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Occlusion of the presynaptic action of cannabinoids in rat substantia nigra pars reticulata by cadmium

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Abstract

Whole-cell patch-clamp recordings were made from substantia nigra pars reticulata (SNR) neurones in rat midbrain slices to characterise the presynaptic action of cannabinoids on GABA neurotransmission and to study the involvement of calcium channels. The cannabinoid agonist WIN55212–2 at 10 μM reduced the amplitudes of $GABA_A$ receptor mediated spontaneous postsynaptic currents (IPSCs). This effect was fully reversed by the selective cannabinoid CB1 receptor antagonist SR141716A (20 μM). WIN5521 2-2 also caused a reduction in the evoked IPSC amplitude, which was associated with a significant increase in the paired pulse ratio over a 50 ms interval. In addition, the reduction in spontaneous IPSC amplitude by WIN55212–2 could be largely occluded by the presence of extracellular Cd^{2+} (200 μM). These observations suggest that cannabinoids exert a presynaptic inhibition on GABAergic transmission to SNR neurones via CB1 receptor, an action which involves inhibition of Cd^{2+}-sensitive presynaptic Ca^{2+} channels.

Keywords: Cannabinoids; Substantia nigra pars reticulata; SR141716A; Cadmium; Ca^{2+} channels

Cannabinoids refer to a group of highly-abused psychoactive compounds obtained from marijuana. Recent experimental advances have accelerated our understanding of the structures and functions of cannabinoids and their receptors in the brain. These include the identification and cloning of the central receptor [12], the synthesis of high-affinity agonists and antagonists [5,16], and the discovery of the endogenous ligand anandamide [6]. In addition to their psychoactive property. cannabinoids also induce changes in motor function [1]. In this context. a notable finding from binding studies is that one of the output centres of the basal ganglia, namely the substantia nigra pars reticulata (SNR). contains a very high density of binding sites for cannabinoids[9]. These data suggest that the motor effects induced by cannabinoids may be mediated by the basal ganglia circuit. This hypothesis is supported by behavioural studies [17], and more recently by extracellular and intracellular recordings from SNR [2,13,19]. These studies reveal that a major action of cannabinoids is to inhibit presynaptically the GABAergic transmission in SNR leading to increased excitability of SNR neurones. However. the mechanism by which cannabinoids inhibit the release of GABA from presynaptic terminals in SNR is unknown. Previous studies on cell lines and cultures of central neurones suggest that cannabinoids inhibit voltage-dependent calcium channels [11, 14,201. However, such a mechanism has so far not been demonstrated in native central neurones. In the present study, the action of cannabinoids in SNR at the membrane level was further characterised by whole-cell patch-clamp recording, and the effect of the selective CB1 receptor antagonist SRI 417 16A was studied. In addition, we provide data showing that the presynaptic action of cannabinoids in SNR is sensitive to cadmium, suggesting the involvement of voltage-gated calcium channels.

Midbrain slices (200-250 μm) from deeply-anaesthetised Sprague-Dawley rats (2–3 weeks old) containing the substantia nigra were prepared by a vibrating microtome. Whole-cell patch-clamp recordings from SNR GABA neurones were obtained under visual control. as has been described in detail before [3]. In the present study. whole-cell pipettes were filled with an internal solution of the

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following composition (in mM): KCl, 130; EGTA, 1.0; $MgCl_2$, 1.0; Na_2-ATP, 2.0; HEPES, 10. The inclusion of 130 mM of KCl in the recording pipettes reversed the polarity of $GABA_A$ receptor-mediated currents from outward to inward and enhanced their detection. Evoked postsynaptic currents were also elicited intranigrally. Identical paired stimuli (<0.2 ms, <99 V, interpulse interval 50 ms) were applied every 1.6 s. Fifty to one hundred consecutive responses were averaged for analysis. Normally no series resistance compensation was applied but a cell was rejected if the series resistance increased significantly (>20%) during recording. AP5 (20 μM) and CNQX (20 μM) were included in the artificial cerebrospinal fluid (ACSF) to eliminate glutamate-mediated synaptic currents.

The evoked and spontaneous IPSCs were analysed using a program developed in our laboratory [3]. Once a synaptic current is detected, information on the time of occurrence and peak amplitude are generated automatically. For spontaneous IPSCs (sIPSCs), histogram and cumulative probability distributions are produced, and statistical comparison of two cumulative probability distributions can be carried out by the Kolmogorov-Smimov (K-S) test. All events detected with the computed parameters are allowed visual inspection before acceptance.

