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European Journal of Pharmacology 459 (2003) 75–81
www.elsevier.com/locate/ejphar

R(+)-methanandamide inhibits tracheal response to endogenously released acetylcholine via capsazepine-sensitive receptors

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Received 15 November 2002; accepted 22 November 2002

Abstract

The effects of cannabinoid drugs on the cholinergic response evoked by electrical field stimulation (0.2 ms pulse width, 20 V amplitude, 10 Hz, 7.5 s train duration) in guinea-pig tracheal preparations were investigated. The stable analogue of the endocannabinoid anandamide, *R*(+)-methanandamide (10^{-7}–10^{-4} M), produced a dose-dependent inhibition (up to 27 ± 5% of control) of electrical field stimulation-mediated atropine-sensitive response. This effect was not blocked by the selective 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^{-6} M), and was not reproduced with the cannabinoid CB_1/CB_2 receptor agonist *R*(+)-[2,3-dihydro-5-methyl-[(morpholinyl)methyl]pyrrolo [1,2,3-de]-1,4-benzoxazin-6-yl]-(1-naphthalenyl)methanone mesylate) (WIN 55,212-2; 10^{-8}–10^{-5} M) or the cannabinoid CB_2 receptor selective agonist 1-propyl-2-methyl-3-(1-naphthoyl)indole (JWH-015; 10^{-8}–10^{-5} M); it was, on the contrary, antagonized by the vanilloid antagonist 2-[2-(4-chlorophenyl)ethyl-amino-thiocarbonyl]-7,8-dihydroxy-2,3,4,5-tetrahydro-1*H*-2 benzazepine (capsazepine; 10^{-6} M). At the postjunctional level, neither *R*(+)-methanandamide nor WIN 55,212-2 nor JWH-015 did affect tracheal contractions induced by exogenous acetylcholine (10^{-6} M). An inhibitory vanilloid receptor-mediated effect on the cholinergic response evoked by electrical stimulation was confirmed with the vanilloid agonist capsaicin, at doses ($3–6 \times 10^{-8}$ M) which poorly influenced the basal smooth muscle tone of trachea. In conclusion, our data indicate that in guinea-pig trachea (a) neither CB_1 nor CB_2 cannabinoid receptor-mediated modulation of acetylcholine release occurs; (b) vanilloid VR1-like receptors appear involved in *R*(+)-methanandamide inhibitory activity on the cholinergic response to electrical field stimulation.

Keywords: Cannabinoid; Vanilloid; Cholinergic; (Guinea-pig); Trachea

1. Introduction

For centuries, Hashish and Marijuana, derived from *Cannabis sativa*, have been used for both their medicinal and psychotomimetic effects. Δ^9-Tetrahydrocannabinol, the major psychopharmacologically active component of *Cannabis*, has been long considered only a non-specific agent like ethanol while now it is recognised to be a specific ligand for membrane receptors. Devane et al. (1988) first demonstrated that some cannabinoid effects, at the central level, are receptor-mediated. Nowadays, two types of cannabinoid receptors have been clearly identified: CB_1, cloned in 1990 (Matsuda et al., 1990), mostly present in central nervous system, but also found in peripheral tissues (Pertwee, 1997), and CB_2, cloned in 1993 (Munro et al., 1993) particularly expressed in immune cells (Galiegue et al., 1995) but also suggested to be located on peripheral nerve endings (Griffin et al., 1997). Very recently, a third type of cannabinoid receptor has been suggested in brain (Breivogel et al., 2001).

The discovery of endogenous ligands (endocannabinoids), such as anandamide and 2-arachidonoylglycerol (Devane et al., 1992; Di Marzo et al., 1998a; Sugiura and Waku, 2000) and the development of specific synthetic agonists and antagonists for cannabinoid receptors, opened the possibility of therapeutic applications of new cannabinoids in different fields of medicine. One possible use, suggested by popular medicine already before the discovery of the endocannabinoid system, is in asthma pathology (Hollister, 1986). In particular, the evidence of a potent bronchodilating property of marijuana or of its active ingredient Δ^9-tetrahydrocannabinol (Tashkin et al., 1973, 1977; Vachon et al., 1973), suggested that new molecules acting in

