Effect of the cannabinoid receptor agonist WIN55212-2 on sympathetic cardiovascular regulation

¹Nathalie Niederhoffer & ^{*,1}Bela Szabo

¹Pharmakologisches Institut der Albert-Ludwigs-Universität, Hermann-Herder-Strasse 5, D-79104 Freiburg i. Br., Germany

1 The aim of the present study was to analyse the cardiovascular actions of the synthetic CB₁/CB₂ cannabinoid receptor agonist WIN55212-2, and specifically to determine its sites of action on sympathetic cardiovascular regulation.

2 Pithed rabbits in which the sympathetic outflow was continuously stimulated electrically or which received a pressor infusion of noradrenaline were used to study peripheral prejunctional and direct vascular effects, respectively. For studying effects on brain stem cardiovascular regulatory centres, drugs were administered into the cisterna cerebellomedullaris in conscious rabbits. Overall cardiovascular effects of the cannabinoid were studied in conscious rabbits with intravenous drug administration.

3 In pithed rabbits in which the sympathetic outflow was continuously electrically stimulated, intravenous injection of WIN55212-2 (5, 50 and 500 $\mu\text{g kg}^{-1}$) markedly reduced blood pressure, the spillover of noradrenaline into plasma and the plasma noradrenaline concentration, and these effects were antagonized by the CB₁ cannabinoid receptor-selective antagonist SR141716A. The hypotensive and the sympathoinhibitory effect of WIN55212-2 was shared by CP55940, another mixed CB₁/CB₂ cannabinoid receptor agonist, but not by WIN55212-3, the enantiomer of WIN55212-2, which lacks affinity for cannabinoid binding sites. WIN55212-2 had no effect on vascular tone established by infusion of noradrenaline in pithed rabbits.

4 Intracisternal application of WIN55212-2 (0.1, 1 and 10 $\mu\text{g kg}^{-1}$) in conscious rabbits increased blood pressure and the plasma noradrenaline concentration and elicited bradycardia; this latter effect was antagonized by atropine.

5 In conscious animals, intravenous injection of WIN55212-2 (5 and 50 $\mu\text{g kg}^{-1}$) caused bradycardia, slight hypotension, no change in the plasma noradrenaline concentration, and an increase in renal sympathetic nerve firing. The highest dose of WIN55212-2 (500 $\mu\text{g kg}^{-1}$) elicited hypotension and tachycardia, and sympathetic nerve activity and the plasma noradrenaline concentration declined.

6 The results obtained in pithed rabbits indicate that activation of CB₁ cannabinoid receptors leads to marked peripheral prejunctional inhibition of noradrenaline release from postganglionic sympathetic axons. Intracisternal application of WIN55212-2 uncovered two effects on brain stem cardiovascular centres: sympathoexcitation and activation of cardiac vagal fibres. The highest dose of systemically administered WIN55212-2 produced central sympathoinhibition; the primary site of this action is not known.

Keywords: Baroreflex; blood pressure; cannabinoid receptor; cardiovascular regulation; conscious rabbit; heart rate; plasma noradrenaline; pithed rabbit; presynaptic receptor; sympathetic nerve activity

Abbreviations: CB, cannabinoid; CP55940, (–)-cis-3-[2-hydroxy-4-(1,1-dimethylheptyl)phenyl]-trans-4-(3-hydroxypropyl)cyclohexanol; i.c., intracisternal; PRE, average of initial values (before drug application); SR141716A, N-piperidino-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-3-pyrazole-carboxamide; WIN55212-2, R(+)-[2,3-dihydro-5-methyl-3-[(morpholinyl)methyl]pyrrolo[1,2,3-de]-1,4-benzoxazinyl]-(1-naphthalenyl)methanone mesylate

Introduction

Cannabinoids produce their typical effects by activation of specific G-protein-coupled receptors. Two cannabinoid receptors have been identified, CB₁ and CB₂ (Matsuda *et al.*, 1990; Munro *et al.*, 1993; for review see Howlett, 1995; Compton *et al.*, 1996; Pertwee, 1997). The CB₁ receptor is preferentially located on neurons, whereas the CB₂ receptor occurs mainly on peripheral non-neuronal cells.

Pharmacological effects of cannabinoids in animals include hypokinesia, analgesia, catalepsy and hypothermia (for review see Howlett, 1995; Compton *et al.*, 1996; Pertwee, 1997). Cannabinoids also elicit cardiovascular changes (for review see Dewey, 1986; Compton *et al.*, 1996). In most experiments in anaesthetized animals, cannabinoids lowered blood pressure

and heart rate, and this was generally attributed to depression of sympathetic tone and enhancement of cardiac vagal activity (rat: Graham & Li, 1973; Adams *et al.*, 1976; Estrada *et al.*, 1987; Varga *et al.*, 1995, 1996; Vidrio *et al.*, 1996; Lake *et al.*, 1997a,b; dog: Caverio *et al.*, 1973a,b, 1974; Jandhyala & Hamed, 1978; cat: Vollmer *et al.*, 1974). In conscious animals, cannabinoids either caused moderate cardiovascular depression (rat: Birmingham, 1973; Vidrio *et al.*, 1996; rabbit: Stark & Dews, 1980; monkey: Fredericks *et al.*, 1981), or they had no effect or elicited hypertension or tachycardia (rat: Osgood & Howes, 1977; Kawasaki *et al.*, 1980; Stein *et al.*, 1996; Lake *et al.*, 1997b; dog: Jandhyala & Hamed, 1978). In conscious humans, acute administration of cannabinoids elicited marked tachycardia accompanied by a small increase in blood pressure (Benowitz *et al.*, 1979; Huestis *et al.*, 1992), whereas long-term cannabinoid application produced hypotension and bradycar-

* Author for correspondence.

dia (Benowitz & Jones, 1975). In the majority of cardiovascular studies, Δ^9 -tetrahydrocannabinol (the main active component from *Cannabis sativa*) or anandamide (a putative endogenous cannabinoid) was used as the agonist. Both compounds are agonists at CB₁ and CB₂ receptors (Felder *et al.*, 1995; Showalter *et al.*, 1996; see Pertwee, 1997) but also elicit effects independent of cannabinoid receptors (see Martin, 1986; Lake *et al.*, 1997a,b).

