

# Effects of Cannabinoids on Sympathetic and Parasympathetic Neuroeffector Transmission in the Rabbit Heart

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## ABSTRACT

Cannabinoids elicit marked cardiovascular responses. It is not clear how peripheral effects on the autonomic nervous system contribute to these responses. The aim of the present study was to characterize the peripheral actions of cannabinoids on the autonomic innervation of the heart. Experiments were carried out on pithed rabbits. In the first series of experiments, postganglionic sympathetic cardioaccelerator fibers were stimulated electrically. The synthetic cannabinoid receptor agonists WIN55212-2 (0.005, 0.05, 0.5, and 1.5 mg kg<sup>-1</sup> i.v.) and CP55940 (0.003, 0.03, 0.3, and 1 mg kg<sup>-1</sup> i.v.) dose dependently inhibited the electrically evoked cardioacceleration. The inhibition by WIN55212-2 (0.5 mg kg<sup>-1</sup> i.v.) was prevented by the CB<sub>1</sub> cannabinoid receptor antagonist SR141716A (0.5 mg kg<sup>-1</sup> i.v.). WIN55212-2 (0.5 mg kg<sup>-1</sup> i.v.) did not change the increase in heart rate evoked by injection of isoprenaline. In the

second series of experiments, preganglionic vagal fibers were stimulated electrically. WIN55212-2 (0.005, 0.05, and 0.5 mg kg<sup>-1</sup> i.v.) and CP55940 (0.003, 0.03, and 0.3 mg kg<sup>-1</sup> i.v.) dose dependently inhibited the stimulation-evoked decrease in heart rate. The inhibition produced by WIN55212-2 (0.005, 0.05, and 0.5 mg kg<sup>-1</sup> i.v.) was antagonized by SR141716A (0.5 mg kg<sup>-1</sup> i.v.). The results indicate that cannabinoids, by activating CB<sub>1</sub> cannabinoid receptors, inhibit sympathetic and vagal neuroeffector transmission in the heart. The mechanism of the sympathoinhibition is probably presynaptic inhibition of noradrenaline release from postganglionic sympathetic neurons. The mechanism of the inhibition of vagal activity was not clarified: cannabinoids may have an inhibitory action on both pre- and postganglionic vagal neurons.

It is well known that cannabinoid agonists cause euphoria, analgesia, change in locomotion, catalepsy, temperature reduction, and memory disturbance in humans and experimental animals (for review see Dewey, 1986; Howlett, 1995; Compton et al., 1996; Pertwee, 1997). The cardiovascular effects of cannabinoids are less known, although they are strong. In conscious humans smoking cigarettes containing  $\Delta^9$ -tetrahydrocannabinol or intravenous injection of  $\Delta^9$ -tetrahydrocannabinol causes, in addition to euphoria, a heart rate increase by as much as 60 beats/min (Benowitz et al., 1979; Perez-Reyes et al., 1982; Huestis et al., 1992). Depending on the species and the state of consciousness, cannabinoids can both increase and decrease blood pressure and heart rate, and the mechanisms of these effects are only partly understood (Dewey, 1986; Compton et al., 1996; Wagner et al., 1998; Niederhoffer and Szabo 1999, 2000).

The present study deals with the effects of cannabinoids on heart rate regulation. In anesthetized animals, system-

ically administered cannabinoids generally lower heart rate (e.g., rat: Varga et al., 1995; Vidrio et al., 1996; Lake et al., 1997a; dog: Cavero et al., 1973; cat: Vollmer et al., 1974). In conscious humans and monkeys, cannabinoids increase heart rate (Benowitz et al., 1979; Fredericks et al., 1981; Perez-Reyes et al., 1982; Huestis et al., 1992). In conscious rats, cannabinoids cause either bradycardia (Vidrio et al., 1996), no change in heart rate (Lake et al., 1997b), or tachycardia (Osgood and Howes, 1977). Cannabinoids elicit bradycardia in conscious dogs (Jandhyala and Hamed, 1978). In conscious rabbits, low doses of cannabinoids lower heart rate, whereas higher doses tend to increase it (Niederhoffer and Szabo, 1999). In most of these experiments, the sites of action of cannabinoids on heart rate regulation were not determined. Therefore, it is not clear how peripheral and central effects on cardiac sympathetic and parasympathetic pathways contribute to the overall effect on heart rate.

The aim of the present study was to analyze the peripheral effects of cannabinoids on the autonomic nerves innervating the heart. In pithed rabbits, heart rate changes were evoked by electrical stimulation of the right postganglionic sympathetic cardioaccelerator nerves and the right vagus nerve,

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and the influence of cannabinoid receptor ligands on the evoked responses was evaluated. To determine whether the peripheral effects play a role in vivo, identical doses of the cannabinoids were given in conscious rabbits, and blood pressure and heart rate responses were compared with those observed in pithed animals.

## Materials and Methods

Experiments were carried out on rabbits of a local breed (obtained from Ketterer, Reute, Germany); rabbits were of either sex and weighed 1.4 to 3.3 kg. The experiments conformed to the German law regulating animal experiments and were approved by a local ethical commission.

### Pithed Rabbits

**Surgical Preparation.** Rabbits were deeply anesthetized with sodium pentobarbitone ( $75 \text{ mg kg}^{-1}$  i.v.). The trachea was cannulated and artificial respiration with room air, at a rate of  $45 \text{ min}^{-1}$ , commenced. Both carotid arteries were ligatured. The proximal stump of the right carotid artery was cannulated for recording arterial pressure. Blood pressure was measured with a Statham P23 Db transducer coupled to a bridge amplifier (Hugo Sachs, Hugstetten, Germany). Heart rate was calculated from the pulsating pressure signal by a cardi tachometer (Hugo Sachs, Hugstetten, Germany). Both jugular veins were ligatured, and the proximal stumps were cannulated for administration of drugs. Neuromuscular transmission was blocked by gallamine triethiodide ( $5 \text{ mg kg}^{-1}$ ) or succinylcholine ( $1 \text{ mg kg}^{-1}$ ). A hole was made in the parietal bone, and the rabbits were pithed by inserting a stainless steel rod (diameter, 3.5 mm; length, 300 mm) into the spinal canal; the rod destroyed the medulla oblongata and the spinal cord. To fully destroy the brain and to stop bleeding, the skull was firmly stuffed with soft paper.