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PII: S0014-2999(02)02831-5

airways as Δ^9-tetrahydrocannabinol, but without psychopharmacological activity, might represent possible new drugs for asthma. The exact mechanism for bronchial relaxation by Δ^9-tetrahydrocannabinol is actually not understood although a direct cannabinoid action on smooth muscle appears unlikely (Ackerman, 1977; Stengel et al., 1998). Plant and synthetic cannabinoids can modulate the release of classical neurotrasmitters and a neuromodulatory role has been shown also for *endocannabinoids* (Pertwee, 1997; Di Marzo et al., 1998a). Also at the airway level, effects of cannabinoids on smooth muscle neural control have been reported: anandamide inhibits bronchospasm evoked by the chemical irritant capsaicin (Calignano et al., 2000) and poorly stimulates sensory nerves, evoking bronchospasm per se when a constricting tone is absent (Calignano et al., 2000; Tucker et al., 2001); moreover, *R*(+)-[2,3-dihydro-5-methyl-[(morpholinyl)methyl]pyrrolo [1,2,3-de]-1,4-benzoxazin-6-yl]-(1-naphthalenyl)methanone mesylate (WIN 55,212-2) inhibits noradrenaline release in guinea-pig lung (Vizi et al., 2001). As concerns the cholinergic system, which is crucial in airway smooth muscle control (Barnes, 1986), possible interference by cannabinoids has been investigated but not clearly defined in previous works, in which a cannabinoid CB_2 receptor-mediated inhibition of acetylcholine release has been suggested but not confirmed by using CB_2 selective agonists or antagonists (Spicuzza et al., 2000; Yousif and Oriowo, 1999).

Recent evidences demonstrate that anandamide acts also as agonist at vanilloid receptors in different cells or tissues (Zygmunt et al., 1999; Smart et al., 2000; Di Marzo et al., 2001; Malinowska et al., 2001; Ralevic et al., 2001; Al-Hayani et al., 2001; Jacobsson et al., 2001; De Petrocellis et al., 2001; Begg et al., 2002); vanilloid receptors are a class of membrane associated proteins present on peripheral sensory nerves and recently suggested on parasympathetic and sympathetic fibers (Szallasi and Blumberg, 1999; Delany et al., 2001).

In the present study, electrical field stimulation-mediated cholinergic response of guinea-pig trachea was evaluated by comparing the effect of *R*(+)-methanandamide, the stable analogue of the endocannabinoid anandamide (Pertwee et al., 1995), with that exerted by the selective cannabinoid CB_2 receptor agonist 1-propyl-2-methyl-3-(1-naphthoyl)indole (JWH-015) and the non-selective cannabinoid receptor agonist WIN 55,212-2, in order to clarify the role of cannabinoid receptors, particularly the CB_2 subtype, and other receptors, i.e. vanilloid ones, in the cholinergic neural control of this tissue.

2. Materials and methods

2.1. General procedure

Male Dunkin–Hartley guinea-pigs (300–400 g) were employed. The experiments were carried out in accordance with the legislation of the Italian authorities (D.L. 27/01/1992 no. 116); the protocol was approved by the institutional ethics committee and complies with the European Community guidelines (CEE Directive 86/609) for the use of experimental animals.

Guinea-pigs were killed after light ether anaesthesia by cervical dislocation and bleeding. Immediately, the tracheobronchial tree was removed 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). Following removal of adherent fat and connective tissue, two preparations, four cartilage rings long were obtained from proximal trachea, by the method of Emmerson and Mackay (1979).

Tracheal preparations were then suspended, under a resting tension of 0.5 g, between two platinum electrodes in a 20-ml organ bath containing the above saline solution maintained at 37 °C and oxygenated with carbogen (95% O_2 and 5% CO_2). Isometric contractile responses were recorded via a force-displacement transducer (mod. FT03D, Grass Instruments, USA) connected to a polygraph (mod. WR 3101, Graphtec, Japan).

