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Effects of $GABA_B$ receptor antagonists on learning and memory retention in a rat model of absence epilepsy

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Abstract

A variety of animal models of absence epilepsy have been described and among these exists a genetically susceptible strain of rat (genetic absence epilepsy rats of Strasbourg (GAERS)). These rats produce periods of behavioural arrest with simultaneous production of cortical spike and wave discharges (SWD). $GABA_B$ receptor antagonists suppress completely the production of these spike and wave discharges. $GABA_B$ receptor ligands have also been reported to affect cognitive performance in rodents. The present study examined the cognitive performance of GAERS and the influence of $GABA_B$ receptor antagonists on this activity. Rats were injected intraperitoneally once per day with saline or a $GABA_B$ receptor antagonist (CGP 36742 (3-amino-propyl-*n*-butyl-phosphinic acid) 100 mg/kg; CGP 56433 ([3-{1-(*S*)-[{3-(cyclohexylmethyl)hydroxy phosphinyl]-2-(*S*) hydroxy propyl] amino]ethyl]benzoic acid) ([3-{1-(*S*)-[{3-(cyclohexylmethyl)hydroxy phosphinyl]-2-(*S*) hydroxy propyl] amino]ethyl]benzoic acid) 1 mg/kg or CGP 61334 ([3-{[3-[(diethoxymethyl)hydroxy phosphinyl]propyl] amino}methyl]-benzoic acid (1 mg/kg). A two-way active avoidance test paradigm with negative reinforcement was used. Untreated GAERS performed significantly better than non-epileptic rats ($P < 0.05$) and this enhancement in cognitive performance was sustained in rats treated with the $GABA_B$ receptor antagonists.

Keywords: $GABA_B$ receptor antagonism; Cognition; GAERS (genetic absence epilepsy rat of Strasbourg); Absence epilepsy

1. Introduction

Petit mal or generalized absence seizures differ clinically and experimentally from other seizure types. Clinically, absence seizures occur in children and have the classic electroencephalogram (EEG) abnormality of 3/s spike wave discharges associated with behavioural arrest, occasional automatisms and the absence of an aura or postictal state (Snead, 1992). The spike and wave discharges originate from thalamocortical pathways. Pharmacologically, absence seizures respond to ethosuximide, valproate and trimethadione, and are exacerbated by phenytoin and carbamazepine. A number of criteria for experimental animal models of generalized absence seizures have been suggested (Snead, 1988, 1995) which emphasize the difference of absence models from those of other seizure types. These include EEG and behavioural similarities with the human condition; reproducibility, predictability, pharmacological specificity for anti-absence drugs, a distinct developmental profile, potentiation by GABA receptor agonists; and involvement of thalamocortical mechanisms.

One of the defining features of generalized absence seizures is their exacerbation by increased GABAergic activity in the brain. Enhancement of GABAergic activity potentiates clinical and all experimental forms of generalized absence seizure activity and may even be sufficient to produce bilaterally synchronous spike and wave discharges under certain conditions (Gloor and Fariello, 1988).

A variety of animal models of absence epilepsy have been described and among these exist an genetically susceptible strain of rat (genetic absence epilepsy rats of Strasbourg (GAERS)) first described by Marescaux and colleagues in Strasbourg (Vergnes et al., 1982; Marescaux et al., 1992a). These rats, which have been inbred through more than 20 generations, produce periods of behavioural

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arrest with the simultaneous production of EEG spike and wave discharges. These events occur spontaneously and provide an excellent model of non-convulsive seizures, which manifest in humans as absence epilepsy (Liu et al., 1991). GABAergic transmission, particularly within the thalamus, appears to play a vital role in the production of spike and wave discharges in GAERS as well as in other rodent models of absence epilepsy. The receptor subtype $GABA_B$ has been implicated in the generation process as $GABA_B$ receptor antagonists suppress completely the production of spike and wave discharges and associated behavioural changes whilst $GABA_B$ receptor agonists exacerbate the symptoms (Marescaux et al., 1992b). $GABA_B$ receptor mechanisms may also influence cognitive processing in the mammalian brain since $GABA_B$ receptor ligands have been reported to affect cognitive performance in primates as well as rodents (Mondadori et al., 1993). The present study therefore examined the cognitive performance of GAERS and the influence of $GABA_B$ receptor antagonists on this activity.

