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ORIGINAL ARTICLE

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Analysis of the respiratory effects of cannabinoids in rats

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Abstract The objective of the present study was to evaluate the respiratory effects of cannabinoids and their influence on cardiovascular homeostasis.

In spontaneously breathing urethane-anaesthetised rats, intravenous injection of the two synthetic cannabinoid receptor agonists WIN55212-2 and CP55940 strongly and dose-dependently lowered mean arterial pressure, heart rate and the plasma noradrenaline concentration. The cardiovascular depressive effects were associated with a large decrease in respiratory rate, hypoxia, hypercapnia and blood acidosis. All depressor effects of WIN55212-2 were abolished by the selective CB_1 cannabinoid receptor antagonist SR141716A. The bradycardia elicited by WIN55212-2 was inhibited by the muscarinic acetylcholine receptor antagonist methylatropine. The natural agonist Δ^9-tetrahydrocannabinol also elicited cardiovascular and respiratory depression. In contrast, WIN55212-3, an enantiomer of WIN55212-2 lacking affinity for cannabinoid receptors, had no effect. The cannabinoid-evoked decreases in blood pressure and heart rate were much more pronounced in spontaneously breathing than in artificially ventilated urethane-anaesthetised rats. In contrast, the plasma noradrenaline concentration was lowered equally in both preparations.

Our results show that activation of CB_1 cannabinoid receptors not only induces cardiovascular depression, but also markedly impairs ventilation. The second major finding of the present study is that the respiratory depression evoked by cannabinoids largely amplifies the cardiovascular depression.

Keywords Anaesthetised rats · Blood pressure · Cannabinoid receptor · Heart rate · Plasma noradrenaline · Respiratory regulation · Δ^9-Tetrahydrocannabinol

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Introduction

Natural and synthetic cannabinoid agonists induce major changes in cardiovascular homeostasis (for reviews, see Dewey 1986; Compton et al. 1996; Wagner et al. 1998; Kunos et al. 2000). In humans, the most prominent effect of the major active component of *Cannabis sativa*, Δ^9-tetrahydrocannabinol, is a tachycardia (Malit et al. 1975; Huestis et al. 1992; Mathew et al. 1992). In anaesthetised animals, pronounced cardiovascular depression is usually reported (Cavero et al. 1972; Graham and Li 1973; Moss and Friedman 1976; Doherty et al. 1983; Varga et al. 1995, 1996; Vidrio et al. 1996; Lake et al. 1997a, 1997b).

In our previous study in artificially ventilated anaesthetised rats, we showed that cannabinoid agonists induce sympathoinhibition (acting mainly at peripheral sites) and enhancement of cardiac vagal tone (the primary site of action for this latter effect remains to be identified); both effects are mediated by CB_1 receptors and lead to cardiovascular depression (Niederhoffer et al. 2003). A surprising finding of this study was that the decreases in blood pressure and heart rate elicited by cannabinoids were much less pronounced in artificially ventilated than in spontaneously breathing anaesthetised rats. Thus, the cardiovascular depressive action of cannabinoids depends strongly on the respiratory state of the animals. On the basis of this observation, we hypothesised that cannabinoid agonists may also affect respiration, and that this respiratory depression could enhance the cardiovascular depression.

Previous studies on the respiratory effect of Δ^9-tetrahydrocannabinol in humans gave inconsistent results: no change, decreases or increases in respiratory rate or tidal volume were observed (Johnstone et al. 1975; Malit et al. 1975; Mathew et al. 1992). In contrast, in animals, Δ^9-tetrahydrocannabinol generally elicited a marked respiratory depression (Phillips et al. 1971; Cavero et al. 1972; Graham and Li 1973; Rosenkrantz et al. 1974; Moss and Friedman 1976; Doherty et al. 1983; Estrada et al. 1987). However, it is not yet clear whether specific cannabinoid receptors were involved in the respiratory effects of Δ^9-tetrahydro-

