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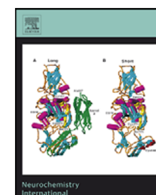

Neurochemical evidence that stimulation of CB1 cannabinoid receptors on GABAergic nerve terminals activates the dopaminergic reward system by increasing dopamine release in the rat nucleus accumbens

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ABSTRACT

We examined the effect of cannabinoid receptor activation on basal and electrical field simulation-evoked (25 V, 2 Hz, 240 shocks) [^{3}H]dopamine efflux in the isolated rat nucleus accumbens in a preparation, in which any effect on the dendrites or somata of ventral tegmental projection neurons was excluded. The cannabinoid agonist (R)-(+)-[2,3-dihydro-5-methyl-3-(4-morpholinylmethyl)pyrrolo[1,2,3-de]-1,4-benzoxazin-6-yl]-1-naphthalenylmethanone mesylate (WIN55,212-2, 100 nM) significantly enhanced stimulation-evoked [^{3}H]dopamine release in the presence of the selective dopamine transporter inhibitor 1-[2-[bis-(4-fluorophenyl)methoxy]ethyl]-4-(3-phenylpropyl)piperazine dihydrochloride (GBR12909, 100 nM). GBR12909 (100 nM–1 μM), when added alone, increased the evoked [^{3}H]dopamine efflux in a concentration-dependent manner. The stimulatory effect of WIN55,212-2 on the evoked tritium efflux was inhibited by the selective CB1 cannabinoid receptor antagonist N-(piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide (AM251, 100 nM) and by the $GABA_A$ receptor antagonist bicuculline (10 μM). Repeated application of N-methyl-D aspartate (1 mM) under Mg^{2+}-free conditions, which directly acts on dopaminergic terminals, reversibly increased the tritium efflux, but WIN55,212-2 did not affect N-methyl-D aspartate-evoked [^{3}H]dopamine efflux, indicating that WIN55,212-2 has no direct action on dopaminergic nerve terminals. AM251 (100 nM) alone also did not have an effect on electrical stimulation-evoked [^{3}H]dopamine efflux. Likewise, the selective CB2 receptor antagonist 6-iodo-2-methyl-1-[2-(4-morpholinyl)ethyl]-1H-indol-3-yl](4-methoxyphenyl)methanone (AM630, 0.3 μM) and the anandamide transport inhibitor (5Z,8Z,11Z,14Z)-N-(4-hydroxy-2-methylphenyl)-5,8,11,14-eicosatetraenamide (VDM11, 10 μM) had no significant effect on electrically evoked [^{3}H]dopamine release.

This is the first neurochemical evidence that the activation of CB1 cannabinoid receptors leads to the augmentation of [^{3}H]dopamine efflux via a local $GABA_A$ receptor-mediated disinhibitory mechanism in the rat nucleus accumbens.

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Natural cannabinoids are among the most popular recreational drugs of abuse, regularly consumed by approximately 4% of the adult population worldwide. Although the addictive properties of cannabinoids are not as strong as opiates and strong psychostimulants, there is now unequivocal evidence that they induce a behavioural pattern that is characteristic of drug addiction, including self-administration and drug-seeking behaviour (Fattore et al., 2008). Consequently, there is a recognized need for medical treatment to facilitate the cessation of cannabis use. On the other hand, cannabinoids are known to modify the addictive properties of other abused substances, such as alcohol, opiates, and psychostimulants, and the cannabinoid system has therefore been proposed to be a potential therapeutic target in drug addiction (Lopez-Moreno et al., 2008; Maldonado et al., 2006; Parolaro et al., 2005).

Similar to other drugs of abuse, cannabinoids are presumed to exert their addictive effects through the motivation and reward system of the brain. This system consists of the mesolimbic dopaminergic pathway: the dopaminergic neurons in the ventral tegmental area (VTA) and their projections and connections in the nucleus accumbens (NAc), prefrontal cortex, and other structures of the limbic system such as the substantia nigra pars reticulata (SNr), the hippocampus, the amygdala and the olfactory tubercle.

