Pergamon

Neuropharmacology 41 (2001) 1000–1005

NEURO PHARMACOLOGY

www.elsevier.com/locate/neuropharm

# The endogenous cannabinoid anandamide activates vanilloid receptors in the rat hippocampal slice

A. Al-Hayani, K.N. Wease, R.A. Ross, R.G. Pertwee, S.N. Davies *

*Department of Biomedical Sciences, Institute of Medical Sciences, University of Aberdeen Aberdeen AB25 2ZD, Scotland, UK*

Received 2 May 2001; received in revised form 1 August 2001; accepted 5 September 2001

**Abstract**

We have previously reported that the synthetic cannabinoid receptor agonist WIN55,212-2 causes a selective reduction in paired-pulse depression of population spikes in the CA1 region of the rat hippocampal slice. This effect is consistent with the observation that activation of cannabinoid receptors inhibits GABA release in the hippocampus. We have now investigated the actions of the putative endogenous cannabinoids 2-arachidonoyl-glycerol (2-AG) and anandamide in this system. 2-AG mimicked the effect of WIN55,212-2 by selectively reducing paired-pulse depression at concentrations of 1–30 μM. In contrast, anandamide caused a selective increase in paired-pulse depression at concentrations of 1–30 μM. This effect was mimicked by the vanilloid receptor agonists capsaicin and resiniferatoxin, and blocked by the vanilloid receptor antagonist capsazepine, but not by the cannabinoid receptor antagonist AM281. These results are the first to demonstrate a clear functional vanilloid receptor-mediated effect in the hippocampus, and further, that anandamide but not 2-AG acts at these receptors to increase paired-pulse depression of population spikes. 

*Keywords:* Anandamide; Cannabinoid; Vanilloid; Capsaicin; Hippocampus; Paired-pulse depression

## 1. Introduction

Capsaicin, the pungent extract of hot chilli peppers, excites a subset of primary afferent neurones by binding to vanilloid receptors (Szallasi and Blumberg, 1999). The receptor has been cloned (VR1) and it forms a $Ca^{2+}$ permeable ion channel that can be gated by reduced pH and high temperature (Caterina et al., 1997). VR1 is present in a subset of dorsal root ganglion neurones that give rise predominantly to C-fibres, consistent with its proposed role in nociception (Guo et al., 1999). Damaging stimuli such as low pH and high temperature have been considered as 'endogenous agonists' of VR1, but whether a true endogenous ligand also exists is still a matter of debate (Piomelli, 2000). One candidate is the putative endogenous cannabinoid anandamide (Zygmunt et al., 1999), which shows considerable structural similarity to other VR1 ligands such as capsaicin (Szallasi and Di Marzo, 2000). In support of this, VR1 located on sensory neurones has been reported to mediate the vasodilator effect of anandamide (Zygmunt et al., 1999), and anandamide was shown to activate VR1-mediated responses in rat dorsal root ganglion cells (Smart et al., 2000) and in host cells expressing cloned rat (Zygmunt et al., 1999; Ross et al., 2001) or human VR1 (Smart et al., 2000).

Whether VR1 is present in the brain is somewhat controversial. Northern blot analysis originally suggested that VR1 mRNA was absent from brain tissue (Caterina et al., 1997) but subsequent PCR based strategies have shown that VR1 mRNA is present (Sasamura et al., 1998; Mezey et al., 2000) and is especially high in the CA1 and CA3 regions of the hippocampus (Mezey et al., 2000). Similarly, initial autoradiographic studies using [$^3$H]resiniferatoxin failed to detect specific binding sites in the central nervous system (Acs et al., 1994; Szallasi et al., 1995), but a modified technique later showed significant specific $^3$[H]resiniferatoxin binding in both rat and human brain (Acs et al., 1996). Most recently, immunoreactivity for antibodies raised against the N and C termini of VR1 has been reported in many areas of

* Corresponding author. Tel.: +44-1224-273086; fax: +44-1224-273019.

*E-mail address:* s.n.davies@abdn.ac.uk (S.N. Davies).

0028-3908/01/$ - see front matter 
PII: S0028-3908(01)00145-9

the brain, including the hippocampus (Mezey et al., 2000). The evidence regarding any functional effects mediated by central VR1 receptors is no less clear cut. Microinjection of capsaicin into the hypothalamus or substantia nigra causes, respectively, a dose-dependent fall in temperature (Jansco-Gabor et al., 1970) or an increase in motor activity (Dawbarn et al., 1981), and systemically administered capsaicin is reported to increase the firing rate of neurones in the locus coeruleus (Hajós et al., 1987). However, the lack of any suitable vanilloid receptor antagonist at the time of these experiments leaves open the possibility that these responses may have been a product of non-specific effects such as membrane perturbation (Aranda et al., 1995; Feigin et al., 1995). A notable more recent exception is therefore the demonstration that capsaicin triggers release of glutamate from hypothalamic tissue slices in a capsazepine-sensitive manner (Sasamura, 1998).

