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Effect of cannabinoids on synaptic transmission in the rat hippocampal slice is temperature-dependent

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Abstract

We have previously reported that the synthetic cannabinoid R-(+)-[2,3-dihydro-5-methyl-3-[(morpholinyl)methyl]pyrrolo[1,2,3-de]-1,4-benzoxazin-yl]-(1-naphthalenyl)methanone mesylate (WIN55,212-2) causes a selective inhibition of paired pulse depression of population spikes recorded from the CA1 region of rat hippocampal slices maintained at 28–30 °C. We now show that this effect is highly temperature-dependent and that WIN55,212-2 actually increases paired pulse depression of population spikes recorded from slices maintained at 35 °C. This temperature dependence was found to correlate with the effects of the known γ-amino butyric acid (GABA)-uptake inhibitors, nipecotic acid and guvacine, which were without effect at 28–30 °C, but increased paired pulse depression at 35 °C. The results show that the effects of cannabinoids on synaptic transmission in the hippocampal slice are highly temperature-dependent and it is suggested that this is due to the presence of increased GABA uptake at higher temperatures.

Keywords: WIN55,212-2; Cannabinoid; Hippocampus; Temperature; GABA (γ-aminobutyric acid), uptake

1. Introduction

The proposed use of cannabinoids for therapeutic purposes has fuelled interest in the cognitive effects of cannabinoids and hence in their mechanism of action on synaptic transmission in the central nervous system. This study focuses on the effects of a synthetic cannabinoid on population spikes recorded from the CA1 region of the rat hippocampal slice. In this region, paired pulse stimulation delivered at short intervals is commonly seen to result in paired pulse depression where the amplitude of the second population spike (PS2) is smaller than that of the first (PS1) (Stanford et al., 1995; Fathollahi et al., 1997; Paton et al., 1998). This paired pulse plasticity may be affected by several factors, but in the intact slice, it is dominated by the strength of γ-aminobutyric acid (GABA)ergic feedback inhibition that is evoked by the first stimulus (Knowles and Schwartzkroin, 1981). We have previously reported that in the adult rat hippocampal slice the synthetic cannabinoid *R*-(+)-[2,3-dihydro-5-methyl-3-[(morpholinyl)methyl]pyrrolo[1,2,3-de]-1,4-benzoxazin-yl]-(1-naphthalenyl)methanone mesylate (WIN55,212-2) has no effect on PS1, but increases the amplitude of PS2 and therefore decreases paired pulse depression (Paton et al., 1998; Al-Hayani and Davies, 2000). This effect is consistent with the very well reported ability of cannabinoids, acting through cannabinoid CB_1 receptors, to inhibit release of GABA from hippocampal neurones (Katona et al., 1999; Hájos et al., 2000a; Hoffman and Lupica, 2000; Irving et al., 2000) and with the immunohistochemical localisation of cannabinoid CB_1 receptors to the terminals of a subset of GABAergic neurones in the hippocampus (Katona et al., 1999; Tsou et al., 1998). All of our previous experiments have been performed at 28–30 °C, however, we have now repeated some of these at more physiological temperatures and found dramatic differences in the effects of the cannabinoids on paired pulse depression.

2. Materials and methods

The experimental protocol was approved by the University of Aberdeen Ethical Committee. Halothane-anaesthetized female Sprague–Dawley rats (5–7 weeks old) were given a blow to the thorax prior to being decapitated.

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Transverse hippocampal slices (400 μm) were cut using a McIlwain tissue chopper and incubated in artificial cerebrospinal fluid (ACSF) in a gassed (95% O_2–5% CO_2) holding chamber for at least 1 h before use. The ACSF contained (in mM): NaCl 124, KCl 3, $NaHCO_3$ 26, NaH_2PO_4 1.25, D-glucose 10, $MgSO_4$ 1 and $CaCl_2$ 2, saturated with 95% O_2–5% CO_2. The slices were then transferred to an interface-type recording chamber which was continually perfused at 1.5 ml/min with ACSF that was maintained at a constant 28–29 °C unless otherwise stated.