2. Materials and methods

Experiments were performed on male non-epileptic Wistar and GAERS rats (250–300 g, 8 rats per group), which were habituated to a standard shuttle box (Ugo Basile) prior to any saline or drug injection. After habituation the rats were injected with saline or a $GABA_B$ receptor antagonist (CGP 36742 (3-amino-propyl-*n*-butyl-phosphinic acid) 100 mg/kg; CGP 56433 ([3-{1-(*S*)-[{3-(cyclohexylmethyl)hydroxy phosphinyl]-2-(*S*) hydroxy propyl] amino]ethyl]benzoic acid) 1 mg/kg or CGP 61334 ([3-{[3-[(diethoxymethyl)hydroxy phosphinyl]propyl] amino}methyl]-benzoic acid) 1 mg/kg) (Froestl and Mickel, 1996), kindly obtained from Ciba-Geigy (Basle, Switzerland), intraperitoneally once per day for 12 days.

A two-way active avoidance test with negative reinforcement was performed for the first 5 of the 12 days (Gozzani and Izquierdo, 1976). Each rat received 30 trials per day. The retention test was then performed 7 days later (i.e., on day 12). The stimulus employed was 6 s light and buzzer (70 dB + 670 Hx) followed within 3 s by 0.4 mA footshock.

The parameters measured were: (1) number of correct responses to the aversive stimulus, i.e., number of avoidances; (2) number of escapes from footshock; (3) reaction latencies; (4) number of footshocks received (taken as a measure of the lack of reaction).

3. Results

Both the saline-treated GAERS and Wistar non-epileptic control rats showed improved cognitive performance during the 5-day training period (Figs. 1 and 2). Both

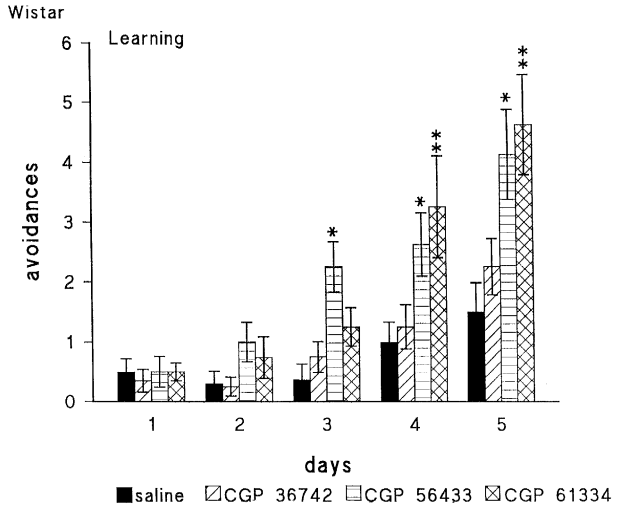

Fig. 1. Effects of $GABA_B$ receptor antagonists CGP 36742, CGP 56433 and CGP 61334 on active avoidance with negative reinforcement in Wistar rats. Ordinate: number of avoidances in 5-day (30 trials per day) learning session. Abscissa: number of training days. Solid bars: controls (treated with saline); hatched bars: treated with $GABA_B$ receptor antagonists CGP 36742 (100 mg/kg), CGP 56433 (1 mg/kg) or CGP 61334 (1 mg/kg). * $P < 0.05$ and ** $P < 0.01$ as compared to saline-treated rats on the same day.

groups of rats produced a significantly greater number of avoidances on day 5 compared to the session on day 1. However, the most striking difference was between the GAERS and control groups. By the fifth day the mean number of avoidances produced by the GAERS was 6 per

