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INHIBITION OF GABAERGIC INHIBITORY POSTSYNAPTIC CURRENTS BY CANNABINOIDS IN RAT CORPUS STRIATUM

B. SZABO.* L. DÖRNER. C. PFREUNDTNER. W. NÖRENBERG and K. STARKE

Institut für Pharmakologie und Toxikologie. Albert-Ludwigs-Universität. D-79104 Freiburg i. Br., Germany

Abstract—Electrophysiological consequences of activation of cannabinoid receptors have been mostly investigated on neuronal cell lines and on cells transfected with cannabinoid receptors. The aim of the present experiments was to study cannahinoid effects on identified neurons *in situ*. Electrically-evoked postsynaptic currents and voltage-dependent calcium currents were investigated in the principal neurons of the corpus striatum. the medium spiny neurons. with the patch-clamp method for brain slices. These neurons were chosen because they produce messenger RNA for cannahinoid receptors and because the density of cannabinoid binding sites in the striatum is high. Activation of muscarinic receptors by carbachol (10^{-5}M) reduced inhibitory postsynaptic current amplitude by 67%. The synthetic cannabinoid receptor agonist R(+)-[2,3-dihydro-5-methyl-3-[(morpholinyl)methyl]pyrrolo[1,2,3-de]-1,4-benzoxazin-yl]-(1-naphtalenyl)methanone (WIN55212-2: 10^{-8} to 10^{-5}M) dose-dependently reduced striatal inhibitor!, postsynaptic currents: the maximum effect. inhibition by 52%. was observed at 10^{-6} M. Another cannabinoid agonist. (−)-*cis*-3-[2-hydroxy-4-(1,1-dimethylheptyl)phenyl]-*trans*-4-(3-hydroxypropyl)cyclohexanol (CP55940: 10^{6} M). also reduced inhibitor! postsynaptic currents. by 50%. The CB1 cannnabinoid receptor antagonist N-piperidino-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-3-pyrazolecarboxamide (SR141716A: 10^{-6}M) had no effect when given alone but abolished the effect of WIN55212-2 (10^{-6} M). WIN55212-2 (10^{-6} M) did not change the current evoked by the $GABA_A$-receptor agonist muscimol (10^{-6}M). Activation of muscarinic receptors by carbachol (10^{-5}M) inhibited voltage-dependent calcium currents by 21%, but the cannabinoid receptor agonist WIN55212-2 (10^{-6}M) was without effect.

The results show that activation of CB1 cannabinoid receptors reduces GABAergic inhibitor! postsynaptic currents in medium spiny neurons of the corpus striatum: the likely mechanism is presynaptic inhibition of GABA release from terminals of recurrent axons of the medium spiny neurons themselves.
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Keywords: calcium channel. cannabinoid receptor corpus striatum, inhibitor!. postsynaptic current. medium spiny neuron. presynaptic inhibition.

Marijuana and hashish are products from *Cannabis sativa* and are widely used by humans for their euphoric effect. Cannabinoids may also be used therapeutically-. e.g., for the treatment of nausea, anorexia. glaucoma and chronic pain.[1,16,38a] The typical pharmacological effects of cannabinoids in animals are antinociception. hypokinesia, catalepsy and hypothermia.[1,16,38a] The mechanisms of cannabinoid effects on the nervous system are not well understood at present. Two G-protein-coupled receptors have been identified as the primary targets of cannabinoids.[16,29,34,38a] The CB1 cannabinoid receptor is preferentially located on neurons. whereas the CB2 receptor occurs typically in peripheral non-neuronal cells.

The typical neurochemical effect ofcannabinoids is inhibition of adenylate cyclase.[4] Electrophysiological effects of cannabinoids have been mostly studied on neuroblastoma (N18) and neuroblastoma-glioma (NG108-15) cell lines and on cells transfected with the cannabinoid CBI receptor. In these artificial systems. activation of CBI receptors leads to inhibition of voltage-gated calcium channels[3,24–26,36] and activation of potassium channels.[13,26] Cannabinoid effects have also been observed on "normal" neurons. Thus. cannabinoids enhanced potassium currents in neurons isolated from rat hippocampus,[5] and the! inhibited currents elicited by 5-HT3

*To whom correspondence should be addressed.

Abbreviations: CNQX. 6-cyano-7-nitroquinoxaline-2,3-dione: CP55940, (−)-*cis*-3-[2-hydroxy-4-(1,1-dimethylheptyl)phenyl]-*trans*-4-(3-hydroxypropyl)cyclohexanol: DL-AP5. *dl*-2-amino-5-phosphonopentanoic acid: DMSO. dimethylsulphoxide: EGTA, ethyleneglycolbis-(aminoethylether)tetra-acetate: HEPES. *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulphonic acid: IPSC′. inhibitory postsynaptic current: PRE. average of initial values (before drug application) of IPSCs or calcium currents: SR141716A. N-piperidino-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-3-pyrazolecarboxamide HCl: WIN55212-2. R(+)-[2,3-dihydro-5-methyl-3-[(morpholinyl)methyl]pyrrolo[1,2,3-de]-1,4-benzoxazin-yl]-(1-naphtalenyl)methanone mesylate.