In the majority of the above mentioned cardiovascular studies only blood pressure and heart rate were measured, and only few experiments were carried out which permitted determination of the site of interaction of cannabinoids with the cardiovascular system. To our knowledge, effects on sympathetic nerve activity have been determined only by Vollmer *et al.* (1974) and effects on the plasma concentration of catecholamines have not been examined. The information on cardiovascular effects of centrally administered cannabinoids is also limited (Cavero *et al.*, 1973a,b; Vollmer *et al.*, 1974). The aim of the present study was to determine the sites of interaction of cannabinoids with the sympathetic nervous system. To reach this goal, four kinds of experiment were carried out. (i) Peripheral prejunctional effects on noradrenaline release from sympathetic neurons were studied in pithed rabbits with electrically stimulated sympathetic outflow. (ii) Peripheral postjunctional vascular effects were studied in pithed rabbits which received a pressor infusion of noradrenaline. (iii) Effects on cardiovascular centres in the brain stem were examined by administration of cannabinoids into the cisterna cerebellomedullaris of conscious rabbits. (iv) Finally, the overall effect of systemically administered cannabinoids on cardiovascular regulation was studied in conscious rabbits; in these experiments, the electrical activity of renal postganglionic sympathetic axons was recorded by means of a chronically implanted electrode.

In most experiments, we used the synthetic aminoalkylindole compound WIN55212-2 as a cannabinoid agonist. This compound possesses affinity for both CB₁ and CB₂ cannabinoid receptors (in this respect it is similar to Δ^9 -tetrahydrocannabinol and anandamide), its affinity for these receptors is high, and more importantly, its lack of affinity for a great number of neurotransmitter receptors and ion channels has been documented (Felder *et al.*, 1995; Showalter *et al.*, 1996; Kuster *et al.*, 1993; for review see Pertwee, 1997). In a few experiments, the effects of WIN55212-2 were compared with the effects of WIN55212-3, the enantiomer of WIN55212-2, which in binding studies has very low affinity for cannabinoid receptors, and with the effects of CP55940, a mixed CB₁/CB₂ cannabinoid receptor agonist with a chemical structure markedly different from WIN55212-2.

Methods

Experiments were carried out on 40 rabbits of a local breed (derived from 'Deutscher Riesenscheck', obtained from Ketterer, Reute, Germany); rabbits were of either sex and weighed 1.4–2.9 kg. Four different experimental preparations were used.

Pithed rabbits with electrically stimulated sympathetic outflow

The method was basically as in Szabo *et al.* (1987). Briefly, rabbits were deeply anaesthetized with pentobarbitone (75 mg kg⁻¹) and artificially ventilated. The left carotid artery was cannulated for recording arterial pressure with a Statham

P23Db transducer coupled to a bridge amplifier (Hugo Sachs Elektronik, Hugstetten, Germany). The heart rate was calculated from the pulsating blood pressure signal by an integrator (Hugo Sachs Elektronik, Hugstetten, Germany). The right carotid artery was also cannulated and served for blood sampling. Both jugular veins were cannulated and they served for administration of drugs. After relaxation of skeletal muscles by gallamine triethiodide (5 mg kg⁻¹), animals were pithed using a 3-mm-thick and 30-cm-long uninsulated stainless steel rod. The entire sympathetic outflow was continuously stimulated through the pithing rod (2 Hz, 100–140 mA, 0.5 ms square-wave pulses). This stimulation markedly increased blood pressure (from 57 ± 3 mmHg [*n* = 14] to 83 ± 4 mmHg [*n* = 14]) and the plasma noradrenaline concentration (from values lower than 10 pg ml⁻¹ (see Urban *et al.*, 1995, for plasma noradrenaline values in unstimulated pithed rabbits) to 310 ± 56 pg ml⁻¹ [*n* = 14]); heart rate increased only moderately (from 233 ± 10 min⁻¹ [*n* = 14] to 259 ± 9 min⁻¹ [*n* = 14]). Thirty minutes after the beginning of electrical stimulation, an infusion of [³H]noradrenaline was started (80 nCi kg⁻¹ min⁻¹; 0.23 ng kg⁻¹ min⁻¹); this tracer infusion did not change blood pressure and heart rate. Parameters were first determined 30–45 min (*t* = 0 min in subsequent text) after the beginning of the [³H]noradrenaline infusion.

Pithed rabbits receiving a pressor infusion of noradrenaline

Rabbits were pithed as described above. Instead of electrical stimulation, vascular tone was raised by i.v. infusion of noradrenaline (2 µg kg⁻¹ min⁻¹). This infusion markedly increased blood pressure (from 53 ± 4 mmHg [*n* = 8] to 79 ± 6 mmHg [*n* = 8]) but did not change heart rate (258 ± 11 min⁻¹ [*n* = 8] before infusion and 261 ± 14 min⁻¹ [*n* = 8] during infusion). Blood was not sampled. Parameters were first determined 30 min (*t* = 0 min) after the beginning of noradrenaline infusion.

Conscious rabbits with intracisternal drug administration

The method was basically as in Szabo *et al.* (1995). Briefly, under halothane anaesthesia (1.5–4%) in spontaneously breathing rabbits, a polyethylene catheter (0.28 mm i.d., 0.61 mm o.d., 25 cm length) was inserted 8 mm into the cisterna cerebellomedullaris through a hole in the atlanto-occipital membrane. The free end of the catheter was tunnelled to an incision in the neck. The wounds were sutured and at least 2 weeks were allowed for recovery before the first experiment was carried out in the conscious animal. Three experiments were carried out on one rabbit at intervals of 7 days. The order of treatments was randomized and no animal received a given kind of treatment twice. After the last experiment, the animals were killed by an overdose of pentobarbitone.

Before the experiments in conscious animals, the central ear artery (for recording arterial pressure and heart rate and for blood sampling) and a marginal ear vein (for administration of drugs) were cannulated under local anaesthesia. Also under local anaesthesia, the intracisternal catheter was recovered from under the skin. Parameters were first determined 45 min (*t* = 0 min) after recovery of the catheter.

Conscious rabbits receiving drugs intravenously

The method was basically as in Szabo *et al.* (1993). Briefly, an electrode was implanted under halothane anaesthesia (1.5–4%) in spontaneously breathing rabbits. The left kidney was

approached retroperitoneally and two sympathetic nerve trunks accompanying the renal artery were dissected free and slipped into the spirals of a stainless steel bipolar electrode. The nerves and electrode were then embedded in silicone gel. The free end of the electrode was tunnelled to an incision in the neck. The wounds were sutured and 3–4 days were allowed for recovery before the first experiment was carried out in the conscious animal. Two to three experiments were carried out on one rabbit at intervals of 3–4 days. The order of treatments was randomized and no animal received a given kind of treatment twice. After the last experiment, the animals were killed by an overdose of pentobarbitone.

Before the experiments in conscious animals, the central ear artery (for recording arterial pressure and heart rate and for blood sampling) and a marginal ear vein (for administration of drugs) were cannulated under local anaesthesia. Also under local anaesthesia, the electrode leads were recovered from under the skin. Parameters were first determined 45 min ($t = 0$ min) after recovery of the electrode leads.

