harrison et al., 1992). Notably, the two pools of presynaptic D2 receptors exert opposite effects on the activity of the GABAergic neurons; agonist binding to D2 receptors on dopaminergic terminals will reduce dopamine release that will indirectly increase cortico-striatal drive and GABAergic cellular activity (Calabresi et al., 1993; Gubellini et al., 2002), whereas agonist binding to D2 receptors on glutamate terminals will reduce glutamate release, and hence reduce GABAergic cellular activity. Consistent with these mechanisms, the D2 agonist quinpirole has a biphasic effect on limbic forebrain anandamide content, i.e., a low dose of quinpirole produced a robust increase in anandamide content, whereas higher doses produced progressively smaller increases. This pattern is consistent with our hypothesis because at low doses we expect quinpirole to preferentially reduce dopaminergic transmission and therefore indirectly increase striatal or cortical neuronal activity, and anandamide content (Fig. 9c). As the quinpirole dose increases, D2-mediated inhibition of glutamate release predominates, which reduces the excessive glutamatergic input and reduces the elevated anandamide content (Fig. 9d). This hypothesis is supported by data suggesting that the two presynaptic pools of D2 receptors exhibit differential sensitivity to dopamine, with the receptor on dopaminergic terminals being most sensitive to agonists (Skirboll et al., 1979). Interestingly, the D2 receptor antagonist eticlopride did not alter anandamide content, suggesting either that the presynaptic D2 receptors are quiescent during normal dopaminergic transmission or that the combined effect of this inhibitor on the functionally diverse D2 receptor pools results in no overall change in anandamide content. Alternatively, because blockade of D2 receptors increases dopamine release, it is possible that activation of D1 receptors by dopamine could actively suppress anandamide synthesis as outlined above.

We have also found that the limbic forebrain content of a second endocannabinoid, 2-AG, is modulated by changes in dopaminergic transmission. The pattern of regulation shares with anandamide a dependence on dopaminergic tone, but suggests differences in the dopamine receptors involved. 2-AG content was significantly reduced when synaptic dopamine was increased by methylphenidate and was slightly reduced by GBR 12909. Interestingly, cocaine, a nonselective monoamine uptake inhibitor, produced a significant elevation in 2-AG content at a dose of 20 mg/kg. Although untested, these data suggest that increases in either norepinephrine and/or serotonin could increase 2-AG content.

Unlike anandamide, 2-AG content was not affected by the
D1 receptor antagonist, but inhibition of D2 receptors with eticlopride resulted in a doubling of 2-AG content in the limbic forebrain. These data are consistent with an overall tonic suppression of 2-AG synthesis by activation of D2 receptors. Unlike the D1 antagonist SCH 23390, eticlopride does not affect mean firing rate of striatal neurons (Kiyatkin and Rebec, 1999) but does increase expression of the immediate early gene c-fos within the striatal complex (S. Patel and C. J. Hillard, unpublished data; Keefe and Adams, 1998). Fos expression is dependent upon changes in neuronal calcium concentrations and is likely increased by D2 antagonists secondary to suppression of dopamine-mediated inhibition of glutamate release, increased NMDA receptor activation, and calcium influx (Morgan and Curran, 1988; Keefe and Adams, 1998). It is our current hypothesis that a detectable increase in 2-AG content requires sustained depolarization and prolonged elevations in intracellular calcium induced by NMDA, metabotropic, or neuropeptide receptor activation. Our finding that the D1 agonist increased 2-AG content supports this contention, because D1 receptor activation results in increased NMDA-mediated postsynaptic responses (Cepeda et al., 1993; Wang and O’Donnell, 2001). Similarly, 2-AG, but not anandamide, is produced by cortical neurons in response to NMDA receptor activation in vitro (Stella and Piomelli, 2001), and NMDA-dependent long-term potentiation induction by high-frequency stimulation of Schaffer collaterals results in selective synthesis of 2-AG in hippocampal slice preparations (Abraham and Huggett, 1997; Stella et al., 1997).

We suggest that the modulatory effects of dopamine on endocannabinoid content are mediated via alterations in glutamatergic transmission, which in turn, drives endocannabinoid synthesis. However, effects of dopamine receptor activation on endocannabinoid degradation or metabolism cannot be excluded from the present data. In addition, the effects of dopaminergic compounds on regions outside the limbic forebrain, for example, the substantia nigra, could contribute to the alterations in endocannabinoid content observed in this study.

The functional and clinical implications of dopamine modulation of endocannabinoid signaling within striatum and prefrontal cortex are far-reaching. Exogenous activation of striatal CB1 receptors profoundly inhibits movement (Gough and Olley, 1978), and elevations in endocannabinoids have been demonstrated in several animal models of Parkinson’s disease (Di Marzo et al., 2000b; Gubellini et al., 2002). Thus, converging data indicate that elevations in endocannabinoid content and CB1 receptor activity are associated with hypokinetic states (for review, see Romero et al., 2002). Our data indicate that decreased D1 and D2 receptor activation would result in increased endocannabinoid content, which could contribute to the motor dysfunction associated with Parkinson’s disease and/or D1 and D2 receptor antagonists. Interestingly, it has recently been reported that coadministration
of the D2 agonist quinpirole and the CB1 receptor antagonist SR141716 produced greater locomotor stimulation than quinpirole alone (Giuffrida et al., 1999; Di Marzo et al., 2000b). Furthermore, we have found that psychomotor stimulant administration, which dramatically increases locomotor activity, results in a decrease in endocannabinoid content, and others have reported that exogenous administration of CB1 agonists attenuates amphetamine-induced hyperactivity (Pryor et al., 1978). These data suggest that reduced endocannabinoid signaling could play a permissive role in the expression of locomotor activity.

Alterations in dopaminergic transmission also contribute to the pathoetiology of schizophrenia. Specifically, decreased mesocortical dopaminergic transmission and decreased activation of D1 receptors is associated with diminished working memory function, a hallmark negative symptom of the disease (Okubo et al., 1997; Abi-Dargham et al., 2002). Because our data indicate that decreased dopaminergic transmission through D1 receptors is associated with increased anandamide content, and exogenous administration of CB1 receptor agonists decreases cognitive function in animals and humans (for review, see Lichtman et al., 2002), it is tempting to speculate that elevated anandamide content contributes to negative schizophrenic symptoms. In fact, increased anandamide content has been demonstrated in the cerebral spinal fluid of schizophrenic patients (Leweke et al., 1999). This hypothesis would predict that pharmacological manipulations that reduce anandamide content or CB1 receptor activation represent a novel approach to the treatment of negative schizophrenic symptoms (Mortimer, 1997).

In summary, these data indicate that anandamide and 2-AG are differentially modulated by dopamine, via activation of D1 and D2 receptors, respectively. These data suggest that anandamide and 2-AG are not redundant molecules and that separate, overlapping anandamide and 2-AG signaling systems could operate within the limbic forebrain. These data provide a basis for understanding the relationship between dopaminergic transmission and endocannabinoid signaling and thus could represent a useful framework upon which to develop endocannabinoid-based treatments for dopaminergic-related neuropsychiatric disorders, including Parkinson’s disease and schizophrenia.

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