Available online at www.sciencedirect.com
SCIENCE DIRECT
European Journal of Pharmacology 525 (2005) 150–153
www.elsevier.com/locate/ejphar

Modulation of ATP-mediated contractions of the rat vas deferens through presynaptic cannabinoid receptors

Simone Tambaro [a,*], Raymond Mongeau [a], Christian Dessi [a], Luca Pani [a,b], Stefania Ruiu [a]

[a] *Neuroscienze-PharmaNess S.c.ar.l, Via Palabanda 9, 09124 Cagliari, Italy*
[b] *C.N.R., Institute of Neurogenetics and Neuropharmacology, Cagliari, Italy*

Received 1 August 2005; received in revised form 16 September 2005; accepted 21 September 2005
Available online 3 November 2005

Abstract

The effect of *R*-(+)-[2,3-dihydro-5-methyl-3-[(morpholiny)methyl]pyrolol[1,2,3-de]-1,4-benzoxazin-yl]-(1-naphthalenyl)methanone mesylate (WIN 55,212-2; a cannabinoid receptor agonist) was investigated on contractions of the bisected (epididymal and prostatic portions) rat vas deferens to assess the role of cannabinoid receptors in sympathetic ATP neurotransmission. WIN 55,212-2 inhibited the electrically induced contractions in both portions of the rat vas deferens. In the presence of the α_1-adrenoreceptor antagonist prazosin, electrical stimulation produces a contraction mediated exclusively by ATP. In this condition, WIN 55,212-2 in the prostatic portion elicited a concentration-dependent inhibition that was antagonized by *N*-piperidinyl-[8-chloro-1-(2,4-dichlorophenyl)-1,4,5,6-tetrahydrobenzo[6,7]cyclohepta[1,2-c]pyrazole-3-carboxamide] (NESS 0327), a selective cannabinoid CB_1 receptor antagonist. NESS 0327 caused a parallel dextral displacement of the WIN 55,212-2 concentration–response curve. It is suggested that activation of pre-junctional cannabinoid receptors on sympathetic nerves of the vas deferens modulates ATP neurotransmission.

Keywords: Cannabinoid CB_1 receptor; Sympathetic nervous system; P2X receptor; NESS 0327

1. Introduction

Cannabis is of interest not only because it is a drug of abuse, but also because it produces effects that are potentially therapeutic (Pertwee, 2000). Cannabinoid CB_1, but not CB_2, receptors are located predominantly in the central nervous system and are also located pre-junctionally on some peripheral autonomic nerves (Howlett, 1998; Casu et al., 2003). A cannabinoid-mediated modulation of neurotransmitter release in the peripheral nervous systems is well documented for neurotransmitters such as acetylcholine and noradrenaline (Ralevic, 2003), but there were no available data for other neurotransmitters such as ATP.

There is substantial evidence that ATP, beside its role in cellular metabolism, acts as a potent extracellular messenger in various tissues (Bodin and Burnstock, 2001). ATP exerts effects on nerve terminals and on postsynaptic cells through the activation of P2 receptors (ionotropic P2X receptors and metabotropic P2Y receptors; Ralevic and Burnstock, 1998).

In the present study, we investigated the effect of the cannabinoid receptor agonist WIN 55,212-2 on ATP-mediated-smooth muscle contraction using the rat vas deferens preparation. An inhibitory action of cannabinoid CB_1 receptors on the electrically induced contractions in the whole rat vas deferens was previously described (Christopoulos et al., 2001), but the purinergic neurotransmitter system that, together with noradrenaline, mediate contractions of the rat vas deferens was not considered. Stimulation-evoked contraction with a single electrical pulse consists of a biphasic response: the earliest phases is linked to ATP and is prevalent in the prostatic portion, whereas the second phase is mediated by α_1-adrenoceptors and is predominant in the epididymal portion (Amobi and Smith, 1987; Sneddon and Machaly, 1992). In the present study, the effect of WIN 55,212-2 was investigated on the first phase of the twitch response, in conditions of noradrenergic blockade following pre-treatment

* Corresponding author. Tel.: +39 070 924 2026/2025; fax: +39 070 924 2206.
E-mail address: simone.tambaro@pharmaness.it (S. Tambaro).