**Nerve Stimulation.** In the experiments in which sympathetic nerves were stimulated, gallamine triethiodide was used to block neuromuscular transmission, and muscarinic acetylcholine receptors were blocked by methylatropine ( $1 \text{ mg kg}^{-1}$  bolus injection followed by an infusion of  $2 \text{ mg kg}^{-1} \text{ h}^{-1}$  until the end of the experiment). The right sympathetic cardioaccelerator nerves were electrically stimulated by a bipolar platinum/iridium electrode coupled to a stimulator (Hugo Sachs, Hugstetten, Germany). The tip of the electrode was positioned with the help of a micromanipulator in the space lying laterally to the trachea, anterior to the vertebral column and rostrally to the rib cage.

In the experiments with vagus nerve stimulation, succinylcholine was used to block neuromuscular transmission; gallamine triethiodide was avoided because it blocks muscarinic receptors in the heart (Maclagan and Faulkner, 1989). In these experiments  $\beta$ -adrenoceptors were blocked by propranolol ( $1 \text{ mg kg}^{-1}$  bolus injection followed by an infusion of  $1 \text{ mg kg}^{-1} \text{ h}^{-1}$  until the end of the experiment). The right vagus nerve in the neck was separated from other accompanying nerves and from the common carotid artery, and stimulated by a bipolar platinum/iridium electrode coupled to a stimulator.

**Protocol.** The cardioaccelerator and the vagus nerves were usually stimulated every 2 min. The evoked responses became stable about 45 min after surgical preparation; the time then was set to  $t = 0$  min, and the corresponding stimulation was defined as the 1st stimulation. Solvent or increasing doses of cannabinoid agonists were given after the 7th, 14th, and 21st stimulation periods (i.e., at  $t = 13, 27,$  and  $41$  min) (see, e.g., Fig. 5 for protocol). In each experiment, the responses to the first seven stimulations (between  $t = 0$  and 12 min) were averaged to yield the baseline reference values (PRE), and all values were expressed as percentages of PRE. Different protocols were used to characterize the experimental mod-

els and interactions with antagonists; these protocols will be described under *Results*.

### Conscious Rabbits

Two to three experiments were carried out on one rabbit at intervals of 4 to 5 days. No animal received a given kind of treatment twice. After the last experiment, the animals were killed by an overdose of pentobarbitone.

**Surgical Preparation.** During the experiments, the rabbits were quietly sitting in a plastic box. The central ear artery was cannulated with an Abbocath catheter (22 gauge) under local anesthesia (lidocaine 2%). The artery served for recording arterial blood pressure and heart rate. A marginal ear vein was cannulated with an Abbocath catheter (24 gauge) for administration of drugs.

**Protocol.** Blood pressure and heart rate were determined every 2 min. The values became stable about 45 min after cannulation of blood vessels; the time then was set to  $t = 0$  min. Solvent or increasing doses of WIN55212-2 were injected at  $t = 19, 37,$  and  $55$  min (see Fig. 6 for protocol). In experiments in which the interaction with methylatropine was studied,  $1 \text{ mg kg}^{-1}$  of the antagonist was injected as a bolus at  $t = -14$  min, and an infusion of  $2 \text{ mg kg}^{-1} \text{ h}^{-1}$  followed until the end of the experiment. In each experiment, the eight blood pressure and heart rate values measured between  $t = 0$  and 14 min were averaged to yield PRE, and all values were expressed as percentages of PRE.

### Statistics

Means  $\pm$  S.E. of  $n$  experiments are given throughout. Differences between groups and within groups were evaluated with the nonparametric Mann-Whitney and Wilcoxon signed-rank tests, respectively.  $p < 0.05$  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: (–)-*cis*-3-[2-hydroxy-4-(1,1-dimethylheptyl)phenyl]-*trans*-4-(3-hydroxypropyl)cyclohexanol (CP55940) from Pfizer (Groton, CT); gallamine triethiodide from Sigma (Deisenhofen, Germany); 2-hydroxypropyl- $\beta$ -cyclodextrin from Fluka (Neu-Ulm, Germany); methylatropine bromide from Sigma; *N*-piperidino-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-3-pyrazole-carboxamide (SR141716A) from Sanofi Recherche (Montpellier, France); propranolol hydrochloride from Sigma; *R*(+)-[2,3-dihydro-5-methyl-3-[(morpholinyl)methyl]pyrrolo[1,2,3-*de*]-1,4-benzoxazinyl]-(1-naphthalenyl)methanone mesylate (WIN55212-2) from RBI (Köln, Germany); succinylcholine chloride from Curamed (Karlsruhe, Germany); and yohimbine hydrochloride from Roth (Karlsruhe, Germany).

WIN55212-2 and CP55940 were dissolved and further diluted in a 19% 2-hydroxypropyl- $\beta$ -cyclodextrin solution (w/v in distilled water). SR141716A was dissolved in 50% ethanol (v/v in saline). Methylatropine, propranolol, and yohimbine were dissolved in saline. Doses refer to the salts. All drugs were administered i.v. Injections generally had a volume of  $0.5 \text{ ml kg}^{-1}$ , infusions a rate of  $1.9 \text{ ml h}^{-1}$ .

