Pharmacology Biochemistry & Behavior, Vol. 17, pp. 99–106, 1982. Printed in the U.S.A.

Effects of γ-Vinyl GABA on Food Intake of Rats

SYLVIE HUOT AND MICHAEL G. PALFREYMAN

Centre de Recherche Merrell International, 16, rue d'Ankara, 67084 Strasbourg Cedex, France

Received 27 January 1982

HUOT, S. AND M. G. PALFREYMAN. *Effects of γ-vinyl GABA on food intake of rats.* PHARMAC. BIOCHEM. BEHAV. 17(1) 99–106, 1982.—γ-Vinyl GABA, an inhibitor of GABA-transaminase, produced a dose-related reduction of food intake in rats, after both single, (125–1000 mg/kg IP or 500 mg/kg PO) and repeated (250 mg/kg/day IP) administration. No tolerance was observed to the effect of repeated injections. Catecholamine and indoleamine systems in the CNS do not appear to be implicated in this anorexic effect. Combination of γ-vinyl GABA with amphetamine (2.5 mg/kg) enhanced the anorexic effects of the latter compound whilst attenuating its stimulant effects. The data suggest an important role for GABA in the control of food intake.

Feeding behaviour Locomotor activity GABA 6-Hydroxydopamine Metergoline Amphetamine Fenfluramine

ALTHOUGH the importance of noradrenaline (NA), dopamine (DA) and serotonin (5HT) in the central control of food intake has been investigated extensively in the last two decades [6, 11, 21], it is only recently that a role for GABA in this regulation has been suggested. This assumption was based on the studies of Kimura and Kuriyama [20] and Cattabeni *et al.* [2] who reported that in rats, the GABA content of the hypothalamus, a part of the brain known to play a major role in the control of food intake, varied with the nutritional state of the animal. Since then, several workers have investigated the effects of manipulation of the GABA systems on food intake in different parts of the rat hypothalamus. Grandison and Guidotti [10] reported that the GABA-agonist muscimol stimulated food intake when injected into the ventromedial hypothalamus (VMH), a structure thought to function as a "satiety center" as its stimulation causes a suppression in food intake, and that this effect was blocked by bicuculline. Kelly and collaborators [17, 18, 19] confirmed these results by showing that GABA itself increased food intake when injected into the rat VMH and that the GABA-receptor antagonist bicuculline increased food intake when injected into the lateral hypothalamus (LH), a structure thought to contain the "hunger center" as its stimulation causes an increase in food intake. These experiments and others [5, 24, 25, 29] are consistent with the hypothesis that GABA has an inhibitory function in both feeding centers. However, the overall effects of changes of whole brain GABA on food intake are more controversial. Several authors have reported an inhibition of food intake and growth following a GABA-supplemented diet [30], after administration of GABA intracerebroventricularly (ICV) [27], following IP administration of muscimol [14], or after IP or ICV administration of GABA-transaminase inhibitors, [28]: Ethanolamine O-sulphate (EOS) [4, 14, 27], aminooxyacetic acid (AOAA) [14] or γ-vinyl GABA (GVG) [8]. On the other hand, two recent reports [26,27] have shown that ICV muscimol induced an increase in rat food intake.

In this report we have investigated in more detail the effects of elevating brain GABA concentrations on food intake and growth of rats by using γ-vinyl GABA, a specific enzyme-activated irreversible inhibitor of GABA-transaminase [16]. Furthermore, the catecholaminergic and serotoninergic neurotransmitter systems known to be involved in the anorexic effects of all currently used appetite-suppressants were examined for their relationship to anorexia produced by GVG.

METHOD

Male Sprague-Dawley rats (200–400 g) from Charles River, France were used throughout. The rats were housed individually in a temperature- and humidity-controlled room with free access to water. Unless otherwise stated, a 12 hr night-day cycle (light: 600–1800 hr) was maintained. Food consisted of standard rat pellets (aliment CRF) and was presented in the normal food reservoir in the cover of individual cages (except Experiment 7 where the same type of pellets was placed on the floor of the cages). Food intake was assessed by the difference in weight of food pellets.

Drug Injections

GVG, dexamphetamine sulphate and fenfluramine were dissolved in saline. 6-Hydroxydopamine (6 OH-DA) and metergoline were dissolved in ascorbic acid solution (0.1% and 1%, respectively). The injection volume was 1 ml/kg except for the doses of GVG greater than 500 mg/kg where the volume was 5 ml/kg.

Experiment 1. Effects of a single injection of GVG on free-feeding rats. After 3–4 days of habituation for baseline

food intake measurements, rats were injected IP at 1500 hr with saline or various doses of GVG and whole night food intake measured.

Experiment 2. Effects of a single injection of GVG on food-deprived rats. Rats were deprived of food for 24 hr and GVG was injected IP at 1000 hr at doses of 125 and 250 mg/kg. Four hr later food was presented and the food intake was measured over the next 2 hr.

Experiment 3. Effects of sub-acute GVG on food intake and locomotor activity on free-feeding rats. Animals were housed singly in cages placed on an Animex activity-meter. Food was available between 1700 hr and 900 hr. Rats were weighed every morning; activity was recorded for the afternoon and whole night periods, and counts printed out every two hours. After 3–4 days of habituation for measurement of baseline food intake and locomotor activity, the drug treatment was started. GVG was given IP at the dose of 250 mg/kg at 1400 hr daily for 13 consecutive days to 5 rats. The other 5 animals received a daily IP injection of saline.