2.2. Experimental protocol

After a 20-min stabilization period, the preparations were pretreated with the following drugs: (1) indomethacin (3 μM) to avoid the production of cyclooxygenase products during electrical field stimulation; (2) propranolol (1 μM), a non-selective β-adrenoceptor antagonist, to block the adrenergic response to electrical stimulation; (3) N^G-nitro-L-arginine methyl ester (L-NAME) (1 μM), an agent which blocks nitric oxide-synthase; (4) α-chymotrypsin, (2 U/ml), a peptidase which degrades VIP; the latter two drugs were used to block the i-NANC (inhibitory non-adrenergic non-cholinergic) responses to electrical stimulation (Tucker et al., 1990).

Fifteen minutes after the pretreatment, an electrical field stimulation was applied by means of a digital stimulator (mod. BM ST 6, Biomedica Mangoni, Italy); single trains (0.2 ms pulse width; 20 V amplitude (supramaximal); 10 Hz; 7.5 s train duration) were repeated every 4 min.

Preliminary experiments verified the repeatability of the response under the above described conditions and confirmed the sensitivity to presynaptic inhibition by using the α_2-adrenergic agonist clonidine (about 70% inhibition by 10^{-6} M). In each preparation a single concentration–response curve to cannabinoid receptor agonists was obtained by adding a single dose in the time interval between two consecutive trains and allowing its maximal effect to occur (two consecutive equal responses to electrical stimulation).

The antagonists were incubated for 10 min before the administration of the agonist. The responses to drugs were always compared to blank controls obtained with vehicle administration.

The effect of cannabinoid receptor agonists was also assessed against the contractile response to exogenously administered acetylcholine; the dose 10^{-6} M was chosen since it produced a response, in percentage of the contractile activity of KCl 40 mM, not significantly different from that obtained with electrical field stimulation (data unshown). A single response to acetylcholine in the presence of the cannabinoid drug (5 min incubation) or its vehicle was obtained in each preparation. In addition, in some experiments the effects of cannabinoids on KCl 40 mM-induced contractions were tested; in this case, two responses to KCl were obtained in the same preparations, the second being in the absence (control) or in the presence of cannabinoid molecules (5 min incubation).

2.3. Drugs

Indomethacin, (±)-propranolol hydrochloride, α-chymotrypsin, L-NAME (L-N^G-nitro-arginine methyl ester), acetylcholine chloride (Ach), capsaicin (8-methyl-*N*-vanillyl-6-nonenamide) and capsazepine (2-[2-(4-chlorophenyl)ethyl-amino-thiocarbonyl]-7,8-dihydroxy-2,3,4,5-tetrahydro-1*H*-2 benzazepine) were obtained from Sigma; KCl was obtained from Carlo Erba; *R*(+)-methanandamide, WIN 55,212-2 (*R*(+)-[2,3-dihydro-5-methyl-[(morpholinyl)-methyl]pyrrolo [1,2,3-de]-1,4-benzoxazin-6-yl]-(1-naphthalenyl)methanone mesylate) and JWH-015 (1-propyl-2-methyl-3-(1-naphthoyl)indole) were purchased from Tocris (UK); SR141716A (*N*-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide hydrochloride) was a kind gift by Sanofi-Recherche (Montpellier, France).

Propranolol, L-NAME, α-chymotrypsin, Ach and KCl were dissolved in distilled water; indomethacin, capsaicin and capsazepine were dissolved in ethanol (2×10^{-3} M); *R*(+)-methanandamide was purchased as ethanol solution (1.38×10^{-2} M); WIN 55,212-2, JWH-015 and SR 141716A were dissolved in dimethyl sulfoxide (2×10^{-3} M). All dilutions were in distilled water except the first dilution of *R*(+)-methanandamide (to 1×10^{-2} M) which was in ethanol.

2.4. Data evaluation and statistics

The response to electrical stimulation obtained before agonist administration, represented the 100% reference value.

The responses to exogenous acetylcholine in the presence or in the absence of cannabinoid drugs were expressed as percentage of the contraction by KCl (40 mM), obtained in the same preparation at the end of the experiment.

All data are reported in graph as mean ± S.E.M. of *n* experiments. Comparisons between means was performed by Student *t*-test for unpaired data (two groups) or analysis of variance (ANOVA) and Bonferroni post-test (more than two groups). A P value ≤ 0.05 was taken to be significant.