0014-2999/$ - see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2005.09.058

with the α_1-adrenoceptor antagonist prazosin. The new potent cannabinoid CB_1 receptor antagonist NESS 0327 (Ruiu et al., 2003) was used to show the specificity of WIN 55,212-2 for cannabinoid receptors in the vas deferens.

2. Materials and methods

Male Sprague-Dawley rats weighing 250–350 g (Harlan, Italy) were housed at 22±2 °C on a 12 h light/dark cycle (light on at 7:00 a.m., off at 7:00 p.m.), with food and water available ad libitum. All experimental protocols were performed in strict accordance with the EC regulations for the care and use of experimental animals (EEC No. 86/609).

2.1. Electrical stimulation

Rats were sacrificed by stunning and exsanguination. Vas deferens were excised and cleaned of adhering connective tissues. Each vas deferens was bisected transversely and prostatic and epididymal segments were set up in separate 10 ml organ baths containing Krebs-bicarbonate solution. The solution was kept at 37 °C and bubbled with 95% O_2 and 5% CO_2. The initial tension of the vas deferens was adjusted to 9.80 mN (1 g) and the preparation was allowed to equilibrate for 45 min to obtain a steady tension (approximately 3–5 mN) before the start of the experiment. Isometric contractions were evoked by stimulation with 1 ms-pulses delivered at 0.05 Hz at supramaximal voltage (45–55 V) through a platinum electrode. Stimuli were generated by a Grass S88K stimulator and then amplified (Multiplexing pulse booster 316S, Ugo Basile, Comerio, Italy). Contractions were monitored by a computer using a date recording and analysis system (PowerLab 400), linked via preamplifiers (QuadBridge) to an F10 transducer (2Biological Instruments, Besozzo, Italy). Preparations were exposed to cumulatively increasing concentrations of WIN 55,212-2 (at 30 min intervals) to obtain concentration–response curves either in the absence or in the presence of prazosin (1μM) and NESS 0327 (1, 5, 10 nM), which were added 30 min before WIN 55,212. At the beginning of the experiments, a single pulse was applied for 5 min at intervals of 20 s and the arithmetic mean of the contractions was considered as the control (100%), in the absence of agonist or in the presence of antagonists. Furthermore, WIN 55,212-2 (1 μM) was added to the organ bath 30 min prior the ATP (1 mM) to observe the effect of WIN 55,212-2 on exogenous ATP-induced contraction.

2.2. Drugs and chemical

WIN 55,212-2 and prazosin were purchased from Tocris Cookson Ltd. NESS 0327 was synthesized by Prof. G.A. Pinna from the Department of Pharmacology, Chemistry and Toxicology of the University of Sassari, Italy. DMSO (dimethyl sulfoxide), suramin and ATP (adenosine 5′-triphosphate) were obtained from Sigma-Aldrich. WIN 55,212-2 and NESS 0327 were dissolved in DMSO (final concentration 0.01%), whereas, prazosin and ATP were dissolved in 0.01 M HCl solution.

2.3. Statistical analysis

The concentration–response curves were analyzed by a nonlinear regression analysis using GraphPad (San Diego, CA) and the determination of pD_2 ($-\log EC_{50}$) values were done with Prism. The pA_2 values for competitive antagonist were calculated by Schild regression analysis (Arunlakshana and Schild, 1959). Data were plotted as log antagonist concentrations (molar) versus log (concentration ratio −1). Statistical analyses were carried out using the Student's *t*-test or a two-way ANOVA (analysis of variance) with repeated measures.

