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Comparative in vivo and in vitro studies with the potent $GABA_B$ receptor antagonist, CGP 56999A

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Abstract

CGP 56999A ([3-[1-(*R*)-[(3-cyclohexylmethyl)hydroxyphosphinyl]-2-(*S*)-hydroxy-propyl]amino]ethyl]-benzoic acid) is a potent $GABA_B$ receptor antagonist showing much more pronounced convulsant features in mice than do other previously studied $GABA_B$ receptor antagonists. The goal of this study was to elucidate the physiological mechanisms underlying this effect. In mice a dose of 0.6 mg/kg intraperitoneal (i.p.) CGP 56999A elicited behavioral activation and stereotypy with periods of intensive scratching and grooming. At 1 mg/kg i.p. most mice displayed myoclonic seizure-like episodes lasting several min. Pretreatment with the lower dose of 0.6 mg/kg i.p. also induced seizures after treatment with a subthreshold dose of pentylenetetrazole (40 mg/kg i.p.). In rats a dose of 3 mg/kg CGP 56999A (i.p.) induced convulsions of tonic–clonic nature. Intracellular sharp microelectrode recordings from rat cortical neurons in slices revealed no paroxysmal actions of CGP 56999A (10 μM). Similar to other $GABA_B$ receptor antagonists, CGP 56999A suppressed the late inhibitory postsynaptic potential (i.p.s.p.), but had no effect on the excitatory postsynaptic potential (e.p.s.p.) in the cortex. In cortical slices exposed to picrotoxin (10 μM), the compound evoked pronounced, spontaneous and intense epileptiform discharges. In conclusion, these findings demonstrated that the convulsive feature of the potent $GABA_B$ receptor antagonist, CGP 56999A, may be due to suppression of the late i.p.s.p., which becomes apparent in the intact brain only, whereas this action remains undetected in untreated brain slices. This remarkable discrepancy between in vitro and in vivo may be a consequence either of disruption of neuronal circuits during slice preparation or of the pronounced hyperpolarization of pyramidal neurons, at least in the case of cortical slice preparations. © 1997 Elsevier Science B.V.

Keywords: $GABA_B$ receptors; CGP 56999A; Epilepsy (rat); brain slices

1. Introduction

Centrally active $GABA_B$ receptor antagonists used in previous behavioral studies have shown few prominent behavioral effects in rodents. In disease models, the most marked effect was in animals with absence-type seizures, where a number of centrally active $GABA_B$ receptor antagonists suppressed the spontaneous 3 Hz spike and wave discharges in a dose-dependent manner (Marescaux et al., 1992; Klebs et al., 1992). There is also considerable evidence for a possible role of $GABA_B$ receptors in cognition since several antagonists elicited positive effects on cognitive functions in various models (Froestl et al., 1996).

We now studied a new and potent $GABA_B$ receptor antagonist, CGP 56999A, which has a significantly higher affinity for the $GABA_B$ receptor than the antagonists previously used in vivo (Waldmeier et al., 1994). It has an apparent IC_{50} value of 2 nM as determined in a radioligand test using [^{3}H]CGP27492 as ligand (Bittiger, personal communication). Surprisingly, this compound elicited prominent behavioral effects not seen to such an extent with other $GABA_B$ receptor antagonists, e.g., convulsions and behavioral activation including increased locomotion and stereotypy. The convulsive events prompted us to look into the physiological effects of this compound at the single-cell level in order to elucidate its mechanism of action.

$GABA_B$ receptors are widely distributed throughout the central nervous system, affecting neuronal activity presynaptically by interfering with transmitter release and postsynaptically by opening potassium channels (Bowery, 1993). In principle, the convulsive features of CGP 56999A might result from the drug's pre- and/or postsynaptic

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actions. Since presynaptic $GABA_B$ receptors are located at GABA and glutamatergic nerve terminals regulating the release of GABA and glutamate respectively, CGP 56999A might induce convulsions by increasing the size of the glutamate-mediated excitatory postsynaptic potential (e.p.s.p.). However, there is no evidence that CGP 56999A does not also act on GABAergic autoreceptors, which upon blockade would increase GABA release and thereby suppress convulsions. In addition, we expected to observe a reduction or complete suppression of the late i.p.s.p., which has been observed with several $GABA_B$ receptor antagonists (Olpe et al., 1993). These possibilities were explored by studying the impact of the drug on cortical neurons in vitro, using intracellular recording techniques. In addition, a series of behavioral experiments were performed in order to characterize the actions of this compound on the intact central nervous system.