It is well known that virtually all known drugs abused by humans stimulate dopamine release from the axon terminals of

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VTA dopaminergic neurons (Spanagel and Weiss, 1999; Crespo et al., 2006). However, the mechanism by which they release dopamine from the nucleus accumbens is not uniform; they can act directly at the terminal level, like amphetamine and cocaine, or indirectly, by stimulating the firing rate of dopaminergic neurons in the VTA, such as opiates and ethanol, or through both mechanisms, like nicotine (Vizi, 2000; Vizi et al., 2004a). Cannabinoid agonists enhance the firing rate of dopaminergic neurons of the VTA (Gessa et al., 1998), and there is compelling evidence that systemic administration of cannabinoids releases dopamine in the nucleus accumbens. THC and other cannabinoids, applied either intraperitonally or intravenously, dose-dependently enhance the extracellular level of dopamine in the nucleus accumbens (Cheer et al., 2004; Chen et al., 1990; Tanda et al., 1997), similar to the effect of cannabinoid self-administration (Fadda et al., 2006; Lecca et al., 2006). Although dopaminergic neurons in general may express the CB1 receptor protein, at least at low levels (Hernandez et al., 2000; Kofalvi et al., 2005; Lau and Schloss, 2008), adult VTA dopaminergic neurons do not contain detectable levels of CB1 receptor mRNA (Matsuda et al., 1993) or protein (Matyas et al., 2006; Pickel et al., 2006; Tsou et al., 1998). Therefore, cannabinoids are assumed to have an indirect effect on dopaminergic transmission, exerted primarily by the modulation of glutamatergic and GABAergic inputs onto the dendrites and somata of VTA neurons (Szabo et al., 2002). Supporting this theory, activation of CB1 receptors in the VTA suppresses $GABA_A$ receptor-mediated inhibitory postsynaptic currents (IPSCs) (Szabo et al., 2002). In addition, the application of cannabinoids did not modulate single pulse evoked dopamine release in the *in vitro* nucleus accumbens, as measured by fast cyclic voltammetry (Szabo et al., 1999). In contrast, cannabinoid receptor agonists inhibit depolarization-evoked glutamate release and N-methyl-D aspartate (NMDA)-evoked acetylcholine and GABA release, respectively (Schoffelmeer et al., 2006), and they inhibit GABAergic transmission in the nucleus accumbens via a presynaptic mode of action (Manzoni and Bockaert, 2001). Likewise, CB1 receptor activation presynaptically inhibits excitatory transmission in accumbal glutamatergic synapses, which receive afferents from the prefrontal cortex (Robbe et al., 2001). This effect also contributes to long-term depression (LTD), a measure of synaptic plasticity and potentially the basis of the neuroadaptation of the brain reward system to marijuana abuse (Robbe et al., 2003, 2002). Therefore, cannabinoid receptor activation could also indirectly release dopamine or modulate dopamine release within the NAc by the activation of CB1 cannabinoid receptors expressed on GABAergic/glutamatergic nerve terminals (Matyas et al., 2006; Pickel et al., 2006). However, this connection has not been proven.

Therefore, in this study, we examined whether the exogenous or endogenous activation of cannabinoid receptors is able to release dopamine or to modify the basal and electrical stimulation-evoked release of tritium in the isolated rat nucleus acccumbens preloaded with [^{3}H]dopamine.

1. Experimental procedures

All animal experiments were performed in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee of the Hungarian Academy of Sciences.