## Results

### Stimulation of Cardiac Sympathetic Nerves in Pithed Rabbits

**Stimulation with Long Trains (30 s) of Electrical Pulses.** Cardiac sympathetic nerves were stimulated every 2 min using the following parameters: frequency, 1 Hz; pulse width, 2 ms; pulse amplitude, 20 mA; and train duration, 30 s (30 pulses). Heart rate was increased by  $38 \pm 4 \text{ beats min}^{-1}$  (PRE;  $n = 12$ ). The stimulation-evoked increase in heart rate was only transiently affected by the ganglion blocking agent

mecamylamine ( $10 \text{ mg kg}^{-1}$ ) but was abolished by propranolol ( $1 \text{ mg kg}^{-1}$ ) (Fig. 1A). This indicates that the cardioacceleration was due to direct stimulation of postganglionic sympathetic fibers. The  $\alpha_2$ -adrenoceptor antagonist yohimbine ( $0.1$  and  $0.5 \text{ mg kg}^{-1}$ ) dose dependently enhanced the effect of electrical stimulation (Fig. 1B), suggesting that inhibition of noradrenaline release via  $\alpha_2$ -autoreceptors operated even under this mild stimulation condition.

In control experiments, the solvent for cannabinoids, 2-hydroxypropyl- $\beta$ -cyclodextrin, was injected three times (Fig. 1C); the stimulation-evoked tachycardia increased slightly during the course of these experiments. The synthetic cannabinoid receptor agonist WIN55212-2 ( $0.005$ ,  $0.05$ , and  $0.5 \text{ mg kg}^{-1}$ ) dose dependently inhibited the heart rate re-

sponses (Fig. 1C). The maximum effect was a 19% decrease in the evoked cardioacceleration (compared with the solvent group).

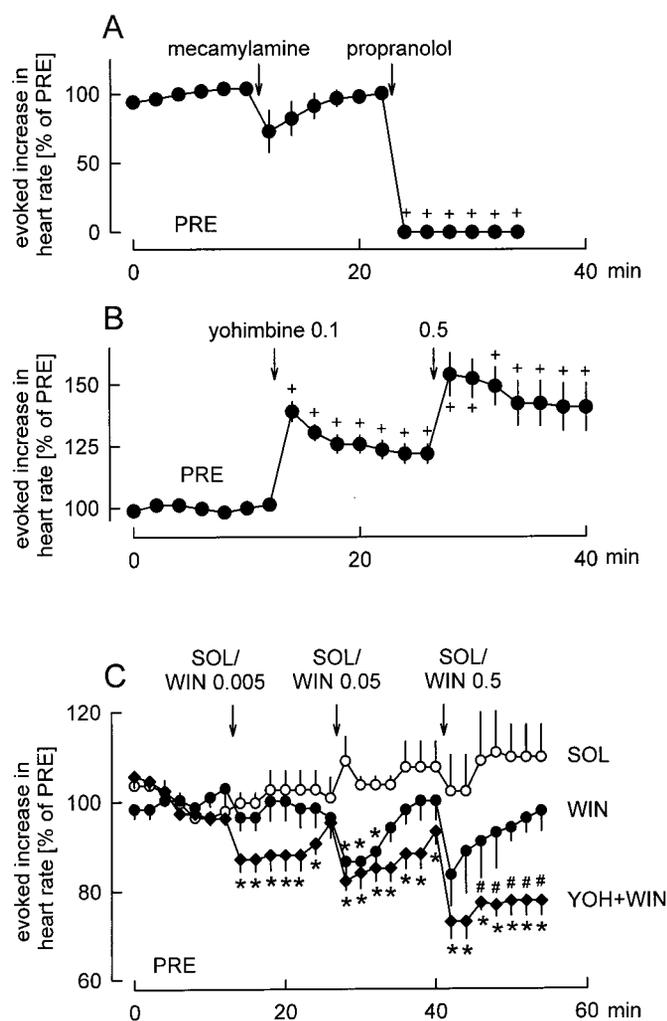
A group of animals was pretreated with yohimbine ( $0.5 \text{ mg kg}^{-1}$ ) at  $t = -14 \text{ min}$  to study the effect of WIN55212-2 in the absence of  $\alpha_2$ -adrenoceptor-mediated autoinhibition of transmitter release. In the presence of yohimbine, electrical stimulation increased heart rate by  $53 \pm 7 \text{ beats min}^{-1}$  (PRE;  $n = 4$ ). The magnitude and the duration of the inhibitory effect of WIN55212-2 ( $0.005$ ,  $0.05$ , and  $0.5 \text{ mg kg}^{-1}$ ) on the stimulation-evoked tachycardia were augmented in yohimbine-pretreated animals (Fig. 1C).

**Stimulation with Short Trains (<1.6 s) of Electrical Pulses.** The aim was to stimulate the cardioaccelerator nerves in a fashion to avoid development of autoinhibition of transmitter release. To this end, pulses were delivered at high frequency ( $50 \text{ Hz}$ ), and the duration of pulse trains was kept short ( $0.04$ – $1.6 \text{ s}$ ). Indeed, under these conditions, the  $\alpha_2$ -adrenoceptor antagonist yohimbine ( $0.5 \text{ mg kg}^{-1}$ ) had no effect on the stimulation-evoked heart rate response (Fig. 2A), indicating lack of autoinhibition (see Illes and Starke, 1983; and Limberger and Starke, 1984, for lack of autoinhibition during short trains of stimulation pulses). For further experiments, the following stimulation parameters were used: frequency,  $50 \text{ Hz}$ ; pulse width,  $2 \text{ ms}$ ; pulse amplitude,  $20 \text{ mA}$ ; and train duration,  $0.4 \text{ s}$  ( $20 \text{ pulses}$ ). This stimulation increased heart rate by  $66 \pm 3 \text{ beats min}^{-1}$  (PRE;  $n = 18$ ). Mecamylamine ( $10 \text{ mg kg}^{-1}$ ) inhibited the response by about 25% and propranolol ( $1 \text{ mg kg}^{-1}$ ) abolished it (Fig. 2B), indicating that the cardioacceleration was predominantly due to stimulation of postganglionic sympathetic fibers.

