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Research Report

The CB_1 receptor antagonist, SR141716A, prevents high-frequency stimulation-induced reduction of feedback inhibition in the rat dentate gyrus following perforant path stimulation *in vivo*

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ABSTRACT

Endocannabinoids acting through CB_1 receptors are thought to regulate GABAergic and glutamatergic neurotransmission and may modulate long-term potentiation (LTP). High-frequency stimulation (HFS) of the medial perforant path to induce LTP was studied in the dentate gyrus with or without the selective CB_1 receptor antagonist, SR141716A in isoflurane-anaesthetised rats. HFS significantly increased the slope of the field excitatory post-synaptic potential (fEPSP) and the amplitude of the population spike (PS; $P<0.001$ in each case; $n=6$). Following administration of SR141716A, HFS no longer increased fEPSP slope, whereas PS amplitude potentiation remained significant ($P<0.0001$; $n=6$). Paired-stimuli revealed that HFS significantly reduced inhibition observed at intervals of 10 ms ($P<0.01$; $n=6$), and produced a leftward shift of the interval–inhibition curve ($P<0.05$; $n=6$). Following administration of SR141716A, HFS no longer reduced inhibition at the 10 ms interval, but a leftward shift in the interval–inhibition curve was still observed ($P<0.05$, $n=6$). These results indicate that LTP in the dentate gyrus reduces local circuit inhibition, consistent with a reduction of GABA release and/or duration of the post-synaptic GABA-receptor mediated response. Selective effects of SR141716A on the degree, but not the timecourse, of paired-pulse inhibition suggest that the reduction in GABA release following LTP induction is due to CB_1 activation. Results also suggest that CB_1 receptors contribute to HFS-induced potentiation of the fEPSP, but not to the mechanism underlying potentiation of PS amplitude. We suggest that CB_1 activation during HFS of the medial perforant path increases glutamate release from perforant path synapses, but inhibits release of GABA from local circuit interneurons.

1. Introduction

Cannabinoid 1 (CB_1) receptors in the CNS are thought to regulate both GABAergic and glutamatergic neurotransmission (Szabo and Schlicker, 2005). Neuroanatomical studies have provided strong evidence for the presence of CB_1 receptors on presynaptic glutamatergic terminals (Katona et al., 2006). Furthermore, functional CB_1 receptors have been shown to co-localise with

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Abbreviations: fEPSP, field excitatory post-synaptic potential; PS, population spike; LTP, long-term potentiation; HFS, high-frequency

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glutamate transporter 1 on glutamatergic cell terminals in the dentate gyrus and to protect against kainic-acid induced seizures (Monory et al., 2006). However, pharmacological studies have provided mixed results. For example, Takahashi and Castillo (2006) reported that the non-selective cannabinoid agonist, WIN 55,212-2 reduced glutamatergic synaptic transmission, an effect that was not present in CB_1 knockout mice; whereas, Hajos et al. (2001) found that WIN 55,212-2 had an equal effect on glutamatergic and GABAergic neurotransmission in $CB_1^{+/+}$ and $CB_1^{-/-}$ mice. More recent studies using a combination of electrophysiological and anatomical techniques demonstrated that activation of CB_1 receptors, located on presynaptic terminals in the hippocampus, reduced excitatory responses evoked by stimulation of glutamatergic afferents (Domenici et al., 2006; Kawamura et al., 2006). Furthermore, Domenici et al. (2006) showed that the effect was present in mice lacking CB_1 exclusively on GABAergic neurons, thus excluding the involvement of CB_1 receptors on GABAergic neurons in this case. Other studies have investigated the longer term effects of CB_1 receptor activation on glutamatergic transmission, and suggest that the endocannabinoid system confers a novel form of activity-dependent synaptic plasticity in which post-synaptic release of endocannabinoids leads to retrograde activation of presynaptic CB_1 receptors. These presynaptic receptors are proposed to suppress both inhibitory and excitatory neurotransmitter release; recently reviewed by Chevaleyre et al (2006).