1.1. [^{3}H]Dopamine release experiments

[^{3}H]Dopamine release experiments were performed according to the method described earlier (Vizi et al., 2004b). Briefly, male Wistar rats weighing 120–150 g were anaesthetised by brief CO_2 inhalation and decapitated. Slices of the microdissected tissue samples from the nucleus accumbens were loaded with [7,8-^{3}H]dopamine (Amersham International Plc, UK, specific activity: 39 Ci/mmol) for 40 min at 37 °C in Krebs solution and aerated with 95% O_2 and 5% CO_2. Tissue slices were then superfused with Krebs solution at 37 °C at a rate of 1 ml/min. After the preperfusion period (60 min), 3 min samples of the effluent were collected and assayed for radioactivity. Electrical field stimulations (EFS, S1, S2) were delivered by a Grass S88 stimulator twice, during the 3rd and 33rd sample of the collection period using the following parameters: 25 V, 1 ms, 2 Hz, 240 shocks. In other experiments, two 5 min perfusions with the glutamatergic agonist N-methyl-D-aspartic acid as the stimulus, instead of EFS, were applied using a similar protocol. GBR12909 was perfused 21 min before the second stimulation period (S2) and onwards, whilst WIN55,212-2 was administered in all experiments 15 min before S2 and onwards, i.e., in the presence of GBR12909. Cocaine-HCl was applied into the perfusion solution 15 min before the S2 stimulation in the absence of GBR12909. The CB1 receptor antagonist AM251 and the $GABA_A$ receptor antagonist bicuculline were preperfused from the beginning of the collection period. In other experiments AM251, CB2 receptor antagonist AM630, and the anandamide transport inhibitor VDM11 were applied 15 min before S2 and onwards, in the presence of GBR12909. At the end of the experiment, tissues were homogenized in 0.5 ml 10% trichloroacetic acid. A 0.5 ml aliquot of the superfusate and 0.1 ml of the tissue supernatant were added to 2 ml of scintillation cocktail (Ultima Gold, Packard). Tritium was measured with a Packard 1900 TR liquid scintillation counter using an internal standard. The release of tritium was calculated in Bq/g and expressed as the percentage of the amount of radioactivity in the tissue at the sample collection time (fractional release, %). The net release evoked by EFS or NMDA (FRS1, FRS2) was calculated by the area-under-the-curve (AUC) method, i.e., subtracting the resting release calculated from the prestimulation period from the release measured during and after EFS/NMDA application. The effect of the drugs on EFS/NMDA-evoked release of [^{3}H]dopamine was expressed as FRS2 over FRS1 (FRS2/FRS1) ratios. For the calculation of resting efflux, the mean of the tritium content of the two samples collected immediately before the respective stimulation was taken into account. The tissue tritium uptake was determined as the total release + the tissue content after the experiment and expressed in Bq/g, which reflects the content of radioactivity of the slices after the 60 min washout period, i.e., the radioactivity that is specifically taken up by the tissue.

Preliminary analyses of the released tritium by HPLC-EC, according to the previously described method (Milusheva et al., 2005), showed that the majority of the tritium-labelled compounds released by electrical field stimulation represent dopamine ($52.09 \pm 1.79\%$, $n = 7$) and its metabolites, dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA). Therefore, for the sake of simplicity, we will refer to tritium release as [^{3}H]dopamine release.

2. Materials

The following materials were used: [^{3}H]dopamine (Amersham, Little Chalfont, UK), (R)-(+)-[2,3-dihydro-5-methyl-3-(4-morpholinylmethyl)pyrrolo[1,2,3-de]-1,4-benzoxazin-6-yl]-1-naphthalenylmethanone mesylate (WIN55,212-2), N-(piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide (AM251), 6-iodo-2-methyl-1-[2-(4-morpholinyl)ethyl]-1H-indol-3-y l](4-methoxyphenyl)methanone (AM630), cocaine hydrochloride, 1-[2-[bis-(4-fluorophenyl)methoxy]ethyl]-4-(3-phenylpropyl)piperazine dihydrochloride (GBR12909), (5Z,8Z,11Z,14Z)-N-(4-hydroxy-2-methylphenyl)-5,8,11,14-eicosatetraenamide (VDM11) (all from Tocris Bioscience, Bristol, UK), N-methyl-D-aspartic acid (NMDA), bicuculline (Sigma, St. Louis, MO, USA). AM630 and AM251 were dissolved in dimethyl sulfoxide (DMSO), WIN55,212-2 was dissolved in 0.1 M HCl, and VDM11 was dissolved in TocrisolveTM100. The maximal concentrations of vehicles used had no significant effect on the release of [^{3}H]dopamine.

The composition of the Krebs solution was the following (in mM): NaCl 113, KCl 4.7, $CaCl_2$ 2.5, KH_2PO_4 1.2, $MgSO_4$ 1.2, $NaHCO_3$ 25, Na_2EDTA 0.03, ascorbic acid 0.3, and glucose 11.5. All solutions were prepared on the day of use.