2. Materials and methods

2.1. Behavioral observation test and pentylenetetrazole threshold test

In the experiments performed on male mice (Tif: MAGf (SPF), 19–25 g body weight, breeding facility Sisseln, Switzerland), the animals were kept in groups of 5. CGP 56999A was dissolved in physiological saline and administered intraperitoneally (i.p.) in doses of 0.3, 0.6 and 1.0 mg/kg (10 ml/kg). In the first experiment, 5 animals were used per dose. In a second experiment, a dose of 1.0 mg/kg of CGP 56999A was administered to 10 mice. All animals receiving the same dose were replaced in their home cages and observed during the subsequent 180 min. For the pentylenetetrazole test CGP 56999A was administered at a dose of 0.6 mg/kg i.p. to 20 mice. After 30 min, 40 mg/kg pentylenetetrazole was given i.p. Behavioral analysis was performed during the 30 min following the administration of pentylenetetrazole.

In the experiments performed on male Sprague-Dawley rats (Tif. RAlf, SPF; 100–110 g body weight, breeding facility Sisseln, Switzerland), the animals were kept in 3 groups of 10. CGP 56999A was dissolved in physiological saline and administered in doses of 0.6, 1.0 and 3 mg/kg i.p. Behavior was observed using the same procedure as for mice.

2.2. L-Baclofen-induced hypothermia

Experiments were conducted with male mice (Tif: MAGf (SPF), weighing 19–25 g, breading facility Sisseln, Switzerland). The animals were housed at 21–22°C in colony cages with free access to food and water on a 12 h light–dark cycle. Each mouse was marked and body temperature was measured rectally using an electrical probe (1.5×20 mm; Type TE3, Elektrolaboratoriet, Copenhagen, Denmark). Thereafter, compounds or placebo were administered either subcutaneously (s.c.), where compounds were dissolved in 0.9% NaCl, or orally, with compounds suspended in 0.5% methyl cellulose and given in a volume of 10 ml/kg body weight to groups of 10 mice each. Drug effects on L-baclofen-induced hypothermia were analyzed for statistical significance by Analysis of Variance (ANOVA). Statistically significant results are presented as mean temperature ± standard error.

Three groups of 10 mice each were used for the experiments with s.c. drug administration. One group received placebo and the other 2 groups were treated with 0.5 and 1.0 mg/kg CGP 56999A, respectively. Thirty minutes later 10 mg/kg s.c. of racemic baclofen was administered to all groups. Symptoms such as hypoactivity, flat body position, exophtalmus, dyspnoea and spastic reactions were assessed by observation. Body temperatures were measured 90 min after injection of baclofen. The mean body temperatures of the treated group prior to drug administration and after L-baclofen administration were compared to the mean body temperatures of the placebo group, prior to placebo and after baclofen treatment.

Four groups of 10 mice each were used for the experiments performed with oral drug administration. One group received placebo and the remaining groups were treated with CGP 56999A at doses of 3, 10 and 30 mg/kg, respectively. After 45 min, drug-induced symptoms such as hypoactivity, flat body position, exophtalmus, dyspnoea and spastic reactions were assessed by observation and body temperature was measured. After 60 min a dose of 10 mg/kg s.c. of racemic baclofen was administered to all groups and behavioral symptoms such as hypoactivity, flat body position, exophtalmus, dyspnoea and spastic reactions were scored as previously described 30, 60 and 90 min thereafter. Body temperatures were measured 90 min after injection of baclofen. Data were evaluated as described for s.c. drug administration.

2.3. Preparation of cortical slices

Male Sprague–Dawley rats (Tif. RAlf, SPF; weighing 140–180 g, breading facility Sisseln, Switzerland), were decapitated under isoflurane anaesthesia (mixed with 90% oxygen). The brains were rapidly dissected out and immersed in a cooled (3–5°C) artificial cerebrospinal fluid (ACSF) pregassed with 95% O_2/5% CO_2. The cerebral cortex and hippocampus were carefully isolated by removing the prefrontal cortex rostrally and the cerebellum caudally using a razor blade and discarding the brain parts ventral to the third ventricle. Transverse slices of 400 μm thickness were made using a vibratome. The standard ACSF had the following composition: 124 mM NaCl, 2.5 mM KCl, 1.2 mM KH_2PO_4, 2.0 mM $MgSO_4$, 2.5 mM $CaCl_2$, 26 mM $NaHCO_3$ and 10 mM D-glucose. Slices were placed on a nylon net located on a gas–liquid interface recording chamber and were maintained at 33°C

for at least 30 min with the ACSF level adjusted to their upper surface. Warm humidified air was continuously circulated over the slices and they were subsequently superfused with gassed ACSF at a rate of 1.5–2 ml/min.