Experiment 4. Effects of ICV 6 OH-DA pretreatment on amphetamine- and GVG-induced anorexia. Twelve rats received a bilateral injection of 6 OH-DA (150 μg/5 μl/side) into the lateral ventricles (coordinates from Bregma: P +1 mm, L ±2.5 mm, D 3.5 mm), under pentobarbital anaesthesia (40 mg/kg). Five of them were pretreated with desmethylimipramine (DMI) IP at 25 mg/kg 45 min before 6 OH-DA. The control rats received an equivalent volume of ascorbic acid solution into the lateral ventricles. All animals received 50 mg/kg pargyline IP 30 min before ICV injection.

Following surgery, the animals were trained to eat all their daily food intake between 1400 hr and 1700 hr. When this was achieved (3 weeks later), drug treatment was initiated. Animals were subjected to a three-way crossover design at 5 to 7 day intervals, such that each animal was treated once with saline, once with dexamphetamine (1.5 mg/kg) and once with GVG (250 mg/kg) in a randomized order. GVG was injected IP 4 hr before, saline and dexamphetamine 15 min before food presentation and the subsequent 1 hr food intake was measured.

One week after the last test, a second series of experiments were started on the same animals. Two experiments were carried out separated by a one week interval, so that each rat received once saline and once GVG 500 mg/kg, in a random order. GVG and saline were injected IP 4 hr before presentation of food.

At the end of the experiment (1½ months after 6 OH-DA), all rats were decapitated and their brains removed for noradrenaline and dopamine determinations according to the method of Wagner *et al.* [31].

Experiment 5. Effects of metergoline pretreatment on GVG- and fenfluramine-induced anorexia. Twenty rats were food-deprived for 24 hr and divided into 5 equal groups which received one of the following IP treatments: (1) Saline; (2) Fenfluramine; (3) Metergoline + fenfluramine; (4) GVG; (5) GVG + metergoline. GVG was injected at a dose of 250 mg/kg, metergoline at 1 mg/kg and fenfluramine at 4 mg/kg. The times of injection of GVG, metergoline and fenfluramine were 3 hr, 2 hr and 30 min, respectively, before the animals had access to food. Food was presented at 1700 hr and food intake was measured during the following 3 hr.

Experiment 6. Sub-acute treatment with GVG (250 mg/kg) alone or in combination with dexamphetamine (2.5 mg/kg). Twenty rats were trained to eat their daily food intake between 1400 and 1700 hr, with a light cycle modified to give a dark period from 1400 hr to 200 hr. After 2 weeks of training,

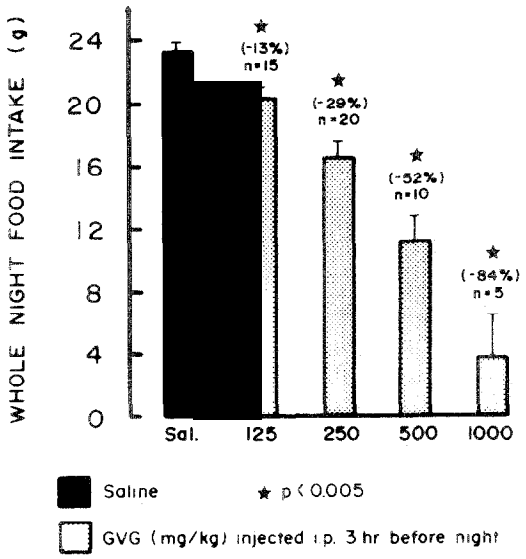

FIG. 1. Effect of γ-vinyl GABA (GVG) on whole night food intake of rats. Saline, (black column, n=50) and GVG, (dotted columns) were injected IP 3 hr before the nighttime period (1800–600 hr). Doses of GVG are indicated under the columns in mg/kg. Results are expressed as means ±S.E.M.; n is the number of animals per group. *$p<0.005$.

the animals were divided into 4 groups and treated for 8 consecutive days with one of the following schedules: the control group received two daily injections of saline (1000 hr and 1400 hr), a second group received GVG IP (250 mg/kg/day) at 1000 hr, a third group received dexamphetamine IP (2.5 mg/kg/day) at 1345 hr and the last group received GVG and dexamphetamine at the doses and times indicated above. The locomotor activity of half the animals was recorded daily such that each animal was on the activity-meter every other day. Stereotypies were assessed by an observer unaware of the treatment given to the animals according to the following rating scale: 0=normal; 1= increased locomotion + intermittent sniffing; 2=continuous sniffing + intermittent gnawing; 3=continuous gnawing and licking, no locomotion. The animals were observed for 1 min every 15 min.

Experiment 7. Influence of the mode of presentation of food and of the route of administration of GVG on GVG-induced anorexia. Twelve male Sprague-Dawley rats of about 300 g were divided into two groups of six, one which had food available in the cover of the cage, and the other one which had food available on the floor of the cage. Normal food intake was recorded for one night and on the next day at 1400 hr all the animals were dosed with GVG 500 mg/kg; 3 in each group were dosed IP, the other 3 were dosed orally. The food intake for the subsequent night was calculated.

