British Journal of Pharmacology (1997) 120, 1397–1398 © 1997 Stockton Press All rights reserved 0007–1188/97 $12.00

SPECIAL REPORT

Paradoxical action of the cannabinoid WIN 55,212-2 in stimulated and basal cyclic AMP accumulation in rat globus pallidus slices

[1]Yannick P. Maneuf & Jonathan M. Brotchie

Division of Neuroscience, School of Biological Sciences, University of Manchester, Manchester M13 9PT

The effects of the synthetic cannabinoid WIN 55,212-2 on forskolin-stimulated and basal adenosine 3′:5′-cyclic monophosphate (cyclic AMP) accumulation in globus pallidus slices were investigated. WIN 55,212-2 caused a concentration-dependent decrease in forskolin-stimulated cyclic AMP accumulation in globus pallidus slices (maximum inhibition 36% for 30 μM). This effect was blocked by the cannabinoid receptor antagonist SR 141716A (100 μM). WIN 55,212-2 alone caused a concentration-dependent increase in cyclic AMP levels in unstimulated slices (maximum increase 52.6% for 100 μM). This effect was also blocked by SR 141716A (100 μM). In either forskolin-stimulated or unstimulated conditions SR 1417161A (100 μM) did not affect cyclic AMP levels.

Keywords: Cannabinoids; CB_1 receptor; globus pallidus; cyclic AMP; WIN 55,212-2; SR 141716A

Introduction Recent advances in cannabinoid pharmacology have led to a considerable increase in our understanding of cannabinoid physiology (for review see Pertwee, 1993). The brain cannabinoid receptor (CB_1) is a member of the superfamily of G-protein linked receptors. Among the various physiological actions of cannabinoids, they have been shown to inhibit adenylyl cyclase activity in different systems leading to a reduction in adenosine 3′:5′-cyclic monophosphate (cyclic AMP) levels (for review see Childers & Deadwyler, 1996). However, other studies have suggested that cannabinoids have opposite effects under certain conditions. Thus, cannabinoids have been shown to increase basal cyclic AMP accumulation in various systems (for review see Pertwee, 1988). However, up to date, there is no evidence of a dual action of cannabinoids on cyclic AMP levels in the same system. In this study, we investigated the effects of the cannabinoid receptor agonist WIN 55,212-2 on basal and on forskolin-stimulated cyclic AMP accumulation in a slice preparation of the globus pallidus.

Methods Male Sprague-Dawley rats were killed and their brains rapidly removed and separated into hemispheres. Globus pallidus slices (400 μm) were obtained with a McIllwain tissue chopper and the globus pallidus was punched out. Pallidal slices were then transferred to artificial cerebrospinal fluid (aCSF: composition (mM): NaCl 118, KCl 4.8, $CaCl_2$ 1.3, $MgSO_4$ 1.2, $NaHCO_3$ 25, KH_2PO_4 1.2, ascorbic acid 0.6, glucose 11) constantly aerated with 95% O_2/5% CO_2 at 22°C containing the cyclic AMP phosphodiesterase inhibitor Ro 20-1724 (100 μM, RBI) and either drug or appropriate vehicle for 60 min. At the end of the incubation period, slices were boiled in HEPES buffer containing 4 mM EDTA for 15 min. The slices were then sonicated and the homogenates were centrifuged at 12000 *g* for 10 min. The cyclic AMP content of the supernatants was assayed with a Scintillation Proximity Assay kit (RPA 538, Amersham). The protein content was determined by the method of Bradford (1976).

Forskolin (Sigma, U.K.), WIN 55,212-2 (R(+)-[2,3-Dihydro-5-methyl-3-[(morpholinyl)methyl]pyrrolo[1,2,3-de]-1,4-benzoxazin-yl]-(1-naphthalenyl)methanone mesylate, RBI, U.K.), SR 141716A (N-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide, Sanofi Recherche, Montpellier, France) and Ro 20-1724 (4-[3-Butoxy-4-methoxyphenyl)methyl]-2-imidazolidinone, RBI, U.K.) were dissolved in DMSO and then diluted in a CSF to the final concentration (final concentration of DMSO: 0.04%). Results were expressed as a percentage of the cyclic AMP levels measured in slices incubated in the presence of the vehicle. Statistical analysis was performed by a one way analysis of variance followed by the Tukey-Kramer *post hoc* test where appropriate. Significance was assigned when $P<0.05$.

