ELSEVIER

European Journal of Pharmacology 308 (1996) 161–164

ejp

Short communication

Activation of the cannabinoid receptor by Δ^9-tetrahydrocannabinol reduces γ-aminobutyric acid uptake in the globus pallidus

Yannick P. Maneuf *, Joanne E. Nash, Alan R. Crossman, Jonathan M. Brotchie

Room 1.124, Division of Neuroscience, School of Biological Sciences, University of Manchester, Manchester M13 9PT, UK

Received 29 February 1996; revised 11 April 1996; accepted 19 April 1996

Abstract

The interaction between GABA (γ-aminobutyric acid) and cannabinoids in the globus pallidus was investigated by evaluating the effects of Δ^9-tetrahydrocannabinol on [^{3}H]GABA uptake into slices of rat globus pallidus. Δ^9-Tetrahydrocannabinol caused a concentration-dependent decrease in GABA uptake (51% decrease at 100 μM Δ^9-tetrahydrocannabinol, IC_{50} = 18.95 μM). This effect was reversed in a concentration-dependent manner (IC_{50} = 11.9 μM) by the cannabinoid receptor antagonist SR 141716A (*N*-(piperidin-1-yl-)5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-arboxiamidehydrochloride. SR 141716A alone did not affect GABA uptake. These results show that cannabinoid receptor activation reduces GABA uptake in the globus pallidus.

Keywords: Tetrahydrocannabinol; GABA (γ-aminobutyric acid) transport; Pallidal slice; SR 141716A

1. Introduction

The recent description of both an endogenous ligand at the cannabinoid receptor, anandamide (Devane et al., 1992), and of the first potent and selective cannabinoid receptor antagonist, SR 141716A (Rinaldi-Carmona et al., 1994) has refocused attention on the mechanism by which cannabinoids such as Δ^9-tetrahydrocannabinol modulate behaviour. Ligand-binding studies revealed the distribution of cannabinoid receptors within the brain and their presence in the basal ganglia (Herkenham et al., 1991). The observation that, within the globus pallidus, cannabinoid receptors are located pre-synaptically on terminals arising from the striatum that use GABA as a transmitter has raised the question of their possible role in modulating GABAergic transmission (Herkenham et al., 1991; Glass et al., 1993). It is generally well acccepted that cannabinoids act to 'facilitate' GABAergic transmission in the brain (Pertwee, 1988) possibly by increasing the free extracellular concentrations of GABA by blockade of the uptake mechanism or by increasing the neuronal release of GABA (Wickens and Pertwee, 1995). Previous studies have suggested that cannabinoid-induced catalepsy is mediated via an enhancement of GABAergic transmission in the globus pallidus (Pertwee and Wickens, 1991). Furthermore, enhancement of GABAergic transmission in the globus pallidus is a key component of the neural mechanisms responsible for causing the akinetic symptoms seen in movement disorders such as Parkinson's disease (Maneuf et al., 1994). In this study, we have tested the hypothesis that cannabinoid receptor activation reduces GABA uptake in globus pallidus slices and that this effect is cannabinoid receptor mediated.

2. Materials and methods

Male Sprague-Dawley rats were killed and their brains rapidly removed and separated into hemispheres. 400 μm globus pallidus slices were obtained using a McIlwain tissue chopper and the globus pallidus dissected out. Pallidal slices were then transferred to an incubation medium (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 12, ascorbic acid 0.6, glucose 11) constantly aerated with 95% O_2/5% CO_2 at room temperature and containing either drug or vehicle for 40 min. After this period, 0.5 μM [^{3}H]GABA (70 Ci/mmol, NEN) was added to the medium for another 30 min incubation period.

* Corresponding author. Tel.: +44 161 275-5275; fax: +44 161 275-5363; e-mail: ymaneuf@fs2.scg.man.ac.uk