3. Data analysis

All data are expressed as means ± S.E.M. of *n* observations. The statistical analysis was made by ANOVA followed by the Dunnett test (multiple comparisons), or Student's *t*-test (pair-wise comparisons). *P* values of less than 0.05 were considered statistically significant.

4. Results

After 60 min of preperfusion, the tissue radioactivity uptake was $1.81 \pm 0.19 \times 10^6$ Bq/g ($n = 8$), and the resting [^{3}H]dopamine efflux was $1.04 \pm 0.08 \times 10^4$ Bq/g, $0.58 \pm 0.03\%$ of the actual radioactivity in the tissue ($n = 8$). When low frequency electrical field stimulation (25 V, 2 Hz, 240 shocks) was used to mimic neuronal activity, it elicited a rapid and reproducible efflux of [^{3}H]dopamine (FRS1: $4.86 \pm 0.64 \times 10^4$ Bq/g, $n = 8$), resulting in an FRS2/FRS1 ratio of 0.87 ± 0.02 ($n = 8$) under control conditions (Fig. 1A). The cannabinoid agonist WIN55,212-2 was applied at a concentration

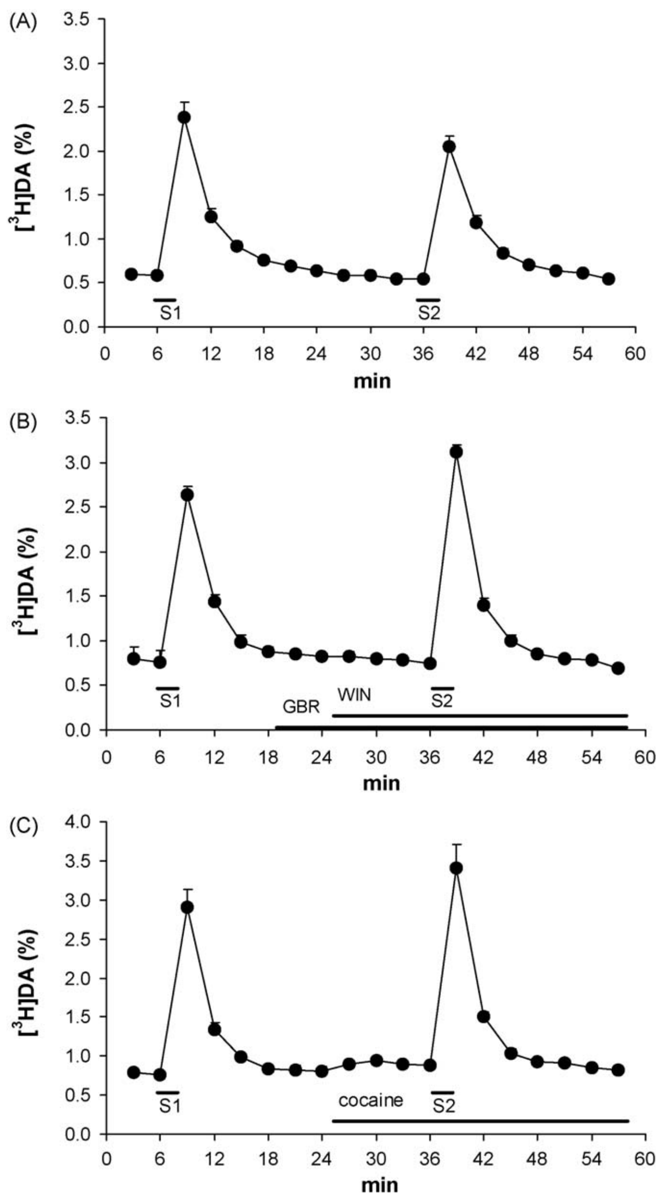

Fig. 1. Effect of the cannabinoid agonist WIN55,212-2 (WIN, 100 nM) (B) and cocaine HCl (30 μM) (C) on the basal and EFS-evoked [^{3}H]dopamine efflux from the rat nucleus accumbens. Collection of 3 min samples was started after 40 min of preincubation in the presence of [^{3}H]dopamine, and 60 min of preperfusion. Electrical field stimulation was applied twice (S1, S2) with 2 Hz for 2 min. (A) Control experiment; (B) WIN55,212-2 (WIN, 100 nM) was applied into the perfusion solution 15 min before S2 stimulation period in the presence of GBR12909 (100 nM, GBR), which was preperfused 21 min before S2, as indicated by the horizontal bars. (C) Cocaine HCl (30 μM) was applied into the perfusion solution 15 min before the S2 stimulation period, as indicated by the horizontal bar. [^{3}H]Dopamine release was expressed in fractional release (%) (for calculation see Section 1). Curve shows the mean ± S.E.M. of 8 (A), 6 (B), and 5 (C) identical experiments.

of 100 nM, at which it activates cannabinoid receptors but does not elicit non-cannabinergic actions (Kofalvi et al., 2003, 2005, 2007). WIN55,212-2 was perfused between the 1st and 2nd stimulation periods in the presence of the specific dopamine transporter (DAT) inhibitor GBR12909 (100 nM) (Andersen, 1989). Uptake inhibitor was applied throughout the experiments in order to preserve the total amount of [^{3}H]dopamine released into the extracellular space. Using this application protocol, WIN55,212-2 significantly enhanced stimulation-evoked [^{3}H]dopamine efflux (Fig. 