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Review

Cannabinoid modulation of peripheral autonomic and sensory neurotransmission

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Abstract

Cannabinoids are cell membrane-derived signalling molecules that are released from nerves, blood cells and endothelial cells, and have diverse biological effects. They act at two distinct types of G-protein-coupled receptors, cannabinoid CB_1 and CB_2 receptors. Cannabinoid CB_1 receptors are highly localised in the central nervous system and are also found in some peripheral tissues, and cannabinoid CB_2 receptors are found outside the central nervous system, in particular in association with immune tissues. Novel actions of cannabinoids at non-CB_1 non-CB_2 cannabinoid-like receptors and vanilloid VR1 receptors have also recently been described. There is growing evidence that, among other roles, cannabinoids can act at prejunctional sites to modulate peripheral autonomic and sensory neurotransmission, and the present article is aimed at providing an overview of this. Inhibitory cannabinoid CB_1 receptors are expressed on the peripheral terminals of autonomic and sensory nerves. The role of cannabinoid receptor ligands in modulation of sensory neurotransmission is complex, as certain of these (anandamide, an ''endocannabinoid'', and *N*-arachidonoyl-dopamine, an ''endovanilloid'') also activate vanilloid VR1 receptors (coexpressed with cannabinoid CB_1 receptors), which excites sensory nerves and causes a release of sensory neurotransmitter. The fact that the activities of anandamide and *N*-arachidonoyl-dopamine span two distinct receptor families raises important questions about cannabinoid/vanilloid nomenclature, and as both compounds are structurally related to the archetypal vanilloid capsaicin, all three are arguably members of the same family of signalling molecules. Anandamide is released from nerves, but unlike classical neurotransmitters, it is not stored in and released from nerve vesicles, but is released on demand from the nerve cell membrane. In the central nervous system, cannabinoids function as retrograde signalling molecules, inhibiting via presynaptic cannabinoid CB_1 receptors the release of classical transmitter following release from the postsynaptic cell. At the neuroeffector junction, it is more likely that cannabinoids are released from prejunctional sites, as the neuroeffector junction is wide in some peripheral tissues and cannabinoids are rapidly taken up and inactivated. Understanding the actions of cannabinoids as modulators of peripheral neurotransmission is relevant to a variety of biological systems and possibly their disorders.

Keywords: Cannabinoid; Neurotransmission; Anandamide

1. Introduction

Cannabinoids are a family of cell membrane-derived signalling molecules that are released from nerves, blood cells and endothelial cells, and have diverse biological effects, including actions on the immune, cardiovascular, and central and peripheral nervous systems. They act at two distinct types of G-protein-coupled receptors, cannabinoid CB_1 and CB_2 receptors (Pertwee, 1993, 1997, 1999). Cannabinoid CB_1 receptors are highly localised in the central nervous system and are also found in some peripheral tissues. Cannabinoid CB_2 receptors are found outside the central nervous system, in particular in association with immune tissues. There is emerging evidence, however, that current cannabinoid receptor classification may be incomplete with the identification of non-CB_1 non-CB_2 cannabinoid-induced responses in a variety of tissues (Járai et al., 1999; Wagner et al., 1999; Kunos and Batkai, 2001; White et al., 2001; Zygmunt et al., 2002). Further complexity was added with the discovery that the archetypal ''endocannabinoid'', anandamide (Devane et al., 1992), is an agonist at the vanilloid VR1 receptor (Zygmunt et al., 1999; Smart et al., 2000) which is highly expressed on sensory nerves. More recently, *N*-arachidonoyl-dopamine, a compound orig-

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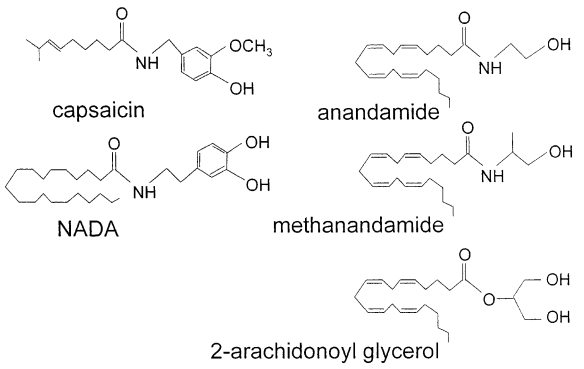

Fig. 1. Structures of the endocannabinoids anandamide and 2-arachidonoyl glycerol, the endovanilloid *N*-arachidonoyl-dopamine (NADA), capsaicin (archetypal vanilloid) and methanandamide (metabolically stable analogue of anandamide).

inally synthesised as an agonist selective for cannabinoid CB_1 over CB_2 receptors (K_i values 0.25 and 12 μM, respectively) (Bisogno et al., 2000), was found to be a naturally occurring capsaicin-like substance (''endovanilloid'') with potent activity at vanilloid VR1 receptors (Huang et al., 2002). Both anandamide and *N*-arachidonoyl-dopamine are structurally similar to capsaicin (Fig. 1), the archetypal vanilloid, raising important questions about nomenclature as these compounds are arguably members of the same family of signalling molecules.

Much of the interest in cannabinoids has focussed on their actions in the central nervous system, which is appropriate since their pronounced psychoactive effects have been known for centuries through the medicinal and recreational use of the cannabis plant *Cannabis sativa* (Butrica, 2002). The main psychoactive compound in the cannabis plant, Δ^9-tetrahydrocannabinol (Fig. 2), was isolated by Gaoni and Mechoulam (1964). There is now growing evidence that cannabinoids can also modulate pre- and postjunctionally neurotransmission in the periphery. There is substantial evidence for a role of the cannabinoid CB_1 receptor in inhibitory modulation of peripheral sympathetic, parasympathetic, enteric and sensory neurotransmitter release, and preliminary evidence for an involvement of non-CB_1 non-CB_2 cannabinoid-like receptors. The role of cannabinoid receptor ligands in modulation of sensory neurotransmission is complex, as certain of these (anandamide and *N*-arachidonoyl-dopamine to date) also activate vanilloid VR1 receptors (coexpressed with cannabinoid CB_1 receptors), which excites sensory nerves and causes a release of sensory neurotransmitter. A number of recent reviews have considered the synthesis, uptake and enzymatic degradation of endocannabinoids, and the structure and properties of the various cannabinoid receptor agonists and antagonists now available (Pertwee, 1997; Di Marzo et al., 1998; Mechoulam et al., 1998; Giuffrida et al., 2001; Howlett et al., 2002). The present review discusses the roles of cannabinoids as prejunctional modulators of peripheral autonomic and sensory neurotransmission.

2. Neuroeffector junction

Autonomic nerves have extensive varicose regions (1–2 μm diameter) free of Schwann cell envelopments separated by narrow (0.1–0.3 μm diameter) intervaricose regions (Burnstock, 1986). The varicose regions contain vesicles (which concentrate at the region of close apposition with the target cell) and mitochondria, and are the sites of neurotransmitter release. The prejunctional varicosity membranes are sometimes thickened, but there are rarely postjunctional specialisations, thus differing from the well-defined pre- and postsynaptic specialisations of the skeletal neuromuscular junction and ganglionic synapses. Studies of the relationship of autonomic nerve fibres to smooth muscle have shown that the neuroeffector cleft width (separation between individual varicosities and target cells) may vary from as little as 15–20 nm in some densely innervated tissues (like vas deferens or iris) to as much as 2000 nm in some large elastic arteries (Burnstock, 1986; Hirst et al., 1996), but even within a single tissue, the range between the smallest and largest neuroeffector cleft widths may be considerable (up to 30-fold difference). The cleft is often filled with a single layer of basal lamina. The individual muscle cells are electrically coupled via low resistance gap junctions which allow electrotonic spread of activity within the tissue, such that when a muscle cell becomes depolarised/hyperpolarised by the

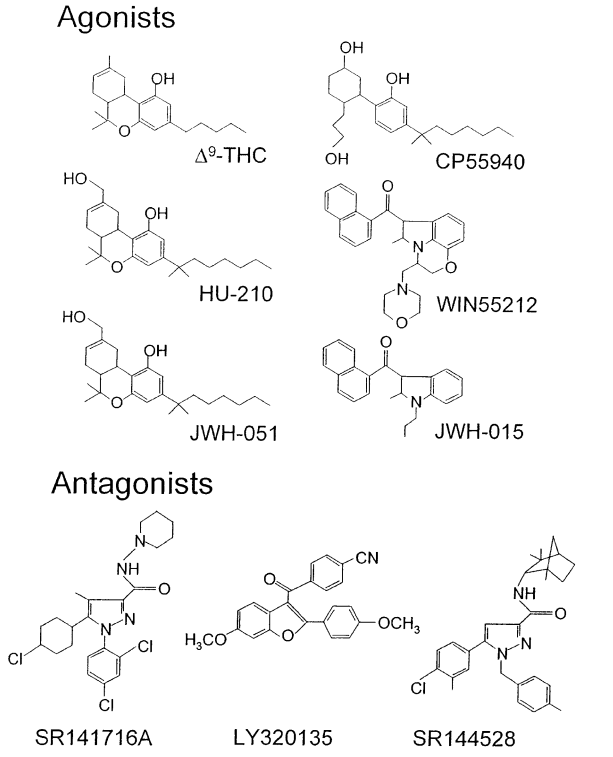

Fig. 2. Structures of the cannabis constituent Δ^9-tetrahydrocannabinol (Δ^9-THC), and cannabinoid receptor agonists and antagonists mentioned in this review. Of the agonists, JWH-051 is CB_2-selective. Of the antagonists, SR141716A and LY320135 are cannabinoid CB_1 receptor-selective and SR144528 is cannabinoid CB_2 receptor-selective.

action of the neurotransmitter, this is spread between the muscle cells. Neurotransmission can be modulated by locally produced and circulating substances acting both at prejunctional sites on the nerve varicosity and/or postjunctional sites on the target cell.

3. Sympathetic nerves

There is molecular evidence for the expression of cannabinoid receptors in sympathetic nerves. Cannabinoid CB_1 receptor, but not CB_2 receptor, mRNA is expressed in embryonic and adult sympathetic ganglia (Ishac et al., 1996; Buckley et al., 1998). Cannabinoid CB_1 and CB_2-like receptor mRNA was detected in mouse vas deferens, a tissue richly innervated with sympathetic nerves (Griffin et al., 1997).

