

Cannabinoids Cause Central Sympathoexcitation and Bradycardia in Rabbits¹

NATHALIE NIEDERHOFFER and BELA SZABO

Institut für Pharmakologie und Toxikologie, Albert-Ludwigs-Universität, Freiburg, Germany

Accepted for publication April 10, 2000 This paper is available online at <http://www.jpet.org>

ABSTRACT

Systemically administered cannabinoids elicit marked cardiovascular effects, and the role of the central and the peripheral nervous system in these effects is not clarified. The aim of this study was to characterize the actions of cannabinoids on cardiovascular regulatory centers in conscious rabbits. A catheter for administration of drugs into the cisterna cerebellomedullaris and an electrode for recording renal sympathetic nerve activity were implanted under halothane anesthesia. Experiments were carried out later in conscious animals. Two cannabinoid receptor agonists were injected intracisternally: the aminoalkylindole WIN55212-2 (0.1, 1, and 10 $\mu\text{g kg}^{-1}$) and the bicyclic Δ^9 -tetrahydrocannabinol analog CP55940 (0.1, 1, and 10 $\mu\text{g kg}^{-1}$). WIN55212-2 and CP55940 dose dependently increased renal sympathetic nerve activity and the plasma noradrenaline concentration and also lowered the heart rate. The highest doses of

WIN55212-2 and CP55940 increased blood pressure. In contrast, intracisternal injection of WIN55212-3 (0.1, 1, and 10 $\mu\text{g kg}^{-1}$), an enantiomer of WIN55212-2 with very low affinity for cannabinoid binding sites, had no effects. The CB₁ cannabinoid receptor antagonist SR141716A (0.5 mg kg^{-1} , i.v.) attenuated the effects of intracisternally administered WIN55212-2 (0.1, 1, and 10 $\mu\text{g kg}^{-1}$). The results indicate that cannabinoids, acting directly on cardiovascular regulatory centers, elicit sympathoactivation and bradycardia. These effects were likely mediated by CB₁ cannabinoid receptors, because they were elicited by two cannabinoid agonists belonging to different chemical classes (WIN55212-2 and CP55940), but not by the inactive enantiomer WIN55212-3, and because they were attenuated by the CB₁ cannabinoid receptor antagonist SR141716A.

Several effects of natural and synthetic cannabinoid agonists are well characterized in humans and experimental animals, e.g., euphoria, analgesia, change in locomotion, cataplexy, temperature reduction, and memory disturbance (for review, see Dewey, 1986; Howlett, 1995a; Compton et al., 1996; Pertwee, 1997).

In contrast, although cannabinoids elicit prominent cardiovascular effects, relatively little is known on the mechanism of these effects (for review, see Dewey, 1986; Compton et al., 1996; Wagner et al., 1998). In conscious humans, cardiovascular responses to acute administration of Δ^9 -tetrahydrocannabinol include a marked tachycardia and a small increase in blood pressure (Benowitz et al., 1979; Huestis et al., 1992). In anesthetized animals, systemically administered cannabinoids generally lower blood pressure and heart rate [e.g., rat (Vidrio et al., 1996; Lake et al., 1997), dog (Jandhyala and Hamed, 1978), and cat (Vollmer et al., 1974)]. In conscious animals, cannabinoids either cause moderate cardiovascular depression [e.g., rat (Vidrio et al., 1996); dog (Stark and Dews, 1980)] or, more often, have no effect or elicit hyperten-

sion or tachycardia [e.g., rat (Osgood and Howes, 1977; Kosersky, 1978; Kawasaki et al., 1980; Stein et al., 1996; Lake et al., 1997), dog (Jandhyala and Hamed, 1978; Stark and Dews, 1980)]. In most of these studies, only blood pressure and heart rate were measured, and the location of the effects to peripheral and central components of the cardiovascular regulatory system was not possible.

Only a few experiments were carried out to characterize the effects of cannabinoids on cardiovascular regulatory centers. In previous studies in anesthetized animals, centrally administered Δ^9 -tetrahydrocannabinol caused central sympathoinhibition and enhancement of cardiac vagal tone (Cavero et al., 1973a,b; Vollmer et al., 1974), whereas systemically administered anandamide had no central cardiovascular effect (Varga et al., 1996). In a recent study (Niederhoffer and Szabo, 1999), we injected the synthetic cannabinoid receptor agonist WIN55212-2 into the cisterna cerebellomedullaris of conscious rabbits to observe its effects on cardiovascular centers in the medulla oblongata. WIN55212-2 increased the plasma noradrenaline concentration, indicating central sympathoexcitation; consequently, blood pressure increased. WIN55212-2 also caused marked bradycardia. All these studies did not allow a conclusion to be

Received for publication January 18, 2000.

¹ This work was supported by grants from the Deutsche Forschungsgemeinschaft (Sz 72/2-2) and the Institut National de la Santé et de la Recherche Médicale (France).

ABBREVIATIONS: PRE, average of initial values (before administration of cannabinoid agonists); i.c., intracisternal.

made on the involvement of specific cannabinoid receptors in the central cardiovascular effects of cannabinoids.