1B, FRS2/FRS1 = 1.22 ± 0.006, n = 6, $P < 0.01$). In order to compare the effect of WIN55,212-2 with other drugs of abuse that are known to release dopamine from the nucleus accumbens, we examined the effect of cocaine (30 μM) using the same experimental paradigm. Confirming previous observations (Vizi et al., 2004a), cocaine (30 μM) significantly increased both the resting (0.88 ± 0.04%, n = 5, $P < 0.01$) and electrical stimulation-evoked efflux of [^{3}H]dopamine from nucleus accumbens slices (Fig. 1C, FRS2/FRS1 = 1.13 ± 0.006, n = 5, $P < 0.01$). GBR12909 (100 nM–1 μM), when added alone, increased EFS-evoked [^{3}H]dopamine efflux in a concentration-dependent manner ($FRS2/FRS1_{100\ nM}$ = 0.98 ± 0.04, n = 6, $P > 0.05$; $FRS2/FRS1_{1\ \mu M}$ = 1.32 ± 0.07, n = 5, $P < 0.01$ vs. control).

In order to identify the receptor responsible for the effect of WIN55,212-2 (100 nM), we also examined its effect in the presence of the selective CB1 cannabinoid receptor antagonist AM251 (100 nM) (Fig. 2). The stimulatory effect of WIN55,212-2 on EFS-evoked tritium efflux was no longer detected under these conditions, indicating the involvement of the CB1 cannabinoid receptors in this effect (Fig. 2). However, the activation of CB1 receptors in general inhibits, rather than enhances, the release of different neurotransmitters. Considering that the release of the inhibitory transmitter GABA is negatively modulated by CB1 cannabinoid receptors in the nucleus accumbens (Schoffelmeer et al., 2006), a potential explanation is that the facilitation of dopamine release is indirect and is mediated by a disinhibitory mechanism. To test this possibility, we examined the effect of WIN55,212-2 (100 nM) in the presence of the $GABA_A$ receptor antagonist bicuculline (10 μM). The enhancement of EFS-evoked [^{3}H]dopamine efflux was abolished in the presence bicuculline (Fig. 2).

We also examined the effect of WIN55,212-2 on [^{3}H]dopamine efflux evoked by the activation of NMDA receptors. Perfusion of the slices with NMDA (1 mM) for 5 min under Mg^{2+} free conditions reversibly increased the tritium efflux (FRS_{1NMDA} = 9.94 ± 1.86 × 10^3 Bq/g, n = 6), and a subsequent identical stimulus elicited a similar, but smaller efflux of radioactivity (FRS2/FRS1 = 0.46 ± 0.05, n = 6). Using this paradigm, WIN55,212-2 (100 nM–3 μM) did not affect NMDA-evoked [^{3}H]dopamine efflux at any tested concentration ($FRS2/FRS1_{100\ nM}$ = 0.34 ± 0.04, n = 6, $P > 0.05$; $FRS2/FRS1_{3\ \mu M}$ = 0.35 ± 0.08, n = 6, $P > 0.05$).