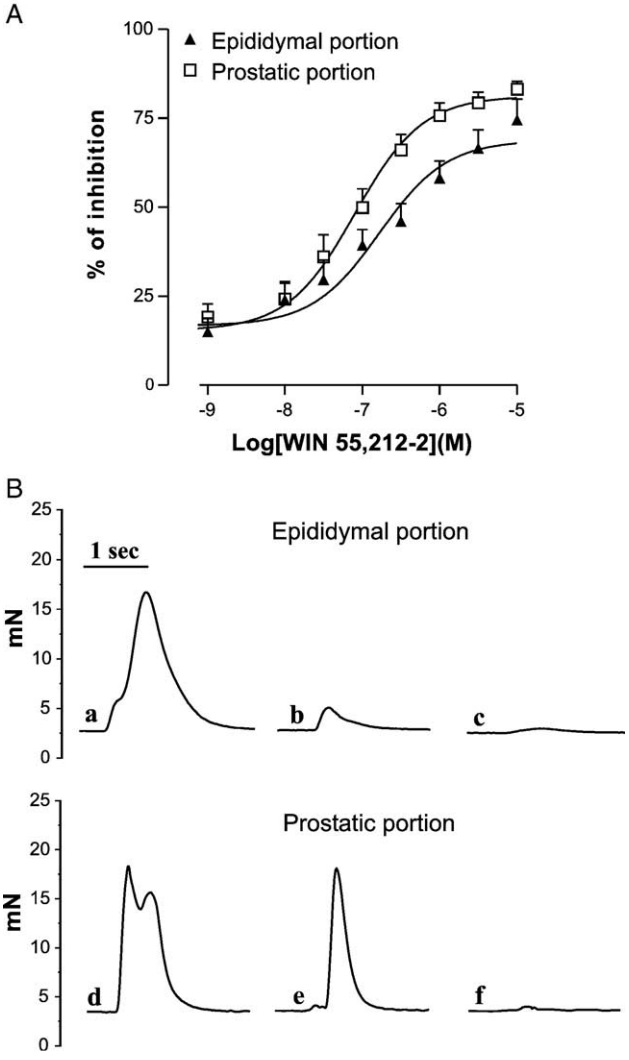

Fig. 1. Dose-dependent inhibitory effect of WIN 55,212-2 on the contractile response to nerve-mediated stimulation (A). Responses are expressed as percentage inhibition of the basal twitch amplitude. Each curve represents the mean values±S.E.M. of 12 experiments. The representative tracings (B) illustrate features of the stimulation-evoked contractions in the epididymal and prostatic portions of the rat vas deferens when the noradrenergic and the purinergic components are successively blocked: control (a, d), in the presence of prazosin 1 μM (b, e) and in the presence of prazosin 1 μM and suramin 300 μM (c, f).

3. Results

WIN 55,212-2 induced a concentration-dependent inhibition of field-stimulation-induced contractions in both portions of the rat vas deferens, but a two-way ANOVA with repeated measures did not show any significant interaction between the tissue and the dose–response parameters [$F_{tissue}(1,161)=5.61$, $P<0.05$; $F_{dose}(8,161)=75.21$, $P<0.01$; $F_{interaction}(8,161)=1.39$, $P>0.05$ (Fig. 1A)]. Nevertheless, a nonlinear regression analysis yielded a pD_2 value for WIN 55,212-2 that was significantly higher ($P<0.05$; Student's t-test) in the prostatic portion (7.10±0.10; $n=12$) than in the epididymal portion (6.76±0.12; $n=12$).

Experiments in the presence of prazosin (1 μM) were done to analyse the effect of WIN 55,212-2 on the ATP-mediated component. As expected, prazosin inhibited the second phase of the twitch response, while not affecting the first phase produced by ATP through post-junctional P2X receptors (Fig. 1B). The ATP-mediated response was completely suppressed by subsequent administration of the P2X receptor antagonist suramin (300 μM; Fig. 1B). As shown in Fig. 1B, amplitude of ATP-mediated contractions were very low in the epididymal portion, while in contrast the ATP component remained prominent in the prostatic portion. For this reason it was not possible to quantify the effects of WIN 55,212-2 following noradrenergic blockade in the epididymal portion.