Determination of noradrenaline plasma concentration and kinetic parameters

The method was basically as in Szabo & Schultheiss (1990). Briefly, the plasma concentrations of endogenous noradrenaline and [^3H]noradrenaline were determined in the plasma from 2-ml blood samples by alumina chromatography followed by high pressure liquid chromatography, electrochemical detection and liquid scintillation counting. The values were used to calculate the [^3H]noradrenaline plasma clearance and the rate of total body noradrenaline spillover into plasma (see also Esler *et al.*, 1990). Since none of the treatments changed the [^3H]noradrenaline plasma clearance, values of this parameter are not given.

Protocol and evaluation of experiments

In pithed rabbits, either solvent (0.5 ml kg^{-1}) or increasing doses of WIN55212-2 (5, 50 and $500\text{ }\mu\text{g kg}^{-1}$), WIN55212-3 (5, 50 and $500\text{ }\mu\text{g kg}^{-1}$) or CP55940 (5, 50 and $500\text{ }\mu\text{g kg}^{-1}$) were injected i.v. at $t = 19, 37$ and 55 min. One group of rabbits was pretreated at $t = -10$ min with the cannabinoid antagonist SR141716A ($500\text{ }\mu\text{g kg}^{-1}$; i.v.).

In conscious rabbits with an intracisternal catheter, either solvent ($25\text{ }\mu\text{l kg}^{-1}$) or increasing doses of WIN55212-2 ($0.1, 1$ and $10\text{ }\mu\text{g kg}^{-1}$) were injected intracisternally (i.c.) at $t = 19, 37$ and 55 min. One group of rabbits was pretreated i.v. at $t = -10$ min with atropine (1 mg kg^{-1}).

In conscious rabbits with renal nerve recording, either solvent (0.5 ml kg^{-1}) or increasing doses of WIN55212-2 (5, 50 and $500\text{ }\mu\text{g kg}^{-1}$) was injected i.v. at $t = 19, 37$ and 55 min. One group of rabbits was pretreated at $t = -10$ min with SR141716A ($500\text{ }\mu\text{g kg}^{-1}$; i.v.).

In all experimental groups, blood pressure and heart rate (and in some groups also renal sympathetic nerve activity) were read every 2 min from $t = 0$ to 68 min. Blood was sampled at $t = 0, 14, 32, 50$ and 68 min for the determination of the plasma noradrenaline concentration (and in some groups also for the determination of the [^3H]noradrenaline plasma concentration). In each experiment, values measured at $t = 0$ and 14 min were averaged (PRE), and all values were expressed as percentages of PRE.

Statistics

Means $\pm$ s.e. mean of n experiments are given throughout. Differences between groups were evaluated with the non-

parametric two-tailed Mann-Whitney test; in the case of multiple comparisons the Bonferroni correction was employed. $P < 0.05$ was taken as the limit of statistical significance and only this level is indicated even if P was < 0.01 or < 0.001 .

Drugs

Drugs were obtained from the following sources: atropine sulphate and (–)-noradrenaline (+)-bitartrate from Sigma (Deisenhofen, Germany); (–)-cis-3-[2-hydroxy-4-(1,1-dimethylheptyl)phenyl]-trans-4-(3-hydroxypropyl)cyclohexanol (CP55940) from Pfizer (Groton, CT, U.S.A.); 2-hydroxypropyl- β -cyclodextrin from Fluka (Neu-Ulm, Germany); N-piperidino-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-3-pyrazole-carboxamide (SR141716A) from Sanofi (Montpellier, France); R(+)-[2,3-dihydro-5-methyl-3-[(morpholinyl)methyl]pyrrolo[1,2,3-de]-1,4-benzoxazinyl]-(1-naphthalenyl)methanone mesylate (WIN55212-2) and S(–)-[2,3-dihydro-5-methyl-3-[(morpholinyl)methyl]pyrrolo[1,2,3-de]-1,4-benzoxazinyl]-(1-naphthalenyl)methanone mesylate (WIN55212-3) from RBI (Köln, Germany); (–)-[ring-2,5,6- ^3H]noradrenaline (specific activity, 59 Ci mmol^{-1}) from DuPont NEN (Bad Homburg, Germany).

WIN55212-2, WIN55212-3 and CP55940 were dissolved in 19% (i.v. injection) or 7.5% (i.c. injection) w/v solutions of 2-hydroxypropyl- β -cyclodextrin; further dilutions were made with the same solvent. SR141716A was dissolved in 66% DMSO. Atropine and (–)-noradrenaline were dissolved in 0.9% saline. [^3H]Noradrenaline was diluted with 0.02 M acetic acid to final concentration. I.v. and i.c. injections had a volume of 0.5 ml kg^{-1} and $25\text{ }\mu\text{l kg}^{-1}$, respectively. [^3H]Noradrenaline and (–)-noradrenaline were infused at a rate of 1.92 ml h^{-1} . Doses refer to the salts.

Results

After an initial stabilization period, parameters were determined twice (at $t = 0$ and 14 min), and the values were averaged to obtain the PRE values. Values recorded later in any given experiment were expressed as percentages of PRE. The PRE values in the four experimental models are given in Table 1. Values recorded from unpretreated rabbits are similar to those obtained in the corresponding preparations in previous studies (compare with Szabo *et al.*, 1987; 1993; 1995). Pretreatment with the cannabinoid antagonist SR141716A ($500\text{ }\mu\text{g kg}^{-1}$; i.v.) had no significant effect on the recorded parameters. Pretreatment with atropine (1 mg kg^{-1} ; i.v.) produced the expected cardioacceleration.

Pithed rabbits with electrically stimulated sympathetic outflow (Figure 1)

An experimental sympathetic tone was maintained in these rabbits by electrical stimulation of preganglionic sympathetic neurons with an electrode in the spinal canal. Intravenous injection (0.5 ml kg^{-1}) of the solvent for WIN55212-2 caused short-lasting blood pressure increases without influencing heart rate. The plasma noradrenaline concentration and the spillover of noradrenaline into plasma decreased slightly in the solvent group. Intravenous injection of three increasing doses of the cannabinoid agonist WIN55212-2 (5, 50 and $500\text{ }\mu\text{g kg}^{-1}$) dose-dependently and greatly reduced blood pressure, the plasma noradrenaline concentration and the spillover of noradrenaline into plasma; heart rate was not changed.

Pretreatment with SR141716A ($500 \mu\text{g kg}^{-1}$; i.v.) nearly abolished the hypotension produced by the two lower doses of WIN55212-2 and attenuated the hypotensive effect of the highest dose. SR141716A also attenuated the reduction of the plasma noradrenaline concentration and the noradrenaline spillover into plasma caused by WIN55212-2.