In order to reveal whether endocannabinoids exert a direct or indirect effect on [^{3}H]dopamine efflux, we utilized antagonists of

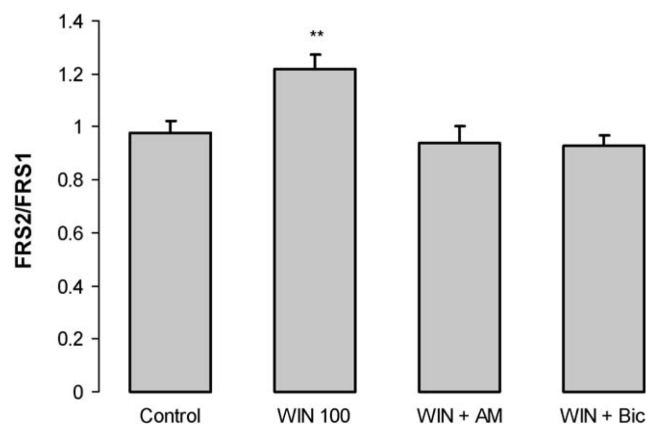

Fig. 2. The reversal of the effect of WIN55,212-2 (100 nM) on evoked [^{3}H]dopamine release by the selective CB1 receptor antagonist AM251 and by the $GABA_A$ receptor antagonist bicuculline. WIN55,212-2 was applied in all experiments in the presence of GBR12909 (100 nM, GBR12909) according to the protocol shown in Fig. 1B. In the presence of GBR12909, WIN55,212-2 significantly enhanced EFS-evoked [^{3}H]dopamine efflux, which is expressed as an FRS2/FRS1 ratio. This effect was abolished when the preparations were preperfused from the beginning of the collection period with AM251 (100 nM, WIN + AM) or with the $GABA_A$ receptor antagonist bicuculline (10 μM, WIN + Bic). Data show mean ± S.E.M. of 6–8 identical experiments. Asterisks indicate significant difference from the respective control ($P < 0.01$).

cannabinoid receptors. The selective CB1 cannabinoid receptor antagonist AM251 (3 μM), when perfused in the presence of GBR12909 (100 nM), did not significantly affect electrical stimulation-induced [^{3}H]dopamine efflux (FRS2/FRS1 = 0.96 ± 0.05, n = 6, $P > 0.05$). Although the CB1 cannabinoid receptor is the major cannabinoid receptor in the brain, recent studies have shown that a subpopulation of CB2 cannabinoid receptors is also expressed in the CNS (Onaivi et al., 2006). The relatively selective CB2 receptor antagonist AM630 (0.3 μM), however, did not significantly change the [^{3}H]dopamine efflux evoked by electrical field stimulation (FRS2/FRS1 = 0.97 ± 0.09, n = 6, $P > 0.05$). As an alternative approach to influence the extracellular level of endocannabinoids, VDM11 (10 μM), an anandamide transport inhibitor, was also tested, but it did not exert a significant effect on EFS-evoked [^{3}H]dopamine efflux (FRS2/FRS1 = 0.79 ± 0.02, n = 6, $P > 0.05$).

5. Discussion

In our experiments, low-frequency electrical stimulation inducing the efflux of [^{3}H]dopamine was used to assess the cannabinergic modulation of dopamine release in the superfused *in vitro* nucleus accumbens. The principal finding of this study is that the cannabinoid agonist WIN55,212-2 significantly augmented EFS-evoked [^{3}H]dopamine release, provided that the fast re-uptake of released dopamine was prevented by the DAT inhibitor GBR12909. This effect was comparable in its magnitude to that obtained by the application of the psychostimulant cocaine, which increases the extracellular level of dopamine by the inhibition of its re-uptake (Vizi et al., 2004a). These findings indicated that WIN55,212-2 activates the reward system within the nucleus accumbens at a relatively low concentration, which is relevant for the activation of CB1 cannabinoid receptors. Although numerous studies have previously demonstrated that systemic application of cannabinoids results in the elevation of extracellular levels of dopamine in the nucleus accumbens (Cheer et al., 2004; Chen et al., 1990; Tanda et al., 1997), these studies proposed that the site of action of cannabinoids was outside the nucleus accumbens, most likely in the ventral tegmental area, where they increase the overall firing rate of dopaminergic neurons (French et al., 1997; Gessa et al., 1998) by the inhibition of GABA release from the inhibitory terminals arriving onto dopaminergic neurons (Szabo et al., 2002). Our data do not contradict, but rather extend these observations by showing that cannabinoids also promote dopamine efflux within the nucleus accumbens.