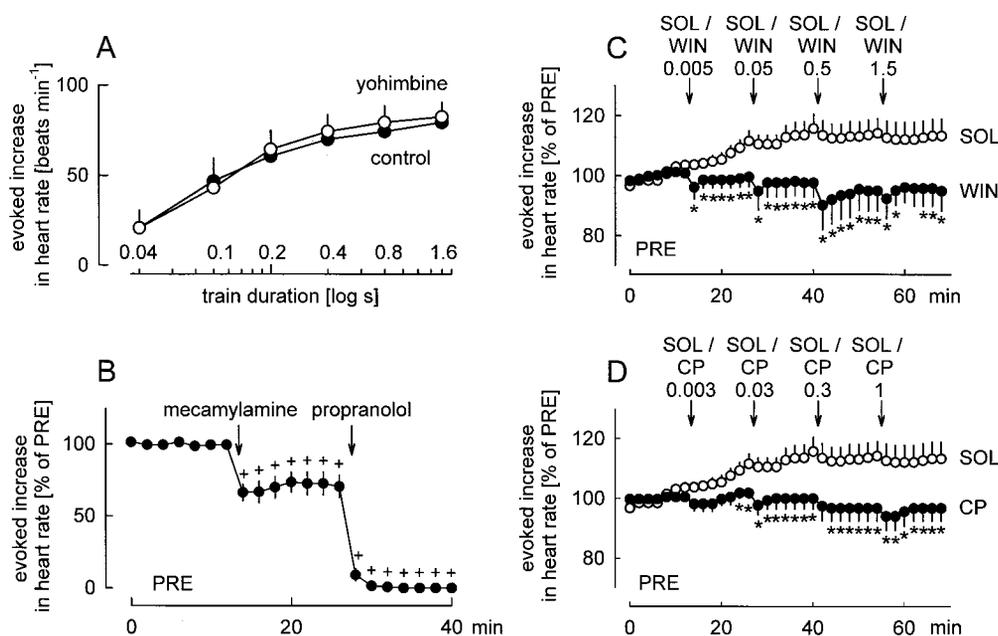
In control experiments, the solvent for cannabinoids was injected four times (Fig. 2C); the stimulation-evoked tachycardia increased during the course of these experiments. WIN55212-2 ( $0.005$ ,  $0.05$ ,  $0.5$ , and  $1.5 \text{ mg kg}^{-1}$ ) dose dependently inhibited the evoked cardioacceleration (Fig. 2C). Compared with the solvent group, the maximum inhibition was 22%. The other synthetic cannabinoid receptor agonist, CP55940 ( $0.003$ ,  $0.03$ ,  $0.3$ , and  $1 \text{ mg kg}^{-1}$ ), also inhibited the electrically evoked increase in heart rate (Fig. 2D). The maximum inhibition by CP55940, again compared with the solvent group, was 17%.

A group of animals was treated with the  $\text{CB}_1$  cannabinoid receptor antagonist SR141716A ( $0.5 \text{ mg kg}^{-1}$ ; Fig. 3). The evoked tachycardia decreased slightly and transiently after administration of the antagonist. When WIN55212-2 ( $0.5 \text{ mg kg}^{-1}$ ) was injected after SR141716A, it did not change the stimulation-evoked increase in heart rate.

**Stimulation of the Heart by Isoprenaline.** The interaction of WIN55212-2 with isoprenaline was studied in four pithed rabbits. Instead of stimulation of cardioaccelerator nerves, these rabbits received bolus injections of isoprenaline ( $0.5 \mu\text{g kg}^{-1}$ ). Isoprenaline increased heart rate by  $106 \pm 8 \text{ beats min}^{-1}$  ( $n = 4$ ). WIN55212-2 ( $0.5 \text{ mg kg}^{-1}$ ) had no effect on the isoprenaline-evoked tachycardia. Thus, 5 and 13 min after administration of the cannabinoid agonist, isoprenaline evoked heart rate increases that were  $104 \pm 3$  and  $105 \pm 2\%$  ( $n = 4$ ), respectively, of the increase measured before WIN55212-2 administration.



**Fig. 1.** Effects of drugs on heart rate increases evoked by stimulation of cardiac sympathetic nerves in pithed rabbits with long trains (30 s) of electrical pulses. Cardiac sympathetic nerves were stimulated at a frequency of  $1 \text{ Hz}$  for 30 s. Mecamylamine ( $10 \text{ mg kg}^{-1}$ ), propranolol ( $1 \text{ mg kg}^{-1}$ ), yohimbine (YOH;  $0.1$  and  $0.5 \text{ mg kg}^{-1}$ ), solvent (SOL;  $0.5 \text{ ml kg}^{-1}$ ), and WIN55212-2 (WIN;  $0.005$ ,  $0.05$ , and  $0.5 \text{ mg kg}^{-1}$ ) were administered i.v., as indicated by the arrows. In C, one of the WIN55212-2 groups (YOH + WIN) was pretreated with yohimbine ( $0.5 \text{ mg kg}^{-1}$  i.v.) at  $t = -14 \text{ min}$ . Values are given as percentages of PRE. Means  $\pm$  S.E. from five (mecamylamine and propranolol), four (yohimbine,  $0.1$  and  $0.5 \text{ mg kg}^{-1}$ ), four (SOL), four (WIN), and four (YOH + WIN) experiments. Differences from PRE: \* $p < 0.05$ ; differences from SOL: \* $p < 0.05$ ; differences from WIN: # $p < 0.05$ .