Intravenous injection of the inactive enantiomer WIN55212-3 (5, 50 and $500 \mu\text{g kg}^{-1}$; same doses as of WIN55212-2)

transiently increased blood pressure (Figure 2), an effect resembling the one occurring after injection of solvent (see Figure 1) and did not change the plasma noradrenaline concentration (Figure 2). Intravenous injection of another cannabinoid agonist, CP55940 (5, 50 and $500 \mu\text{g kg}^{-1}$), produced effects (Figure 2) very similar to those of WIN55212-2: dose-dependent hypotension and a dose-dependent decrease of the plasma noradrenaline concentration.

Table 1 Absolute values of parameters before injection of solvent or WIN 55212-2 (PRE values^a)

Pretreatment	n	Mean arterial pressure (mmHg)	Heart rate (min ⁻¹)	Renal sympathetic nerve activity (impulses s ⁻¹)	Plasma noradrenaline concentration (pg ml ⁻¹)	Noradrenaline spillover rate (ng kg ⁻¹ min ⁻¹)
<i>Pithed rabbits with electrically stimulated sympathetic outflow</i>						
—	10	81 ± 6	245 ± 9		250 ± 44	19 ± 0.4
SR141716A i.v. ^b	4	87 ± 5	269 ± 20		372 ± 160	34 ± 17
<i>Pithed rabbits receiving a pressor infusion of noradrenaline</i>						
—	8	79 ± 6	261 ± 14			
<i>Conscious rabbits with intracisternal drug administration</i>						
—	16	75 ± 2	196 ± 8		149 ± 19	
Atropine i.v. ^c	9	71 ± 3	244 ± 17*		170 ± 40	
<i>Conscious rabbits receiving drugs intravenously</i>						
—	13	83 ± 3	207 ± 7	29 ± 4	153 ± 26	
SR141716A i.v. ^b	7	90 ± 4	192 ± 8	27 ± 4	199 ± 70	

^aPRE values are averages of values measured at $t=0$ and 14 min. ^bSR141716A ($500 \mu\text{g kg}^{-1}$) was injected i.v. at $t=-10$ min.

^cAtropine (1 mg kg^{-1}) was injected i.v. at $t=-10$ min. *Significant difference ($P<0.05$) from unpretreated rabbits.

Figure 1 Effects of i.v. injections of solvent (SOL) and WIN 55212-2 (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 $500 \mu\text{g kg}^{-1}$) were injected as indicated by arrows. One of the two WIN-2-groups was pretreated at $t=-10$ min with SR141716A (SR; $500 \mu\text{g kg}^{-1}$; i.v.). Values are given as percentages of PRE values (Table 1). Means $\pm$ s.e. mean from six (SOL), four (WIN-2) and four (SR + WIN-2) experiments. Differences from SOL: * $P<0.05$; differences from WIN-2: + $P<0.05$.

Figure 2 Effects of i.v. injections of either WIN55212-3 (WIN-3) or CP55940 (CP) on mean arterial pressure (MAP) and plasma noradrenaline concentration (PL-NA) in pithed rabbits with electrically stimulated sympathetic outflow. WIN-3 (5, 50 and 500 $\mu\text{g kg}^{-1}$) and CP (5, 50 and 500 $\mu\text{g kg}^{-1}$) were injected as indicated by arrows. Representative curves from three (WIN-3) and three (CP) experiments with similar results.

Pithed rabbits receiving a pressor infusion of noradrenaline (Figure 3)

An artificial vascular tone was maintained in these rabbits by intravenous infusion of noradrenaline. Intravenous injection of solvent caused short-lasting blood pressure increases and no change in heart rate. The effects of WIN55212-2 (5, 50 and 500 $\mu\text{g kg}^{-1}$) were similar: short-lasting blood pressure increases and no change in heart rate were observed. The effects of WIN55212-2 differed remarkably in the two pithed rabbit models: blood pressure decreased in animals with electrically stimulated sympathetic outflow (Figure 1), whereas only blood pressure increases (effect of the solvent) were observed in animals with a pressor infusion of noradrenaline (Figure 3).

Conscious rabbits with intracisternal drug administration (Figure 4)

Injection of the solvent (25 $\mu\text{l kg}^{-1}$) into the cisterna cerebellomedullaris caused no change in blood pressure, heart rate and the plasma noradrenaline concentration. When three increasing doses of WIN55212-2 (0.1, 1 and 10 $\mu\text{g kg}^{-1}$) were injected intracisternally, blood pressure was not changed by the two lower doses but was elevated after the highest dose. Central application of WIN55212-2 elicited pronounced and dose-dependent bradycardia and a dose-dependent increase in the plasma noradrenaline concentration.

When rabbits were pretreated with atropine (1 mg kg^{-1} ; i.v.), the hypertension elicited by the highest dose of WIN55212-2 (10 $\mu\text{g kg}^{-1}$) was unchanged; in these animals,

Figure 3 Effects of i.v. injections of solvent (SOL) and WIN 55212-2 (WIN-2) on mean arterial pressure and heart rate in pithed rabbits receiving a pressor infusion of noradrenaline. SOL (0.5 ml kg^{-1}) and WIN-2 (5, 50 and 500 $\mu\text{g kg}^{-1}$) were injected as indicated by arrows. Values are given as percentages of PRE values (Table 1). Means $\pm$ s.e. mean from four (SOL) and four (WIN-2) experiments.

Figure 4 Effects of i.c. injections of solvent (SOL) and WIN 55212-2 (WIN-2) on mean arterial pressure, heart rate and plasma noradrenaline concentration in conscious rabbits. SOL ($25 \mu\text{g kg}^{-1}$) and WIN-2 (0.1 , 1 and $10 \mu\text{g kg}^{-1}$) were injected as indicated by arrows. One of the two WIN-2-groups was pretreated at $t = -10$ min with atropine (ATR; 1 mg kg^{-1} ; i.v.). Values are given as percentages of PRE values (Table 1). Means $\pm$ s.e. mean from seven (SOL), nine (WIN-2) and nine (ATR + WIN-2) experiments. Differences from SOL: * $P < 0.05$; differences from WIN-2: + $P < 0.05$.

even a lower dose of WIN55212-2 ($1 \mu\text{g kg}^{-1}$) tended to increase blood pressure. Pretreatment with atropine abolished the bradycardia elicited by the lower doses of WIN55212-2 (0.1 and $1 \mu\text{g kg}^{-1}$) and attenuated the bradycardia elicited by the highest dose ($10 \mu\text{g kg}^{-1}$). Atropine also attenuated the increase in the plasma noradrenaline concentration produced by WIN55212-2.