Direct evidence for the existence of prejunctional cannabinoid CB_1 receptors on sympathetic nerves has come from studies showing that cannabinoids inhibit the release of noradrenaline from these nerves (see Table 1). The most robust evidence for this is in the mouse vas deferens where the nonselective cannabinoid receptor agonists WIN 55,212-2 and CP 55,940 potently inhibited the electrically evoked overflow of [^{3}H]noradrenaline (EC_{50} values 2.7 and 0.27 nM, respectively) and this action was blocked by nanomolar

Table 1
Cannabinoid prejunctional modulation of sympathetic neurotransmitter release

Species	Tissue	Effect of Agonist	Agonist(s)	Antagonist(s) blocking agonist effect	References
Human	Heart	↓ [^{3}H]NA release	CP 55,940, anandamide (both 1 and 10 μM)	SR141716A, LY320135 (both 1 μM); rauwloscine (30 μM)	Molderings et al., 1999
Rabbit	Pithed rabbit—electrically stimulated sympathetic outflow	↓ plasma NA and BP	WIN 55,212 (5–500 μg kg^{-1} i.v.)	SR141716A (0.5 mg kg^{-1} i.v.)	Niederhoffer and Szabo, 1999
			CP 55,940 (5–500 μg kg^{-1} i.v.)	–	
			(−)WIN 55,212 inactive (up to 500 μg kg^{-1} i.v.)	–	
	Heart (of pithed rabbit)—electrically evoked cardioacceleration	↓ HR	WIN 55,212 (5–1500 μg kg^{-1} i.v.)	SR141716A (0.5 mg kg^{-1} i.v.)	Szabo et al., 2001
			CP 55,940 (3–1000 μg kg^{-1} i.v.)	–	
Rat	Pithed rat—electrically stimulated sympathetic outflow	↓ BP	WIN 55,212, CP 55,940 (both 0.1 μmol kg^{-1} i.v.)	SR141716A (0.03 μmol kg^{-1} i.v.)	Malinowska et al., 1997
	Heart	↓ [^{3}H]NA release	Δ^9-THC, anandamide (both 0.3–10 μM)	SR141716A (1–10 μM)	Ishac et al., 1996
	Vas deferens	↓ [^{3}H]NA release	Δ^9-THC, anandamide (both 0.3–10 μM)	SR141716A (1–10 μM)	Ishac et al., 1996
	Tail artery	No effect on [^{3}H]NA overflow	WIN 55,212, CP 55,940 (both 1 μM)	–	Malinowska et al., 1997
	Renal artery	↓ [^{3}H]NA release (KCl-stimulated)	anandamide (1 μM)	SR141716A (1 μM) L-NAME (0.1 mM)	Deutsch et al., 1997
	Mesenteric arterial bed	↓ NA release	HU210 (3 μM)	LY320135 (1 μM)	Ralevic and Kendall, 2002
			HU211 inactive (3 μM)	SR144528 (1 μM) inactive	
Mouse	Vas deferens	↓ [^{3}H]NA release	WIN 55,212 (EC_{50} 2.7 nM, E_{max} 79%)	SR141716A (0.001 and 0.01 μM)	Trendelenburg et al., 2000
			CP 55,940 (EC_{50} 0.3 nM, E_{max} 64%)	SR141716A (0.01 and 0.1 μM) pK_d = 9.7–10.5	
	Vas deferens (1-day-old mice)	↓ [^{3}H]NA release	WIN 55,212 (0.0001–1 μM)	–	Schelb et al., 2001
	Atria	↓ [^{3}H]NA release	WIN 55,212 inactive (up to 1 μM)	–	Trendelenburg et al., 2000
	Spleen	↓ [^{3}H]NA release	WIN 55,212 inactive (up to 1 μM)	–	Trendelenburg et al., 2000
	Sympathetic chain neurones in culture	↓ [^{3}H]NA release	WIN 55,212 (0.01–10 μM; EC_{50} 282 nM, E_{max} 75%)	SR141716A (0.001 and 0.01 μM) pK_d = 9.3–9.9	Göbel et al., 2000

Unless indicated otherwise, [^{3}H]NA and NA release was evoked by electrical stimulation.
Unless indicated otherwise, all tissues/animals are adults.
BP, blood pressure; HR, heart rate; NA, noradrenaline.

concentrations of SR141716A, a selective cannabinoid CB_1 receptor antagonist (Trendelenburg et al., 2000) (Fig. 3). The effective concentrations of the ligands were similar to those known to bind recombinant cannabinoid CB_1 receptors and endogenous cannabinoid CB_1 receptors in the brain (Pertwee, 1997, 1999). The release study of Trendelenburg et al. (2000) is in line with earlier functional studies in the mouse vas deferens, which measured neurotransmitter release indirectly via the end organ response. In this tissue, nanomolar concentrations of the archetypal cannabinoids Δ^9-tetrahydrocannabinol and anandamide, and synthetic cannabinoids, inhibited the electrically evoked twitch response, without affecting the response to exogenous noradrenaline, and this effect was antagonised by SR141716A, indicating an action at prejunctional cannabinoid CB_1 receptors (Pertwee et al., 1992, 1996b; Rinaldi-Carmona et al., 1994; Lay et al., 2000; Ross et al., 2001b). Inhibitory cannabinoid CB_2-like receptors may also be expressed on sympathetic nerves in the mouse vas deferens, as nanomolar concentrations of the cannabinoid CB_2 receptor-selective agonists JWH-015 and JWH-051 blocked electrically evoked sympathetic neurogenic contractions, and this action was insensitive to SR141716A (Griffin et al., 1997). Consistent with the presence of cannabinoid receptors in the mouse vas deferens is the observation that in vivo pretreatment of mice with Δ^9-tetrahydrocannabinol can induce cannabinoid tolerance (reduction in the slope and maximal response of the concentration response curve to Δ^9-tetrahydrocannabinol indicating a reduction in receptor reserve) without affecting the sensitivity of the twitch response to inhibition by the α_2-adrenoceptor agonist clonidine and to opioid receptor agonists (Pertwee and Griffin, 1995).

Prejunctional cannabinoid CB_1 receptors have also been identified on sympathetic nerves in the rat vas deferens. In this tissue, Δ^9-tetrahydrocannabinol and anandamide (0.3–10 μM) inhibited electrically evoked [^{3}H]noradrenaline release in an SR141716A-sensitive manner (Ishac et al., 1996). In line with this, WIN 55,212-2 and CP 55,940 inhibited the electrically evoked twitch response (pEC_{50} values 8 and 6.8, respectively) in an SR141716A- and LY321035-sensitive manner, and with pK_B estimates similar to the activities of the antagonists at cannabinoid CB_1 receptors (Christopoulos et al., 2001). Moreover, the inhibitory effects of CP 55,940 were stereoselective (Pertwee, 1997).

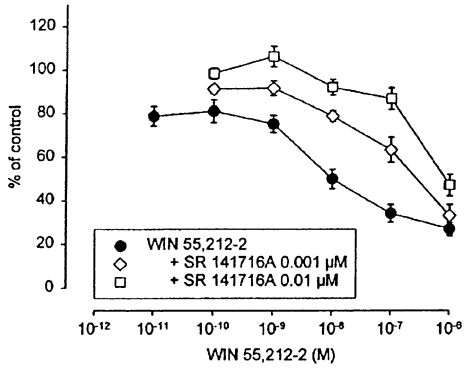

Fig. 3. Effect of the cannabinoid receptor agonist WIN 55,212-2 on the evoked overflow of tritium from pieces of mouse vas deferens, and interaction with the antagonist SR 141716A. S_1 to S_6 each consisted of 20 pulses at 50 Hz. WIN 55,212 was added at increasing concentrations before S_2–S_6. WIN 55,212 was given either alone or with SR 141716A, which was present throughout superfusion. Ordinates, evoked overflow of tritium, calculated from S_n/S_1 ratios and expressed as a percentage of control. Values are means ± S.E.M. from 6 to 10 tissue pieces (from Trendelenburg et al., 2000, with permission).

Cannabinoid inhibition of noradrenaline release from sympathetic nerves has been shown in a variety of other tissues including isolated heart, blood vessels and sympathetic neurones in culture (Table 1). Typically, only SR141716A was used in a number of the studies, and unfortunately, this compound has been shown to have actions in addition to inhibition of cannabinoid receptors at concentrations of >1 μM, including inhibition of gap junctions (Chaytor et al., 1999), vanilloid VR1 receptors (Zygmunt et al., 1999; DePetrocellis et al., 2001) and imidazoline receptors (Molderings et al., 1999). SR141716A can also act as an inverse agonist at cannabinoid CB_1 receptors (Bouaboula et al., 1997; MacLennan et al., 1998; Pan et al., 1998), which may complicate interpretation regarding a possible involvement of endogenous cannabinoids. It is interesting that with few exceptions, most notably the mouse vas deferens, the concentrations of cannabinoid ligands used to inhibit noradrenaline release from sympathetic nerves (Table 1) are several fold higher than the nanomolar affinity demonstrated for agonists and antagonists at recombinant and endogenous cannabinoid receptors (Pertwee, 1997; Howlett et al., 2002). However, as the concentrations of both agonists and antagonists used were uniformly relatively high across a number of the studies (Table 1), and as there is no good reason why sympathetic nerves in the mouse vas deferens should be different from those in other tissues in terms of their expression of prejunctional receptors (but see Martin et al., 2000), this may reflect differences in ligand penetration, ligand affinity, stimulus–response coupling mechanisms or methodological differences.

A recent study in the rat mesenteric arterial bed has shown that electrically evoked release of noradrenaline is inhibited by HU210, but not by the inactive (+) stereoisomer HU211, in an LY321035-sensitive and SR144528-insensitive manner, indicating an involvement of prejunctional cannabinoid CB_1 receptors as modulators of sympathetic neurotransmission in this tissue (Ralevic and Kendall, 2002). In contrast, both HU210 and HU211 attenuated sympathetic neurogenic contractions of the rat mesenteric arterial bed, and this was unaffected by cannabinoid CB_1 and CB_2 receptor antagonists (Ralevic and Kendall, 2002). As HU210 and HU211 (and CP 55,940 and methanandamide) inhibited contractions to exogenous noradrenaline and KCl, this suggests that their inhibitory actions on sympathetic neurogenic contractions may reflect principally effects mediated at a postjunctional site;

the lack of stereoselectivity and antagonist insensitivity may indicate receptor-independent actions at the smooth muscle, or actions at a novel cannabinoid receptor. Spicuzza et al. (2000) have also reported a mismatch between observed pre- and postjunctional effects of cannabinoids (there was a block of acetylcholine release from parasympathetic nerves, but not cholinergic neurogenic contraction), in the guinea-pig trachea. This underlines the importance of measuring neurotransmitter release in order to characterise prejunctional actions.

There is a lack of consensus between different researchers about the effects of cannabinoids on sympathetic neurotransmission in the heart, which may be due to differences between species studied and methodology. Ishac et al. (1996) showed that Δ^9-tetrahydrocannabinol and anandamide concentration-dependently inhibited electrically evoked [^{3}H]noradrenaline release from rat atria in an SR141716A-sensitive manner, indicating an action at prejunctional cannabinoid CB_1 receptors. Moreover, in pithed rats and rabbits, WIN 55,212-2 and CP 55,940 inhibited the increase in heart rate mediated by electrical stimulation of postganglionic sympathetic cardioaccelerator fibres in an SR141716A-sensitive manner, but had no effect on cardioacceleration evoked by isoprenaline, indicating a prejunctional action via cannabinoid CB_1 receptors (Malinowska et al., 2001; Szabo et al., 2001). The likely mechanism is prejunctional inhibition of noradrenaline release from terminals of the postganglionic sympathetic nerves. However, in mouse atria, electrically evoked [^{3}H]noradrenaline release was not blocked by micromolar concentrations of WIN 55,212-2 (Trendelenburg et al., 2000), and in both rat and mouse atria, sympathetic nerve-induced tachycardia was not blocked by micromolar concentrations of endogenous and synthetic cannabinoids (Lay et al., 2000).