Statistics

Data of experiments 1 and 2 were analysed with Student's *t*-test, and in experiments 3, 4, 5 and 6 a two-way analysis of variance with repeated measures on one factor was used.

Drugs

GVG was synthesized in our Center. Fenfluramine was a

TABLE 1

EFFECT OF γ-VINYL GABA (GVG) IP ON FOOD INTAKE IN FOOD-DEPRIVED RATS

Dose of GVG (mg/kg)	Food intake (g/rat/2 hr) Saline	GVG	% Inhibition
125	5.6 ± 0.6	4.05 ± 0.5	28
250	6.6 ± 0.5	2.3 ± 0.4	65*

Each value represents the mean ± S.E.M. of 17 animals.
*$p<0.001$.

TABLE 2

EFFECT OF ICV 6-HYDROXYDOPAMINE (6 OH-DA) AND IP DESMETHYLIMIPRAMINE (DMI) PRETREATMENT ON WHOLE BRAIN CATECHOLAMINE LEVELS

Pretreatment			NA	DA
Vehicle		(6)	424 ± 19	824 ± 28
6 OH-DA +	Saline	(6)	60 ± 7†	155 ± 39†
	DMI	(5)	347 ± 19*	169 ± 51†

See text for details of doses/pretreatment schedules.
Results are expressed in ng/g wet tissue, (mean ± S.E.M.). Number of animals in each group is indicated in parentheses.
*$p<0.025$, compared to vehicle treated controls.
†$p<0.005$, compared to vehicle treated controls.

gift from Servier laboratories. Metergoline was a gift from Pharmitalia. Dexamphetamine sulphate was obtained commercially. Pargyline was purchased from Sigma.

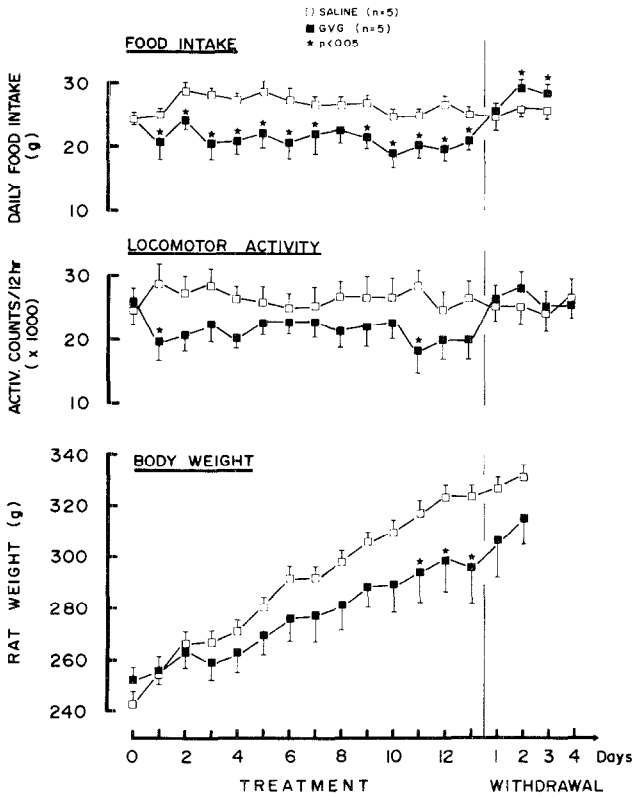

FIG. 2. Effect of repeated doses of γ-vinyl GABA (GVG) on rat food intake, locomotor activity and body weight. GVG was injected at 1400 hr at a dose of 250 mg/kg/day. Nighttime locomotor activity was recorded between 1800 hr and 600 hr. There was no tolerance to the effect on food intake, as indicated by the lack of significant interaction between the duration of treatment and the anorexic effect of GVG, $F=0.637$; $p<0.8$. Interaction between the duration of treatment and the sedative effect of GVG almost reached significance, $F=1.80$; $p<0.06$.

RESULTS

Experiment 1. Effects of a Single dose of GVG on Free-Feeding Rats: Dose-Response Curve

GVG produced a dose-related decrease in whole night food intake (Fig. 1). This decrease was significant at 125 mg/kg and reached 85% at 1000 mg/kg. Parallel decreases in water intake and locomotor activity were also observed, the effects being significant at 250 mg/kg (data not shown).

Experiment 2. Effects of Acute Dosing with GVG on Food-Deprived Rats

GVG decreased food intake of hungry rats at both doses tested and the effect reached significance at 250 mg/kg ($p<0.001$) (Table 1).

Experiment 3. Effects of Sub-Acute Dosing with GVG, 250 mg/kg/Day for 13 Days

As shown in the upper part of Fig. 2, repeated treatment gave a constant and significant decrease in whole night food intake of rats. This effect did not show any tolerance during the 13 days of treatment. After withdrawal, there was a slight but significant rebound. This anorexic effect was associated with a small but constant decrease in the activity counts recorded during the whole night, (middle part of the figure). This decrease represented a depression of the activity during the first 4 or 5 hours of the night (about 50% reduction). For the rest of the night, the activity was similar to that of control rats (data not illustrated). This effect on locomotor activity did not show any tolerance and was statistically significant on days 1 and 11 of the treatment. There was no statistically significant rebound after withdrawal. Repeated treatment with GVG, 250 mg/kg, reduced the rate of growth of rats as compared to controls, (lower part of Fig. 2). This effect reached significance after 11 days of treatment.