Conscious rabbits receiving drugs intravenously (Figure 5)

Intravenous injection of solvent (0.5 ml kg^{-1}) did not change blood pressure, heart rate, renal sympathetic nerve activity and the plasma noradrenaline concentration. Three increasing doses of WIN55212-2 (5 , 50 and $500 \mu\text{g kg}^{-1}$) were injected intravenously. The two lower doses caused a slight hypotension, bradycardia and an increase in the firing rate of the renal sympathetic nerves. The increase in the plasma noradrenaline concentration observed after these doses of WIN55212-2 was not significant. The pattern of effects changed after the highest dose of WIN55212-2 ($500 \mu\text{g kg}^{-1}$): blood pressure decreased on average (see below), a tachycardia appeared, and sympathetic nerve activity and plasma noradrenaline returned to the baseline. The response to the highest dose of WIN55212-2 was not uniform in all the rabbits. One rabbit (from $n = 7$) was behaviourally excited and tried to jump in the cage; in this rabbit, blood pressure increased after the highest dose of WIN55212-2.

Pretreatment with SR141716A ($500 \mu\text{g kg}^{-1}$; i.v.) changed the effects of intravenously administered WIN55212-2 as follows. The bradycardia elicited by the two lower doses of WIN55212-2 (5 and $50 \mu\text{g kg}^{-1}$) was markedly reduced. The enhancement of sympathetic nerve activity by these doses of

WIN55212-2 was not changed by the antagonist. The effects produced by the highest WIN55212-2 dose ($500 \mu\text{g kg}^{-1}$) were modified by SR141716A to a greater extent. The hypotension produced by WIN55212-2 was slightly, but not significantly, attenuated. Instead of a tachycardia, the highest dose of WIN55212-2 produced a slight (non-significant) bradycardia. In the pretreated animals WIN55212-2 greatly increased renal sympathetic nerve firing and tended to increase the plasma noradrenaline concentration.

Discussion

The present results show that the mixed CB_1/CB_2 cannabinoid receptor agonist WIN55212-2 interferes with cardiovascular regulation by at least four mechanisms: prejunctional inhibition of transmitter release from postganglionic sympathetic neurons, central sympathoexcitation at the level of the brain stem, activation of cardiac vagal efferents at the level of the brain stem, and (at high systemic doses) central sympathoinhibition.

Peripheral prejunctional inhibition

In pithed rabbits with electrically stimulated sympathetic outflow, WIN55212-2 greatly reduced the spillover of noradrenaline into plasma, the plasma noradrenaline concentration and the blood pressure. The effects were mediated by specific cannabinoid receptors, because they were also elicited by another cannabinoid agonist from a different chemical class, CP55940, but not by WIN55212-3, the enantiomer of WIN55212-2 possessing very low affinity for cannabinoid binding sites. Antagonism of the effect of

Figure 5 Effects of i.v. injections of solvent (SOL) and WIN 55212-2 (WIN-2) on mean arterial pressure, heart rate, renal sympathetic nerve activity and plasma noradrenaline concentration in conscious rabbits. SOL (0.5 ml kg^{-1}) and WIN-2 (5, 50 and $500 \mu\text{g kg}^{-1}$) were injected as indicated by arrows. One of the two WIN-2-groups was pretreated at $t = -10 \text{ min}$ with SR141716A (SR; $500 \mu\text{g kg}^{-1}$; i.v.). Values are given as percentages of PRE values (Table 1). Means $\pm$ s.e. mean from six (SOL), seven (WIN-2) and seven (SR + WIN-2) experiments. Differences from SOL: * $P < 0.05$; differences from WIN-2: + $P < 0.05$.

WIN55212-2 by the CB_1 -selective antagonist SR141716A (Rinaldi-Carmona *et al.*, 1994; Pertwee, 1997) verifies involvement of CB_1 cannabinoid receptors. Lack of full antagonism of the effects of the higher WIN55212-2 doses is probably due to insufficient tissue concentration of the antagonist during this late phase of the experiments. However, involvement of CB_2 cannabinoid receptors in the effects of the higher WIN55212-2 doses cannot be ruled out (see Griffin *et al.*, 1997, for peripheral neuronal CB_2 receptors). The blood pressure decrease seen after administration of WIN55212-2 was solely due to diminished release of transmitter, since WIN55212-2 did not influence vascular tone raised by an infusion of noradrenaline.

The diminished release of the sympathetic transmitter was probably not due to ganglionic inhibition, since it was mediated by cannabinoid receptors (see above) and since agonists of CB_1 and CB_2 cannabinoid receptors (Δ^9 -tetrahydrocannabinol and anandamide) have no effect on ganglionic transmission (Cavero *et al.*, 1973a,b; Vollmer *et al.*, 1974; Varga *et al.*, 1996). The likely mechanism of the inhibition of transmitter release is activation of inhibitory presynaptic receptors on axon terminals of postganglionic sympathetic neurons. Such a mechanism is supported by *in vitro* observations: in mouse and rat vas deferens and rat heart, cannabinoids cause presynaptic inhibition (Pertwee *et al.*, 1992; Ishac *et al.*, 1996). Peripheral presynaptic inhibition of the release of sympathetic transmitter by cannabinoids in intact animals has been recently suggested by two groups. The suggestion by Varga *et al.* (1996) was based on their finding that cannabinoids reduced the increase in blood pressure, but not the increase in the sympathetic nerve firing rate, produced by electrical stimulation of presympathetic neurons in the

rostral ventrolateral medulla oblongata. In the experiments of Malinowska *et al.* (1997) on pithed rats, cannabinoids reduced the electrical stimulation-evoked increase in blood pressure, but not the noradrenaline-evoked increase in blood pressure. It is interesting to note that results fully compatible with peripheral presynaptic inhibition of noradrenaline release by Δ^9 -tetrahydrocannabinol were obtained by Cavero *et al.* already in 1973 (Cavero *et al.*, 1973b): in an anaesthetized dog preparation, in which the effect of Δ^9 -tetrahydrocannabinol was restricted to peripheral tissues, Δ^9 -tetrahydrocannabinol produced hypotension, and intact sympathetic innervation of the tissues was necessary for this effect. The authors did not conclude to peripheral presynaptic inhibition of noradrenaline release, probably because this type of neuromodulation was little known in 1973. Our results are the first demonstration of presynaptic inhibition of transmitter release from sympathetic neurons in a whole animal preparation by direct measurement of the transmitter. The inhibition of noradrenaline release and the hypotension was not accompanied by bradycardia in the present experiments. Bradycardia would be expected if noradrenaline release from cardiac sympathetic neurons were inhibited by a presynaptic mechanism. The explanation for the lack of a bradycardic response probably is the fact that cardiac sympathetic neurons are only weakly stimulated in our pithed rabbit preparation (see Szabo *et al.*, 1987).