2.4. Intracellular recordings from cortical neurons

Intracellular recordings were made from rat cortical pyramidal neurons in layers II–III using glass electrodes filled with 2 M potassium methylsulphate (resistance 80–110 MΩ). Pyramidal neurons were identified after impalement on the basis of their firing pattern (Connors and Gutnick, 1990) observed in response to subthreshold depolarizing current pulses. The input resistance of the neurons was determined by injecting constant depolarizing and hyperpolarizing current pulses (0.1 nA steps; 100, 200 and 700 ms width) through the recording electrode. Neuron-fired action potentials in response to threshold stimulation and their amplitudes were displayed on the oscilloscope. The resting membrane potential was determined at the end of each recording by estimating the difference between extracellular and intracellular potentials after withdrawing the recording electrode. Postsynaptic potentials were evoked by constant current stimulation (50–600 μA, 0.2 ms width, 0.03 Hz) delivered through a bipolar stainless steel electrode positioned close to the recording electrode. Recording and stimulation electrodes were placed along one line parallel to the slice surface in order to stimulate and record within the same cellular layer. During stimulation, a depolarizing current pulse was injected into the recorded neuron in order to reduce the strongly negative membrane potential of cortical neurons by 20–30 mV. This was a prerequisite for the activation and visualization of i.p.s.ps. Evoked responses were amplified using an Axoclamp-2A amplifier in bridge mode, displayed on a storage oscilloscope (Le Croy-Model 9310), averaged (4 sweeps) and plotted on a pen recorder (Kontron 340). Evoked responses were only recorded after stable impalement of the neuron. During data acquisition, the membrane potential was checked for stability. In case of baseline shifts exceeding 3–5 mV during recordings lasting 2–3 h, the recordings were terminated since this indicated a change in neuronal properties.

All drugs were dissolved in ACSF and added to the perfusion chamber at known concentrations via the main perfusion line. In some experiments 10 μM picrotoxin was added to induce epileptiform burst discharges, while 40 μM AP5 (DL-2-amino-5-phosphonopentanoic acid) and 14 μM CNQX (6-cyano-7-nitroquioxaline-2,3-dione) was added to obtain monosynaptic inhibitory potentials. Recovery from the effects of CGP 56999A was not observed, because the drug does not wash out within 1 h. In all experiments in which more than one drug was applied, the second drug was added to the ACSF containing the first drug in order to maintain the desired concentration throughout the experiment.

2.5. Materials

CGP 56999A [3-[1-(*R*)-[(3-cyclohexylmethyl)hydroxy-phosphinyl]-2-(*S*)-hydroxy-propyl]amino]ethyl]-benzoic acid, monolithium salt and L-baclofen were synthesized in the chemical laboratories of Ciba-Geigy (Basel, Switzerland). Picrotoxin and AP 5 (DL-2-amino-5-phosphonopentanoic acid) were purchased from Sigma (St. Louis, MO, USA). CNQX (6-cyano-7-nitroquioxaline-2,3-dione) was purchased from Tocris Neuramin (Bristol, UK).

3. Results

3.1. Behavioral effects of CGP 56999A

At the lowest dose, 0.3 mg/kg i.p., mice ($n = 5$ per group) showed moderate motor hyperactivity lasting approximately 30 min. At 0.6 mg/kg, 4 out of five animals showed marked signs of hyperactivity lasting more than 2 h. Simultaneously, pronounced signs of stereotypy were observed, characterized by intensive grooming behavior. One animal displayed an atypical epileptic fit. This myoclonic seizure-like episode was characterized by generalized, rhythmic twitches of the extremities. It was accompanied by body rotation and started immediately after a grooming phase. This animal died after a tonic convulsion. At 1 mg/kg i.p. the behavioral effects were very pronounced, starting with stereotypy and increased motor activity. Forty minutes after injection 7 out of the 10 animals tested displayed myoclonic seizure-like episodes lasting several min. Between these fits the animals showed intensive scratching and grooming behavior. After 2 h the epileptic fits ceased and short phases of hyperactivity occurred occasionally. At 3 h after injection no behavioral abnormalities were noticed.

Rats treated with a dose of 0.6 or of 1 mg/kg i.p. showed no behavioral abnormalities. At a dose of 3 mg/kg i.p. repeated chewing movements occurred before the onset of convulsions. At 45 min after injection 7 out of 10 rats displayed pronounced convulsions. Two of these animals showed tonic convulsions only, while 5 showed tonic–clonic convulsions. Convulsions lasted for a few minutes and the animals were killed 50 min after injection because of the severity of the convulsions.

