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# Suppression of Haloperidol-Induced Oral Dyskinesias in Rats by Vigabatrin

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SEILER, N., C. GRAUFFEL, J. ELANDS, M. VAN DEN BUUSE, B. KNÖDGEN, S. SARHAN, P. MORAN AND S. GOBAILLE. *Suppression of haloperidol-induced oral dyskinesias in rats by vigabatrin.* PHARMACOL BIOCHEM BEHAV **50**(2) 181–189, 1995.—Acute and chronic administration of vigabatrin, a selective inactivator of GABA-T, suppresses haloperidol-induced dyskinesias at low doses without preventing the enhancement of striatal dopamine $D_2$ receptor density or the development of vacuous chewing movements. The long-term administration of vigabatrin does not attenuate its effect. The observations presented in this work support the GABA hypothesis of haloperidol-induced vacuous chewing behavior in rats, and suggest that vigabatrin is an appropriate means to enhance nigral GABAergic activity.

Haloperidol Dyskinesia Rats Vigabatrin GABA

THE DEVELOPMENT of dopaminergic supersensitivity in the striatum after the chronic administration of neuroleptics has been presumed to underlie tardive dyskinesia (5,38,51). However, there is also evidence from clinical studies for impaired GABAergic function in this disease: Lumbar GABA concentrations were reduced in schizophrenic patients with tardive dyskinesia, but not in those without tardive dyskinesia (4,53), and administration of GABA agonists reduced the hyperkinetic movements in tardive dyskinesia (3,48,52).

In particular, the fact that the GABA-T inactivator, 4-amino-5-hexenoic acid (vigabatrin), a successful drug for the treatment of therapy-resistent epilepsies (9,41) ameliorated in selected patients symptoms of tardive dyskinesia (19,21,48,49) prompted us to study effects of vigabatrin in an animal model of tardive dyskinesia.

## METHOD

### *Chemicals*

Haloperidol was obtained from Aldrich-Chemie (Steinheim, Germany). Vigabatrin is a product of Marion Merrell Dow (Strasbourg, France). $^{125}$I-sulpiride (specific activity 2200 Ci/mmol) was from Amersham (Les Ulis, France); apomorphine hydrochloride and polyethylenimine were from Sigma Chemical Co. (St. Louis, MO).

### *Animals*

Male Sprague-Dawley rats, initially weighing 202 ± 5 g, were obtained from Charles River (St. Aubin-les-Elbeuf, France) (Experiments 1 and 2), or from Iffa-Credo (L'Arbresle, France) (Experiment 3). They were kept in groups of four under standardized conditions (21°C; 60% relative humidity, 12 h light–12 h dark cycle) and had free access to standard diet (RF 20%; Charles River, Epinay-sur-Orge, France), drug-containing drinking fluid, and tap water.

### *Drug Treatment*

Stock solutions containing a 10-fold concentration of the various drugs were prepared twice per month and were stored in a refrigerator. The actual drinking fluids were prepared twice per week by 1 : 10 dilution of the stock solutions with tap water.

### *Experiment 1*

Treatment groups consisted of 12 or 13 animals.

*Group A (haloperidol).* A solution of 2.5 mg haloperidol/100 ml tap water was given during the first 18 days of treatment. After this, a solution containing 2.0 mg haloperidol/100 ml was given, to adjust to the lower fluid intake of the

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group receiving vigabatrin and haloperidol. The average fluid intake after adjustment was 37 ± 3 ml/rat per day, corresponding to a daily intake of 0.70 ± 0.04 mg haloperidol/rat.

*Group B (vigabatrin).* A solution of 75 mg vigabatrin/100 ml tap water was given until treatment day 18. Then, the concentration of the drug was reduced to 60 mg/100 ml, and was further reduced from day 70 to 42.5 mg/100 ml, to adjust the drug consumption of this group to that receiving haloperidol plus vigabatrin. The average fluid intake after adjustment of this group was 48 ± 4 ml/rat per day, corresponding to a daily intake of 23 ± 3 mg vigabatrin/rat.

*Group C (haloperidol plus vigabatrin).* These animals received a solution in tap water containing 2.5 mg haloperidol and 75 mg vigabatrin/100 ml. The average fluid intake (after a few days of adaptation) was 32 ± 3 ml, corresponding to an intake of 0.74 ± 0.06 mg haloperidol, and 22 ± 2 mg vigabatrin/rat per day.

*Group D (untreated controls).* Treatment of all groups was continued until day 116. Then, haloperidol was removed from the drinking fluids for 3 days, but administration of vigabatrin was continued. Brains were isolated on day 120 of vigabatrin administration.

### *Experiment 2*

Thirty rats received a solution of 2.5 mg haloperidol/100 ml tap water as sole drinking fluid. The average daily fluid intake per animal was 34 ± 4 ml, and the haloperidol intake 0.81 ± 0.05 mg Haloperidol administration was interrupted for certain periods (see Fig. 2). During the drug-free periods the animals had access to tap water ad lib.

### *Experiment 3*

A group of 13 rats received a drinking solution containing 2.5 mg haloperidol and 150 mg vigabatrin per 100 ml tap water. The average daily fluid intake was 27 ± 3 ml, corresponding to 0.67 ± 0.09 mg haloperidol and 40 ± 5 mg vigabatrin/day per rat. After 209 days of treatment, vigabatrin was omitted, but administration of haloperidol was continued, and spontaneous orofacial movements were assessed during the following days.