In these experiments, we further analyzed the effects of cannabinoids on medullary cardiovascular centers in conscious rabbits. The first aim was to study whether specific cannabinoid receptors are involved in the effects of intracisternally given cannabinoid agonists. To this end, the effects of WIN55212-2 were compared with the effects of WIN55212-3 and CP55940. WIN55212-3 is an enantiomer of WIN55212, and its affinity for cannabinoid binding sites is very low (Kuster et al., 1993; Pertwee, 1997). The chemical structure of CP55940 (a bicyclic compound structurally resembling Δ^9 -tetrahydrocannabinol) differs markedly from that of WIN55212-2 (an aminoalkylindole) (Pertwee, 1997). The interaction between WIN55212-2 and the CB₁ cannabinoid receptor antagonist SR141716A (Rinaldi-Carmona et al., 1994; Pertwee, 1997) was also studied. The second aim was to observe centrally elicited changes in sympathetic tone directly; to this end, sympathetic nerve activity was recorded.

Materials and Methods

Experiments were carried out on 21 rabbits of a local breed (derived from Deutscher Riesenscheck, obtained from Ketterer, Reute, Germany). Rabbits were of both sexes and weighed 1.8 to 3.3 kg.

Implantation of Catheters and Electrodes. In a first operation, a catheter was implanted into the cisterna cerebellomedullaris under halothane anesthesia (1.5–4%) in spontaneously breathing rabbits (for details, see Szabo et al., 1995). Briefly, a polyethylene catheter (i.d., 0.28 mm; o.d., 0.61 mm; length, 25 cm) was inserted 8 mm into the cisterna cerebellomedullaris through a hole in the atlanto-occipital membrane. The free end of the catheter was tunneled to an incision in the neck, and the wounds were sutured.

A second operation was performed after 2 weeks of recovery. Under halothane anesthesia (1.5–4%) in spontaneously breathing rabbits, an electrode was implanted for recording activity of postganglionic renal sympathetic nerves (for details, see Szabo et al., 1993). Briefly, 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 the electrode were embedded in silicone gel. The free end of the electrode was tunneled to an incision in the neck, and the wounds were sutured.

Experiments in Conscious Rabbits. The first experiment in the conscious animal was carried out 3 to 4 days after implantation of the renal nerve electrode. Two to three experiments were carried out on one rabbit at 3- to 4-day intervals. No animal received a given treatment twice. After the last experiment, the animals were sacrificed by an overdose of pentobarbitone.

On the day experiments were performed in conscious animals, the central ear artery was cannulated under local anesthesia for recording arterial blood pressure and heart rate and for blood sampling. Arterial pressure was recorded with a Statham P23Db transducer coupled to a bridge amplifier (Hugo Sachs Elektronik, Hugstetten, Germany); heart rate was calculated from the pulsating blood pressure signal by an integrator (Hugo Sachs Elektronik). A marginal ear vein was also cannulated; this served for reinjection of erythrocytes (resuspended in saline) of blood samples and, in some experiments, for administration of the CB₁ cannabinoid receptor antagonist SR141716A. Also under local anesthesia, the intracisternal (i.c.) catheter and electrode leads were recovered from under the skin.

The plasma noradrenaline concentration was determined as previously described (Szabo and Schultheiss, 1990). Briefly, 2-ml blood samples were obtained, and plasma catecholamines were determined by alumina chromatography followed by HPLC and electrochemical detection.

Protocol and Statistics. Parameters were first determined 45 min ($t = 0$ min) after recovery of the catheter and electrode leads. Either solvent (SOL) ($25 \mu\text{l kg}^{-1}$) or increasing doses of WIN55212-2 ($0.1, 1, \text{ and } 10 \mu\text{g kg}^{-1}$), WIN55212-3 ($0.1, 1, \text{ and } 10 \mu\text{g kg}^{-1}$), or CP55940 ($0.1, 1, \text{ and } 10 \mu\text{g kg}^{-1}$) were injected i.c. at $t = 19, 37, \text{ and } 55$ min (for protocol, see Fig. 1). In experiments with the CB₁ cannabinoid receptor antagonist SR141716A, the antagonist (0.5 mg kg^{-1}) was injected i.v. at $t = -10$ min; WIN55212-2 ($0.1, 1, \text{ and } 10 \mu\text{g kg}^{-1}$) was injected i.c. at $t = 19, 37, \text{ and } 55$ min. In all experiments, blood pressure and heart rate (and in most groups also renal sympathetic nerve activity) were read every 2 min from $t = 0$ to 68 min. Blood was sampled at $t = 0, 14, 32, 50, \text{ and } 68$ min for the determination of the plasma noradrenaline concentration. In each experiment, values measured at $t = 0$ and 14 min were averaged to yield the average of initial values before administration of cannabinoid