3.2. CGP 56999A induces clonic seizures in pentylenetetrazole treated mice

The purpose of these experiments was to investigate whether CGP 56999A induces seizures in mice treated with a subthreshold dose of pentylenetetrazole. Pentylenetetrazole at 40 mg/kg i.p. did not itself induce any convulsions. In animals pretreated with 0.6 mg/kg CGP 56999A the subthreshold dose of the convulsant pentylenetetrazole induced clonic convulsions in 55% of

the animals ($n = 20$). The clonic fits were always followed by compulsive periods of scratching and grooming. At 30 min after pentylenetetrazole, 20% of the animals had died, all following tonic convulsions.

3.3. CGP 56999A reverses L-baclofen-induced hypothermia

The purpose of these experiments was to determine the bioavailability of CGP 56999A by examining whether CGP 56999A interacts with the effects of the systemically administered $GABA_B$ receptor agonist, L-baclofen, in vivo in mice. In the control group the mean temperature reduction induced by L-baclofen administration s.c. was 5.9°C (37.3 ± 0.01 vs. 31.4 ± 0.63°C) but in the mice treated with 0.5 mg/kg of CGP 56999A the temperature drop was 0.3°C (37.0 ± 0.14 vs. 36.7 ± 0.12°C) only. In a second experiment, 1.0 mg/kg of CGP 56999A s.c. completely prevented the action of L-baclofen (36.7 ± 0.13 vs. 37.7 ± 0.09°C). In addition, side effects such as hyperactivity and stereotypy occurred; 4 out of 10 animals displayed myoclonic seizure-like episodes.

Following oral administration of 3 and 10 mg/kg of CGP 56999A the baclofen-induced temperature reduction was not significantly changed (data not shown). However, at 30 mg/kg the temperature reduction was almost completely reversed. Whereas the control group had a mean reduction in rectal temperature of 6.5°C (37.9 ± 0.15 vs. 31.4 ± 0.84°C), the animals treated with CGP 56999A showed a mean decrease in temperature of 1.0°C (37.5 ± 0.21 vs. 36.5 ± 0.26°C).

3.4. Effects of CGP 56999A on cortical pyramidal neurons

Recordings were made from a total of 60 cells located in cortical layers II–III. These were regular spiking neurons with a mean membrane potential of 79.8 ± 0.4 mV ($n = 43$); the mean input resistance amounted to 36.4 ± 3.6 MΩ ($n = 5$). The values of these parameters indicate that the cells were mainly pyramidal and not spiny, stellate neurons (Connors and Gutnick, 1990). In order to study postsynaptic potentials it was necessary to inject depolarizing current pulses into these neurons inducing polarizations reaching between 20 and 30 mV. These current pulses remained constant throughout the experiment while the stimulation intensity at the stimulation electrode was variable. Low-intensity stimulation evoked only a fast e.p.s.p. At higher stimulation intensities the postsynaptic potential became triphasic, i.e. the fast e.p.s.p. was followed by an early and late i.p.s.p. The effect of CGP 56999A on input resistance was examined in 5 neurons, using subthreshold hyper-and depolarizing current pulses. The I/V plots obtained by linear regression indicated that CGP 56999A did not affect the input resistance ($n = 5$, data not shown).