**Fig. 2.** Effects of drugs on heart rate increases evoked by stimulation of cardiac sympathetic nerves in pithed rabbits with short trains (<1.6 s) of electrical pulses. Cardiac sympathetic nerves were stimulated at a frequency of 50 Hz for 0.04 to 1.6 s (A) or for 0.4 s (B–D). In the experiments shown in A, the train duration-response relationship was studied in animals before (control) and after treatment with yohimbine (0.5 mg kg<sup>-1</sup> i.v.). Mecamylamine (10 mg kg<sup>-1</sup>), propranolol (1 mg kg<sup>-1</sup>, solvent (SOL; 0.5 ml kg<sup>-1</sup>), WIN55212-2 (WIN; 0.005, 0.05, 0.5, and 1.5 mg kg<sup>-1</sup>), and CP55940 (CP; 0.003, 0.03, 0.3, and 1 mg kg<sup>-1</sup>) were administered i.v. as indicated by the arrows. Values are given as percentages of PRE. Means  $\pm$  S.E. from five (control), five (yohimbine), seven (mecamylamine and propranolol), seven (SOL), seven (WIN) and four (CP) experiments. Differences from PRE: \* $p$  < 0.05; differences from SOL: \* $p$  < 0.05.

### Stimulation of the Right Vagus Nerve in Pithed Rabbits

The right vagus nerve was stimulated using the following parameters: frequency, 10 Hz; pulse width, 0.2 ms; and pulse amplitude, 5 mA. The vagally evoked slowing of the heart rate depended on the duration of impulse trains (Fig. 4A). Further experiments were carried out using 5-s-long trains (50 pulses). Such stimulation trains lowered heart rate by  $79 \pm 5$  beats min<sup>-1</sup> (PRE;  $n = 15$ ). The response was abolished by mecamylamine (10 mg kg<sup>-1</sup>) (Fig. 4B), indicating that the stimulation was preganglionic.

In control experiments, the solvent for cannabinoids was injected three times (Fig. 4C); the vagally mediated decrease in heart rate remained very constant in these experiments. Injection of the cannabinoid receptor agonist WIN55212-2 (0.005, 0.05, and 0.5 mg kg<sup>-1</sup>) dose dependently inhibited the evoked bradycardia (Figs. 4C and 5A). Injection of the other cannabinoid receptor agonist, CP55940 (0.003, 0.03, and 0.3

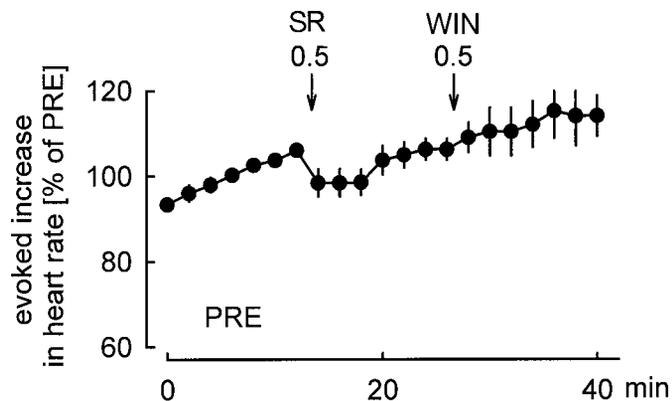
mg kg<sup>-1</sup>), caused a dose-dependent inhibition as well (Figs. 4D and 5B).

A group of animals was pretreated with the CB<sub>1</sub> cannabinoid receptor antagonist SR141716A (0.5 mg kg<sup>-1</sup>) at  $t = -14$  min. In antagonist-pretreated animals, the evoked decrease in heart rate was  $74 \pm 6$  beats min<sup>-1</sup> (PRE;  $n = 5$ ), not significantly different from the decrease observed in animals without pretreatment (see above). SR141716A significantly attenuated, however, the inhibitory effect of WIN55212-2 (0.005, 0.05, and 0.5 mg kg<sup>-1</sup>) on vagally mediated bradycardia (Fig. 4C).

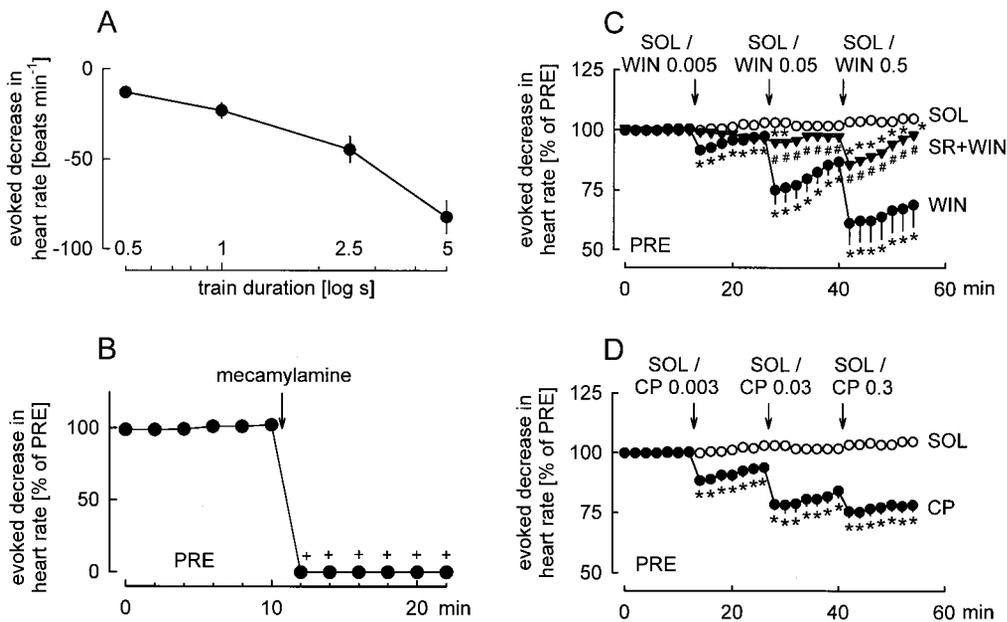
### Conscious Rabbits

In conscious rabbits, mean arterial pressure and heart rate were  $72 \pm 2$  mm Hg and  $246 \pm 11$  beats min<sup>-1</sup>, respectively (PRE;  $n = 8$ ). In control experiments, the solvent for cannabinoids was injected three times. Mean arterial pressure remained constant in these experiments, whereas heart rate tended to decrease (Fig. 6A and B). Three doses of the cannabinoid receptor agonist WIN55212-2 (0.005, 0.05, and 0.5 mg kg<sup>-1</sup>) were administered. None of the doses elicited significant blood pressure changes (Fig. 6A). Effects on heart rate were biphasic (Fig. 6B): the two lower doses caused significant and dose-dependent bradycardia; after the highest dose, the heart rate rose above the control value.

