Pharmacological Research 123 (2017) 51–61

Contents lists available at ScienceDirect

Pharmacological Research

journal homepage: www.elsevier.com/locate/yphrs

Perspective

Pregnenolone does not interfere with the effects of cannabinoids on synaptic transmission in the cerebellum and the nucleus accumbens

Anna Krohmer[a,c], Martin Brehm[a], Volker Auwärter[b], Bela Szabo[a,*]

[a] Institut für Experimentelle und Klinische Pharmakologie und Toxikologie, Medizinische Fakultät, Albert-Ludwigs-Universität Freiburg, Germany
[b] Institut für Rechtsmedizin, Medizinische Fakultät, Albert-Ludwigs-Universität Freiburg, Germany
[c] Institut für Biologie I, Fakultät für Biologie, Albert-Ludwigs-Universität Freiburg, Germany

ARTICLE INFO

Article history:
Received 26 February 2016
Accepted 26 April 2017
Available online 24 June 2017

Keywords:
CB_1 cannabinoid receptor
Endocannabinoid
Neurosteroid
Pregnenolone
Synaptic transmission
Δ^9-Tetrahydrocannabinol
THDOC

ABSTRACT

The steroid hormone pregnenolone attenuates several in vivo behavioural and somatic effects of the phytocannabinoid Δ^9-tetrahydrocannabinol, and it was suggested that pregnenolone can protect the brain from cannabis intoxication. The primary neuronal cannabinoid action behind most of the behavioural and somatic effects of cannabinoids is presynaptic inhibition of synaptic transmission. Therefore, the hypothesis of the present study was that pregnenolone attenuates the inhibition of synaptic transmission elicited by cannabinoids.

Brain slices containing the cerebellum or the nucleus accumbens were prepared from brains of mice and rats. Spontaneous and electrically evoked GABAergic inhibitory postsynaptic currents (sIPSCs and eIPSCs) and evoked glutamatergic excitatory postsynaptic currents (eEPSCs) were recorded in superfused brain slices with patch-clamp electrophysiological techniques.

Pregnenolone (10^{-7} M) did not affect the spontaneous GABAergic synaptic input (sIPSCs) to Purkinje cells in mouse cerebellar slices. The synthetic mixed CB_1/CB_2 receptor agonists JWH-210 (5×10^{-6} M) and JWH-018 (5×10^{-6} M) inhibited the spontaneous GABAergic synaptic input (sIPSCs) to Purkinje cells. This inhibition was not affected by pregnenolone (10^{-7} M). Tetrahydrodeoxycorticosterone (THDOC; 10^{-7} M), an in vivo metabolite of pregnenolone, also did not affect the inhibition of the GABAergic synaptic transmission by JWH-018. The depolarization of the Purkinje cells induced suppression of the GABAergic input to Purkinje cells; pregnenolone (10^{-7} M) did not affect this endocannabinoid-mediated form of synaptic suppression. In rat nucleus accumbens slices, glutamatergic and GABAergic synaptic input to medium spiny neurons was activated by electrical stimulation of axons. Δ^9-Tetrahydrocannabinol (2×10^{-5} M), which is a partial agonist of both CB_1 and CB_2 receptors, suppressed the glutamatergic and GABAergic synaptic transmission in the rat nucleus accumbens. These suppressive effects of Δ^9-tetrahydrocannabinol were not changed by pregnenolone (10^{-7} M). The suppression of the GABAergic synaptic transmission by Δ^9-tetrahydrocannabinol in the rat nucleus accumbens was also not affected by THDOC (10^{-7} M).

The results indicate that pregnenolone, a neurosteroid, does not affect GABAergic synaptic transmission. The inhibition of GABAergic and glutamatergic synaptic transmission elicited by synthetic, endogenous and phyto-cannabinoids is also not changed by pregnenolone. Therefore, it is unlikely that interference with cannabinoid-induced inhibition of synaptic transmission is the mechanism by which pregnenolone attenuates behavioural and somatic effects of Δ^9-tetrahydrocannabinol in vivo.