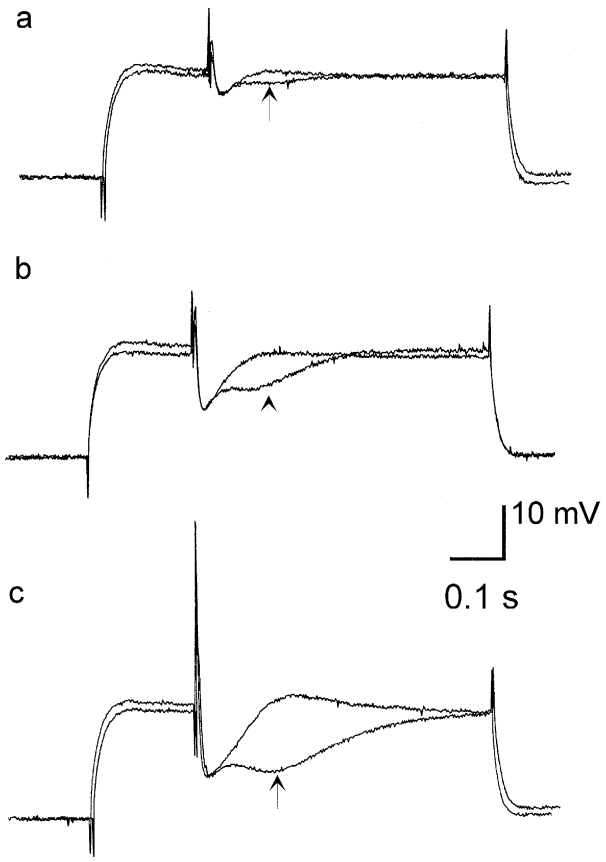

Fig. 1. The effect of CGP 56999A (10 μM) on the postsynaptic response of a cortical pyramidal neuron. This pyramidal neuron (action potential 80 mV, membrane potential 85 mV) was depolarized by a constant current pulse of 0.6 nA. Each panel depicts the average of 4 sweeps and shows, superimposed, the control response and the response observed 10–15 min following the administration of CGP 56999A. The arrow indicates the late i.p.s.p. (a) Subthreshold stimulation (70 μA) evoked an e.p.s.p. as well as small early and late i.p.s.ps. (b) Stimulation with 80 μA increased the amplitudes of the e.p.s.p. and early i.p.s.p., while it increased both the amplitude and duration of the late i.p.s.p. (c) Suprathreshold stimulation (200 μA) generated an orthodromic spike arising from the e.p.s.p. and increased the amplitudes of the early and late i.p.s.p. CGP 56999A completely abolished the late i.p.s.p. in the three panels.

In the absence of a convulsant agent, bath-applied CGP 56999A (10 μM) consistently blocked the late i.p.s.p. ($n = 13$, Fig. 1) without affecting the $GABA_A$ receptor-mediated early i.p.s.p. or the fast e.p.s.p. If stimulation was strong enough to elicit an action potential, CGP 56999A was not 'epileptogenic', since it neither induced additional action potentials nor evoked paroxysmal discharges.

3.5. CGP 56999A facilitates epileptic-like activity of cortical neurons induced by picrotoxin

Since CGP 56999A shows marked convulsive features in vivo, we explored whether this compound interfered with the paroxysmal discharges evoked by picrotoxin. Picrotoxin (10 μM), which partly blocks $GABA_A$ recep-

tors, was investigated on six neurons. At all stimulation intensities, the e.p.s.p. was broadened, and multiple instead of single orthodromic spikes were elicited. This phenomenon is interpreted to represent epileptiform burst activity. Fig. 2 depicts the evoked response elicited in a cortical neuron by stimulating cortical tissue nearby. On addition of 10 μM picrotoxin, the early i.p.s.p. was suppressed, the e.p.s.p. became considerably broader and the late i.p.s.p. increased in amplitude. The area of the late i.p.s.p. increased on the average by a factor of 1.5 ± 0.18 in the presence of the convulsant ($n = 6$). On addition of CGP 56999A (10 μM) to the perfusion line the late i.p.s.p. was eliminated, indicating, that $GABA_B$ receptors were blocked, and spontaneous, intense and prolonged epileptiform discharges were generated by the compound in all neurons investigated ($n = 5$, Fig. 2).

It was subsequently investigated whether CGP 56999A elicited the same effects in slices in which excitatory glutamatergic transmission was interrupted. To this end, slices were exposed to AP 5 (14 μM), CNQX (40 μM) and picrotoxin (75 μM), a cocktail of blockers used to evoke monosynaptic late i.p.s.ps. Recordings were taken 20–30 min following drug administration. Under these circumstances CGP 56999A (10 μM) had no effect on the epileptiform activity, which was manifested in the multiple spikes generated by suprathreshold stimulation (400 μA)