A bipolar stimulating electrode was placed in stratum radiatum of the CA1 region to allow orthodromic stimulation of the Schaffer-collateral commissural fibres and population spikes were recorded from stratum pyramidale using a borosilicate glass capillary electrode filled with 3 M NaCl (resistance 2–9 MΩ). The slice was stimulated every 15 s and stimulus intensity was set to evoke a population spike of approximately half-maximal amplitude. Population spike amplitudes were measured and monitored using the LTP data program (Anderson and Collingridge, 1997; http:\\www.LTP-program.com). To facilitate pooling of data, the control amplitude of the first population spike (PS1) was calculated as the mean of the responses 15 min immediately before drug addition, and drug effects on PS1 were expressed as percentage of control ± standard error of the mean (S.E.M.). The degree of paired pulse depression of the second population spike (PS2) was calculated using the formula 100 × PS2/PS1. Statistical analysis was performed using the INSTAT programme and a paired Student's *t*-test. $P < 0.05$ was taken to indicate significance. In all cases, *n* numbers refer to the number of slices and these were taken from separate animals. Drugs were applied by addition to the perfusion medium.

R-(+)-[2,3-dihydro-5-methyl-3-[(morpholinyl)methyl] pyrrolo[1,2,3-de]-1,4-benzoxazin-yl]-(1-naphthalenyl)methanone mesylate (WIN55,212-2), and *S*-(−)-[2,3-dihydro-5-methyl-3-[(morpholinyl)methyl]pyrrolo[1,2,3-de]-1,4-benzoxazin-yl]-(1-naphthalenyl)methanone mesylate (WIN55,212-3) were obtained from RBI; guvacine hydrochloride, (±)-nipecotic acid, and 1-(2,4-dichlorophenyl)-5-(4-iodophenyl)-4-methyl-*N*-4-morphonyl-1*H*-pyrazole-3-carboxamide (AM281) from Tocris, UK; 5-α-pregnan-3α-ol-20-one, pentobarbital and picrotoxin from Sigma-Aldrich. Stock solutions of WIN55,212-2 and WIN55,212-3 were made up in alcohol at 1 mg/ml and stored at 4 °C. When required, they were mixed with Tween 80 (two parts Tween 80 to one part drug solution) and the ethanol was evaporated off under a steam of nitrogen gas. Saline was then added in aliquots of 0.05 ml and vortexed before being diluted with ACSF to obtain the required concentration. Other stock solutions at least 1000 × the final concentration required were made up in either di-methyl sulphoxide (DMSO) (AM281, 5-α-pregnan-3α-ol-20-one) or equimolar NaOH (pentobarbital, nipecotic acid, guvacine) and diluted directly in ACSF.

3. Results

Stimulation at interpulse intervals between 5 and 200 ms usually caused a paired pulse depression of PS2 at intervals up to 40 or 50 ms and paired pulse facilitation at intervals above that (Fig. 1A,B). Slices that showed weak paired pulse depression often also showed a secondary population spike in response to a single stimulus. For this reason, only those slices that showed control paired pulse depression that persisted up to the 40-ms interpulse interval were included in this study. Control experiments showed that the degree of paired pulse depression remained constant over the recording periods used in these experiments.

3.1. Effects of cannabinoid receptor ligands

The cannabinoid receptor agonist WIN55,212-2 caused a decrease in paired pulse depression. Perfusion of 500 nM WIN55,212-2 for 20 min had no effect on PS1 but caused a gradual increase in the amplitude of PS2. At the 20-ms interstimulus interval, PS2 increased from 40 ± 7% to 86 ± 17% of PS1, $n = 6$, Fig. 2A). This effect was most pronounced at interstimulus intervals of up to 50 ms and no recovery was observed after over 60-min wash.

Neither the inactive isomer WIN55,212-3, nor the drug vehicle (Tween 80) which was used to disperse the cannabinoids had any effect on either PS1 or PS2. At the 20-ms

