Pharmacology Biochemistry and Behavior, Vol. 41, pp. 603-606, 1992
Printed in the U.S.A.

0091-3057/92 $5.00 + .00

RAPID COMMUNICATION

Antagonism of Phencyclidine-Induced Hyperactivity in Mice by Elevated Brain GABA Concentrations

N. SEILER[1] AND C. GRAUFFEL

Marion Merrell Dow Research Institute, 67009 Strasbourg, Cédex, France

Received 14 August 1991

SEILER, N. AND C. GRAUFFEL. *Antagonism of phencyclidine-induced hyperactivity in mice by elevated brain GABA concentrations.* PHARMACOL BIOCHEM BEHAV **41**(3) 603-606, 1992.—Vigabatrin and (S)-4-allenylGABA (MDL 72483), two anticonvulsant GABA-T inhibitors, partially antagonize phencyclidine (PCP)-induced hyperactivity in mice at doses that do not affect spontaneous motor activity. The PCP antagonism is related to whole-brain GABA concentrations. The results indicate the potential use of GABA-T inhibitors in the therapy of PCP intoxications and perhaps also in the treatment of certain forms of endogenous psychoses.

Phencyclidine GABA Vigabatrin (R/S)-4-VinylGABA (S)-4-AllenylGABA Hyperactivity GABA-T inhibitors

PHENCYCLIDINE [1-(1-phenylcyclohexyl)piperidine; PCP] has been shown to induce behavioral changes in animals and humans and is an often used drug of abuse (1). Prolonged toxic reactions to PCP lead in many cases to hospital admissions for schizophrenia-like psychoses (10,29) and may last for as long as several weeks, probably due to accumulation of the drug in adipose tissue and brain (14).

Vigabatrin [(R/S)-4-vinylGABA; (R/S)-4-aminohex-5-enoic acid] and (S)-4-allenylGABA [(S)-4-amino-5,6-heptadienoic acid (MDL 72483)] are inactivators of GABA-T (6,7) and anticonvulsants that exert their anticonvulsant effects due to elevation of brain GABA concentrations (21,23,26). Vigabatrin is an antiepileptic drug effective even in some therapy-resistant epilepsies (22).

Defects in brain GABA systems have repeatedly been suggested as potential pathogenetic causes of schizophrenia (13, 18,19,24). It seemed, therefore, logical to explore the effects of GABA-T inhibitors with regard to their effects on PCP-induced hyperactivity in mice.

METHOD

Determination of Locomotor Activity

Male CD1 albino mice (weighing 34–40 g) (Charles River, St. Aubin-les-Elbeuf, France) were kept under standardized conditions (22°C; 60% relative humidity; 12 L:12 D cycle; water and standard diet ad lib).

Locomotor activity was determined using the Event-Counter of Columbus Instr. Corp. (Columbus, Ohio) as follows: Animals (six per cage) were brought into the room with the actimeter to allow adaptation to the new environment for several hours. PCP was given 10 min or 5 h after the GABA-T inhibitors. The dose of PCP (5 μg/g in 10 μl physiol. saline, IP) was the same as used by others previously (3,32). Immediately after PCP administration (or of the same volume of physiol. saline in the case of controls), animals were placed individually into standard plastic cages (23 × 17 cm) and the actimeter was set into action. Total time of observation was 35 min. The scores of 5-min intervals between 20 and 30 min were integrated and stored by the computer.

Determination of GABA, Glycine, and Other Nonessential Amino Acids

Amino acids were determined in perchloric acid brain extracts by a previously published isocratic separation of their ion pairs with *n*-dodecylsulfate on a reversed-phase column and postcolumn derivative formation with *o*-phthalaldehyde/2-mercaptoethanol (25).

Calculations

For the calculation of the significance of differences between PCP + GABA-T inhibitor-treated and PCP-treated

[1] Requests for reprints should be addressed to N. Seiler, Marion Merrell Dow Research Institute, 16, rue d'Ankara, 67009 Strasbourg, Cédex, France.

mice, analysis of variance (ANOVA) for single-factor experiments (34) was used. Except one group, consisting of 6 mice, the PCP + GABA-T inhibitor-treated groups consisted of 12 mice. The PCP-treated controls were 18 animals per group. A $p \leq 0.05$ was considered significant.

