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Effects of cannabinoids on non-adrenergic non-cholinergic-mediated relaxation in guinea-pig trachea

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Abstract

The effects of cannabinoid receptor agonists on the non-adrenergic non-cholinergic (NANC) inhibitory responses to electrical field stimulation in guinea-pig trachea were assessed. R-(+)-[2,3-dihydro-5-methyl-3-[(morpholilinyl) methyl]pyrrolo [1,2,3-de]-1,4-benzoxazin-6-yl]-(1-naphthalenyl)methanone mesylate (WIN 55,212-2; 10^{-5} M) significantly enhanced the frequency-dependent response to electrical stimulation. The same concentration of R-(*N*)-(2-hydroxy-1-methylethyl)-5*Z*,8*Z*,11*Z*,14*Z*-eicosatetraenamide (R(+)methanandamide) and 1-propyl-2-methyl-3-(1-naphthoyl)indole (JWH-015) did not affect significantly the electrically induced inhibitory NANC responses. The effect of WIN 55,212-2 was not modified by the cannabinoid CB_1 and CB_2 receptor-selective antagonists, *N*-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide hydrochloride (SR141716A; 10^{-5} M) and *N*-(1S)-endo-1,3,3-trimethyl bicyclo [2.2.1] heptan-2-yl]-5-(4-chloro-3-methylphenyl)-1-(4-methylbenzyl)-pyrazole-3-carboxamide (SR 144528; 10^{-5} M), respectively. Moreover, the nitric oxide synthase inhibitor, L-N^G-nitro-arginine methyl ester (L-NAME; 10^{-4} M), but not the peptidase, α-chymotrypsin (2 U/ml), blocked the effect of WIN 55,212-2. Postsynaptically, WIN 55,212-2 did not produce any change of tracheal smooth muscle tone, either basal or histamine-induced, and did not interfere with the relaxant activity of the nitric oxide donor, sodium nitroprusside (10^{-8}–10^{-4} M). In conclusion, our results suggest that (a) cannabinoid CB_1 and CB_2 receptor stimulation does not alter the inhibitory NANC transmission in guinea-pig trachea, and (b) WIN 55,212-2 potentiates the NO-mediated component of the NANC relaxant response to electrical stimulation through a cannabinoid receptor-independent mechanism.

Keywords: NANC (Non-adrenergic non-cholinergic) inhibitory; Cannabinoid; WIN 55,212-2; Trachea; (Guinea-pig); Nitric oxide (NO)

1. Introduction

Cannabinoids modulate neurotransmission at a central and a peripheral level; their ability to modify neural activity has also been demonstrated in airways on cholinergic (Yousif and Oriowo, 1999; Spicuzza et al., 2000; Nieri et al., 2003), adrenergic (Vizi et al., 2001) and excitatory non-adrenergic non-cholinergic (NANC) (Calignano et al., 2000; Tucker et al., 2001) fibers. In addition to the above mentioned neural pathways, the inhibitory NANC system plays a relevant role in the control of airway smooth muscle tone in most mammals, notably the human species in which there is evidence for a scanty adrenergic innervation of the tracheobronchial tree (Barnes, 1986). NANC relaxations, evoked by electrical field stimulation in guinea-pigs (Tucker et al., 1990; Li and Rand, 1991), are mediated by vasoactive intestinal peptide (VIP) and related peptides, by nitric oxide (NO) and probably by other neurotransmitters not yet clearly identified (Canning and Fischer, 2001). The aim of the present study was to assess the action of exogenous cannabinoids on the electrical field stimulation-mediated NANC relaxant response in guinea-pig trachea by evaluating the effects of R(+)-methanandamide, the stable analogue of the endocannabinoid, anandamide (Pertwee et al., 1995), and of other cannabinoid receptor agonists and antagonists for the two types of receptor so far discovered, CB_1 and CB_2 (Howlett et al., 2002).

2. Materials and methods

The experiments were carried out in conformity with the legislation of the Italian authorities (D.L. 27/1/1992 no. 116) and the European Community Directive 86/609, concerning the care and use of laboratory animals. Male Dunkin–Hartley guinea-pigs (250–300 g) were killed after light ether anaes-

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thesia by cervical dislocation and bleeding. The trachea was removed immediately and placed in Krebs–Henseleit solution (composition mM: NaCl 118, KCl 4.7; $CaCl_2$ 2.5, KH_2PO_4 1.2, $MgSO_4$ 1.2, $NaHCO_3$ 25.0, glucose 11.5), freed from adherent fat and connective tissue and cut according to the method of Emmerson and Mackay (1979). Trachea preparations were suspended between two platinum electrodes in a 20-ml organ bath containing the Krebs–Henseleit solution maintained at 37 °C, gassed with carbogen (95% O_2 and 5% CO_2), set-up under 0.5-g resting tension and tied to isometric transducers (mod. FT03D, Grass Instruments, USA), connected to a polygraph (mod. WR 3101, Graphtec Corp., Japan).