Results WIN 55,212-2 (3–100 μM) caused a significant ($F=8.951$; d.f. = 6, $P<0.0001$) decrease in cyclic AMP accumulation in forskolin-stimulated globus pallidus slices. A significant decrease was observed with 10 μM, 30 μM and 100 μM WIN 55,212-2 (31.4%, 34.8% and 33.9%, respectively). This effect was blocked by the addition of the cannabinoid receptor

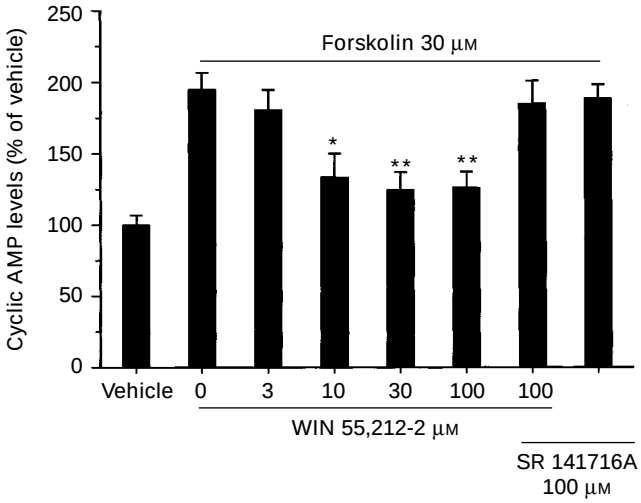

Figure 1 Effect of the cannabinoid receptor agonist WIN 55,212-2 on forskolin-stimulated (30 μM) cyclic AMP accumulation in a slice preparation of the globus pallidus. Forskolin caused a 94.7% increase in cyclic AMP levels. This effect was reduced by increasing concentrations of WIN 55,212-2 (*$P<0.05$; **$P<0.01$). The cannabinoid receptor antagonist SR141716A (100 μM) blocked the effect of 100 μM WIN 55,212-2 and had itself no effect on cyclic AMP accumulation. Results were drawn from at least 4 separate experiments with 18–25 slices per condition.

[1] Author for correspondence.

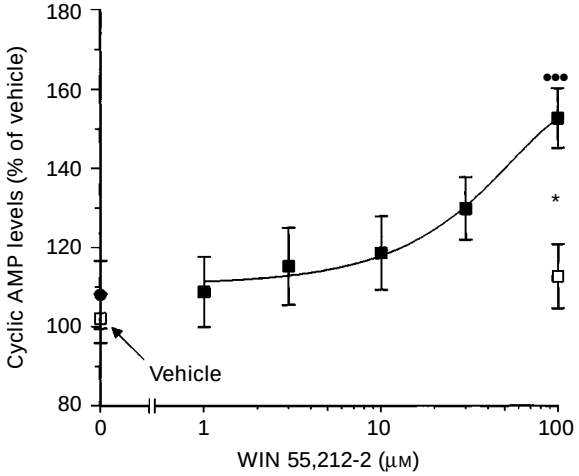

Figure 2 The effect of the cannabinoid receptor agonist WIN 55,212-2 on basal cyclic AMP accumulation was examined in a slice preparation of globus pallidus. A concentration-dependent increase in cyclic AMP levels was observed; $EC_{50}=32.8\,\mu M$. This effect was significant for $100\,\mu M$ WIN 55,212-2 (•••$P<0.001$). (□) SR 141716A ($100\,\mu M$) blocked the effect of $100\,\mu M$ WIN 55,212-2, *$P<0.05$. Results were drawn from at least 4 separate experiments with 20–28 slices per condition. (●) SR 141716A ($100\,\mu M$) alone had no significant effect on basal cyclic AMP levels.

antagonist SR141716A (100 μM). SR 141716A (100 μM) alone did not affect forskolin-stimulated cyclic AMP levels (Figure 1). WIN 55,212-2 (1–100 μM) caused a concentration-dependent increase in unstimulated cyclic AMP accumulation measured in the absence of forskolin ($F=4.049$; d.f.$=6$; $P<0.0009$). This effect was significant for 100 μM WIN 55,212-2 (52.5% increase, $P<0.001$). The calculated EC_{50} was 32.8 μM. The effect of WIN 55,212-2 was blocked by 100 μM SR141716A ($P<0.05$ vs 100 μM WIN 55,212-2 alone) (Figure 2). SR 141716A (100 μM) was without any effect on unstimulated cyclic AMP levels ($P<0.05$ when compared with the vehicle). The average protein content was 0.37 ± 0.04 mg ml^{-1}.

