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Effects of Vinconate, a Novel Vinca Alkaloid, on Spatial Learning Deficits Induced by the Basal Forebrain Lesion in Rats

HIROSHI KINOSHITA,*† TSUTOMU KAMEYAMA,*
TAKAAKI HASEGAWA† AND TOSHITAKA NABESHIMA†[1]

**Department of Chemical Pharmacology, Faculty of Pharmaceutical Sciences, Meijo University, Nagoya 468 Japan*
†Department of Neuropsychopharmacology and Hospital Pharmacy, Nagoya University School of Medicine, Nagoya 466, Japan

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KINOSHITA, H., T. KAMEYAMA, T. HASEGAWA AND T. NABESHIMA. *Effects of vinconate, a novel vinca alkaloid, on spatial learning deficits induced by the basal forebrain lesion in rats.* PHARMACOL BIOCHEM BEHAV **42**(1) 19–23, 1992.—We investigated the effects of vinconate, a novel vinca alkaloid, on spatial learning deficits induced by the basal forebrain (BF) lesion in rats. Bilateral BF lesions were produced by injecting ibotenic acid (6 μg/0.5 μl/side). In BF-lesioned rats, impairment of spatial learning in escaping onto the platform during training and decrease in spatial bias during the spatial probe trial in Morris's water maze task were both observed. Vinconate (5 and 10 mg/kg) treatment shortened the increase of escape latency to the platform in BF-lesioned rats and significantly reversed the decrease in spatial bias induced by the BF lesion. Vinconate (10 mg/kg) attenuated the decrease in choline acetyltransferase activity in the frontoparietal cortex caused by the BF lesion. The present study suggests that vinconate has an antiamnesic effect on the BF-lesion-induced amnesia by ameliorating the dysfunction in cholinergic neurons.

Vinconate Basal forebrain lesion Morris's water maze Spatial learning
Choline acetyltransferase activity Frontoparietal cortex Vinca alkaloid Rats

VINCONATE (OM-853; (±)-methyl 3-ethyl-2,3,3*a*,4-tetrahydro-1*H*-indolo[3,2,1,-de][1,5]naphthyridine-6-carboxylate hydrochloride) is a novel vinca alkaloid developed as a cerebral metabolic ameliorator. It increases oxygen utilization and glucose uptake or activates neurotransmission. In a previous study, vinconate significantly extended consciousness and delayed the appearance of electroencephalographic (EEG) disturbances under hypoxic conditions (27). Araki et al. reported that vinconate has a protective effect on the delayed neuronal death of hippocampus CA1 after ischemia in rats and gerbils (1,2). In light of these observations, it has been suggested that vinconate may be effective in the treatment of cerebral disorders induced by hypoxic conditions such as ischemia.

For elderly subjects, oral administration of vinconate may be followed by improvements in psychomotor activity, complex reactions, and cognitive functions, as well as by a significant improvement in attention and concentration (26). Furthermore, it has been reported that vinconate improves scopolamine-induced amnesia in mice during passive avoidance tasks (25) and inhibits [^{3}H]quinuclidinyl benzilate binding to muscarinic cholinergic (ACh) receptors (14). These data indicate that vinconate is a drug that may be used to alleviate geriatric memory deficits by activating the AChergic system.

One approach to the study of pharmacologically active drugs, with the aim of ameliorating learning and memory deficits induced by the dysfunction of the AChergic system, is to lesion several brain regions that are involved in spatial learning. Lesions of the basal forebrain (BF), in which the cell bodies of the AChergic system are located, have been shown to impair performance on more complex spatial learning tasks such as the radial arm maze and Morris's water maze (3,6, 12,28), as well as behavioral responses to a variety of simple tasks such as passive avoidance (3,8,16). Moreover, a selective decrease in choline acetyltransferase (ChAT) in the cerebral cortex is induced by the BF lesion (3,11,12). Therefore, in the present study we evaluated the effects of vinconate on spatial learning impairments induced by the BF lesion in rats using Morris's water maze task.

[1] To whom reprint requests should be addressed.

METHOD

Animals

Male Kbl Wistar rats (Kyoto Institute, Kitayama Laboratories Co., Ltd. Kyoto, Japan) weighing 280–320 g, 9 weeks old at the beginning of the experiments, were housed in wire-mesh cages in groups of three to four and given free access to food and water. Rats were maintained at a fixed temperature (24 ± 1°C) and relative humidity (50 ± 10%), and on a 12 L:12 D cycle (with a 12-h light cycle starting at 8:00 a.m.).