We have previously reported that while the synthetic cannabinoid receptor (CB) agonist WIN55,212-2 has no effect on a single population spike evoked in the CA1 region of the adult rat hippocampal slice, it does cause a powerful reduction in the paired-pulse depression of a second population spike evoked at short interpulse intervals after the first (Paton et al., 1998; Al-Hayani and Davies, 2000). This probably reflects a reduction in feedback inhibition (Knowles and Schwartzkroin, 1981) caused by a selective inhibition of GABA release (Katona et al., 1999; Hoffman and Lupicia, 2000). In the following experiments, we have examined the actions of the putative endogenous cannabinoids, anandamide and 2-arachidonoyl-glycerol (2-AG), and compared these with the effects of the vanilloid receptor agonists, capsaicin and resiniferatoxin.

## 2. Methods

Young adult Sprague Dawley rats (aged 4–6 weeks) were decapitated under deep halothane anaesthesia and the brain was rapidly removed into ice cold artificial cerebrospinal fluid (ACSF). The hippocampi were dissected out and 400 μm thick transverse slices were cut using a McIlwain tissue chopper. Slices were stored in a holding chamber at room temperature before being transferred as required to an interface type recording chamber which was continuously perfused with ACSF at a rate of 1.5 ml/min and a temperature of 29–30°C. 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, and was continuously bubbled with 95%$O_2$–5%$CO_2$. A glass extracellular recording electrode was placed in stratum pyramidale of the CA1 region to record population spikes evoked by a stimulating electrode placed in stratum radiatum at the CA1–CA3 border. Stimulus strength was always set to elicit a population spike of approximately half the maximum amplitude. Paired-pulse stimulation at interpulse intervals between 5 and 200 ms was used in order to identify effects on both the amplitude of the first population spike (PS1), and the extent of paired-pulse depression of the second population spike (PS2). Anandamide, capsaicin, and AM281 were obtained from Tocris (UK); 2-AG and capsazepine from Sigma-Aldrich (UK); resiniferatoxin from Semat International Ltd (UK). All drugs were applied by addition to the perfusion medium. Anandamide was supplied as a soluble emulsion and was dispersed directly into the perfusion medium. All other drugs were dispersed using either DMSO or Tween 80. Control experiments using Tween 80, DMSO or the oil used to make the anandamide emulsion at equivalent concentrations to those used in the experiments, all showed no effect on the synaptic responses. All statistical tests were made using Student's paired *t*-test where $P<0.05$ was considered significant.

## 3. Results

Paired-pulse stimulation at interpulse intervals between 5 and 200 ms resulted in a powerful inhibition of PS2 which usually persisted up to the 40 ms interpulse interval, and at longer intervals, a paired-pulse facilitation. Perfusion of the putative endogenous cannabinoid anandamide for 20 min at concentrations of 1, 10 and 30 μM had no effect on the amplitude of PS1 but caused a concentration-dependent decrease in the amplitude of PS2, i.e. the extent of paired-pulse depression was increased. This effect was most evident at interpulse intervals between 15 and 50 ms (using the 20 ms interpulse interval, 10 μM anandamide caused the amplitude of PS2 to fall from 53±10 to 31±6% of PS1, $n$=6, Fig. 1(a)–(c)). This effect is in contrast to the synthetic cannabinoid receptor agonist WIN55,212-2 which, in slices prepared from adult rats, also has no effect on PS1 but causes the amplitude of PS2 to increase, thus decreasing the extent of paired-pulse depression (Paton et al., 1998; Al-Hayani and Davies, 2000). The effect of anandamide on PS2 still persisted after perfusion of the $CB_1$ receptor antagonist AM281 (500 nM) (using the 20 ms interpulse interval, after treatment with AM281, perfusion of 30 μM anandamide caused the amplitude of PS2 to fall from 51±5 to 26±6% of PS1, $n$=4, Fig. 1(d)). In contrast, perfusion of the VR antagonist capsazepine (10 μM) prior to application of anandamide totally blocked the effect of subsequently applied anandamide on PS2 (using the 20 ms interpulse interval, PS2 was 71±3% of PS1 in 10 μM anandamide plus 10 μM capsazepine, compared to 65±4% of PS1 before drug administration, $n$=4, Fig. 1(d) and (e)). It is noteworthy that after blockade of vanilloid receptors with capsazepine, the effect of anandamide was more cannabinoid-like, in that