Discussion Our results suggest that cannabinoid receptor stimulation can increase cyclic AMP accumulation in globus pallidus slices in the absence of forskolin. This finding is in contrast with the well-accepted notion that cannabinoids act to decrease cyclic AMP levels in forskolin-stimulated conditions (see Childers & Deadwyler, 1996, for review). The inhibitory actions of cannabinoids are thought to be mediated through a G_i protein-mediated inhibition of adenylyl cyclase. However, under unstimulated conditions, other studies have suggested that cannabinoids can increase stimulated cyclic AMP accumulation.

Previously, Δ^9-THC at low concentrations was shown to increase cyclic AMP levels and cyclic AMP-dependent protein kinase activity in human cultured lung cells (Kelly & Butcher, 1979) and basal adenylyl cyclase activity in mouse cerebral cortical homogenates (Hillard & Bloom, 1983). We have shown similar effects with the synthetic cannabinoid WIN 55,212-2 in the globus pallidus and been able to demonstrate the involvement of a receptor-mediated action by virtue of blockade by the selective cannabinoid receptor antagonist SR 141716A.

It has previously been hypothesized that cannabinoid-induced increase in adenylyl cyclase activity could be mediated through an increase in prostaglandin levels as the increase in adenylyl cyclase activity seen following incubation of cortical homogenates with Δ^9-THC was blocked by indomethacin, an irreversible prostaglandin synthase inhibitor. More recently, preliminary findings showing evidence of a differential regulation of cannabinoid receptor coupling pathways that could either stimulate or inhibit adenylyl cyclase were demonstrated in CHO cells expressing the CB_1 receptor and in primary striatal cultures (Glass & Felder, 1996). In this study, the endogenous cannabinoid receptor agonist anandamide reduced forskolin-stimulated cyclic AMP production. However, pretreatment of the cells with pertussis toxin unmasked a stimulating effect of anandamide on adenylyl cyclase. Both effects were as in the present study blocked by a cannabinoid receptor antagonist. It thus appears likely that the cannabinoid receptor can functionally couple to G proteins to exert a diversity of functional responses. We suggest that, in the presence of forskolin, the cannabinoid receptor appears to couple to pertussis toxin-sensitive G protein that inhibit adenylyl cyclase activity (presumably G_i or similar). However, in other situations it appears that the cannabinoid receptor can activate pertussis-toxin-insensitive G proteins which stimulate cyclic AMP levels. The identity of such a G protein is unclear but it may be G_s which would directly activate adenylyl cyclase. Understanding the regulation of cannabinoid receptor-G protein coupling may prove to be of key importance if we are to appreciate fully the role of cannabinoids as chemical signals in the CNS.

This work was funded by the Medical Research Council. We are grateful to Sanofi Recherche, Montpellier, France for the gift of SR 141716A.

References

BRADFORD, M.M. (1976). A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.*, **72,** 218–226.

CHILDERS, S.R. & DEADWYLER, S.A. (1996). Role of cyclic AMP in the actions of cannabinoid receptors. *Biochem. Pharmacol.*, **52,** 819–827.

GLASS, M. & FELDER, C.C. (1996). Concurrent stimulation of cannabinoid CB1 and dopamine D_2 receptors results in an augmentation of forskolin-stimulated cAMP in striatal neurones. *Soc. Neurosc. Abst.*, **615,** 4.

HILLARD, C.J. & BLOOM, A.S. (1983). Possible role of prostaglandin in the effects of the cannabinoids on adenylyl cyclase activity. *Eur. J. Pharmacol.*, **91,** 21–27.

KELLY, L.A. & BUTCHER, R.W. (1979). Effects of delta-1-tetrahydrocannabinol on cyclic AMP in cultured human diploid fibroblasts. *J. Cyclic Nucleotide Res.*, **5,** 303–313.

PERTWEE, R.G. (1988). The central neuropharmacology of cannabinoids. *Pharmacol. Ther.*, **36,** 189–261.

PERTWEE, R.G. (1993). The evidence for the existence of cannabinoid receptors. *Gen. Pharmacol.*, **24,** 811–824.

(Received December 20, 1996
Accepted January 28, 1997)