1. Introduction

The primary neuronal target of the phytocannabinoid Δ^9-tetrahydrocannabinol and the endogenous cannabinoids (endocannabinoids) is the CB_1 cannabinoid receptor [1]. It is widely distributed in the central and the peripheral nervous systems, and within the neurons it is strongly concentrated in the axon terminals [2–6]. Accordingly, the typical and most frequently described

Abbreviations: ACSF, artificial cerebrospinal fluid; IPSC, inhibitory postsynaptic current; EPSC, excitatory postsynaptic current; PRE, initial reference period; PREG, pregnenolone; SOL, solvent; THDOC, tetrahydrodeoxycorticosterone.

* Corresponding author at: Institut für Experimentelle und Klinische Pharmakologie und Toxikologie, Albert-Ludwigs-Universität, Albertstrasse 25, D-79104 Freiburg i. Br., Germany.
E-mail address: szabo@pharmakol.uni-freiburg.de (B. Szabo).

http://dx.doi.org/10.1016/j.phrs.2017.04.032
1043-6618/

neuronal effect following activation of CB_1 receptors is presynaptic inhibition of synaptic transmission [7–10]. At many synapses, endogenous cannabinoids are released by postsynaptic neurons, diffuse retrogradely to presynaptic axon terminals and inhibit transmitter release from the terminals [10–12].

Steroid hormones, synthesized in peripheral tissues like the adrenal cortex, ovaries and testicles and injected exogenous steroids, usually affect the functions of neurons by acting on nuclear steroid hormone receptors. Steroids can also be synthesized in the brain and they can then influence neurons in an autocrine or paracrine manner. For example, cerebellar Purkinje cells synthesize progesterone and estradiol, and these steroids, by activating nuclear progesterone and estrogen receptors, enhance dendritic growth, spinogenesis and synaptogenesis [13]. Steroids can also elicit rapid neuronal effects which are independent of nuclear receptors [14]. Tetrahydroprogesterone (THPROG; also called allopregnanolone) and tetrahydrodeoxycorticosterone (THDOC) are physiological metabolites of progesterone and deoxycorticosterone, respectively, and they can also be produced in the brain [15,16]. THPROG and THDOC rapidly potentiate $GABA_A$ receptor-mediated synaptic transmission [15,17–19]. The intravenously administered synthetic general anaesthetic agent alphaxalone also rapidly potentiates the effect of GABA on $GABA_A$ receptors [20]. Several sites on the $GABA_A$ receptor were identified as targets of the endogenous and synthetic neuroactive steroids [21]. The blood and brain concentrations of endogenous neurosteroids increase during stress and pregnancy, and it is thought that the concentrations are sufficiently high to affect $GABA_A$ receptor function [19,22,23].

Recently, Vallée et al. [24] has shown that injection of Δ^9-tetrahydrocannabinol and synthetic cannabinoids elicits an increase in pregnenolone concentration in many brain regions. CB_1 cannabinoid receptors were involved in this effect. In the brain, Δ^9-tetrahydrocannabinol increased the expression of cytochrome P450 side chain cleavage enzyme (also called cholesterol desmolase or CYP11A1), the enzyme which converts cholesterol into pregnenolone. It is, therefore, probable that Δ^9-tetrahydrocannabinol increased the brain concentration of pregnenolone by increasing its production in the brain. Injected pregnenolone and endogenously produced pregnenolone markedly attenuated the four typical effects of Δ^9-tetrahydrocannabinol ("tetrad"): inhibition of locomotion, decrease in body temperature, catalepsy and analgesia [24]. Pregnenolone also counteracted the effects of Δ^9-tetrahydrocannabinol on the firing rate of neurons in the ventral tegmental area and the release of dopamine in the nucleus accumbens. Finally, the inhibition of glutamatergic synaptic transmission by Δ^9-tetrahydrocannabinol in brain slices containing the nucleus accumbens was also attenuated by pregnenolone [24].

As mentioned above, presynaptic inhibition of synaptic transmission is the principal cannabinoid effect in the nervous system, and it is very likely the primary neuronal effect of Δ^9-tetrahydrocannabinol, when it elicits the "tetrad" of effects and when it increases the firing rate of neurons in the ventral tegmental area and dopamine release in the nucleus accumbens [9,25,26]. The hypothesis of the present study is, that the basis of the in vivo interaction between pregnenolone and Δ^9-tetrahydrocannabinol is an interaction between pregnenolone and Δ^9-tetrahydrocannabinol on synaptic transmission. To test this hypothesis, we studied the effects of pregnenolone on synaptic inhibition elicited by synthetic-, endogenous- and phyto-cannabinoids in vitro in brain slices. Parts of the results have been published in abstract form [27]. It has been recently shown that pregnenolone does not interfere with endocannabinoid-mediated synaptic inhibition in cultured autaptic hippocampal neurons [28].