The results obtained in pithed rabbits receiving a pressor infusion of noradrenaline permit two additional conclusions. Firstly, that WIN55212-2 has no direct effect on the heart. Secondly, and more importantly, that it does not cause direct vasoconstriction or vasodilatation. It was recently suggested that an endogenous cannabinoid may be involved in

endothelium-dependent vasorelaxation (Randall *et al.*, 1996; Randall & Kendall, 1997). Lack of vasodilatation by an exogenous mixed CB₁/CB₂ cannabinoid receptor agonist makes it unlikely (at least in the rabbit) that an endogenous cannabinoid can serve as a vasodilator.

Central sympathoexcitation

Injection of WIN55212-2 into the cisterna cerebellomedullaris, i.e. into the vicinity of medullary and pontine cardiovascular regulatory centres, increased sympathetic tone as indicated by the elevated plasma concentration of noradrenaline. This sympathoactivation led to hypertension even despite marked bradycardia (see below). Experiments with antagonists were not carried out in this part of the study. Yet, involvement of cannabinoid receptors in the sympathoexcitation is likely, since very low doses of the selective (see Introduction) cannabinoid agonist WIN55212-2 elicited the response. Moreover, intracisternally applied CP55940 also increases blood pressure and the firing rate of renal sympathetic nerves (Niederhoffer & Szabo, unpublished observation). Cardiovascular effects of cannabinoids applied directly into the central nervous system have been studied, to our knowledge, three times. In cats, injection of Δ^9 -tetrahydrocannabinol into the lateral cerebral ventricle elicited bradycardia without any change in blood pressure (Vollmer *et al.*, 1974). In dogs with isolated and perfused cerebral circulation, cerebral administration of Δ^9 -tetrahydrocannabinol caused bradycardia and hypotension (Cavero *et al.*, 1973a,b). Thus, direct excitation of centres regulating sympathetic tone by cannabinoids was first observed in the present study, and it was demonstrated by measurement of the sympathetic transmitter in the blood. Increases in blood pressure, heart rate or vascular resistance after systemic administration of Δ^9 -tetrahydrocannabinol have been repeatedly observed (Osgood & Howes, 1977; Jandhyala & Hamed, 1978; Benowitz *et al.*, 1979; Kawasaki *et al.*, 1980; Huestis *et al.*, 1992); the sympathoexcitation shown in our study may be the basis of these changes.

Central activation of cardiac vagal efferents

Injection of low doses of WIN55212-2 into the cisterna cerebellomedullaris led to dose-dependent and strong bradycardia. Enhancement of cardiac vagal tone is the likely mechanism, since atropine antagonized the effect. Preliminary results show that intracisternally injected CP55940 also produces bradycardia (Niederhoffer & Szabo, unpublished observation). The bradycardia was also observed after systemic administration of WIN55212-2 (5 and 50 $\mu\text{g kg}^{-1}$), and this latter effect was attenuated by SR141716A. The results, thus, indicate that activation of CB₁ cannabinoid receptors in the brain stem enhances cardiac vagal activity. The nucleus tractus solitarius and the nucleus dorsalis nervi vagi possess cannabinoid binding sites (Mailleux & Vanderhaeghen, 1992) and, hence, are likely primary sites of action of cannabinoids for eliciting bradycardia. As mentioned above, bradycardia after central nervous application of cannabinoids was also observed by Vollmer *et al.* (1974) and Cavero *et al.* (1973a), and it was attributed to a decrease in cardiac sympathetic tone (Vollmer *et al.*, 1974) and to enhanced cardiac vagal tone and to a decrease in cardiac sympathetic tone (Cavero *et al.*, 1973a). Bradycardia has also been observed after systemic administration of cannabinoids and was thought to be partly due to an increase in cardiac vagal tone, since atropine, methylatropine or vagotomy attenuated the effect (Graham & Li, 1973; Varga *et al.*, 1995; Vidrio *et al.*, 1996).

Cooperation of primary actions after the two lower doses of WIN55212-2

Three primary effects of the cannabinoid agonist WIN55212-2 on cardiovascular function have been discussed separately above. Here we try to explain how the three effects combine to produce the overall cardiovascular response to this drug after systemic administration in conscious rabbits. The two lower doses of WIN55212-2 (5 and 50 $\mu\text{g kg}^{-1}$) are considered at first. Peripheral prejunctional inhibition of noradrenaline release by WIN55212-2 certainly operated in this model, yet blood pressure decreased only minimally, and the plasma noradrenaline concentration did not decrease. The explanation is the simultaneously occurring central sympathoexcitation, evident from the increase in firing of the renal sympathetic nerves; the primary site of action is probably the brain stem. The baroreceptor reflex probably also operates and counteracts the depressive effect of the peripheral prejunctional inhibition on blood pressure. The bradycardia observed after systemic administration is most probably due to enhancement of efferent cardiac vagal activity with a primary action in the brain stem.

Cooperation of primary actions after the highest dose of WIN55212-2: indication for central sympathoinhibition

The effects of the highest i.v. dose of WIN55212-2 (500 $\mu\text{g kg}^{-1}$) in conscious rabbits are more complex and not completely understood. Though blood pressure decreased markedly after this dose, sympathetic activity did not increase further (which would be expected from the central sympathoexcitation and from the function of the baroreflex), but declined toward the baseline which it reached at the end of the 14-min observation period; plasma noradrenaline decreased simultaneously. We interpret this decline in sympathetic activity as central sympathoinhibition. It is probably mediated by CB₁ receptors, because in animals pretreated with SR141716A, sympathetic nerve activity and the plasma noradrenaline concentration increased after the highest dose of WIN55212-2. The origin of the sympathoinhibition produced by WIN55212-2 is not known: sites of action rostrally or caudally from the brain stem, including the spinal cord, are all possible. Ganglionic inhibition cannot be excluded but seems unlikely, as discussed above. Not only sympathetic nerve activity and plasma noradrenaline changed their response pattern after the highest dose of WIN55212-2. The heart rate response changed as well: heart rate decreased after the two lower doses but increased after the highest dose. The mechanism of this tachycardia is not known, but baroreflex-mediated withdrawal of vagal tone in response to the hypotension is one possibility. As mentioned in the Introduction, Δ^9 -tetrahydrocannabinol decreased blood pressure and heart rate in many experimental models (mostly anaesthetized animals) and this was often attributed to a central sympathoinhibition. The sympathoinhibition has been demonstrated directly only by Vollmer *et al.* (1974) by measurement of cardiac sympathetic nerve activity.