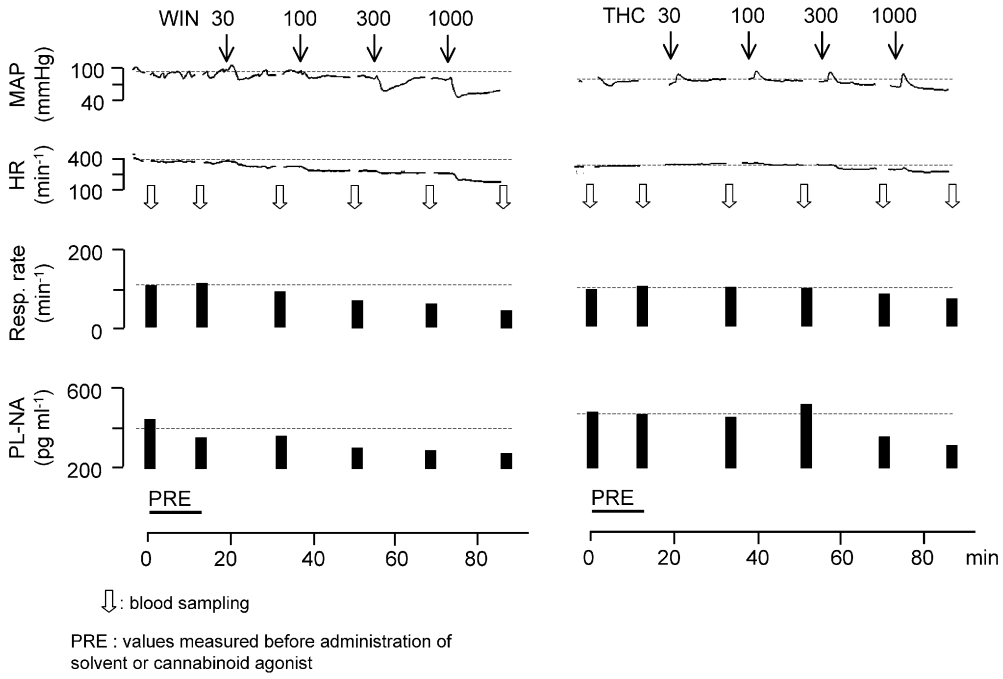

Fig. 1 Effects of WIN55212-2 (*WIN*) and Δ^9-tetrahydrocannabinol (*THC*) on mean arterial pressure (*MAP*), heart rate (*HR*), respiratory rate (*Resp. rate*) and the plasma noradrenaline concentration (*PL-NA*) in two spontaneously breathing anaesthetised rats. WIN (30, 100, 300 and 1,000 µg kg^{-1}) and THC (30, 100, 300 and 1,000 µg kg^{-1}) were injected i.v. as indicated by the *arrows*. Note that injection of THC and WIN elicited a transient increase in mean arterial pressure (attributable to the solvent for cannabinoid agonists; see Fig. 2), followed by longer-lasting decrease in mean arterial pressure (the proper cannabinoid effect). The two agonists also decreased heart rate, respiratory rate and the plasma noradrenaline concentration. The original tracings represent eight (WIN) and three (THC) experiments with similar results

cannabinol. First, this compound can also elicit effects independent of cannabinoid receptors (Martin 1986; Lake et al. 1997b). Second, no cannabinoid receptor antagonist was used, since not available, in these early experiments. In a recent publication, the synthetic cannabinoid WIN55212-2 was used as an agonist (Vivian et al. 1998): no change in the respiratory rate of conscious monkeys was observed but tidal volume was lowered, suggesting that cannabinoid agonists may impair respiration by acting on specific cannabinoid receptors.

Therefore, the first objective of our study was to identify the receptor involved in the respiratory effect of cannabinoids in anaesthetised rats. To this end, we analysed the effects of the natural cannabinoid agonist, Δ^9-tetrahydrocannabinol, and of two synthetic cannabinoid receptor agonists, WIN55212-2 and CP55940. WIN55212-2 and CP55940 belong to different chemical classes, but are similar to each other in that they possess high affinity for CB_1 and CB_2 receptors and lack affinity for other receptors and ion channels (Felder et al. 1995; Showalter et al. 1996; Pertwee 1999). Interaction experiments with the CB_1-selective antagonist SR141716A (Pertwee 1999) were also carried out. The second objective of our study was to determine whether the respiratory depression elicited by cannabinoids could contribute to cardiovascular depression. To this end, cardiovascular responses obtained in spontaneously breathing anaesthetised rats were compared to those observed previously in artificially ventilated anaesthetised rats (Niederhoffer et al. 2003).

Materials and methods

Experiments were carried out on 51 Wistar rats (250–350 g) obtained from Charles River, Kisslegg, Germany.

Surgical procedures. Rats were anaesthetised with urethane i.p. (1.5 g kg^{-1}). A polyethylene catheter (0.5 mm i.d., 1 mm o.d.) was implanted into the right femoral artery and connected to a low volume pressure transducer (Baxter, Bentley Laboratories Europe, Uden, The Netherlands) coupled to a bridge amplifier (Hugo Sachs Elektronik, Hugstetten, Germany) for recording blood pressure. Heart rate was calculated from the pulsating blood pressure signal using a tachometer (EKA-PULS, Hugo Sachs Elektronik, Hugstetten, Germany). The arterial catheter also served for blood sampling. A second catheter was implanted into the right femoral vein for intravenous (i.v.) administration of drugs. In some animals, a third catheter was implanted into the left femoral vein for continuous i.v. infusion. A tracheotomy was performed to maintain an open airway. At the end of the surgery, additional urethane was given i.v. (0.2 g kg^{-1}). Body temperature was maintained at 37°C throughout the experiment using a heating pad controlled by a rectal probe.