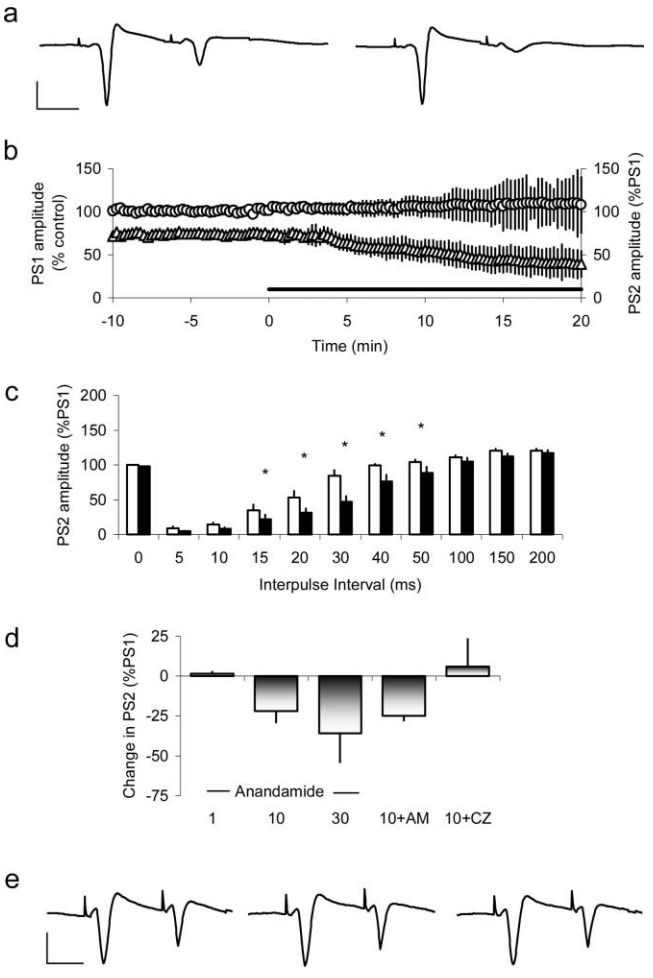


Fig. 1. Anandamide decreases PS2 amplitude in the hippocampal slice and this is blocked by capsazepine but not by AM281. (a) Example synaptic responses recorded from a single slice stimulated with paired pulses at an interpulse interval of 20 ms. The response on the left was recorded under control conditions whereas the response on the right was recorded after perfusion of 10 μM anandamide for 20 min. Note no change in PS1, but a reduction in the amplitude of PS2. The scale bar represents 10 ms and 2 mV. (b) Slices were continually stimulated with paired pulses at an interpulse interval of 20 ms to illustrate the time course of action of 10 μM anandamide, perfused for the period indicated by the bar, on PS1 (circles) and on PS2 (triangles). Points represent mean±standard error, *n*=6. (c) The effect of 10 μM anandamide on the full range of interpulse intervals as recorded from the same slices at the start (open bars) and end (filled bars) of the period is illustrated in (b). In this, and in similar figures, * indicates a significant difference from control at that specific interpulse interval. (d) Histograms represent the change in PS2 amplitude evoked by 1, 10, and 30 μM anandamide, and by 10 μM anandamide after treatment with 500 nM AM281 or 10 μM capsazepine. (e) Example synaptic responses show, from left to right, recordings from a single slice under control conditions, in the presence of 10 μM capsazepine, and in the presence of 10 μM anandamide after treatment with capsazepine. Note no effect of capsazepine alone, or of capsaicin after treatment with capsazepine.

it increased the amplitude of PS2. This effect was apparent at interpulse intervals between 10 and 150 ms although it only reached statistical significance at the 30 ms interval. The antagonist had no effect on either PS1 or PS2 on its own.

2-AG, another putative endogenous ligand for the cannabinoid receptor, behaved more like the cannabinoid receptor agonist WIN55,212-2. Perfusion of 2-AG at concentrations of 1, 10 and 30 μM for 20 min again had no effect on PS1, but caused a concentration-dependent increase in the amplitude of PS2, i.e. paired-pulse depression was decreased (using the 20 ms interpulse interval 10 μM 2-AG caused the amplitude of PS2 to increase from 59±11 to 105±4% of PS1, *n*=4, Fig. 2(a)–(c)). This effect of 10 μM 2-AG was completely blocked by prior perfusion of AM281 (using the 20 ms interpulse interval, PS2 was 58±9% of PS1 in 10 μM 2-AG plus 500 nM AM281, compared to 60±6% of PS1 before drug administration, *n*=4, Fig. 2(d) and (e)).