receptor activation in neurons isolated from the rat nodose ganglion.[7]

The aim of the present experiments was to characterize cannubinoid effects on identified neurons in the rat brain. The principal neuron of the corpus striatum. medium spiny neuron. was selected for this purpose. Medium spiny neurons produce mRNA for the CB1 receptor."-'" A high density of cannabinoid binding sites is found in the corpus striatum itself and in the projection regions of medium spiny neurons. i.e. the substantia nigrapars reticulata. the globus pallidus and the entopeduncular nucleus.[10,14,18,27,50] Cannabinoid agonists activate G-proteins[46] and inhibit adenylate cyclase[20] in striatal neurons. Effects of cannabinoids on electrophysiological properties of medium spiny neurons were studied with the patch-clamp method for brain slices. We studied effects on inhibitory postsynaptic currents (IPSCs) elicited by electrical stimulation of the striatum and effects on voltage-dependent calcium currents.

EXPERIMENTAL PROCEDURES

Patch-clamp recording from neurons in brain slices was performed as described by Edwards and Konnerth and Stuart *et al.*[6,47]

Preparation and storage of brain slices

Eight to IX-day-old Wistar rats (obtained from Charles River. Sulzfeld. Germany) of either sex were used. Brain slices were prepared and stored in artificial cerebrospinal fluid of the following composition ("standard" extracellular solution; mM): NaCl 126. NaH_2PO_4 1. KCl 3. $MgCl_2$ 1, $CaCl_2$ 2.5, $NaHCO_3$ 26.2. glucose 10. pH 7.3-7.4 (after the solution was gassed with 95% O_2/5% CO_2). After decapitation the brain was immediately immersed in ice-cold cerrbrospinal fluid. A block of brain containing the corpus striatum was glued with cyanoacrylate glue to a stage fitting into a vibrating tissue slicer (Vibratome 1000: Plano. Marburg, Germany). Five to six coronal slices. 300 μm, were cut. The slices were stored in a Gibb chamber until superfusion started up to 8 h later: the temperature of the chamber during the first 40 min was 35°C. thereafter 20–24°C.

Superfusion and optical setup

For patch-clamp recording slices were transferred into a superfusion chamber and fixed at the glass bottom of the chamber with a nylon grid on a platinum frame. Slices were superfused at 20–24°C at a speed of 1–2 ml/min with a roller pump (Ismatec IPC-I: Bender & Hobein. Bruchsal. Germany). They were trans-illuminated with red light (λ_{max} = 780 nM) and viewed with a Zeiss Axioskop FS microscope (Zeiss. Göttingen. Germany) equipped with a water-immersion lens (40 × 0.75 W: Ph 2) and differential interference-contrast optics. The microscope was connected to a video camera sensitive to infrared light (Newvicon C 1400-07-C: Hamamatsu. Herrsching. Germany).

Patch-clamping

Pipettes were pulled from borosilicate glass (2 mm outer diameter, 0.3 mm wall thickness) with a vertical puller (L/M-3P-A; List. Darmstadt. Germany); they had a resistance of 3–5 MΩ when filled with intracellular solution. The patch pipette and the stimulating electrode were moved by step motor-driven micromanipulators (Luigs & Neumann. Ratingen. Germany). The same pipette was used for cleaning of the surface of the recorded neuron and for establishment of a gigaseal (2–10 GΩ) with the neuronal membrane ("one-step procedure"; see Stuart *et al.*[47]). Patch-clamp recordings were obtained nith an EPC-9 amplifier (List. Darmstadt, Germany) and evaluated with the TID.4 for Windows software (HEK.4 Elektronik. Lambrecht. Germany). Series resistance compensation of 50–60% was applied. Except where remarked. voltage values were not corrected for liquid junction potential. Data were filtered at 1 kHz and recorded at a sampling rate of 5 kHz. In neurons contained in brain slices. current analysis is complicated by extensive dendritic arborization: part of the dendritic tree probably escapes from somatic voltage-clamp (see 23). and this probably influences absolute values of calcium currents and inhibitory postsynaptic currents also in the present experiments. With this proviso. the method still permits examination of drug effects on these currents.

Recording of inhibitory postsynaptic currents

For recording of IPSCs standard extracellular solution containing 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX; 10 μM) and DL-2-amino-5-phosphonopentanoic acid (DL-AP5; 25 μM) was used. The composition of the intracellular solution was (mM): CsCl 107. CsF 35. $MgCl_2$ I. HEPES 10. EGTA IO. ATP-Na_2 4. pH 7.4 (adjusted with CsOH). Cs^+ was applied intracellularly in order to prevent contamination of synaptic responses by potassium currents: membrane potential was held at −40 mV in order to prevent sodium currents (see 23.33). Single rectangular electrical pulses (0.1 ms wide. 2–12 V) were delivered by an isolated stimulator (ST-02/ISO-100; Experimetria. Budapest. Hungary). Slices were stimulated either by stainless steel concentric bipolar electrodes (inserted 20–40 μm into the slice at a horizontal distance of 100–200 μm from the recorded neuron) or by platinum parallel bipolar electrodes (placed onto the surface of the slice so that the recorded neuron lay between the two arms of the electrode). Results obtained with the two kinds of electrodes were similar and hence pooled. Input resistance was continuously monitored during the experiments by observing the current response to a 10 mV hyperpolarizing pulse: experiments were eliminated if input resistance increased more than 30%.