The present study reveals complex effects of a mixed CB₁/CB₂ cannabinoid receptor agonist on the cardiovascular system, including excitatory and inhibitory components. Enhancement and depression of cardiovascular function are both observed in humans consuming *Cannabis* products or receiving the main active component of *Cannabis*, Δ^9 -tetrahydrocannabinol. For example, recrea-

tional doses of cannabinoids usually produce tachycardia with a slight increase in blood pressure (Benowitz *et al.*, 1979; Huestis *et al.*, 1992). In contrast, high doses or long-term application of cannabinoids can elicit orthostatic hypotension, hypotension and bradycardia (Benowitz & Jones, 1975).

References

- ADAMS, M.D., CHAIT, L.D. & EARNHARDT, J.T. (1976). Tolerance to the cardiovascular effects of Δ^9 -tetrahydrocannabinol in the rat. *Br. J. Pharmacol.*, **56**, 43–48.
- BENOWITZ, N.L. & JONES, R.T. (1975). Cardiovascular effects of prolonged delta-9-tetrahydrocannabinol ingestion. *Clin. Pharmacol. Ther.*, **18**, 287–297.
- BENOWITZ, N.L., ROSENBERG, J., ROGERS, W., BACHMAN, J. & JONES, R.T. (1979). Cardiovascular effects of intravenous delta-9-tetrahydrocannabinol: Autonomic nervous mechanisms. *Clin. Pharmacol. Ther.*, **25**, 440–446.
- BIRMINGHAM, M.K. (1973). Reduction by Δ^9 -tetrahydrocannabinol in the blood pressure of hypertensive rats bearing regenerated adrenal glands. *Br. J. Pharmacol.*, **48**, 169–171.
- CAVERO, I., LOKHANDWALA, M.F., BUCKLEY, J.P. & JANDHYALA, B.S. (1974). The effect of (–)- Δ^9 -trans-tetrahydrocannabinol on myocardial contractility and venous return in anesthetized dogs. *Eur. J. Pharmacol.*, **29**, 74–82.
- CAVERO, I., SOLOMON, T., BUCKLEY, J.P. & JANDHYALA, B.S. (1973a). Studies on the bradycardia induced by (–)- Δ^9 -trans-tetrahydrocannabinol in anesthetized dogs. *Eur. J. Pharmacol.*, **22**, 263–269.
- CAVERO, I., SOLOMON, T., BUCKLEY, J.P. & JANDHYALA, B.S. (1973b). Studies on the hypotensive activity of (–)- Δ^9 -trans-tetrahydrocannabinol in anesthetized dogs. *Res. Comm. Chem. Pathol. Pharmacol.*, **6**, 527–540.
- COMPTON, D.R., HARRIS, L.S., LICHTMAN, A.H. & MARTIN, B.R. (1996). Marijuana. In *Pharmacological Aspects of Drug Dependence. Handbook of Experimental Pharmacology*. eds. Schuster, C.R. & Kuhar, M.J. pp. 83–158. Springer: Heidelberg.
- DEWEY, W.L. (1986). Cannabinoid pharmacology. *Pharmacol. Rev.*, **38**, 151–178.
- ESLER, M., JENNINGS, G., LAMBERT, G., MEREDITH, I., HORNE, M. & EISENHOFER, G. (1990). Overflow of catecholamine neurotransmitters to the circulation: source, fate, and functions. *Physiol. Rev.*, **70**, 963–985.
- ESTRADA, U., BRASE, D.A., MARTIN, B.R. & DEWEY, W.L. (1987). Cardiovascular effects of Δ^9 - and $\Delta^9(11)$ -tetrahydrocannabinol and their interaction with epinephrine. *Life Sci.*, **41**, 79–87.
- FELDER, C.C., JOYCE, K.E., BRILEY, E.M., MANSOURI, J., MACKIE, K., BLOND, O., LAI, Y., MA, A.L. & MITCHELL, R.L. (1995). Comparison of the pharmacology and signal transduction of the human cannabinoid CB₁ and CB₂ receptors. *Molec. Pharmacol.*, **48**, 443–450.
- FREDERICKS, A.B., BENOWITZ, N.L. & SAVANAPRIDI, C.Y. (1981). The cardiovascular and autonomic effects of repeated administration of Δ -9-tetrahydrocannabinol to rhesus monkeys. *J. Pharmacol. Exp. Ther.*, **216**, 247–253.
- GRAHAM, J.D.P. & LI, D.M.F. (1973). Cardiovascular and respiratory effects of cannabis in cat and rat. *Br. J. Pharmacol.*, **49**, 1–10.
- GRIFFIN, G., FERNANDO, S.R., ROSS, R.A., McKAY, N.G., ASHFORD, M.L.J., SHIRE, D., HUFFMAN, J.W., YU, S., LAINTON, J.A.H. & PERTWEE, R.G. (1997). Evidence for the presence of CB₂-like cannabinoid receptors on peripheral nerve terminals. *Eur. J. Pharmacol.*, **339**, 53–61.
- HOWLETT, A.C. (1995). Pharmacology of cannabinoid receptors. *Ann. Rev. Pharmacol. Toxicol.*, **35**, 607–634.
- HUESTIS, M.A., SAMPSON, A.H., HOLICKY, B.J., HENNINGFIELD, J.E. & CONE, E.J. (1992). Characterization of the absorption phase of marijuana smoking. *Clin. Pharmacol. Ther.*, **52**, 31–41.
- ISHAC, E.J.N., JIANG, L., LAKE, K.D., VARGA, K., ABOOD, M.E. & KUNOS, G. (1996). Inhibition of exocytotic noradrenaline release by presynaptic cannabinoid CB₁ receptors on peripheral sympathetic nerves. *Br. J. Pharmacol.*, **118**, 2023–2028.
- JANDHYALA, B.S. & HAMED, A.T. (1978). Pulmonary and systemic hemodynamic effects of Δ^9 -tetrahydrocannabinol in conscious and morphine-chloralose-anesthetized dogs: anesthetic influence on drug action. *Eur. J. Pharmacol.*, **53**, 63–68.
- KAWASAKI, H., WATANABE, S. & UEKI, S. (1980). Effects of chronic administration of Δ^9 -tetrahydrocannabinol on the cardiovascular system, and pressor and behavioral responses to brain stimulation in freely moving rats. *Eur. J. Pharmacol.*, **65**, 63–69.
- KUSTER, J.E., STEVENSON, J.L., WARD, S.J., D'AMBRA, T.E. & HAYCOCK, D.A. (1993). Aminoalkylindole binding in rat cerebellum: selective displacement by natural and synthetic cannabinoids. *J. Pharmacol. Exp. Ther.*, **264**, 1352–1363.
- LAKE, K.D., COMPTON, D.R., VARGA, K., MARTIN, B.R. & KUNOS, G. (1997a). Cannabinoid-induced hypotension and bradycardia in rats is mediated by CB₁-like cannabinoid receptors. *J. Pharmacol. Exp. Ther.*, **281**, 1030–1037.
- LAKE, K.D., MARTIN, B.R., KUNOS, G. & VARGA, K. (1997b). Cardiovascular effects of anandamide in anesthetized and conscious normotensive and hypertensive rats. *Hypertension*, **29**, 1204–1210.
- MAILLEUX, P. & VANDERHAEGHEN, J.J. (1992). Distribution of neuronal cannabinoid receptor in the adult rat brain: a comparative receptor binding radioautography and *in situ* hybridization histochemistry. *Neuroscience*, **48**, 655–668.
- MALINOWSKA, B., GODLEWSKI, G., BUCHER, B. & SCHLICKER, E. (1997). Cannabinoid CB₁ receptor-mediated inhibition of the neurogenic vasopressor response in the pithed rat. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **356**, 197–202.
- MARTIN, B.R. (1986). Cellular effects of cannabinoids. *Pharmacol. Rev.*, **38**, 45–74.
- MATSUDA, L.A., LOLAIT, S.J., BROWNSTEIN, B.J., YOUNG, A.C. & BONNER, T.I. (1990). Structure of a cannabinoid receptor and functional expression of the cloned cDNA. *Nature*, **346**, 561–564.
- MUNRO, S., THOMAS, K.L. & ABU-SHAAR, M. (1993). Molecular characterization of a peripheral receptor for cannabinoids. *Nature*, **365**, 61–65.
- OSGOOD, P.F. & HOWES, J.F. (1977). Δ^9 -tetrahydrocannabinol and dimethyl-heptylpyran induced tachycardia in the conscious rat. *Life Sci.*, **21**, 1329–1336.
- PERTWEE, R.G. (1997). Pharmacology of cannabinoid CB₁ and CB₂ receptors. *Pharmacol. Ther.*, **74**, 129–180.
- PERTWEE, R.G., STEVENSON, L.A., ELRICK, D.B., MECHOULAM, R. & CORBETT, A.D. (1992). Inhibitory effects of certain enantiomeric cannabinoids in the mouse vas deferens and the myenteric plexus preparation of guinea-pig small intestine. *Br. J. Pharmacol.*, **105**, 980–984.
- RANDALL, M.D., ALEXANDER, S.P.H., BENNETT, T., BOYD, E.A., FRY, J.R., GARDINER, S.M., KEMP, P.A., MCCULLOCH, A.I. & KENDALL, D.A. (1996). An endogenous cannabinoid as an endothelium-derived vasorelaxant. *Biochem. Biophys. Res. Comm.*, **229**, 114–120.
- RANDALL, M.D. & KENDALL, D.A. (1997). Involvement of a cannabinoid in endothelium-derived hyperpolarizing factor-mediated coronary vasorelaxation. *Eur. J. Pharmacol.*, **335**, 205–209.
- RINALDI-CARMONA, M., BARTH, F., HEAULME, M., SHIRE, D., CALANDRA, B., CONGY, C., MARTINEZ, S., MARUANI, J., NELIAT, G., CAPUT, D., FERRARA, P., SOUBRIE, P., BRELIERE, J.C. & LE FUR, G. (1994). SR141716A, a potent and selective antagonist of the brain cannabinoid receptor. *FEBS Lett.*, **350**, 240–244.
- SHOWALTER, V.M., COMPTON, D.R., MARTIN, B.R. & ABOOD, M.A. (1996). Evaluation of binding in a transfected cell line expressing a peripheral cannabinoid receptor (CB₂): identification of cannabinoid receptor subtype selective ligands. *J. Pharmacol. Exp. Ther.*, **278**, 989–999.
- STARK, P. & DEWS, P.B. (1980). Cannabinoids. II. Cardiovascular effects. *J. Pharmacol. Exp. Ther.*, **214**, 131–138.