In contrast, the definitive VR ligand capsaicin, and the ultrapotent VR agonist resiniferatoxin, both mimicked the effect of anandamide. Perfusion of capsaicin for 20 min at concentrations of 0.5, 0.75 and 1 μM had no significant effect on the amplitude of PS1, but caused a concentration-dependent decrease in the amplitude of PS2 (using the 20 ms interpulse interval, in the presence of 1 μM capsaicin the amplitude of PS2 was reduced from 66±12 to 17±8% of PS1, *n*=4, Fig. 3(a)–(c)). Perfusion of 10 μM capsazepine for 20 min prior to administration of capsaicin blocked the effect of capsaicin on paired-pulse depression (using the 20 ms interpulse interval, PS2 was 55±2% of PS1 in 1 μM capsaicin plus 10 μM capsazepine, compared to 51±3% of PS1 before drug administration, *n*=4, Fig. 3(d) and (e)). Resiniferatoxin perfused for 20 min at concentrations of 0.5, 1 and 10 nM also had no effect on PS1, but again increased paired-pulse depression (using the 20 ms interpulse interval, 10 nM resiniferatoxin perfused for 20 min reduced the amplitude of PS2 from 48±4 to 35±9% of PS1, *n*=4, Fig. 4(a)–(c)). The inhibition of PS2 by 10 nM resiniferatoxin was apparently smaller than that produced by anandamide or capsaicin, however, higher concentrations of resiniferatoxin produced no greater inhibition of PS2. The effect of 10 nM resiniferatoxin was completely blocked by prior perfusion of capsazepine (using the 20 ms interpulse interval, PS2 was 57±2% of PS1 in 10 nM resiniferatoxin plus 10 μM capsazepine, compared to 53±5% of PS1 before drug administration, *n*=4, Fig. 4(d) and (e)).

## 4. Discussion

Anandamide was the first putative endogenous ligand for cannabinoid receptors to be identified, however, there is some overlap between the structures of anandamide

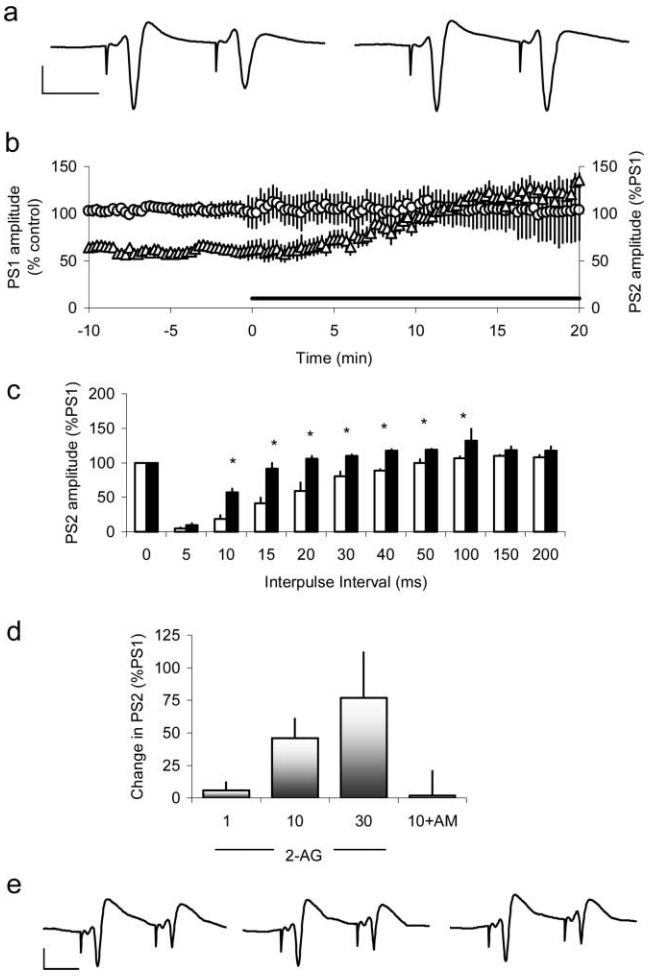


Fig. 2. 2-AG increases PS2 amplitude. (a) Example synaptic responses recorded from a single slice recorded under control conditions and after perfusion of 10 μM 2-AG for 20 min. Note no change in PS1, but an increase in the amplitude of PS2. The scale bar represents 10 ms and 2 mV. (b) Time course data showing that perfusion of 10 μM 2-AG for 20 min increases the amplitude of PS2 (and therefore reduces paired pulse depression) recorded at the 20 ms interpulse interval, $n$=4. (c) Data from the same slices showing the effect of 2-AG on the full range of interpulse intervals. (d) PS2. Change in PS2 amplitude evoked by 1, 10, and 30 μM 2-AG, and by 10 μM 2-AG after treatment with 500 nM AM281. (e) Example synaptic responses show, from left to right, recordings from a single slice under control conditions, in the presence of 500 nM AM281, and in the presence of 10 μM 2-AG after treatment with AM281. Note no effect of AM281 alone, or of 2-AG after treatment with AM281.