In human atria, Molderings et al. (1999) showed that CP 55,940 and anandamide produced a concentration-dependent inhibition of [^{3}H]noradrenaline overflow that was inhibited by both SR141716A and LY320135 (both at 1 μM). However, SR141716A and LY320135 also abolished the inhibitory actions of imidazolines on evoked [^{3}H]noradrenaline release, and rauwolscine, an α_2-adrenoceptor antagonst, abolished the inhibitory effects of CP 55,940 and anandamide on evoked [^{3}H]noradrenaline release. This effect was not restricted to human atria as SR141716A (1 μM) was also a potent antagonist at prejunctional imidazoline receptors in the rat vena cava and aorta, and rabbit pulmonary artery (Molderings and Göthert, 1998, 1999). Moreover, rauwolscine, an α_2-adrenoceptor antagonist (used at 30 μM, a concentration that blocks imidazoline receptors), also blocked the responses to CP 55,940 and anandamide (Molderings et al., 1999). These findings may indicate interactions between cannabinoid and imidazoline receptors, or common binding sites of the receptors for these antagonists.

In mouse spleen, WIN 55,212-2 (up to 1 μM) did not change the evoked overflow of [^{3}H]noradrenaline, although there was evidence for release-enhancing α_2-adrenoceptors and bradykinin B_2 receptors in this tissue (Trendelenburg et al., 2000). Cannabinoid receptors have been evidenced in rat spleen by [^{3}H]CP 55,940 binding (Lynn and Herkenham, 1994), and cannabinoid CB_2 receptor mRNA has been detected in rat spleen (Munro et al., 1993), but in association with immune cells. WIN 55,212-2 and CP 55,940 also failed to affect the electrically evoked overflow of [^{3}H]noradrenaline and vasoconstriction in the rat tail artery (Malinowska et al., 1997).

Cannabinoid CB_1 receptors thus join α_2-adrenoceptors, adenosine A_1, neuropeptide Y and opioid receptors, as inhibitory receptors expressed on the prejunctional terminals of sympathetic nerves. Noradrenaline and ATP are cotransmitters in sympathetic nerves, released in varying proportions depending on the species and blood vessel (Burnstock, 1990; Burnstock and Ralevic, 1996), and there is some debate about whether release of the two neurotransmitters can be differentially modulated (Ellis and Burnstock, 1989; von Kügelgen and Starke, 1991). In this regard, whether cannabinoids have differential actions on the release of noradrenaline and ATP from sympathetic nerves has not been investigated yet.

3.1. Involvement of nitric oxide in cannabinoid modulation of sympathetic neurotransmission

Indirect actions of cannabinoids may be involved in their modulatory effects on sympathetic neurotransmission. Anandamide inhibited KCl-stimulated [^{3}H]noradrenaline release in renal artery segments of the rat, and this was essentially abolished by SR141716A (1 μM) and by N^G-nitro-L-arginine methyl ester, an inhibitor of nitric oxide synthase, which suggests that activation of cannabinoid CB_1 receptors in this tissue might lead to the release of nitric oxide (Deutsch et al., 1997). Moreover, anandamide caused a nitric oxide-dependent and SR141716A-sensitive vasorelaxation of isolated juxtamedullary afferent arterioles, and stimulated nitric oxide release, sensitive to SR141716A, in rat renal arterial segments, and nitric oxide release from cultured renal microvascular endothelial cells (Deutsch et al., 1997). Finally, reverse-transcriptase polymerase chain reaction showed that renal endothelial cells contain mRNA for cannabinoid CB_1, but not CB_2 receptors (Deutsch et al., 1997). Anandamide (1 μM) inhibited the potassium-evoked release of [^{3}H]dopamine, but not [^{3}H]5-HT, in invertebrate ganglia, and this effect was mimicked by *S*-nitroso-*N*-acetyl-DL-penicillamine, a nitric oxide donor, and blocked by SR141716A (1 μM) and N^G-nitro-L-arginine methyl ester, indicating a role of nitric oxide (Stefano et al., 1997). Although anandamide, at the concentration used in these studies, activates vanilloid VR1 receptors, and SR141716A can act as a vanilloid VR1 receptor antagonist (at least at $\geq$ 3 μM) (Zygmunt et al., 1999; DePetrocellis et al., 2001), it is unlikely that the nitric oxide response involves vanilloid VR1 receptors as these receptors are

located on sensory nerves and not on endothelial cells. The anandamide-induced nitric oxide-mediated response described by Deutsch et al. (1997) appears to be distinct from a novel endothelial site for anandamide and abnormal cannabidiol in the rat mesenteric arterial bed, which is blocked by SR141617A and unaffected by capsazepine, but is unaffected by blockade of nitric oxide synthase (Járai et al., 1999).

3.2. Endocannabinoid modulation of sympathetic neurotransmission?

In the rat vas deferens, both SR141716A and LY320135 significantly increased the basal contractile response of the tissues to field stimulation, by about 100% at 0.1 μM SR141716A (Christopoulos et al., 2001). This may be due to inverse agonist effects in the presence of constitutively active cannabinoid CB_1 receptors, as shown in cell lines and brain (Landsman et al., 1997; MacLennan et al., 1998; Pan et al., 1998), or inhibition of endogenous agonist-mediated receptor activity. As the amidase inhibitor phenylmethylsulphonyl fluoride, which inhibits the degradation of anandamide, attenuated the basal electrically evoked contractile response, there may be a tonic activation of cannabinoid receptors due to ongoing basal release of an endocannabinoid such as anandamide in the rat vas deferens (Christopoulos et al., 2001). However, this has not been confirmed in release studies carried out by other investigators. Both Ishac et al. (1996) and Trendelenburg et al. (2000) reported no effect of SR141716A on basal and stimulated outflow of [^{3}H]noradrenaline from rat vas deferens. SR141716A also had no effect on [^{3}H]noradrenaline outflow from human and rat atria (Ishac et al., 1996; Molderings et al., 1999). These investigators were able to demonstrate agonist-evoked inhibition of [^{3}H]noradrenaline release, and the concentrations of SR141716A used were similar to those used by Christopoulos et al. (2001), so methodological differences are unlikely to explain these different findings. Unfortunately, no information was provided by Christopoulos et al. (2001) about whether or not augmentation of the sympathetic neurogenic contractile response involved postjunctional actions of phenylmethylsulphonyl fluoride and the cannabinoid CB_1 receptor antagonists.

4. Parasympathetic nerves

Cannabinoid CB_1 receptor, but not CB_2 receptor, mRNA is expressed in embryonic parasympathetic ganglia (Buckley et al., 1998).

In guinea-pig trachea, CP 55,940 inhibited electrically evoked acetylcholine release from parasympathetic nerves in an SR141716A-insensitive manner, but paradoxically had no effect on cholinergic contractile responses evoked by electrical field stimulation (Spicuzza et al., 2000). This underlines the fact that caution must be used in drawing conclusions about the prejunctional actions of cannabinoids from assay of the end organ response. CP 55,940 also did not relax precontracted tracheal strips or affect contractile responses to exogenous acetylcholine, consistent with it acting at a prejunctional site (Spicuzza et al., 2000). An involvement of cannabinoid CB_2 receptors was suggested. Similarly, in guinea-pig trachea, inhibitory effects of anandamide and WIN 55,212 on electrically evoked contractions were not attenuated by SR141716A, indicating a possible involvement of cannabinoid CB_2 receptors (Yousif and Oriowo, 1999). This effect was also likely mediated via a prejunctional site of action as anandamide did not relax precontracted tracheal segments.

In pithed rabbits, intravenous administration of WIN 55,212-2 and CP 55,940 inhibited the decrease in heart rate produced by electrical stimulation of preganglionic vagal fibres in an SR141716A-sensitive manner, indicating an action at cannabinoid CB_1 receptors (Szabo et al., 2001). The mechanism of the vagal inhibition was not investigated further, and inhibitory actions of cannabinoids on both pre- and postganglionic vagal neurones were possible. In pithed rats, WIN 55,212-2 or CP 55,940 had no effect on bradycardia caused by electrical stimulation of the vagus nerve, although this was decreased by the opioid receptor agonist nociceptin (Malinowska et al., 2001), a difference which may be due to species differences or differences in experimental conditions.

In mammalian bladders, neurogenic contraction is mediated by acetylcholine and ATP activation of postjunctional muscarinic and purine P2X receptors. In mouse urinary bladder, Δ^9-tetrahydrocannabinol, anandamide, CP 55,244 and WIN 55,212-2 concentration-dependently inhibited electrically evoked contractions, but not those to exogenous muscarinic agonists or α,β-methylene ATP CaP2X receptor agonist, indicating a prejunctional site of action (Pertwee and Fernando, 1996; Martin et al., 2000). Responses to cannabinoids were blocked by nanomolar concentrations of SR141716A, indicating an action at cannabinoid CB_1 receptors (Pertwee and Fernando, 1996; Martin et al., 2000). In the rat bladder, responses to both cannabinoid CB_1 and CB_2 receptor agonists were also blocked by the cannabinoid CB_2 receptor-selective antagonist SR144528, suggesting pharmacological differences between the rat and mouse orthologues of the cannabinoid CB_1 receptor, or a mixed population of cannabinoid CB_1 and CB_2 receptors in the rat bladder (Martin et al., 2000). In the rat and mouse bladder, no parasympathetic ganglia are located within the urinary bladders, indicating an action of the cannabinoids at prejunctional cannabinoid receptors at the neuroeffector junction (Martin et al., 2000). Interestingly, WIN 55,212-2 had no effect on electrically evoked contractions in bladders from dog, pig, cynomolgus monkey and human, suggesting that the inhibitory effect of cannabinoids in the mouse and rat bladder is not common to all mammalian species (Martin et al., 2000).

4.1. Endocannabinoid modulation of parasympathetic neurotransmission?

SR141716A caused a significant increase in the amplitude of electrically evoked contractions of the mouse urinary bladder, suggesting release of an endogenous cannabinoid receptor agonist, or an inverse agonist effect at precoupled cannabinoid receptors (Pertwee and Fernando, 1996).

5. Enteric neurones/gastrointestinal tract

The role of cannabinoids in the gastrointestinal tract has recently been comprehensively reviewed (Pertwee, 2001a) and an overview only is presented here.

