Beneficial Effect of Idebenone (CV-2619) on Cerebral Ischemia-Induced Amnesia in Rats

Naoki YAMAZAKI, Yomei TAKE, Akinobu NAGAOKA and Yuji NAGAWA
Central Research Division, Takeda Chemical Industries, Ltd., Juso-Honmachi, Yodogawa-ku, Osaka 532, Japan
Accepted July 19, 1984

Abstract—An experimental model of amnesia induced by cerebral ischemia after one-trial passive avoidance learning was established to test the effects of a novel compound, 6-(10-hydroxydecyl)-2,3-dimethoxy-5-methyl-1,4-benzoquinone (idebenone, CV-2619), and some commonly used drugs in rats. One day after the vertebral artery was electrocauterized bilaterally, the common carotid artery was transiently occluded bilaterally to produce cerebral ischemia. The amnesia was estimated by the response latency for a rat to step from a light safety compartment to a dark compartment in which a foot-shock was given. The results of the retention test given 24 hr after the ischemia indicated that amnesia was successfully produced when the 200–600 sec ischemia was provided within 20 min after the avoidance learning. The effects of drugs on the amnesia induced by a 200-sec ischemia immediately after the avoidance learning were as follows: CV-2619 (10, 30 mg/kg, i.p. or p.o.) given before the retention test significantly increased the response latency, indicating a reversal effect on the amnesia. Physostigmine (0.1, 0.2 mg/kg, i.p.) and arginine-vasopressin (10 μg/kg, s.c.) were also effective, and calcium hopantenate (500 mg/kg, p.o.) showed a slight reversal action. Furthermore, CV-2619 (10 mg/kg, i.p.), given before or after the ischemia, significantly inhibited the appearance of amnesia. These findings suggest that CV-2619 exerts an ameliorating effect on memory disturbance induced by cerebral ischemia in rats.

Neurons in the central nervous system are vulnerable to ischemia. During cerebral ischemia, abrupt loss of neurological function occurs, which, if serious, leads to death. The neurological disfunction is naturally accompanied by deterioration of behavioral performance or memory function. In fact, cerebral ischemia induced by an injection of microspheres results in poor retention of active avoidance response in rats (1). Recently, Pulsinelli and Brierley (2) introduced a new method to produce cerebral ischemia in unanesthetized rats. The method consists of temporarily occluding the common carotid arteries and permanently interrupting the vertebral arteries. In the present study, this method was used to investigate whether amnesia is induced experimentally by the post-learning ischemia and, if so, whether it can be improved by drug treatment. The main purpose of the study was to examine in detail the effects of a novel compound, 6-(10-hydroxydecyl)-2,3-dimethoxy-5-methyl-1,4-benzoquinone (idebenone, CV-2619), on amnesia. The compound has an ameliorating effect on neurological symptoms caused by stroke in stroke-prone spontaneously hypertensive rats (in preparation). The other drugs used in this study were an acetylcholine-esterase inhibitor, physostigmine; a neuropeptide, arginine-vasopressin; and calcium hopantenate, which are known to be effective, to some extent, on experimentally induced amnesia or senile dementia (3–5).

Materials and Methods
Animals: Male Wistar rats weighing 270–
320 g, 9 weeks old, were individually housed and maintained in an air-conditioned room controlled for temperature (23–24°C), humidity (50–60%), and light (7:00–19:00); and given food and water ad libitum.

**Apparatus:** The rats were trained in a conventional step-through type passive avoidance training box divided into two compartments (an illuminated side with dimensions of $12 \times 12 \times 25$ cm and a dark side with dimensions of $12 \times 31 \times 25$ cm) by a sliding door and a 3 cm hurdle (6). The safe compartment, painted grey, had a flat floor and was illuminated by a 15 W fluorescent lamp located 1 cm outside the transparent wall. The dark side had a grid floor of 2 mm stainless rods, spaced 1.1 cm apart, used for giving a foot shock delivered by means of a scrambled dc constant current shock generator (Lehigh Valley Electronics, Model 113-04). The experimental room was kept as dark as possible and the masking noise of a fan was generated throughout the experiment.