### *Assessment of Vacuous Chewing Movements*

Individual rats were observed in a cylindrical Plexiglas jar (21 cm in diameter and 21 cm high). The animals were allowed at least 2 min to accommodate to the rating cage. The jaw movements were counted during 2 min, stopping whenever the rat began locomotion, grooming, and so forth. Two types of jaw movements were recorded: vacuous chewing movements and bursts of jaw tremor. For the calculation of total scores, each burst of jaw tremor was regarded as being equal to two vacuous chewing movements, in accordance with previously published scoring methods (11,17).

### *Brain Dissection*

The animals were decapitated, the heads dropped in ice-cold physiologic saline, and the brains rapidly isolated. Cortical tissue, striata, and cerebellum—and in one group, S. nigra—were dissected on a cooled plate, following the method of Vayer et al. (54).

### *Analytical Methods*

GABA was determined by isocratic separation of the perchloric acid brain tissue extracts as an ion pair with *n*-octane sulfonate on a reversed-phase column, postcolumn derivative formation with *o*-phthalaldehyde-2-mercaptoethanol, and fluorimetry (40). Vigabatrin was determined as described elsewhere (47), with a method similar to that used for GABA. GABA-T activity was estimated spectrophotometrically by coupling of the transamination reaction with succinic semialdehyde dehydrogenase (43). GAD activity was assayed using (1-$^{14}$C)L-glutamate as substrate, by a $^{14}CO_2$ trapping method (58).

### *Dopamine $D_2$ Receptor Assay*

Samples of striata were homogenized in 10 ml ice-cold incubation buffer, containing 50 mM Tris-HCl, 120 mM NaCl, 5 mM KCl, 1 mM $CaCl_2$, and 1 mM $MgCl_2$ (pH 7.4), and centrifuged. The pellet was resuspended in incubation buffer at a final tissue concentration of 20 mg/ml. The incubation tubes contained 100 μl buffer, 50 μl apomorphin solution (10 μM final concentration), 100 μl $^{125}$I-sulpiride solution (0.005–1.3 nM final concentration), and 200 μl of the membrane fraction. After 1 h (at room temperature) each incubation was terminated by rapid filtration through Whatman GF/B glass fiber filters presoaked in 0.1% polyethylenimine. The filters were rinsed three times with 3 ml of 50 mM Tris-HCl buffer (pH 7.4) and counted in a γ-spectrometer. Specific binding of the radioligand was determined (after addition of apomorphin) as the difference between total and nonspecific binding. $K_D$ and $B_{max}$-values of sulpiride binding were evaluated by Scatchard analysis (31,37); protein content was determined according to Lowry et al. (25).

### *Calculations*

All data showed normal distribution. For their statistical evaluation, analysis of variance followed by individual *t*-tests with Bonferroni correction for α-slippage was used (Statview version 4.0 for MacIntosh PC; Abacus Concepts, Berkeley, CA). The binding data (see Table 2) were evaluated by Scheffé's multiple range test (57).

## RESULTS

### *Experiment 1: Prevention of Spontaneous Orofacial Movement by Chronic Administration of Vigabatrin*

*Food and drug consumption.* The daily food consumption per rat of the haloperidol and the haloperidol plus vigabatrin-treated groups (22.9 and 24.6 g, respectively) was somewhat lower than that of the control (25.6 g) or vigabatrin-treated group (26.5 g); their fluid intake varied considerably: control animals consumed 54 ± 3 ml/rat per day; vigabatrin-treated 48 ± 4 (89%) ml/rat per day; haloperidol-treated 37 ± 3 (69%) ml/rat per day; and haloperidol plus vigabatrin-treated 32 ± 3 (59%) ml/rat per day.

Nevertheless, the rats of the different treatment groups exhibited nearly identical growth curves. Only the rats receiving haloperidol remained somewhat behind the other groups (Fig. 1).

The daily drug consumption per animal was practically constant during the entire treatment period, because the concentrations of the drugs in the drinking fluid were adjusted for changes in fluid intake. However, based on the body weight, the daily dose of the different drugs decreased gradually with the increasing body weight, to 32–38% of the initial dose. Despite the decreasing dose of haloperidol, the total scores for the vacuous chewing movements remained remark-