In guinea-pig myenteric plexus-longitudinal muscle, direct evidence of a prejunctional action of cannabinoids via cannabinoid CB_1 receptors was provided with the demonstration that WIN 55,212 and CP 55,940 inhibited electrically evoked acetylcholine release, and this effect was blocked by SR141716A (Pertwee et al., 1996a; Coutts and Pertwee, 1997). Cannabinoids also inhibited the twitch response, in an SR141716A-sensitive manner, but not contractions to exogenously applied acetylcholine, consistent with a prejunctional action at cannabinoid CB_1 receptors (Coutts and Pertwee, 1997). The enantiomer (−)-WIN 55,212 was approximately 300 times less active than the (+) enantiomer, indicating that the responses were selective and receptor-mediated (Coutts and Pertwee, 1997). Pronounced stereoselectivity has also been reported for enantiomers of Δ^9-tetrahydrocannabinol, HU210 and CP 55,940 (Roth, 1978; Pertwee et al., 1992, 1996a). There is also functional evidence for the expression of prejunctional cannabinoid CB_1 receptors in human myenteric plexus-longitudinal muscle (Croci et al., 1998).

Anandamide and WIN 55,212-2 reduced the cholinergic twitch response in rat gastric fundus, and were without effect on the activity of the smooth muscle at baseline or in preconstricted preparations, indicating actions at a prejunctional site (Storr et al., 2002). Interestingly, the effects of anandamide, but not WIN 55,212-2, were reversed by the cannabinoid receptor antagonist AM630, indicating a possible involvement of more than one receptor type (Storr et al., 2002). Anandamide and WIN 55,212 also reduced neurogenic non-adrenergic non-cholinergic relaxant responses caused by electrical stimulation of the rat gastric fundus, an effect that could be reversed by AM630 (cannabinoid CB_2 receptor-selective antagonist/inverse agonist) for anandamide, but not for WIN 55,212, indicating a possible involvement of more than one receptor type (Storr et al., 2002). Neither compound had an effect on the baseline or preconstricted smooth muscle activity, consistent with actions at a prejunctional site.

The inhibitory effect of WIN 55,212 and anandamide on non-adrenergic non-cholinergic contraction of the circular muscle of the guinea-pig ileum was antagonised by SR141716A (Izzo et al., 1998). Neither compound modified the smooth muscle contractile response to exogenous substance P.

In line with the functional studies, there is abundant evidence for the expression of cannabinoid receptors throughout the gastrointestinal tract. Specific binding sites for cannabinoids, with similar affinity to recombinant cannabinoid CB_1 receptors and endogenous cannabinoid CB_1 receptors in the central nervous system, have been described in the myenteric plexus (Pertwee, 1997, 1999). Binding studies showed that [^{3}H]CP 55,940 underwent specific binding to homogenates of guinea-pig myenteric plexus-longitudinal muscle with a similar dissociation constant to its binding in guinea-pig brain, and that was similarly displaced in both preparations by low nanomolar concentrations of SR141716A (Ross et al., 1998). Cannabinoid CB_1 receptor, but not CB_2-like receptor, mRNA has been localised in the guinea-pig myenteric plexus and in the myenteric and submucosal plexus of rat embryo digestive tract (Griffin et al., 1997; Buckley et al., 1998). Both cannabinoid CB_1 and CB_2-like receptor mRNA was detected in guinea-pig whole gut (Griffin et al., 1997). Cannabinoid CB_1 and CB_2 receptor mRNA was detected in the stomach (Storr et al., 2002). Cannabinoid CB_1 receptor mRNA has been detected in human colon (Shire et al., 1995). Cannabinoid CB_1 receptor immunoreactivity was localised in the myenteric and submucosal ganglionated plexuses of porcine ileum and colon (Kulkarni-Narla and Brown, 2000). In both the ileum and distal colon, cannabinoid CB_1 receptor-immunoreactive neurones expressed immunoreactivity to choline acetyltransferase, a cholinergic marker, but not immunoreactivity to vasoactive intestinal polypeptide or nitric oxide synthase (Kulkarni-Narla and Brown, 2000).

5.1. Endocannabinoid modulation of enteric/gastrointestinal neurotransmission?

SR141716A alone produced an increase in the release of acetylcholine and enhanced contractile responses elicited by electrical stimulation of guinea-pig myenteric plexus-longitudinal muscle and circular muscle, but not contractions produced by acetylcholine (Coutts and Pertwee, 1997; Izzo et al., 1998). Thus, an endocannabinoid may inhibit cholinergic neurotransmission in the intestine. SR141716A alone also enhanced non-adrenergic non-cholinergic contraction of the circular muscle of the guinea-pig ileum, indicating a possible release of an endocannabinoid that modulates neurotransmission (Izzo et al., 1998). However, an inverse agonist effect of SR141716A at precoupled cannabinoid CB_1 receptors could also account for these effects. AM630 alone caused an increase in the neurogenic non-adrenergic non-cholinergic relaxant response of the rat gastric fundus (Storr et al., 2002), indicating a possible role of endocannabinoids, although this cannabinoid CB_2 receptor-selective antagonist is also an inverse agonist (Howlett et al., 2002).

6. Sensory nerves

The effects of cannabinoids on the peripheral efferent (motor) function of sensory nerves are discussed in this section. The roles of cannabinoid and vanilloid receptors in nociception have been recently reviewed and will not be considered further here (Walker et al., 1999, 2000; Fuentes et al., 1999; Elphick and Egertová, 2001; Pertwee, 2001b; Di Marzo et al., 2002; Hohmann, 2002). It is noteworthy that changes in the function of the sensory nervous system can influence the function of autonomic nerves, interactions between these two systems having been described. For example, there is evidence that both acute and chronic (capsaicin treatment of neonates) inhibition of sensory neurotransmission leads to augmentation of sympathetic neurotransmission (Ralevic et al., 1995; Ziganshin et al., 1995; Wardle et al., 1996).

6.1. Cannabinoid receptors

An in situ hybridization study of cannabinoid CB_1 receptor mRNA distribution revealed subpopulations of rat dorsal root ganglion cells, predominantly medium and large-sized cells, capable of synthesising cannabinoid receptors and capable of inserting them on peripheral terminals (Hohmann and Herkenham, 1999a,b). Cannabinoid CB_2 receptors do not appear to be expressed on sensory nerves. Hohmann and Herkenham (1999a) reported that dorsal root ganglion cells do not synthesise cannabinoid CB_2 mRNA and Ross et al. (2001a) showed that the cannabinoid CB_1 receptor antagonist SR141716A, but not the cannabinoid CB_2 receptor antagonist SR144528, blocked the inhibition of voltage-activated Ca^{2+} currents by WIN 55,212 in cultured dorsal root ganglion neurones.

Inhibitory effects of cannabinoids on sensory neurotransmission have been reported in the rat isolated mesenteric arterial bed. In this tissue, electrical field stimulation of sensory nerves evokes a release of calcitonin gene-related peptide (CGRP) and vasorelaxation (Kawasaki et al., 1988). WIN 55,212 and CP 55,940 inhibited mesenteric arterial sensory neurogenic vasorelaxation (Duncan et al., 2001, 2002). These synthetic cannabinoids are inactive at vanilloid VR1 receptors (Zygmunt et al., 1999; Smart et al., 2000). Their actions were blocked by LY320135 and SR141716A, selective cannabinoid CB_1 receptor antagonists, but not by SR144528, a selective cannabinoid CB_2 receptor antagonist (Duncan et al., 2001, 2002). Moreover, the stereoisomer (−)-WIN 55,212 was inactive, indicating that the action was selective and receptor-mediated. WIN 55,212 and CP 55,940 had no significant effect on vasorelaxation mediated by exogenous CGRP, indicating that they were acting at prejunctional cannabinoid CB_1 receptors. This is in line with immunohistochemical and molecular evidence for the expression of cannabinoid CB_1 receptors on sensory nerve cells (Hohmann and Herkenham, 1999a,b; Ahluwalia et al., 2000; Ross et al., 2001a).

A non-CB_1 non-CB_2 cannabinoid receptor may be expressed on sensory nerves in rat mesenteric arteries, along with the cannabinoid CB_1-like receptor described above. HU210 (Fig. 4), Δ^9-tetrahydrocannabinol and the endocannabinoid noladin ether (Hanuš et al., 2001) attenuated mesenteric arterial sensory neurogenic relaxation evoked by electrical field stimulation, but this inhibitory action was unaffected by cannabinoid CB_1 and CB_2 receptor antagonists, and for noladin ether, the response was blocked by pertussis toxin pretreatment of rats, indicating a possible involvement of a novel $G_{i/o}$-protein-coupled cannabinoid receptor (Ralevic and Kendall, 2001; Duncan et al., 2003 unpublished observations). None of the cannabinoids affected vasorelaxation to exogenous CGRP, indicating a prejunctional site of action (Ralevic and Kendall, 2001; Duncan et al., 2003). Moreover, they are inactive or only weak agonists at vanilloid VR1 receptors. Although it is not an agonist at vanilloid VR1 receptors, the inhibitory effect of HU210 was essentially abolished by ruthenium red (1 μM) Unpublished observations. Interestingly, Zygmunt et al. (2002) have described a cannabinoid CB_1 and CB_2 receptor-independent, vanilloid VR1 receptor-independent, activation of CGRP release by Δ^9-tetrahydrocannabinol, which was blocked by ruthenium red, in rat and mouse mesenteric arteries. These authors also suggested the possi-

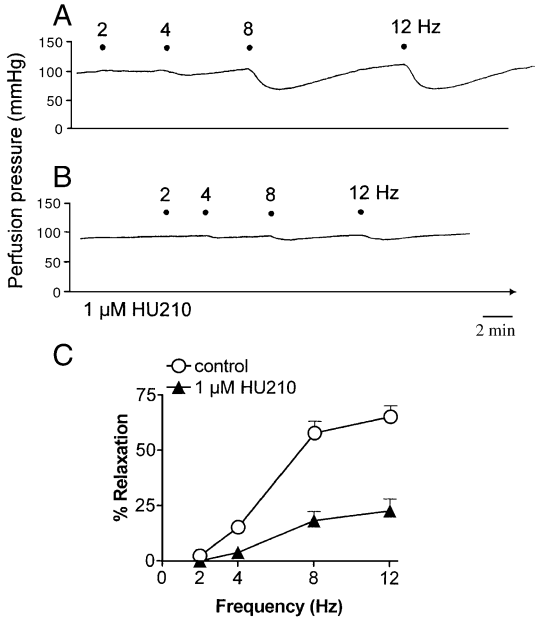

Fig. 4. (A, B) Representative trace showing the effect of HU210 on frequency-dependent sensory neurogenic vasorelaxation of the rat isolated mesenteric arterial bed. Electrical field stimulation (2–12 Hz, 0.1 ms, 60 V, 30 s) evoked frequency-dependent vasorelaxation and is shown in: (A) control conditions; (B) the presence of HU210 (1 μM). The perfusate contained guanethidine (5 μM) to block sympathetic neurotransmission and methoxamine (5 μM) to preconstrict the preparation. Relaxation to electrical field stimulation was attenuated by HU210. (C) Inhibition of sensory neurogenic relaxation by 1 μM HU210 ($n = 6–7$) (from Ralevic and Kendall, 2001, with permission).

ble involvement of a novel cannabinoid receptor, possibly coupled to a transient receptor potential ion channel to evoke CGRP release.