After a 20-min stabilization period, the bathing medium was supplemented with indomethacin (3×10^{-6} M) to avoid the production of cyclooxygenase products during electrical stimulation, and with capsaicin (10^{-5} M) to deplete C-fiber endings (Jancsò et al., 1967). After capsaicin-induced contraction, an interval of 45–50 min was allowed so that the trachea preparations could return to their basal tone. After the adrenergic and cholinergic responses to electrical field stimulation had been blocked by 15-min pretreatment with propanolol and atropine (10^{-6} M both), tracheal tissues were pre-contracted with histamine (10^{-5} M) until a stable plateau was reached; then a relaxing frequency–response curve was obtained by electrical field stimulation (digital stimulator, BM ST 6 Biomedica Mangoni, Italy): single trains (0.5-ms pulse width; 20-V amplitude; 15-s train duration) were repeated with increasing frequency (1–100 Hz) every 5 min. In each preparation, a single frequency–response curve was obtained. On histamine pre-contracted and electrically or chemically stimulated preparations, the following protocols were performed:

Protocol 1: The cannabinoid receptor agonists, R-(*N*)-(2-hydroxy-1-methylethyl)-5*Z*,8*Z*,11*Z*,14*Z*-eicosatetraenamide (R(+)-methanandamide; slightly selective for CB_1), R-(+)-[2,3-dihydro-5-methyl-3-[(morpholilinyl) methyl]-pyrrolo [1,2,3-de]-1,4-benzoxazin-6-yl]-(1-naphthalenyl)methanone mesylate (WIN 55,212-2; slightly selective for CB_2) or 1-propyl-2-methyl-3-(1-naphthoyl)indole (JWH-015; CB_2 selective), (10^{-7}–10^{-5} M), were added after reaching the plateau response to histamine and 5-min before electrical stimulation.

Protocol 2: The cannabinoid CB_1 receptor antagonist *N*-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide hydrochloride (SR 141716A; 10^{-5} M) or the cannabinoid CB_2 receptor antagonist *N*-(1S)-endo-1,3,3-trimethyl bicyclo [2.2.1] heptan-2-yl]-5-(4-chloro-3-methylphenyl)-1-(4-methylbenzyl)-pyrazole-3-carboxamide (SR 144528; 10^{-5} M) was added to the bathing medium 10-min before cannabinoid agonist (WIN 55,212-2) administration (see protocol 1).

Protocol 3: L-N^G-Nitro-arginine methyl ester (L-NAME; 10^{-4} M) or α-chymotrypsin (2 U/ml) was incubated for 10 min before cannabinoid receptor agonist (WIN 55,212-2) administration (see protocol 1).

Protocol 4: WIN 55,212-2 (10^{-5} M) was tested on a concentration–response curve for the nitric oxide-donor sodium nitroprusside (10^{-8}–10^{-4} M).

Drug responses were always compared to controls with drug vehicle alone.

Electrical field stimulation- and sodium nitroprusside-induced relaxations were expressed as percent reversal of