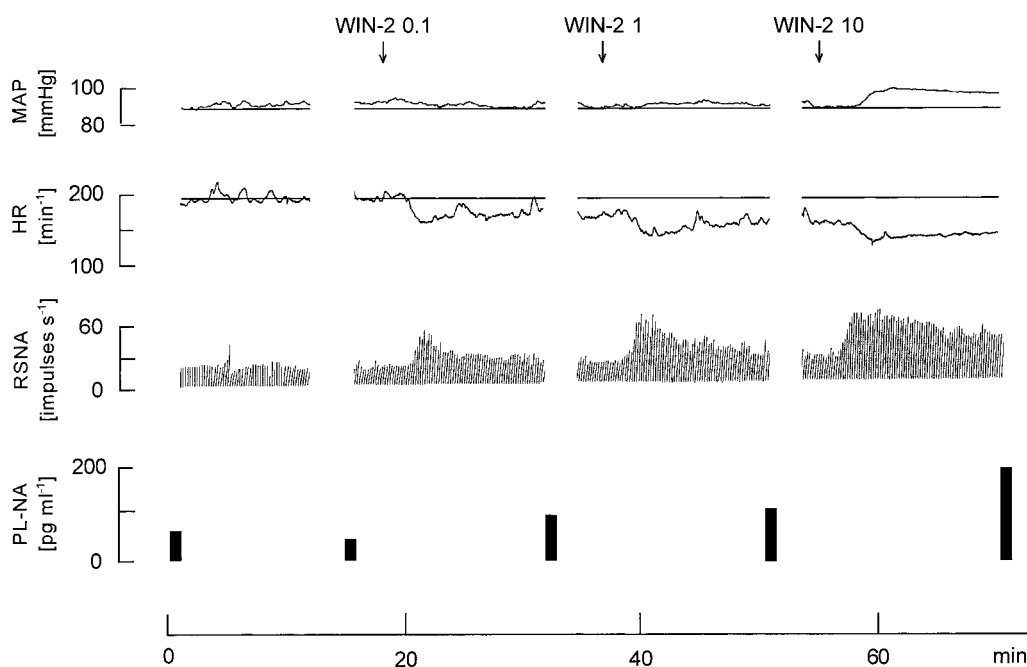


Fig. 1. Effect of WIN55212-2 (WIN-2) on mean arterial pressure (MAP), heart rate (HR), renal sympathetic nerve activity (RSNA), and plasma noradrenaline concentration (PL-NA) in a conscious rabbit (original tracings). WIN55212-2 ($0.1, 1, 10 \mu\text{g kg}^{-1}$) was injected i.c., as indicated by the arrows.

agonists (PRE values), and all values were expressed as percentages of PRE.

Data were analyzed with the SPSS for Windows statistical program (version 8.0.0; SPSS, Chicago, IL). Means $\pm$ S.E. of n experiments are given throughout. Two-way ANOVA coupled with Scheffé's test was used to identify significant differences; $P < .05$ was taken as the limit of statistical significance, and only this level is indicated even if P was $<.01$ or $<.001$.

Drugs. Drugs were obtained from the following sources: (–)-*cis*-3-[2-hydroxy-4-(1,1-dimethylheptyl)phenyl]-*trans*-4-(3-hydroxypropyl)cyclohexanol (CP55940) from Pfizer (Groton, CT); 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 Recherche (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 Research Biochemicals International (Köln, Germany).

WIN55212-2, WIN55212-3, and CP55940 were dissolved and further diluted in a 7.5% 2-hydroxypropyl- β -cyclodextrin solution (w/v in distilled water); they were injected i.c. in a volume of $25 \mu\text{l kg}^{-1}$ within 1 min. SR141716A was dissolved in a vehicle containing ethanol/Emulphor (Rhône-Poulenc, Cranberry, NJ)/saline (1:1:18, v/v); it was injected i.v. in a volume of 0.5 ml kg^{-1} . Doses refer to the salts.

Results

Baseline Parameters (PRE Values). After an initial stabilization period, baseline parameters were determined twice (at $t = 0$ and 14 min), and the values were averaged to obtain the PRE values. Mean PRE values in animals without

pretreatment were: mean arterial pressure, $70 \pm 1 \text{ mm Hg}$ ($n = 32$); heart rate, $211 \pm 6 \text{ min}^{-1}$ ($n = 32$); renal sympathetic nerve activity, $33 \pm 3 \text{ impulses s}^{-1}$ ($n = 24$); and plasma noradrenaline concentration, $237 \pm 30 \text{ pg ml}^{-1}$ ($n = 32$). Similar values were obtained in previous studies using similar preparations (Szabo et al., 1993, 1995; Niederhoffer and Szabo, 1999). For further evaluation, values in any given experiment were expressed as percentages of PRE.

Control Experiments. Injection of the solvent (three times $25 \mu\text{l kg}^{-1}$) into the cisterna cerebellomedullaris caused no change in mean arterial pressure, heart rate, renal sympathetic nerve activity, or the plasma noradrenaline concentration (Fig. 2).

Effects of WIN55212-2 and WIN55212-3. The effects of the aminoalkylindole cannabinoid agonist WIN55212-2 are shown in Fig. 1 (original tracings) and Fig. 2 (statistical evaluation). Three increasing doses (0.1, 1, and $10 \mu\text{g kg}^{-1}$) were injected i.c. Blood pressure was not changed after the two lower doses but was significantly elevated after the highest dose. Central application of WIN55212-2 also elicited pronounced and dose-dependent bradycardia and dose-dependent increases in renal sympathetic nerve activity and plasma noradrenaline concentration. Effects of WIN55212-2 were compared with those of its inactive stereoisomer, WIN55212-3 (Fig. 2). Increasing doses of WIN55212-3 (0.1, 1, and $10 \mu\text{g kg}^{-1}$; same doses as of WIN55212-2) had no effect on blood pressure, heart rate, and plasma noradrenaline concentration.