Drug Treatment

Vinconate hydrochloride (Tokyo Tanabe Co., Ltd., Japan) was dissolved in distilled water (5 ml/kg) and administered at doses of 5 and 10 mg/kg. The administration of vinconate was begun 3 weeks after BF lesion and was given orally by intubation immediately after each trial in Morris's water maze task for 10 days. The tenth administration of vinconate was given immediately after the probe trial, and vinconate was administered for 4 subsequent days after completion of the behavioral testing. The controls and BF-lesioned rats were also intubated with distilled water.

Surgery

Surgery was conducted under pentobarbital anesthesia (50 mg/kg, IP). The rat was placed in a stereotaxic apparatus with a tooth bar 3.3 mm below the interaural line. The following coordinates for placement of the microsyringe needle were used: 0.8 mm posterior to the bregma, 2.8 mm laterally to the midline, and 7.3 mm ventral from the dura, according to the stereotaxic atlas of Paxinos and Watson (24). Bilateral BF lesions were made by injecting ibotenic acid on each one side at an interval of 3 days. Ibotenic acid (Sigma) was dissolved in a small volume of 0.5 N NaOH and brought up to a concentration of 12 μg/μl in phosphate buffer, pH 7.4. The final pH of the solution was adjusted to 7.4 with 0.5 N HCl. It was injected in a volume of 0.5 μl over 5 min through a microsyringe. The microsyringe was left for a further 5 min. Control rats received phosphate buffer alone. Behavioral testing was conducted beginning 3 weeks after the second injection of ibotenic acid.

Morris's Water Maze

Apparatus. A Morris's water maze task apparatus consisting of a transparent circular pool (1.4 m in diameter and 45 cm high) was used (21). The pool was filled to a depth of 30 cm with water (maintained at 23 ± 3°C). A platform (10 cm in diameter and 28 cm high) made of transparent vinyl chloride was arranged so that its surface was submerged 2 cm below the surface of the water. Four points on the rim of the pool were arbitrarily designated north, south, east, and west, dividing the surface of the pool into four quadrants: northeast (NE), northwest (NW), southeast (SE), and southwest (SW). The platform was located in a constant position in the middle of one quadrant, equidistant from the center and edge of the pool. Spatial cues were placed in the room, and these remained in fixed positions throughout the experiment (e.g., TV camera stand, pictures, lamps, etc.). The escape latency and the distance the animal swam to find the platform were monitored by a TV camera (BTA-2A; Muromachi Kikai Co., Ltd., Japan) and analyzed by a computer (PC-9801; NEC Co., Ltd., Japan).

Procedure. Each rat was placed into the water facing the wall of the pool at one of four starting points that divided the pool into four quadrants. The sequence of starting points was randomly selected. If the rat found the platform, it was allowed to stay there for 30 s and was then returned to its home cage. Rats that failed to find the platform within 120 s were placed on it for 30 s and a maximum score of 120 s was assigned. Each trial was carried out once daily for 10 consecutive days. One h after the final trial, spatial bias in the rat's search pattern was tested through a spatial probe trial conducted without the platform. Spatial bias was expressed as a percentage of the total latency swum in the previous training quadrant during the spatial probe trial. The same behavioral study repeated twice, using different groups of animals to confirm the drug effects. Each group consisted of seven rats.

ChAT Activity

The rat was sacrificed by decapitation 24 h after the last administration of vinconate and the brain was removed rapidly and dissected into various brain portions, including the frontoparietal cortex. The tissue was stored at −80°C until assayed. ChAT activity was measured by the method of Fonnum (9). The tissue was homogenized (4% w/v) in 50 mM phosphate buffer (pH 7.4). The homogenate was activated with 1.5% Triton X-100 to ensure release of the enzyme. The sample was incubated (37°C, 30 min) in a medium containing unlabeled acetyl CoA, [^{14}C]acetyl CoA (for a final concentration of 0.4 mM acetyl CoA), choline chloride (10 mM), physostigmine sulphate (0.1 mM), NaCl (300 mM), Na phosphate buffer (pH 7.4; 50 mM), and EDTA-2Na (pH 7.4). The reaction was terminated by the addition of sodium tetraphenylborate/acetonitrile (5 mg/ml), which extracts the [^{14}C]ACh. Radioactivity in the top organic phase was determined by liquid scintillation spectrometry. Protein was measured by the method of Lowry et al. (15).