Experiment 4. Effects of 6 OH-DA Pretreatment on GVG- and Dexamphetamine-induced Anorexia

In order to investigate if DA or NA were involved in the anorexic effect of GVG, as has been shown for dexamphetamine [3], selective and nonselective lesions of catecholamine pathways were produced by ICV 6 OH-DA, with and without DMI pretreatment respectively. As can be seen from Table 2, 6 OH-DA alone depleted NA and DA concentrations significantly in rat brains, (86% for NA, 81% for

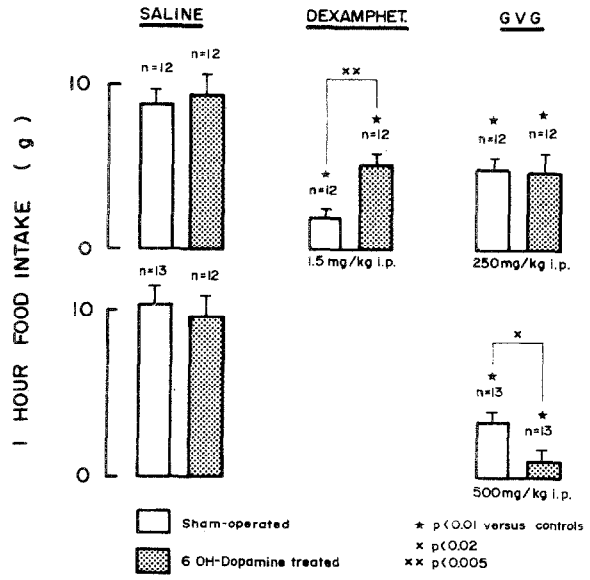

FIG. 3. Effect of 6-Hydroxydopamine (6 OH-DA) pretreatment on anorexia induced by dexamphetamine (DEXAMPHET.) and γ-vinyl GABA (GVG). Since DMI plus 6 OH-DA did not vary from 6 OH-DA alone in its effect on the anorexic action of either dexamphetamine or GVG, the data have been combined. Dexamphetamine was injected 15 min and GVG 4 hr before food presentation. Results are expressed as the mean±S.E.M.; n is the number of animals per group.

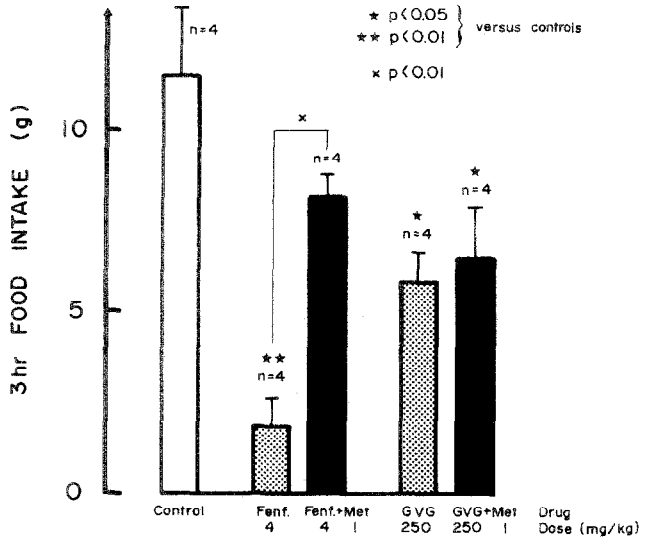

FIG. 4. Effect of metergoline (Met) pretreatment on anorexia induced by fenfluramine (Fenf.) and γ-vinyl GABA (GVG). GVG was given 3 hr, metergoline 2 hr and fenfluramine 30 min before food presentation. All drugs were injected IP. Results are expressed as means±S.E.M. n is the number of animals per group.

TABLE 3

EFFECT OF ICV 6-HYDROXYDOPAMINE (6 OH-DA) AND IP DESMETHYLIMIPRAMINE (DMI) PRETREATMENT ON THE ANOREXIC EFFECTS OF DEXAMPHETAMINE AND γ-VINYL GABA (GVG)

			Food Intake (g/rat/1 hr)		
Pretreatment			Saline	Dexamphetamine 1.5 mg/kg IP	GVG 250 mg/kg IP
Vehicle (13)			8.8 ± 0.5	2.0 ± 0.4*	5.0 ± 0.6*
6-OH-DA +	Saline	(6)	9.9 ± 0.9	5.3 ± 1.0*	5.2 ± 1.0*
	DMI	(5)	8.6 ± 1.4	5.2 ± 0.6*	4.2 ± 1.0*

See text for details of doses/pretreatment schedules.

Each value represents the mean ± S.E.M. Number of animals in each group is indicated in parentheses.

*$p<0.005$, compared with rats given saline (column 1).

DA). In the same rats, the marked anorexic effect of 1.5 mg/kg of dexamphetamine was significantly attenuated, whilst the anorexic effect of 250 mg/kg GVG was unaffected. Surprisingly, the anorexia produced by a higher dose of GVG (500 mg/kg) was potentiated by the 6 OH-DA lesion (Fig. 3).

The other group of rats pretreated with DMI showed, as expected, a significant decrease in DA (79%), but only minimal depletion of NA (18%), (Table 2). In these rats, a reduction of the anorexic effect of dexamphetamine was still observed; that due to GVG was unchanged (Table 3).