Experimental protocol. Experiments were started after 60 min of stabilisation (t=0 min). After a 15-min baseline period (PRE period), solvent (0.5 ml kg^{-1}) or increasing doses of the cannabinoid receptor ligands WIN55212-2, WIN55212-3, CP55940 and Δ^9-tetrahydrocannabinol (30, 100, 300 and 1,000 µg kg^{-1}) were injected i.v. at t=19, 37, 55 and 73 min (see also Fig. 1 for protocol). A second WIN55212-2 group was treated from t=–10 min onwards with the peripherally acting muscarinic acetylcholine receptor antagonist methylatropine (1 mg kg^{-1} bolus injection+2 mg kg^{-1} h^{-1} infusion). In a further series of experiments, rats were pretreated at t=–10 min with the CB_1 cannabinoid receptor antagonist SR141716A (2 mg kg^{-1}) and received a single dose of WIN55212-2 (300 µg kg^{-1}) at t=19 min.

Evaluation and statistics. Blood pressure and heart rate were recorded continuously from t=0 min until the end of the experiment. Values given at each time point are averages of values recorded over a 10-s interval. The respiratory rate was evaluated by counting the respiratory movements over a 15-s period. In some animals, partial pressure of oxygen (pO_2) and carbon dioxide (pCO_2) and pH in arterial blood were measured in 85-µl blood samples using a blood gas analyser (Radiometer Copenhagen ABL 510, Brønshøj, Denmark). The plasma noradrenaline concentration was determined from 0.5-ml arterial blood samples by alumina chromatography followed by HPLC and electrochemical detection as previously detailed (Szabo et al. 2001a).

Mean arterial pressure and heart rate were read every 2 min. The respiratory rate (and in some animals, arterial blood pH, pO_2 and pCO_2) and the plasma noradrenaline concentration were evaluated twice during the PRE-period (at t=0 and 14 min) and then 15 min after each drug administration. In each experiment, values for mean arterial pressure, heart rate, respiratory rate and the plasma noradrenaline concentration measured at t=0 and 14 min were averaged to yield initial absolute values for parameters before administration of solvent or cannabinoid ligands (PRE). All further values were then expressed as percentages of PRE.

Means ± SEM of n experiments are given throughout. Differences between groups were evaluated using the non-parametric two-tailed Mann-Whitney test. In all experiments, $P<0.05$ 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, USA); Emulphor from Rhone-Poulenc (Cranberry, NJ, USA); N-piperidino-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-3-pyrazole-carboxamide hydrochloride (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 RBI (Cologne, Germany); 2-hydroxypropyl-ß-cyclodextrin (ß-CDX) from Fluka (Neu-Ulm, Germany); Δ^9-tetrahydrocannabinol, urethane and methylatropine sulfate from Sigma (Deisenhofen, Germany).

WIN55212-2, WIN55212-3, CP55940 and Δ^9-tetrahydrocannabinol were dissolved in 45% (w/v) solutions of ß-CDX; further dilutions were made with the same solvent. SR141716A was dissolved in a vehicle containing ethanol/Emulphor/saline (1:1:8; v:v:v). Urethane and methylatropine were dissolved in 0.9% saline. Doses refer to the salts. All drugs were administered intravenously in a volume of 0.5 ml kg^{-1}. Methylatropine was infused at a rate of 1.9 ml h^{-1}.

Results

Baseline parameters (PRE values)

Values for parameters measured at t=0 and 14 min were averaged to yield initial absolute values before administration of solvent or cannabinoid ligands (PRE; see Table 1). In unpretreated rats, mean PRE values for mean arterial pressure and the plasma noradrenaline concentration were similar to those previously obtained, whereas heart rate was slightly lower (Szabo et al. 2001a; Niederhoffer et al. 2003). In rats pretreated with the cannabinoid receptor antagonist SR141716A (2 mg kg^{-1}), the plasma noradrenaline concentration was significantly higher and mean arterial pressure tended to be higher than in unpretreated animals. However, mean arterial pressure was higher in these animals already at the beginning of experiments, i.e., during the stabilisation period, and was not significantly enhanced by SR141716A (not shown). Therefore, the high blood pressure and plasma noradrenaline values measured after SR141716A administration probably do not reflect an effect of the antagonist, but are due to the accidentally high sympathetic tone in this group of animals. In animals treated with methylatropine (1 mg kg^{-1} bolus injection + 2 mg kg^{-1} h^{-1} infusion), heart rate was increased and the respiratory rate was lowered.