Effects of CP55940. The effects of the bicyclic analog of Δ^9 -tetrahydrocannabinol, CP55940, are shown in Fig. 3. Three increasing doses (0.1, 1, and $10 \mu\text{g kg}^{-1}$) were injected

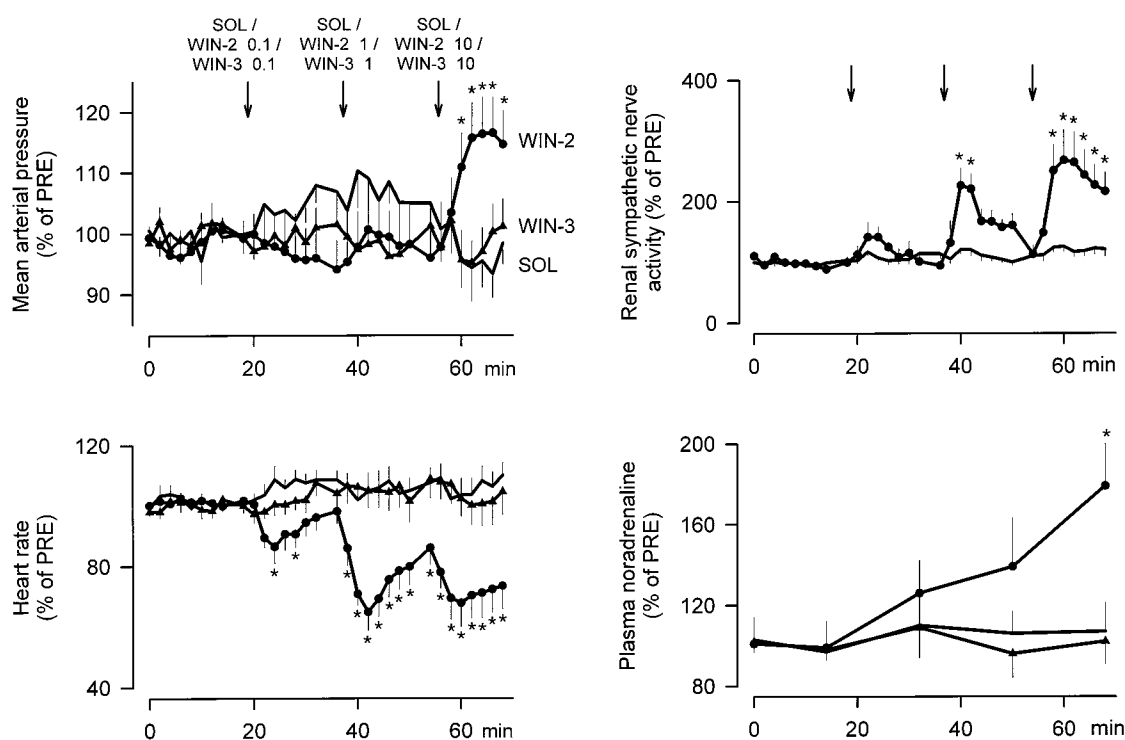


Fig. 2. Effects of solvent (SOL), WIN55212-2 (WIN-2), and WIN55212-3 (WIN-3) on mean arterial pressure, heart rate, renal sympathetic nerve activity, and plasma noradrenaline concentration in conscious rabbits. SOL ($25 \mu\text{l kg}^{-1}$), WIN-2 (0.1, 1, and $10 \mu\text{g kg}^{-1}$), and WIN-3 (0.1, 1, and $10 \mu\text{g kg}^{-1}$) were injected i.c., as indicated by the arrows. Values are given as percentages of PRE. Means $\pm$ S.E. are from eight (SOL), eight (WIN-2), and eight (WIN-3) experiments. Differences from SOL: * $P < .05$. Renal sympathetic nerve activity was not determined in the group that received WIN-3.