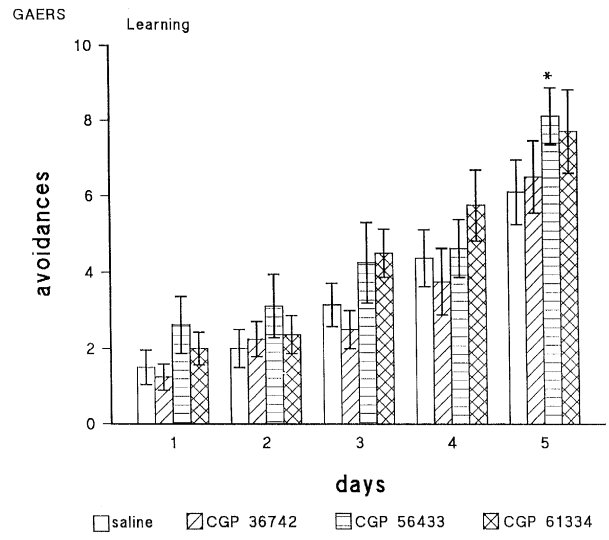

Fig. 2. Effects of $GABA_B$ receptor antagonists CGP 36742, CGP 56433 and CGP 61334 on active avoidance with negative reinforcement in GAERS rats. Ordinate: number of avoidances in 5-day training (30 trials per day) learning session. Abscissa: number of training days. Open bars: controls (treated with saline); hatched bars: treated with $GABA_B$ receptor antagonists CGP 36742 (100 mg/kg), CGP 56433 (1 mg/kg) or CGP 61334 (1 mg/kg). * $P < 0.05$ as compared to saline-treated GAERS on the same day.

Table 1
Mean number of footshocks (±S.E.M.) received of 30 elicited each day to each group of 8 rats

Day	Saline	CGP 36742 100 mg/kg	CGP 56433 1 mg/kg	CGP 61334 1 mg/kg
Non-epileptic group				
1	27.50±2.50	28.30±1.50	26.50±2.65	27.57±3.50
2	25.75±2.15	27.45±1.80	22.57±1.58	26.30±2.71
3	24.62±2.72	26.35±2.07	21.00±1.57	24.85±2.34
4	23.25±2.35	25.15±2.66	20.50±1.44	23.50±2.01
5	21.25±3.23	23.75±2.33	18.37±2.44	18.50±1.44
12	23.37±3.89	25.75±2.19	18.50±3.96	24.25±1.97
Genetic absence epilepsy rats of Strasbourg (GAERS)				
1	22.37±2.78	24.12±1.65	23.00±3.39	22.75±2.11
2	18.87±3.41	23.50±1.79	21.00±2.66	20.37±2.01
3	17.37±2.52	22.25±2.18	20.75±2.69	18.37±2.21
4	16.75±2.92	20.00±2.57	19.50±1.39	16.00±1.41
5	13.75±2.05	18.12±1.18	16.50±1.69	14.75±1.11
12	17.75±3.05	22.25±2.22	14.62±3.67	16.62±3.24

test session whereas only 1.5 was achieved by the control group (Figs. 1 and 2).

Control rats treated with the $GABA_B$ receptor antagonist, CGP 56433 (1 mg/kg) showed an increased number of avoidances on day 3, day 4 and day 5 compared with the saline-treated group. CGP 61334 (1 mg/kg) similarly increased the number of avoidances on the fourth and fifth days (Fig. 1). The number of avoidances produced by the GAERS was maintained in the $GABA_B$ receptor antagonist-treated animals and even increased on the fifth day in the CGP 56433-treated group (Fig. 2). The number of footshocks received by each group of rats is summarized in Table 1. These results concur with the avoidance data.