Recording of calcium currents

For recording of calcium currents extracellular solution of the following composition was used (mM): NaCl 144. KCl 3. $MgCl_2$ 1. $CaCl_2$ 5. HEPES 10. glucose 10. tetrodotoxin 0.0002. pH 7.4 (adjusted with NaOH). The composition of the intracellular solution was (mM): CsCl 75. tetraerhylammonium-chloride 10, $MgCl_2$ 4. HEPES 40. EGTA IO. glucose 10. ATP-Mg 5. GTP-Tris 0.3. creatine phosphate-Na_2 14. creatine phosphokinase 50 U ml^{-1}, pH 7.4 (adjusted with CsOH). Calcium currents were elicited by voltage ramps from a holding potential of −80 mV to +60 mV. Ramps had a speed of 0.5 mV ms^{-1} and were applied every 30 s. For determination of linear leak currents. hyperpolarizing voltage ramps from -80 mV to −108 mV were applied 5s after each depolarizing ramp. The maximum leak-subtracted current measured during a ramp was used for statistical evaluation.

Experimental protocol. data evaluation and statistics

Recordings started 15 min after establishnient of the whole-cell configuration. Zero time in the figures is the time when recording began. Calcium currents were elicited every 30 s. IPSCs every 15 s.

Recordings lasted for 6–20 min. Slices were superfused initially with control solution for 3-5 min. Either drugs or control solution were then superfused for the remainder of the experiment. Currents measured during the initial 3-5 min were averaged and this average is referred to as

PRE: all values of an experiment were expressed as percentages of PRE. In most experiments currents measured in consecutive 0.5 min (two IPSCs) or 2.5 min periods (five calcium currents or 10 IPSCs) were averaged and these averages were also expressed as percentages of PRE.

Means ± S.E.M. are given throughout. Differences between groups were evaluated with the non-parametric two-tailed Mann-Whitney test: in appropriate cases Bonferroni correction was applied. $P<0.05$ was taken as the limit of significance and only this level is indicated even if P was <0.01 or 0.001.

Materials

Drugs were obtained from Calbiochem (Bad Soden. German!.): tetrodotoxin: Fluka (Buchs. Switzerland): muscimol: Merck (Darmstadt. Germany): tetraethylammonium-chloride: Pfizer (Groton. CT. U.S.A.): (−)-*cis*-3-[2-hydroxy -4-(1,1-dimethylheptyl)phenyl]-*trans*-4-(3-hydroxypropyl)cyclohexanol (CP55940): RBI (Köln. Germany): R(+)-[2,3-dihydro-5-methyl-3-[(morpholinyl) methyl]pyrrolo[1,2,3-de]-1,4-benzoxazin-yl]-(1 -naphtalenyl) methanone mesylate (WIN55212-2): Sanofi (Montpellier. France): N-piperidino-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-3-pyrazolecarboxamide HCl (SR141716A): Sigma (Deisenhofen, Germany): ATP-Mg. ATP-Na,. bicuculline. bovine serum albumin (fatty acid free), carbachol–HCl. creatine phosphate-Na,. creatine phosphokinasr and GTP-Tris: and Tocris Cookson (Bristol. England): CNQX and DL-AP5.

The cannabinoid ligands WIN55212-2, CP55940 and SR 14 1716A were dissolved in SO- 100% dimethglsulphoxide (DMSO) and stock solutions were stored at − 20°C. Further dilutions were made with superfusion buffer containing 1 mg/ml bovine serum albumin (in order to prevent adsorption of cannabinoids to the tubing); the final concentration of DMSO was 0.05–0.1%. Control solutions always contained the appropriate concentration of DMSO and bovine serum albumin (but only DMSO is indicated in the figures). CNQX and bicuculline were dissolved in 100% DMSO. carbachol. DL-4P5. tnuscimol and tetrodotoxin in distilled water: stock solutions were stored at − 70°C. Further dilutions were made with superfusion buffer.