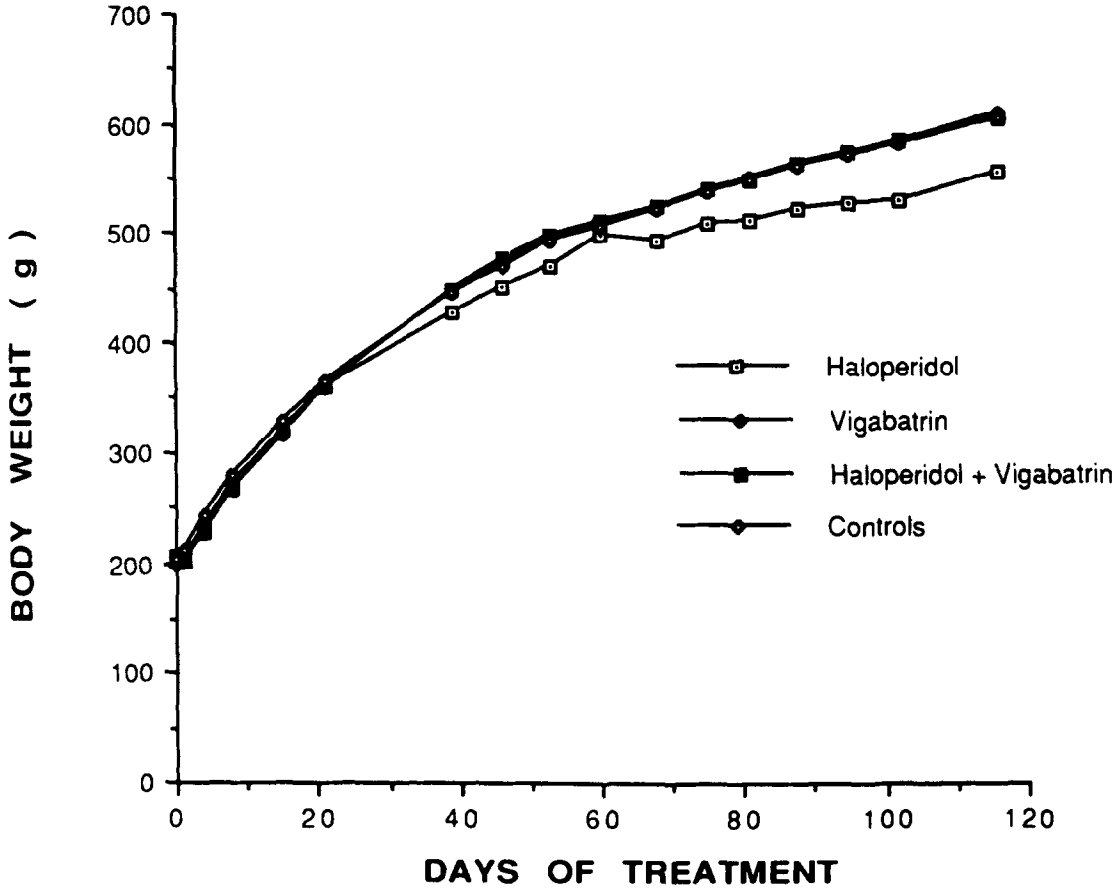


FIG. 1. Mean increase in body weight of rats of Experiment 1. For details of drug regimen, see Method.

ably constant during the observation period between days 60 and 116 (Table 1). The observation period was chosen because Kaneda et al. (17) found that under similar conditions, the syndrome fully developed after about 10 weeks of haloperidol administration, and high scores remained constant for more than 12 mo.

The scores observed for the same animal at different rating sessions varied considerably. However, in the haloperidol group, only one of 13 rats exhibited scores consistently < 10—that is, > 90% of the rats acquired orofacial dyskinesias as a result of haloperidol treatment. In the nontreated controls one rat showed in each rating session scores consistently >

TABLE 1

VACUOUS CHEWING MOVEMENTS (TOTAL SCORES) OF RATS OF EXPERIMENT 1

| Time of Treatment (days) | Treatment | | | |
|---|---|---|---|---|
| | None | Haloperidol | Vigabatrin | Haloperidol + Vigabatrin |
| 60 | 4 ± 3 | 15 ± 8 | 4 ± 3 | 8 ± 7 |
| 68 | 3 ± 3 | 14 ± 11 | 5 ± 4 | 5 ± 5 |
| 77 | 3 ± 3 | 14 ± 8 | 4 ± 4 | 6 ± 5 |
| 81 | 5 ± 5 | 17 ± 13 | 7 ± 5 | 11 ± 5 |
| 88 | 6 ± 5 | 20 ± 10 | 2 ± 2 | 11 ± 9 |
| 95 | 3 ± 3 | 16 ± 12 | 7 ± 6 | 11 ± 11 |
| 102 | 4 ± 4 | 21 ± 15 | 8 ± 6 | 9 ± 9 |
| 116 | 8 ± 8 | 18 ± 10 | 8 ± 6 | 12 ± 9 |

Mean total scores ± SD ($n$ = 12 or 13). The statistical analysis of the overall group differences gave the following results: haloperidol vs. control, $p < 0.0001$; haloperidol vs. haloperidol plus vigabatrin, $p < 0.0001$; vigabatrin vs. control, not significant; vigabatrin vs. haloperidol plus vigabatrin, not significant; haloperidol vs. vigabatrin, $p < 0.0001$.

TABLE 2

$D_2$-RECEPTOR DENSITY AND AFFINITY OF $^{125}$I-SULPIRIDE IN THE STRIATA OF THE RATS OF EXPERIMENT 1

| Treatment | $K_d$ nM | $B_{max}$ fmol/mg protein |
|---|---|---|
| Control | 0.92 ± 0.05 | 235 ± 8 |
| Haloperidol | 1.4 ± 0.1 | 337 ± 9* |
| Vigabatrin | 1.21 ± 0.09 | 193 ± 10 |
| Haloperidol plus vigabatrin | 1.12 ± 0.06 | 365 ± 13* |

Mean values ± SEM ($n$ = 6). *Significant difference ($p < 0.05$) between treated and controls.

