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Effects of an Inhibitor of GABA-Aminotransferase (γ-Vinyl-GABA) on the Spatial Navigation Deficit Induced by Muscarinic Blockade

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MAZURKIEWICZ, M., J. SIRVIÖ AND P. J. RIEKKINEN. *Effects of an inhibitor of GABA-aminotransferase (γ-vinyl-GABA) on the spatial navigation deficit induced by muscarinic blockade.* PHARMACOL BIOCHEM BEHAV **43**(1) 91-96, 1992. – The present study investigated whether stimulation of the GABAergic system affects spatial navigation [water-maze (WM)] deficit induced by muscarinic blockade (scopolamine). The effects of various doses of γ-vinyl-GABA (GVG) (50, 150, and 300 mg/kg) and scopolamine (0.4 and 0.1 mg/kg) were examined alone and in combination. GVG at 50 and 150 mg/kg alone did not impair the performance of rats in the WM yask. At 300 mg/kg, GVG caused slight impairment, increasing latency and total distance swum during training trials. Scopolamine at 0.4 mg/kg clearly impaired the performance of rats in the WM task. When the two drugs were coadministered, no interaction between scopolamine and GVG was observed. Our results do not provide support for any interaction between cholinergic muscarinic and GABAergic mechanisms.

γ-Vinyl-GABA GABAergic system Cholinergic system Spatial learning Water maze task

THE involvement of the cholinergic system in cognitive functions, such as learning and memory, has received support due to correlation between diminished cholinergic activity and impaired memory in aged subjects and patients with Alzheimer's disease (2,3,9,24,41). Furthermore, impaired performance of experimental animals has been observed in procedures designed to investigate memory and learning functions after animals have been challenged with lesions or pharmacological agents that directly interfere with cholinergic transmission (4,9,21,26,43). Centrally acting muscarinic antagonists such as scopolamine and atropine have been reported to impair an animal's performance in several learning tasks, for example, Morris water maze, spontaneous alternation, and spatial matching to sample (5,6,8,20,31,32,38).

It has been observed that interactions between cholinergic and other neurotransmitter systems (serotonergic, noradrenergic, dopaminergic, GABAergic, peptidergic) may have a role to play in learning and memory processes (14,18,19,27,36,44). Anatomical correlates to a GABAergic/cholinergic interaction has been demonstrated by Zaborsky et al. (45), who showed that GAD-immunopositive terminals make synaptic contacts with cholinergic neurons of the basal forebrain, and Sarter and Schneider (28), who showed that there is a high density of GABA/benzodiazepine receptor binding sites in substantia innominata. The inhibitory nature of the GABAergic input has been shown by experiments in which GABA, a GABA agonist, or a benzodiazepine agonist inhibits the activity of the basal forebrain neurons (7,13,39). Other experiments have demonstrated that chronic GABA infusions induce a set of deficits associated with a reduction of the animal's spatial ability (16,29,34,43).

It has been proposed that GABAergic neurons may be preserved in subcortical structures (e.g., nucleus basalis of Meynert) in patients with Alzheimer's disease (23) and that the remaining neurons are subjected to increased GABAergic inhibition in senile dementia (35). Furthermore, previous experiment data have shown that lesions of the basal forebrain system combined with pharmacological GABAergic activation produce more robust learning and memory deficits than basal forebrain lesions alone (11).

The present experiments were undertaken to further study the interactions between the GABAergic and cholinergic systems in learning and memory. Our study investigated whether the pharmacological activation of the GABAergic system affects the spatial learning and memory deficit induced by cholinergic dysfunction. We used γ-vinyl-GABA (GVG) (5-

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TABLE 1
GROUPS USED IN THE PRESENT EXPERIMENTS

Experiment 1:	Effects of GVG at 50 and 150 mg/kg on the Spatial Navigation Deficit Induced by Muscarinic Blockade		
	Dose	Number of Rats	Abbreviation
Saline	–	7 (7)	SAL-SAL
Saline + scopolamine	0.4 mg/kg	8 (7)	SAL-SCOP
GVG + scopolamine	50 mg/kg		
	0.4 mg/kg	7	GVG50-SCOP
GVG + scopolamine	150 mg/kg		
	0.4 mg/kg	7	GVG150-SCOP
GVG + saline	50 mg/kg		
	–	7	GVG50-SAL
GVG + saline	150 mg/kg		
	–	7	GVG150-SAL

