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Blockade of $GABA_B$ receptors accelerates amygdala kindling development

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Abstract. The aim of this study was to investigate the putative role of $GABA_B$ receptors in the development of amygdala kindling in rats. The effects of the $GABA_B$ blocker CGP 35348 and the $GABA_B$ agonist baclofen on the progressive development of behavioural seizure symptoms (stages 1–5 classified by Racine) and duration of afterdischarges (AD) were studied. CGP 35348 at a dose of 300 mg/kg i.p, which blocks central $GABA_B$ receptors, moderately but consistently accelerated the development of behavioural seizure symptoms. CGP 35348 had no marked effect on the duration of ADs corresponding to the different seizure stages. L-baclofen (6 mg/kg i.p.) had a dual effect on kindling development. It retarded the development of the behavioural symptoms, but increased the duration of AD. In conclusion, the results suggest that synaptically-released GABA activated $GABA_B$ receptors and thereby exerted a depressant effect on kindling development.
Key words. $GABA_B$ receptors; amygdala kindling; CGP 35348; baclofen; rat.

The roles of central gamma-aminobutyric acid ($GABA_B$) receptors are not well understood. The main source of information are in vitro studies demonstrating that presynaptically located $GABA_B$ receptors regulate the release of GABA [1]; postsynaptically they mediate a late inhibitory potential via activation of potassium channels [2, 3]. There is however a paucity of information on the functions of $GABA_B$ receptors in vivo. It is still unknown whether there is a '$GABA_B$ tone' under physiological conditions. It has been questioned recently whether endogenously released GABA activates pre- and/or postsynaptic $GABA_B$ receptors under physiological conditions [4]. In an attempt to obtain more insight into the function of $GABA_B$ receptors in vivo we investigated whether the blockade of $GABA_B$ receptors via the selective blocker CGP 35348 [5] or receptor stimulation with the $GABA_B$ agonist baclofen interfere with kindling development.

Kindling refers to the progressive development of behavioural and electroencephalographic epileptiform manifestations, triggered by repetitive delivery of an initially subconvulsive stimulus train [6]. The expression of epileptogenesis proceeds through characteristic behavioural stages which were classified by Racine [7]. Although the mechanisms that underlie the kindling process are not clear, the inhibitory neurotransmitter GABA appears to have an important role. It has been reported that drugs that augment GABA levels, such as GABA transaminase inhibitors (e.g. gamma-vinyl GABA [8]) and GABA uptake inhibitors (e.g. SK & F 89976-A [9]), may delay or block the kindling development in rats. Furthermore, drugs that increase GABAergic transmission by potentiating the effect of GABA at the postsynaptic $GABA_A$ receptor complex, e.g. benzodiazepines and phenobarbital [10], have also been shown to suppress kindling development. Conversely, drugs that decrease GABA levels by inhibition of GABA synthesis (3-mercaptopropionic acid) or block the postsynaptic $GABA_A$ receptor (bicuculline) have been reported to accelerate the kindling process [11].

CGP 35348 blocks pre- [12] and postsynaptic $GABA_B$ receptors [5] selectively up to a concentration of 1 mM. Since there are no pharmacological means available yet to affect pre- or postsynaptic $GABA_B$ receptors selectively, we chose rather high doses of the agonist and antagonist respectively which would presumably affect both types of receptors. The dose of baclofen could not be raised further because of its muscle-relaxing activity. The dose of CGP 35348 used has previously been shown to block central $GABA_B$ effects elicited by baclofen applied locally or systemically [5].

Materials and methods

Male rats (Tif: RAlf SPF) weighing 280–320 g at the time of surgery were anaesthetized with pentobarbital. Monopolar stainless steel depth electrodes were