A group of animals was pretreated with the muscarinic receptor antagonist methylatropine at  $t = -14$  min (1 mg kg<sup>-1</sup> bolus followed by infusion of 2 mg kg<sup>-1</sup> h<sup>-1</sup>). This treatment increased the heart rate to  $304 \pm 12$  beats min<sup>-1</sup> (PRE;  $n = 4$ ). Blood pressure,  $74 \pm 2$  mm Hg (PRE;  $n = 4$ ), was not changed. In methylatropine-pretreated animals, the lowest dose of WIN55212-2 (0.005 mg kg<sup>-1</sup>) did not change the blood pressure, whereas the two higher doses (0.05 and 0.5 mg kg<sup>-1</sup>) caused pronounced hypertension (Fig. 6A). Pretreatment with methylatropine prevented the bradycardia caused by the two lower doses of WIN55212-2 (0.005 and 0.05 mg kg<sup>-1</sup>). The slight tachycardia caused by the highest dose



**Fig. 3.** Interaction of SR141716A (SR) with WIN55212-2 (WIN) on heart rate increases evoked by stimulation of cardiac sympathetic nerves in pithed rabbits with short trains (0.4 s) of electrical pulses. Cardiac sympathetic nerves were stimulated at a frequency of 50 Hz for 0.4 s. SR (0.5 mg kg<sup>-1</sup>) and WIN (0.5 mg kg<sup>-1</sup>) were administered i.v. as indicated by the arrows. Values are given as percentages of PRE. Means  $\pm$  S.E. from four experiments.



**Fig. 4.** Effects of drugs on heart rate decreases evoked by stimulation of the right vagus nerve in pithed rabbits. The vagus nerve was stimulated at a frequency of 10 Hz for 0.5 to 5 s (A) or for 5 s (B–D). Mecylamine (10 mg kg<sup>-1</sup>, solvent (SOL; 0.5 ml kg<sup>-1</sup>), WIN55212-2 (WIN; 0.005, 0.05, and 0.5 mg kg<sup>-1</sup>) and CP55940 (CP; 0.003, 0.03, and 0.3 mg kg<sup>-1</sup>) were administered i.v. as indicated by the arrows. In C, one of the WIN55212-2 groups (SR + WIN) was pretreated with SR141716A (0.5 mg kg<sup>-1</sup> i.v.) at *t* = -14 min. Values are given as percentages of PRE. Means ± S.E. from five (A), five (mecylamine), five (SOL), five (WIN), five (SR + WIN), and five (CP) experiments. Differences from PRE: <sup>+</sup>*p* < 0.05; differences from SOL: <sup>\*</sup>*p* < 0.05; differences from WIN: <sup>#</sup>*p* < 0.05.

of WIN55212-2 (0.5 mg kg<sup>-1</sup>) persisted in the presence of methylatropine (Fig. 6B).

## Discussion

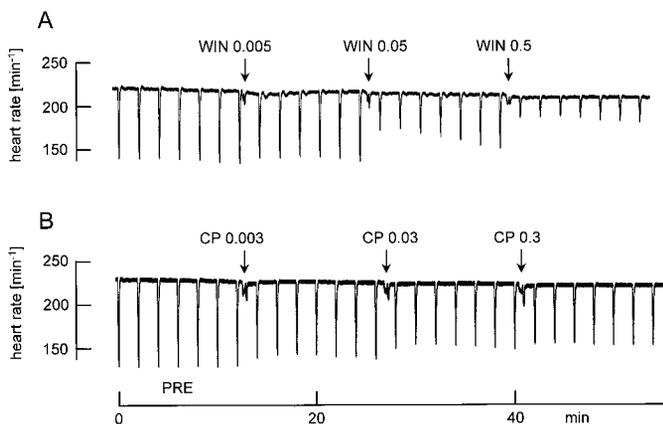
WIN55212-2 and CP55940 are synthetic cannabinoid receptor agonists belonging to different chemical classes; WIN55212-2 is an aminoalkylindole and CP55940 is a bicyclic compound resembling Δ<sup>9</sup>-tetrahydrocannabinol (for review, see Howlett, 1995; Pertwee, 1999). WIN55212-2 and CP55940 are selective for cannabinoid receptors, but do not distinguish between CB<sub>1</sub> and CB<sub>2</sub> cannabinoid receptors (Kuster et al., 1993; Felder et al., 1995; Showalter et al., 1996). Both agonists inhibited the evoked cardioacceleration in rabbits, and the inhibition by WIN55212-2 was prevented by the CB<sub>1</sub> cannabinoid receptor-selective antagonist SR141716A (Rinaldi-Carmona et al., 1994; Felder et al., 1995; Showalter et al., 1996; Pertwee, 1999). This pattern

suggests that CB<sub>1</sub> receptors were involved in the inhibition of the cardioacceleration.