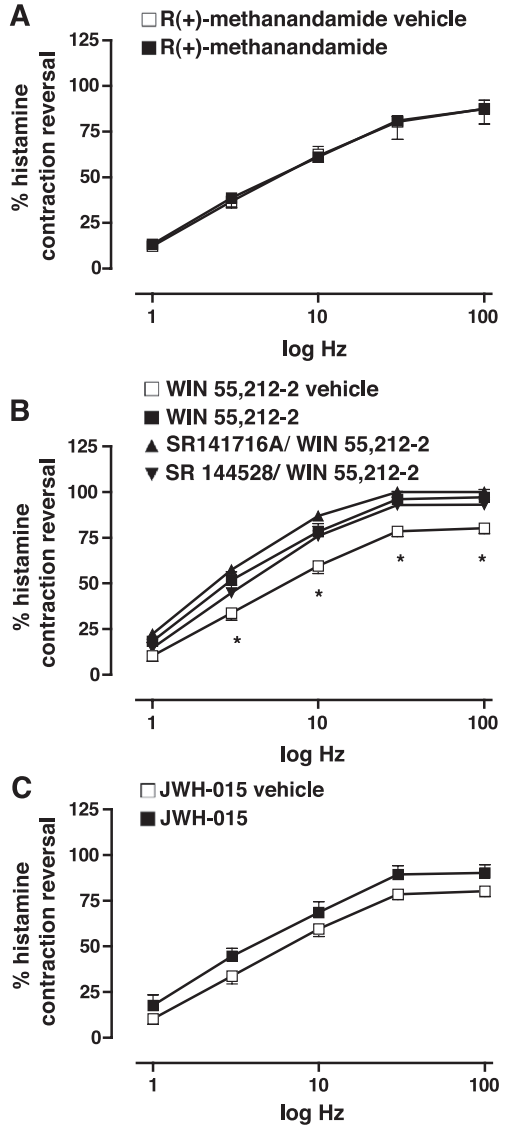

Fig. 1. Frequency-dependent relaxant response of pre-contracted (histamine 10^{-5} M) and electro-stimulated (0.5-ms pulse width; 20-V amplitude; 15-s train duration; 1–100 Hz) guinea-pig trachea, in the presence of: (A) R(+)-methanandamide 10^{-5} M (■) or R(+)-methanandamide vehicle (EtOH 0.1%) (□); (B) WIN 55,212-2 (■), WIN 55,212-2 vehicle (DMSO 0.5%) (□) or WIN 55,212-2 after administration of SR 141716A 10^{-5} M (▲) and SR 144528 10^{-5} M (▼); (C) JWH-015 (■) or JWH-015 vehicle (□). Each point represents the mean of five to seven experiments and vertical lines represent standard error of the mean. *$P \leq 0.05$ from Student's *t* test for unpaired data.

the histamine-induced tone. GraphPad Prism 3.0 (GraphPad Software, USA) was used for the computer-fitting procedure. All data are showed in graphs as means ± S.E.M. of *n* experiments. Statistical evaluations were performed using Student's *t* test for unpaired data (two groups). $P \leq 0.05$ was taken to be significant.

3. Results

The frequency-dependent relaxant response obtained in guinea-pig trachea with electrical field stimulation had a neural origin since it was blocked by tetrodotoxin (10^{-6} M).

The cannabinoid CB_1>CB_2 receptor agonist R(+)-methanandamide at concentrations up to 10^{-5} M did not affect the frequency–response curve (Fig. 1A). The cannabinoid CB_2>CB_1 receptor agonist, WIN 55,212-2, on the contrary, at 10^{-5} M, but not at lower concentrations, significantly enhanced the frequency–response curve to electrical stimulation; this effect was not antagonized by the cannabinoid CB_1 or CB_2 receptor-selective antagonists, SR 141716A and SR 144528 (10^{-5} M), respectively (Fig. 1B) (Table 1).

The cannabinoid CB_2 receptor-selective agonist, JWH-015 (10^{-7}–10^{-5} M), did not significantly modify the frequency–response curve for electrical stimulation (Fig. 1C). In some experiments, WIN 55,212-2 potentiating activity on the response to electrical stimulation was evaluated in the presence of L-NAME (10^{-4} M) or α – chymotrypsin (2 U/ml); in these experiments, L-NAME, but not α – chymotrypsin, was able to block the potentiation under study (Fig. 2A and B) (Table 1).

When WIN 55,212-2 was tested on the relaxant response to the NO-generating agent, sodium nitroprusside, no change of the concentration–response curve to the NO donor was seen (Fig. 2C).

None of the cannabinoid receptor agonists employed in this study showed a significant effect on tracheal tone, both under basal conditions and in the presence of the histamine-induced contraction. Moreover, there was no observable effect of the antagonists, SR 141716A and SR 144528, tested alone on the relaxant responses to electrical stimulation.

Table 1
Maximal response, as percent reversal of histamine-induced tone, obtained with electrical field stimulation

Treatment	E_{max} ± S.E.M.
Vehicle (EtOH)	87.6 ± 4.6
R(+)-methanandamide	87.4 ± 8.2
Vehicle (DMSO)	80.2 ± 3.0
JWH-015	90.2 ± 4.5
WIN 55,212-2 (WIN)	97.1 ± 4.3[a]
SR141716A/WIN	100.0 ± 0.2[a]
SR144528/WIN	93.1 ± 4.4[a]
L-NAME/WIN vehicle	68.0 ± 6.5
L-NAME/WIN	70.1 ± 2.2
α-Chymotrypsin/WIN vehicle[c]	68.1 ± 4.0
α-Chymotrypsin/WIN	88.0 ± 5.1[b]

[a] Significantly different ($P \leq 0.05$) from vehicle.
[b] Significantly different ($P \leq 0.05$) from its control ([c]).