SR141716A was obtained as a gift from Research Triangle Institute (Research Triangle Park. NC, USA) via NIH. All other drugs were obtained from Research Biochemicals (Natick, MA, USA). SR141716A and WIN 55212–2 were dissolved in dimethyl sulfoxide and ethanol. respectively, to produce concentrated stocks, which were freshly diluted to the final concentrations described below, The solvents did not produce any effect on the synaptic currents. Numerical data are expressed as mean ± SEM. Statistical comparisons. except the K-S test, were carried out by the Student's t-test.

Data were obtained from 44 cells exhibiting the electrophysiological characteristics of GABA output neurones [8]. They were characterised by a weak hyperpolarization-activated inward rectification, brief action potentials (<2 ms) and a high firing frequency (8.2 ± 1.0 Hz, n = 5). As reported previously [2,3], in the presence of AP5 (20 μM) and CNQX (20 μM), spontaneous IPSCs were observed at potentials close to the resting membrane potential (approx. –50 mV). These currents were inward-going and sensitive to 1 μM bicuculline (not shown). Thus, they were reversed

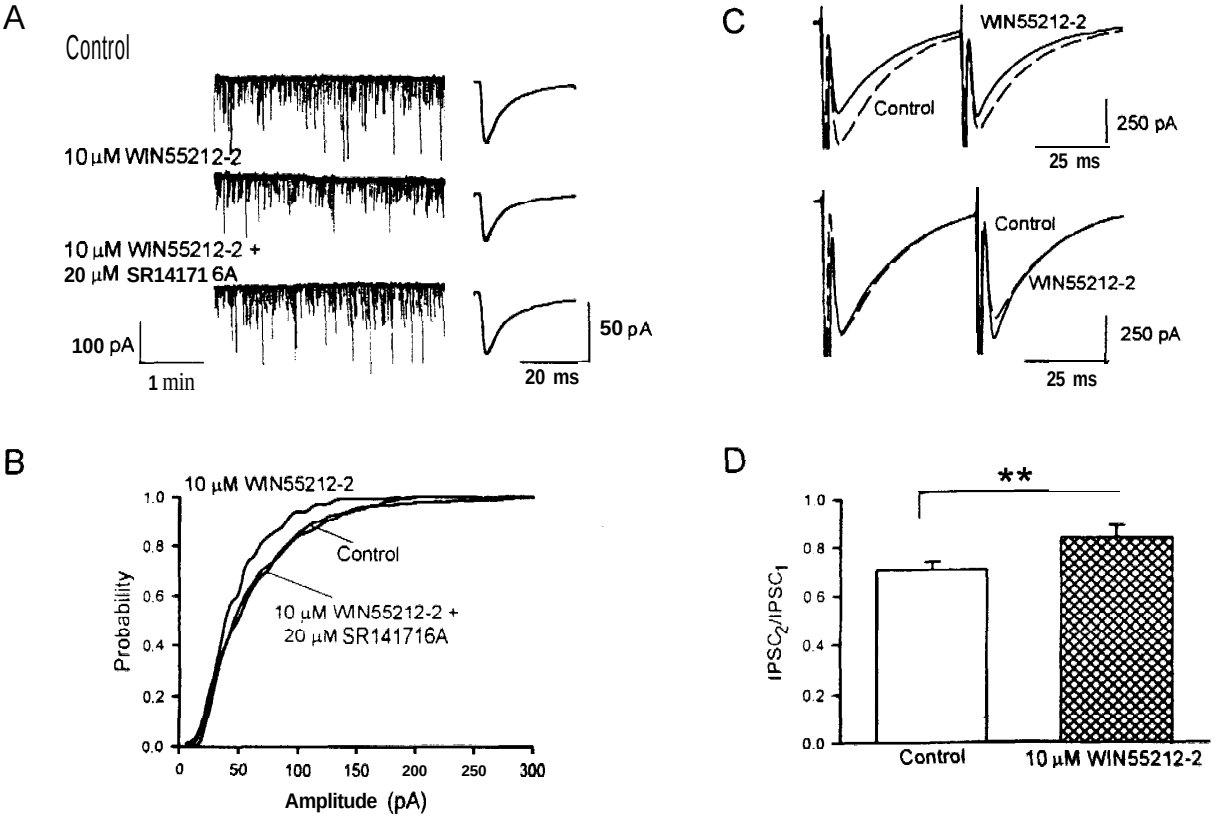

Fig. 1. (A) In this SNR GABA neurone, bath application of 10 μM WIN55212–2 reduced the amplitudes of sIPSCs while addition of 20 μM SR141716A antagonised this effect. The synaptic currents shown on the right were obtained by averaging 100 sIPSCs. (B) Corresponding cumulative distributions of the sIPSCs amplitude revealed that a significant reduction was induced by WIN55212–2 ($P < 0.01$, K-S test), and was reversed by SR141716A. The neurone was clamped at –58 mV. (C) Evoked IPSCs were produced by intranigral stimulation. The upper trace shows averaged evoked IPSCs in response to identical paired stimuli separated by 50 ms before (dotted line) and during (solid line) application of 10 μM WIN55212–2. The traces normalised to the first IPSC are shown in the bottom and reveal a reduction in the paired pulse depression. The neurone was clamped at -70 mV. (D) The mean values obtained from six neurones revealed that WIN55212-2 significantly increased the mean paired pulse ratio ($P < 0.01$).**

$GABA_A$-receptor mediated IPSCs. Bath application of WIN55212–2, a cannabinoid receptor agonist, significantly reduced the amplitude of sIPSCs and this inhibitory effect could be antagonised by SR141716A. Fig. 1A,B shows a typical example in which the effects of 10 μM WIN55212–2 with or without the presence of 20 μM SR141716A were studied. In eight neurones, the mean control sIPSC amplitude was 43.7 ± 6.8 pA, which was significantly reduced by 10 μM WIN55212–2 to 36.5 ± 6.0 pA ($P < 0.001$). SR 141716A at 20 μM restored the sIPSC amplitude to 