10, indicating the spontaneous development of oral dyskinesias in this rat.

*Effect of vigabatrin on orofacial dyskinesias.* The consumption of daily 23 ± 3 mg vigabatrin (group B) had no effect on vacuous chewing movements. The mean scores observed in the vigabatrin-treated group were the same as those in the untreated controls. In this group one animal showed constantly high scores (>10) from week 10 of treatment onward. We assume that in this rat the development of oral dyskinesias was spontaneous, not drug-related.

Rats receiving haloperidol and vigabatrin (group C) exhibited significantly less vacuous chewing movements and bursts of jaw movements than those receiving only haloperidol (group A) ($p < 0.0001$). However, two of 13 animals showed scores consistently > 16. The difference between haloperidol and haloperidol plus vigabatrin-treated groups remained significant, when bursts of jaw tremor were neglected.

*Biochemical observations.* Using $^{125}$I-sulpiride as ligand, dopamine $D_2$ receptor binding in the striatum was determined. The group receiving haloperidol showed a significantly ($p < 0.05$) higher receptor density than controls or viagabatrin-treated rats, but the treatment did not affect binding affinity. Coadministration of vigabatrin did not influence the haloperidol effect on sulpiride binding (Table 2).

Measurement of GABA and vigabatrin concentrations, and of the activities of GAD and GABA-T in the cerebellum of the rats (Table 3)carried out to obtain an impression of the magnitude of the effects of vigabatrin on brain GABA metabolic parameters. GABA was elevated by 55%. Not unexpectedly (16), GAD activity was reduced, although by only 8%. Administration of haloperidol, alone or in combination with vigabatrin, had no significant effect on parameters of GABA metabolism in cerebellum, nor on the concentration of vigabatrin. More precisely, after 3 days of haloperidol deprivation, there was no significant difference between haloperidol-treated and nontreated groups, with respect to GABA, GAD, or GABA-T.

GAD activity was also determined in the S. nigra of a separate group of five rats, after 380 days' exposure to haloperidol. A 24% increase ($p > 0.005$) of GAD activity over control value was observed in these animals.

To compare the effects of the chronic dosing of vigabatrin with a single dose, groups of five rats (weighing 560 ± 30 g) received either saline or 38 $mg \cdot kg^{-1}$ vigabatrin, IP. This dose corresponds to that consumed in average by the rats at the end of the chronic treatment period. Twenty-four hours later, the brains were isolated. Under these conditions cerebellar GABA-T activity was significantly reduced. GAD activity and GABA concentrations were not significantly different from control values, and vigabatrin concentrations were below the detection limit ($< 2$ nmol $\times$ $g^{-1}$) of our method. These results indicate an accumulation of vigabatrin in the brains during chronic oral administration of the drug, a cumulative effect on GABA-T activity, with corresponding enhancement of GABA concentration as mandatory events. However, the elevation of brain GABA had no effect on motor behavior or body temperature.

### *Experiment 2: Influence of Interrupted Haloperidol Administration and of Single Doses of Vigabatrin on Orofacial Movements*

The growth and general behavior of the rats in Experiment 2 were not different from the similarly treated animals in Experiment 1. Total scores > 20 were observed in a group of 30 rats after about 90 days of exposure to a daily dose of 0.81 ± 0.05 mg haloperidol. At this time haloperidol administration was interrupted in 16 rats, whereas the remaining animals received haloperidol as usual. Vacuous chewing gradually decreased in the drug-free group, to reach control scores after 3 weeks. When the administration of haloperidol was recommenced, scores > 20 were observed again after 25–30 days (Fig. 2).

With the second group, haloperidol withdrawal was started after 205 days of exposure to the drug. The decrease in vacuous chewing behavior was as rapid as in the first group. After 52 days of abstinence, haloperidol was administered again and resulted in the similar enhancement of orofacial movements, as was observed for the first group (Fig. 2). However, in the group receiving haloperidol for 205 days, the animals were more excited after drug withdrawal than in the first group with a shorter exposure to haloperidol. It seems evident from these observations that the length of the period of haloperidol administration does not significantly affect the rates of loss and recovery of vacuous chewing behavior because of withdrawal and readministration of the drug.

TABLE 3

GABA AND VIGABATRIN CONCENTRATIONS, AND GAD AND GABA-T ACTIVITIES IN THE CEREBELLUM OF THE RATS OF EXPERIMENT 1, AFTER 120 DAYS OF EXPOSURE TO VIGABATRIN

| Treatment | GABA ($\mu mol \cdot g^{-1}$) | Vigabatrin ($\mu mol \cdot g^{-1}$) | GABA-T ($\mu mol \cdot g^{-1} \cdot h^{-1}$) | GAD ($\mu mol \cdot g^{-1} \cdot h^{-1}$) |
|---|---|---|---|---|
| None | 1.16 ± 0.09 | | 110 ± 13 | 10.4 ± 0.6 |
| Haloperidol | 1.22 ± 0.09 | | 119 ± 9 | 10.3 ± 0.8 |
| Vigabatrin | 1.8 ± 0.2* | 9 ± 4 | 44 ± 11 | 8.8 ± 0.8* |
| Vigabatrin plus haloperidol | 1.7 ± 0.2* | 9 ± 2 | 41 ± 5 | 8.4 ± 0.5* |

Mean values ± SD ($n$ = 12 or 13). *Statistically significant difference ($p \leq 0.05$) between treated and control rats.