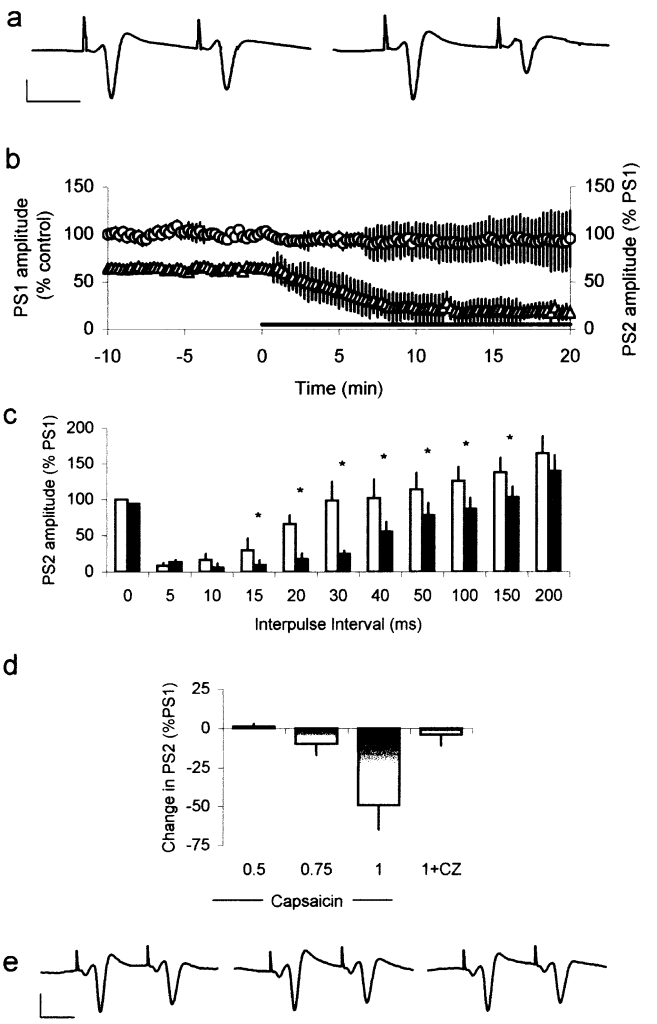


Fig. 3. The VR1 agonist capsaicin mimics the effect of anandamide. (a) Example synaptic responses recorded from a single slice recorded under control conditions and after perfusion of 1 μM capsaicin for 20 min. Note no change in PS1, but a decrease in the amplitude of PS2. The scale bar represents 10 ms and 2 mV. (b) Using continuous stimulation at an interpulse interval of 20 ms perfusion of 1 μM capsaicin for 20 min has no effect on PS1 (circles), but causes a decrease in PS2 (triangles), $n$=4. (c) Data from the same slices showing the effect of capsaicin over all interpulse intervals. (d) Histograms represent the effect of 0.5, 0.75 and 1 μM capsaicin on PS2, and 1 μM capsaicin after treatment with 10 μM capsazepine. (e) Example synaptic responses show recordings from a single slice, from left to right, under control conditions, in the presence of 10 μM capsazepine, and in the presence of 1 μM capsaicin after treatment with capsazepine. Note no effect of capsazepine alone, or of capsaicin after treatment with AM281.

and vanilloid receptor ligands such as capsaisin, (Szallasi and Di Marzo, 2000) and therefore attention was focussed on whether anandamide may also act as an endogenous vanilloid receptor ligand (Premkumar and Ahern, 2000). Because the primary role of vanilloid receptors has previously been considered as being in peripheral nociception, these experiments have been directed towards studies on peripheral sensory neurones. In contrast, early attempts to demonstrate the existence of central vanilloid receptors were largely unsuccessful (Acs et al., 1994; Szallasi et al., 1995). However, improved techniques have consistently shown evidence for the existence of central vanilloid receptors in several areas (Acs et al., 1996; Mezey et al., 2000), including the hippocampus (Mezey et al., 2000).