41.7 ± 6.3 pA which showed no significant difference from the control value ($P > 0.05$). On the other hand, SR141716A alone at the same concentration did not have significant effect on the sIPSC amplitude, indicating that the cannabinoid receptors are not tonically activated. In four cells studied, the control sIPSC amplitude was 64.7 ± 10.1 pA, which became 73.2 ± 16.9 pA ($P > 0.05$) in the presence of SR141716A.

WIN55212–2 also reduced the amplitude of the intranigrally-evoked IPSC. In the present study, identical stimuli separated by an interval of 50 ms were applied and the effect of WIN55212–2 on the paired pulse ratio ($IPSC_2$ amplitude/ $IPSC_1$ amplitude) was determined. Under control conditions, the mean ratio was 0.71 ± 0.04 ($n = 6$), with paired-pulse depression being observed. Bath application of 10 μM WIN55212–2 decreased the paired pulse depression and increased the mean paired pulse ratio to 0.83 ± 0.01 (an 18.4 ± 3.8% increase, $P < 0.01$, $n = 6$, Fig. 1D). A typical example is shown in Fig. 1C.

The involvement of Ca^{2+} channels in the inhibitory effect of cannabinoids was examined by the inclusion of Cd^{2+} in the superfusing ACSF. In ten neurones tested, 200 μM of Cd^{2+} itself reduced the sIPSC amplitude from 60.4 ± 7.9 pA to 37.0 ± 3.6 pA. In the presence of Cd^{2+}, WIN55212–2 at 10 μM only slightly reduced the sIPSC amplitude to 35.0 ± 3.7 pA (a 5.1 ± 6.6% decrease) which showed no significant difference from the sIPSC amplitude with Cd^{2+} alone ($P > 0.05$). A set of typical data is shown in Fig. 2. In contrast, in 18 other neurones, WIN55212–2 at the same concentration alone caused a 17.6 ± 2.6% reduction of the sIPSC amplitude ($P < 0.01$)

In a previous report [2], we demonstrated that WIN 55212–2, but not the inactive enantiomer WIN55212–3, reduced both the spontaneous and evoked IPSCs in rat SNR. These effects of WIN55212–2 could be observed at a concentration as low as 1 μM. In the present study, we routinely applied a high concentration of WIN55212–2 (10 μM) in order to generate a maximum response. The dependence of the effects of WIN55212–2 on cannabinoid CB1 receptor is indicated by the action of SR141716A, which fully reversed the effect of WIN55212–2. SR141716A has been shown to effectively antagonise the biochemical, behavioural and electrophysiological effects of cannabinoid agonists [4,19,20], and has been used to characterise the CB1 receptor in neuronal tissue [10]. These data are therefore consistent with results of studies on cannabinoid binding sites [9] and in particular with the recent finding that SNR shows an intense immunoreactive staining of CB1 protein [15], and thus confirm the functional significance of the CB1 receptor in SNR.