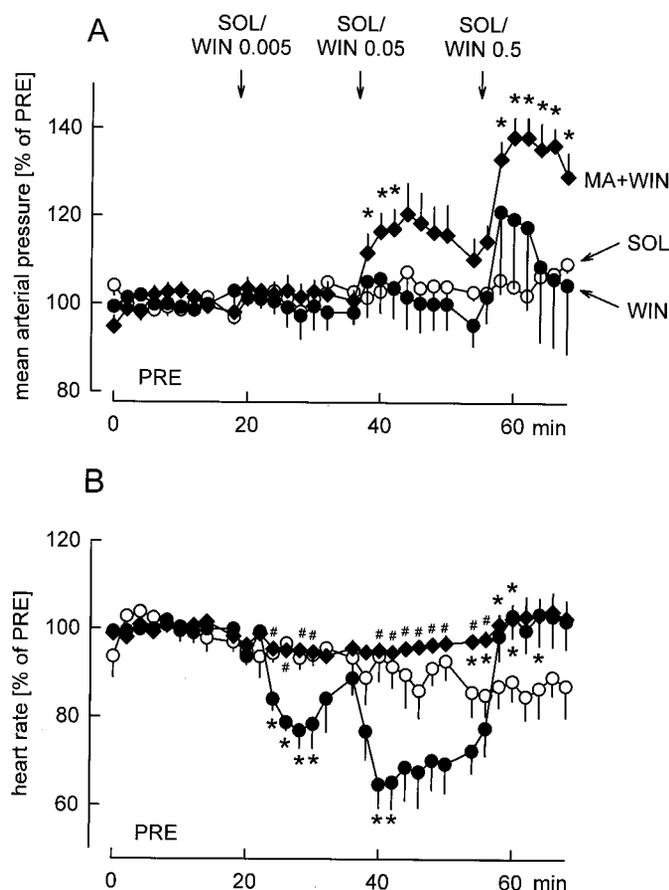
The likely mechanism of the inhibition of the electrically evoked cardioacceleration is presynaptic inhibition of noradrenaline release from terminals of postganglionic sympathetic axons. Postganglionic sympathetic axons of the heart originate in the cervical sympathetic ganglia, and CB<sub>1</sub> cannabinoid receptor mRNA was detected in the superior cervical ganglion (Ishac et al., 1996). It is reasonable to assume that the receptor is transported to the axon terminals of the sympathetic neurons in the heart, where it could presynaptically modulate transmitter release. A presynaptic action in the heart is also supported by recent observations: cannabinoids inhibited the field stimulation-evoked release of [<sup>3</sup>H]noradrenaline in isolated human (Molderings et al., 1999) and rat heart preparations (Ishac et al., 1996); however, there was no inhibition in the mouse heart (Lay et al., 2000; Trendelenburg et al., 2000).

A postsynaptic, direct heart effect of cannabinoids in our experiments is unlikely, because WIN55212-2 did not influence the cardioacceleration evoked by isoprenaline. WIN55212-2 also had no effect on the resting heart rate, suggesting that it does not interfere with the function of cardiac ion channels involved in generation and propagation of pacemaker impulses. The localization of cannabinoid receptors also indicates that a postsynaptic direct effect on the heart is unlikely. No CB<sub>1</sub> cannabinoid receptor mRNA (Ishac et al., 1996) or specific binding of the cannabinoid ligand [<sup>3</sup>H]CP55940 (Lynn and Herkenham, 1994) was observed in the heart (note: presynaptic receptors are frequently not detected by autoradiography in peripheral tissues, because of their small number).

In experiments with long trains of electrical pulses, the effect of WIN55212-2 was potentiated by yohimbine, most likely because yohimbine eliminated the simultaneously operating α<sub>2</sub>-adrenoceptor-mediated autoinhibition. It has been previously shown that the function of presynaptic heteroreceptors (e.g., opioid κ-receptors and adenosine A<sub>1</sub> receptors)



**Fig. 5.** Effects of WIN55212-2 (WIN) and CP55940 (CP) on heart rate decreases evoked by stimulation of the right vagus nerve in pithed rabbits. The vagus nerve was stimulated at a frequency of 10 Hz for 5 s. WIN (0.005, 0.05, and 0.5 mg kg<sup>-1</sup>) and CP (0.003, 0.03, and 0.3 mg kg<sup>-1</sup>) were administered i.v. as indicated by the arrows.



**Fig. 6.** Effects of solvent (SOL), WIN55212-2 (WIN), and methylatropine (MA) on mean arterial pressure and heart rate in conscious rabbits. SOL (0.5 ml kg<sup>-1</sup>) and WIN (0.005, 0.05, and 0.5 mg kg<sup>-1</sup>) were injected i.v. as indicated by the arrows. One of the WIN groups (MA + WIN) was pretreated with MA (1 mg kg<sup>-1</sup> bolus + 2 mg kg<sup>-1</sup> h<sup>-1</sup> infusion, i.v.) at *t* = -14 min. Values are given as percentages of PRE. Means ± S.E. from four (SOL), four (WIN), and four (MA + WIN) experiments. Differences from SOL: \**p* < 0.05; differences from WIN: #*p* < 0.05.

is attenuated by the physiologically functioning  $\alpha_2$ -adrenoceptor-mediated autoinhibition (Ramme et al., 1986; Limberger et al., 1988). Both CB<sub>1</sub> cannabinoid receptors and  $\alpha_2$ -adrenoceptors couple to G<sub>i/o</sub> proteins. During physiological  $\alpha_2$ -adrenoceptor-mediated autoinhibition, the transducer proteins and mechanisms, which finally cause presynaptic inhibition, are partly activated. Under this condition, stimulation of a second receptor, using the same or a very similar intracellular transmission pathway, will elicit a blunted presynaptic response.

Cannabinoids strongly lower the spillover of noradrenaline into the blood in pithed rabbits in which the entire sympathetic outflow is electrically stimulated, suggesting strong presynaptic inhibition in several major organs (Niederhoffer and Szabo, 1999). In contrast, cannabinoids inhibited the electrically evoked tachycardia in the present study by about 20%. In the same preparation, the  $\alpha_2$ -adrenoceptor agonists rilmenidine and moxonidine inhibit the evoked tachycardia by more than 60% (U. Nordheim, N. Niederhoffer, and B. Szabo, unpublished observation). Thus, compared with other sympathetic neurons, the sympathetic neurons of the heart seem to be relatively insensitive to the presynaptic effects of cannabinoids.