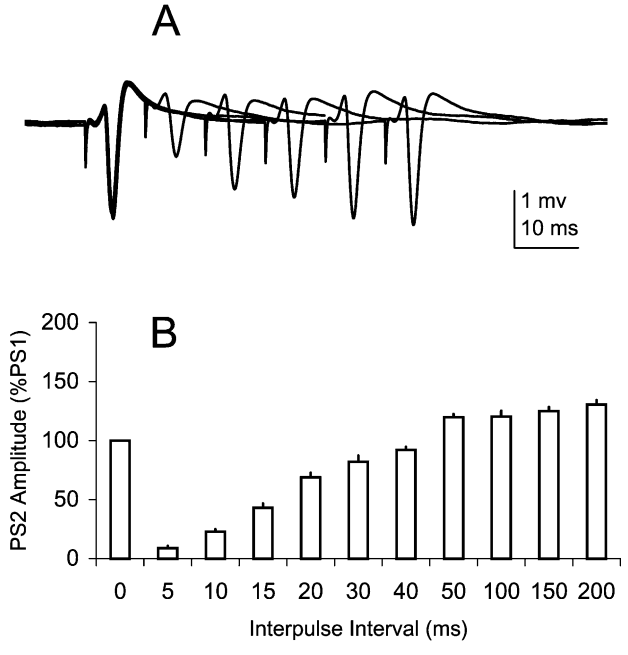

Fig. 1. Paired pulse depression in the rat hippocampal slice. (A) Superimposed synaptic responses from a single slice stimulated at interpulse intervals between 10 and 50 ms. The stimulus strength was set so that the amplitude of the first population spike (PS1) was approximately half the maximum amplitude. (B) Pooled data from eight slices where paired pulse depression is calculated as 100 × PS1 amplitude/PS2 amplitude.

interstimulus interval, PS2 went from 38 ± 10% to 40 ± 9% of PS1 in the presence of 500 nM WIN55,212-3 ($n = 5$), and from 65 ± 4% to 62 ± 3% of PS1 in the presence of Tween 80 when perfused at the concentration used to disperse the cannabinoids ($n = 5$).

The effect of WIN55,212-2 was blocked by the cannabinoid CB_1 receptor antagonist, AM281. Perfusion of 500 nM AM281 for 20 min had no significant effect on PS1 or PS2 (at the 20-ms interpulse interval, the amplitude of PS2 went from 68 ± 6% to 63 ± 8% of PS1 in the presence of AM281), but totally blocked the effect of 500 nM WIN55,212-2 perfused for 20 min immediately after (at the 20-ms interpulse interval, PS2 was 70 ± 8% of PS1 in the presence of WIN55,212-2, $n = 4$, Fig. 2B).

In dramatic contrast, with the slices maintained at 35 °C the previous effect of WIN55,212-2 in reducing paired pulse depression was totally lost, and this was replaced by a small but significant increase in paired pulse depression. Perfusion of WIN55,212-2 (500 nM) for 20 min had no effect on the amplitude of PS1 but caused a decrease in the amplitude of PS2, thus increasing paired pulse depression (at the 20-ms interpulse interval, PS2 fell from 60 ± 13% to 41 ± 10% of PS1, $n = 4$, Fig. 2C). This effect was significant only at the 20- and 30-ms interpulse intervals. The WIN55,212-2 induced increase in paired pulse depression was attenuated, but not entirely blocked, by prior perfusion of 500 nM AM281 (at the 20-ms interpulse interval, the amplitude of PS1 fell from 70 ± 2% to 65 ± 5% of PS1, $n = 4$, Fig. 2D).

The effect of WIN55,212-2 on paired pulse depression at the higher temperature is consistent with it causing an increase in GABAergic transmission. Apart from inhibiting release of GABA, there is also evidence that cannabinoids inhibit uptake of the transmitter (Banerjee et al., 1975; Hershkowitz et al., 1977; Maneuf et al., 1996; Coull et al., 1997). We therefore investigated the action of known inhibitors of GABA uptake to establish whether such an effect could account for the actions of WIN55,212-2.

3.2. Effects of known modulators of GABAergic transmission

Nipecotic acid and guvacine both inhibit the uptake of GABA and may therefore be expected to potentiate GABAergic synaptic transmission and so increase paired pulse depression. However, in slices maintained at 28–30 °C, perfusion of neither nipecotic acid (500 μM) nor guvacine (50 μM) for 20 min had any significant effect on PS1 or on PS2 (at the 20-ms interstimulus interval, PS2