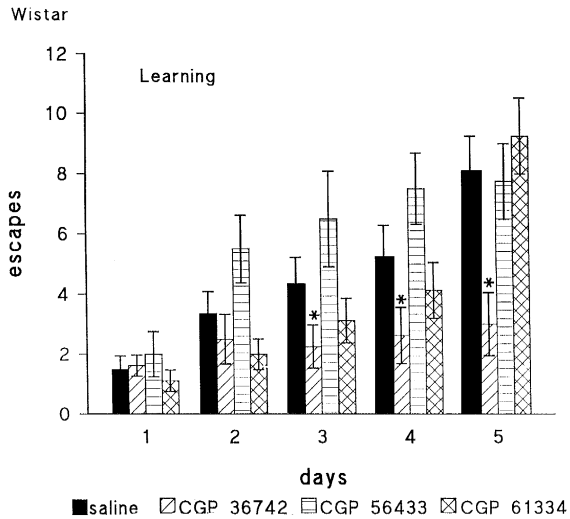

Fig. 3. Effects of $GABA_B$ receptor antagonists CGP 36742, CGP 56433 and CGP 61334 on active avoidance with negative reinforcement in Wistar rats. Ordinate: number of escapes in 5-day (30 trials per day) learning session. Abscissa: number of training days. Solid bars: controls (treated with saline); hatched bars: treated with $GABA_B$ receptor antagonists CGP 36742 (100 mg/kg), CGP 56433 (1 mg/kg) or CGP 61334 (1 mg/kg). * $P < 0.05$ as compared to saline-treated rats on the same day.

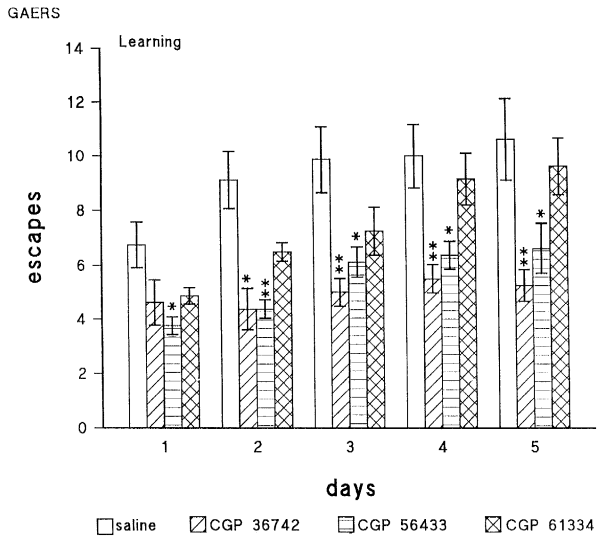

Fig. 4. Effects of $GABA_B$ receptor antagonists CGP 36742, CGP 56433 and CGP 61334 on active avoidance with negative reinforcement in GAERS rats. Ordinate: number of escapes in 5-day training (30 trials per day) learning session. Abscissa: number of training days. Open bars: controls (treated with saline); hatched bars: treated with $GABA_B$ receptor antagonists CGP 36742 (100 mg/kg), CGP 56433 (1 mg/kg) or CGP 61334 (1 mg/kg). * $P < 0.05$ and ** $P < 0.01$ as compared to saline-treated GAERS on the same day.

Non-epileptic control animals treated with saline made more escapes on the fifth day than on day 1 (Fig. 3). Rats treated with CGP 36742 (100 mg/kg) made fewer escapes on the third, fourth and fifth days compared with saline-treated control animals. GAERS treated with saline maintained a consistent number of escapes during the 5-day training session (Fig. 4). GAERS treated with CGP 36742