In our previous study in pithed rabbits in which the entire sympathetic outflow was continuously stimulated by an electrode in the spinal canal, WIN55212-2 had no effect on heart rate (Niederhoffer and Szabo, 1999), most likely because preganglionic sympathetic neurons of the heart were only weakly stimulated in that experimental model (see Szabo et al., 1987).

In summary, the present study shows for the first time that cannabinoids presynaptically inhibit the sympathetic cardioaccelerator response in the rabbit heart. This was demonstrated under relatively physiological conditions in a whole animal preparation, using orthodromic nerve stimulation and the functional consequence of the presynaptic effect could be directly seen.

The bradycardia evoked by stimulation of the right vagus nerve was dose dependently inhibited by the mixed CB<sub>1</sub>/CB<sub>2</sub> cannabinoid receptor agonists WIN55212-2 and CP55940 and the inhibition by WIN55212-2 was prevented by the CB<sub>1</sub> cannabinoid receptor antagonist SR141716A. This pattern suggests involvement of CB<sub>1</sub> cannabinoid receptors. To our knowledge, this is the first demonstration that cannabinoids inhibit neuroeffector transmission between the vagus nerve and the heart.

The mechanism of the inhibition of vagal transmission was not clarified in the present study. Theoretically, at least four mechanisms could play a role. 1) Cannabinoids could inhibit ganglionic transmission by inhibiting acetylcholine release from preganglionic vagal neurons. The dorsal motor nucleus of the vagus in the medulla oblongata synthesizes CB<sub>1</sub> mRNA (Matsuda et al., 1993), and receptor protein is also present in this nucleus (Mailleux and Vanderhaeghen, 1992). If the receptor is transported to the axon terminals of preganglionic vagal neurons in the heart, it could mediate the inhibition of acetylcholine release. 2) Cannabinoids could inhibit ganglionic transmission by acting on somadendritic receptors of postganglionic vagal neurons. 3) Cannabinoids could presynaptically inhibit transmitter release from axon terminals of postganglionic vagal neurons. Presynaptic inhibition of acetylcholine release from pre- and/or postganglionic vagal neurons by cannabinoids would be in line with results showing that cannabinoids inhibit acetylcholine release from peripheral (Pertwee et al., 1996) and central neurons (Gifford and Ashby, 1996). 4) Cannabinoids could interfere with the effect of released acetylcholine postsynaptically, at the level of the sinus node cells. This latter mechanism is the least likely, because, as already mentioned above, the heart itself does not synthesize cannabinoid receptors (Lynn and Herkenham, 1994; Ishac et al., 1996). We attempted to study the interaction between WIN55212-2 and intravenously injected acetylcholine on heart rate. However, the experiments were not successful, because injection of heart rate-lowering doses of acetylcholine always irreversibly damaged the pithed rabbit preparation.

In conscious rabbits, WIN55212-2 lowered heart rate at low doses, and this effect was prevented by the peripherally acting muscarinic receptor antagonist methylatropine. A new observation of the present study is that the bradycardia evoked by systemically administered WIN55212-2 was solely mediated by the vagus nerve. In our previous study (Niederhoffer and Szabo, 1999), the bradycardia produced by intracisternally administered WIN55212-2 was antagonized by intravenously injected atropine. The site of interference of

atropine with the effect of WIN55212-2 was not clear: atropine could block vagal transmission peripherally, or, since it penetrates the blood-brain barrier, could influence cardiovascular regulation at the level of the medulla oblongata.

WIN55212-2 did not change blood pressure in untreated animals. After elimination of vagally mediated effects by methylatropine, WIN55212-2 caused marked hypertension, probably because the central sympathoexcitatory effect of the drug was not balanced by the vagally mediated cardiodepression (see Niederhoffer and Szabo, 1999, 2000, for central sympathoexcitation by cannabinoids).

The peripheral effects of cannabinoids on the autonomic innervation of the heart occurred at the same doses that elicit marked effects on heart rate, blood pressure, sympathetic nerve activity, and the plasma noradrenaline concentration after systemic administration in conscious animals (Niederhoffer and Szabo, 1999; present study). What is the contribution of the peripheral effects to the overall effects of cannabinoids on heart rate regulation? In conscious rabbits, heart rate effects of WIN55212-2 were mostly prevented by methylatropine, indicating that modulation of cardiac sympathetic tone by cannabinoids is of minor importance. The small increase in heart rate in methylatropine-treated animals after the highest dose of WIN55212-2 is indicative of central sympathoexcitation (see Niederhoffer and Szabo, 1999, 2000). The weak peripheral presynaptic inhibition of cardiac sympathetic transmission probably attenuated the centrally elicited sympathoexcitation. WIN55212-2 (0.005 and 0.05 mg kg<sup>-1</sup> i.v.) lowers heart rate in conscious rabbits by enhancing cardiac vagal tone with a primary central nervous action (see Niederhoffer and Szabo, 1999, 2000). Peripheral inhibition of vagal transmission is certainly operating after these WIN55212-2 doses, and it counteracts the central excitation of the vagus nerve. The heart rate increase in conscious rabbits after the highest dose of WIN55212-2 (0.5 mg kg<sup>-1</sup> i.v.) is primarily due to removal of cardiac vagal tone. Peripheral inhibition of vagal transmission may be the mechanism of this effect.

As described in the Introduction, acute administration of cannabinoids causes marked tachycardia in humans (Benowitz et al., 1979; Perez-Reyes et al., 1982; Huestis et al., 1992). The effect is antagonized by propranolol and atropine, indicating involvement of sympathetic and parasympathetic pathways. The results of the present study suggest that peripheral inhibition of cardiac vagal neuroeffector transmission may be one mechanism of the tachycardia in humans.

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