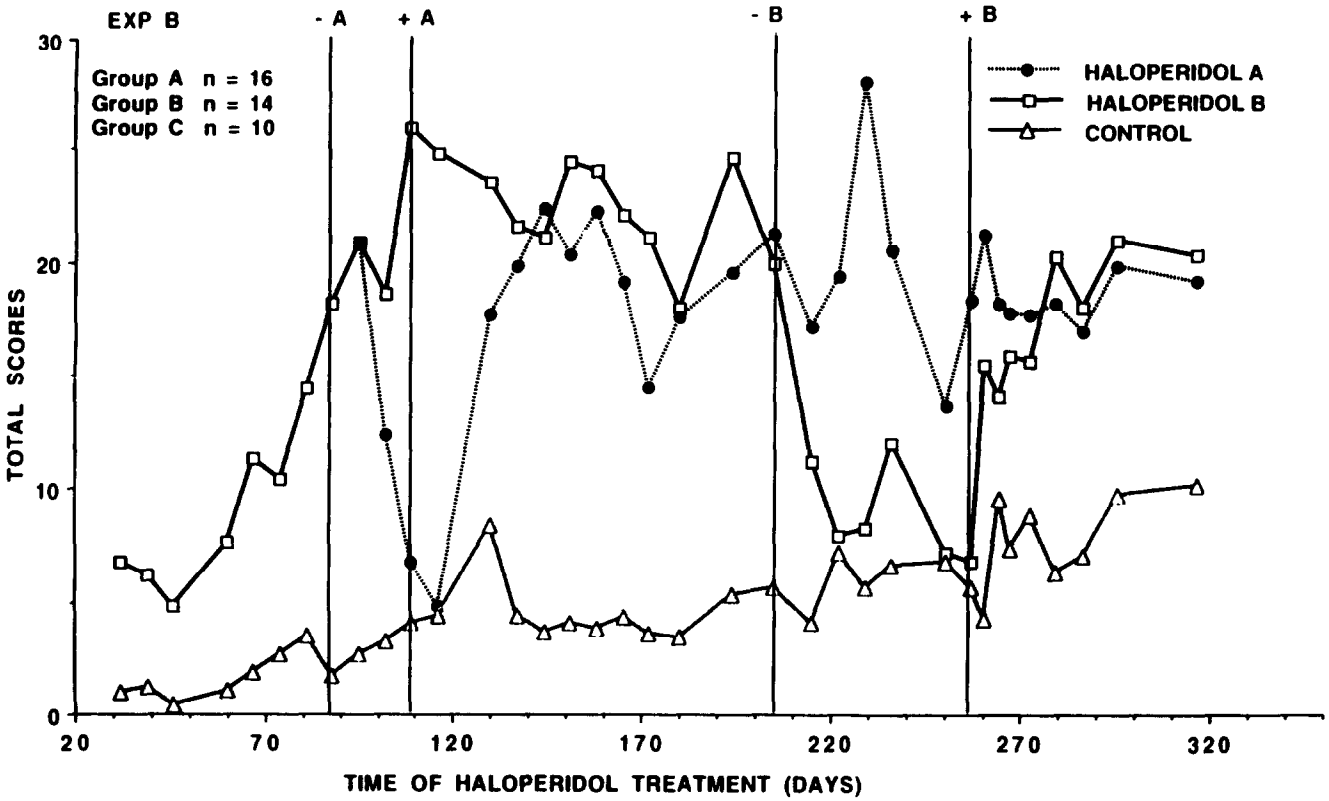


FIG. 2. Total scores (mean values) of oral dyskinetic movements of the rats of Experiment 2, between days 30 and 320 of treatment. Effects of removal and readministration of haloperidol. – A; – B: Haloperidol removed from the drinking water of group A and group B, respectively. +A and +B: Haloperidol (2.5 mg/100 ml) was added back to the drinking water of groups A and B. The difference in the activity of orofacial dyskinetic movements between controls and haloperidol-treated rats was statistically significant ($p < 0.001$) for group A from days 140–320 and for group B from days 80–190 and 270–320. The increase in orofacial movements of untreated controls between days 30 and 320 was statistically significant ($p < 0.01$).

The gradual increase of vacuous chewing movements in the control group (Fig. 2) indicates an age-dependent phenomenon.