RESULTS

Patch-clamp recordings were made from the principal neurons. the medium spiny neurons. of the corpus striatum. The perikarya of these neurons were spindle-formed and measured 12-l 6 μm along the longer axis. Cell capacitance. input resistance. series resistance and membrane time constant were calculated (see 38) using standard extracellular solution and the CsCl-based intracellular solution used for IPSC recording: they were 52 ± 4 pF. 1263 ± 206 MR. 31 ± 1 MΩ and 1.1 IO.1 ms. respectively (n=15). Resting membrane potential. measured in current-clamp mode using standard extracellular solution and a potassium gluconate-based intracellular buffer (see e.g.. 21). was ~ 74 ± 3 mV15 min after establishment of the whole-cell configuration (n= 13: corrected for liquid junction potential): it remained constant for at least 20 min (not shown). Cells did not fire spontaneously, but depolarizing current injections (100 pA. 500 ms) induced regular firing at a rate of 24 ± 1 Hz (n= 15). These morphological and electrophysiological properties of medium spiny neurons are as observed previously.[11,19,35]

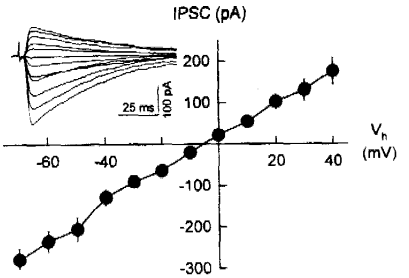

Fig.1. Relationship between amplitude of inhibitor! postsynaptic currents (IPSCs) and holding potential (V_h). Neurons were held at a given V_h for 6 s. and IPSCs were evoked by a single pulse. Means ± S.E.M. of nine experiments. The inset shows original tracings from one experiment.

Characterization of inhibitory postsynaptic currents

IPSCs were evoked by single rectangular electrical pulses. In the first series of experiments the holding potential was varied from − 70 mV to +40 mV in order to obtain the current–voltage relationship (Fig. 1). The current–voltage relationship was linear. The reversal potential was − 10.5 mV (corrected for liquid junction potential). fairly close to the calculated reversal potential of Cl^- under our experimental conditions. − 5.6 mV.

In all further experiments neurons were clamped at − 40 mV and IPSCs evoked by single electrical pulses every 15 s. At the beginning of the experiments, the IPSC amplitude was 219 ± 9 pA (n= 160: PRE). The IPSC amplitude decreased slightly in control experiments in which normal buffer w-as superfused (Fig. 3).

Superfusion of the sodium channel blocker tetrodotoxin (2×10^{-7} M) for 10 min strongly reduced IPSC amplitude. The maximum response was observed at the end of the 10 min superfusion. a decrease to 19 ± 7% of PRE (n=10; $P<0.05$: not shown). 5 min after the end of tetrodotoxin application there was a slight recovery to 38 ± 7% of PRE. IPSCs were nearly abolished by the $GABA_A$-receptor antagonist bicuculline (2×10^{-5} M) (Fig. 2). The maximum effect was reached within 2 min. Finally. the muscarine receptor agonist carbachol (10^{-5} M) greatly reduced the IPSC amplitude (Fig. 3). The maximum effect developed within *4* min.

Effect of cannabinoids on inhibitory postsynaptic currents

As in the experiments with tetrodotoxin. bicuculline and carbachol. neurons were clamped at − 40 mV and IPSCs evoked by single pulses every 15s. The IPSC amplitude decreased to 64–82% of PRE in control experiments in which 0.1% DMSO was superfused (Figs 4-h).

Two synthetic cannabinoid agonists. WIN55212-2 (10^{-6} M) and CP55940 (10^{-6} M), significantly decreased the amplitude of IPSCs (Fig. 1). At least 5 min were necessary for development of the

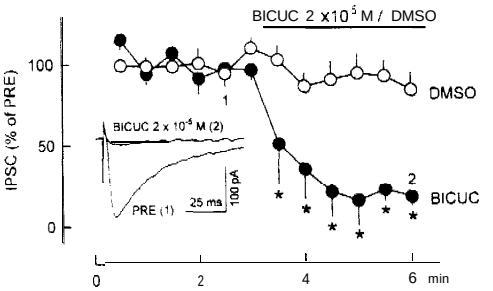

Fig. 2. Effect of bicuculline (BICUC) on inhibitory postsynaptic currents (IPSCs). IPSCs were evoked every 15 s by a single pulse. BICUC (2×10^{-5} M) was superfused from $t=3$ to $t=6$ min. The control group (DMSO) was superfused with buffer containing DMSO (0.1%) during this period. Values measured during the first 3 min were averaged (PRE). Values were averaged every 0.5 min and these averages were expressed as percentages of PRE. Means ± S.E.M. of six (BICUC) and 13 (DMSO) experiments. Significant difference from DMSO: $P<0.05$. The inset shows original tracings from an experiment with BICUC: tracings were obtained at time-points 1 and 2.