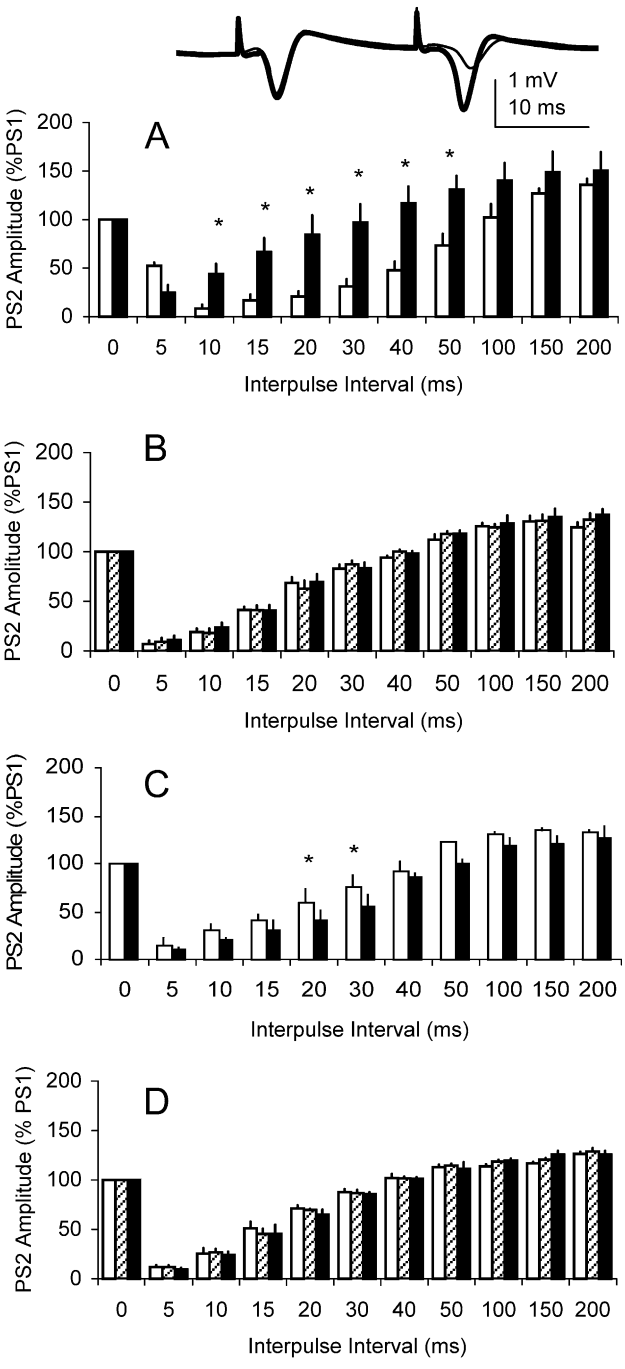

Fig. 2. Effects of WIN55212-2 on paired pulse depression. In this and all the remaining figures, the graphs plot pooled data for the effect of each drug on paired pulse depression at interstimulus intervals between 5 and 200 ms. Open bars show data collected during the control period, and closed bars that collected after drug perfusion. (A) In slices maintained at 28–30 °C, 500 nM WIN55,212-2 ($n = 6$) had no effect on PS1 but decreased paired pulse depression. The inset shows an example synaptic recording using the 20-ms interpulse interval. The thin line shows the control recording and the thick line a recording in the presence of WIN55,212-2. (B) The effect of WIN55,212-2 was blocked by prior perfusion of 500 nM AM281 ($n = 4$). In this case, the three bars of the histogram represent control paired pulse depression (open bars), that recorded in the presence of AM281 (hatched bars), and that recorded during the subsequent perfusion of WIN55,212-2. (C) In slices maintained at 35 °C, 500 nM WIN55,212-2 ($n = 4$) had no effect on PS1 but caused a small increase in the extent of paired pulse depression. (D) The WIN55,212-2 induced increase in paired pulse depression recorded at 35 °C was attenuated by 500 nM AM281 ($n = 4$). Open bars represent control paired pulse depression, hatched bars that recorded in the presence of AM281, and filled bars that recorded after subsequent perfusion of 500 nM WIN55,212-2.

went from 67 ± 10% to 88 ± 7% of PS1 in nipecotic acid, $n = 4$, and from 70 ± 5% to 77 ± 7% of PS1 in guvacine, $n = 8$, Fig. 3A,B).