RESULTS

Mice showed during the first minutes of the observation period an enhanced locomotor activity due to intense exploratory behavior. A gradual decline of locomotor activity followed with time. Stable locomotor activity was always observed between 20 and 30 min after PCP (or physiol. saline). Therefore, this period was used for the evaluation of drug actions.

To test "direct" effects of the drugs, locomotor activity was determined starting 10 min after IP administration of 150 mg/kg (S)-4-allenylGABA and 400 mg/kg vigabatrin, respectively.

As appears from the results of Table 1, neither spontaneous nor PCP-induced locomotor behavior was significantly affected by this treatment.

The locomotor behavior of mice with elevated brain GABA concentrations was tested as follows: Vigabatrin and (S)-4-allenylGABA were administered IP in a dose range from 100–800 mg/kg and 50–300 mg/kg, respectively. Within this dose range, whole-brain GABA concentrations, as observed 5 h postinjection, were between 2.5 and 10 μmol/g (Fig. 1). The concentration of none of the other nonessential amino acids was changed significantly by this treatment (not shown).

Spontaneous locomotor activity was not affected markedly by vigabatrin, although there was a tendency toward lower values with increasing dose (Table 2). In PCP-treated mice, a significant decrease of locomotor activity by vigabatrin was noticed in all animals receiving a dose of 200–700 mg/kg (Table 2). The lowest value (observed after administration of 400 mg/kg vigabatrin) was 55% that of PCP-treated controls. Increase of the dose of vigabatrin above 400 mg/kg caused a tendency to increase rather than decrease locomotor activity.

As appears from the results in Table 3, administration of (S)-4-allenylGABA in a dose range from 50–300 mg/kg did not affect spontaneous locomotor activity of mice. At the very high dose of 600 mg/kg, animals were sedated and revealed little motor activity (not shown). In PCP-treated hyperactive animals, a dose-related decrease of locomotor activity could be observed. At the highest dose given (300 mg/kg), PCP-induced hypermotility was reduced by 75%. A statistically significant difference between (S)-4-allenylGABA-pretreated mice and mice receiving only PCP was seen at a dose of ≥ 100 mg/kg (S)-4-allenylGABA.

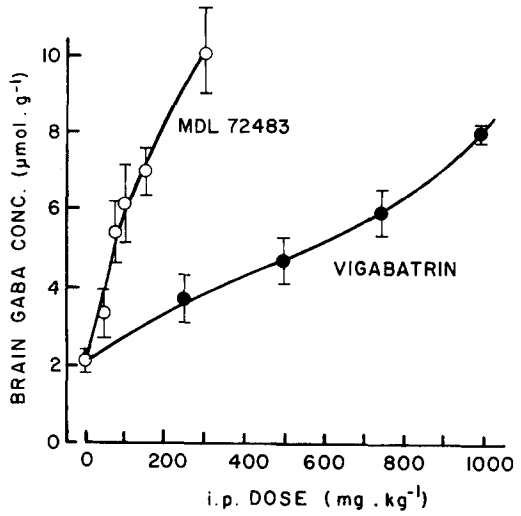

FIG. 1. GABA concentrations in the brains of CD1 albino mice 5 h after IP administration of vigabatrin and (S)-4-allenylGABA, respectively. Mean values ± SD (n = 3).

Since high doses of glycine are known to antagonize PCP-induced hypermotility (32) and since coadministration of glycine potentiates the anticonvulsant effects of GABA agonists against seizures induced by a functional GABA deficit (27,28) the effect of glycine–GABA-T inhibitor combinations on PCP hyperactivity was tested.