The pD_2 value of WIN 55,212-2 obtained in presence of prazosin (7.12±0.11; $n=10$; Fig. 2A) was not different ($P>0.05$) from the pD_2 value obtained in absence of prazosin (7.10±0.10; $n=12$). Furthermore, WIN 55,212-2 (1 μM) did not change the contractions produced by exogenous ATP (1 mM) suggesting that its site of action was not post-junctional (the contractions produced by exogenous ATP were 2.31±0.15 mN in controls and 2.20±0.21 mN in the presence of WIN 55,212-2, $n=6$; $P>0.05$; Fig. 2B).

NESS 0327 produced a concentration-dependent rightward and parallel shift of the concentration-response curve of WIN 55,212-2, with a pA_2 value of 8.52±0.02 and with a Schild plot slope not significantly different from the unity (0.95±0.04; $P>0.05$; Fig. 2A). Importantly, a high dose of NESS 0327 (10 nM) had no effect by itself on the electrically induced contraction (Fig. 2A, insert).

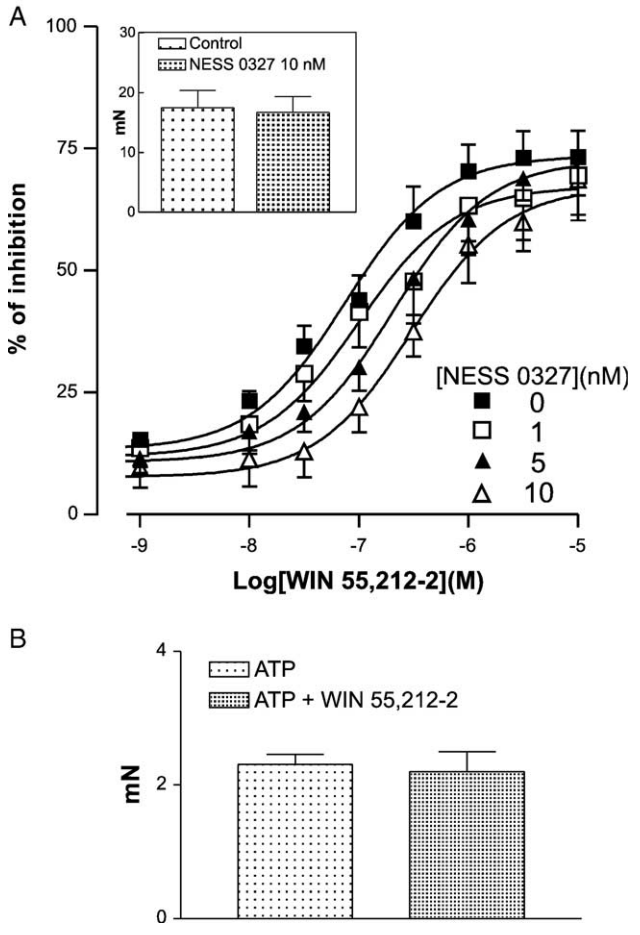

Fig. 2. Effect of WIN 55,212-2 on contractions mediated by intrasynaptically release ATP and by exogenous ATP. Cumulative concentration–response curves (A) show the effect of WIN 55,212-2 in the prostatic portion of the rat vas deferens, observed in the absence or in the presence of various concentrations of NESS 0327. Each curve represents the mean value±S.E.M. of 10 experiments. Responses are expressed as percentage inhibition of the basal twitch amplitude. All experiments were done in the presence of prazosin 1 μM. The insert shows that NESS 0327 did not produce any effect by itself on the basal electrically evoked contractile response in the prostatic portion of the rat vas deferens ($P>0.05$). Histograms show the effect of ATP (1 mM) alone and in the presence of WIN 55,212-2 (1 μM) in the prostatic portion (B). Values are expressed as differences between electrically induced contraction and baseline tension (mN). Bars represent the mean±S.E.M. of 8 experiments (A) and of 6 experiments (B).