Statistics

Data from Morris's water maze task were analyzed by repeated-measure analysis of variance (ANOVA). Biochemical data were analyzed using one-way ANOVA. All analyses were followed by Tukey's test.

RESULTS

Morris's Water Maze

The effects of vinconate on the prolongation of escape latency induced by the BF lesion in rats are shown in Fig. 1. The escape latency for all groups decreased with training, $F(9,520) = 27.0$, $p < 0.001$. The escape latencies of BF-lesioned rats on the first trial were not different from those of the control group. However, throughout training BF-lesioned rats were slower to escape onto the platform than were animals in the control group, $F(1,260 = 80.8$, $p < 0.001$. Post-training administration of vinconate (5 or 10 mg/kg) shortened the prolongation of the escape latency induced by the BF lesion, $F(2,390) = 5.9$, $p < 0.01$. Significant differences in the escape latency were observed between BF-lesioned and control rats during the sixth to ninth trials (Tukey's test). Such differences were not observed between vinconate-treated and control rats except at the eighth trial at a dose of 5 mg/kg. Therefore, vinconate significantly attenuated the impairment of learning in BF-lesioned rats. Results from the distance that the animal swam were also similar to those for the escape latency (data not shown).

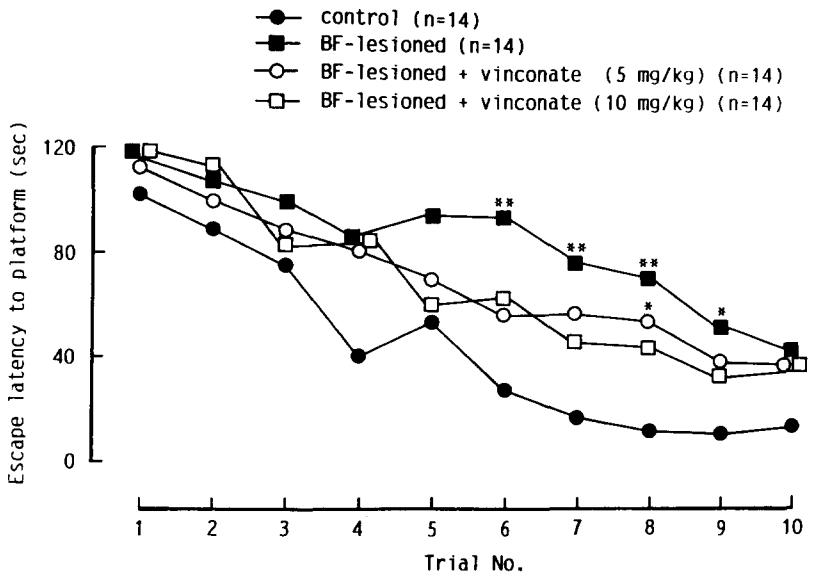

FIG. 1. Effects of vinconate on the deficit in learning induced by the basal forebrain (BF) lesion in rats in Morris's water maze task. Vinconate was given immediately after each trial except for the tenth administration. Each point indicates the mean. $*p < 0.05$, $**p < 0.01$; compared with the control group (Tukey's test).

The spatial probe trial was carried out 1 h after the tenth trial to examine if the rat had learned the position of the platform. In the spatial probe trial, the percentage of the total time (60 s) swum in the quadrant where the platform was previously located was used as an index of memory (Fig. 2). The spatial probe trial revealed impaired spatial bias in BF-lesioned rats. In comparison with the control group, BF-lesioned rats showed a significant decrease in the time swum in the previous training quadrant ($p < 0.01$). Posttraining administration of vinconate (5 or 10 mg/kg) significantly prolonged the decrease in the time swum in the previous training quadrant induced by the BF lesion ($p < 0.01$).