Table 1 Absolute initial values for parameters before injection of solvent or cannabinoid ligands (PRE[a])

Pretreatment	n	Mean arterial pressure (mmHg)	Heart rate (min^{-1})	Respiratory rate (min^{-1})	Plasma noradrenaline concentration (pg ml^{-1})
–	43	84±1	372± 7	128±3	581± 36
SR141716A[b]	4	95±7	383± 8	136±2	1,091±127*
Methylatropine[c]	4	83±6	428±16*	95±3*	710±167

[a]Average of values measured at t=0 and 14 min
[b]SR141716A (2 mg kg^{-1}) was injected at t=–10 min
[c]Methylatropine (1 mg kg^{-1} bolus injection + 2 mg kg^{-1} h^{-1} infusion) was given from t=–10 min
*$P<0.05$ vs. unpretreated animals

Control experiments

Administration of the solvent for cannabinoids elicited transient increases in mean arterial pressure with no change in the other parameters (Figs. 2, 3).

Effects of cannabinoid ligands

Increasing doses of the synthetic cannabinoid agonists WIN55212-2 and CP55940 (30, 100, 300 and 1,000 µg kg^{-1}) markedly and dose-dependently lowered mean arterial pressure, heart rate and the plasma noradrenaline concentration (Fig. 2; see also an original tracing in Fig. 1). These cardiovascular effects were accompanied by a large decrease in respiratory rate (Figs. 1, 2). CP55940 decreased, in the arterial blood, the partial pressure of oxygen and the pH, whereas it increased the partial pressure of carbon dioxide (Fig. 3). In the CP55940 group, two of nine animals stopped breathing immediately after administration of the highest dose (1,000 µg kg^{-1}) and were not included in the statistics; in the same group, another animal stopped breathing shortly before the end of the experiment, so that the four last values for mean arterial pressure and heart rate (Fig. 2), the last values for plasma noradrenaline and respiratory rate (Fig. 2), and the last values for arterial blood pH and partial pressures of oxygen and carbon dioxide (Fig. 3) could not be determined.

The natural cannabinoid agonist, Δ^9-tetrahydrocannabinol (30, 100, 300 and 1,000 µg kg^{-1}) also decreased mean arterial pressure, heart rate, the plasma noradrenaline con-