The activity-dependent release of endogenous cannabinoids from the post-synaptic neuron has been linked to the prior activation of metabotropic glutamate 1 receptors and post-synaptic voltage gated calcium channels. Consequently, the release of endocannabinoids might be expected during the induction of classical NMDA receptor-dependent long-term potentiation (LTP). Therefore, in the present study, we investigated the induction of LTP in the dentate gyrus following high-frequency stimulation (HFS) of the medial perforant path in the absence and presence of the clinically-used CB_1 selective receptor antagonist, SR141716A (Rinaldi-Carmona et al., 1994) in isoflurane-anaesthetised rats. Furthermore, we used paired stimuli to evaluate changes in local circuit inhibition before and after induction of LTP, and in the absence and presence of the CB_1 antagonist as demonstrated *in vitro* by Chevaleyre and Castillo (2003). Thus the aim of the study was to evaluate the contribution of CB_1 receptors to the induction of LTP, and to determine whether effects might be due to modulation of glutamate release from perforant path synapses or modulation of the activity of inhibitory circuits within the dentate gyrus.

2. Results

2.1. Current–response relationship

Fig. 1 shows the field response to electrical stimulation of the perforant path. Each response consisted of a positive-going field excitatory post-synaptic potential (fEPSP) with a superimposed negative-going population spike (PS). The maximum fEPSP slope that could be evoked under basal conditions was 6.98±0.40 mV ms^{-1} (at 1000 μA; $n=17$; Fig. 2A). The maximum PS that could be evoked under basal conditions was 21.53±1.58 mV (at 1000 μA; $n=17$, Fig. 2B).

2.2. Response to paired-pulse stimulation

Fig. 1C and 1D show representative traces of paired-pulse recordings at interpulse intervals of 10 ms (Fig. 1C) and 50 ms (Fig. 1D). Under baseline conditions, paired-pulse stimulation evoked a characteristic profile of inhibition followed by slight facilitation (Fig. 3A). At intervals between 10 and 40 ms the second population spike was inhibited relative to the first, and at intervals greater than 50 ms the second PS was facilitated. Near-complete inhibition of the second PS was observed with the 10 ms interpulse interval (−98.18±0.90%, $n=20$; Figs. 1C and 3A).

2.3. Effect of high-frequency stimulation

2.3.1. Effect of high-frequency stimulation on the current–response relationship

Fig. 4 shows the timecourse of the effects of HFS of the perforant path on the fEPSP slope and PS amplitude in the presence of vehicle or SR141716A. In the presence of vehicle, HFS induced a rapid and sustained increase in fEPSP slope (Fig. 4A; baseline: 5.38±0.62 to 7.11±0.62 mV/ms; $P=0.0015$; $n=5$) and PS amplitude (Fig. 4B; baseline: 6.59±0.90 to 27.12±3.36 mV; $P=0.001$; $n=5$). Analysis of the current–response relationship also demonstrated that potentiation was still significantly increased 30 min after the HFS (fEPSP slope $P<0.01$; $n=5$; Fig. 2C, and PS amplitude $P=0.0001$; $n=5$; Fig. 2D) and was unaffected by the presence of drug vehicle (Figs. 2E and F).

2.3.2. Effect of high-frequency stimulation on local circuit inhibition

Thirty minutes after HFS of the perforant path there was a reduction in the degree of paired-pulse inhibition at short (10–50 ms) intervals (Fig. 3B). HFS significantly reduced the degree of paired-pulse inhibition at the 10 ms interval (−96.68±3.32 to −82.09±2.44%, $P<0.01$; $n=5$). HFS also shifted the interval–inhibition curve to the left such that the interval at which 50% inhibition of the second population spike would occur was reduced (baseline: 42.9±5.5 ms; post-HFS: 26.8±1.0 ms, $P<0.05$, $n=5$).

2.4. Effect of high-frequency stimulation in presence of SR141716A

In a subsequent series of experiments, HFS stimulation was performed 20 min after administration of SR141716A (1 mg kg^{-1}, i.v.; Fig. 4) and current–response (Fig. 2) and paired-pulse profiles (Fig. 3) were determined 30 min later. These were then compared to the baseline profiles prior to injection of SR141716A.

Injection of SR141716A alone (1 mg kg^{-1}, i.v.) was without effect on fEPSP slope, population spike amplitude or paired-pulse inhibition (data not shown; $n=6$) at 20 or 50 min after administration.

HFS in the presence of vehicle caused a significant increase in the fEPSP slope ($P<0.001$; $n=6$; Fig. 2E). In the presence of