The effect of WIN55,212-2 was sensitive to inhibition by the CB1-selective antagonist AM251, indicating that it is mediated by CB1 cannabinoid receptors. AM251 has been reported to also have non-cannabinergic actions; for example, it inhibits voltage-dependent sodium channels (Liao et al., 2004), even though these actions are seen only at higher concentrations than those used in our study.

Cannabinoids are known to exert their effects presynaptically via CB1 receptors located mainly on glutamatergic and GABAergic nerve terminals (Cinar et al., 2008; Freund et al., 2003; Katona et al., 1999; Schlicker and Kathmann, 2001). Importantly, in our experiments the facilitatory effect of WIN55,212-2 was abolished after the blockade of $GABA_A$ receptors by bicuculline. Therefore, it is reasonable to assume that the activation of CB1 cannabinoid receptors – which is known to inhibit GABAergic transmission in the nucleus accumbens (Manzoni and Bockaert, 2001; Schoffelmeer et al., 2006) – relieved the inhibitory tone on dopamine efflux and thereby caused the augmentation of [^{3}H]dopamine efflux (Fig. 3). Although the majority of GABAergic neurons in the nucleus accumbens are projection neurons or synapse onto other GABAergic interneurons, previous microdialysis studies have repeatedly shown that the release of dopamine is under the tonic

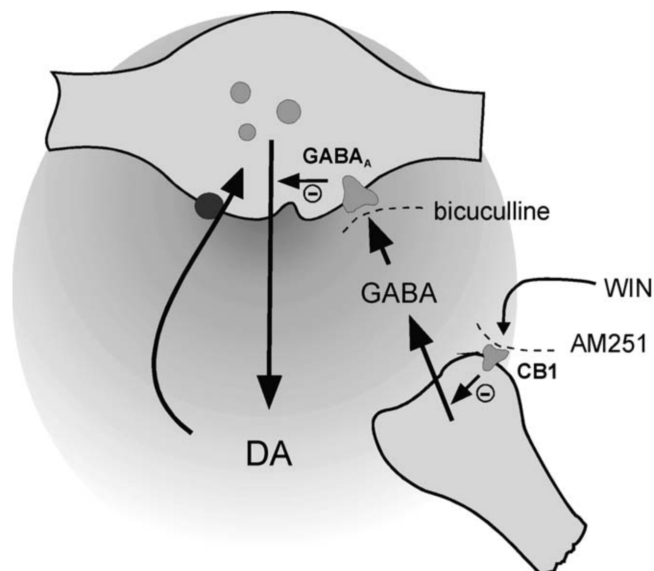

Fig. 3. Cannabinergic disinhibition of GABAergic inhibitory transmission onto dopaminergic terminals in the nucleus accumbens. Schematic drawing showing the site of action of cannabinoids to influence dopamine release. Dopamine is released in response to depolarization of dopaminergic axon terminals originating in the ventral tegmental area. CB1 cannabinoid receptors are located on the nerve terminals of GABAergic interneurons in the nucleus accumbens. GABA released from these nerve terminals exerts a tonic inhibitory influence on dopamine release. The activation of CB1 receptors by WIN55,212-2 (WIN) decreases the release of GABA and thereby relieves dopaminergic terminals from this inhibition. Because GABAergic nerve terminals do not synapse onto dopaminergic nerve terminals, this is an example of non-synaptic communication (Vizi, 2000). The selective CB1 receptor antagonist AM251 and the $GABA_A$ receptor antagonist bicuculline counteract the cannabinergic disinhibitory mechanism.