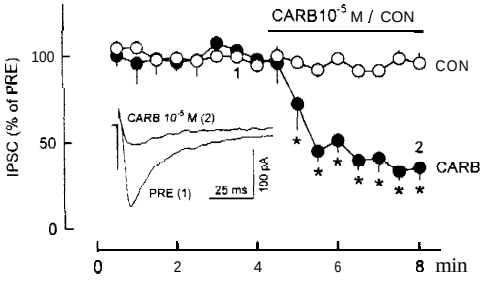

Fig. 3. Effect of carbachol (CARB) on inhibitory postsynaptic currents (IPSCs). IPSCs were evoked every 15 s by a single pulse. CARB (10^{-5} M) was superfused from $t=4$ to $t=8$ min. The control group (CON) was superfused with normal buffer. Values measured during the first 4 min were averaged (PRE). Values were averaged every 0.5 min and these averages were expressed as percentages of PRE. Means ± S.E.M. of nine (CARB) and 11 (CON) experiments. Significant difference from CON: $P<0.05$. The inset shows original tracings from an experiment with CARB: tracings were obtained at time-points 1 and 2.

inhibition. 15 min after commencement of superfusion of WIN55212-2 (10^{-6} M) and CP55940 (10^{-6} M), IPSC amplitude decreased to 40 ± 4% and 50 ± 4% of PRE, respectively (corrected for decrease in the appropriate control group). The effect of WIN55212-2 was not reversible on washout for 10 min (not shown), probably because this highly lipophilic drug is not readily washed out from the brain slice. But, long-lasting cannabinoid effects have been observed also in experiments on isolated neurons." WIN55212-2 was administered at four concentrations (10^{-8}–10^{-5} M) in order to study the concentration-reponse relationship (Fig. 5). WIN55212-2 (10^{-8} M) had no effect. The tendency for an effect of WIN55212-2 (10^{-7} M) was not significant whereas WIN55212-2 (10^{-6} and 10^{-5} M) significantly reduced the IPSC amplitude. The

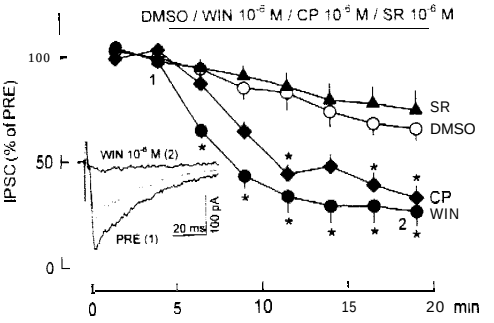

Fig. 4. Effect of WIN55212-2 (WIN), CP55940 (CP) and SR141716A (SR) on inhibitory postsynaptic currents (IPSCs). IPSCs were evoked every 15 s by a single pulse. WIN (10^{-6} M), CP (10^{-6} M) and SR (10^{-6} M) were superfused from $t=5$ to $t=20$ min. The control group (DMSO) was superfused with buffer containing DMSO (0.1%) during this period. Values measured during the first 5 min were averaged (PRE). Values were averaged every 2.5 min and these averages were expressed as percentages of PRE. Means ± S.E.M. of six (WIN), six (CP), 13 (SR) and 17 (DMSO) experiments. Significant difference from DMSO: $P<0.05$. The inset shows original tracings from an experiment with WIN; tracings were obtained at time-points 1 and 2; the thin line shows the PRE (1) curve scaled down according to the average decrease observed in the DMSO group (decrease to 64% of PRE).

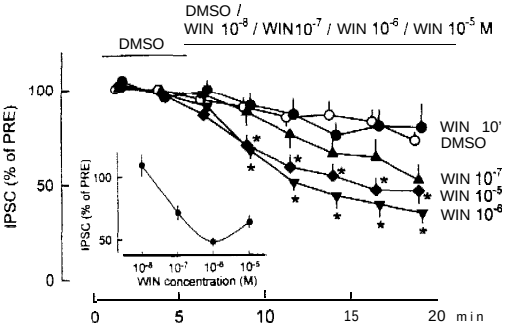

Fig. 5. Dose-dependent effect of WIN55212-2 (WIN) on inhibitory postsynaptic currents (IPSCs). IPSCs were evoked every 15 s by a single pulse. The WIN groups were superfused with buffer containing DMSO (0.1%) until $t=5$ min, and with buffer containing WIN (10^{-8} M, 10^{-7} M, 10^{-6} M or 10^{-5} M) from $t=5$ to $t=20$ min. The control group (DMSO) was superfused throughout with buffer containing DMSO (0.1%). Values measured during the first 5 min were averaged (PRE). Values were averaged every 2.5 min and these averages were expressed as percentages of PRE. Means ± S.E.M. of 10 (WIN 10^{-5} M), eight (WIN 10^{-7} M), seven (WIN 10^{-6} M), eight (WIN 10^{-5} M) and 11 (DMSO) experiments. Significant difference from DMSO: $P<0.05$. The inset shows the concentration–response relationship: values measured at the end of the superfusion were used and they were corrected for the decrease observed in the DMSO group (decrease to 73% of PRE).

maximum effect, an inhibition by 52% (corrected for decrease in the appropriate control group), was observed at 10^{-6} M. The data permit a rough estimation of the EC_{50} value of around 9×10^{-8} M.