**Ischemia treatment and avoidance learning:** Each experiment was repeated several times. The rats were anesthetized with pentobarbital, and their vertebral arteries were cauterized bilaterally (2) by a bipolar coagulator (Mizuhoika Kogyo, MICRO-1D), and threads were placed around their common carotid arteries. A sham-cauterized control group was also prepared. On the next day, the rats that behaved normally were used in the following experiments. The rats were trained in a one-trial passive avoidance task. In a habituation trial, 5 sec after the animal was placed in the safe compartment, the door was opened and the time required (response latency) to step through from the safe compartment to the dark compartment was measured. When all four paws were in the dark compartment, the door was closed; the rat was removed from the dark compartment 5 sec later. After 30–60 min, an acquisition trial was carried out in the same way except that when the rat was in the dark compartment, an inescapable foot shock (2 mA, 3 sec) was delivered. Rats that received no foot shock served as a non-shocked control group to assess the effect of drugs on motor function. After the acquisition trial, each rat was restrained by hand, and the bilateral common carotid arteries were exposed, by pulling the threads, and occluded with clips. The rats receiving vertebral artery cauterization and the carotid artery occlusion lost the righting reflex during the period of ischemia; these animals were assigned to the ischemic group. The sham-cauterized rats did not lose the righting reflex during the carotid occlusion and were assigned to the non-ischemic group.

In the test trial given 24 hr after the ischemia, the rat was again placed in the safe compartment and the response latency to enter the dark compartment was measured. Animals that did not enter the dark compartment within 300 sec were removed and assigned a ceiling score of 300 sec.

**Drugs:** The following drugs were used: 6-(10-hydroxydecyl)-2,3-dimethoxy-5-methyl-1,4-benzoquinone (idebenone, CV-2619, Takeda), physostigmine (Eserine salfate, Wako), arginine-vasopressin (Vaso-pressin, 465 I.U./mg, Sigma), calcium hopantenate (Tanabe). CV-2619 was suspended in a 5% gum arabic solution, and other drugs were dissolved in physiological saline. A volume of 0.2 ml/100 g body weight was administered; control rats were given an equal volume of saline. CV-2619 was given intraperitoneally or orally 30 min before the test trial and also intraperitoneally 30 min before or immediately after the ischemia. Physostigmine was given intraperitoneally 30 min, arginine-vasopressin subcutaneously 60 min, and calcium hopantenate orally 30 min before the test trial.

**Statistical analysis:** The degree of memory impairment was evaluated by the response latency and the percentage of rats showing latencies longer than a 100 or 200 sec criterion in the test trial. Median response latency in the test trial was analyzed by the two-tailed Mann-Whitney $U$ test. The percentage of rats achieving the criteria was analyzed by a two-tailed $z^2$ test.

**Results**

In all the experiments, no statistical differences were detected among the different groups in response latency in the acquisition trial.
Characteristics of cerebral ischemia-induced amnesia: In the intact rats (n=9), the response latencies in the acquisition and test trials were 6.3 and 215.9 sec, respectively, and the percentages of rats achieving the 100-sec criterion were 0 and 67, respectively, indicating a good avoidance performance. Under this condition of avoidance learning, the effect of duration of the ischemia on the retention of avoidance response was tested. As shown in Fig. 1, the response latency in the test trial decreased as the duration of the ischemia increased. In particular, when the ischemia lasted over 200 sec, the response latencies were significantly shorter than those of non-ischemic controls that had median latencies over 300 sec. The rats with the different durations of the ischemia did not show any abnormality in gross behavior at the test trial. Thus a retrograde amnesia was induced by an ischemia longer than 200 sec, whereas retention of the avoidance response was not affected by carotid occlusion alone.

The effect of varying the interval between the avoidance learning and the production of the ischemia was studied in animals subjected to ischemia for 5 min. As shown in Fig. 2, response latencies in the test trial were reduced when the interval was not longer than 60 min, whereas it was not reduced when the interval was prolonged to 120 min.

The persistence of amnesia was studied 1, 2, 4 and 8 days following a 5-min ischemia given immediately after the acquisition trial. On all the test days, the response latencies in the non-ischemic rats (n=11–12) were over 248.7 sec, and those in the ischemic rats (n=9–11) were below 17.9 sec (P<0.002), indicating that amnesia was consistently found and no spontaneous recovery from amnesia had occurred within 8 days. Thus, as a 5-min ischemia produced a severe amnesia, the duration of the ischemia was reduced to 200 sec in the subsequent pharmacological experiments.