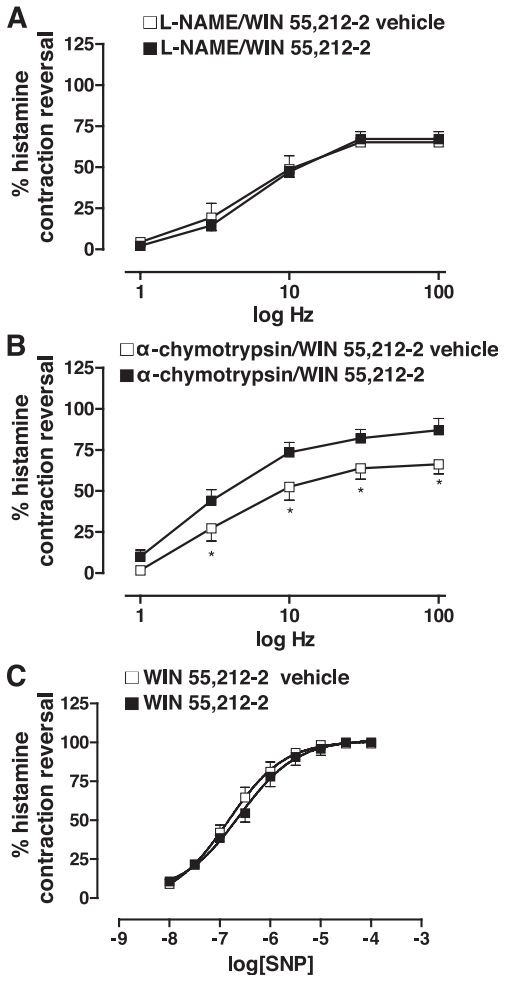

Fig. 2. Effects of WIN 55,212-2 (■) or WIN 55,212-2 vehicle (□) on the responses of pre-contracted (histamine 10^{-5} M) guinea-pig trachea to: (A) to electrical field stimulation (0.5-ms pulse width; 20-V amplitude; 15-s train duration; 1–100 Hz) in the presence of L-NAME (10^{-4} M); (B) to electrical field stimulation (0.5-ms pulse width; 20-V amplitude; 15-s train duration; 1–100 Hz) in the presence of α-chymotrypsin (2 U/ml); (C) to sodium nitroprusside (10^{-8}–10^{-4} M). Each point represents the mean of five to seven experiments and vertical lines represent standard error of the mean. *$P \leq 0.05$ from Student's *t* test for unpaired data.

4. Discussion

WIN 55,212-2, but not R(+)-methanandamide and JWH-015, modified the inhibitory non-adrenergic non-cholinergic (NANC) tracheal response to electrical field stimulation. This effect consisted of a significant potentiation of the relaxing response, although at a high concentration of the cannabinoid receptor agonist. This result suggests that cannabinoid receptors are not involved in WIN 55,212-2 activity; this finding was confirmed by the lack of antagonism by the cannabinoid

CB_1 and CB_2 receptor-selective antagonists, SR141716A and SR 144528, respectively. The potentiating activity of WIN 55,212-2 seems to involve the nitric oxide (NO)-mediated component of the inhibitory NANC response, while the VIP-mediated one appears not to be involved. The WIN 55,212-2 effect disappeared, in the presence of L-NAME, a NO synthase inhibitor (Rees et al., 1990), while it was maintained in the presence of α-chymotrypsin, a peptidase able to degrade VIP (Altiere and Diamond, 1985). The results obtained with the NO donor, sodium nitroprusside, suggest that WIN 55,212-2 does not alter NO-induced relaxation at the postsynaptic level.

An increase of NO generation by cannabinoids has already been reported for different cells and tissues, but linked to the stimulation of cannabinoid CB_1 or vanilloid VR1 receptors (Howlett and Mukhopadhyay, 2000; Howlett et al., 2002; Mukhopadhyay et al., 2002). The pre-treatment with a high concentration of capsaicin carried out in our experiments could explain the lack of effect of R(+)-methanandamide via VR1 receptors, while the absence of cannabinoid receptor-mediated effects is consistent with the evidence of no detectable specific binding for the cannabinoid CB_1/CB_2 receptor agonist, CP 55,940, in guinea-pig trachea (Spicuzza et al., 2000).

There are only scant data on the activity of cannabinoids on inhibitory NANC pathways innervating smooth muscle and they appear not to be conclusive. Facilitation by methanandamide, blocked by cannabinoid CB_1 receptor antagonists, in guinea-pig intestinal tract (Heinemann et al., 1999) and inhibitory activity of anandamide and WIN 55,212-2 in the rat gastric fundus, blocked by cannabinoid receptor antagonists only in the case of the endogenous cannabinoid (Storr et al., 2002), have been reported. Our results show for the first time a facilitating effect on the synthesis and/or release of NO by a cannabinoid molecule through a mechanism unaffected by cannabinoid CB_1 or CB_2 receptor antagonists. NO released following nerve stimulation is probably linked to the activity of the neural nitric oxide synthase (nNOS) reported to be expressed in guinea-pig trachea (Samb et al., 2001). Therefore, the effect of WIN 55,212-2 can be interpreted as positive modulation of nNOS activity and/or facilitation of NO release directly from neurones. However, since the effect of WIN 55,212-2 was weak and obtained with a high concentration of the compound, a potential role of WIN 55,212-2-like molecules as therapeutic bronchodilating drugs cannot be suggested.

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