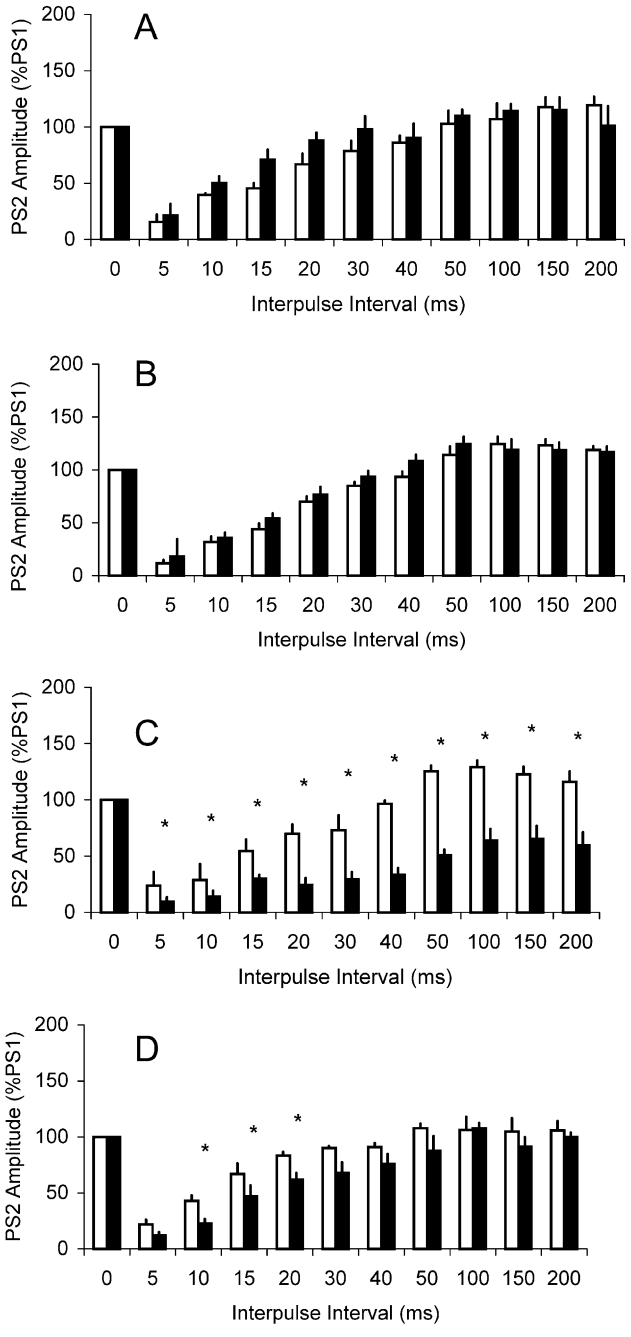

Fig. 3. Effects of modulators of GABAergic transmission on paired pulse depression in slices maintained at 28–30 °C. Neither 500 μM nipecotic acid (A, $n = 4$), nor 50 μM guvacine (B, $n = 8$), had any effect on PS1 or on paired pulse depression. In contrast, 100 μM pentobarbital (C, $n = 4$) and 500 nM α-pregnanolone (D, $n = 4$) both had no effect on PS1 but increased paired pulse depression. All experiments were performed at 28–29 °C. In this and other similar figures, error bars represent ± S.E.M. and the stars indicate a significant difference between the two conditions at the specified interstimulus interval as measured using a paired Student's *t*-test (* indicates $P < 0.05$).

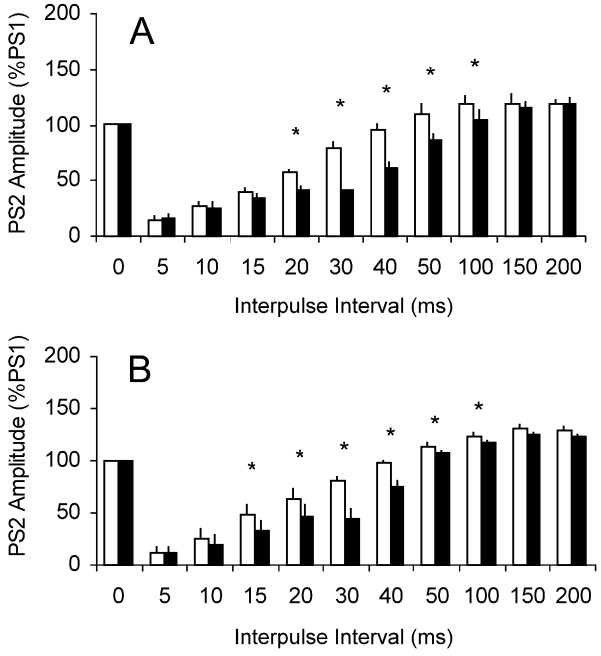

Fig. 4. Inhibitors of GABA uptake increase paired pulse depression in slices maintained at 34–35 °C. 500 μM nipecotic acid (A, $n = 4$) and 50 μM guvacine (B, $n = 4$) both had no effect on PS1, but reduced the amplitude of PS1 and therefore caused an increase in the extent of paired pulse depression.