The conclusion that cannabinoids have a presynaptic action on GABA transmission in SNR is based on a number of observations. For example, cannabinoid binding sites in SNR were largely abolished by lesioning the caudate nucleus [9]. More direct evidence was obtained from electrophysiological studies in which administration of cannabinoids increased the firing rate of SNR neurones and decreased the amplitude of their IPSCs without affecting the response to exogenously-applied GABA [2,19]. In the current study, cannabinoids decreased the paired-pulse depression of the evoked IPSCs, an effect which has been regarded as indicative of a presynaptic locus of action [18]. Thus, the present data provide alternative evidence for the presynaptic action of cannabinoids in SNR.

It has been demonstrated that cannabinoids inhibit voltage-dependent calcium channels in cells transfected with

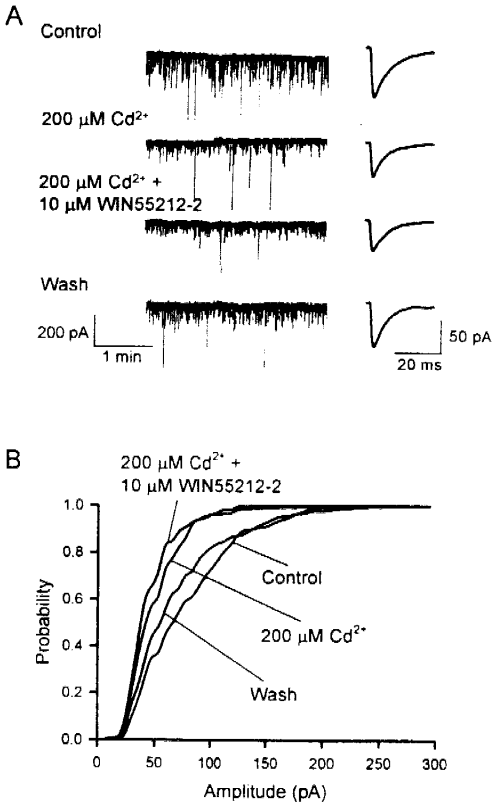

Fig. 2. (A) In this set of typical recordings from an SNR GABA neurone, 200 μM Cd^{2+} caused a reduction in the amplitudes of sIPSCs. In the presence of Cd^{2+}, a saturating concentration of WIN55212–2 (10 μM) only slightly reduced the sIPSC amplitudes. The traces on the right are averaged sIPSC from 100 records. (B) Cumulative distribution plots of the sIPSC amplitudes from the corresponding periods shown in (A). In the presence of 200 μM Cd^{2+}, 10 μM WIN 55212–2 did not cause significant reduction in sIPSC amplitudes. The effects of Cd^{2+} and WIN55212–2 could be washed out. The neurone was clamped at –61 mV.

CB1 receptor [14] and in cultured rat hippocampal neurones [20]. In the present study, we test whether such a mechanism can be demonstrated in native SNR neurones in brain slices. Cd^{2+} at a concentration greater than 100 μM has been employed as a standard tool to block voltage-dependent calcium channels and in the study of calcium channel involvement in synaptic transmission [7]. The present finding that Cd^{2+} largely occluded the effect of cannabinoids on the sIPSC amplitude suggests that cannabinoids reduce the release of GABA by inhibiting Cd"-sensitive presynaptic Ca^{2+} channels. Although the subtype of calcium channels involved was not elucidated in the present study, it is possible that N- and Q/P-type channels are involved, as they play important roles in neurotransmitter release in general and are inhibited by cannabinoids in cultured hippocampal neurones [20]. However. since the effect of WIN 55212–2 cannot be occluded completely by Cd^{2+}, other mechanisms, such as activation of potassium channels [11], or processes downstream of calcium influx, may also be involved in the presynaptic action of cannabinoids.

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