In rat hindpaw skin, activation of vanilloid receptors on sensory nerves induces plasma extravasation and vasodilatation. Anandamide inhibited capsaicin-evoked plasma extravasation in rat hindpaw skin, and this action was blocked by SR141716A, indicating an involvement of cannabinoid CB_1 receptors (Richardson et al., 1998). Anandamide (1 nM) also inhibited capsaicin-evoked CGRP release in this model (Richardson et al., 1998), presumably via the same cannabinoid CB_1 receptor, but had no effect on basal release of CGRP (i.e. at vanilloid VR1 receptors) (at up to at least 1 nM). In all biological systems studied to date, anandamide has been shown to be less potent, or at best equipotent with capsaicin at vanilloid VR1 receptors, and the EC_{50} for capsaicin in this model was 30 μM. Thus, anandamide is considerably more potent at cannabinoid CB_1 receptors than at vanilloid VR1 receptors in rat hindpaw skin.

6.2. Vanilloid receptors

The vanilloid VR1 receptor is a ligand-gated ion channel which acts as a polymodal nociceptor as it is activated by a variety of noxious stimuli including capsaicin, heat and protons (Caterina et al., 1997, 2000; Szallasi and Blumberg, 1999; Davis et al., 2000; Caterina and Julius, 2001). The VR1 receptor has a relatively restricted distribution, as it is found principally on a population of capsaicin-sensitive primary afferent nerve fibres, namely thin, unmyelinated C fibres and large diameter myelinated Aδ fibres (Holzer, 1991; Szallasi and Blumberg, 1999). However, the wide distribution of these nerves, and therefore of vanilloid VR1 receptors, suggests that vanilloid VR1 receptors also have other functions (Szallasi and Blumberg, 1999; Mezey et al., 2000), including the modulation of cardiovascular function (Maggi and Meli, 1988; Holzer, 1992; Zygmunt et al., 1999; Ralevic et al., 2000, 2002; Malinowska et al., 2001).

The link between the vanilloid and cannabinoid systems was first identified when two groups reported that the vanilloid agonist olvanil was a potent inhibitor of the anandamide transporter (Di Marzo et al., 1998; Beltramo and Piomelli, 1999). Anandamide, the archetypal cannabinoid, shares structural similarities with olvanil and capsaicin. This prompted Zygmunt et al. (1999) to investigate the role of vanilloid receptors in the vascular actions of anandamide. In a seminal study, they reported that vasorelaxation to anandamide was abolished by desensitization of the sensory nerves with capsaicin in guinea-pig basilar, rat hepatic and rat mesenteric arteries. In addition, the anandamide-induced vasorelaxation was blocked by a vanilloid receptor antagonist, capsazepine, and by a CGRP receptor antagonist, CGRP-[8–37]. They, and subsequently others, also demonstrated that anandamide was a full agonist at rat (Zygmunt et al., 1999; Ralevic et al., 2001) and human (Smart et al., 2000, 2001) recombinant vanilloid VR1 receptors with similar kinetic and electrophysiological properties to capsaicin (Fig. 5). Thus, anandamide can act at vanilloid VR1 receptors to evoke the release of neurotransmitters from sensory nerves leading to vasorelaxation. Similar observations have been made with a metabolically stable analogue of anandamide, methanandamide, which causes capsaicin- and capsazepine-sensitive vasorelaxation in the rat mesenteric arterial bed and isolated mesenteric arteries (Ralevic et al., 2000). However, in the same vascular bed, Harris et al. (2002) reported that vasorelaxation to anandamide was only partly sensitive to capsaicin pretreatment.

Anandamide has been shown to activate depolarising currents (Smart et al., 2000; Morisset et al., 2001), promote Ca^{2+} influx (Tognetto et al., 2001) and stimulate the release of substance P (Tognetto et al., 2001) in capsaicin-sensitive

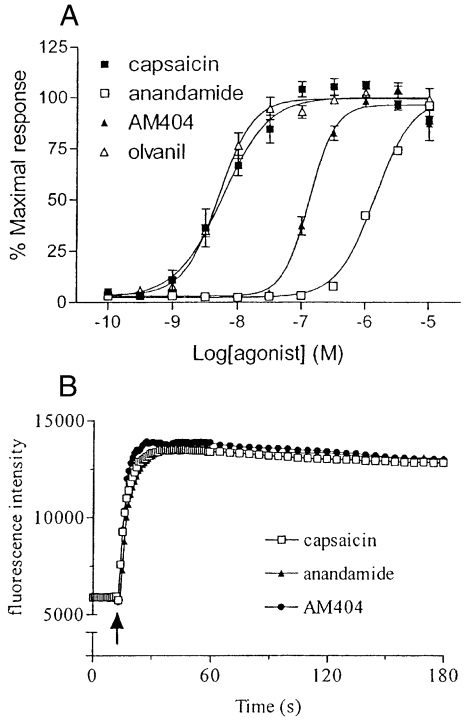

Fig. 5. (A) The AM404- and anandamide-induced Ca^{2+} responses are concentration-dependent in rat vanilloid VR1 receptor-expressing HEK293 cells. $[Ca^{2+}]_i$ was monitored using Fluo-3AM in rat vanilloid VR1 receptor-expressing HEK293 cells before and after the addition of capsaicin, anandamide, AM404 or olvanil (all at 100 pM to 10 μM). Responses were measured as peak increase in fluorescence minus basal, expressed relative to the maximum capsaicin response and are given as means ± S.E.M., where $n = 8$. (B) AM404- and anandamide-induced Ca^{2+} responses have the same kinetics in rat vanilloid VR1 receptor-expressing HEK293 cells. Intracellular Ca^{2+} concentrations (as fluorescence intensity) were measured in rat vanilloid VR1 receptor-expressing HEK293 cells before and after the addition (at arrow) of capsaicin (100 nM), AM404 (300 nM) or anandamide (1 μM). Data shown are representative traces, typical of at least $n = 20$ (from Ralevic et al., 2001, with permission).

neurones in dorsal root ganglia. Indeed, both the kinetics (Smart et al., 2000; Olah et al., 2001) and the electrophysiological properties (Smart et al., 2000; Morisset et al., 2001) of the anandamide-induced responses in dorsal root ganglion cells are very similar to those evoked by capsaicin. Moreover, the anandamide-induced responses are blocked by capsazepine (Smart et al., 2000; Olah et al., 2001; Tognetto et al., 2001) and ruthenium red (Morisset et al., 2001), but not by cannabinoid receptor antagonists (Morisset et al., 2001; Tognetto et al., 2001). Furthermore, the capsaicin- and anandamide-induced responses cross-desensitise (Tognetto et al., 2001), and other cannabinoid ligands, e.g. WIN 55,212-2, do not activate the same vanilloid VR1 receptor-mediated currents as anandamide in dorsal root ganglion neurones (Morisset et al., 2001). Taken collectively, these data are very similar to those found in recombinant systems (Smart et al., 2000; Smart and Jerman, 2000).

There is a now a considerable body of evidence showing that anandamide activates endogenous vanilloid VR1 receptors in a variety of native tissues, most notably dorsal root ganglion cells (Smart et al., 2000) and sensory nerves in vascular beds (Zygmunt et al., 1999; Ralevic et al., 2000), as well as sensory neurones in the lung (Craib et al., 2001; Tucker et al., 2001), ileum (Mang et al., 2001), vas deferens (Ross et al., 2001b), knees (Gauldie et al., 2001), and trigeminals (Szoke et al., 2000). Anandamide has also been shown to activate vanilloid VR1 receptors in the hippocampus (Al-Hayani et al., 2001) and various immune cells (Maccarrone et al., 2000; Jacobsson et al., 2001). More recently, *N*-arachidonoyl-dopamine, a compound originally synthesised as an agonist selective for cannabinoid CB_1 over CB_2 receptors (K_i values 0.25 and 12 μM, respectively) (Bisogno et al., 2000), was found to be a naturally occurring capsaicin-like substance (''endovanilloid'') with potent activity at recombinant rat and human (EC_{50} ~ 50 nM) and native rat (EC_{50} ~ 800 nM) vanilloid VR1 receptors (Huang et al., 2002). Both anandamide and *N*-arachidonoyl-dopamine are structurally similar to capsaicin. 2-Arachidonoyl glycerol, a macrophage-derived endocannabinoid (Di Marzo et al., 1999), has only very weak effects at rat recombinant vanilloid VR1 receptors (Zygmunt et al., 1999) and the endocannabinoid noladin ether is a weak agonist at human recombinant vanilloid VR1 receptors (Smart, unpublished data). The synthetic cannabinoid agonists WIN 55,212-2, HU210 and CP 55,950 are also without effect at rat and human vanilloid VR1 receptors (Zygmunt et al., 1999; Smart et al., 2000).

There is considerable interest in the biological significance of the interaction of anandamide and *N*-arachidonoyl-dopamine with both vanilloid VR1 receptors and cannabinoid CB_1 receptors, and one area that has received some attention in this respect is a consideration of their relative potencies at the two receptors. In model cell systems transfected with receptors, anandamide is generally more potent at causing activation of cannabinoid CB_1 receptors (pEC_{50} 5.7–7.1 for inhibition of cAMP formation) (Pertwee, 1997; Bonhaus et al., 1998) than activation of vanilloid VR1 receptors (pEC_{50} 5.6–5.9 for Ca^{2+} entry) (Zygmunt et al., 1999; Smart et al., 2000; Ralevic et al., 2001). Similarly, anandamide is more potent at endogenous cannabinoid CB_1 receptors than at vanilloid VR1 receptors in rat hindpaw skin (Richardson et al., 1998) and dorsal root ganglion neurones (Smart and Jerman, 2000; Morisset et al., 2001; Tognetto et al., 2001). This indicates that low doses of anandamide may inhibit neurotransmission via cannabinoid CB_1 receptors while higher doses are excitatory in a vanilloid VR1 receptor-dependent manner, the latter possibly being involved in sensory nerve excitation in pathophysiological conditions. In contrast, *N*-arachidonoyl-dopamine is similarly potent at endogenous cannabinoid CB_1 receptors (pEC_{50} ~ 6.2–6.6) (Bisogno et al., 2000) and endogenous vanilloid VR1 receptors (pEC_{50} ~ 6.1) (Huang et al., 2002). Clearly, *N*-arachidonoyl-dopamine, and certain concentrations of anandamide, will activate simultaneously vanilloid VR1 and cannabinoid CB_1 receptors, which may lead to complex actions in cells in which these receptors are coexpressed (see also Section 10).