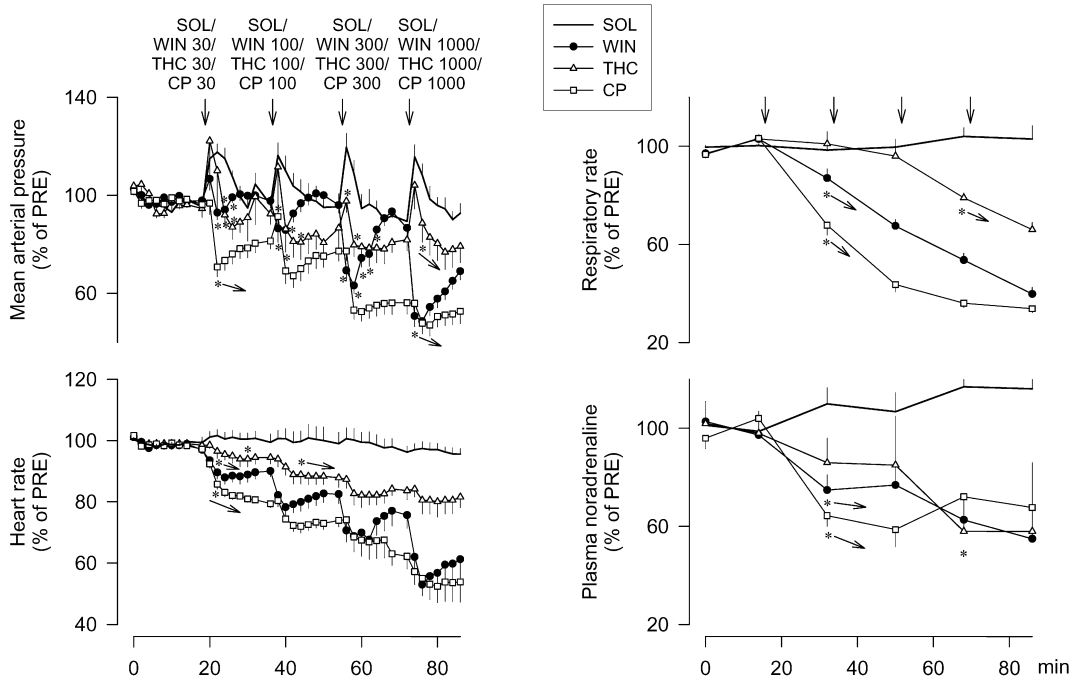

Fig. 2 Effects of solvent (*SOL*), WIN, THC and CP55940 (*CP*) on mean arterial pressure, heart rate, respiratory rate and the plasma noradrenaline concentration in spontaneously breathing anaesthetised rats. SOL (0.5 ml kg^{-1}), WIN (30, 100, 300 and 1,000 µg kg^{-1}), THC (30, 100, 300 and 1,000 µg kg^{-1}) and CP (30, 100, 300 and 1,000 µg kg^{-1}) were injected i.v. as indicated by the *arrows*. Values are given as percentages of PRE. Means ± SEM of eight (SOL), eight (WIN), three (THC) and seven (CP) experiments. In the CP group, 1 of the 7 animals stopped breathing shortly before the end of the experiment, so that the four last values for mean arterial pressure and heart rate, and the last values for respiratory rate and the plasma noradrenaline concentration could not be determined in this animal. **P*<0.05 vs. SOL (*arrows* in the graphs indicate that all further values were significantly different from SOL)

centration and respiratory rate (Fig. 2; see also an original tracing in Fig. 1).

In contrast, the inactive enantiomer WIN55212-3 (30, 100, 300 and 1,000 µg kg^{-1}) elicited transient increases in mean arterial pressure similar to those observed after solvent injection, with no change in the other measured parameters (mean arterial pressure: 100±9, heart rate: 91±3, respiratory rate: 96±6, and plasma noradrenaline concentration: 104±17% of PRE after WIN55212-3 1,000 µg kg^{-1}; *n*=8).

Interaction experiments

Cardiovascular and respiratory responses to a single dose of WIN55212-2 (300 µg kg^{-1}) were abolished by pretreatment with SR141716A (2 mg kg^{-1}; Fig. 4).

In the presence of methylatropine (1 mg kg^{-1} bolus injection + 2 mg kg^{-1} h^{-1} infusion), the bradycardic effect of WIN55212-2 was greatly attenuated, the decrease in mean arterial pressure was slightly enhanced, whereas the decrease in the plasma noradrenaline concentration was not changed (Fig. 5). Methylatropine prevented the respiratory depression caused by WIN55212-2 (30 µg kg^{-1}), but did not change the respiratory depression caused by the higher doses (Fig. 5).

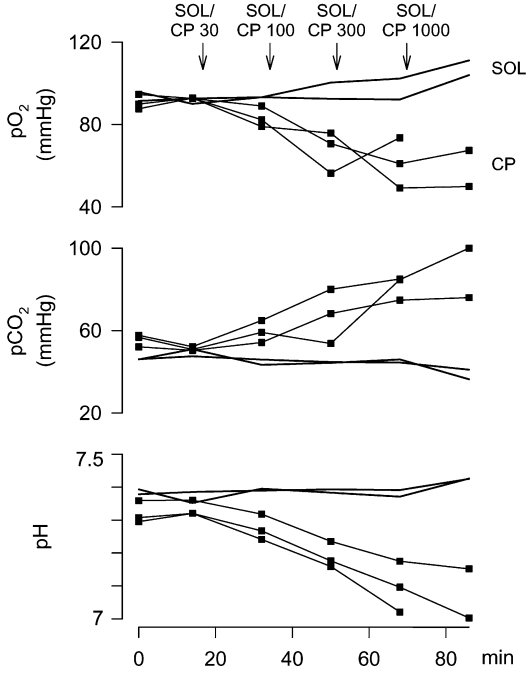

Fig. 3 Effects of SOL and CP on partial pressures of oxygen (pO_2) and carbon dioxide (pCO_2), and on pH in arterial blood from spontaneously breathing anaesthetised rats. SOL (0.5 ml kg^{-1}) and CP (30, 100, 300 and 1,000 µg kg^{-1}) were injected i.v. as indicated by the *arrows*. In the CP group, 1 of the 3 animals stopped breathing shortly before the end of the experiment, so that the last values for pO_2, pCO_2 and pH could not be determined in this animal