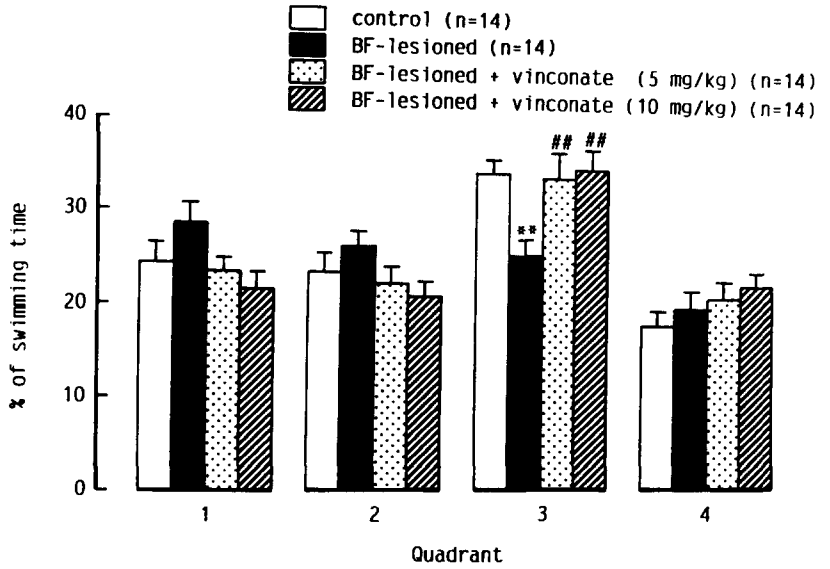

FIG. 2. Effect of vinconate on the deficit in the probe trial induced by the basal forebrain (BF) lesion in rats in Morris's water maze task. The probe trial was carried out 1 h after the tenth trial. The platform had been located in the middle of the third quadrant. Vinconate was given immediately after each trial except for the tenth administration. The tenth administration was carried out immediately after the probe trial. Each column indicates the mean ± SEM. $**p < 0.01$; compared with the control group (Tukey's test). $\#\#p < 0.01$; compared with the BF-lesioned group (Tukey's test).

ChAT Activity

ChAT activity in BF-lesioned rats 24 h after the fourteenth administration of vinconate is shown in Fig. 3. ChAT activity in the frontoparietal cortex significantly decreased in untreated and vinconate-(5 mg/kg) treated BF-lesioned rats by 17 and 19%, respectively ($p < 0.01$). However, a significant decrease in ChAT activity was not observed in vinconate-(10 mg/kg) treated BF-lesioned rats as compared with that in the control group, and there was a tendency to reverse the ChAT depletion caused by the BF lesion.

DISCUSSION

A major AChergic input to the cerebral cortex originates in the BF, which is known in primates as the nucleus basalis magnocellularis or nucleus basalis of Meynert. Lesions of the BF produce severe impairment in the performance of learning tasks (8,11,16). Several reports have observed that the BF lesion leads to impairment in performance on passive avoidance and Morris's water maze tasks, which require reference memory (6,19,28), and on radial arm maze tasks, which require working memory (3,12). Moreover, a selective decrease in ChAT activity in the cerebral cortex is produced by the BF lesion (3,11,12). In this study, we confirmed that BF lesion impaired the spatial reference memory during Morris's water maze tasks and decreased ChAT activity in the frontoparietal cortex. The decrease in ChAT activity in BF-lesioned rats in the present experiment was smaller than that of other reports using ibotenic acid since injection of ibotenic acid was carried out at each one side at an interval of 3 days to avoid death and weight loss.

In the present study, vinconate ameliorated spatial learning impairment, especially the disruption of the spatial probe trial, which was produced by the BF lesion. Since BF-lesioned rats in this study did not show any changes in motor activity and in swim speed as compared with the control rats (data not shown), it seems unlikely that the amelioration produced by vinconate was due to a secondary factor such as improvement in motor disturbance. During the spatial probe trial, none of the untreated BF-lesioned rats acquired the exact spatial location of the platform, as indicated by the decrease in the searching time spent in the platform quadrant as compared with that of the control group. Vinconate-treated BF-lesioned rats spent a significantly longer time in this region. These data indicate that vinconate-treated BF-lesioned rats learned a simple strategy to find the hidden platform. Since vinconate was administered immediately after each trial, the ameliorative effects on spatial learning deficits resulted from enhancement of the processes of consolidation and retrieval in learning and memory.