Superfusion with the cannabinoid antagonist SR141716A (10^{-6} M) for 15 min did not change the

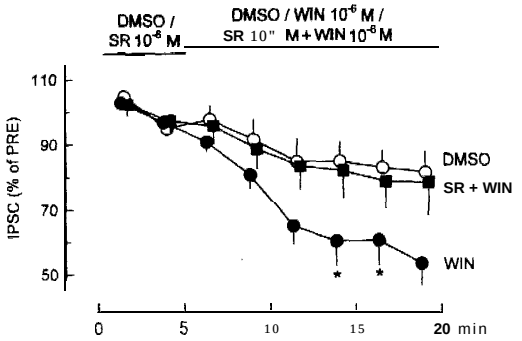

Fig. 6. Interaction of WIN55212-2 (WIN) with SR111716.4 (SR) on inhibitory postsynaptic currents (IPSCs). IPSCs were evoked every 15 s by a single pulse. The WIN group was superfused with buffer containing DMSO (0.1%) until $t=5$ min. and with buffer conraining WIN (10^{-6} M) from $t=5$ to $t=20$ min. In the combination group, SR (10^{-6} M) was superfused until 5 min. and SR (10^{-6} M) and WIN (10^{-6} M) were superfused together from $t=5$ to $t=20$ min. The control (DMSO) group was superfused throughout with buffer containing DMSO (0.1%). Values measured during the first 5 min were averaged (PRE). Values were averaged every 2.5 min and these averages were expressed as percentages of PRE. Means ± S.E.M. of 11 (WIN). 11 (SR+WIN) and eight (DMSO) experiments. Significant difference from DMSO: $P<0.05$.

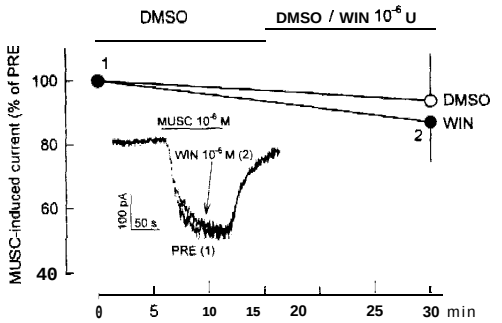

Fig. 7. Effect of WIN55212-2 (WIN) on currents evoked by muscimol (MUSC). MUSC (10^{-6} M) was superfused twice for 60–110 s. The WIN group was superfused with buffer containing DMSO (0.1%) until $t=15$ min. and with buffer containing WIN (10^{-6} M) from $t=15$ to $t=30$ min. The control group (DMSO) was superfused throughout with buffer containing DMSO (0.1%). The second MUSC-evoked current was expressed as a percentage of the first MUSC-evoked current (PRE). Means ± S.E.M. of six (WIN) an seven (DMSO) experiments. The inset shows original tracings from an experiment with WIN: tracings were obtained at time-points 1 and 2

IPSC amplitude (Fig. 4). SR141716A (10^{-6} M) abolished, however. the effect of WIN55212-2 (10^{-6} M) (Fig. 6).

Effect of cannabinoids on muscimol-evoked currents

A possible interference of WINS521 2-2 with the $GABA_A$-receptor ion channel complex was studied in the next series of experiments (Fig. 7). The $GABA_A$-receptor agonist muscimol (10^{-6} M) was superfused twice. The first superfusion elicited a current with an

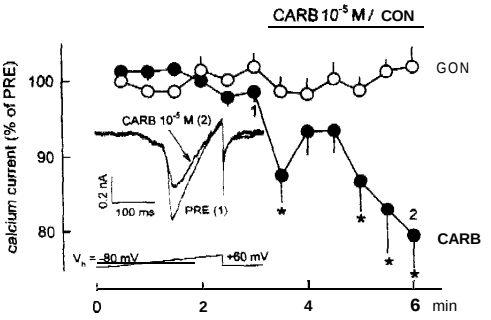

Fig. 8. Effect of carbachol (CARB) on calcium currents. Calcium currents were evoked every 30 s by voltage ramps (−80 mV to +60 mV). CARB (10^{-5} M) was superfused from $t=3$ to $t=6$ min. The control group (CON) was super-fused with normal buffer. Values measured during the firsr 3 min were averaged (PRE); all values were expressed as percentages of PRE. Means ± S.E.M. of seven (CARB) and 10 (CON) experiments. Significant difference from CON: $P<0.05$. The inset shows original tracings (leak-subtracted) from an experiment with CARB; tracings were obtained at time-points 1 and 2.

amplitude of 314 ± 48 pA ($n=13$: PRE). The muscimol-evoked current decreased slightly in control experiments with 0.1% DMSO. Superfusion of WIN55212-2 (10^{-6} M) for 15 min had no significant effect on the muscimol-evoked current.

Effects of cannabinoids on voltage-dependent calcium channels

Striatal medium spiny neurons were held at a holding potential of −80 mV. Voltage-dependent calcium channels were activated by ramp depolarization. The mean amplitude of calcium currents at the beginning of the experiments was 0.68 ± 0.05 nA ($n=52$: PRE). Calcium current amplitude remained fairly constant in control experiments in which normal buffer or 0.1% DMSO (Fig 8. 9) was superfused.