Experiment 5. Effect of Metergoline on Fenfluramine- and GVG-Induced Anorexia

The anorexia produced by fenfluramine was significantly antagonized by 1 mg/kg of metergoline (Fig. 4). However, metergoline was without effect on GVG-induced anorexia.

Experiment 6. Sub-Acute Treatment with GVG (250 mg/kg/Day) Alone or Associated with Dexamphetamine (2.5 mg/kg/Day)

As already demonstrated in Experiment 2, repeated

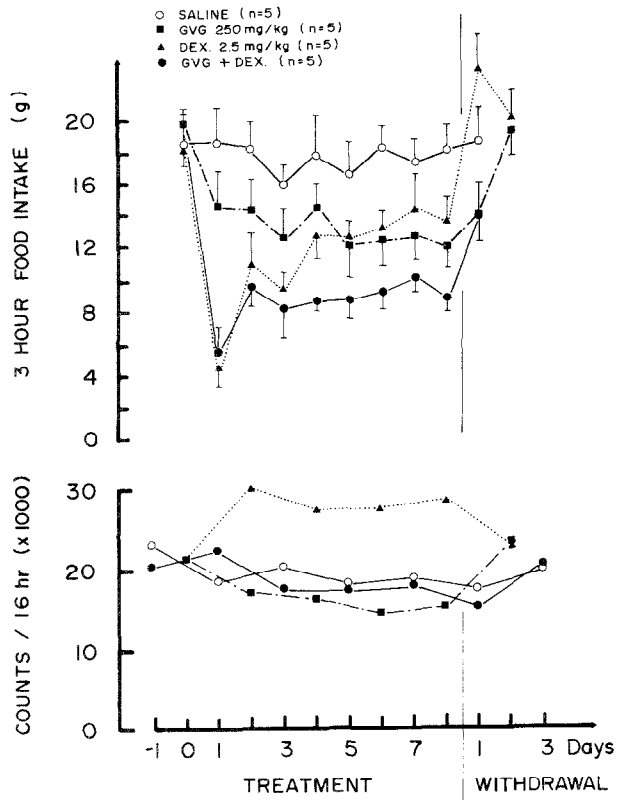

FIG. 5. Repeated treatment with γ-vinyl GABA (GVG) alone or in combination with dexamphetamine (DEX); effects on food intake and locomotor activity. GVG was injected 4 hr and dexamphetamine 15 min before food presentation. The 2 drugs were injected IP. Locomotor activity was recorded from 1400 hr to 600 hr. Results are expressed as means±S.E.M. for food intake; n is the number of animals per group. For clarity, S.E.M.s (<16% of the mean) have been omitted on the lower part of the figure. The interaction between duration of treatment and anorexic effect is significant only in the animals treated with dexamphetamine alone, $F=9.25$, $p<0.001$, which indicates a marked tolerance. In the other three groups, there is no significant tolerance: (Saline: $F=0.77$, $p<0.61$; GVG alone: $F=1.073$, $p<0.38$; GVG + DEX: $F=1.71$, $p<0.11$).

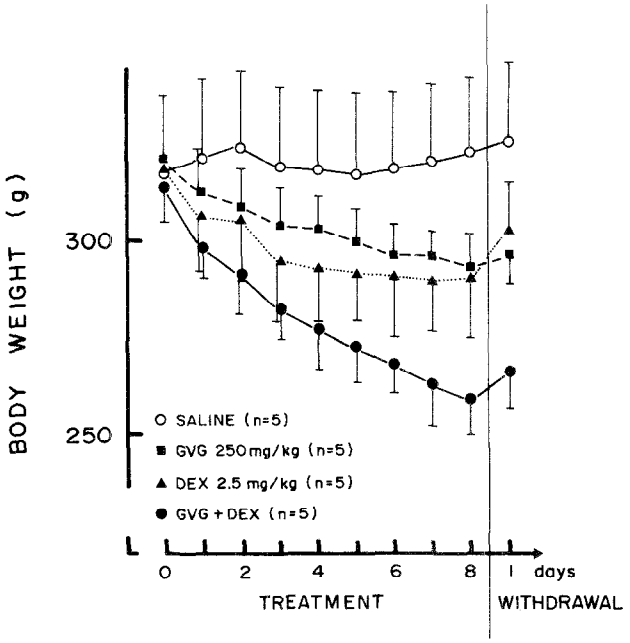

FIG. 6. Repeated treatment with γ-vinyl GABA (GVG) alone or in combination with dexamphetamine (DEX); effects on body weight. For details of protocol, see Fig. 5.

treatment with GVG 250 mg/kg/day, produced a constant reduction in food intake which did not show any tolerance during the 8 day period of the experiment. In contrast, the pronounced anorexic effect which was observed after the first dose of dexamphetamine waned rapidly with repeated injections (Fig. 5). The combination of the two treatments produced profound anorexia, which showed less rapid tolerance than treatment with dexamphetamine alone, and which induced a marked fall in the body weight of rats (Fig. 6). As shown on the lower part of Fig. 5, the locomotor activity of rats treated with GVG alone was slightly reduced compared to controls whereas rats treated with dexamphetamine showed a constant and marked increase in activity counts, which corresponded to a combination of increased locomotor activity and mild stereotypy. Interestingly, the activity counts of animals treated with the combination of the two drugs were quite similar to controls. This antagonism of the amphetamine-induced hyperactivity of rats by GVG could be due to a reduction of locomotor activity or of stereotypies. In order to elucidate this point, we scored the stereotypies of rats treated with dexamphetamine alone or with dexamphetamine + GVG (5 per group) and observed a slight reduction in peak intensity of stereotypies and a clear reduction in the duration of stereotypies in GVG + dexamphetamine-treated rats (total score for 2 hr: Saline=0.5; Dexamphetamine alone=50; Dexamphetamine + GVG=36.5).