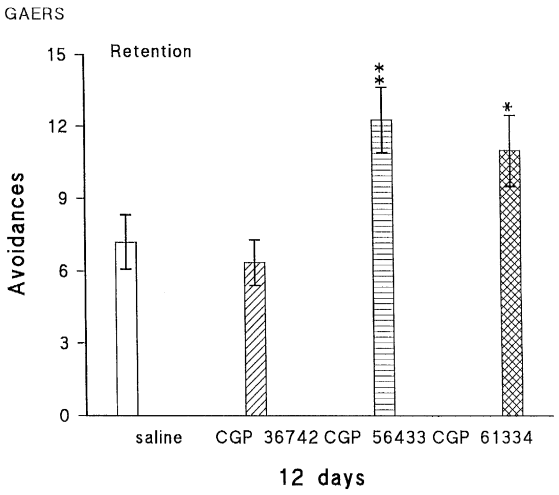

Fig. 5. Effects of $GABA_B$ receptor antagonists CGP 36742, CGP 56433 and CGP 61334 on active avoidance with negative reinforcement in GAERS rats: retention of learned behaviour. Ordinate: number of avoidances on 12th day (30 trials) of retention session. Abscissa: open bars: controls (treated with saline); hatched bars: treated with $GABA_B$ receptor antagonists CGP 36742 (100 mg/kg), CGP 56433 (1 mg/kg) or CGP 61334 (1 mg/kg). * $P < 0.05$ and ** $P < 0.01$ as compared to saline-treated GAERS on day 12.

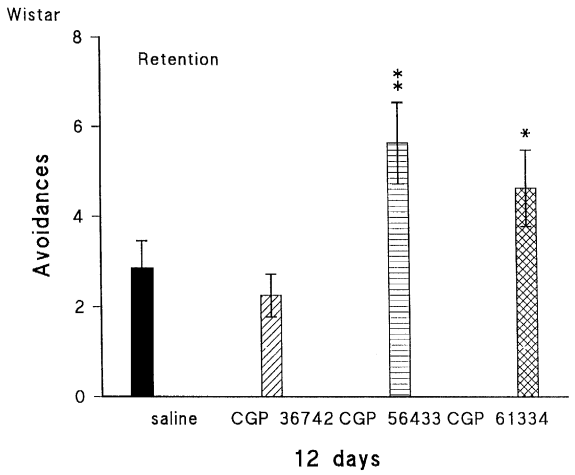

Fig. 6. Effects of $GABA_B$ receptor antagonists CGP 36742, CGP 56433 and CGP 61334 on active avoidance with negative reinforcement in Wistar rats: retention of learned behaviour. Ordinate: number of avoidances on 12th day (30 trials) of retention session. Abscissa: solid bars: controls (treated with saline); hatched bars: treated with $GABA_B$ receptor antagonists CGP 36742 (100 mg/kg), CGP 56433 (1 mg/kg) and CGP 61334 (1 mg/kg). * $P < 0.05$ and ** $P < 0.01$ as compared to saline-treated rats on day 12.

(100 mg/kg) and CGP 56433 (1 mg/kg) made significantly fewer escapes on days 2, 3, 4 and 5 compared with the saline group (Fig. 4).

In the retention session on day 12 (7 days after the last training session) the saline-treated GAERS maintained the number of avoidances achieved by the fifth day. Similarly GAERS treated with CGP 56433 or CGP 61334 and non-epileptic control rats treated with the antagonists maintained the increase in the number of avoidances compared with saline-treated animals (Figs. 5 and 6).

The mean reaction latency of each group of animals is summarized in Table 2. In all groups there was a clear trend towards a decrease in reaction latency with time.

Table 2
Mean response latency (s) (±S.E.M.) for each 30-trial period

Day	Saline	CGP 36742 100 mg/kg	CGP 56433 1 mg/kg	CGP 61334 1 mg/kg
Non-epileptic group				
1	174.44±3.79	176.31±8.55	162.27± 6.55	166.90±6.75
2	171.36±4.05	173.67±4.57	156.26± 8.64	164.98±8.29
3	166.15±5.05	174.53±4.60	152.41± 7.45	163.12±6.08
4	163.70±6.08	173.72±5.84	143.47± 7.74	160.08±8.82
5	164.45±5.05	170.17±4.71	144.52± 9.91	158.56±7.76
12	156.11±4.39	170.17±4.72	141.85± 8.82	163.12±6.08
Genetic absence epilepsy rats of Strasbourg (GAERS)				
1	164.63±4.07	167.56±3.66	163.76± 8.73	169.21±8.20
2	153.82±7.50	169.75±4.24	161.10± 8.00	155.37±6.33
3	149.05±6.03	158.77±6.31	154.36± 5.97	139.50±5.79
4	143.88±7.50	146.83±7.66	149.13± 6.98	137.62±5.92
5	142.07±8.08	138.71±7.31	132.15± 6.06	129.38±5.78
12	145.74±9.14	158.71±6.31	121.85±11.27	135.97±9.53