To test the effect of single doses of vigabatrin on vacuous chewing behavior, rats that had received haloperidol for 98–133 days were injected IP with either saline or 50, 100, or 200 mg × $kg^{-1}$ vigabatrin. Orofacial movements were recorded at 30 min, 6 h, and 24 h after injection. As shown in Table 4, vacuous chewing was considerably reduced after the injections, including, however, that of saline. A significant drug-related reduction of spontaneous orofacial movements was evident only 6 h after the administration of 100 or 200 mg·$kg^{-1}$ vigabatrin. Intraperitoneal doses of 50 and 100 mg·$kg^{-1}$ vigabatrin did not affect body temperature or spontaneous motor behavior; however, 6 h after a dose of 200 mg·$kg^{-1}$ vigabatrin to haloperidol-treated rats, their body

TABLE 4

EFFECT OF A SINGLE IP DOSE OF VIGABATRIN ON VACUOUS CHEWING BEHAVIOR OF HALOPERIDOL-TREATED RATS

| Treatment | Scores Before Vigabatrin Administration | Scores After Vigabatrin Administration | | |
|---|---|---|---|---|
| | | 0.5 h | 6 h | 24 h |
| Physiologic saline | 24 ± 11 | 13 ± 7 | 13 ± 9 | 19 ± 9 |
| 50 mg · $kg^{-1}$ vigabatrin | 25 ± 14 | 15 ± 10 | 12 ± 8 | 13 ± 7 |
| 100 mg · $kg^{-1}$ vigabatrin | 26 ± 10 | 15 ± 7 | 4 ± 3* | |
| 200 mg · $kg^{-1}$ vigabatrin | 23 ± 15 | 18 ± 9 | 1 ± 1* | 14 ± 10 |

Mean total scores ± SD ($n = 14$). Groups of 14 rats were pretreated for 98–133 days with haloperidol (2.5 mg/100 ml drinking fluid). In intervals of about 8 days, they received either physiologic saline or vigabatrin (IP) (0.1 ml/100 g body wt.). At 0.5, 6, and 24 h after vigabatrin, vacuous chewing movements were assessed. In addition, the body temperature was determined at 6 and 24h. *Statistically significant difference ($p < 0.01$) between treated and control animals.

temperatures had decreased by 1.5°C and were again normal at 24 h.

In control rats 50 $mg \cdot kg^{-1}$ vigabatrin had no significant effect on whole brain GABA concentration, but 100 and 200 $mg \cdot kg^{-1}$ of the drug enhanced GABA by 23 and 55%, respectively, and caused a decrease of GABA-T activity by 39 and 52%, respectively (Table 5). The vacuous chewing scores observed at 24 h after vigabatrin are presumably drug related, because the elevation of brain GABA by vigabatrin is long-lasting (16).

*Experiment 3: Effect of Withdrawal of Vigabatrin on Spontaneous Orofacial Movements after Long-Term Treatment With Haloperidol.*

The thirteen rats of this group seemed to be somewhat less sensitive to vigabatrin than those of Experiment 1, presumably indicating slight genomic differences. Therefore, the daily dose of vigabatrin was enhanced to 40 ± 5 mg/rat. This dose of vigabatrin, together with a daily dose of 0.67 ± 0.09 mg haloperidol, affected the animals' growth only slightly. On day 205 of treatment, the average body weight of the group receiving the drug combination was lower by 9%, compared with untreated controls. The haloperidol-treated group had a body weight deficit of 4%. As was the case in Experiment 1, the differences in vacuous chewing movements between the groups receiving haloperidol and haloperidol plus vigabatrin were statistically significant ($p < 0.001$) (Fig. 3). After the termination of vigabatrin administration, spontaneous orofacial movements increased rapidly (Fig. 3).

## DISCUSSION

Chronic administration of neuroleptics to rats is known to cause the development of spontaneous orofacial movements (5,7,11,15,36,50). Although there is some disagreement about the validity (56), vacuous chewing movements of rats have now been widely accepted as a model of tardive dyskinesia, because they have a similar etiology, phenomenology, and pharmacology, even though wide-ranging effects in terms of magnitude, types, and emergence of movements have been observed by different authors.

We were mainly interested in a direct comparison of the effects of vigabatrin with those of progabide, a potential GABA agonist. Therefore, we used the same treatment with haloperidol as was used by Kaneda et al. (17). No doubt, the oral administration of drugs is limited in terms of the precision of daily drug intake, but it is less stressful than daily injections, especially if these have to be carried out over a very long period. The reproducibility of our observations retrospectively justified our experimental approach, especially when it turned out that even the injection of small volumes of saline had significant effects on the dyskinetic behavior of rats.

TABLE 5

GABA CONCENTRATION AND GABA-T ACTIVITY IN THE BRAINS OF RATS 6 h AFTER A SINGLE IP DOSE OF VIGABATRIN

| Dose of Vigabatrin ($mg \cdot kg^{-1}$) | GABA Concentration ($\mu mol \cdot g^{-1}$) | GABA-T Activity ($\mu mol \cdot g^{-1} \cdot h^{-1}$) |
|---|---|---|
| 0 | 2.2 ± 0.1 | 96 ± 3 |
| 50 | 2.3 ± 0.2 | 70 ± 4* |
| 100 | 2.7 ± 0.2* | 59 ± 6* |
| 200 | 3.4 ± 0.3* | 46 ± 1* |

Mean values ± SD ($n = 4$). *Statistically significant ($p \leq 0.01$) difference between treated and control groups. The haloperidol-treated animals were exposed to this drug for 98–113 days (daily oral dose 0.81 ± 0.05 mg/rat).