Experiment 7. Influence of the Mode of Presentation of Food and of the Route of Administration of GVG on GVG-Induced Anorexia

As shown in Table 4, the rats that had food available on the floor ate slightly more than the other group during the control night (27.4 g as compared to 25.1 g). However, after GVG treatment, the degree of inhibition was quite similar (49 and 48%). On the other hand, there was no difference between the food intake of the animals that had been treated orally and those that had been treated IP. Therefore it can be concluded that the anorexic effect of GVG is independent of the food presentation and of the route of administration of GVG.

DISCUSSION

γ-Vinyl-GABA has been shown to be a specific irreversible inhibitor of GABA-transaminase capable of markedly elevating brain GABA concentration in experimental animals [1, 16, 28]. This increase has been shown to be maximal and stable between 4 and 24 hr after injection of GVG in mice [16]. We have confirmed these observations in rats (unpublished data) and have demonstrated that a significant increase in brain GABA is detectable 4 hours after GVG in-

TABLE 4

LACK OF INFLUENCE OF THE MODE OF PRESENTATION OF FOOD AND OF THE ROUTE OF ADMINISTRATION OF GVG ON GVG-INDUCED ANOREXIA IN RATS

Food Presentation		Food intake (g) Control night	Route of Administration	Food intake (g) after GVG 500 mg/kg	% Inhibition for the group
Cover	n=3	25.1 ± 2	IP	12.2 ± 4	49%
(n=6)	n=3	25.2 ± 1	PO	13.5 ± 2	
Floor	n=3	27.5 ± 2	IP	14.3 ± 4	48%
(n=6)	n=3	27.3 ± 1	PO	14.3 ± 1	

The normal food intake of 12 rats was recorded for one night, six of them having food available in the grid cover of the cage, the other six having food available on the floor of the cage. The following day, all rats were dosed with 500 mg/kg of GVG at 1400 hr (4 hrs before the beginning of night). In each group, 3 animals received GVG IP and the 3 others orally, using the same volume. Food intake was measured for the following night.

jected IP at doses as low as 62.5 mg/kg [15]. We thus chose to inject GVG 3 or 4 hr before the beginning of the night, to maintain a maximal increase in brain GABA throughout the period of measurement. In all experimental conditions used in this report, GVG inhibits rat food intake and growth. Our data is consistent with that of Tews *et al.* [30], Cooper *et al.* [4], Howard *et al.* [14] and Gale and Iadarola [8] who measured a decrease in food intake or growth rate of rats after elevating brain GABA concentrations or by administering GABA or GABA-agonists. All these results would be consistent with the hypothesis that GABA has inhibitory effects on the two structures or systems that trigger or suppress feeding, but that the inhibition of feeding is dominant when the two systems are inhibited together. However, two recent papers reporting the effects of an intracerebroventricular injection of muscimol are not consistent with this hypothesis, since both Olgiati *et al.* [27] and Morley *et al.* [26] report an increase in rat food intake after ICV muscimol. It is not clear why muscimol given ICV should have an effect opposite to that seen after an IP injection since Howard *et al.* [14] and Cooper *et al.* [4] reported a decrease in milk consumption (indicative of an anorexic effect, according to them), after the IP injection of 0.5 to 2 mg/kg of muscimol. One possible explanation would be that the distribution of muscimol among the different hypothalamic structures is not the same when the drug is given ICV or IP. This hypothesis needs experimental confirmation. Alternatively, muscimol administered IP is known to be rapidly metabolized [7] and the effects on food intake may thus be due to a metabolite.

The inhibitory effect on food intake, following GVG treatment, is associated with a concomitant reduction of water intake, and a decrease in locomotor activity. A similar spectrum of activity has been reported by Cooper *et al.* [4] after 400 μg EOS ICV. It is thus difficult to exclude the possibility that the anorexia seen after GVG or EOS is due to a general depression of behaviour, to physical incapacitation or to muscular incoordination. However, the results of Experiment 7 show that the anorexic effect of GVG is exactly the same if the rats must reach the food on the top of the covers or if they just have to pick it up from the floor of their cage; moreover, experiments done with mice on the rotorrod have shown that there is no significant loss of muscular coordination at doses up to 2 g/kg (J. Fozard and M. Host, personal communication). These two results suggest that physical incapacitation or muscle incoordination are of little importance in the anorexic effect of GVG. In addition, from Experiment 6 reported here, where GVG was associated with amphetamine, the animals receiving both drugs had activity counts similar to controls. In contrast, the effects on food intake and body weight were additive. Thus in this situation at least, the inhibitory effects of GVG on food intake can be shown to occur associated with normal locomotor activity. It is interesting to note that GVG also antagonized the stereotyped behaviour induced by this dose of dexamphetamine (2.5 mg/kg), which is in agreement with the results reported by Hammerstadt *et al.* [12] after comparable doses of amphetamine, but a higher dose of GVG (1000 mg/kg).