For comparison, we also tested in slices maintained at 28–30 °C the effects of two other drugs that increase GABAergic transmission, but by a different mechanism. Pentobarbital, which potentiates the action of GABA on $GABA_A$ receptors, caused an increase in paired pulse depression. Perfusion of 100-μM pentobarbital for 20 min again had no effect on PS1, but caused a marked decrease in the amplitude of PS2, thus increasing paired pulse depression (at the 20-ms interpulse interval, pentobarbital caused the amplitude of PS2 to decrease from 70 ± 8% to 24 ± 6% of PS1, $n = 4$, Fig. 3C). The effect of pentobarbital was significant at all interstimulus intervals and was fully reversed after 20-min wash (data not shown). Like pentobarbital, the steroid 5-α-pregnanolone also potentiates the action of GABA on $GABA_A$ receptors, and this too caused an increase in paired pulse depression. Perfusion of 500 nM 5-α-pregnanolone for 20 min again had no effect on PS1, but caused a decrease in PS2, thus causing an increase in paired pulse depression (at the 20-ms interpulse interval, the amplitude of PS2 decreased from 83 ± 3% to 62 ± 6% of PS1, $n = 4$, Fig. 3D). In contrast to pentobarbital, the effect of 5-α-pregnanolone was much more selective, only being statistically significant at the 10-, 15- and 20-ms interpulse intervals.

The results indicate that modulation of GABAergic transmission does indeed influence the strength of paired pulse depression, but also that inhibition of GABA uptake does not affect paired pulse depression recorded at 28–30 °C. One interpretation of this result is that evoked release of

GABA is sufficient to saturate $GABA_A$ receptors, and hence that reducing GABA uptake can have no further effect. However, since uptake of GABA is temperature-dependent, it is possible that uptake may limit the postsynaptic action of GABA at higher, more physiological, temperatures. Some of the previous experiments were therefore repeated at a higher temperature. When the slices were maintained at 35 °C, both nipecotic acid and guvacine caused an increase in paired pulse depression. Nipecotic acid (500 μM) or guvacine (50 μM) perfused for 20 min had no effect on the amplitude of PS1, but caused a decrease in the amplitude of PS2, thus increasing the strength of paired pulse depression (at the 20-ms interpulse interval, the amplitude of PS2 fell from 58 ± 2% to 42 ± 4% of PS1, $n = 4$, and from 64 ± 9% to 46 ± 12% of PS1, $n = 4$, for nipecotic acid and guvacine respectively, Fig. 4A,B).

4. Discussion

4.1. Paired pulse depression

The extent of paired pulse depression that we observed is greater than that reported in some other studies in the CA1 region in vitro. This may partly be due to the way we selected slices for study. Those slices that showed a tendency to fire a secondary population spike in response to a single high intensity stimulus also had weak paired pulse depression. We therefore equated poor paired pulse depression with an unhealthy slice showing reduced GABAergic inhibition and so discarded any slices not showing paired pulse depression that persisted up to the 40-ms interpulse interval.

Paired pulse plasticity of synaptic responses could be affected by a multitude of factors including changes in presynaptic release probability (Andreasen and Hablitz, 1994), activity of presynaptic auto- (or hetero-) receptors on inhibitory or excitatory terminals (Davies et al., 1990; Scanziani et al., 1997), modulation of postsynaptic conductances (Deadwyler et al., 1995; Twitchell et al., 1997) and the presence of powerful GABAergic inhibition following the excitatory postsynaptic potential (EPSP) (Knowles and Schwartzkroin, 1981). In line with previous reports in the CA1 region of the hippocampal slice (Pearce, 1996; Stanford et al., 1995), our results suggest that the last of these plays a major role in mediating paired pulse depression of population spikes. Hence, drugs which potentiate postsynaptic actions of GABA on $GABA_A$ receptors increase paired pulse depression. Specifically, we used the barbiturate pentobarbital and the steroid 5-α-pregnanolone which both act primarily by promotion of a long open state of the associated Cl-ion channel (MacDonald and Olsen, 1994; Lambert et al., 1995). 5-α-pregnanolone caused a relatively selective increase in paired pulse depression which was most evident over interpulse intervals between 10 and 50 ms; a range which corresponds well with the time course of the pharmacologically isolated $GABA_A$ receptor mediated inhibitory postsynaptic potential (IPSP) recorded under similar conditions (Davies et al., 1990; Pearce, 1996). The effect of pentobarbital was far less specific and was still evident at the longest interpulse interval tested of 200 ms, suggesting that its effect is not solely due to an action on fast $GABA_A$ receptor mediated transmission. Note that 5-α-pregnanolone and pentobarbital both act downstream of GABA binding to $GABA_A$ receptors and therefore would still potentiate the postsynaptic effect of GABA, even if $GABA_A$ receptors were saturated.