Experiment 2:	Effects of GVG (300 mg/kg) on the Spatial Navigation Deficit Induced by Muscarinic Blockade		
	Dose	Number of Rats	Abbreviation
Saline	–	7	SAL-SAL
Saline + scopolamine	0.4 mg/kg	7	SAL-SCOP
GVG + scopolamine	300 mg/kg		
	0.4 mg/kg	7	GVG300-SCOP
GVG + saline	300 mg/kg		
	–	7	GVG300-SAL

Experiment 3:	Effects of GVG and Scopolamine (0.1 mg/kg) on Acquisition of the Water Maze Task		
	Dose	Number of Rats	Abbreviation
Saline	–	8	SAL-SAL
Saline + scopolamine	0.1 mg/kg	7	SAL-SCOP 0.1
GVG + scopolamine	150 mg/kg		
	0.1 mg/kg	7	GVG-SCOP 0.1
GVG + saline	150 mg/kg		
	–	7	GVG-SAL

Experiment 4:	Effects of GVG and *N*-Methyl Scopolamine on Acquisition of the Water Maze Task		
	Dose	Number of Rats	Abbreviation
Saline	–	8	SAL-SAL
Saline + *N*-Methyl scopolamine	0.4 mg/kg	8	SAL-MET
GVG + *N*-Methyl scopolamine	150 mg/kg		
	0.4 mg/kg	8	GVG-MET
GVG + saline	150 mg/kg		
	–	7	GVG-SAL

amino-4-hexenoic-acid), an inhibitor of GABA transaminase that increases GABA levels in brain (15,22). The effects of GVG treatment were studied in groups of scopolamine- and saline-treated rats using the water maze task.

METHOD

Animals

One-hundred thirty-one adult, male Kuo-Wistar rats, weighing 240–410 g, were used. Rats were housed in cages in groups of two or three with food and water freely available. The light period was 14 h (lights on 0700–2100 h). Room temperature was +20°C and humidity 50–60%.

Drugs

The drug treatment consisted of the muscarinic antagonist scopolamine (Sigma Chemical Co., St. Louis, MO) (0.1 and 0.4 mg/kg) and GVG (Vigabatrin, Merrel Dow, UK) (50, 150, and 300 mg/kg) or the combination of the two (Table 1).

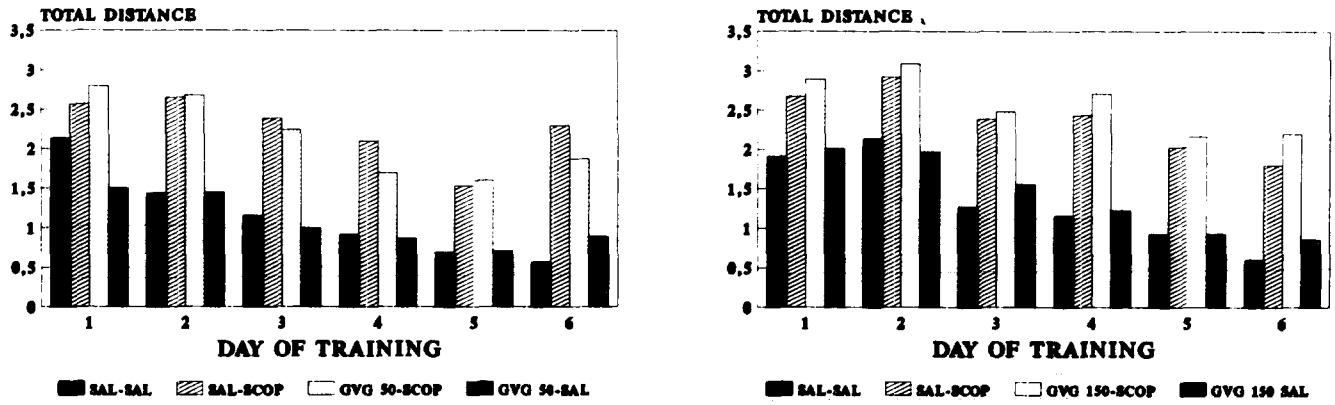

FIG. 1. Effects of administration of GVG (50 or 150 mg/kg) on the spatial navigation deficit induced by muscarinic blockade (scopolamine 0.4 mg/kg) (distance is expressed in arbitrary units—thousands of pixels).