Our interest in this field arose when we found that the putative endogenous cannabinoid anandamide did not mimic the effect of the synthetic cannabinoid WIN55,212-2 on paired-pulse depression of population spikes recorded in the CA1 region of the rat hippocampal

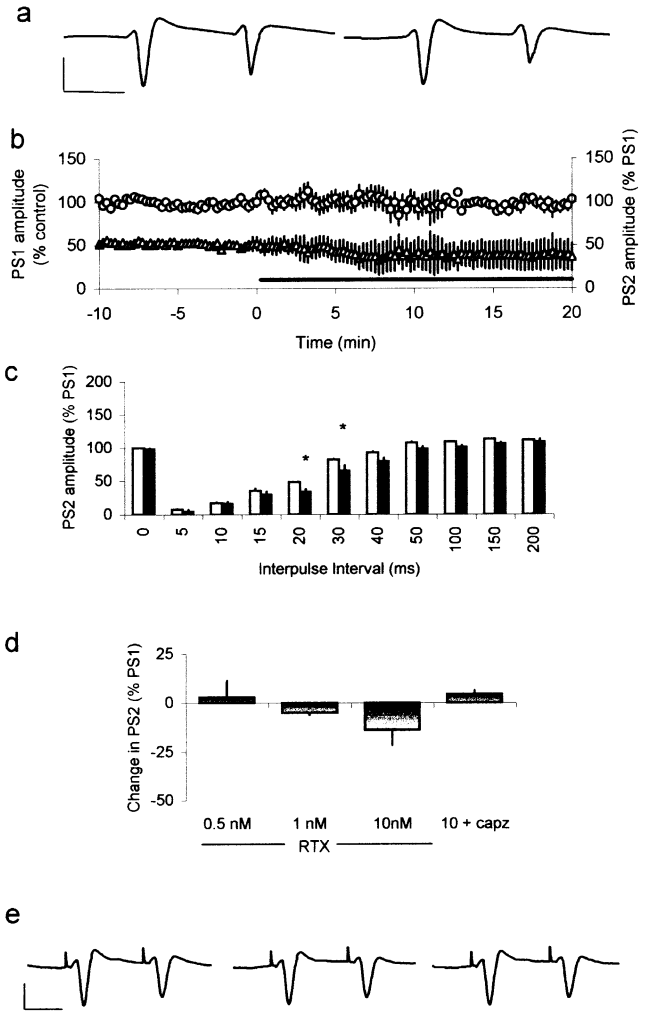


Fig. 4. The VR1 agonist resiniferatoxin mimics the effect of anandamide. (a) Example synaptic responses recorded from a single slice recorded under control conditions and after perfusion of 10 nM resiniferatoxin for 20 min. Note no change in PS1, but a decrease in the amplitude of PS2. The scale bar represents 10 ms and 2 mV. (b) Using continuous stimulation at an interpulse interval of 20 ms perfusion of 10 nM resiniferatoxin for 20 min has no effect on PS1 (circles), but causes a decrease in PS2 (triangles), $n$=4. (c) Data from the same slices showing the effect of resiniferatoxin over all interpulse intervals. (d) Histograms represent the effect of 0.5, 1 and 10 nM resiniferatoxin on PS2, and 10 nM resiniferatoxin after treatment with 10 μM capsazepine. (e) Example synaptic responses show, from left to right, recordings from a single slice under control conditions, in the presence of 10 nM capsazepine, and in the presence of 10 nM resiniferatoxin after treatment with capsazepine. Note no effect of capsazepine alone, or of resiniferatoxin after treatment with capsazepine.

slice. The term paired-pulse depression originates from the observation that the second of a pair of population spikes evoked at short interpulse intervals is powerfully depressed. Several factors contribute to paired-pulse plasticity, but in slices with intact synaptic circuitry this depression is largely due to a strong GABAergic feedback inhibition of the CA1 pyramidal cells (Knowles and Schwartzkroin, 1981). The study of paired-pulse depression has proved a useful screen for the effects of cannabinoids since, in adult animals, $CB_1$ agonists have little effect on the population spike evoked by a single stimulus, but cause a consistent decrease in paired-pulse depression (Paton et al., 1998; Al-Hayani and Davies, 2000). In contrast, the results presented here show that anandamide caused a dramatic increase in paired-pulse depression, i.e. a decrease in the amplitude of the PS2, with little or no effect on PS1. The findings that this effect was mimicked by capsaicin and resiniferatoxin at appropriate concentrations (Szallasi and Blumberg, 1999), and blocked by capsazepine, suggest that the effect is mediated by vanilloid receptors. The maximum effect of resiniferatoxin was, however, substantially smaller than that evoked by anandamide or capsaicin. It is notable that Caterina et al. (1997) also reported that resiniferatoxin produced a somewhat smaller maximal activation of membrane currents in VR1 transfected oocytes than capsaicin. In addition, we have found (Gibson, 2000) that the maximum inhibition of electrically evoked contractions of the mouse isolated vas deferens by resiniferatoxin is significantly lower than that of capsaicin. Therefore, it is possible that resiniferatoxin, although a very potent agonist, may have lower efficacy than capsaicin. Alternatively, these findings may be explained by different desensitization characteristics of these compounds (Szallasi and Blumberg, 1999).