In Table 4, results of the effect of 10 mmol/kg glycine on locomotor behavior are summarized. In these experiments, glycine was given SC 1 h before the actimeter was started. Vigabatrin (400 mg/kg, IP) was given 4 h before glycine. At the time of PCP administration, brain glycine concentrations were elevated by 53 ± 4%. Ten mmol/kg glycine had no significant effect on spontaneous or PCP-induced locomotor activity. If given in combination with vigabatrin, locomotor

TABLE 1

EFFECT OF ADMINISTRATION OF VIGABATRIN AND (S)-4-ALLENYLGABA (MDL 72483) ON SPONTANEOUS AND PCP-INDUCED LOCOMOTOR ACTIVITY OF MICE

Drug	Dose (mg/kg,IP)	Spontaneous Locomotor Activity	PCP-Induced Locomotor Activity
None	–	399 ± 235 (11)	2730 ± 1270 (11)
Vigabatrin	400	528 ± 239 (6)	2460 ± 1220 (11)
MDL 72483	150	688 ± 308 (6)	2680 ± 1040 (11)

Mean scores ± SD (number of animals in parentheses).
Vigabatrin and MDL 72483 were given 10 min before PCP (5 mg/kg) and physiol. saline, respectively.

TABLE 2

EFFECT OF PRETREATMENT WITH VIGABATRIN ON SPONTANEOUS AND PCP-INDUCED LOCOMOTOR ACTIVITY OF MICE

Vigabatrin Dose (mg/kg)	Spontaneous Locomotor Activity	PCP-Induced Locomotor Activity
0	597 ± 348 (24)	3080 ± 1200 (18)
100	–	2360 ± 1100 (12)
200	505 ± 344 (6)	1970 ± 730 (12)*
400	471 ± 306 (12)	1690 ± 1385 (12)*
600	361 ± 139 (6)	2180 ± 410 (12)*
700	441 ± 298 (6)	1780 ± 1080 (12)*
800	435 ± 275 (6)	2100 ± 1780 (12)

Mean scores ± SD (number of animals in parentheses).
Vigabatrin was given IP. 5 h before PCP (5 mg/kg).
*Statistically significant difference ($p \leq 0.05$) between controls and vigabatrin-treated mice [ANOVA for single-factor experiments (34)].

activity was decreased by about 23% further than after administration of vigabatrin alone, but this difference was statistically not significant.

DISCUSSION

Pretreatment of mice with either vigabatrin or (S)-4-allenylGABA prevented PCP-induced hyperactivity to a considerable degree, but complete antagonism of the PCP effect was not possible at doses of the GABA-T inhibitors that do not affect significantly spontaneous motor activity.

The effects of vigabatrin and (S)-4-allenylGABA on PCP-induced hypermotility are indirect, as appears from the absence of an effect on locomotor activity 15 min after administration of optimum doses of the drugs at a time when brain GABA concentrations are still below 3 μmol/g (6,21). One has to assume that both the anticonvulsant effects of these drugs (21,23,26) and the antagonism of hyperactivity are due to the increase of brain GABA concentration. The difference in the potency of the two drugs to antagonize PCP hypermotility is probably a direct consequence of the difference in their ability to elevate brain GABA concentration (see Fig. 1). Unclear is why at the highest dose used (S)-4-allenylGABA produced a further decrease of locomotor activity of PCP-treated mice, whereas vigabatrin did not, in spite of not too different brain GABA concentrations. This observation is, however, analogous to the decreased anticonvulsant effect of this drug at doses > 750 mg/kg, and one has to remember that the pharmacology of various selective GABA-T inhibitors differs greatly in spite of the same basic mechanism, that is, their ability to enhance brain GABA concentrations (23,26).

We consider the fact that (S)-4-allenylGABA exhibited a direct dose–response relationship as a potential therapeutic advantage of this drug.

Antagonism of PCP-induced hyperactivity in mice has been demonstrated for glycine (31) and for compounds of several other primary actions, such as cholinergic, dopaminergic, serotoninergic, and GABAergic drugs (2,3). Imidazole acetic acid [a GABA agonist (5)] and chlorpromazine were suggested as potential drugs for treatment of PCP intoxication based on an extensive screening program in which dipropylacetate, baclofen, and diazepam (GABA-related drugs) among other drugs turned out to be ineffective (3).