Scopolamine hydrochloride was dissolved in 0.9% NaCl and injected IP 30 min before training. GVG was dissolved in 0.9% NaCl and injected IP. Saline injections of equal volume served as controls. GVG and saline administration were started 4 days before behavioural testing and continued daily until training was over. Injections were given 2–3 h after behavioural training. The intention was to perform the test during a steady drug level, which could not be guaranteed with injections before training. Injections given immediately after training could have an influence on learning and memory consolidation processes. Intraperitoneal injections of methylscopolamine (0.4 mg/kg; 30 min before testing) were used for peripheral controls.

Morris Water Maze

Animals were tested in the water maze apparatus (San Diego Instruments, San Diego, CA) which has been described previously (25). Briefly, the water maze pool was a circular, black, fiberglass tank filled with water (temperature 20°C). The platform was always located in the southwest quadrant and its surface was 1.5 cm below the water line. A daily training consisted of three trials (maximum 70 s each). If the rat found the platform, it was allowed to stay on it for 10 s. The recovery time between trials was 30 s. The starting locations were east or north every day and west or south every second day. The swim paths were monitored by a videocamera linked to a computer through an image analyzer. The computer calculated the total distance swum, swimming speed, and latency of rats in the water maze task.

Statistics

Main group effect and group comparisons of the training trial data (path length, swimming speed) were analysed with analysis of variance (ANOVA) using training trial as a variant.

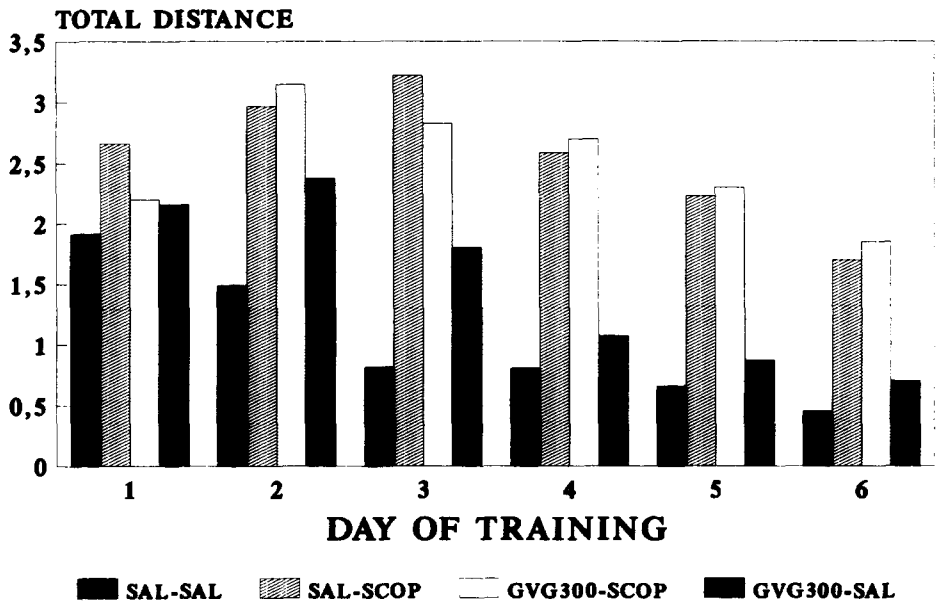

FIG. 2. Effects of GVG (300 mg/kg) on the spatial navigation deficit induced by muscarinic blockade (scopolamine 0.4 mg/kg) (distance is expressed in arbitrary units—thousands of pixels).