2-AG is another putative endogenous cannabinoid, but it is apparently not an effective vanilloid receptor agonist (Zygmunt et al., 1999). This is reflected in the fact that it in our experiments it acted like a cannabinoid and not like a vanilloid receptor agonist by causing a decrease in paired-pulse depression. The observation that in the presence of capsazepine, anandamide had only a very small WIN55,212-2-like effect is intriguing. Anandamide is the subject of enzymatic breakdown by fatty acid amide hydrolase (FAAH; Deutsch and Chin, 1993) and in some systems addition of FAAH inhibitors is required to show effects of anandamide. This suggests that anandamide, rather than a breakdown product, is the effective cannabinoid receptor ligand. In contrast, robust vanilloid receptor-mediated effects of anandamide were observed in the absence of any enzyme inhibition, which makes it possible that it is some breakdown product of anandamide rather than anandamide itself, which activates vanilloid receptors (Hwang et al., 2000; Piomelli, 2000).

The results show, for the first time, a functional electrophysiological effect of vanilloid receptors in the hippocampus, and also that anandamide acts as an effective vanilloid receptor agonist. Our experiments have not addressed the mechanism of action of these vanilloid receptor-mediated effects of anandamide, but they are consistent with activation of vanilloid receptors causing a selective increase in inhibitory GABAergic synaptic transmission in the hippocampus. Further experiments using pharmacologically isolated synaptic responses would be required to clarify this point. Neither is it clear

whether the actions of vanilloid receptors in the hippocampus are related to their involvement with nociception in the periphery, or whether this represents some totally unrelated physiological function. It is clear, however, that activation of vanilloid receptors in the hippocampus has powerful effects on synaptic transmission. This therefore opens up avenues for the investigation of the physiological role of these receptors in the CNS, and their possible exploitation for therapeutic ends.

## Acknowledgements

We thank the Wellcome Trust and the PPP Healthcare Trust for support.

## References

Acs, G., Palkovits, M., Blumberg, P.M., 1994. Comparison of [H-3] resiniferatoxin binding by the vanilloid (capsaicin) receptor in dorsal root ganglia, spinal cord, dorsal vagal complex, sciatic and vagal nerve and urinary bladder of the rat. Life Sciences 55, 1017–1026.

Acs, G., Palkovits, M., Blumberg, P.M., 1996. Specific binding of $^{3}$[H]resiniferatoxin by human and rat preoptic area, locus ceruleus, medial hypothalamus, reticular formation and ventral thalamus membrane preparations. Life Sciences 59, 1899–1908.

Al-Hayani, A., Davies, S.N., 2000. Developmental regulation of CB1 receptor mediated inhibition of synaptic transmission in the rat hippocampal slice. British Journal of Pharmacology 131, 663–665.

Aranda, F.J., Villalaína, J., Gómez-Fernándeza, J.C., 1995. Capsaicin affects the structure and phase organization of phospholipid membranes. Biochimic Biophysica Acta 1234, 225–234.

Caterina, M.J., Schumacher, M.A., Tominaga, M., Rosen, T.A., Levine, J.D., Julius, D., 1997. The capsaicin receptor: a heat activated ion channel in the pain pathway. Nature 389, 816–824.

Dawbarn, D., Harmar, A.J., Pycock, C.J., 1981. Intranigral injection of capsaicin enhances motor activity and depletes nigral 5-hydroxytryptamine but not substance P. Neuropharmacology 20, 341–346.

Deutsch, D.G., Chin, S.A., 1993. Enzymatic — synthesis and degredation of anandamide, a cannabinoid receptor agonist. Biochemical Pharmacology 46, 791–796.

Feigin, A.M., Aronov, E.V., Bryant, B.P., Teeter, J.H., Brand, J.G., 1995. Capsaicin and its analogs induce ion channels in planar lipid bilayers. Neuroreport 6, 2134–2136.