Effect of CV-2619 on amnesia: CV-2619, administered intraperitoneally prior to the ischemia, did not affect response latencies in the acquisition trial, indicating that the compound did not disturb motor function. CV-2619 administered before the ischemia had a protective effect against amnesia; the dose-response relation was bell-shaped (Fig. 3). CV-2619 at a dose of 10 mg/kg significantly increased both response latencies (Fig. 3) and the percentage of rats achieving the 100-sec criterion (Table 1). This effective dose of CV-2619 did not affect the performance in the test trial of non-shocked control
animals that were rendered ischemic but did receive a foot shock. The differences between the shocked and non-shocked groups given 10 mg/kg of CV-2619 were statistically significant for both indices.

CV-2619 administered after the ischemia was produced also reversed the amnesia in a bell-shaped dose-response relation. A
peak effect on the response latencies was obtained with 10 mg/kg, and the percentage of rats achieving the criterion also increased significantly (Fig. 3, Table 1). The same dose of CV-2619 did not influence the response latency in the non-shocked control.

CV-2619 (10 and 30 mg/kg) administered intraperitoneally or orally before the test significantly increased, in a dose-related manner, the response latencies and the percentage of rats achieving the criterion (Fig. 4, Table 2). The same doses of CV-2619 did not increase response latencies in the non-shocked control animals.

Effects of physostigmine, arginine-vasopressin and calcium hopantenate on amnesia: The effects of the commonly used drugs on amnesia are summarized in Table 3. Physostigmine administered at doses of 0.1 and 0.2 mg/kg before the test significantly increased response latencies and the percentage of rats achieving the criteria, but 0.05 mg/kg did not show any effect on the indices. Similar improvement was observed when arginine-vasopressin was administered

![Fig. 4. Effect of CV-2619 administered intraperitoneally (i.p.) or orally (p.o.) before the test on the retention of passive avoidance response in rats. The 200-sec ischemia was produced immediately after the acquisition trial. The drug was given 30 min before the test trial performed 24 hr after the ischemia was produced. The number of rats used was 9–28. Abbreviations, cf Fig. 3. *P<0.05, **P<0.01, ***P<0.002 (U test) vs. CONT. +P<0.05, **+P<0.002 (U test) vs. the shocked group with the same dose.]

Table 2. Effect of CV-2619 administered intraperitoneally or orally before the test on the retention of passive avoidance response in ischemic rats (percentage of rats showing latencies longer than 100 or 200 sec)

| Dose (mg/kg) | Criteria (sec) |
|-------------|----------------|
|              | 100  | 200 |
|              | BCAO | CONT | BCAO | CONT |
| Intraperitoneal |     |      |     |      |
| CV-2619      |     |      |     |      |
| 0            | (11) | 100*** | 100*** |
| 3            | (10) | 40 | 0 |
| 10           | (11) | 64 | 0 |
| 30 (no FS)   | (9)  | 22 | 11 |
| 30 (no FS)   | (9)  | 22 | 11 |
| Oral         |     |      |     |      |
| CV-2619      |     |      |     |      |
| 3            | (15) | 27 | 13 |
| 10           | (17) | 59 | 24 |
| 30 (no FS)   | (18) | 67* | 28* |
| 30 (no FS)   | (9)  | 0*** | 0 |

Data are expressed as the percentage of rats achieving the criteria of the response latency in the retention test. Ischemia for 200 sec was produced immediately after the passive avoidance learning. The drug was given 30 min before the retention test trial made 24 hr after the ischemia was produced. BCAO: non-ischemic control group with saline, CONT: ischemic control with saline, no FS: rats without experience of a foot shock. *P<0.05, ***P<0.002 (χ² test) vs. CONT. #P<0.002 (χ² test) vs. the shocked group with the same dose.
at a dose of 10 μg/kg, 3 μg/kg had no effect. Calcium hopantenate at a dose of 500 mg/kg improved only the response latencies, but not the percentage of rats achieving the criteria; 200 mg/kg did not show significant effects on both indices. None of the drugs at effective doses affected either index in the non-shocked control animals.