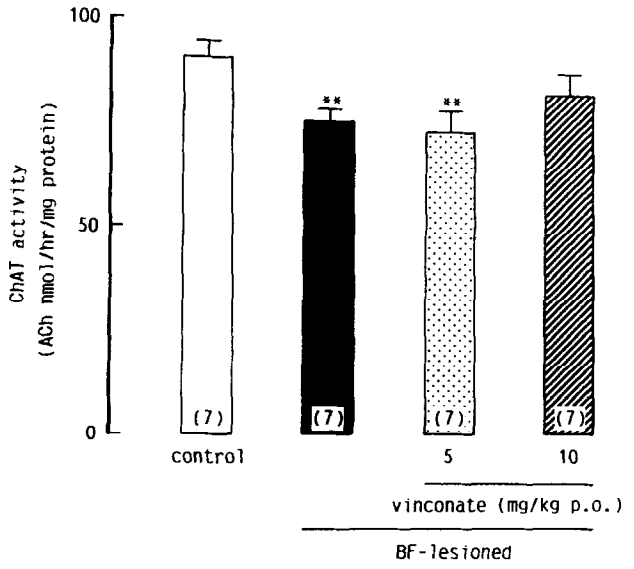

FIG. 3. Effect of vinconate on the decrease in ChAT activity in the frontoparietal cortex induced by the basal forebrain (BF) lesion in rats. The rat was sacrificed by decapitation 24 h after the 14th administration. Each column indicates the mean ± SEM. $^{**}p < 0.01$; compared with the control group (Tukey's test). See Fig. 2 for key.

Recently, reversal of BF-lesion-induced learning and memory deficits has been demonstrated on a variety of tasks using drugs that activate the central AChergic function (4,5,10,18). For example, continuous infusion of physostigmine, an ACh esterase inhibitor, produces marked improvement in the acquisition and retention of several learning tasks in rats with the BF lesion (17,20). In a previous study, it was observed that vinconate improves scopolamine-induced amnesia in mice during passive avoidance tasks (25) and inhibits [^{3}H]quinuclidinyl benzilate binding to muscarinic AChergic receptors in a striatal particulate fraction more effectively than carbachol (14). These data suggest that the antiamnesic effects of vinconate on spatial learning impairment observed in this study may result from activation of the AChergic system.

Although vinconate could not completely reverse the decrease in ChAT activity caused by the BF lesion in the frontoparietal cortex, there was a tendency to recover the ChAT depletion at a dose of 10 mg/kg. Previous studies that examined the effects of AChergic drugs on BF-lesion-induced amnesia have shown that AChergic drugs that do not have nerve growth factor (NGF)-like activity can improve spatial learning deficits in the same manner as observed in this study but do not cause reversal of the ChAT depletion (4,17). On the contrary, Nabeshima et al. (22,23) recently reported that staurosporine, possessing an NGF-like action, not only attenuated amnesia induced by the BF lesion but also completely reversed the cortical ChAT depletion. These data indicate that the survival of a subpopulation of BF neurons leads to the complete recovery of ChAT depletion. Therefore, future studies should attempt to determine if vinconate has an NGF-like action.

In preliminary experiments, we confirmed that there are no changes in monoamine contents and glutamate decarboxylase activity in BF-lesioned rats, but we did not examine the effect of vinconate on these neurotransmitter systems yet. However, in a previous study, Koda et al. (13,14) suggested that facilitation of the metabolic turnover of dopamine and 5-hydroxytryptamine by vinconate may be responsible for the therapeutic effect of vinconate on various neuropsychiatric disorders such as impairment of psychomotor reaction, complex reaction, and concentration, as observed in elderly subjects. Moreover, Dunnet et al. (7) suggested that the disruption of passive avoidance retention reflects a decline in cortical AChergic function, but a non-AChergic system may be involved in the acquisition deficit in passive avoidance. Therefore, learning and memory deficits induced by injections of ibotenic acid into the BF may result from dysfunctions not only in AChergic but also in other neuronal systems. Based on these observations, another possible mechanism for improvement in spatial

learning deficits induced by the BF lesion is that vinconate activates other neuronal systems.

In conclusion, the results of the present study indicate that vinconate produces marked recovery from learning impairment induced by the BF lesion, especially disruption of the spatial probe trial, through AChergic and other neuronal systems and that vinconate is an interesting drug for possible use in clinical trials of treatment strategies designed for geriatric memory disorders. Further studies will be necessary to establish biochemical changes that occur after the amelioration of spatial learning deficits.

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