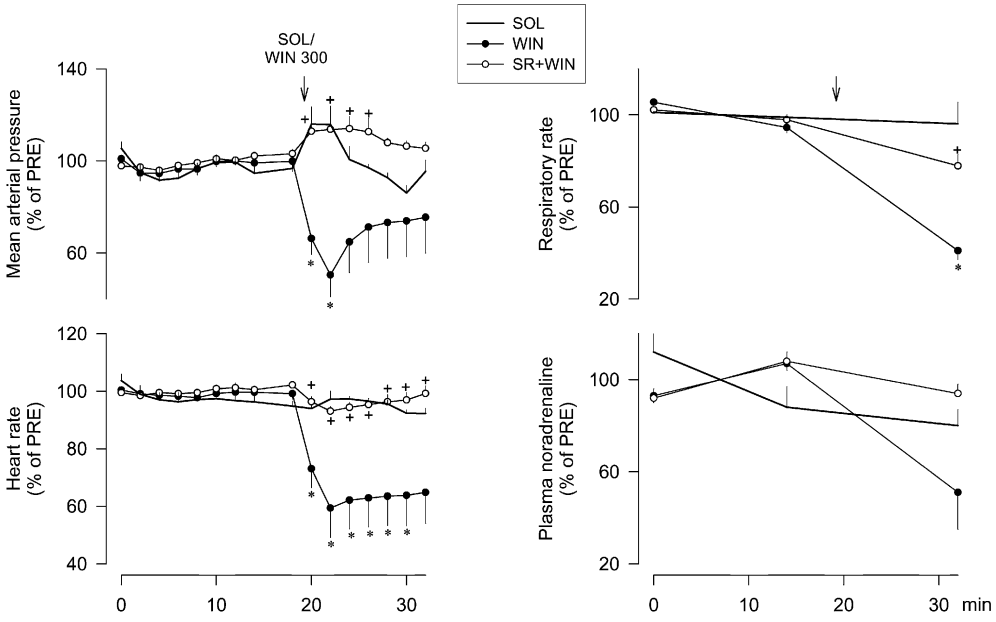

Fig. 4 Interaction between WIN and SR141716A (*SR*) in spontaneously breathing anaesthetised rats. SOL (0.5 ml kg^{-1}) and WIN (300 µg kg^{-1}) were injected i.v. as indicated by the *arrows*. One of the two WIN groups was pretreated at *t*=–10 min with SR (2 mg kg^{-1}). Values are given as percentages of PRE. Means ± SEM of four (SOL), five (WIN) and four (SR+WIN) experiments. **P*<0.05 vs. SOL; +*P*<0.05 vs. WIN alone

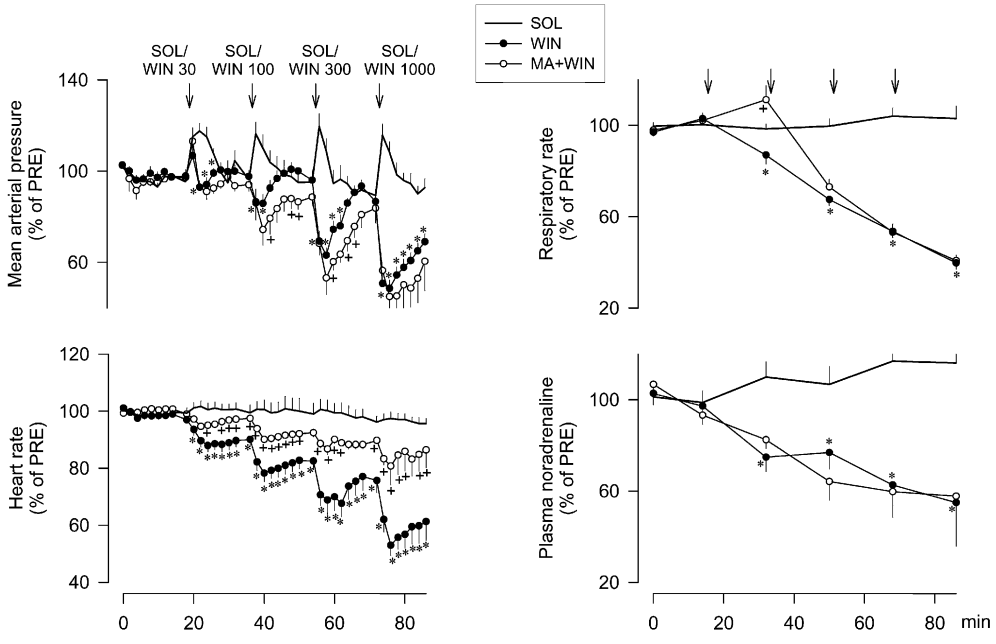

Fig. 5 Interaction between WIN and methylatropine (*MA*) in spontaneously breathing anaesthetised rats. SOL (0.5 ml kg^{-1}) and WIN (30, 100, 300 and 1,000 µg kg^{-1}) were injected i.v. as indicated by the *arrows*. One of the two WIN groups was treated from *t*=–10 min with MA (1 mg kg^{-1} bolus injection + 2 mg kg^{-1} h^{-1} infusion). Values are given as percentages of PRE. Means ± SEM of eight (SOL), eight (WIN) and four (MA+WIN) experiments. **P*<0.05 vs. SOL; +*P*<0.05 vs. WIN alone

Comparison of cardiovascular responses to cannabinoids in artificially ventilated and spontaneously breathing rats

Cardiovascular effects of the two agonists WIN55212-2 and CP55940 in artificially ventilated anaesthetised rats were described in detail in our previous publication (Niederhoffer et al. 2003).

The decreases in mean arterial pressure and heart rate after CP55940 were greatly enhanced in spontaneously breathing rats as compared to those observed in artificially ventilated rats; in contrast, there was no significant difference between effects on the plasma noradrenaline concentration in the two preparations (Fig. 6). Blood pressure and heart rate responses to WIN55212-2 were also enhanced in spontaneously breathing rats compared to those observed in artificially ventilated rats (see Fig. 5 in Niederhoffer et al. 2003).