Anandamide may have complex effects in tissues that are innervated by both sympathetic and sensory nerves, potentially having both excitatory and inhibitory effects on sensory nerves, as well as causing inhibition of sympathetic neurotransmission. Capsazepine, a vanilloid receptor antagonist, blocked a part of the inhibitory action of anandamide on the sympathetic neurogenic twitch response in the mouse vas deferens, indicating an involvement of vanilloid VR1 receptors (Ross et al., 2001b). WIN 55,212 also caused an inhibition of the twitch response, but this was unaffected by capsazepine, indicating an involvement only of cannabinoid CB_1 receptors (Ross et al., 2001b), consistent with evidence that WIN 55,212 is not an agonist at vanilloid VR1 receptors (Zygmunt et al., 1999). Ross et al. (2001b) noted that similar concentrations of anandamide were effective at eliciting both cannabinoid CB_1 (SR141716A-sensitive) and vanilloid VR1 (capsazepine-sensitive) receptor-mediated inhibitory responses in the mouse vas deferens, despite the reported lower affinity of anandamide for recombinant vanilloid VR1 over cannabinoid CB_1 receptors (Zygmunt et al., 1999; Smart et al., 2000; Ross et al., 2001b), and suggested that this might be due to synergistic interactions between vanilloid VR1 and cannabinoid CB_1 receptors. As vanilloid VR1 receptors are not expressed on sympathetic nerves, it seems likely that the VR1-mediated inhibitory effect of anandamide on sympathetic neurotransmission involves indirect actions at a postjunctional site on the smooth muscle, possibly via CGRP released from the sensory nerves. Indeed, exogenous CGRP and capsaicin both cause inhibition of the twitch response/contraction of the vas deferens likely via a postjunctional site (Saito et al., 1987; Maggi et al., 1987,

1991; Filippelli et al., 1999). Thus, if synergistic interactions are involved these are likely to be complex, involving actions of neurotransmitters released from sensory and/or sympathetic nerves. There may be an additional level of complexity involved due to desensitization of vanilloid VR1 receptors, as when this occurs sympathetic neurotransmission may be augmented (Ralevic et al., 1995; Ziganshin et al., 1995; Wardle et al., 1996).

6.3. Endocannabinoid modulation of sensory neurotransmission?

A recent study has shown that stimulation of dorsal root ganglion cells in culture with either capsaicin (via vanilloid VR1 receptors) or KCl induces the production and release of anandamide (>500 nM), indicating that andandamide may be a locally produced endogenous regulator of the excitability of capsaicin-sensitive sensory nerves (Ahluwalia et al., 2003). Depending on the anandamide concentration released, which may be different in physiological and pathophysiological conditions, and on the post-translational modification state of vanilloid VR1 receptors (see Section 9), it can be envisaged that the anandamide released would either cause further anandamide release via vanilloid VR1 receptors and amplification of the response, or reduce the excitability of the cells via cannabinoid CB_1 receptors.

SR141716A was reported to augment neurogenic vasorelaxation mediated by electrical field stimulation of capsaicin-sensitive sensory nerves in the rat isolated mesenteric arterial bed, indicating that there may be a release of endocannabinoids from the sensory nerves, which then act at a prejunctional site in a negative feedback manner to inhibit sensory neurotransmitter release (Ralevic and Kendall, 2001). The facilitatory effect observed in the presence of SR141716A would thus be due to disinhibition. Although SR141716A can act as an inverse agonist, it is unlikely that this mechanism is involved, as there was no observed change in the tone of the preparations and enhancement was observed only for neurogenic relaxant responses generated during electrical field stimulation.

Ishioka and Bukoski (1999) demonstrated that sensory nerve-dependent Ca^{2+}-induced relaxation of rat mesenteric arteries was blocked by SR141716A, and suggested that an endocannabinoid was released from the nerves and mediated the relaxation. Although this suggestion is broadly similar to that of Ralevic and Kendall (2001) it differs in detail in that the SR141716A-sensitive action of the endocannabinoid was suggested to mediate relaxation at a postjunctional site, and not via prejunctional inhibition of neurotransmitter release. Recently, however, the SR141716A sensitivity of the Ca^{2+}-induced vasorelaxation has been shown to be maintained in mesenteric arteries of cannabinoid CB_1/CB_2 receptor knockout mice, indicating the involvement of a novel cannabinoid receptor or a cannabinoid receptor-independent effect of the antagonist (Bukoski et al., 2002).

7. Whole animals

Cannabinoids elicit complex cardiovascular effects in whole animals, including both an increase and decrease in blood pressure and heart rate, which may be the result of actions at multiple sites (pre- and postjunctional), different species, and state of consciousness.

Niederhoffer and Szabo (1999, 2000) carried out a comprehensive series of experiments in pithed and conscious rabbits and identified a number of sites at which WIN 55,212-2 can modulate cardiovascular regulation. In pithed rabbits with electrically stimulated sympathetic outflow, WIN 55,212-2 (5, 50 and 500 μg kg^{-1}) dose-dependently reduced blood pressure, the plasma noradrenaline concentration and the spillover of noradrenaline into plasma, with no change in heart rate, and this action was blocked by the cannabinoid CB_1 receptor antagonist SR141716A (Niederhoffer and Szabo, 1999, 2000) (Fig. 6). The sympathoinhibitory actions of WIN 55,212-2 were mimicked by CP 55,940, but not by the inactive enantiomer WIN 55,212-3. Furthermore, WIN 55,212-2 had no effect on vascular tone established by infusion of noradrenaline in pithed rats. Collectively, these results indicate an involvement of inhibitory cannabinoid CB_1 receptors located on postganglionic sympathetic neurones. In conscious rabbits, different effects of intravenous administration of WIN 55,212-2 were observed, and these also differed depending on the dose. At 5 and 50 μg kg^{-1}, WIN 55,212 caused bradycardia, hypotension, no change in plasma noradrenaline levels and an increase in renal sympathetic nerve firing (Niederhoffer and Szabo, 1999). In contrast, the highest dose of WIN 55,212 (500 μg kg^{-1}) elicited tachycardia, hypotension, and a decrease in sympathetic nerve activity and plasma noradrenaline concentration. Both bradycardia and tachycardia were blocked by SR141716A. Injection of WIN 55,212 into the cisterna cerebellomedullaris (vicinity of medullary and pontine cardiovascular regulatory centres) in conscious rabbits caused sympathoexcitation (hypertension and an elevated plasma noradrenaline concentration) and atropine-sensitive bradycardia, indicating direct excitation of centres regulating sympathetic tone by cannabinoids (Niederhoffer and Szabo, 2000). The authors concluded that at low doses there is central sympathoexcitation and activation of cardiac vagal fibres (both at the level of the brain stem), and at high doses central sympathoinhibition, by systemically administered WIN 55,212-2 in conscious rabbits (Niederhoffer and Szabo, 1999). In the overall cardiovascular response to WIN 55,212, these central effects likely act together with the peripheral prejunctional inhibition of noradrenaline release demonstrated in anaesthetised pithed rabbits.

With regard to cannabinoids and brainstem cardiovascular regulatory centres, the levels of cannabinoid CB_1 receptor mRNA (Matsuda et al., 1993) and [^{3}H]CP 55,940 binding (Herkenham et al., 1991; Mailleux and Vanderhaeghen, 1992) detected in the brainstem are low. However, this does not rule out an involvement and indeed cardiovascular

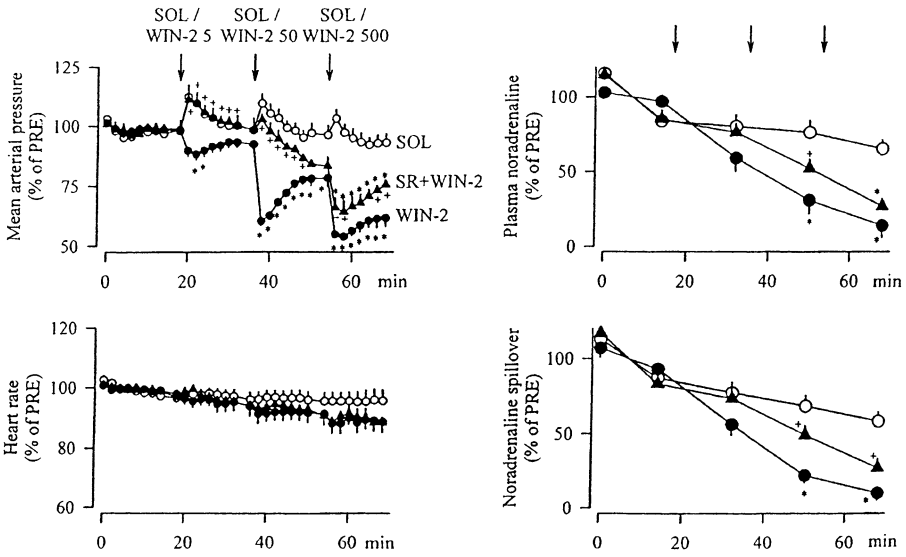

Fig. 6. Effects of i.v. injections of solvent (SOL) and WIN 55,212 (WIN-2) on mean arterial pressure, heart rate, plasma noradrenaline concentration and noradrenaline spillover rate in pithed rabbits with electrically stimulated sympathetic outflow. SOL (0.5 ml kg^{-1}) and WIN-2 (5, 50 and 500 μg kg^{-1}) were injected as indicated by arrows. One of the two WIN-2 groups was pretreated at $t = -10$ min with SR141716A (SR; 500 μg kg^{-1}; i.v.). Values are given as percentages of PRE values (Table 1). Means ± S.E.M. from six (SOL), four (WIN-2) and four (SR + WIN-2) experiments. Differences from SOL: $^{*}P < 0.05$; differences from WIN-2; $^{+}P < 0.05$ (from Niederhoffer and Szabo, 1999, with permission).

actions (hypertension, elevated plasma noradrenaline concentration and bradycardia) have been observed on intracisternal administration of cannabinoids in conscious rabbits (Niederhoffer and Szabo, 2000). CP 55,940 and WIN 55,212 modulated the firing rate (activated and inhibited) of neurones in the nucleus tractus solitarius in rat brain slices (Himmi et al., 1998) and immunoreactivity for cannabinoid CB_1 receptors was detected in many of the fibres (Tsou et al., 1998). Furthermore, microinjection of SR141716A, but not WIN 55, 212−2, into the nucleus tractus solitarius increased baroreceptor duration in dogs (Rademacher et al., 2003).

Detailed in vivo pharmacological studies of the respective roles of vanilloid VR1 and cannabinoid CB_1 receptors in the cardiovascular effects of anandamide have revealed a complex picture. Administration of anandamide to anaesthetised rats in vivo causes bradycardia (with secondary hypotension) and a transient pressor effect, followed by a delayed but maintained reduction in blood pressure (Lake et al., 1997; Malinowska et al., 2001). The initial bradycardia and associated short-lasting hypotension is believed to be vagally mediated, via the Bezold–Jarish reflex, and involves vanilloid receptors on the sensory nerves, as it is mimicked by capsaicin (and methanandamide), and blocked by atropine treatment or cervical vagotomy (Varga et al., 1995, 1996) as well as by the vanilloid VR1 receptor blockers capsazepine and ruthenium red, but not by the cannabinoid receptor antagonist SR141716A (Malinowska et al., 2001). The pressor effect of anandamide observed on intravenous administration appears to be independent of the central nervous system and of cannabinoid CB_1 receptors as, in rats, this was not blocked by α-adrenoceptor blockade, cervical spinal cord transection, or SR141716A (Varga et al., 1996; Lake et al., 1997). Thereafter, both cannabinoids, but not capsaicin, induced a considerably more prolonged decrease in blood pressure (second depressor phase).