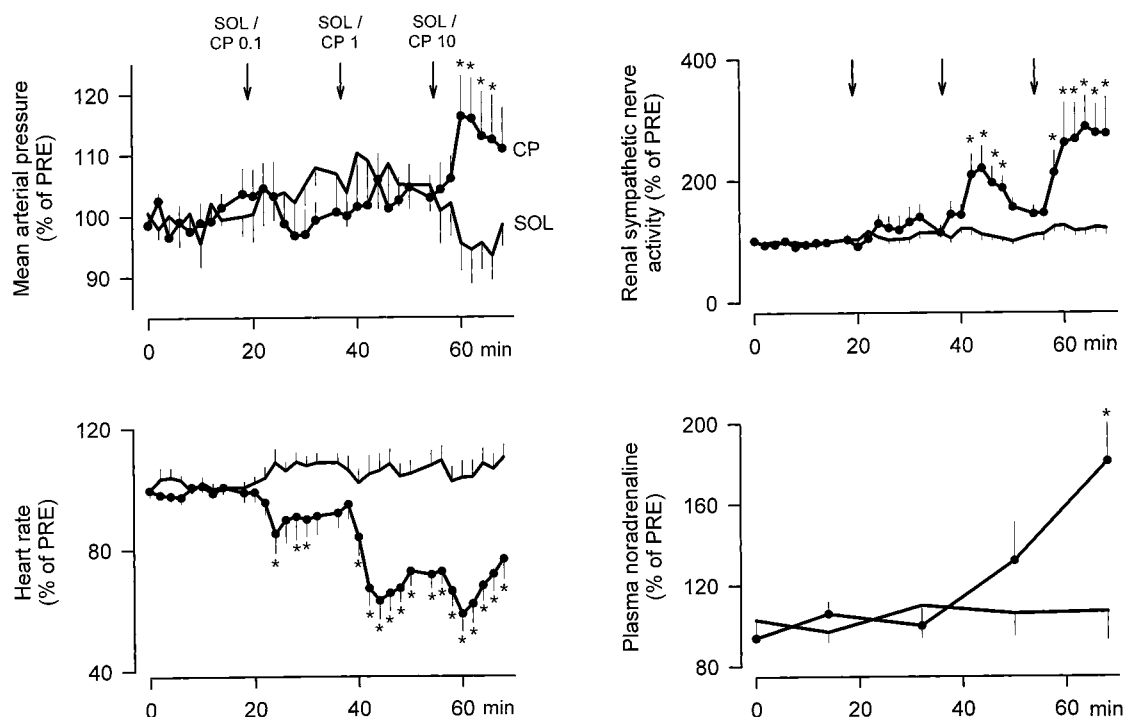


Fig. 3. Effects of solvent (SOL) and CP55940 (CP) on mean arterial pressure, heart rate, renal sympathetic nerve activity, and plasma noradrenaline concentration in conscious rabbits. SOL ($25 \mu\text{l kg}^{-1}$) and CP (0.1 , 1 , and $10 \mu\text{g kg}^{-1}$) were injected i.c., as indicated by the arrows. Values are given as percentages of PRE. Means $\pm$ S.E. are from eight (SOL) and eight (CP) experiments. Differences from SOL: $*P < .05$. The SOL group is identical with the SOL group in Fig. 2.

i.c. The pattern of effects was very similar to that observed after administration of WIN55212-2. Thus, blood pressure increased significantly after the highest dose. CP55940 also elicited a dose-dependent bradycardia, a dose-dependent increase in renal sympathetic nerve firing, and a dose-dependent increase in plasma noradrenaline concentration.

Interaction between SR141716A and WIN55212-2. The CB_1 cannabinoid receptor antagonist SR141716A (0.5 mg kg^{-1}) was injected i.v. at $t = -10 \text{ min}$. It had no effect on the measured parameters. Thus, the PRE values of blood pressure, $70 \pm 3 \text{ mm Hg}$ ($n = 8$), heart rate, $223 \pm 11 \text{ min}^{-1}$ ($n = 8$), RSNA, $37 \pm 5 \text{ impulses s}^{-1}$ ($n = 7$), and plasma noradrenaline concentration, $209 \pm 31 \text{ pg ml}^{-1}$ ($n = 8$) did not significantly differ from the values measured in untreated animals (see above). However, pretreatment with SR141716A attenuated the effects of WIN55212-2 (Fig. 4). The highest dose of WIN55212-2 no longer significantly increased blood pressure in the SR141716A-pretreated animals. The bradycardia and the increase in sympathetic nerve activity observed after WIN55212-2 at 1 and $10 \mu\text{g kg}^{-1}$ were attenuated. The increase in plasma noradrenaline concentration observed after the highest dose of WIN55212-2 was also significantly antagonized by SR141716A.

Discussion

Administration of cannabinoids into the vicinity of cardiovascular centers in the medulla oblongata elicited two primary effects: sympathetic tone increased and heart rate decreased. The new finding of this study is that CB_1 cannabinoid receptors are involved in these effects.

An increase in sympathetic tone was indicated by changes in two parameters, plasma noradrenaline concentration and

RSNA. These results confirm our previous observation of a centrally elicited increase in plasma noradrenaline concentration after i.c. administration of a cannabinoid agonist (Niederhoffer and Szabo, 1999). The increase in plasma noradrenaline concentration indicates enhanced noradrenaline release in many sympathetically innervated tissues; it is likely that vascular resistance increases in these tissues. This study demonstrates for the first time the central sympathoexcitatory effect of cannabinoids by direct electrical measurement of sympathetic nerve activity. We measured activity of renal sympathetic nerves because they reflect the function of many baroreceptor reflex-controlled sympathetic nerves. The functional consequence of the increased activity of renal sympathetic nerves is an increase in renal vascular resistance (Malpas et al., 1996).

Involvement of the central nervous system in the effects of cannabinoids on sympathetic tone and blood pressure has been seldom studied previously. Using the head cross-circulation experimental model in dogs, Cavero et al. (1973a) concluded that Δ^9 -tetrahydrocannabinol centrally depresses sympathetic tone. Blood pressure decreased in cats after injection of Δ^9 -tetrahydrocannabinol into the lateral cerebral ventricle, indicating central sympathoinhibition (Vollmer et al., 1974). In rats, the putative endogenous cannabinoid anandamide elicited hypotension through peripheral sites of action; no centrally elicited effect on the firing rate of presympathetic sympathoexcitatory neurons in the rostral ventrolateral medulla oblongata and of splanchnic sympathetic nerve fibers was evident (Varga et al., 1996). The three studies noted above were carried out on anesthetized animals. Central sympathoexcitation was observed only in our experiments in conscious animals (Niederhoffer and Szabo,