3. Results

Preliminary experiments confirmed the completely cholinergic nature of the response to EFS since it was abolished by atropine 2×10^{-6} M.

3.1. Studies with cannabinoid receptor ligands

R(+)-methanandamide (10^{-7}–10^{-4} M) induced a significant decrease of the response to electrical field stimulation with an inhibition up to 27 ± 5% ($n = 8$) of control at 10^{-4} M (Fig. 1). The cannabinoid CB_1 receptor antagonist SR 141716A (10^{-6} M) failed to reverse this effect, giving only a poor and not significant reduction of the inhibitory activity observed with 3×10^{-5} and 10^{-4} M of *R*(+)-methanandamide (Fig. 2); moreover, SR 141716A (10^{-6} M) did not give any significant change of the response to electrical stimulation in the absence of *R*(+)-methanandamide: 120 ± 7% ($n = 4$) vs. 110 ± 9% ($n = 4$) of control response to electrical stimulation in SR141716A- and vehicle-treated tissues, respectively.

The cannabinoid CB_1/CB_2 receptor agonist WIN 55,212-2 (10^{-8}–10^{-5} M) and the selective cannabinoid CB_2 receptor agonist JWH-015 (10^{-8}–10^{-5} M) gave an enhancement of the control response to electrical stimulation which was not significantly different from that observed with vehicle alone (Fig. 3); these data indicate that the solvent (dimethyl sulfoxide), and not the cannabinoid agonists, is able to increase the cholinergic response to electrical stimulation in guinea-pig trachea.

In order to investigate whether cannabinoids directly affect smooth muscle responsiveness to acetylcholine, the

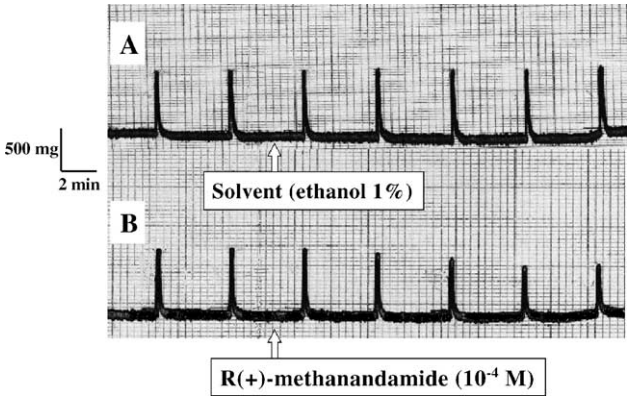

Fig. 1. Representative trace showing the cholinergic response of guinea-pig trachea to trains of stimuli (20 V, 0.2 ms pulse width, 10 Hz; 7.5 s), repeated every 4 min, as modified (A) by *R*(+)-methanandamide vehicle (ethanol 1%) and (B) by *R*(+)-methanandamide (10^{-4} M).

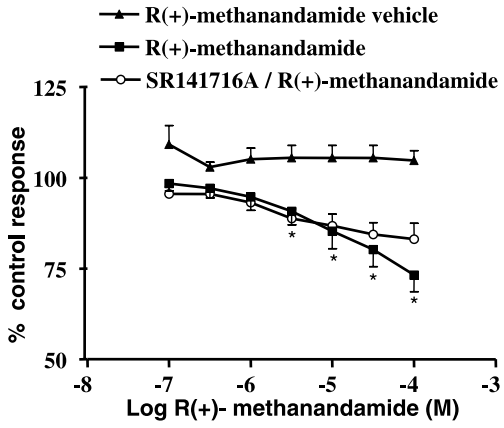

Fig. 2. Cumulative concentration–effect curves to *R*(+)-methanandamide in the absence or presence of SR141716A (10^{-6} M) and to *R*(+)-methanandamide vehicle (ethanol 0.001–1%) on electro-stimulated guinea-pig trachea (20 V, 0.2 ms pulse width, 10 Hz; 7.5 s). Control response is the stable contraction to electrical field stimulation just prior to *R*(+)-methanandamide or vehicle addition. Each point represents the mean ± S.E.M. of six to eight experiments. *$P \leq 0.05$ by the Student *t*-test comparison of the effects of each concentration of *R*(+)-methanandamide and of the respective vehicle.