The second depressor effect of anandamide is believed to be mediated by cannabinoid CB_1 receptor prejunctional inhibition of sympathetic outflow in the periphery as, in anaesthetised rats, the effect was attenuated by cervical spinal transection, α-adrenoceptor and cannabinoid receptor antagonists, but not by capsazepine (Malinowska et al., 2001; Lake et al., 1997; Varga et al., 1995, 1996). Also, in pithed rats, WIN 55,212-2 and CP 55,940, but not (−)-WIN 55,212-2, inhibited the increase in blood pressure caused by electrical stimulation of preganglionic sympathetic nerve fibres, but not that caused by exogenous noradrenaline, and this effect was blocked by SR141716A, indicating an action at cannabinoid CB_1 receptors located prejunctionally on postganglionic sympathetic nerves (Malinowska et al., 1997). The second depressor response to anandamide does not appear to involve central effects (although its magnitude is dependent on the level of sympathetic outflow), as there was only an increase in the activity of barosensitive presympathetic rostral ventrolateral medulla neurones in intact rats, and activity remained unchanged in barodenervated rats (Varga et al., 1996). The inhibitory response was greater in spontaneously hypertensive rats compared with normotensive controls, perhaps reflecting the higher level of sympathetic tone in the former (Lake et al., 1997). However, not all investigators are agreed that there is an

involvement of the sympathetic nervous system in the hypotensive actions of cannabinoids (Vidrio et al., 1996).

In conscious rats, anandamide causes complex regional haemodynamic changes (Gardiner et al., 2002a). At all doses used, anandamide provoked a transient increase in mean arterial blood pressure associated with vasoconstriction in mesenteric, renal and hindquarters vascular beds. At doses of 2.5 and 3 mg kg^{-1}, anandamide caused a marked bradycardia preceding the hypertension and the hindquarters vasoconstriction was followed by vasodilatation. None of the effects were inhibited by the cannabinoid CB_1 receptor antagonist AM 251, but the bradycardia was atropine-sensitive and the hindquarters vasodilatation was blocked by the β_2-adrenoceptor antagonist ICI 118551. It is possible that anandamide could cause the release of adrenaline from the adrenal medulla by activating vanilloid VR1 receptors on the chromaffin cells (Zhou and Livett, 1991). In conscious rats, the synthetic cannabinoids WIN 55,212 and HU210 had pressor, renal and mesenteric vasoconstrictor and hindquarters vasodilator actions, which were blocked by AM251, indicating an involvement of cannabinoid CB_1 receptors (Gardiner et al., 2002b). The hindquarters vasodilator actions appeared to involve also β_2-adrenoceptors as these were inhibited by ICI 118551 (Gardiner et al., 2002b). In conscious humans, acute administration of cannabinoids causes tachycardia and a small increase in blood pressure (Benowitz et al., 1979; Huestis et al., 1992). In other conscious animals, both tachycardia and hypertension (Jandhyala and Hamed, 1978; Stein et al. 1996; Lake et al., 1997) and cardiovascular depression (Birmingham, 1973; Vidrio et al., 1996; Fredericks et al., 1981; Niederhoffer and Szabo, 1999) have been described.

In mice, both anandamide and synthetic cannabinoid receptor agonists cause biphasic hypotension, which is thought to be entirely cannabinoid CB_1 receptor-mediated as the responses are absent in cannabinoid CB_1 receptor knockout mice (Ledent et al., 1999). It is interesting to note that a comparison of the timings of the blood pressure measurements in the earlier pharmacological and cannabinoid CB_1 receptor knockout mice studies (Járai et al., 1999; Lake et al., 1997; Ledent et al., 1999) to more recent data (Malinowska et al., 2001) shows that the former were taken during Malinowska's phase III and so would have missed the short-term effects associated with the VR1-mediated Bezold–Jarisch reflex. It is also worth noting that more recent pharmacological data (White et al., 2001), including studies using cannabinoid CB_1 receptor knockout mice (Járai et al., 1999), indicate that there is a third cannabinoid receptor involved in the vasorelaxant properties of the endocannabinoids, which is distinct from cannabinoid CB_1 and CB_2 or vanilloid VR1 receptors, and is expressed on the endothelium (Kunos and Batkai, 2001; White et al., 2001).

A recent study by Smith and McQueen (2001) has shown that administration of anandamide and capsaicin into rat hindlimb induces a fall in blood pressure and an increase in ventilation that was blocked by capsaicin pretreatment and the vanilloid antagonist capsazepine, but not by the cannabinoid CB_1 receptor antagonist SR141716A, indicating an action at vanilloid VR1 receptors on sensory nerves. Moreover, the cardiovascular and respiratory responses to capsaicin and anandamide were blocked by sectioning of the femoral and sciatic nerves, indicating the involvement of a reflex action (Smith and McQueen, 2001). Additional local vasodilator actions of anandamide and capsaicin at vanilloid VR1 receptors in the femoral artery, involving the efferent function of sensory nerves, are possible. Indeed, although there was no change in mean arterial blood pressure to anandamide in cannabinoid CB_1 receptor knockout mice (Járai et al., 1999; Ledent et al., 1999), anandamide was still a vasorelaxant in mesenteric beds taken from these mice (Járai et al., 1999), suggesting that vanilloid VR1 receptor-mediated effects of anandamide may be important in regulating regional blood flow without affecting overall blood pressure (White et al., 2001). These local vasodilator effects persist even if the endothelium is removed, but are blocked by capsazepine (Vanheel and Van de Voorde, 2001), indicating that the effect is mediated by vanilloid VR1 receptors on sensory nerve terminals (Kunos and Batkai, 2001), a mechanism first proposed by Zygmunt et al. (1999).

In whole animals, cannabinoids have been shown to cause a depression of gastrointestinal motility, largely through their inhibitory effects on predominantly contractile neurotransmitter release. Cannabinoids have been shown to inhibit the contractile activity of the stomach, and to inhibit intragastric pressure, gastric emptying, gastric acid secretion, gastrointestinal motility and intestinal secretion, which may be a consequence of their actions on the myenteric plexus and vagus nerve (see Izzo et al., 2001; Pertwee, 2001a).

8. Mechanism(s) of cannabinoid inhibition of neurotransmission via cannabinoid receptors

Cannabinoids can modulate neurotransmission via inhibition of neurotransmitter release at prejunctional sites and by postjunctional actions (e.g. functional antagonism caused by endothelium-dependent and -independent smooth muscle relaxation, and inhibition of the release of intracellular Ca^{2+} available for contraction). Mechanisms involved in cannabinoid smooth muscle relaxation have been recently reviewed (Randall et al., 2002) and only prejunctional mechanisms by which cannabinoids modulate neurotransmission are considered here.

Endocannabinoids are rapidly deactivated by being taken up into cells by a carrier-mediated mechanism, and hydrolysed by intracellular membrane-bound anandamide amidohydrolase (Di Marzo et al., 1994, 1998; Di Marzo, 1999; Giuffrida et al., 2001). This implies that

they have a short life extracellularly, which, in turn, implies that for cannabinoids to modulate peripheral neurotransmission, they must be released from sources close to the nerves. Peripheral nerves are one possible source of endocannabinoids. It is known that electrical and other depolarising stimuli can release anandamide from cell membranes of nerves in the central nervous system (Di Marzo et al., 1994; Stella and Piomelli, 2001) and from dorsal root ganglion cells (Ahluwalia et al., 2003). Unlike classical neurotransmitters, anandamide is not stored in and released from vesicles, but rather is thought to be synthesised on demand from the cell membrane by the phospholipase D-mediated hydrolysis of *N*-arachidonyl-phosphatidylethanolamine (Di Marzo et al., 1994, 1998). There is also evidence for a neuronal release of endocannabinoids in the periphery. In rat mesenteric arteries, Ca^{2+}-induced relaxation is mediated by the release of an endocannabinoid, possibly anandamide, from sensory nerves which diffuses to the underlying vascular smooth muscle to cause an SR141716A-sensitive, but non-CB_1 non-CB_2 cannabinoid receptor, mediated relaxation (Ishioka and Bukoski, 1999; Bukoski et al., 2002), and SR141716A has been shown to augment sensory neurogenic vasorelaxation during electrical field stimulation, consistent with the release of an endocannabinoid from perivascular sensory nerves (Ralevic and Kendall, 2001).

In the central nervous system, cannabinoids are released from the postsynaptic cell and function as retrograde signalling molecules, inhibiting presynaptically via cannabinoid CB_1 receptors the release of classical transmitter (Elphick and Egertová, 2001; Wilson and Nicoll, 2001, 2002; Ohno-Shosaku et al., 2001, 2002; Kreitzer and Regehr, 2002; Yoshida et al., 2002). It seems unlikely that this mechanism operates in the periphery, i.e. that prejunctional cannabinoid CB_1 receptors are activated by the retrograde actions of endocannabinoids released from a postjunctional site, namely the smooth muscle, following stimulation by the classical neurotransmitter. This is because the junctional cleft is variable in size, and often wide, and because cannabinoids are rapidly taken up and inactivated (Di Marzo et al., 1994, 1998; Di Marzo, 1999; Giuffrida et al., 2001). It seems more likely that cannabinoids are released from, and act at, prejunctional sites. This would be in line with the actions of other peripheral neuromodulators such as noradrenaline, ATP (via adenosine following enzymatic degradation) and neuropeptide Y, which act at prejunctional inhibitory autoreceptors following release from peripheral nerves. In addition to nerves, blood cells and endothelial cells are local sources of endocannabinoids which could act as modulators of autonomic and sensory neurotransmission at pre- and/or postjunctional sites.

Cannabinoids are highly lipophilic molecules which may directly affect the organisation of neuronal membrane lipids. However, the fact that their effects are, in various peripheral tissues, concentration-dependent, stereoselective, blocked by selective antagonists, and occur at concentrations similar to those in binding studies, indicates a cannabinoid receptor-mediated mechanism. Both cannabinoid CB_1 and CB_2 receptors are coupled through $G_{i/o}$ proteins. Cannabinoid CB_1-mediated inhibition of neurotransmission has been investigated most thoroughly in the central nervous system, where it has been shown that there may be an inhibition of adenylyl cyclase, inhibition of L-type, N-type, P/Q-type and T-type Ca^{2+} channels, inhibition of D-type potassium channels, and activation of A-type and inwardly rectifying potassium channels (see Pertwee 1993, 1997, 1999; Chemin et al., 2001; Howlett et al., 2002). Similar mechanisms of cannabinoid CB_1 receptor inhibition of neurotransmitter release are likely to operate in the periphery. In guinea-pig myenteric plexus-longitudinal muscle, WIN 55,212-2-induced inhibition of electrically evoked contractions was enhanced by lowering the external Ca^{2+} concentration, and attenuated by elevating the external Ca^{2+} concentration or by exposing the tissue to forskolin, 8-bromo-cAMP, or to a phosphodiesterase inhibitor (Coutts and Pertwee, 1998), consistent with negative coupling of cannabinoid CB_1 receptors through $G_{i/o}$ proteins to adenylyl cyclase and Ca^{2+} channels (Pertwee, 1997).