Gibson, M., 2000. Characterisation of cannabinoid receptors and their ligands in isolated smooth muscle preparations. PhD thesis, University of Aberdeen.

Guo, A., Vulchanova, L., Wang, J., Li, X., Elde, R., 1999. Immunocytochemical localization of the vanilloid receptor 1 (VR1): relationship to neuropeptides, the $P2X_3$ purinoceptor and IB4 binding sites. European Journal of Neuroscience 11, 946–958.

Hajós, M., Jansco, G., Engberg, G., 1987. Capsaicin-induced excitation of locus coeruleus neurons. Acta Physiologica Scandinavica 129, 415–420.

Hwang, S.W., Cho, H., Kwak, J. et al., 2000. Direct activation of capsaicin receptors by products of lipoxygenases: endogenous capsaicin-like substances. Proceedings of the National Academy of Sciences of the United States of America 97, 6155–6160.

Hoffman, A.F., Lupicia, C.R., 2000. Mechanisms of cannabinoid inhibition of $GABA_A$ synaptic transmission in the hippocampus. Journal of Neuroscience 20, 2470–2479.

Jansco-Gabor, A., Szolcsanyi, J., Jansco, N., 1970. Irreversible impairment of thermoregulation induced by capsaicin and similar pungent substances in rats and guinea pigs. Journal of Physiology 206, 495–507.

Katona, I., Sperlagh, B., Sik, A., Kafalvi, A., Vizi, E.S., Mackie, K., Freund, T.F., 1999. Presynaptically located CB1 cannabinoid receptors regulate GABA release from axon terminals of specific hippocampal interneurons. Journal of Neuroscience 19, 4544–4558.

Knowles, W.D., Schwartzkroin, P.A., 1981. Local circuit synaptic-interactions in hippocampal brain slices. Journal of Neuroscience 1, 318–322.

Mezey, E., Tóth, Z.E., Cortright, D.N., Arzubi, M.K., Krause, J.E., Elde, R., Guo, A., Blumberg, P.M., Szallasi, A., 2000. Distribution of mRNA for vanilloid receptor subtype 1 (VR1), and VR1-like immunoreactivity, in the central nervous system of the rat and human. Proceedings of the National Academy of Sciences of the United States of America 97, 3655–3660.

Paton, G.S., Pertwee, R.G., Davies, S.N., 1998. Correlation between cannabinoid mediated effects on paired pulse depression and induction of long term potentiation in the rat hippocampal slice. Neuropharmacology 37, 1123–1130.

Piomelli, D., 2000. The ligand that came from within. Trends in Pharmacological Sciences 22, 17–19.

Premkumar, L.S., Ahern, G.P., 2000. Induction of vanilloid receptor channel activity by protein kinase C. Nature 408, 985–990.

Ross, R.A., Gibson, T.M., Brockie, H.C., Leslie, M., Pashmi, G., Craib, S.J., Di Marzo, V., Pertwee, R.G., 2001. Structure–activity relationship for the endogenous cannabinoid, anandamide, and certain of its analogues at vanilloid receptors in transfected cells and vas deferens. British Journal of Pharmacology 132, 631–640.

Sasamura, T., Sasaki, M., Tohda, C., Kuraishi, Y., 1998. Existence of capsaicin-sensitive glutamergic terminals in rat hypothalamus. Neuroreport 9, 2045–2048.

Smart, D., Gunthorpe, M.J., Jerman, J.C., Nasir, S., Gray, J., Muir, A.L., Chmbers, J.K., Randall, A.D., Davis, J.B., 2000. The endogenous lipid anandamide is a full agonist at the human vanilloid receptor (hVR1). British Journal of Pharmacology 129, 227–230.

Szallasi, A., Blumberg, P.M., 1999. Vanilloid (capsaicin) receptors and mechanisms. Pharmacological Reviews 51, 159–211.

Szallasi, A., Di Marzo, V., 2000. New perspectives on enigmatic vanilloid receptors. Trends in Neurosciences 23, 491–497.

Szallasi, A., Nilsson, S., FarkasSzallasi, T., Blumberg, P.M., Hokfelt, T., Lundberg, J.M., 1995. Vanilloid (capsaicin) receptors in the rat: distribution in the brain, regional differences in the spinal cord, axonal transport to the periphery, and depletion by systemic vanilloid treatment. Brain Research 703, 175–183.

Zygmunt, P.M., Peterson, J., Andersson, D.A., Chunag, H., Sorgard, M., Di Marzo, V., Julius, D., Högestätt, E.D., 1999. Vanilloid receptors on sensory nerves mediate the vasodilator action of anandamide. Nature 400, 452–457.