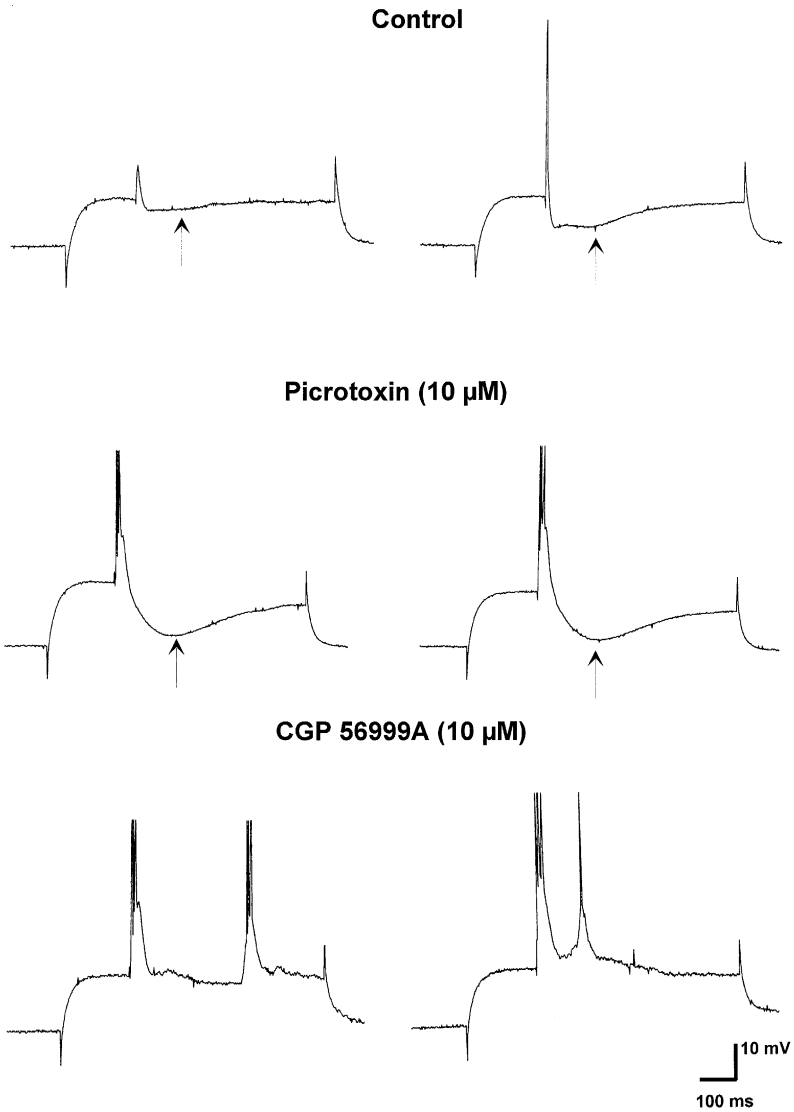

Fig. 2. The effect of picrotoxin (10 μM) and CGP 56999A (10 μM) on the activity of a cortical pyramidal neuron. This pyramidal neuron (action potential 80 mV, membrane potential 77 mV) was depolarized using a constant current pulse of 0.4 nA. Each panel depicts the average of 4 sweeps, except for the response to CGP 56999A, which was depicts only a single sweep. The arrow indicates the peak amplitude of the late i.p.s.p. The left panel depicts the response to subthreshold stimulation (66 μA) which evoked relatively small triphasic postsynaptic potentials when compared to those evoked by threshold stimulation (100 μA) in the right panel. An orthodromic spike was generated instead of the small e.p.s.p. and the amplitude of both i.p.s.ps was increased. Picrotoxin evoked epileptiform burst discharges in both panels (note the broadened area of the e.p.s.p. and the multiple spikes generated in this area). CGP 56999A completely abolished the late i.p.s.p. and evoked spontaneous, prolonged epileptiform discharges in both panels.

during picrotoxin administration only. Subthreshold stimulation (84 μA) completely failed to generate any postsynaptic responses during blockade of glutamatergic transmission (data not shown).

3.6. Interaction between picrotoxin, L-baclofen and CGP 56999A in vitro

The effect of the $GABA_B$ receptor agonist, L-baclofen (100 μM), on cortical postsynaptic potentials in slices exposed to picrotoxin (10 μM) depended on the stimulation intensity with which the synaptic responses were elicited. L-baclofen hyperpolarized the neurons by 8–10 mV ($n = 10$) and increased the action potential threshold by 0.1–0.6 nA. L-baclofen had an inhibitory effect on neuronal excitability at low stimulation intensity, while its effect was disinhibitory at high stimulation intensity ($n = 10$; Fig. 3). L-baclofen reduced the multiple spikes generated by picrotoxin to a small e.p.s.p. at low stimulation intensity (Fig. 3a). At slightly higher stimulation intensity L-baclofen only reduced the number of multiple spikes generated by picrotoxin and also abolished the late i.p.s.p. (Fig. 3b). At suprathreshold stimulation intensity L-baclofen potentiated the epileptiform burst activity of picrotoxin by increasing the number of multiple spikes (Fig. 3c). On addition of CGP 56999A to the bath, in the presence of L-baclofen and picrotoxin, the $GABA_B$ receptor antagonist caused a depolarization of the resting membrane potential by 6–10 mV ($n = 10$). CGP 56999A evoked prolonged, spontaneously occurring epileptiform discharges ($n = 10$). These discharges could be evoked by weak stimulation of afferent fibers that was subthreshold under control conditions (data not shown).