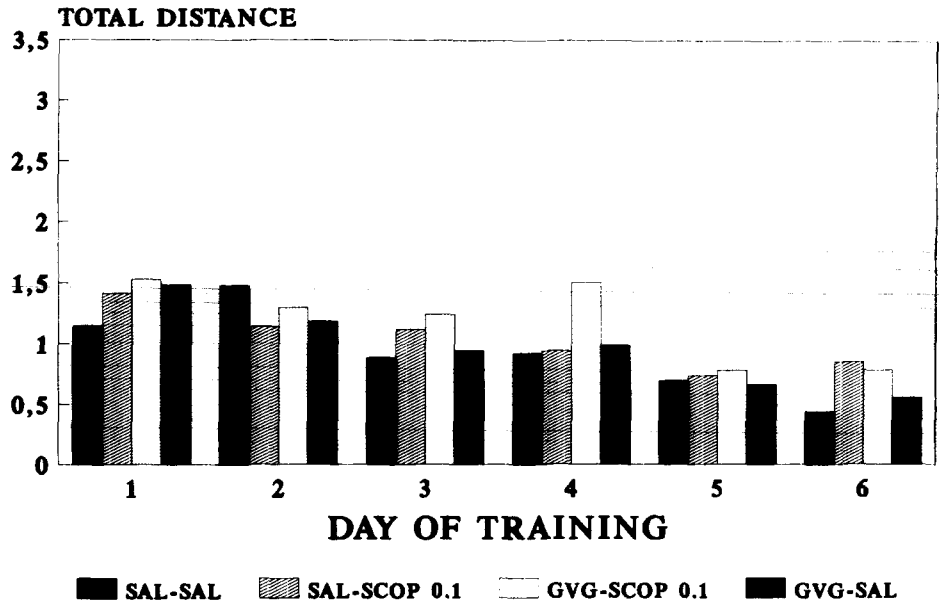

FIG. 3. Effects of GVG (150 mg/kg) and scopolamine (0.1 mg/kg) on acquisition of the water maze task (distance is expressed in arbitrary units—thousands of pixels).

GVG and scopolamine were used as a variants in analysis of interaction between these two drugs using ANOVA.

RESULTS

Previous experiments have shown that scopolamine increases the swimming speed in rats trained in the water maze task. Also, GVG seems to slightly increase speed of swimming (30). Thus, we used escape distance to express the acquisition data.

Experiment 1

The impairment induced by scopolamine (0.4 mg/kg) was statistically significant, $F(1, 518) = 112.6$, $p < 0.001$. At 50 and 150 mg/kg, vigabatrin did not impair acquisition in the

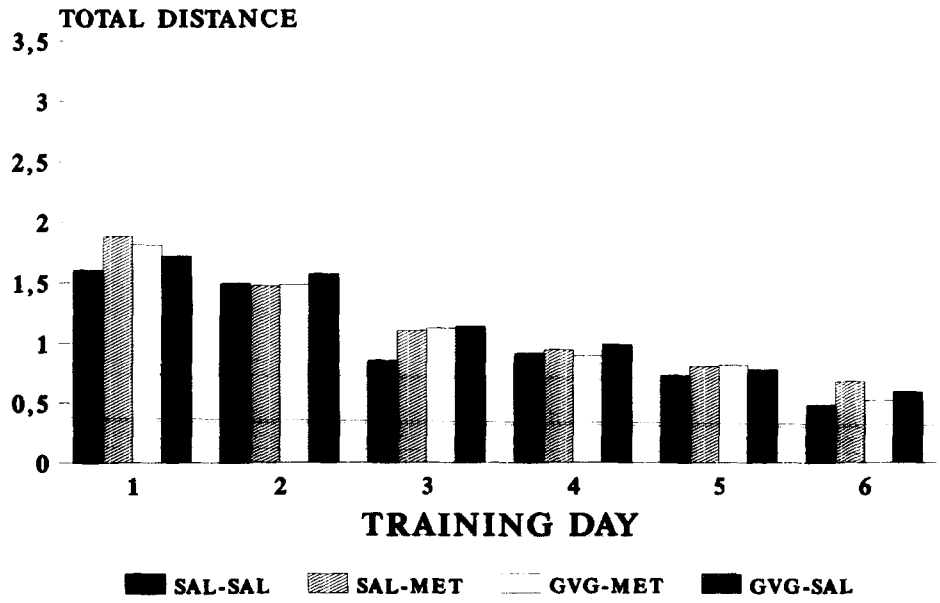

FIG. 4. Effects of GVG (150 mg/kg) and *N*-methyl scopolamine (0.4 mg/kg) on acquisition of the water maze task (distance is expressed in arbitrary units—thousands of pixels).

water maze task ($p > 0.1$ for both doses). The impairment induced by scopolamine (0.4 mg/kg) did not differ between GVG- and saline-treated rats (no significant interaction) $F(1, 518) = 0.0$, $p > 0.1$, for 50 mg/kg and $F(1, 471) = 2.7$, $p > 0.05$, for 150 mg/kg (Fig. 1).