Superfusion of the non-selective calcium channel blocker cadmium (10^{-4} M) for 2 min greatly reduced calcium current amplitude. At the end of the 2 min superfusion. calcium current amplitude was 13 ± 2% of PRE ($n=17$: $P<0.05$; not shown). Calcium currents were only slightly reduced by nifedipine (10^{-6} M; 12–15% inhibition: $n=2$; not shown) and ω-conotoxin GVJA (10^{-7} M: 0–21% inhibition: $n=2$; not shown). The muscarine receptor agonist carbachol (10^{-5} M) significantly inhibited calcium currents (Fig. 8). The current was reduced by 21% after 3 min of carbachol superfusion. but the maximum effect was not yet reached at this time-point (Fig. 8).

Superfusion of the cannabinoid receptor agonist WIN55212-2 (10^{-6} M) for 15 min did not significantly change calcium currents (Fig. 9). The effects of WIN55212-2 were also studied in slices in which muscarine receptors and dopamine $D_{1/5}$ receptors were blocked by atropine (10^{-6} M) and SCH23390

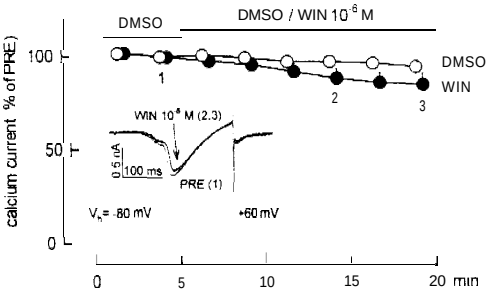

Fig. 9. Effect of WIN55212-2 (WIN) on calcium currents. Calcium currents were evoked every 30 s by voltage ramps (−80 mV to +60 mV). The WIN group was superfused with buffer containing DMSO (0.1%) until $t = 5$ min, and with buffer containing WIN (10^{-6} M) from $t = 5$ to $t = 20$ min. The control group (DMSO) was superfused throughout with buffer containing DMSO (0.1%). Values measured during the first 5 min were averaged (PRE). Values were averaged every 2.5 min and these averages were expressed as percentages of PRE. Means ± S.E.M. of nine (WIN) and eight (DMSO) experiments. The inset ***shows*** original tracings (leak-subtracted) from an experiment with WIN: tracings were obtained at time-points 1-3.

(10^{-7} M), respectively. After 4 min of superfusion with WIN55212−2 (10^{-6} M), the calcium current ampiitude was 102 ± 2% of PRE ($n = 9$; not shown). The value was not significantly different from the value observed after superfusion with 0.1% DMSO for 4 min in the appropriate atropine- and SCH23390-treated control group (106 ± 2% of PRE; $n = 10$; not shown).

DISCUSSION

The major finding of this study is that cannabinoids inhibited IPSCs in the corpus striatum. We interpret this observation as a CB1 cannabinoid receptor-mediated presynaptic inhibition of GABA release from terminals of recurrent axon collaterals of medium spiny neurons. Contrary to our expectation. activation of cannabinoid receptors had no effect on somadendritic voltage-dependent calcium channels in medium spin): neurons.

IPSCs were nearly abolished by tetrodotosin and bicuculline. and the reversal potential of the current was close to the calculated reversal potential of Cl^-. It is. thus. likely that electrical stimulation elicited action potential-dependent release of GABA from presynaptic axon terminals, which in turn activated $GABA_A$-receptors of the postsynaptic neuron. leading to an outward Cl- current. The corpus striatum has no major GABAergic afferent input from other brain regions." 90–95% of striatal neurons are GABAergic medium spiny neurons: the axons of these neurons arborize extensively within the striatum itself and form synaptic contacts with their own cell bodies and with other medium spiny neurons.[12,37] About 5% of striatal neurons are GABAergic interneurons." It is. therefore, likely that striatal IPSCs are mainly due to GABA released from axon terminals of medium spiny neurons. but GABA released from striatal interneurons may also contribute. Carbachol inhibited striatal IPSCs. verifying previous observations with other muscarinic agonists.[48]

Two chemically different synthetic cannabinoid agonists. WIN557 12-2 and CP55940. reduced striatal IPSCs. The effect of WIN55212-2 was dose-dependent and the IC_{50} (9×10^{-8} M) similar to values obtained with WIN552 12 − 2 in other electrophysiological studies.[5,7,13,24,26,36] WIN552 12-2 and CP55940 are agonists for both CB1 and CB2 cannabinoid receptors.[8,45] The effect of WIN55212-2 was antagonized by SK 141716A. which has much higher affinity for CB1 receptors ($K_i = 2$–12 nM) than for CB2 receptors ($K_i = 702$–> 1000 nM),[8,41,45] indicating that CB1 cannabinoid receptors were involved in inhibition of striatal IPSCs.