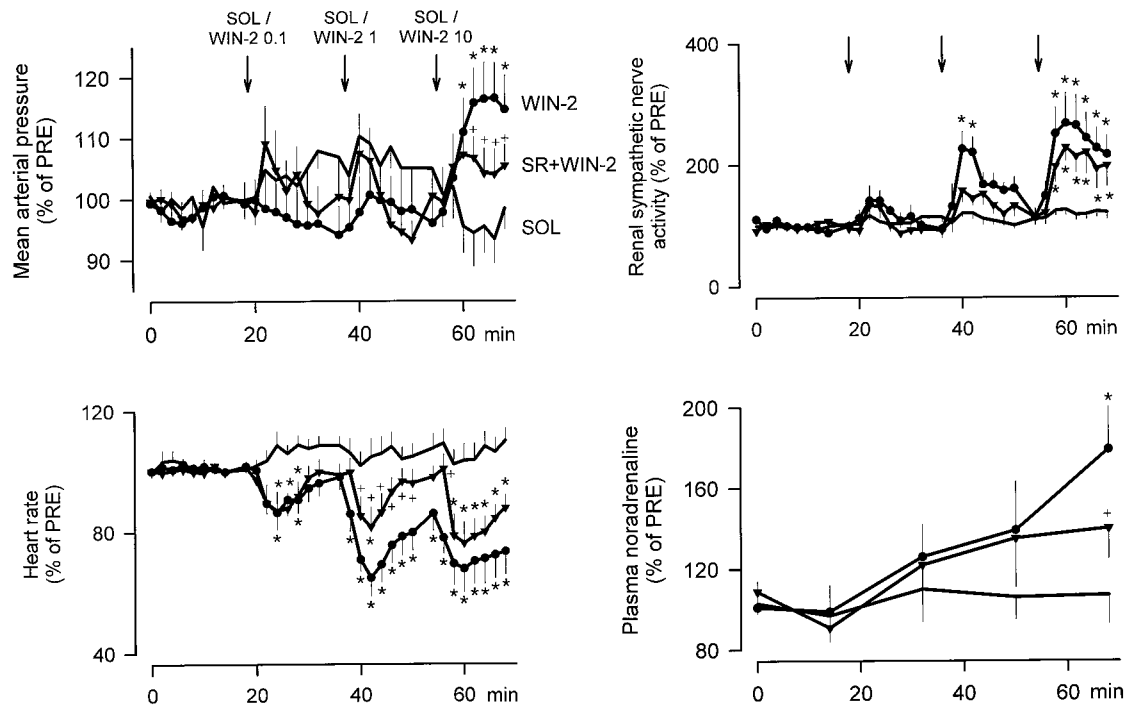


Fig. 4. Interaction between WIN55212-2 (WIN-2) and SR141716A (SR) on mean arterial pressure, heart rate, renal sympathetic nerve activity, and plasma noradrenaline concentration in conscious rabbits. SOL ($25 \mu\text{l kg}^{-1}$) and WIN-2 (0.1, 1, and $10 \mu\text{g kg}^{-1}$) were injected i.c., as indicated by the arrows. One group of animals was pretreated with SR (0.5 mg kg^{-1} , i.v.) at -10 min. Values are given as percentages of PRE. Means $\pm$ S.E. are from eight (SOL), eight (WIN-2), and eight (SR + WIN-2) experiments. Differences from SOL: * $P < .05$; differences from WIN-2: + $P < .05$. The SOL and WIN-2 groups are identical with the SOL and WIN-2 groups in Fig. 2.

1999; and the present study). It is possible that central sympathoexcitation occurs only in the conscious but not in the anesthetized state. In agreement with this latter assumption, when cannabinoids were studied under comparable conditions in anesthetized and conscious animals, they lowered blood pressure under anesthesia but caused no change in awake animals [rats (Lake et al., 1997), dogs (Jandhyala and Hamed, 1978)].

The second effect after central application of the cannabinoid agonists was a marked bradycardia. The effect was attributable to an increase in cardiac vagal activity for two reasons. First, the bradycardia produced by i.c.-administered WIN55212-2 was prevented by i.v.-administered atropine (Niederhoffer and Szabo, 1999). Similarly, the bradycardia produced by i.v.-injected WIN55212-2 was prevented by i.v.-administered methylatropine (B. Szabo, U. Nordheim, and N. Niederhoffer, unpublished observation). Second, the heart rate of conscious rabbits can only be lowered minimally by reducing cardiac sympathetic tone because the resting cardiac sympathetic tone is very low in these animals (McRitchie and Chalmers, 1981). The highest dose of WIN55212-2 and CP55940 ($10 \mu\text{g kg}^{-1}$) increased blood pressure; theoretically, operation of the baroreceptor reflex could contribute to the reduction in heart rate after this dose. Such a contribution of the baroreceptor reflex is, however, unlikely: the bradycardia after the highest dose of the cannabinoids was not greater than the bradycardia elicited by the preceding dose ($1 \mu\text{g kg}^{-1}$), which did not increase blood pressure.