stereotaxically implanted bilaterally into the amygdala and were used for stimulation and recording. The coordinates were taken from the atlas of De Groot[13]: AP = + 5.6 mm, L = 4.5 mm (from the sagittal suture) and V = − 3.0 mm. The incisor bar was adjusted to 5 mm above the interaural line. For the recording of the surface electroencephalogram, stainless steel screws were inserted into the skull above the occipital cortex. The electrode leads were soldered to a socket mounted to the skull, thus allowing connection with a freely moving cable. After a least 10 days of postoperative recovery, the threshold voltage for the induction of a brief afterdischarge (AD) was determined for each rat as follows. The animals were kept in observation cages and the left amygdala was stimulated with a train of 60 Hz square waves of 1-ms pulse width for 3 s. This was repeated with increasing voltages every 60 min until a short AD was elicited. Kindling of the rats was then performed once daily (for 12 days) with constant stimulation parameters, i.e. with a voltage of 0.2 V above the threshold value for AD of each animal. For each rat the duration of AD and the behavioural seizure symptoms were recorded and scored according to the five stages adapted from Racine[7] stage 1: immobility, eye closure, twitching of vibrissae; stage 2: chewing movements; stage 3; head nodding, clonus of one forelimb; stage 4: bilateral forelimb clonus (rearing); stage 5: rearing with loss of balance and falling accompanied by generalized clonic seizures. The animals were divided into 4 groups for the kindling study according to treatment: 1) CGP 35348 300 mg/kg (n = 11), 2) L-baclofen 6 mg/kg (n = 10), 3) L-baclofen 6 mg/kg + CGP 35348 300 mg/kg (n = 11) and 4) saline (n = 10). Each day for 12 days, CGP 35348 and baclofen were given intraperitoneally (i.p.) 30 and 60 min, respectively, before stimulation. Saline-injected (i.p.) animals (control group) and drug-treated animals underwent identical procedures. The number of stimulations required for the different seizure stages to be reached as well as AD durations at these stages were recorded for each rat. All values are given as means ± SEM. Statistical analysis was performed using the Kruskal-Wallis test with a non-parametric follow-up test (Mann-Whitney's U-test) for analysis of behavioural data and one-way analysis of variance with Dunnett's test (to compare one control with several treatments) for analysis of afterdischarge data. To verify electrode placements, the animals were sacrificed by decapitation and the brains were removed. Cryostat sections of the brains were cut and unstained sections investigated by light microscopy.

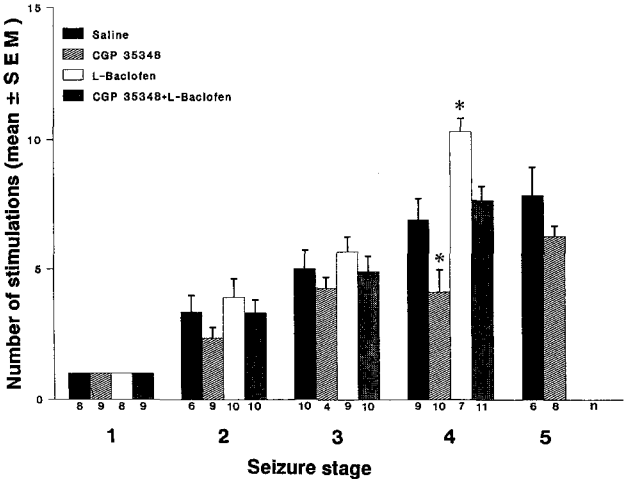

Figure 1. Effect of CGP 35348 and baclofen on the development of behavioural symptoms of kindling seizures in rats. CGP 35348 (300 mg/kg i.p.) accelerated the kindling process, i.e. less stimulations were required to reach the different seizure stages (2, 3, 4 and 5). Baclofen (6 mg/kg i.p) delayed the development of kindling seizures. When administered together, CGP 35348 and baclofen were mutually antagonistic for stages 2, 3 and 4. Seizure stage 5 was not reached during the experiment (>12 stimulations required) by the rats treated with either baclofen alone or baclofen together with CGP 35348. n = the number of rats in each group exhibiting the different seizure stages. * $p < 0.05$ compared to the saline-treated control group.

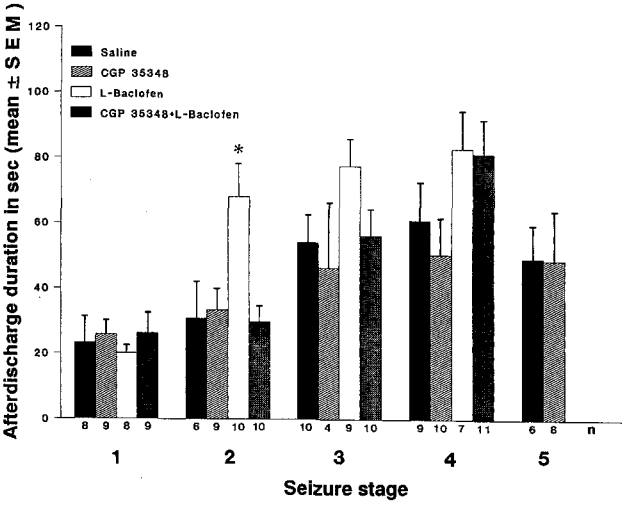

Figure 2. Effect of CGP 35348 and baclofen on AD duration in kindling seizure development in rats. Whereas CGP 35348 (300 mg/kg i.p.) had no marked effect, baclofen (6 mg/kg i.p.) increased the AD durations at the different seizure stages. CGP 35348 antagonized the effect of baclofen at stages 2 and 3. Seizure stage 5 was not reached by the rats treated with either baclofen alone or baclofen together with CGP 35348, n = the number of rats in each group exhibiting the different seizures stages. * $p < 0.01$ compared to saline-treated control and CGP 35348 + L-baclofen groups.