4. Discussion

Interest about cannabinoid drugs has been mainly focussed on their actions in the central nervous system, but there is evidence showing that cannabinoids also have an important action in the peripheral nervous system (Ishac et al., 1996; Pertwee and Fernando, 1996). In this study we investigated the role of prejunctional cannabinoid receptors along the rat vas deferens. The results show that WIN 55,212-2 has the ability to inhibit electrically evoked contractions in the two portions of the vas deferens. The potency (pD_2 value) of WIN 55,212-2 was higher in the prostatic portion then in the epididymal portion. This difference might be due to an unequal distribution of cannabinoid CB_1 receptors along the rat vas deferens. Indeed, previous pharmacological characterization studies have shown that this tissue is not regionally homogeneous in terms of prejunctional receptor modulation. For example, a regional variation in the distribution of α_2-adrenoceptors has been shown along the bisected rat vas deferens (Ventura and Pennefather, 1994).

Christopoulos et al. (2001) had previously shown an inhibitory action of WIN 55,212-2 on the contractions of the whole rat vas deferens in conditions of purinergic blockade with nifedipine. When we used prazosin to isolate the purinergic component, we also observed an inhibitory action of WIN 55,212-2 on twitch contractions in the prostatic portion. This inhibitory effect of WIN 55,212-2 was attenuated by NESS 0327, a new and selective cannabinoid CB_1 receptor antagonist, indicating that cannabinoid CB_1 receptors modulate the ATP-

mediated component of the twitch contraction. Taken together these results suggest that both noradrenergic and purinergic components of contractions are modulated by cannabinoid CB_1 receptors. Interestingly, WIN 55,212-2 displayed an inhibitory potency on the ATP-mediated component that was similar to that obtained in the presence of the noradrenergic component. This observation could be explained by the fact that ATP and noradrenaline are released from the same vesicles (Stjarne, 1989). However, some authors (see Todorov et al., 1996) believe that the different contractions produced by these neurotransmitters can only be explained in terms of release from distinct vesicular sites.

Modulation of ATP release through presynaptic receptors was previously shown for other neurotransmitters (von Kügelgen, 1996), but a modulation of ATP-mediated contractile function through cannabinoid receptors has never been reported before. The role of cannabinoid CB_1-receptors on ATP neurotransmission might be of pharmacological relevance not only in the peripheral nervous system, where ATP mediates pain (Burnstock and Wood, 1996), but also in the central nervous system. Indeed, a role for ATP was shown for many brain functions including cognition, memory and learning (Inoue et al., 1996). Moreover, recent studies suggested an involvement of extracellular ATP in neurodegenerative processes (Le Feuvre et al., 2002). Knowing that cannabinoids have similar inhibitory actions on noradrenergic neurotransmission in the peripheral and in the central nervous system (Schlicker and Kathmann, 2001), it might be useful to consider a similar possibility for ATP-mediated phenomena. The present results, obtained with a simple and reliable bioassay, can thus stimulate further studies to determine whether a CB_1-modulation of ATP neurotransmission through terminal presynaptic receptors could occur elsewhere in the nervous system.

References

Amobi, N.I., Smith, I.C., 1987. Adrenergic and 'Non-adrenergic' contributions to the two-component tetanus in rat vas deferens. Eur. J. Pharmacol. 135, 173–182.

Arunlakshana, O., Schild, H.O., 1959. Some quantitative uses of drug antagonists. Br. J. Pharmacol. 14, 48–58.

Bodin, P., Burnstock, G., 2001. Purinergic signalling: ATP release. Neurochem. Res. 26, 959–969.