One of the major drawbacks of known anorexic drugs is the very rapid induction of tolerance to the effect [13,23]. We did not observe any tolerance to the effect of GVG given daily for 2 weeks and this result has been confirmed by other experiments in our Centre where rats were given GVG for longer periods. After the end of treatment, a small rebound in food intake was observed which was much less marked than that seen with dexamphetamine (see Experiment 6). These observations would argue against the conclusions of a recent paper by Levitsky *et al.* [22], suggesting that tolerance to anorexic drugs is consequent on the loss in body weight.

It has been established recently that most anorexic drugs exert their effect via a stimulation of the catecholaminergic systems (amphetamine and all related psychostimulant anorexics), but that the effect of fenfluramine is due to stimulation of the 5HT system [9]. We therefore tried to determine whether catecholamines or 5HT play any role in the anorexic effect of GVG. Depletion of brain DA, or brain DA and NA, with an ICV injection of 6 OH-DA in rats is known to decrease strongly the anorexic effect of amphetamine. We have confirmed that the integrity of the brain DA system is essential for the effect of amphetamine but did not find any evidence for a role of NA in this effect, a conclusion in agreement with the consensus reviewed by Clineschmidt *et al.* [3]. In both conditions however, (with or without a concomitant depletion of NA), no antagonism of the GVG effect was observed, which would imply that the effect of GVG is not due to an interaction with the DA or NA systems. No clear explanation was found for the potentiation of the anorexic effect after higher doses of GVG and this point needs further experimentation.

Several 5HT receptor blockers have been used to block selectively the anorexic effect of fenfluramine, [3]. In our experiments, metergoline clearly antagonized the effect of fenfluramine, but had no effect on that produced by GVG. Although we have eliminated the catecholaminergic and serotonergic systems as being implicated in the anorexic effect of GVG, it remains to be established if this effect is a direct effect of GABA on hypothalamic feeding centers or if it is mediated by other neurotransmitters or neuromodulators such as opioids, peptides, etc. (see [25]). Furthermore, the central site of GABA action is not proven, since GVG elevates GABA in peripheral as well as in central tissues [1]. Nevertheless, the similarities between the anorexic effects of parenteral GVG and ICV EOS make a purely peripheral site unlikely.

It is likely that the anorexic effects of GVG are species specific. In preliminary clinical studies in patients with a variety of neuropsychiatric conditions, oral treatment with GVG for up to 8 weeks, using doses as high as 10 g/day, was without effect on body weight and appetite suppression was not reported (P. J. Schechter, personal communication). This difference is not due to the route of administration as GVG is also active in rats after oral administration.

In conclusion, our data strongly support the hypothesis of a role for GABA in the central control of food intake in rats. This anorexic effect of GVG does not show tolerance, and the mechanism of action appears different from that of other anorexic drugs, since it does not depend on the integrity of catecholaminergic or serotoninergic systems.