4. Discussion

This is the first report on a $GABA_B$ receptor antagonist showing pronounced convulsive features in mice and rats at low doses. In both species clonic seizures were observed after the injection of CGP 56999A, and rats also showed tonic seizures. This discrepancy remains to be explained

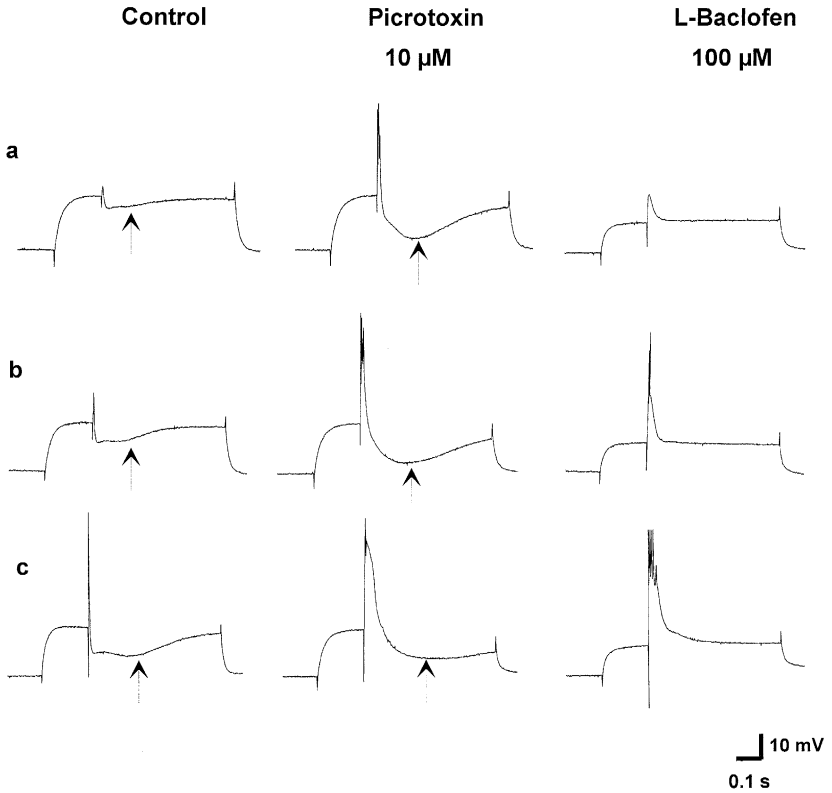

Fig. 3. Inhibitory and disinhibitory effects of L-baclofen on neuronal excitability in the cortex in the presence of picrotoxin. This pyramidal neuron (action potential 80 mV, membrane potential 80 mV) was depolarized with a constant current pulse of 0.45 nA. Each panel depicts the average of 4 sweeps, while the arrow indicates the peak of the late i.p.s.p. (a) Subthreshold stimulation (96 μA) evoked small triphasic postsynaptic potentials. Picrotoxin abolished the early i.p.s.p., increased the amplitude and duration of the late i.p.s.p. and generated multiple spikes. L-baclofen reduced these spikes to a small e.p.s.p. and abolished the late i.p.s.p. (b) Slight increase of the stimulation intensity (120 μA) increased the amplitude of the triphasic postsynaptic potentials. Picrotoxin had the same effect as in a, while L-baclofen reduced the number of multiple spikes in addition to eliminating the late i.p.s.p. (c) Suprathreshold stimulation (300 μA) generated an orthodromic spike arising from the e.p.s.p. and increased the amplitude of both i.p.s.ps. Picrotoxin had the same effect as in (a) and (b), while L-baclofen increased the number of multiple spikes and abolished the late i.p.s.p. The responses to L-baclofen were recorded without manual clamping of the membrane potential.

but it could be based on strain and species differences. The fact that this compound induces convulsions and reverses L-baclofen-induced hypothermia in mice indicates that it enters the brain, although direct evidence for appearance of CGP 56999A in the brain of mice is not yet available. In a previous study, the widely used $GABA_B$ receptor antagonist, CGP 35348, was found to produce a tendency to convulsions, only at the high dose of 1000 mg/kg i.p. (Bittiger et al., 1990). There are several possible explanations for the apparent discrepancy between these two antagonists. The simplest explanation would be a difference in affinity of the two compounds at the $GABA_B$ receptor. Another explanation might be that the two compounds affect differently $GABA_B$ receptor subtypes such as $GABA_B$ auto- versus heteroreceptors, thus having distinct effects on glutamate and GABA release. If CGP 56999A is more active on the heteroreceptors located on glutamatergic terminals, this could explain the drug's convulsive features, since blockade of the heteroreceptor would probably increase the release of glutamate from these terminals.