activity of *R*(+)-methanandamide (10^{-5} M), WIN 55,212-2 (10^{-5} M) and JWH-015 (10^{-5} M) was also assessed on a tracheal contraction induced by the exogenously administered cholinergic mediator (10^{-6} M) and by the reference compound KCl (40 mM); in these experiments none of the cannabinoids significantly affected both the cholinergic response (Table 1) and KCl-induced contraction. Moreover, they did not modify the basal tone of tracheal preparations for all the concentrations used, with the exception of *R*(+)-methanandamide which, in a few experiments, at 3×10^{-5} and 1×10^{-4} M, was poorly contracting; to avoid misinterpretation of results, only the experiments in which no change of tracheal basal tone was observed, was considered for data evaluation.

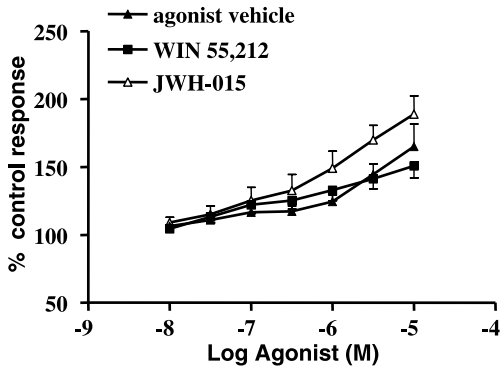

Fig. 3. Cumulative concentration–effect curves to WIN 55,212, JWH-015 and their vehicle (dimethyl sulfoxide 0.0005–0.5%) on the cholinergic response of guinea-pig trachea to electrical field stimulation (20 V, 0.2 ms pulse with, 10 Hz for 7.5 s). Control response is the stable contraction to electrical field stimulation just prior to agonist or vehicle addition. Each point represents the mean ± S.E.M. of 7–10 experiments.

Table 1
Guinea-pig tracheal response to exogenous acetylcholine 10^{-6} M in the absence or in the presence of cannabinoid molecules or their vehicle

	Vehicle	Drug
R(+)-methanandamide (10^{-5} M)	27.93 ± 3.26	27.20 ± 1.00
WIN 55,212-2 (10^{-5} M)	48.80 ± 2.98	52.37 ± 5.18
JWH-015 (10^{-5} M)	48.80 ± 2.98	51.80 ± 0.83

The responses are reported as percentage of a KCl 40 mM contraction obtained in the same tissue and are the mean ± S.E.M. of four experiments. Vehicle of *R*(+)-methanandamide solution is ethanol 0.1% and that of both WIN 55,212-2 and JWH-015 solutions is dimethyl sulfoxide 0.5%.

3.2. Experiments with vanilloid receptor ligands

To investigate the role played by vanilloid receptors in the inhibitory effect by *R*(+)-methanandamide on cholinergic contraction in electro-stimulated guinea-pig trachea, the vanilloid receptor antagonist capsazepine (10^{-6} M) was used. As shown in Fig. 4, capsazepine significantly blocked the *R*(+)-methanandamide-induced inhibition; on the other hand, the vanilloid receptor antagonist did not exert per se any significant effect on the response to electrical stimulation: 134 ± 5% ($n = 4$) vs. 127 ± 7% ($n = 4$) of control response in capsazepine- and vehicle-treated tissues, respectively.

To confirm a role for vanilloid receptors on cholinergic neurotransmission, the activity of the classical vanilloid receptor agonist capsaicin was evaluated; at a concentration, varying from 3×10^{-8} to 6×10^{-8} M between preparations, which only slightly increased tracheal basal tone, capsaicin significantly decreased the cholinergic response