Burnstock, G., Wood, J.N., 1996. Purinergic receptors: their role in nociception and primary afferent neurotransmission. Curr. Opin. Neurobiol. 6, 526–532.

Casu, M.A., Porcella, A., Ruiu, S., Saba, P., Marchese, G., Carai, M.A., Reali, R., Gessa, G.L., Pani, L., 2003. Differential distribution of functional cannabinoid CB_1 receptors in the mouse gastroenteric tract. Eur. J. Pharmacol. 459, 97–105.

Christopoulos, A., Coles, P., Lay, L., Lew, M.J., Angus, J.A., 2001. Pharmacological analysis of cannabinoid receptor activity in the rat vas deferens. Br. J. Pharmacol. 132, 1281–1291.

Howlett, A.C., 1998. The CB_1 cannabinoid receptor in the brain. Neurobiol. Dis. 5, 405–416.

Inoue, K., Koizumi, S., Ueno, S., 1996. Implication of ATP receptors in brain functions. Prog. Neurobiol. 50, 483–492.

Ishac, E.J.N., Jiang, L., Lake, K.D., Varga, K., Abood, M.E., Kunos, G., 1996. Inhibition of exocytotic noradrenaline release by presynaptic cannabonoid CB_1 receptors on peripheral sympathetic nerves. Br. J. Pharmacol. 118, 2023–2028.

Le Feuvre, R., Brough, D., Rothwell, N., 2002. Extracellular ATP and P2X7 receptors in neurodegeneration. Eur. J. Pharmacol. 447, 261–269.

Pertwee, R.G., 2000. Neuropharmacology and therapeutic potential of cannabinoids. Addict. Biol. 5, 37–46.

Pertwee, R.G., Fernando, S.R., 1996. Evidence for the presence of cannabinoid CB_1 receptors in mouse urinary bladder. Br. J. Pharmacol. 118, 2053–2058.

Ralevic, V., 2003. Cannabinoid modulation of peripheral autonomic and sensory neurotransmission. Eur. J. Pharmacol. 472, 1–21.

Ralevic, V., Burnstock, V., 1998. Receptors for purines and pyrimidines. Pharmacol. Ther. 50, 413–492.

Ruiu, S., Pinna, G.A., Marchese, G., Mussino, J.M., Saba, P., Tambaro, S., Casti, P., Vargiu, R., Pani, L., 2003. Synthesis and characterization of NESS 0327: a novel putative antagonist of the CB1 cannabinoid receptor. J. Pharmacol. Exp. Ther. 306, 363–370.

Schlicker, E., Kathmann, M., 2001. Modulation of transmitter release via presynaptic cannabinoid receptors. Trends Pharmacol. Sci. 22, 565–572.

Sneddon, P., Machaly, M., 1992. Regional variation in purinergic and adrenergic responses in isolated vas deferens of rat, rabbit and guinea-pig. J. Auton. Pharmacol. 12, 421–428.

Stjarne, L., 1989. Basic mechanisms and local modulation of nerve impulse-induced secretion of neurotransmitters from individual sympathetic nerve varicosities. Rev. Physiol., Biochem. Pharmacol. 112, 1–137.

Todorov, L.D., Mihaylova-Todorova, S., Craviso, G.L., Bjur, R.A., Westfall, D.P., 1996. Evidence for the differential release of the cotransmitters ATP and noradrenaline from sympathetic nerves of the guinea-pig vas deferens. J. Physiol. 496, 731–748.

Ventura, S., Pennefather, J.N., 1994. Alpha 2-adrenoceptor binding sites vary along the length of the male reproductive tract: a possible basis for the regional variation in response to field stimulation. Eur. J. Pharmacol. 254, 167–173.

von Kügelgen, I., 1996. Modulation of neural ATP release through presynaptic receptors. Semin. Neurosci. 8, 247–257.