PII S0014-2999(96)00326-3

Preliminary experiments showed that the uptake assayed in this preparation is linear for up to 40 min. The calculated kinetic parameters were $K_m = 6.5 \pm 1.8$ μM and $V_{max} = 0.36 \pm 0.06$ nmol/min/mg of wet tissue. The uptake was decreased to 20 ± 3% of that of the control in the presence of the neuronal GABA uptake inhibitor nipecotic acid (300 μM) whereas the selective glial uptake inhibitor 4,5,6,7-tetrahydroisoxazolo[4,5-c]-pyridin-3-ol (THPO) (300 μM) had no effect on the uptake (102 ± 15% of that of the control). The GABA transaminase inhibitor aminooxyacetic acid was not included in the medium since it also blocks the malate-aspartate shuttle, which is the main pathway for the reoxidation of NADH, and so would place the slices under metabolic stress (Nicholls, 1989). Slices were then washed in aCSF, placed into scintillation vials containing 0.5 ml of Triton X100 and the radioactivity was counted overnight in a Packard 1500 β scintillation counter. Δ^9-Tetrahydrocannabinol was dissolved to a concentration of 3 mg/ml in 45% w/v 2-hydroxypropyl-β-cyclodextrin (RBI) and then diluted to the final concentration with aCSF. SR 141716A (Sanofi) was dissolved in dimethyl sulfoxide and then diluted to the final concentration in aCSF (final concentration of dimethyl sulfoxide: 0.04%).

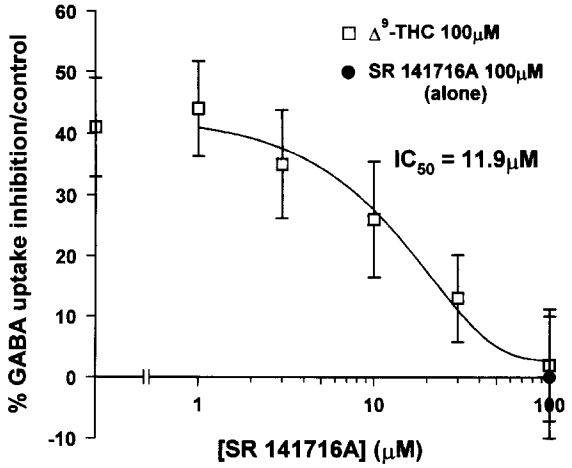

Fig. 2. Effect of increasing concentrations of SR 141716A (1–100 μM) on the inhibition of GABA uptake induced by Δ^9-tetrahydrocannabinol. SR 141716A reduced the inhibition of GABA uptake induced by application of Δ^9-tetrahydrocannabinol (100 μM) in a concentration-dependent manner ($IC_{50} = 11.9$ μM). SR 141716A (100 μM) did not affect the uptake of GABA into pallidal slices in a significant manner (98.8 ± 12.3% of that of control conditions, $P > 0.05$). In this case, results were drawn from 3 different experiments ($n = 6$ animals/experiments, 5–7 replicates/experiment).

3. Results

Δ^9-Tetrahydrocannabinol caused a concentration-dependent decrease in the uptake of [^{3}H]GABA. This effect was significantly different from that of the control following incubation with 50 μM and 100 μM Δ^9-tetrahydrocannabinol (49% and 48.3% inhibition respectively, one-way analysis of variance followed by a Tukey-Kramer test, $P < 0.05$). The IC_{50} was calculated as being 18.95 μM (Fig. 1). The vehicle did not affect the uptake (101 ± 4% of control conditions, $P > 0.05$).

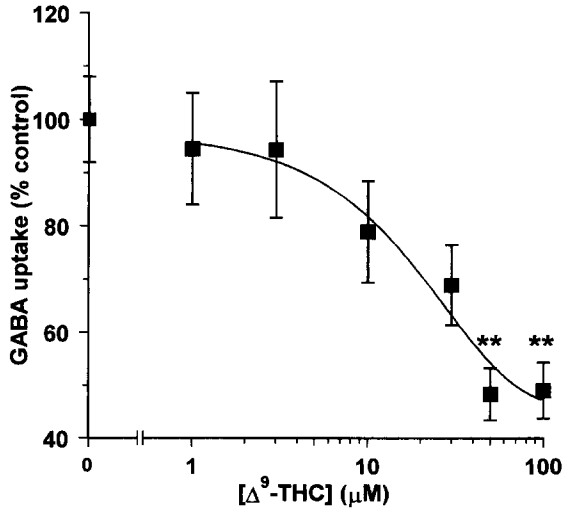

Fig. 1. Inhibition of [^{3}H]GABA uptake into globus pallidus slices by the cannabinoid receptor agonist Δ^9-tetrahydrocannabinol. Δ^9-Tetrahydrocannbinol was applied to the medium 40 min prior to adding [^{3}H]GABA to the medium. Significant reductions in GABA uptake were observed following incubation with 50 μM and 100 μM Δ^9-tetrahydrocannabinol (** $P < 0.01$, one-way analysis of variance followed by Tukey-Kramer test). Data are expressed as a mean percentage ± S.E.M. of the uptake of the control from 7 experiments each with 4 animals (18–24 replicates/experiment).