4. Discussion

The present results suggest that rats genetically susceptible to absence seizures (GAERS) learned faster that non-epileptic control rats and this was not suppressed by $GABA_B$ receptor antagonists which block spike and wave discharges. We could conclude, therefore, that the superior cognitive performance of GAERS may not directly involve the $GABA_B$ receptor. Whilst $GABA_B$ receptor activation appears to be implicated in the generation of spike and wave discharges in the GAERS model of absence epilepsy (Marescaux et al., 1992b) blockade of these receptors does not suppress the cognitive behaviour of these animals. In fact Mondadori (1995) and Mondadori et al. (1993, 1996) have shown that $GABA_B$ receptor antagonists can enhance cognitive performance in primates as well as rodents. Our present data obtained in non-epileptic control Wistar rats would support this with an enhancement in avoidance behaviour being produced by the third day of treatment. Even in the GAERS the high cognitive level achieved by day 5 was even further enhanced by one of the antagonists, CGP 56433 whilst the other antagonists at least maintained the level of cognitive performance. Mondadori et al. (1996) suggest that $GABA_B$ receptor antagonists may enhance cognitive function by facilitating cholinergic transmission which the authors link with the established memory-enhancing effects of cholinomimetics. However, Bernasconi et al. (1992) suggest that modulation of the synaptic release of glutamate may also provide a possible explanation. Whilst the present study does not provide any further information about their mechanism of action the data do show that $GABA_B$ receptor antagonism has a dramatic effect on learning behaviour.

Spike and wave discharges can be suppressed in GAERS by systemic or selective intrathalamic administration of $GABA_B$ receptor antagonists (Marescaux et al., 1992b). Conversely, $GABA_B$ receptor agonists increase the duration and number of spontaneous absence seizures in GAERS (Marescaux et al., 1992b). It has been suggested by Crunelli and Leresche (1991) (see also Coulter et al., 1990) that the long-lasting hyperpolarization produced by $GABA_B$-mediated synaptic transmission in thalamic neurones provokes the generation of low threshold Ca^{2+} currents to produce the spikes which trigger the thalamocortical pathway. Clearly, suppression of this process does not reduce the cognitive performance of rats. It could be, therefore, that the enhanced cognitive activity of GAERS observed in the present study is a consequence of, rather than a link with, the long-term generation of spike and wave discharges. If it can be assumed that the animals do not learn during the absence attacks they must use the seizure-free periods to greater effect than control animals. Based on data from Marescaux et al. (1992b), GAERS spend about 400 in every 1200 s in seizure episodes.

Whether the enhanced cognitive performance in absence epilepsy rats is due to a genetic modification or at

the neurochemical level in the brain is clearly unknown but certainly warrants further investigation. Vergnes et al. (1991) had previously noted that although many behavioural characteristics of GAERS were the same as those of control rats there was a possibility that avoidance learning in GAERS may have been enhanced in a few cases. The present data confirm and extend this observation.

Whether the data obtained in this rat model can be extrapolated to man remains to be seen. It is generally accepted that absence epilepsy may impair learning behaviour in children although treatment with drugs may confound this conclusion. However, a clinical report by Lennox and Lennox (1960) indicated that children with benign absence epilepsy showed no impairment in intellectual performance. In fact it may have even been improved.

In summary, the present study indicates that rats producing spontaneous spike and wave discharges showed an increase in cognitive performance in an active avoidance paradigm. This increase in learning behaviour was not suppressed by $GABA_B$ receptor antagonists even though the doses administered were sufficient to stop the seizure activity. Clearly many more studies are required to determine the basis for this apparent cognitive enhancement.

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