In contrast, in slices maintained at 28–30 °C neither of the known inhibitors of GABA uptake that we used had any significant effect on the extent of paired pulse depression, despite using them at effective concentrations. It therefore appears that in our experimental situation, uptake of transmitter does not limit the action of GABA. This is consistent with the results of modelling studies which suggest that the release of a single vesicle of GABA is sufficient to almost saturate the postsynaptic $GABA_A$ receptors (Busch and Sakmann, 1990; Kullmann and Asztely, 1998). This notion receives mixed support from experiments using the benzodiazepine zolpidem, which increases the affinity of GABA binding to $GABA_A$ receptors (Perrais and Ropert, 1999) and should have no effect at synapses which are already saturated. In some experiments, zolpidem had no effect on miniature inhibitory postsynaptic currents (IPSCs) recorded in the rat hippocampal slice (Mody et al., 1994) suggesting that the postsynaptic receptors were saturated, but in others it was found to potentiate miniature IPSCs recorded in CA1 pyramidal cells (Hájos et al., 2000b). The second observation suggests that $GABA_A$ receptors are not saturated on the CA1 neurones. Possible reasons for this discrepancy include differences in the properties of individual synapses making contact onto the same cell type (Thomson et al., 2000), developmental changes in the efficiency of GABA uptake (Druguhn and Heinemann, 1996), or differences between evoked and spontaneous potentials (Auger and Marty, 1997; Nusser et al., 1999).

With all of these drugs, a selective effect on PS2 was observed at concentrations that had no significant effect on the amplitude of PS1. The selective effect on PS2 would be consistent with the notion that the inhibition is primarily feedback in nature and triggered by the action potentials that constitute PS1, and furthermore, suggests that in our slices any spontaneous activity in the interneurone network does not tonically inhibit CA1 pyramidal cells (Kirby et al., 2000). The lack of effect on PS1 is important since we have previously demonstrated that changes in amplitude of PS1 influence the extent of paired pulse depression (Paton et al., 1998) and are therefore more difficult to interpret.

4.2. Effects of cannabinoid receptor ligands

Consistent with our previous findings (Paton et al., 1998; Al-Hayani and Davies, 2000), at 28–30 °C WIN55,212-2

caused a decrease in the extent of paired pulse depression which is consistent with the well-documented inhibition of GABA release by cannabinoids (Katona et al., 1999; Hájos et al., 2000a; Hoffman and Lupica, 2000; Irving et al., 2000). This effect was most evident over interpulse intervals ranging from 10 to 50 ms, again correlating well with the time course of the pharmacologically isolated $GABA_A$ receptor mediated IPSP (Davies et al., 1990; Pearce, 1996). It was produced in the absence of any effect on PS1 indicating that the cannabinoid had no effect on the release of the excitatory transmitter L-glutamate. This is a controversial subject with some groups finding powerful effects of cannabinoids on excitatory transmission in the CA1 (Ameri et al., 1999; Misner and Sullivan, 1999), others finding no effect (Terranova et al., 1995; Paton et al., 1998), or an effect only in neonatal tissue (Al-Hayani and Davies, 2000). Whatever the reason, the absence of any effect on PS1 in our experiments makes the selective effect on PS2 easier to interpret as a direct reduction in GABAergic inhibition. The effect was not mimicked by the less active stereoisomer WIN55,212-3, and was blocked by prior perfusion of the selective cannabinoid CB_1 receptor antagonist AM281, suggesting that the effect is mediated by cannabinoid CB_1 receptors. AM281 failed to have any effect on its own which is consistent with our previous results using another cannabinoid CB_1 receptor antagonist (*N*-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-arboxiamide hydrochloride (SR141716A) (Paton et al., 1998). This suggests that there is no endogenous tone in the cannabinoid control of paired pulse depression, either through pre-coupled receptors, or through tonic release of endogenous cannabinoids such as anandamide of 2-arachidonoyl glycerol.