Discussion

In agreement with previous studies (see Introduction), administration of cannabinoid agonists in anaesthetised rats strongly lowered mean arterial pressure and heart rate. The agonists also elicited a marked decrease in the plasma noradrenaline concentration. Effects of WIN55212-2 on blood pressure, heart rate and the plasma noradrenaline concentration were abolished by pretreatment with SR141716A.

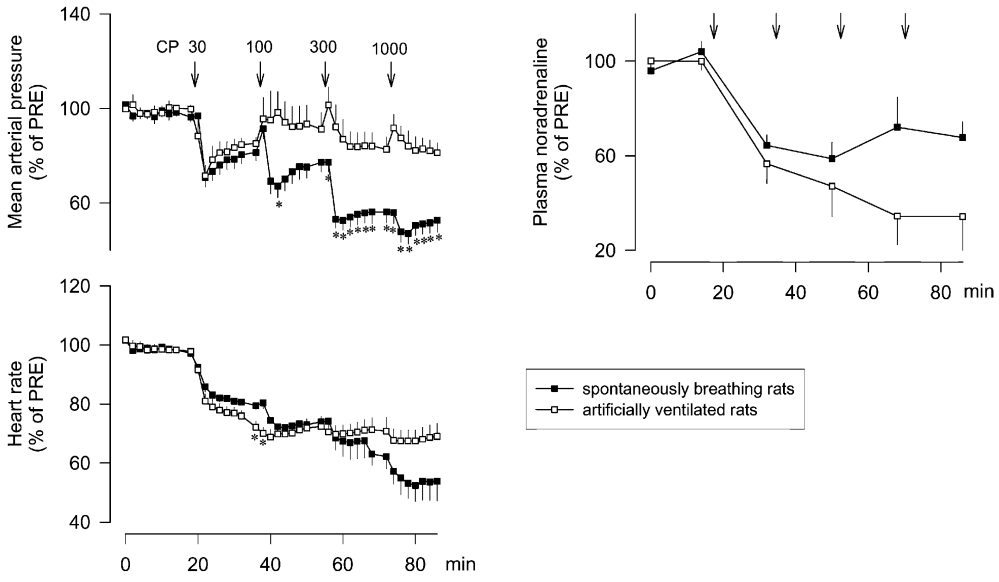

Fig. 6 Comparison of the effects of CP on mean arterial pressure, heart rate and the plasma noradrenaline concentration in spontaneously breathing and artificially ventilated anaesthetised rats. CP (30, 100, 300 and 1,000 µg kg^{-1}) was injected i.v. as indicated by the *arrows*. Values are given as percentages of PRE. Means ± SEM of seven (spontaneously breathing) and six (artificially ventilated) experiments. **P*<0.05 vs. ventilated rats

The bradycardia elicited by WIN55212-2 was almost completely prevented by muscarinic acetylcholine receptor blockade. Thus, the present results support previous data collected by us (Niederhoffer and Szabo 1999, 2000; Niederhoffer et al. 2001, 2003; Szabo et al. 2001b) and other groups (Ishac et al. 1996; Varga et al. 1995, 1996; Vidrio et al. 1996; Lake et al. 1997a, 1997b; Malinowska et al. 1997, 2001) suggesting that the cardiovascular depressive effects of cannabinoids result from CB_1 receptor-mediated sympathoinhibition and enhancement of cardiac vagal tone.

The first major finding of the present study is that cannabinoid agonists – both synthetic and natural – also elicit respiratory depression, as shown by a marked decrease in respiratory rate, hypoxia, hypercapnia and arterial blood acidosis. These effects involved specific cannabinoid receptors of the CB_1 subtype since WIN55212-3, the enantiomer of WIN55212-2 lacking affinity for cannabinoid receptors, was ineffective, and since pretreatment with the CB_1 receptor antagonist SR141716A abolished the effects of WIN55212-2. Respiratory depression in rats receiving Δ^9-tetrahydrocannabinol was reported in early publications (Phillips et al. 1971; Graham and Li 1973; Rosenkrantz et al. 1974; Estrada et al. 1987) but involvement of specific cannabinoid receptors could not be demonstrated. The present study demonstrates for the first time that CB_1 cannabinoid receptors are involved in the respiratory actions of cannabinoids in this species. In a recent study on conscious monkeys, WIN55212-2 decreased tidal and minute volume, whereas respiratory frequency was not changed (Vivian et al. 1998). The discrepancy between these results and ours may be due to the use of different species, different ways of administration of the agonist (i.m. vs. i.v.) and/or state of consciousness (awake vs. anaesthetised).