Discussion

The present experiments showed that cerebral ischemia, produced for 200–600 sec after the acquisition of a passive avoidance response, resulted in the induction of retrograde amnesia in rats. The results are consistent with the well known findings that post-learning treatment with amnesic inducers, such as electroconvulsive shock and anoxia, impairs avoidance performance in the retention test in rats (7, 8). The impairment has been interpreted as a consequence of interference with memory retrieval (9). The results of the present study also indicate that a 5-min ischemia produced within 20 min after the avoidance learning led to amnesia, but ischemia produced later than 1 hr after the learning did not induce amnesia (Fig. 2). Furthermore, the amnesia induced by a 5-min ischemia immediately after the avoidance learning persisted for at least 8 days. This persistence contrasted with the finding of a spontaneous recovery from
cycloheximide-induced amnesia 3 days after the passive avoidance learning in mice (10). The results suggest that a definite time is required to consolidate the information obtained at the acquisition trial, but once long-term memory is established, it is difficult to disrupt by a transient cerebral ischemia.

The present study clearly indicates that CV-2619 reverses the amnesia induced by cerebral ischemia immediately after avoidance learning in rats. Moreover, it is significant that the effect of CV-2619 was observed regardless of whether the drug was administered before or after the ischemia or just before the retention test (Figs. 3, 4; Tables 1, 2). These results suggest that CV-2619 protects against ischemia-induced amnesia and facilitates the recall of stored memory. Since CV-2619 did not affect the test performance of the non-shocked controls, it is evident that the anti-amnesic effect of CV-2619 is not due to peripheral disruptive action on motor function but due to mediation of the central nervous system.

The dose-response relation on the anti-amnesic action of CV-2619 varied with the time of administration. A linear dose response was obtained when the compound was given before the test trial (Fig. 4), and a bell-shaped dose response was observed when it was given before or after the ischemia was produced (Fig. 3). This difference in the dose response pattern is also found in ACTH4_10 (11). It is uncertain whether the difference reflects the interaction between the doses used and the extent of the disturbance in the cerebral metabolism. Although the mechanism underlying the bell-shaped effect of CV-2619 are still unclear, the same response pattern has also been found for drugs such as a nootropic drug, aniracetam (12), an analog of ACTH4_9 (11) and a metabolite of arginine-vasopressin (13), known to improve memory function.

The amnesia in the present study can be interpreted as a result of dysfunction of the memory retrieval process (see the excellent review of experimental amnesia by Miller and Springer (9)). If the ischemia perfectly disrupted a memory consolidation process, no reference memory should exist to be recovered in a subsequent test. However, the fact that CV-2619 and the other drugs, administered before the test trial, successfully recovered the avoidance performance demonstrates that a trace of memory was present even after the ischemic treatment. Therefore, it is implied that CV-2619 and other drugs improve the deteriorated memory retrieval process.

In stroke-prone spontaneously hypertensive rats (SHRSP), CV-2619 protects the brain from the ischemic deficits induced by the bilateral common carotid artery occlusion and inhibits the increment of the lactate content and decrement of the ATP content (in preparation). Therefore, it is reasonable to suggest that the anti-amnesic action of CV-2619 may be related to improvement in cerebral metabolism. This speculation is supported by the fact that cerebral ischemia induced in rats by the injection of microspheres causes a deterioration of active avoidance learning that can be reversed by treatment with metabolic enhancers (1).

It was reported that physostigmine, arginine-vasopressin and some other drugs reversed cycloheximide- or puromycin-induced amnesia in mice (4, 14–16). Physostigmine and arginine-vasopressin also reversed amnesia induced in the model used in our experiments. This suggests that regardless of the inducers, the mechanism of amnesia may be based on common physiological changes in the brain. Clinically, cognitive dysfunction in senile dementia or other organic brain syndromes is reported to be treatable, to some extent, with drugs that include cerebral metabolic enhancers, vasodilators, neuropeptides, neurotransmitters, and their precursors (17, 18). Physostigmine (19), arginine-vasopressin (20) and calcium hopantenate (5) are already used for such treatments. On the basis of the present study, it is expected that CV-2619 would also have an ameliorating effect on the cognitive dysfunction.

Acknowledgements: We are grateful to Dr. J.R. Miller for comments on the manuscript and Katsuichi Ikeda and Emiko Fujiwara for their excellent technical assistance.