Results

CGP 35348 moderately accelerated the development of amygdala kindling in rats (fig. 1). Rats, which had received CGP 35348, required a lower number of stimulations to reach the different behavioural stages (2–5) of kindling compared to controls. A statistically significant difference was seen at stage 4 (CGP 35348: 4.1 ± 0.8 stimulations, n = 10; saline: 6.9 ± 0.8 stimulations, n = 9, $p < 0.05$). CGP 35348 did not have a marked influence on the duration of AD recorded for the different seizures stages (fig. 2). Figure 1 shows that baclofen retarded the rate of kindling development. Rats treated with baclofen required a larger number of stimulations

overall to reach the different seizure stages than did the saline-injected controls. The difference was statistically significant for stage 4 (baclofen: 10.3 ± 0.6 stimulations, n = 7; saline: 6.9 ± 0.8 stimulations, n = 9, $p < 0.05$). None of the baclofen-injected animals reached stage 5 during the experiment (i.e. after 12 stimulations). Figure 2 shows that baclofen tended to induce longer ADs in rats compared to controls. A significant difference was reached only for AD durations recorded for stage 2 (baclofen: 67.9 ± 10.6 s, n = 10; saline: 30.8 ± 11.4 s, n = 6, $p < 0.01$). In the group of animals which received CGP 35348 together with baclofen, no marked differences compared to controls in the number of stimulations required to reach seizure stages 1–4 were observed (fig. 1). As in the group treated with baclofen alone, none of the animals in this group reached stage 5 during the experiment (i.e. after 12 stimulations). For seizure stages 1–3, there were no differences in AD duration between the drug-treated animals and the controls. For stage 4 the duration of ADs tended to be longer than in the control group and reached approximately the value recorded for baclofen alone (Fig. 2).

Behavioural observations of the rats showed that CGP 35348 (300 mg/kg i.p.) did not induce any overt behavioural changes. Baclofen (6 mg/kg i.p.), on the other hand, induced muscle relaxation and a reduction in spontaneous movements. In the rats treated with both CGP 35348 and baclofen, these effects of baclofen were clearly reduced. Histological examination of electrode placements found that all depth electrode tips were within the amygdala.

Discussion

The most interesting finding was the moderate accelerating effect observed with the $GABA_B$ blocker CGP 35348 on the development of the behavioural symptoms of kindling seizures. In a previous pilot kindling study with CGP 35348 (100 and 300 mg/kg i.p.), we had obtained qualitatively similar results as in the present experiments, and effects on the behavioural seizure symptoms were already observed at 100 mg/kg. However, due to a slightly more rostral placement of the electrodes in the pilot study, the data could not be pooled. The lower dose of 100 mg/kg elicited similar although weaker effects. Lower doses were not tested because central $GABA_B$ blockade would be too weak to elicit any effects [5].

Baclofen seemed to have the opposite effect to CGP 35348 on the development of the behavioural seizure symptoms. These baclofen–induced effects are reproducible since similar results had already been obtained with baclofen (6 mg/kg i.p.) in the pilot study mentioned above. Almost identical results had been obtained in a previous investigation performed on immature rats and using a different stimulation procedure (rapid kindling). In this study, in which a lower dose of L-baclofen (2 mg/kg) was used, the development of kindling was also retarded and AD durations were prolonged as well [14].

Since baclofen has multiple sites of action on both inhibitory and excitatory neurons it is difficult to interpret the findings in terms of the drug's mode of action. The mechanism underlying the unexpected prolongation of afterdischarges induced by baclofen remains to be elucidated. In rats which received CGP 35348 and baclofen, the effects of the two drugs on both behavioural symptoms and AD duration seemed to be mutually antagonistic at the lower seizure levels (see fig. 1 and 2). In spite of being able to reverse partially the observed muscle-relaxing effects of baclofen, CGP 35348 did not antagonize the action of baclofen on AD duration corresponding to stage 4 nor did it influence the inability of baclofen-injected rats to reach seizure stage 5. The reason for the failure of CGP 35348 to block the effect of baclofen on the afterdischarge duration might be found by pharmacokinetic studies, since little is known about the long-term effects of these drugs after repeated daily administration.