4.3. Influence of temperature

While the in vitro slice recordings represent a relatively intact preparation compared to those employed in more mechanistic studies, there are several important differences from the in vivo situation. In particular, our experiments were performed at 28–29 °C, whereas in vivo the deep brain temperature would be considerably higher. Manipulating the temperature is likely to affect a number of events including rate of transmitter uptake (Fyske and Fonnum, 1988), transmitter release (Hardingham and Larkman, 1998), the excitability of neurones, the amplitude of individual action potentials (Thompson et al., 1985; Borst and Sakmann, 1988), and the kinetics of transmitter binding to the receptors (Kullmann and Asztelly, 1998). This complexity makes it hard to predict the final outcome on synaptic transmission (Volgushev et al., 2000); however, it is important that at 35 °C both guvacine and nipecotic acid caused a significant increase in paired pulse depression, suggesting that at this temperature postsynaptic GABA receptors are not saturated. This effect of the uptake inhibitors is unlikely to be due to faster deterioration of the slice at higher temperatures for two reasons. First, decreased, rather than increased paired pulsed depression is normally indicative of an unhealthy slice, and secondly, complete washout of the effects of both nipecotic acid and guvacine was observed at the higher temperature. At 35 °C, the previous effect of WIN55,212-2 was reversed and instead the cannabinoid caused a small increase in paired pulse depression which was most evident at the 20- and 30-ms interpulse intervals. This would be consistent with the dominant effect of the cannabinoid at the higher temperature being to increase GABAergic inhibition, for example by inhibiting GABA uptake (Banerjee et al., 1975; Hershkowitz et al., 1977; Maneuf et al., 1996; Coull et al., 1997). In our experiments, this increase in paired pulse depression was also attenuated by AM281 suggesting that it is cannabinoid CB_1 receptor mediated. This is consistent with the demonstration that the Δ^9-THC induced inhibition of GABA uptake in the globus pallidus is sensitive to SR141716A (Maneuf et al., 1996). Such an effect might be expected to alter the time course of miniature IPSCs, however, experiments examining the effects of cannabinoids on miniature IPSCs recorded in the hippocampal slice have shown no effect on the rate of decay of the current (Hoffman and Lupica, 2000). It is significant though, that this work was performed at room temperature and, to the best of our knowledge, such experiments have not been performed on intact hippocampal slices at more physiological temperatures. Repeating these experiments at higher temperatures would be important in identifying the mechanisms underlying the effects on paired pulse depression that we have observed. Recently, it has been suggested that endocannabinoids may mediate depolarisation-induced suppression of inhibition (DSI) (Pitler and Alger, 1992). The hypothesis being that depolarisation of CA1 pyramidal cells might trigger the release of endocannabinoids which act as retrograde messengers to inhibit release of GABA onto those cells (Wilson and Nicoll, 2001; Ohno-Shasuku et al., 2001). This phenomenon was observed as a decrease in the frequency of spontaneous IPSCs, but in the light of the results presented here it would be interesting to establish if DSI is also associated with a temperature-dependent increase in the half time of those IPSPs. The balance between the effects of cannabinoids on release and uptake of GABA may critically influence events such as synaptic long-term potentiation, which is highly dependent on membrane depolarisation.

4.4. Conclusions

Our results indicate that the effects of WIN55,212-2 on synaptic transmission are highly temperature-dependent. At the lower temperature WIN55212,2, decreases paired pulse depression, an effect which is consistent with the widely reported inhibition of GABA release by cannabinoids. At the higher temperature, WIN55,212-2 increased paired pulse depression, an effect which is consistent with the reported inhibition of GABA uptake. Experiments with known

inhibitors of GABA uptake suggest that they only affect paired pulse depression at the higher temperature. We speculate that any effect on inhibition of GABA uptake at the lower temperature is rendered irrelevant since GABA is present in the synaptic cleft at saturating concentrations. However, at higher temperatures uptake is able to limit the action of GABA at postsynaptic $GABA_A$ receptors and so under these conditions cannabinoids increase paired pulse depression, presumably by inhibiting GABA uptake and so potentiating GABAergic transmission. The experiments have important consequences for interpreting in vitro data to predict actions of the cannabinoids in vivo.

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