REFERENCES

1. Böhlen, P., S. Huot and M. G. Palfreyman. The relationship between GABA concentrations in brain and cerebrospinal fluid. *Brain Res.* **167:** 297–305, 1979.
2. Cattabeni, F., A. Maggi, M. Monduzzi, L. De Angelis and G. Racagni. GABA: circadian fluctuations in rat hypothalamus. *J. Neurochem.* **31:** 565–567, 1978.
3. Clineschmidt, B. and P. D. Bunting. Differential effects of pharmacological agents acting on monoaminergic systems on drug-induced anorexia. *Prog. Neuropsychopharmac.* **4:** 327–339, 1980.
4. Cooper, B. R., J. L. Howard, H. L. White, F. Soroko, K. Ingold and R. A. Maxwell. Anorexic effects of ethanolamine-O-sulfate and muscimol in the rat: evidence that GABA inhibits ingestive behavior. *Life Sci.* **26:** 1997–2002, 1980.
5. Coscina, D. V. and K. G. Lloyd. Medial hypothalamic obesity: Association with impaired hypothalamic GABA synthesis. *Brain Res. Bull.* **5:** Suppl. 2, 793–796, 1980.
6. Duhault, J., L. Beregi, P. Gonnard and M. Boulanger. Brain serotonergic system and anorectic drugs. In: *Central Mechanisms of Anorectic Drugs,* edited by S. Garattini and R. Samanin. New York: Raven Press, 1978, pp. 205–215.
7. Enna, S. J., A. Maggi, P. Worms and K. G. Lloyd. Muscimol: Brain penetration and anticonvulsant potency following GABA-T inhibition. *Brain Res. Bull.* **5:** Suppl. 2, 461–464, 1980.
8. Gale, K. and M. Iadarola. Seizure protection and increased nerve-terminal GABA: delayed effects of GABA-Transaminase inhibition. *Science* **208:** 288–291, 1980.
9. Garattini, S., E. Borroni, T. Mennini and R. Samanin. Differences and similarities among anorectic agents. In: *Central Mechanisms of Anorectic Drugs,* edited by S. Garattini and R. Samanin. New York: Raven Press, 1978, pp. 127–143.
10. Grandison, L. and A. Guidotti. Stimulation of food intake by muscimol and β-endorphin. *Neuropharmacology* **16:** 533–536, 1977.
11. Grossman, S. P. Eating or drinking elicited by direct adrenergic or cholinergic stimulation of hypothalamus. *Science* **132:** 301–302, 1960.
12. Hammerstad, J. P., L. Gronke, J. Nutt and D. Casey. The effect of γ-vinyl-GABA on amphetamine stereotypy in rats. *Brain Res. Bull.* **5:** Suppl. 2, 609–612, 1980.
13. Heffner, T. G. and L. S. Seiden. The effect of depletion of brain dopamine by 6-hydroxydopamine on tolerance to the anorexic effect of d-amphetamine and fenfluramine in rats. *J. Pharmac. exp. Ther.* **208:** 134–143, 1979.
14. Howard, J. L., B. R. Cooper, H. L. White, F. Soroko and R. A. Maxwell. A role for GABA in the control of ingestive behavior in rats: Effects of ethanolamine-O-sulfate and muscimol. *Brain Res. Bull.* **5:** Suppl. 2, 595–599, 1980.
15. Huot, S., M. Robin and M. G. Palfreyman. Effect of γ-vinyl GABA alone or associated with diazepam on a conflict test in the rat. In: *Amino Acid Neurotransmitters,* edited by F. V. De Feudis and P. Mandel. New York: Raven Press, 1981, pp. 45–52.
16. Jung, M. J., B. Lippert, B. W. Metcalf, P. Böhlen and P. J. Schechter. Gamma-vinyl GABA (4-amino-hex-5-enoic acid), a new selective irreversible inhibitor of GABA-T: effects on brain GABA metabolism in mice. *J. Neurochem.* **29:** 797–802, 1977.
17. Kelly, J., G. F. Alheid, A. Newberg and S. Grossman. GABA stimulation and blockade in the hypothalamus and midbrain: Effects on feeding and locomotor activity. *Pharmac. Biochem. Behav.* **7:** 537–541, 1977.
18. Kelly, J. and S. P. Grossman. GABA and hypothalamic feeding systems: II A comparison of GABA glycine and acetylcholine agonists and their antagonists. *Pharmac. Biochem. Behav.* **11:** 647–652, 1979.
19. Kelly, J. and S. Grossman. GABA and hypothalamic feeding systems. *Brain Res. Bull.* **5:** Suppl. 2, 237–244, 1980.
20. Kimura, H. and K. Kuriyama. Distribution of GABA in the rat hypothalamus, functional correlates of GABA with activities of appetite-controlling mechanisms. *J. Neurochem.* **24:** 903–907, 1975.
21. Leibowitz, S. F. Identification of catecholamine receptor mechanisms in the perifornical lateral hypothalamus and their role in mediating amphetamine and L-DOPA anorexia. In: *Central Mechanisms of Anorectic Drugs,* edited by S. Garattini and R. Samanin. New York: Raven Press, 1978, pp. 39–82.
22. Levitsky, D. A., B. J. Strupp and J. Lupoli. Tolerance to anorectic drugs: Pharmacological or artifactual. *Pharmac. Biochem. Behav.* **14:** 661–667, 1981.
23. Lewander, T. Experimental studies on anorexigenic drugs: tolerance, cross-tolerance and dependence. In: *Central Mechanisms of Anorectic Drugs,* edited by S. Garattini and R. Samanin. New York: Raven Press, 1978, pp. 343–355.
24. Meeker, R. B. and R. D. Myers. GABA and glutamate: possible metabolic intermediaries involved in the hypothalamic regulation of food intake. *Brain Res. Bull.* **5:** Suppl. 2, 253–259, 1980.
25. Morley, J. E. The neuroendocrine control of appetite: the role of the endogenous opiates, cholecystokinin, TRH, GABA and the diazepam receptors. *Life Sci.* **27:** 355–368, 1980.
26. Morley, J. E., A. S. Levine and J. Kneip. Muscimol-induced feeding: a model to study the hypothalamic regulation of appetite. *Clin. Res.* **29:** 431A, 1981.
27. Olgiati, V. R., C. Netti and F. Guidobond. The central GABAergic system and control of food intake under different experimental conditions. *Psychopharmacology* **68:** 163–167, 1980.

28. Palfreyman, M. G., P. J. Schechter, W. R. Buckett, G. P. Tell and J. Koch-Weser. The pharmacology of GABA-transaminase inhibitors. *Biochem. Pharmac.* **30:** 817–824, 1981.
29. Porrino, L. J. and E. E. Coons. Effect of GABA receptor blockade on stimulation-induced feeding and self-stimulation. *Pharmac. Biochem. Behav.* **12:** 125–130, 1980.
30. Tews, J. K., E. A. Riegel and A. E. Harper. Effect of dietary GABA and protein on growth, food intake and GABA metabolism in the rat. *Brain Res. Bull.* **5:** Suppl. 2, 245–251, 1980.
31. Wagner, J., M. Palfreyman and M. Zraika. Determination of DOPA, dopamine, DOPAC, epinephrine, norepinephrine, α-monofluoromethyldopa and α-difluoromethyldopa in various tissues of mice and rats using reversed-phase ion-pair liquid chromatography with electrochemical detection. *J. Chromat.* **164:** 41–54, 1979.