Heart rate effects of centrally applied cannabinoids have been seldom studied. Based on their head cross-circulation experiments in dogs, Cavero et al. (1973b) concluded that the

central effect of Δ^9 -tetrahydrocannabinol on heart rate is depressive and that both the sympathetic and the parasympathetic nervous system are involved in the response. In their experiments in cats, Vollmer et al. (1974) observed bradycardia and a decrease in cardiac sympathetic nerve activity after systemic injection and bradycardia after lateral cerebral ventricular administration of Δ^9 -tetrahydrocannabinol; the effects were attributed to a centrally elicited reduction of cardiac sympathetic tone. Our studies (Niederhoffer and Szabo, 1999; and the present study) are the first in which the heart rate effects of centrally administered cannabinoids were studied in conscious animals.

It is interesting that cannabinoids influence cardiovascular regulation at many sites (Fig. 5). As shown by our results, medullary centers regulating sympathetic and vagal nerve activity can be directly influenced. These centers are relay stations for the cardiovascular effects elicited by primary actions of cannabinoids in higher brain regions, e.g., in the mesolimbic system and hypothalamus. Cannabinoids have also marked effects on peripheral autonomic neurons. They presynaptically inhibit the release of noradrenaline from many postganglionic sympathetic neurons (Pertwee et al., 1996; Malinowska et al., 1997; Niederhoffer and Szabo, 1999), also in the heart (Ishac et al., 1996). Finally, cannabinoids inhibit vagal neurotransmission in the heart (B. Szabo, U. Nordheim, and N. Niederhoffer, unpublished observation).

How do these primary effects contribute to the overall cardiovascular response to systemic administration of cannabinoids? After systemic administration of cannabinoids in conscious humans and animals, the peripheral presynaptic inhibition of noradrenaline release seems to counteract the simultaneously occurring central sympathoexcitation; there-

their activation changes the firing pattern of neurons (Himmi et al., 1998). These nuclei were likely primary targets when the i.c.-applied cannabinoids elicited sympathoactivation and bradycardia.

In conclusion, centrally applied cannabinoids caused sympathoactivation and bradycardia in conscious rabbits. This is the first demonstration of a central sympathoexcitation by cannabinoids by direct measurement of sympathetic nerve activity. The centrally elicited cardiovascular effects were probably mediated by CB₁ receptors.

Acknowledgments

The expert technical assistance of Claudia Schurr and the support and advice of Klaus Starke are gratefully acknowledged. We thank Pfizer (Groton, CT) and Sanofi Recherche (Montpellier, France) for a generous supply of CP55940 and SR141716A, respectively.