What was the mechanism of the inhibition of IPSCs by cannabinoids? WIN55212-2 (10^{-6} M) had no major effect on voltage-dependent sodium currents (Dörner and Szabo. unpublished observations). ruling out inhibition of action potential propagation. WIN55212-2 does not stimulate the neuronal uptake of GABA. but instead, GABA uptake is inhibited at higher concentrations of WIN55212-2 ($\geq 5 \times 10^{-5}$ M).[28] WIN55212-2 had no effect on the current evoked by muscimol in striatal neurons, and hence a postsynaptic action is also unlikely. The remaining and very likely alternative is that WIN55212-2 inhibited IPSCs by activating presynaptic CB1 cannabinoid receptors at axon terminals of GABAergic neurons. These axons probably are. as mentioned above, recurrent axons of the medium spiny neurons themselves. Medium spiny neurons produce mRNA for CB1 cannabinoid receptors and there is a high density of CB1 receptors in the striatum.[10,14,18,27,50]

For patch-clamp studies. brain slices are best prepared from young animals in order to improve survival and visibility of neurons (see 6). Accordingly. our studies were carried out on brain slices obtained from eight- to 18-days-old rats. Cannabinoid receptors are already expressed at this young age (see McLaughlin and Abood[30] for expression of CB1 receptor mRNA in young animals and Glass *et al.*[10] for the distribution of cannabinoid binding sites in the fetal and newborn brain).

Cannabinoid receptor-mediated presynaptic inhibition of transmitter release was first observed in the mouse vas deferens and in the guinea-pig small intestine.[40] It was later identified also in the mouse urinary bladder[39] and heart atria." Presynaptic inhibition of transmitter release has been recently observed also in the central nervous system. Cannabinoids inhibit the release of acetylcholine and noradrenaline in the hippocampus,[9,43] the release of dopamine from amacrine cells of the retina[42] and the release of glutamate, and hence glutamatergic neurotransmission, in hippocampal cultures.[44] Presynaptic

inhibition of release of several neurotransmitters by cannabinoids may turn out to be a key neuronal effect of cannabinoids.

Results obtained recently in anaesthetized animals indicate thar cannabinoids presynaptically inhibit GABA release from axon terminals of striatal medium spiny neurons in the projection regions substantia nigra and globus pallidus.[31,32,49] These and our observations supplement each other. Presynaptic cannabinoid receptor-mediated inhibition of GAB.4 release seems to operate in three different projection regions of medium spiny neurons: in the substantia nigra, the globus pallidus and the corpus striatum itself (projection site of recurrent axons). It is interesting to note that the presynaptic inhibition at these three sites will influence the output from medium spin) neurons differently. Presynaptic inhibition in the striatum will block recurrent inhibition of medium spiny neurons. and hence. will increase their firing rate and finally the output from these neurons. Presynaptic inhibition in the substantia nigra and the globus pallidus will decrease output from medium spiny neurons.

The cannabinoid antagonist SRI 41716.4 had an effect on its own in several tissues: it enhanced release of neurotransmitter[9,42] or caused a functional response which was attributed to an enhanced release of transmitter.[39,49] These finding were interpreted as indication of a presynaptic inhibition by an endogenous cannabinoid. SR141716A had no effect on its own in our experiments. and this rules out the function of an endogenous cannabinoid under our experimental conditions. It should be noted. however. that the stimulation applied. one pulse every 15 s. possibly was not sufficient to release endogenous cannabinoids.

N-. P- and L-type calcium channels and an unidentified channel contribute 27. 21.31 and <34%. respectively, to the overall depolarization-evoked calcium current in isolated medium spiny neurons.' The weak inhibition of calcium currents produced by w-conotoxin GVIA and nifedipine in the present experiments is in accordance with this observation. Activation of muscarinic receptors leads to a G-protein-dependent inhibition of calcium channels in isolated striatal neurons." Carbachol inhibited calcium currents also in medium spiny neurons in brain slices indicating that the slice model may be suitable for studying receptor-mediated modulation of calcium channels.

The synthetic cannabinoid agonist WIN55212-2. however. had no effect on the calcium current. An inhibiton by cannabinoids was not uncovered even during blockade of dopamine D,,, receptors and muscarinic receptors: these receptors were blocked in order to prevent potential interference of endogenous transmitters with calcium currents. Lack of effect of cannabinoids on somadendritic calcium currents in striatal medium spiny neurons stands in contrast to the inhibition of calcium currents in neuronal cell lines and in cells transfected with cannabinoid receptors:[3,24–26,36] It may be that expression and cellular localization of cannabinoid receptors or their coupling with calcium channels is different in cell lines and transfected cells on the one hand and *in situ* neurons in brain slices on the other. While cannabinoids do not inhibit somadendritic calcium currents, inhibition of calcium currents in axon terminals is one possible mechanism for the observed presynaptic inhibition of GABA release.

CONCLUSION

Catalepsy is a typical cannabinoid effect in animals and is thought to be due to an interference of cannabinoids with extrapyramidal motor systems.[1,16] The observed presynaptic inhibition of GABA release in the striatum and in striatal projection regions should be incorporated in any model aimed to explain extrapyramidal effects of cannabinoids.

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