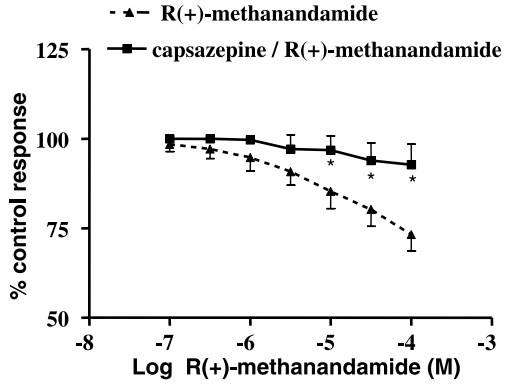

Fig. 4. Cumulative concentration–effect curves to *R*(+)-methanandamide in the absence or in the presence of capsazepine (10^{-6} M) on the cholinergic response of guinea-pig trachea to electrical field stimulation (20 V, 0.2 ms pulse with, 10 Hz for 7.5 s). Control response is the stable contraction to electrical field stimulation just prior to *R*(+)-methanandamide addition. Each point represents the mean ± S.E.M. of six to eight experiments. *$P \leq 0.05$ by the Student *t*-test comparison of the effects of each concentration of *R*(+)-methanandamide alone and in the presence of capsazepine, respectively.

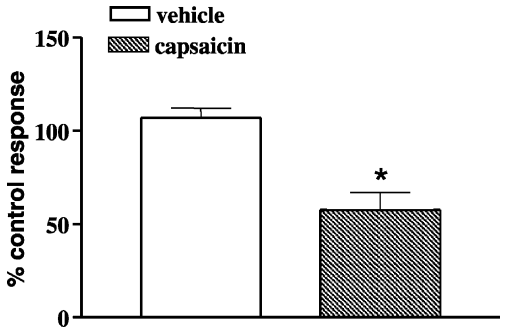

Fig. 5. Effect of capsaicin ($3-6\times10^{-8}$ M) or its vehicle (ethanol 0.0015–0.03%) on the cholinergic response evoked by electrical field stimulation in guinea-pig trachea (20 V, 0.2 ms pulse with, 10 Hz for 7.5 s). Control response is the stable contraction to electrical field stimulation just prior to capsaicin or vehicle addition. Each point represents the mean ± S.E.M. of six experiments. $^*P \leq 0.05$ obtained by Student *t*-test analysis.

to electrical stimulation (up to 42 ± 9% of control response; $n=6$) (Fig. 5).

4. Discussion

Cannabinoids modulate cholinergic transmission both at the central and peripheral level (Croci et al., 1998; Gifford and Ashby, 1996; Mang et al., 2001; Kathmann et al., 2001).

In the present study, *R*(+)-methanandamide, a stable anandamide analogue (Pertwee et al., 1995), induced an inhibitory effect on the cholinergic response of electrostimulated guinea-pig trachea but this effect seems to be not mediated by a cannabinoid CB_1 or CB_2 receptor since the cannabinoid CB_1 receptor selective antagonist SR 141716A (Rinaldi-Carmona et al., 1994) failed to block it and both the cannabinoid receptor non-selective agonist WIN 55,212-2 and the cannabinoid CB_2 receptor selective agonist JWH-015 (Pertwee, 1999) did not exert such an effect. In agreement with our results, Spicuzza et al. (2000) observed that the cannabinoid agonist CP 55,940 did not modify the cholinergic response induced by electrical field stimulation and that no CB_1 and CB_2 receptor binding sites were present in tracheal homogenates. On the contrary, as far as anandamide is concerned, Spicuzza et al. (2000) reported a different result from ours: while they revealed a decrease of tritiated acetylcholine release, they did not show any significant change of the cholinergic contraction to electrical field stimulation in the presence of the cannabinoid agonist. This difference could be due to the use, in our experiment, of the stable analogue *R*(+)-methanandamide, instead of anandamide itself, and a consequent more detectable activity of the non-degraded molecule in the specific functional assay.

Nevertheless, other authors, experimenting on rat trachea (Yousif and Oriowo, 1999), reported an inhibitory activity of both anandamide and WIN 55,212-2, on the cholinergic response to electrical field stimulation, which was not antagonized by SR 141716A. This latter finding is in agreement with the result of the present study with *R*(+)-methanandamide, but in contrast as far as WIN 55,212-2 activity is concerned; a species-related difference between guinea-pig and rat as responsible of this discrepancy may be suggested.