References
1 Le Poncin-Lafitte, M., Grosdemouge, C., Roy-Billon, C., Potrat, P., Lespinasse, P. and Rapin,
J.R.: Short-term memory and cerebral ischemia: Pharmacological application. Eur. Neurol. 20, 265–269 (1981)

2 Pulsinelli, W.A. and Brierley, J.B.: A new model of bilateral hemispheric ischemia in the unanesthetized rat. Stroke 10, 267–272 (1979)

3 Davis, J.W., Thomas, R.K., Jr. and Adams, H.E.: Interaction of scopolamine and physostigmine with ECS and one trial learning. Physiol. Behav. 6, 219–222 (1971)

4 Walter, R., Hoffman, P.L., Flexner, J.B. and Flexner, L.B.: Neurohypophysial hormones, analogues, and fragments: their effect on puromycin-induced amnesia. Proc. Natl. Acad. Sci. U.S.A. 72, 4180–4184 (1975)

5 Miyazaki, M. and Kobayashi, T.: Effect of Ca2+ chelatantate (HOPA) on the senile mental disorders caused by the brain vascular disturbance. Clin. Rep. 13, 179–195 (1979) (in Japanese)

6 Yamazaki, N., Niihama, K. and Imada, H.: Effects of electroconvulsive shock on the retention of avoidance behavior. Japan. J. Psychol. 48, 303–306 (1977) (in Japanese)

7 Chorover, S.L. and Schiller, P.H.: Short-term retrograde amnesia in rats. J. Comp. Physiol. Psychol. 59, 73–78 (1965)

8 Rigter, H., van Riezen, H. and de Wied, D.: The effects of ACTH- and vasopressin-analogue on CO2 induced retrograde amnesia in rats. Physiol. Behav. 13, 381–388 (1974)

9 Miller, R.R. and Springer, A.D.: Amnesia, consolidation, and retrieval. Psychol. Rev. 80, 69–79 (1973)

10 Squire, L.R. and Barondes, S.H.: Variable decay of memory and its recovery in cycloheximide-treated mice. Proc. Natl. Acad. Sci. U.S.A. 69, 1416–1420 (1972)

11 Fekete, M. and de Wied, D.: Dose-related facilitation and inhibition of passive avoidance behavior by the ACTH4-9 analog (ORG2766), Pharmacol. Biochem. Behav. 17, 177–182 (1982)

12 Cumin, R., Bandle, E.F., Gamzu, E. and Haefely, W.E.: Effects of the novel compound aniracetam (Ro13–5057) upon impaired learning and memory in rodents. Psychopharmacology (Berlin) 78, 104–111 (1982)

13 Burbach, J.P.H., Kovacs, G.L., de Wied, D., van Nispen, J.W. and Greven, H.M.: A major metabolite of arginine vasopressin in the brain is a highly potent neuropeptide. Science 221, 1310–1312 (1983)

14 Yamazaki, N., Shintani, M., Saji, Y. and Nagawa, Y.: Interaction with cholinergic drugs in reversal of cycloheximide-induced amnesia by thyrotropin-releasing hormone and its analog DN-1417 in mice. Japan. J. Psychopharmacol. 3, 127–136 (1983) (in Japanese)

15 Stamm, E.R. and Schlesinger, K.: Effects of cycloheximide administered in conjunction with nicotine on retention of a passive avoidance task in mice. J. Comp. Physiol. Psychol. 94, 1184–1190 (1980)

16 Quartermain, D. and Botwinick, C.Y.: Role of the biogenic amines in the reversal of cycloheximide-induced amnesia. J. Comp. Physiol. Psychol. 88, 386–401 (1975)

17 Fisman, M.: Clinical pharmacology of senile dementia. Prog. Neuropsychopharmacol. 5, 447–457 (1981)

18 Kent, S.: Preventing senile dementia: hope for the future. Geriatrics 36, 130–136 (1981)

19 Thal, L.J. and Fuld, P.A.: Memory enhancement with oral physostigmine in Alzheimer’s disease. N. Engl. J. Med. 308, 720 (1983)

20 Weingartner, H., Kaye, W., Gold, P., Smallberg, S., Peterson, R., Gillin, J.C. and Ebert, M.: Vasopressin treatment of cognitive dysfunction in progressive dementia. Life Sci. 29, 2721–2726 (1981)