The affinity of CGP 56999A to the $GABA_B$ receptor was found to be 2 nM in displacement experiments using the $GABA_B$ receptor ligand, [^{3}H]CGP 27492, while the IC_{50} of CGP 35348 was similarly determined to be 35 μM (Bittiger, personal communication). Based on these data CGP 56999A has an affinity roughly 10 000-times higher than that of CGP 35348. Unfortunately, without knowing the brain concentrations the two compounds reached in mice, a strict comparison of the in vivo data is difficult. Nonetheless, the compound induces convulsions in mice at approximately 1 mg/kg (i.p.) for CGP 56999A and 1000 mg/kg (i.p.) for CGP 35348. Therefore, a ratio of 1000 was observed in vivo as compared to a ratio of more than 10 000 observed in vitro. These considerations do not eliminate the possibility that the convulsive features of CGP 56999A reflect the drug's higher affinity at $GABA_B$ receptors. An alternative explanation could be that CGP 56999A acts more effectively on glutamatergic terminals than CGP 35348, thus facilitating the release of glutamate. Such a shift in the balance between excitation and inhibition could well explain the drug's convulsive features in vivo. However, the present in vitro study did not show that CGP 56999A increased excitatory transmission, since evoked e.p.s.p.s were unchanged whereas the convulsant picrotoxin broadened the area of the e.p.s.p. and generated multiple spikes. These results are also in line with results of biochemical release studies (Waldmeier et al., 1994). While confirming that CGP 56999A is the most potent $GABA_B$ receptor antagonist from a large series of compounds tested, these authors found no preference for the hetero- versus autoreceptor in cortical slice preparations when measuring [^{3}H]GABA and glutamate release.

One of the best established models for inducing convulsions is the blockade of $GABA_A$ receptors. Our recordings from cortical pyramidal neurons did not yield any evidence for an inhibitory effect of CGP 56999A on the $GABA_A$ receptor-mediated i.p.s.p.s, even at high concentrations. The only circumstance in which this antagonist showed proconvulsive features in vitro was in disinhibited slices exposed to picrotoxin. Under these conditions, CGP 56999A showed clear-cut proconvulsive features to the extent that spontaneously occurring paroxysmal discharges became apparent. This effect resembled a similar effect demonstrated previously for pyramidal hippocampal neurons, in which the weak $GABA_B$ receptor antagonist, CGP 35348, increased the duration of paroxysmal discharges in slices exposed to bicuculline (Karlsson et al., 1992).

The interpretation of the interaction studies between CGP 56999A and the $GABA_B$ receptor agonist, L-baclofen, is not straightforward since the net effect of L-baclofen depended on stimulation intensity. Controversial effects of L-baclofen in epilepsy models had been observed in previous in vivo and in vitro studies, where L-baclofen was found to be proconvulsive, anticonvulsive or ineffective (for review, see Swartzwelder et al., 1986). These differences were attributed to the binding of L-baclofen either to presynaptic or to pre- and postsynaptic $GABA_B$ receptors, depending on the concentration used. Therefore, we used a high concentration of L-baclofen (100 μM) to ensure its binding both pre- and postsynaptically in order to simplify the interpretation of its action in our epilepsy model. Our findings indicate that the inhibitory effect of L-baclofen observed at low stimulation intensity probably resulted from the suppression of excitatory transmission, which seemed to be more effective than the suppression of GABAergic transmission. The latter was manifested as the complete elimination of the late i.p.s.p. On the other hand, the hyperpolarization of recorded pyramidal neurons in response to L-baclofen might also have contributed to its inhibitory effect. During strong stimulation of afferent fibers, L-baclofen facilitated the epileptiform activity of picrotoxin, probably because of the more effective suppression of inhibitory transmission. This may be attributed either to the fact that a larger number of neurons were activated during strong stimulation or to the fact that activation of glutamatergic terminals occurs more readily, especially during elimination of GABAergic inhibition. CGP 56999A reversed the effect of L-baclofen on membrane potential and increased the action potential threshold, indicating that it antagonized the action of L-baclofen (data not shown). Nevertheless, CGP 56999A evoked spontaneous, prolonged epileptiform burst discharges regardless of the stimulation intensity used. This probably resulted from the effective blockade of presynaptic heteroreceptors increasing the release of excitatory transmitter and the complete elimination of the i.p.s.p by CGP 56999A. However, it is important to stress that this increase in excitatory transmission was only observed in slices treated with picrotoxin.

In conclusion, this is the first extensive study with the very potent $GABA_B$ receptor antagonist, CGP 56999A.

Since $GABA_B$ receptors have not been cloned yet it cannot be determined whether the proconvulsive features of this compound result from the compound's higher affinity to these receptors or from its distinct affinity to $GABA_B$ receptor subtypes. The present study demonstrated a remarkable discrepancy between results of in vivo and of in vitro studies regarding the convulsive features of this $GABA_B$ antagonist. These findings demonstrate that pharmacological studies on slices may be misleading when the epileptogenic features of $GABA_B$ related compounds are investigated. Conceivably, disruption of neuronal circuits during slice preparation may account for these differences.

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