influence of local inhibition by $GABA_A$ receptors in the nucleus accumbens (Ferraro et al., 1996; Yan, 1999) and in the striatum (Smolders et al., 1995; Whitehead et al., 2001). This inhibitory tone is presumably non-synaptic and is mediated by $GABA_A$ receptors located on dopaminergic nerve terminals. Tonic inhibition by GABAergic neurons – as opposed to phasic inhibition – is not a unique phenomenon and has attracted considerable interest in the past years as a physiological mechanism of the control of neuronal excitability (Farrant and Nusser, 2005) and as a central target site of neuroactive substances such as neurosteroids and ethanol (Mody, 2008; Mody and Pearce, 2004). Interestingly, tonic inhibition is predominantly mediated by the high affinity α5 and δ subunit-containing $GABA_A$ receptors (Glykys et al., 2008), located at extrasynaptic sites, while phasic inhibition is mediated by lower affinity synaptic $GABA_A$ receptors composed of different subunits. Because presumptive dopaminergic neurons in the ventral tegmental area are endowed with various $GABA_A$ receptor subunits including the δ subunit (Schwarzer et al., 2001), they could detect extrasynaptic GABA levels at their terminals and the alleviation of tonic inhibition by cannabinoids.

On the other hand, our results also confirm previous observations showing that the resting, depolarization-induced dopamine release is not subject to direct modulation by cannabinoid receptors in the NAc (Szabo et al., 1999). The finding that WIN55,212-2 did not have any effect on NMDA-evoked [^{3}H]dopamine release also supports this assumption. These observations are in line with the paucity of CB1 receptor expression on dopaminergic nerve terminals (Matyas et al., 2006; Pickel et al., 2004) and with the lack of direct modulation of dopamine release by CB1 receptors in other brain areas such as the striatum (de Lago et al., 2004; Kofalvi et al., 2005; Szabo et al., 1999; Sidló et al., 2008). Only

one study is not in agreement with those data (Cadogan et al., 1997). In this study, however, more intensive stimulation parameters (5 ms duration) were used, which probably do not elicit purely action-potential-dependent release.

Because CB1 receptors also control glutamatergic transmission (Robbe et al., 2001; Schoffelmeer et al., 2006) and NMDA-evoked GABA release in the NAc (Schoffelmeer et al., 2006), another possibility is that CB1 cannabinoid receptors expressed on glutamatergic excitatory nerve terminals also participate in this modulation. However, in this case NMDA should decrease, but not increase dopamine efflux, excluding this possibility in our experimental conditions.

It is interesting to consider whether the disinhibition of dopamine release revealed by previous experiments is also activated by endocannabinoids, presumed to be released under neuronal activity from medium spiny neurons in the nucleus accumbens (Robbe et al., 2002). In our experiments, AM251, the selective CB1 receptor antagonist, AM630, the selective CB2 receptor antagonist, and VDM11, the anandamide transport inhibitor, all failed to modify the EFS-evoked efflux of [^{3}H]dopamine. Therefore, endocannabinoid levels in the vicinity of GABAergic nerve terminals might not reach high enough concentrations to activate CB1 cannabinoid receptors and cause downstream modulation of dopamine release.

In conclusion, we demonstrated that the activation of CB1-cannabinoid receptors in the rat nucleus accumbens leads to the augmentation of dopamine efflux via a local, $GABA_A$ receptor-mediated disinhibitory mechanism. Our findings provide new insight into the site of action of cannabinoids in the NAc and support the view that cannabinoids do not directly affect dopamine release but rather fine-tune dopaminergic transmission through trans-synaptic signalling mechanisms involving GABAergic synapses in the basal ganglia and the mesolimbic reward system (van der Stelt and Di Marzo, 2003; Yanovsky et al., 2003). Taking into account the complex interactions between the endocannabinoid signalling system and other drugs of abuse (Fattore et al., 2008; Lopez-Moreno et al., 2008; van der Stelt and Di Marzo, 2003; Katona and Freund, 2008), our findings might have further implications in the pathological neuroadaptive mechanisms leading to drug addiction.

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