TABLE 3

EFFECT OF PRETREATMENT WITH (S)-4-ALLENYLGABA (MDL 72483) ON SPONTANEOUS AND PCP-INDUCED LOCOMOTOR ACTIVITY OF MICE

MDL 72483 Dose (mg)	Spontaneous Locomotor Activity	PCP-induced Locomotor Activity
0	472 ± 303 (18)	3610 ± 1400 (18)
50	306 ± 244 (6)	2760 ± 1420 (12)
100	406 ± 221 (6)	1770 ± 775 (12)*
150	315 ± 304 (6)	1800 ± 1370 (6)*
300	445 ± 420 (6)	880 ± 1000 (12)*

Mean scores ± SD (number of animals in parentheses).
MDL 72483 was given IP 5 h before PCP (5 mg/kg, IP).
*Statistically significant difference ($p = 0.01$) between controls and (S)-4-allenylGABA-treated mice [ANOVA for single-factor experiments (34)].

TABLE 4

EFFECT OF 10 mmol/kg GLYCINE ON SPONTANEOUS AND PCP-INDUCED LOCOMOTOR ACTIVITY IN NAIVE AND VIGABATRIN-PRETREATED MICE

Dose Vigabatrin (mg/kg IP)	Glycine (mg/kg SC)	Spontaneous Locomotor Activity	PCP-Induced Locomotor Activity
–	–	480 ± 370	3300 ± 970
400	–	470 ± 300	2050 ± 1330*
–	750	400 ± 480	3400 ± 1200
400	750	360 ± 460	1570 ± 620*

Mean scores ± SD ($n = 12$).
Vigabatrin was given 5 h, glycine 1 h before PCP (5 mg/kg, IP) (respectively physiol. NaCl).
*Statistically significant difference ($p = 0.01$) between treated and control animals [ANOVA for single-factor experiments (34)].

The partial antagonism of PCP-induced hypermotility by the indirect GABA agonists vigabatrin and (S)-4-allenylGABA is in line with the analogous effects of the direct $GABA_A$ agonists (5,15), muscimol and imidazole acetic acid (3), and suggests that actions may occur via $GABA_A$ receptors. In line with this notion is the fact that baclofen was inactive in the same model (3).

Glycine given intragastrically in a dose range of 10–75 mmol/kg to BALB/c By mice was reported to reduce PCP-induced hyperactivity (32). In our experiments with CD1 mice, no significant effect was seen at a SC dose of 10 mmol/kg, but glycine seemed to enhance somewhat the effect of vigabatrin. This effect is, however, much smaller than the reported (11,27) synergism between glycine and vigabatrin against 3-mercaptopropionic acid induced convulsions.

PCP has only a minor effect on GABA metabolism (9), but it affects a great variety of other neurotransmitters and neuromodulators: It has both cholinergic and anticholinergic effects (12,16), displaces ligands from opiate, muscarinic, and nicotinic receptor binding sites (8,33), inhibits the uptake of catecholamines and serotonin (4,30), and binds to sigma and NMDA receptor complexes (17). None of these observations allow us presently to deduce mechanistic features of GABA agonists in PCP-induced hyperactivity. However, the multiple actions of PCP are presumably a sufficient explanation for the incomplete antagonism of hyperactivity by elevated brain GABA concentrations or $GABA_A$ agonists.

PCP-induced psychoses in man resemble in many respects symptoms observed during hallucinatory phases in certain schizophrenic patients (1) and are probably the best model for this disease presently available, but the relationships between mouse hyperactivity and schizophrenia-like PCP-induced psychoses in humans are not clear. The predictive value of the antagonism of PCP-induced hyperactivity by drugs is, therefore, uncertain with regard to their usefulness in the treatment of PCP intoxication or psychoses.

From recent observations (20), one may perhaps conclude that elevation of whole-brain GABA concentrations by GABA-T inhibitors is a contraindication for psychotic patients. However, in some patients withdrawal of vigabatrin developed psychoses (20). Clarification of the question of the

usefulness of GABA-T inhibitors in the treatment of certain forms of psychoses can only be expected from clinical trials in nonepileptic patients. In a recent study in patients with hyperkinetic involuntary movement disorders, vigabatrin had no effect on the mental status of the patients at dose that ameliorated the diseases (31).

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