The primary site of action of cannabinoids for eliciting respiratory depression was not identified in the present study. The inhibition of the respiration was most likely not a consequence of the cardiovascular depression, since cardiovascular depression – similar to that occurring in our experiments – usually leads to a stimulation of the central ventilatory drive, and consequently, to an increase in respiratory frequency (Schläfke and Koepchen 1996). The cannabinoids inhibited respiration probably by acting directly in the central nervous system. Thus, we recently observed that administration of low doses of cannabinoids into the cisterna cerebello medullaris of anaesthetised rats elicits similar respiratory depression (Szabo et al. 2002). Previous studies also concluded that the decrease in respiratory frequency elicited by Δ^9-tetrahydrocannabinol results primarily from depression of the central respiratory drive rather than from a peripheral action of the drug (Moss and Friedman 1976; Doherty et al. 1983). However, an additional effect at the periphery cannot be ruled out. Cannabinoids could affect the function of different peripheral receptors involved in respiratory regulation (e.g., chemo- and baroreceptors, pulmonary stretch receptors). They could also influence airway resistance acting directly on the bronchi (Calignano et al. 2000).

The second major finding of the present study is that the respiratory depression markedly enhances the cardiovascular depression observed after cannabinoid administration. Hence, the decreases in mean arterial pressure and heart rate elicited by the two cannabinoid agonists WIN55212-2 and CP99540 were much more pronounced in spontaneously breathing rats than in artificially ventilated rats. These findings are in contrast with those of Lake et al. (1997b), who reported similar cardiovascular depressive actions of anandamide in spontaneously breathing and artificially ventilated animals. It was suggested that the mechanisms underlying cardiovascular actions may differ between anandamide and other more potent and efficacious cannabinoid agonists, like WIN55212-2 and CP99540 (Varga et al. 1996; Lake et al. 1997a). This may explain the different influence of artificial ventilation on cardiovascular responses to anandamide and other agonists.

It could be argued that the cannabinoids caused different cardiovascular depression in spontaneously breathing and artificially ventilated rats because baseline respiratory and/or cardiovascular parameters were different in the two preparations. Several observations suggest, however, that this was not the case. First, baseline values for partial pressure of oxygen and carbon dioxide and pH in arterial blood were similar in the two preparations (compare present study with Niederhoffer et al. 2003), and were in agreement with those usually reported in urethane-anaesthetised rats (see for example, Carruba et al. 1987). Second, PRE values for mean arterial pressure and the plasma noradrenaline concentration measured in spontaneously breathing animals (present paper) were similar to those measured in artificially ventilated animals (Niederhoffer et al. 2003), indicating that baseline sympathetic tone was the same in both preparations. Only baseline heart rate was lower in spontaneously breathing rats, suggesting that cardiac vagal tone was higher in these animals than in artificially ventilated rats. Since cannabinoids decrease heart rate mainly through an enhancement of cardiac vagal tone (see above), their action should be greater in animals with a low basal cardiac vagal tone than in animals with a high basal cardiac vagal tone. Consequently, one would expect a larger bradycardic effect of the cannabinoid agonists in artificially ventilated animals than in spontaneously breathing animals. However, the opposite was observed: the cannabinoid-induced fall in heart rate was greater in spontaneously breathing rats than in artificially ventilated rats.

The additional "respiratory-dependent" hypotension measured in spontaneously breathing rats was unrelated to the concomitant decrease in heart rate since it persisted in animals treated with methylatropine. It was also not due to a greater sympathoinhibition since the decreases in the plasma noradrenaline concentration were similar in spontaneously breathing and artificially ventilated rats. One possible explanation is that hypercapnia following hypoventilation leads to vasodilation of resistance vessels, and thus to a decrease in total peripheral resistance (Rothe et al. 1990). In addition, the cannabinoid evoked respiratory depression may also impair the effectiveness of the baroreceptor reflex, resulting in more pronounced cardiovascular depression in spontaneously breathing than in artificially ventilated animals. Indeed, it is known that the function of the baroreceptor reflex is modulated by respiration (Haymet and McCloskey 1975; Davidson et al. 1976).

The additional "respiratory-dependent" bradycardia was mainly due to activation of cardiac vagal tone since it was almost totally abolished by methylatropine. Stimulation of preganglionic vagal fibres innervating the heart may result from prolonged post-inspiratory phases and/or stimulation of arterial chemoreceptors following respiratory depression (De Burgh Daly and Hazzledine 1963; Haymet and McCloskey 1975; Gilbey et al. 1984; Taylor et al. 1999).

In summary, the present study shows that Δ^9-tetrahydrocannabinol and synthetic cannabinoid agonists cause not only cardiovascular depression, but also a marked respiratory depression by acting at CB_1 receptors. This respiratory depression greatly enhances the cardiovascular depression.

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