In studies measuring $D_1$ and $D_2$ receptors in postmortem brains (18,33,55) or in vivo by positron emission tomography (2,24), no support was obtained in favor of the dopamine receptor supersensitivity hypothesis (36,51,55). Therefore, a deficit in GABA-mediated neurotransmission has attracted attention as a pathophysiologic basis in tardive dyskinesia. In addition to the mentioned clinical observations (3,4,19,21, 48,49,52,53), studies with animal models supported the GABA hypothesis. Gunne and Häggström (10) reported the reduction of nigral GAD activity in rats after long-term haloperidol administration, and a negative correlation between GAD activity and orofacial movements. The reduction of GABA levels and a decrease in GAD activity in the subthalamic nucleus, the internal segment of the globus pallidus, and the pars reticulata of the S. nigra were also found in Cebus monkeys after long-term neuroleptic treatment (12). Blockade of nigral GABA receptors by bicuculline, or depletion of GABA by isoniazid infusion into the S. nigra, induced vacuous chewing, whereas infusion of muscimol into the target field of nigro-tegmental projections abolished isoniazid-induced orofacial dyskinesias (13). A single dose of 200 $mg \cdot kg^{-1}$ vigabatrin to Cebus monkeys with neuroleptic-induced persistent dyskinesias reduced involuntary movements to a lesser extent than 4 $mg \cdot kg^{-1}$ THIP [a powerful GABA agonist (20)], but also with fewer side effects (1). These observations, the enhanced GABA binding in S. nigra (8), and the sustained stimulation of GABA receptors (22) after chronic administration of neuroleptics, as well as the prevention of haloperidol-induced oral dyskinesias by coadministration of progabide (17), were considered to be further arguments in favor of a deficient GABAergic neurotransmission. However, See et al. (46) did not observe a relationship between vacuous chewing scores and nigral GABA receptor binding, and Mithani et al. (30) were not able to confirm the previously reported correlation between decreased GAD activity and oral dyskinesias. Our own determinations are also in opposition to the notion that nigral GAD activity is downregulated as a result of haloperidol administration. It appears that after long-term haloperidol administration, the release of striatal dopamine is not changed (44), indicating that the nigro-striatal pathway was not affected by haloperidol under the specific experimental conditions. This implies that GAD activity should not be decreased. The elevation of GAD, as was observed in this work, could indicate a compensatory reaction in a situation of an impaired nigro-striatal dopaminergic system. It should also be kept in mind that GAD activity, as measured ex vivo, may not reflect the in vivo activity of the enzyme. The determination of the various forms of GAD (28) may contribute to the solution of the contradictory results; however, an explanation for the obvious incongruence of the various observations concerning nigral GAD is not available at present. One should remember in this context that even relatively minor differences in doses and dosing schedules may cause differences in the time course and type of orofacial movements (45). These may be observable as differences in biochemical parameters. Presumably, a more important source for the differences in results obtained by different laboratories is variations between rat strains. In our experiments the Sprague-Dawley rats obtained from two sources showed clear differences with regard to sensitivity to haloperidol (50).