Experiment 2

Scopolamine (0.4 mg/kg) impaired acquisition of the water maze task (a significant treatment effect), $F(1, 611) = 94.8$, $p < 0.001$. At 300 mg/kg, vigabatrin affected acquisition of the water maze task, $F(1, 611) = 8.229, p < 0.01$. There were no marked differences between groups receiving combinations of saline and scopolamine or GVG and scopolamine (no significant interaction), $F(1, 611) = 1.832, p > 0.1$ (Fig.2).

Experiment 3

At 0.1 mg/kg, scopolamine did not affect acquisition, $F(1, 547) = 2.0$, $p > 0.1$. There was no interaction between scopolamine and vigabatrin (150 mg/kg) treatment, $F(1, 547) = 0.1, p > 0.1$ (Fig. 3).

Experiment 4

N-Methyl scopolamine (0.4 mg/kg) did not affect acquisition of the water maze task, $F(1, 585) = 0.3$, $p > 0.1$. At 150 mg/kg, vigabatrin also did not impair performance in the water maze, $F(1, 585) = 0.3$, $p > 0.1$. No interaction between those two drugs was noticed, $F(1, 585) = 0.8, p > 0.1$ (Fig. 4).

DISCUSSION

In the present experiments, we investigated whether activation of the GABAergic system (a subchronic administration of GVG) affects the spatial navigation deficit induced by muscarinic blockade (scopolamine). At 0.4 mg/kg, scopolamine significantly impaired the performance of rats. At 50, 150, and 300 mg/kg, GVG did not affect scopolamine-induced spatial navigation deficit. Furthermore, GVG (150 mg/kg) did not interfere with the subthreshold dose of scopolamine (0.1 mg/kg). At the highest dose used (300 mg/kg), GVG alone caused slight impairment of rats' performance. These results suggest that elevation of GABA levels in brain do not aggravate the deficit in the water maze task after treatment with scopolamine.

Our results support previous evidence that muscarinic acetylcholine receptor blockers impair performance of rats in spatial learning tasks, for example, radial arm and water mazes (4,24,32,37,38). The peripherally acting muscarinic antagonist *N*-methyl-scopolamine did not have any effect on water maze acquisition of rats. This agrees with previous data (24) and suggests that scopolamine-induced effects are of central origin. It has been suggested that the hypermobility exhibited by animals receiving scopolamine contributes to the decrease in performance of the task by increasing perseverance and circling behaviour (7,33,40,42). In our experiment, rats treated with scopolamine when first placed in water swam quickly in circles around the pool, with sniffing movements against the wall. They persisted in this behaviour for many trials until they accidently found the correct cue. This "wall-hugging" strategy is probably related to the movement "stereotypies" described by Lindner and Schallert as tigmotaxia (14). It is not clear whether the effects of scopolamine on spatial learning are due to memory impairment or mnemonic dysfunction.

Chronic infusions of GABA into the medial prefrontal cortex, nucleus basalis magnocellularis (NBM), sulcus principalis region, led to cognitive deficits associated with a reduction of spatial mapping ability in rats (16,29,43).

We used a subchronic administration of GVG in the dose range 50–300 mg/kg. At >50 mg/kg, subchronic administration of GVG (>4 days) increased GABA levels in rat brain and cerebrospinal fluid (10,30). For example, during subchronic administration of a daily 100-mg/kg dose of GVG GABA levels are increased by 118% (cortex), 192% (hippocampus), 310% (cerebellum), and 63% (spinal cord), while with a daily 250-mg/kg dose the respective levels of GABA increased by 247, 316, 577, and 126% (10). At 50 and 150 mg/kg, water maze acquisition was not impaired; however, we observed slight performance impairment using 300 mg/kg.

There exists evidence to support direct interaction between GABAergic and cholinergic systems in the basal forebrain (1,27). In one study, the effects of NBM lesions combined with GVG treatment revealed that GVG (50 and 200 mg/kg) aggravated the NBM-lesion-induced water maze deficit (11). In the context of previous results suggesting that NBM-lesion (ibotenic acid)-induced deficit in the water maze task is, at least mainly, due to noncholinergic pathology [see (11) for references], the present results suggest that GVG administration aggravated the noncholinergic component of spatial navigation deficit in GVG-treated, ibotenic-acid-NBM-lesioned rats.

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