The data obtained with the $GABA_B$ blocker which interferes selectively with ongoing GABAergic transmission provide evidence for a modulatory, probably inhibitory role of $GABA_B$ receptors in the regulation of kindling development. The moderate facilitatory effect is in line with previous data obtained from various in vitro models of epilepsy [15, 16]. Although CGP 35348 has no proconvulsive features on its own, it facilitates epileptic-like discharges induced in hippocampal slices by bicuculline, low Mg^{2+} or penicillin [15].

More caution is in place in interpreting the results obtained with the $GABA_B$ agonist baclofen. In contrast to CGP 35348, baclofen presumably affects both synaptic and extrasynaptic $GABA_B$ receptors. Therefore, although the effects are opposite to the ones found with the $GABA_B$ blocker and although they are more robust they may reflect pharmacological rather than physiological effects. Furthermore, although the rats did not show any marked motor impairment, it is difficult to exclude entirely the possibility that the kindling results were affected by the muscle-relaxing property of baclofen.

In conclusion, the results obtained with the centrally active $GABA_B$ blocker CGP 35348 suggest that synaptically-released GABA activates $GABA_B$ receptors and thus exerts a depressant effect on kindling development.

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Carbamazepine inhibits NMDA-induced depolarizations in cortical wedges prepared from DBA/2 mice

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Abstract. There is some doubt as to the mechanism of action of the widely-used anticonvulsant drug, carbamazepine. In cortical wedges prepared from genetically epilepsy-prone DBA/2 mice, carbamazepine at therapeutic concentrations (1-10 μM) markedly reduced the depolarization produced by N-methyl-D-aspartate (NMDA). The NMDA sub-type of glutamate receptor has been implicated in the pathogenesis of epilepsy and the inhibitory action of carbamazepine on this response suggests that the anticonvulsant action of the drug may be due to its blockade of NMDA receptor-mediated events.
Key words. Carbamazepine; NMDA; cortical wedges; DBA/2 mice.

Carbamazepine has been found to be one of the most effective antiepileptic drugs in clinical use for generalised tonic/clonic seizures. It has also been used for the treatment of trigeminal neuralgia and more recently for bipolar illness[1]. However, although it has been in use for more than twenty years, its mechanism of action has still not been proven. There is evidence that carbamazepine blocks sodium channels[2] and also that it facilitates potassium efflux[3], either of these mechanisms would reduce neuronal excitability and could account for its antiepileptic action. A further recent report[4] has shown that carbamazepine blocked NMDA-activated currents in cultured spinal cord neurones.

In this present study we have investigated the action of carbamazepine on NMDA-induced depolarizations in cortical wedges prepared from genetically epilepsy-prone DBA/2 mice.

Materials and methods

Male or female DBA/2 mice aged between 21 and 30 days, which corresponds to the time of peak audiogenic seizure susceptibility, were used throughout. Mice were killed by cervical dislocation and the brain rapidly removed and placed in ice-cold artificial cerebrospinal fluid (aCSF). Coronal slices (500 μm) were cut using a McIlwain tissue chopper, and cortical wedges were prepared as described by Burton et al.[5]. The tissue was placed in a two-compartment bath with a grease seal isolating the grey cortical matter from the callosum. Each compartment was perfused independently with gassed (95% O_2/5% CO_2) aCSF at 2 ml/min at room temperature (20–22 °C). The composition of aCSF in mM was: NaCl 124; KCl 5; NaH_2PO_4 1.25; $MgSO_4$ 2; $CaCl_2$ 2; $NaHCO_3$ 26; glucose 10; pH 7.4. For Mg^{2+}-free aCSF a corresponding increase in NaCl concentration was made.

Following slicing, both compartments were perfused with normal aCSF for 45–60 min to allow the tissue to equilibrate; perfusion of the cortical side was then continued with Mg^{2+}-free aCSF to facilitate NMDA-receptor activation, while the callosal side was perfused with normal aCSF throughout the experiment. The NMDA-induced depolarizations were monitored continuously via Ag/AgCl electrodes and amplified (Flyde 2601A), filtered and displayed on a BBC Goertz-Metrawatt chart recorder. The depolarization of individual neurones by NMDA was recorded as a population response in this preparation. Drugs were perfused into the 'cortical' side of the bath. NMDA (20 μM) was perfused for 2 min at approximately 15-min intervals and once stable recordings were obtained carbamazepine at varying concentrations (0.5–200 μM) was perfused for 10 min prior to, and during, the perfusion with NMDA. In a further series of experiments concentration-response curves were constructed for NMDA alone and in the presence of either 2.5 or 100 μM carbamazepine. Carbamazepine was dissolved in dimethyl sulphoxide (DMSO) and dilut-