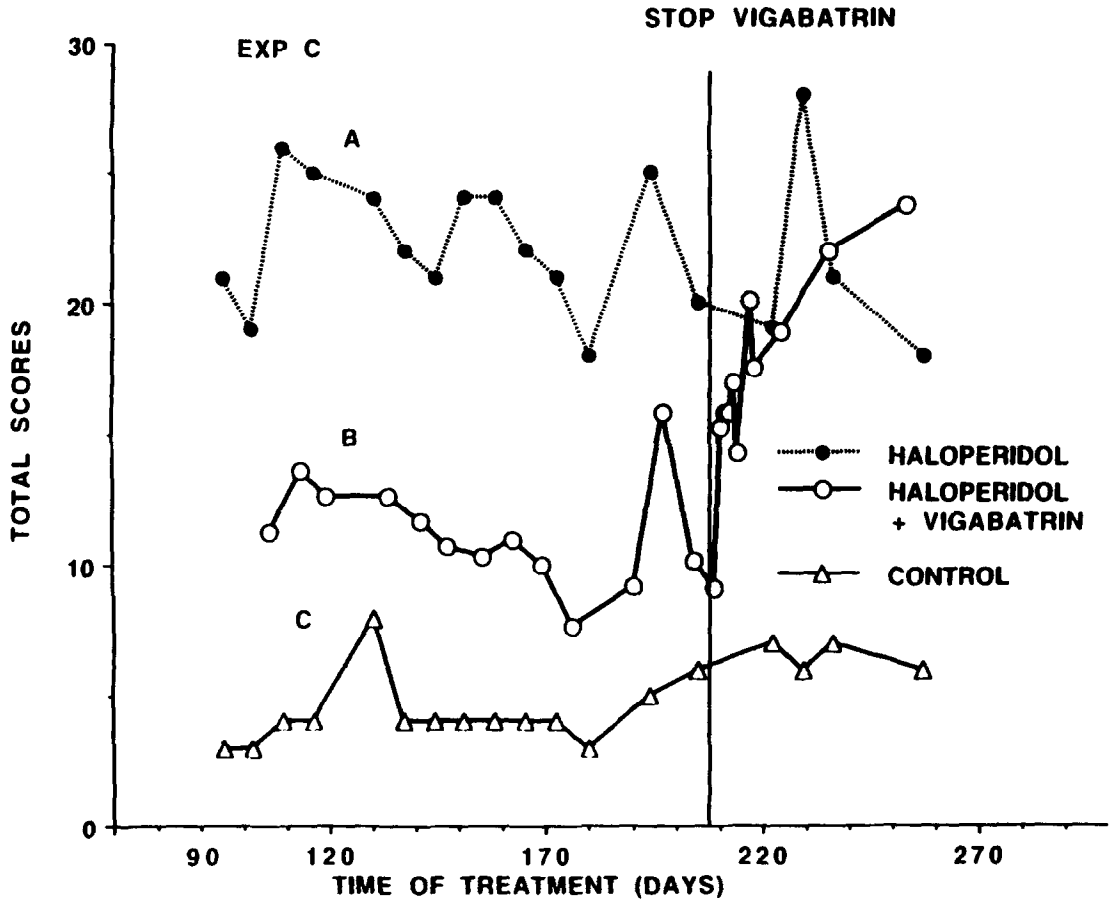


FIG. 3. Effect of the removal of vigabatrin from drinking fluid after the chronic treatment of rats with haloperidol plus vigabatrin (Experiment 3). For details of the drug regimen, see Method. The administration of vigabatrin decreased haloperidol-induced orofacial movements with high significance ($p < 0.0001$). After the removal of vigabatrin from the drinking fluid, dyskinesia scores were elevated to haloperidol-induced level within 10–20 days.

Vigabatrin is a selective inactivator of GABA-T (16,29) that exerts its anticonvulsant effects as a result of the elevation of brain GABA concentrations (42). Therefore, it seems to be the most appropriate compound available for testing the hypothesis of a functional GABA deficit in haloperidol-induced orofacial dyskinesias. It is known that high intracortical doses of vigabatrin produce dyskinesias in rats because of a weak, reversible GABA antagonistic effect (32). As a result of this property of vigabatrin, it is evident that direct effects of this drug can be excluded from being responsible for the observed amelioration of orofacial movements. The low concentration of vigabatrin detected in the brain after long-term administration (Table 3) is additional evidence in favor of the assumption that the behavioral changes observed in this work are a result of elevated GABA concentrations, and not of the accumulation of the drug. That the acute dose of vigabatrin required to antagonize haloperidol-induced orofacial movements is somewhat higher than after chronic treatment with the drug may be explained by the fact that brain GABA concentration was higher after chronic treatment than after a single dose of vigabatrin, a dose that corresponded to the average daily intake of the drug in the chronic experiment. Moreover, vacuous chewing behavior seems sensitive to stress, and presumably also to other factors, as appears from our observation that injection of saline decreased the number of orofacial movements significantly, even 6 h after injection, and from the variation of the scores observed in individual animals during different assessment sessions.

Six hours after acute vigabatrin administration, when whole brain GABA concentrations approximate plateau levels (16), the prevention of vacuous chewing movements seemed dose-dependent. However, 24 h after drug administration, orofacial movements were apparently independent of the dose of vigabatrin (Table 4). We have no clear explanation for this observation; however, the following findings may offer a basis for an explanation: After subchronic oral treatment of gerbils with vigabatrin, the highest GABA concentrations were found in the S. nigra and the hypothalamus 18 h after the last dose, but in 11 brain regions no differences in GABA concentration were found between animals receiving daily 50 or 100 $mg \cdot kg^{-1}$ of the drug (23). The reason for this is most probably because of the GABA concentration-dependent downregulation of GAD. This can be observed after both single and multiple doses of vigabatrin and other GABA-T inhibitors (40). It is also known that the anticonvulsant effect of elevated brain GABA is not simply concentration-dependent, but depends on the availability of functionally active GABA in presynaptic terminals, which changes with time (14,39). Thus, it

is likely that the GABA pools responsible for the antagonism of oral dyskinesias are not significantly different 24 h after the administration of vigabatrin at doses between 50 and 200 $mg \cdot kg^{-1}$.

In agreement with other authors (26,27), we found an increase of $D_2$ receptors in striatum, but the coadministration of haloperidol and vigabatrin had no effect on the development of dopamine $D_2$ receptor density. Although the functional interaction of GABAergic and dopaminergic neurons is undoubted, as has been again shown recently in humans in a single photon emission tomography study (34), it is not surprising that treatment with vigabatrin was unable to prevent the development of haloperidol-induced orofacial movements. Evidence has recently been presented for the formation of neurotoxic products from haloperidol, which may mimic the effect of 1-methyl-4-phenylpiperidinium ($MPP^+$) (35). $MPP^+$ is supposed to accumulate via the dopamine transporter in the terminals of nigro-striatal neurons, where it inhibits the intramitochondrial electron transport chain (6), and thus produces tardive dyskinesia. There is no mechanistic basis for the prevention of the development of $MPP^+$-like neurotoxicity by elevated GABA levels, but there is, of course, a basis for the suppression of dyskinetic movements by enhanced GABAergic function.

The chronic dose of vigabatrin that produced a significant reduction of orofacial movements in rats was lower than was needed to suppress seizures in suitable animal models (39,40, 42). More important, no attenuation of the efficacy of the treatment was observed over the treatment period of > 3 mo. Therefore, it may be warranted to resume clinical studies of tardive dyskinesia with small doses of vigabatrin, or with more advanced inhibitors of GABA-T.

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