2. Methods

2.1. Brain slices

Experiments were performed on mouse and rat brain slices. The experiments conformed to the European Communities Council Directive of 24 November 1986 (86/609/EEC). All efforts were made to minimize the number of animals and their suffering. The methods were similar to those described previously [29,30].

Ten to 18-day-old NMRI mice or Wistar rats were anaesthetized with isoflurane (> 3%) and decapitated. The brains were rapidly removed and placed in ice-cold artificial cerebrospinal fluid (ACSF) of the following composition (mM): NaCl 126, NaH_2PO_4 1.2, KCl 3, $MgCl_2$ 5, $CaCl_2$ 1, $NaHCO_3$ 26, glucose 20, Na-lactate 4, pH 7.3-7.4 (after the solution was gassed with 95% O_2/5% CO_2). 250 μm thick slices were cut with a Leica VT1000S vibrating tissue slicer (Wetzlar, Germany). Mouse brains were used to prepare sagittal slices containing the cerebellar vermis, whereas rat brains were used to prepare coronal slices containing the nucleus accumbens.

After cutting, the slices were stored in a Gibb chamber containing ACSF of the following composition (mM): NaCl 126, NaH_2PO_4 1.2, KCl 3, $MgCl_2$ 1, $CaCl_2$ 2.5, $NaHCO_3$ 26, glucose 10, Na-lactate 4, pH 7.3-7.4. For patch-clamping, brain slices were superfused at a flow rate of 2 ml min^{-1} with ACSF of the following composition (mM): NaCl 126, NaH_2PO_4 1.2, KCl 3, $MgCl_2$ 1, $CaCl_2$ 2.5, $NaHCO_3$ 26, glucose 10, pH 7.3-7.4. The experiments were performed at 20–24 °C, because patch-clamp recordings in brain slices are more stable at these temperatures.

2.2. Patch-clamping

Neurons in slices were visualised with infrared video microscopy, and patch-clamp recordings were obtained with an EPC-9 amplifier under the control of TIDA software (HEKA Elektronik, Lambrecht, Germany). Series resistance compensation of 50% was usually applied. Series resistance was measured before and after recordings and experiments with major changes in series resistance (> 20%) were discarded. Synaptic currents were recorded at a holding potential of −70 mV with pipettes (2.5 − 5 MΩ) containing (mM): CsCl 147, $MgCl_2$ 1, HEPES 10, EGTA 1, ATP-Na_2 4, GTP-Na 0.4, N-ethyl-lidocaine Cl 2, pH 7.4. In cerebellar cortical Purkinje cells, spontaneous GABAergic inhibitory postsynaptic currents (sIPSCs) were recorded in the presence of the NMDA and non-NMDA glutamate receptor antagonists DL-AP5 (2.5×10^{-5} M) and DNQX (10^{-5} M). In nucleus accumbens medium spiny neurons, GABAergic inhibitory postsynaptic currents (eIPSCs) and glutamatergic excitatory postsynaptic currents (eEPSCs) were evoked by electrical stimulation with a tungsten bipolar electrode in the vicinity of the patch-clamped neurons. eIPSCs were recorded in the presence of glutamate receptor antagonists (see above), eEPSCs in the presence of the $GABA_A$ receptor antagonist bicuculline (2×10^{-5} M).

2.3. Protocols and statistics

Recordings started 20 min after establishment of the whole-cell configuration (Fig. 1). sIPSCs were detected with the MiniAnalysis software (version 6.0.3; Synaptosoft, Fort Lee, NJ, USA). The lower limit of the amplitude was usually set to 30 pA. Every trace was inspected by the experimenter, and eventual errors of the automatic analysis were manually corrected. Amplitude and frequency values of sIPSCs were transferred from MiniAnalysis to Sigmaplot (version 12; Systat Software, Erkrath, Germany), and further calculations were performed by a program written by us in Sigmaplot. This program calculated, for example, the cumulative amplitude of sIPSCs by summing up the amplitudes of all sIPSCs within 2 min