The authors gratefully acknowledge the advice and support of Klaus Starke. The help of Claudia Schurr at determining plasma catecholamines was essential. We thank Pfizer (Groton, CT, U.S.A.) and Sanofi (Montpellier, France) for generous supply of CP55940 and SR141716A, respectively. The study was supported by the Deutsche Forschungsgemeinschaft (Sz 72/2-2). Dr Nathalie Niederhoffer is recipient of an Alexander von Humboldt fellowship.

- STEIN, E.A., FULLER, S.A., EDGEMOND, W.S. & CAMPBELL, W.B. (1996). Physiological and behavioural effects of the endogenous cannabinoid, arachidonylethanolamide (anandamide), in the rat. *Br. J. Pharmacol.*, **119**, 107–114.
- SZABO, B., HEDLER, L., LICHTWALD, K. & STARKE, K. (1987). ACTH increases noradrenaline release in pithed rabbits with electrically stimulated sympathetic outflow. *Eur. J. Pharmacol.*, **136**, 391–399.
- SZABO, B. & SCHULTHEISS, A. (1990). Desipramine inhibits sympathetic nerve activity in the rabbit. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **342**, 469–476.
- SZABO, B., URBAN, R., LIMBERGER, N. & STARKE, K. (1995). Cardiovascular effects of agmatine, a 'clonidine-displacing substance', in conscious rabbits. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **351**, 268–273.
- SZABO, B., URBAN, R. & STARKE, K. (1993). Sympathoinhibition by rilmenidine in conscious rabbits: involvement of α_2 -adrenoceptors. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **348**, 593–600.
- URBAN, R., SZABO, B. & STARKE, K. (1995). Involvement of peripheral presynaptic inhibition in the reduction of sympathetic tone by moxonidine, rilmenidine and UK 14304. *Eur. J. Pharmacol.*, **282**, 29–37.
- VARGA, K., LAKE, K.D., HUANGFU, D., GUYENET, P.G. & KUNOS, G. (1996). Mechanism of the hypotensive action of anandamide in anesthetized rats. *Hypertension*, **28**, 682–686.
- VARGA, K., LAKE, K., MARTIN, B.R. & KUNOS, G. (1995). Novel antagonist implicates the CB₁ cannabinoid receptor in the hypotensive action of anandamide. *Eur. J. Pharmacol.*, **278**, 279–283.
- VIDRIO, H., SANCHEZ-SALVATORI, M.A. & MEDINA, M. (1996). Cardiovascular effects of (–)-11-OH- Δ^8 -tetrahydrocannabinol-dimethylheptyl in rats. *J. Cardiovasc. Pharmacol.*, **28**, 332–336.
- VOLLMER, R.R., CAVERO, I., ERTEL, R.J., SOLOMON, T.A. & BUCKLEY, J.P. (1974). Role of the central autonomic nervous system in the hypotension and bradycardia induced by (–)- Δ^9 -trans-tetrahydrocannabinol. *J. Pharm. Pharmacol.*, **26**, 186–192.

(Received June 4, 1998
Revised October 24, 1998
Accepted November 4, 1998)