SR 141716A inhibited the effect of Δ^9-tetrahydrocannabinol in a concentration-dependent manner ($IC_{50} = 11.9$ μM). SR 141716A significantly reduced the inhibitory effect of Δ^9-tetrahydrocannabinol at concentrations of 30 μM and 100 μM (one-way analysis of variance, followed by a Tukey-Kramer test, $P < 0.05$) (Fig. 2). SR 141716A (100 μM) alone did not affect GABA uptake (98.8 ± 12.3%, $P > 0.05$).

4. Discussion

Our results show that activation of the cannabinoid receptor in the globus pallidus can reduce the uptake of GABA in a concentration-dependent manner and are similar to those of Banerjee et al. (1975), in whole brain synaptosomes, but we can now demonstrate that such actions result from activation of cannabinoid receptors.

In these experiments, we have focused on the role of cannabinoids in the globus pallidus. Given the size of the globus pallidus, we have employed a tissue punch method. However, characterization of the uptake shows kinetic and pharmacological properties similar to those published previously when neuronal GABA transporters are expressed

in heterologous systems (Borden et al., 1994; Ikegaki et al., 1994). Glial uptake is probably reduced in our slice preparation due to the use of a McIllwain tissue chopper. Indeed, slices that have been prepared using a McIllwain tissue chopper show extensive damage and > 90% of the cells appear pyknotic (Garthwaite et al., 1979). In contrast, the elements showing the best preservation are the synaptic structures. Thus it appears that chopped slices might be excellent for studying nerve terminals mechanisms.

The globus pallidus is a favoured site to study the interactions between GABA transmission and cannabinoids due to the presynaptic localization of type-1 cannabinoid receptors (CB_1) on the GABAergic terminals of striatal efferents to the globus pallidus. More than 85% of CB_1 sites in the globus pallidus are located on those GABA terminals (Herkenham et al., 1991).

The development of a specific cannabinoid receptor antagonist (Rinaldi-Carmona et al., 1994) allowed us to test whether these effects are cannabinoid-receptor mediated. Although at high concentrations Δ^9-tetrahydrocannabinol has been shown to have non-specific effects, notably on membrane fluidity (Makriyannis and Rapaka, 1990), our finding that the maximum effect of Δ^9-tetrahydrocannbinol was entirely blocked by the cannabinoid receptor antagonist SR 141716A discounts the possibility of non-cannabinoid receptor-mediated effect of Δ^9-tetrahydrocannabinol. The finding that SR 141716A did not affect the uptake of GABA suggests that, in this preparation, cannabinoid receptors are tonically activated.

The mechanism underlying cannabinoid receptor-mediated inhibition of GABA uptake remains to be elucidated. However, cannabinoids are known to decrease cAMP levels (Bidaut-Russel et al., 1990) and to block calcium channels (see Howlett, 1995, for review). Cannabinoids have also been shown to have a direct action on protein kinase A and protein kinase C (Kelly and Butcher, 1979; Hillard and Auchampach, 1994). This modulatory role of cannabinoids on protein kinases activation might have a role in changing the phosphorylation status of sites on the GABA transporter and therefore modify the uptake (Guastella et al., 1990). However, they are not likely to play a role in the interactions reported here as such direct actions would not be antagonized by SR 141716A.

The cataleptic effect of cannabinoids is well documented (see Pertwee, 1988, for review). Furthermore, catalepsy has been reported to be linked with an increase in GABAergic transmission in the globus pallidus (Scheel-Kruger et al., 1981). The findings reported thus offer a mechanism by which cannabinoids might increase pallidal GABA transmission and elicit catalepsy. GABA transmission in the globus pallidus is known to be abnormal in both Parkinson's disease and Huntington's disease, modulation of cannabinoid receptor function may therefore prove a useful therapeutic approach in these and other basal ganglia-related movement disorders. Further characterization of the effects of cannabinoids on GABAergic transmission is necessary to our understanding of how neurotransmission is modulated in the basal ganglia.

Acknowledgements

We are very grateful to Sanofi Recherche (Montpellier, France) for the generous gift of SR 141716A and to the Medical Research Council for funding this research.

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