9. Mechanism of cannabinoid activation of sensory neurotransmission via vanilloid VR1 receptors

The mechanism by which anandamide activates vanilloid VR1 receptors remains unclear. The activation involves binding of the ligand to the receptor, as [^{3}H]anandamide has been shown to bind to vanilloid VR1 receptors in membranes prepared from cells expressing recombinant vanilloid VR1 receptors (Olah et al., 2001). Furthermore, anandamide displaces [^{3}H]resiniferatoxin from vanilloid VR1 receptor-expressing cells (DePetrocellis et al., 2001; Ross et al., 2001b). This appears to be to an intracellular site, as reported for capsaicin (Jung et al., 1999), as inhibition of the anandamide transporter inhibited the anandamide-, but not the capsaicin-, induced response in vanilloid VR1 receptor-expressing HEK293 cells (DePetrocellis et al., 2001). Moreover, inhibition of intracellular anandamide hydrolysis enhanced the anandamide-, but not the capsaicin-, induced response in these cells (DePetrocellis et al., 2001). This evidence for an intracellular site of action has lead to suggestions that anandamide may activate vanilloid VR1 receptors indirectly (Craib et al., 2001), possibly in addition to direct activation of vanilloid VR1 receptors. It has been suggested that anandamide activates vanilloid VR1 receptors via protein kinase C (Premkumar and Ahern, 2000) based on the evidence that inhibition of protein kinase C reduces the response to anandamide. However, inhibition of protein kinase C also inhibits the direct activation of vanilloid VR1 receptors by capsaicin

(Smart et al., 2001; Jerman et al., 2000), suggesting a common modulatory activity rather than a key mechanism.

One of the breakdown products of anandamide is arachidonic acid (Kunos and Batkai, 2001), and recent reports suggest that lipooxygenase metabolites of arachidonic acid, most notably 12-HPETE (12-(*S*)-hydroperoxyeicosatetraenoic acid), activate vanilloid VR1 receptors (Hwang et al., 2000; Piomelli, 2001). Inhibition of fatty acid amide hydrolase, the enzyme responsible for the metabolism of anandamide (Hwang et al., 2000), enhances rather than inhibits the anandamide-induced activation of vanilloid VR1 receptors (Ross et al., 2001b; DePetrocellis et al., 2001; Craib et al., 2001). However, this does not rule out the possibility that rapid intracellular conversion of anandamide to arachidonic acid and, subsequently, to lipooxygenase metabolites may be a means of vanilloid VR1 receptor activation in systems where these enzymes (fatty acid amide hydrolase and lipooxygenases) are both highly expressed.

The vanilloid VR1 receptor is a nonselective cation channel that displays a high permeability for Ca^{2+} (Szallasi and Blumberg, 1999). The inward currents carried by vanilloid VR1 receptors depolarise the cells and this causes repetitive firing of action potentials (Caterina et al., 1997). In addition, the influx of Ca^{2+} causes exocytotic release of peptide neurotransmitters. It has been shown for capsaicin that, depending on the concentration and exposure time, excitation is rapidly followed by intracellular accumulation of Ca^{2+} and Na^{+}, which determines vanilloid toxicity. This may range from a rapid but reversible desensitization, to block of nerve conduction and necrotic cell death (Maggi and Meli, 1988; Holzer, 1991, 1998). Clearly, cannabinoids acting at vanilloid VR1 receptors could have very different effects on sensory neurotransmission depending on their concentration and time of exposure.

The potency of agonists at vanilloid VR1 receptors may be enhanced by other factors present in native tissue. The potency of anandamide and capsaicin at recombinant rat and human vanilloid VR1 receptors is enhanced by protons in a pH-dependent manner (Olah et al., 2001; Ralevic et al., 2001; Smart et al., 2000, 2001; Jerman et al., 2000; Hayes et al., 2001). Activation of protein kinase C with phorbol 12-myristate 13-acetate enhanced the potency of the response to anandamide and capsaicin in rat and human vanilloid VR1 receptors (Premkumar and Ahern, 2000; Vellani et al., 2001). Similarly, activation of protein kinase A enhances both the potency (~ 5-fold) and efficacy of the anandamide-induced response in dorsal root ganglion neurones (DePetrocellis et al., 2001). Other factors can also enhance the anandamide-induced activation of recombinant vanilloid VR1 receptors. Noxious heat (50 °C) increased the magnitude of the anandamide-induced response by ~ 2.5-fold in cells expressing rat vanilloid VR1 receptors (Sprague et al., 2001). Palmitoylethanolamide also enhanced the anandamide-induced response in human vanilloid VR1 receptor-expressing cells, apparently by enhancing anandamide binding to vanilloid VR1 receptors (DePetrocellis et al., 2001).

10. Significance of cannabinoid activation of cannabinoid and vanilloid VR1 receptors

Activation of cannabinoid receptors on sympathetic, parasympathetic and myenteric nerves leads to an inhibition of neurotransmitter release and a decrease in associated motor functions. The role of cannabinoids in modulation of sensory neurotransmission is more complex as this may involve both inhibitory actions via cannabinoid receptors and excitatory actions via vanilloid VR1 receptors (Fig. 7). The biological significance of this is unclear, but a number of possibilities exist.

Cannabinoid CB_1 and vanilloid VR1 receptors have different sensitivities to certain endocannabinoids/endovanilloids so that there may be preferential activation of one or other of the receptors. This seems to be the case in rat hindpaw skin, where anandamide is a much more potent agonist at cannabinoid CB_1 receptors (effective at 1 nM) than at vanilloid VR1 receptors (inactive at 1 nM, and likely up to μM concentrations) and attenuates the capsaicin-evoked neurogenic inflammation response (Richardson et al., 1998). A number of studies in the dorsal horn have also shown activation of cannabinoid CB_1 receptors at low doses and vanilloid VR1 receptors at high doses of anandamide (Szoke et al., 2000; Morisset et al., 2001; Tognetto et al., 2001). Thus, the cannabinoid CB_1 receptor may act as a functional brake on vanilloid VR1 receptor-mediated activation of sensory nerves. Indeed, HU210 blocked vanilloid VR1 receptor-mediated increases in intracellular Ca^{2+} concentration in adult rat dorsal root ganglion cells in an SR141716A-sensitive manner (Millns et al., 2001). This suggests that inhibition of sensory neurotransmission via cannabinoid CB_1 receptors may be the physiological role of endocannabinoids, and that much higher levels, such as might be achieved in pathophysiological conditions, are required for vanilloid VR1 receptor-mediated activation of sensory nerves.

However, the similar potency of *N*-arachidonoyl-dopamine for endogenous cannabinoid CB_1 and vanilloid VR1 receptors (Bisogno et al., 2000; Huang et al., 2002) suggests that other forms of differentiation may exist, perhaps involving differential modulation by factors such as pH and second messengers, or rates of activation and/or inactivation of the response (see Section 9). In this respect, it is known that the vanilloid VR1 receptor response rapidly desensitises and activation may be followed by desensitization and/or neurotransmitter depletion, which renders the sensory nerves refractory to further stimulation. The involvement of non-CB_1 cannabinoid receptors and other endogenous cannabinoids might be important. For example, anandamide is a partial agonist at cannabinoid CB_2 receptors and could thus act as an endogenous antagonist of, for

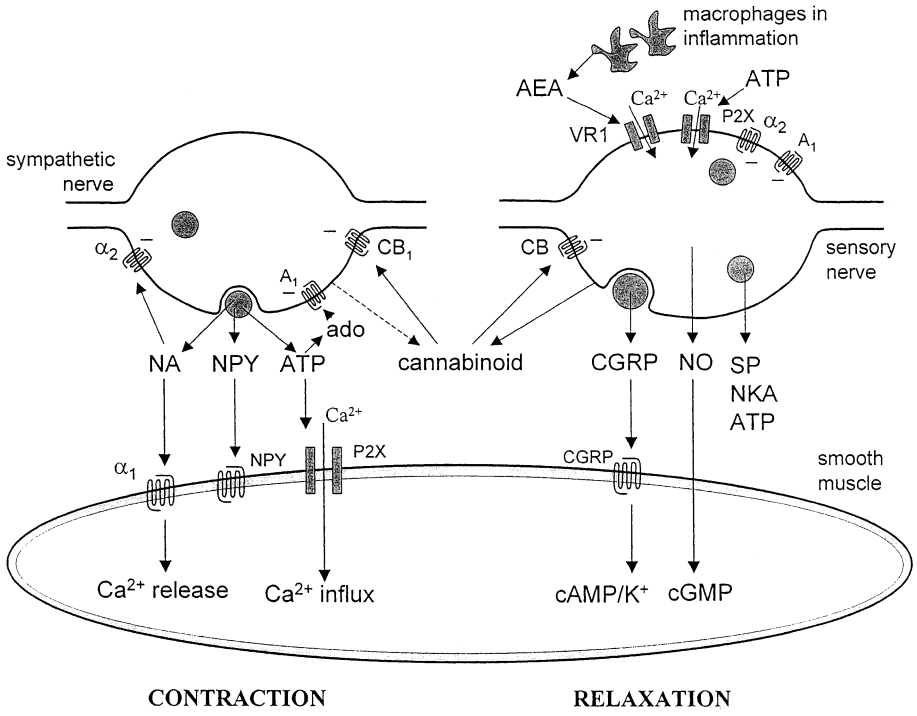

Fig. 7. Diagram showing modulation of peripheral neurotransmission by cannabinoids. Cannabinoids may be released from the cell membrane of sympathetic and sensory nerves, together with classical and other novel neurotransmitters. Rapid uptake means that diffusion is restricted; hence, the principal action is likely to be prejunctional neuromodulation. Cannabinoids can act prejunctionally to inhibit sympathetic and sensory neurotransmitter release. Cannabinoid CB_1 receptors are expressed on sympathetic and sensory nerves. Anandamide (and *N*-arachidonoyl-dopamine) can also act at vanilloid VR1 receptors to activate sensory nerves, leading to a release of sensory neurotransmitter and relaxation. It is possible that this occurs when high levels of these compounds are generated locally, for example, from macrophages in inflammation. A_1, A_1 adenosine receptor; $\alpha_{1/2}$, $\alpha_{1/2}$-adrenoceptor; ado, adenosine; cAMP, cyclic AMP; cGMP, cyclic GMP; NA, noradrenaline; NKA, neurokinin A; NPY, neuropeptide Y; P2X, purine P2X receptor; SP, substance P (from Randall et al., 2002, with permission).

example, the actions of 2-arachidonoyl glycerol (which is inactive at endogenous and recombinant rat vanilloid VR1 receptors) as a full agonist at this receptor (Gonsiorek et al., 2000). Finally, modulation of sensory neurotransmission by cannabinoid CB_1 and vanilloid VR1 receptors may indirectly influence autonomic nerve function as both acute and chronic interactions between these neuronal systems have been described.

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