CB_1 and CB_2 receptor-independent mechanisms have been described for cannabinoids by different authors: in addition to a third cannabinoid receptor suggested in brain (Breivogel et al., 2001), non-receptor mediated effects have been reported (Howlett and Mukhopadhyay, 2000; White et al., 2001); the interaction of cannabinoids with receptors different from the cannabinoid ones is also a mechanism which received great attention. On this regard, many recent papers discuss the role of anandamide as a possible endogenous ligand for vanilloid receptors: Zygmunt et al. (1999) demonstrated a vasodilator action of anandamide, antagonized by the vanilloid receptor antagonist capsazepine, in isolated arteries; moreover, a full agonism activity of anandamide at human vanilloid receptor (hVR_1) (Smart et al., 2000) and a chemical similarity of the endocannabinoid molecule to some synthetic vanilloid agonists have been reported (Di Marzo et al., 1998b; Szallasi and Blumberg, 1999). Also the stable analogue methanandamide is described to produce vanilloid VR1 receptor-mediated response although with a lower efficacy than anandamide (Zygmunt et al., 1999; Smart et al., 2000).

In our experiments, the ability of capsazepine to antagonize the inhibitory action of *R*(+)-methanandamide seems to comfirm such an interaction. *R*(+)-methanandamide did not exert any change on the exogenous acetylcholine-mediated response suggesting that its effect on electrical field stimulation-mediated response does not involve the receptorial or postreceptorial mechanisms activated by acetylcholine on smooth muscle cells. Moreover, our data show that the ability of methanandamide, observed at central level to inhibit the binding of different drugs to muscarinic receptors (Lagalwar et al., 1999) is not confirmed in the tracheal smooth muscle, in agreement with the results obtained by Yousif and Oriowo (1999) and Spicuzza et al. (2000). An inhibitory role for vanilloid receptors on parasympathetic fibers has been already suggested in ovine airways by Mustafa and Oriolo (1999) by employing capsaicin. Also in our experimental model, the use of capsaicin, the pungent ingredient of pepper, induced a decrease of acetylcholine-mediated response to electrical stimulation; this is unlikely due to tachykinins released from sensory neurons, since a potentiation of acetylcholine release by prejunctional tachykinin receptors has been described both in vivo and in vitro in airways and in other tissues (Hey et al., 1996; Colasurdo et al., 1995; John et al., 1993; Guzman et al., 1993). Moreover, an inhibitory effect of capsaicin at postsynaptic level seems to be excluded from literature data reporting a vanilloid agonist-induced muscarinic hyperreactivity in guinea-pig trachea (Van Hoof et al., 1998).

Very recent findings (Delany et al., 2001) suggest the existence in human of a new vanilloid receptor, called

VRL-2, which was found on sympathetic and parasympathetic nerve fibers in several tissues including airways. It should be speculative to suggest an involvement of this specific subtype receptor in our experiments, since its pharmacological profile is actually unknown.

Lipoxygenase participation in the activation of vanilloid receptors by anandamide in the guinea-pig bronchus has been recently suggested by Craib et al. (2001). We cannot exclude a possible involvement of lipoxygenase metabolites in our experiments, while the tissue pre-treatment with indomethacin let us to exclude a participation of cyclo-oxygenase metabolites of arachidonic acid.

Some effects of anandamide are nitric oxide (NO)-mediated, as described in human monocytes (Stefano et al., 1996), rat median eminence (Prevot et al., 1998) and cultured human endothelial cells (Fimiani et al., 1999; Mombouli et al., 1999). NO is a documented mediator of the inhibitory non-adrenergic, non-cholinergic system in airways, is released after electrical field stimulation and its effect counterbalances acetylcholine-mediated contraction of the tracheo-bronchial tree (Canning and Fischer, 2001). Nevertheless, in our experiments, a role for NO in *R*(+)-methanandamide activity can be ruled out since the tissues were pretreated with the nitric oxide synthase inhibitor L-NAME.

In conclusion, our data suggest that cannabinoid selective agonists do not alter the cholinergic component of the contractile response to electrical field stimulation in guinea-pig trachea, while this response may be reduced by *R*(+)-methanandamide via vanilloid VR-1 like receptors.

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