References

- Benowitz NL, Rosenberg J, Rogers W, Bachman J and Jones RT (1979) Cardiovascular effects of intravenous delta-9-tetrahydrocannabinol: Autonomic nervous mechanisms. *Clin Pharmacol Ther* **25**:440–446.
- Cavero I, Solomon T, Buckley JP and Jandhyala BS (1973a) Studies on the hypotensive activity of (–)-Δ⁹-trans-tetrahydrocannabinol in anesthetized dogs. *Res Commun Chem Pathol Pharmacol* **6**:527–540.
- Cavero I, Solomon T, Buckley JP and Jandhyala BS (1973b) Studies on the bradycardia induced by (–)-Δ⁹-trans-tetrahydrocannabinol in anesthetized dogs. *Eur J Pharmacol* **22**:263–269.
- Compton DR, Harris LS, Lichtman AH and Martin BR (1996) Marihuana. Pharmacological aspects of drug dependence, in *Handbook of Experimental Pharmacology* (Schuster CR and Kuhar MJ eds) pp 83–158, Springer, Heidelberg, Germany.
- Dewey WL (1986) Cannabinoid pharmacology. *Pharmacol Rev* **38**:151–178.
- Felder CC, Joyce KE, Briley EM, Mansouri J, Mackie K, Blond O, Lai Y, Ma AL and Mitchell RL (1995) Comparison of the pharmacology and signal transduction of the human cannabinoid CB₁ and CB₂ receptors. *Mol Pharmacol* **48**:443–450.
- Galiegue S, Mary S, Marchand J, Dussosoy D, Carriere D, Carayon P, Bouaboula M, Shire D, Le Fur G and Casellas P (1995) Expression of central and peripheral cannabinoid receptors in human immune tissues and leukocyte subpopulations. *Eur J Biochem* **232**:54–61.
- Himmi T, Perrin J, El Ouazzani T and Orsini JC (1998) Neuronal responses to cannabinoid receptor ligands in the solitary tract nucleus. *Eur J Pharmacol* **359**:49–54.
- Howlett AC (1995a) Pharmacology of cannabinoid receptors. *Annu Rev Pharmacol Toxicol* **35**:607–634.
- Howlett AC (1995b) Cannabinoid compounds and signal transduction mechanisms, in *Cannabinoid Receptors* (Pertwee RG ed) pp 171–204, Academic Press, London.
- Huestis MA, Sampson AH, Holicky BJ, Henningfield JE and Cone EJ (1992) Characterization of the absorption phase of marijuana smoking. *Clin Pharmacol Ther* **52**:31–41.
- Ishac EJN, Jiang L, Lake KD, Varga K, Abood ME and Kunos G (1996) Inhibition of exocytotic noradrenaline release by presynaptic cannabinoid CB₁ receptors on peripheral sympathetic nerves. *Br J Pharmacol* **118**:2023–2028.
- Jandhyala BS and Hamed AT (1978) Pulmonary and systemic hemodynamic effects of Δ⁹-tetrahydrocannabinol in conscious and morphine-chloralose-anesthetized dogs: Anesthetic influence on drug action. *Eur J Pharmacol* **53**:63–68.
- Kawasaki H, Watanabe S and Ueki S (1980) Effects of chronic administration of Δ⁹-tetrahydrocannabinol on the cardiovascular system, and pressor and behavioral responses to brain stimulation in freely moving rats. *Eur J Pharmacol* **65**:63–69.
- Kosersky DS (1978) Antihypertensive effects of Δ⁹-tetrahydrocannabinol. *Arch Int Pharmacodyn Ther* **233**:76–81.
- Kuster JE, Stevenson JI, Ward SJ, D'Ambra TE and Haycock DA (1993) Aminoalkylindole binding in rat cerebellum: Selective displacement by natural and synthetic cannabinoids. *J Pharmacol Exp Ther* **264**:1352–1363.
- Lake KD, Martin BR, Kunos G and Varga K (1997) Cardiovascular effects of anandamide in anesthetized and conscious normotensive and hypertensive rats. *Hypertension* **29**:1204–1210.
- Mailleux P and 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 and 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.
- Malpas SC, Shweta A, Anderson WP and Head GA (1996) Functional response to graded increases in renal nerve activity during hypoxia in conscious rabbits. *Am J Physiol* **271**:R1489–R1499.
- Matsuda LA, Bonner TI and Lolait SJ (1993) Localization of cannabinoid receptor mRNA in rat brain. *J Comp Neurol* **327**:535–550.
- McRitchie RJ and Chalmers JP (1981) Paradoxical inotropic effects of clonidine and labetalol in the conscious rabbit. *J Cardiovasc Pharmacol* **3**:818–827.
- Munro S, Thomas KL and Abu-Shaar M (1993) Molecular characterization of a peripheral receptor for cannabinoids. *Nature (Lond)* **365**:61–65.
- Niederhoffer N and Szabo B (1999) Effect of the cannabinoid receptor agonist WIN55212-2 on sympathetic cardiovascular regulation. *Br J Pharmacol* **126**:457–466.
- Osgood PF and Howes JF (1977) Δ⁹-9-Tetrahydrocannabinol and dimethylheptylpyran induced tachycardia in the conscious rat. *Life Sci* **21**:1329–1336.
- Pertwee RG (1997) Pharmacology of cannabinoid CB₁ and CB₂ receptors. *Pharmacol Ther* **74**:129–180.
- Pertwee RG, Joe-Adigwe G and Hawksworth GM (1996) Further evidence for the presence of cannabinoid CB₁ receptors in mouse vas deferens. *Eur J Pharmacol* **296**:169–172.
- 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 JC and Le Fur G (1994) SR141716A, a potent and selective antagonist of the brain cannabinoid receptor. *FEBS Lett* **350**:240–244.
- Showalter VM, Compton DR, Martin BR and Abood ME (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.
- Skaper SD, Buriani A, Dal Toso R, Petrelli L, Romanello S, Facci L and Leon A (1996) The ALIamide palmitoylethanolamide and cannabinoids, but not anandamide, are protective in a delayed postglutamate paradigm of excitotoxic death in cerebellar granule neurons. *Proc Natl Acad Sci USA* **93**:3984–3989.
- Stark P and Dews PB (1980) Cannabinoids. II. Cardiovascular effects. *J Pharmacol Exp Ther* **214**:131–138.
- Stein EA, Fuller SA, Edgemont WS and Campbell WB (1996) Physiological and behavioural effects of the endogenous cannabinoid, arachidonylethanolamide (anandamide), in the rat. *Br J Pharmacol* **119**:107–114.
- Szabo B and 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 and 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 and Starke K (1993) Sympathoinhibition by rilmenidine in conscious rabbits: Involvement of α₂-adrenoceptors. *Naunyn-Schmiedeberg's Arch Pharmacol* **348**:593–600.
- Tsou K, Brown S, Sanudo-Pena MC, Mackie K and Walker JM (1998) Immunohistochemical distribution of cannabinoid CB₁ receptors in the rat central nervous system. *Neuroscience* **83**:393–411.
- Varga K, Lake KD, Huangfu D, Guyenet PG and Kunos G (1996) Mechanism of the hypotensive action of anandamide in anesthetized rats. *Hypertension* **28**:682–686.
- Vidrio H, Sanchez-Salvadori MA and Medina M (1996) Cardiovascular effects of (–)-11-OH-Δ⁸-tetrahydrocannabinol-dimethylheptyl in rats. *J Cardiovasc Pharmacol* **28**:332–336.
- Vollmer RR, Cavero I, Ertel RJ, Solomon TA and Buckley JP (1974) Role of the central autonomic nervous system in the hypotension and bradycardia induced by (–)-Δ⁹-trans-tetrahydrocannabinol. *J Pharm Pharmacol* **26**:186–192.
- Wagner JA, Varga K and Kunos G (1998) Cardiovascular actions of cannabinoids and their generation during shock. *J Mol Med* **76**:824–836.

Send reprint requests to: Dr. Bela Szabo, Institut für Pharmakologie und Toxikologie, Albert-Ludwigs-Universität, Hermann-Herder-Strasse 5, D-79104, Freiburg i. Br., Germany. E-mail: szabo@uni-freiburg.de