Pergamon

Pharmacology Biochemistry and Behavior, Vol. 50, No. 4, pp. 509–515, 1995

0091-3057/95 $9.50 + .00

0091-3057(94)00326-2

Ameliorating Effect of Clentiazem (TA-3090), a New Ca Antagonist, on the Impaired Learning Ability of Poststroke SHRSP

KIYOSHI KINOSHITA,[1] MICHIO YAMAMURA AND YUZO MATSUOKA

Pharmacological Research Laboratory, Tanabe Seiyaku Co. Ltd., 2-2-50 Kawagishi, Toda-shi, Saitama 335, Japan

Received 4 October 1993

KINOSHITA, K., M. YAMAMURA AND Y. MATSUOKA. *Ameliorating effect of clentiazem (TA-3090), a new Ca antagonist, on the impaired learning ability of poststroke SHRSP.* PHARMACOL BIOCHEM BEHAV **50**(4) 509–515, 1995.—We investigated the influence of clentiazem (8-chloro-diltiazem, (+)(2S,3S)-3-acetoxy-8-chloro-5-[2-(dimethylamino)ethyl]-2,3-dihydro-2-(4-methoxyphenyl)-1,5-benzothiazepin-4(5H)-one maleate, TA-3090) and other Ca antagonists on the impaired learning ability in poststroke spontaneous hypertensive rats stroke prone (SHRSP) using a shuttle box. SHRSP were given 1% NaCl solution as drinking water until the development of stroke (poststroke SHRSP). Active avoidance task was started from the fourth day after the onset of stroke. Over a period of 20 sessions (15 trials/session/day), the mean avoidance rate of the poststroke SHRSP was significantly lower than that of the nonstroke control group that was not given the salt solution. Clentiazem (1, 3, and 10 mg/kg/day) administered orally for 23 days after the development of stroke increased the avoidance rate in a dose-dependent manner. Nimodipine (1 and 10 mg/kg/day) also increased the avoidance rate, but its effect was not dose dependent. We also investigated the influence of clentiazem and other Ca antagonists on the passive avoidance performance by mice using a light-dark box. Clentiazem (3, 10, and 30 mg/kg) and other Ca antagonists, nimodipine (1 mg/kg) and nicardipine (10 mg/kg), all failed to protect either CO_2- or electroconvulsive shock (ECS)-induced avoidance deficit when administered orally 1 h before the acquisition or retention trial. These results may be explained by the possibility that the Ca antagonists may ameliorate the impaired learning ability in poststroke SHRSP through their improving effect on the cerebral circulation disturbance.

Ca-antagonist Clentiazem Poststroke SHRSP Memory impairment Active avoidance

MANY reports have shown that Ca antagonists exert an ameliorating effect on cerebrovascular impairments in various experimental models (5,9,21,30,33,35). Most of them state that these drugs improve cerebrovascular circulatory disorders and minimize the cerebral neuronal damage histologically, and that neurological symptoms or physical functional abnormalities are ameliorated by the Ca antagonist treatment. However, there is a paucity of information concerning the influence of these drugs on the impaired learning ability of experimental animals with cerebrovascular disorders (3,7).

Clentiazem (8-chloro-diltiazem, (+)(2S,3S)-3-acetoxy-8-chloro-5-[2-(dimethylamino)ethyl]-2,3-dihydro-2-(4-methoxyphenyl)-1,5-benzothiazepin-4(5H)-one maleate, TA-3090) was synthesized by Tanabe Seiyaku Co. Ltd. and is currently under development as a novel calcium antagonist (Fig. 1). The new diltiazem derivative has been known to have a potent hypotensive action when orally administered to various hypertensive animals such as spontaneous hypertensive rats (SHR) and deoxycorticosterone acetate (DOCA)-salt hypertensive rats (26). In addition, clentiazem increases vertebral and coronary blood flow in anesthetized dogs by intra-arterial or intravenous administration (23,24). Clentiazem is thought to be a Ca antagonist with a selectivity for the cerebral artery, because its Ca antagonistic action in the basilar artery is more potent than in the coronary, renal, and mesenteric arteries (15). Moreover, the potencies of clentiazem are known to be 10 times greater in the basilar artery and two to three times greater in the other arteries than those of diltiazem in dogs (15).

In spontaneously hypertensive rats stroke prone (SHRSP), chronic administration of clentiazem from the prestroke stage delayed the occurrence of stroke (13). Moreover, in salt-loaded SHRSP, its chronic administration after the occur-

[1] To whom requests for reprints should be addressed.

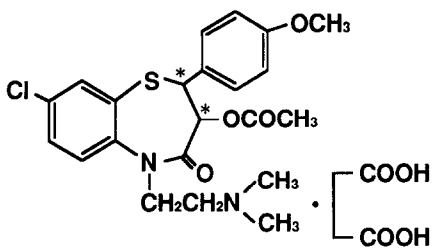

FIG. 1. Chemical structure of clentiazem maleate (TA-3090)*: asymmetric carbon atom.

rence of stroke suppressed to some extent the histological damage of the brain and delayed the development of neurological deficits (14). These results suggest that clentiazem may have protective, prophylactic, and/or even therapeutic effects against the brain damage by stroke.

The aim of our present study is to examine the influence of clentiazem and other Ca antagonists on the impaired learning ability of animals with cerebrovascular disorders, in comparison with their effects on the conventional amnesia of animals without cerebrovascular impairments. Therefore, we investigated the effects of these drugs on the impaired learning ability of poststroke SHRSP performing the active avoidance task and compared them with their effects on the deficit of passive avoidance in mice with amnesia induced by CO_2 gas exposure or electroconvulsive shock (ECS).

METHOD

Experiment 1: Active Avoidance Task in SHRSP

Animals. Male SHRSP (201–302 g, 10 weeks old, n = 12–19) were used. SHRSP, derived from the Okamoto-Aoki strain of SHR, was obtained from Dr. K. Okamoto of Kinki University Medical School, Osaka, Japan, and bred by Marugo Research Service Co., Ltd. (Saitama, Japan). Each animal was housed in one compartment (15 × 25 × 14 cm) of a stainless steel five-compartment wire mesh cage (75 × 25 × 14 cm). They were kept in an air-conditioned room with controlled temperature (23 ± 1°C), humidity (55 ± 5%), and lighting (lights on 0630 through 1830 h). They were allowed free access to a standard pellet diet (CRF-1, Oriental Yeast Co., Ltd.) and tap water.

After SHRSP were divided into seven groups based on their body weights, six groups were given 1% NaCl solution ad lib as drinking water until the development of stroke. The remaining one group was given tap water and served as the nonstroke control.

The animals of the 1% NaCl solution-treated groups were observed daily (0900–1100) for their neurologic symptoms according to Nagaoka's neurologic scoring scale (25) shown in Table 1 until the development of stroke. The stroke development was identified by the appearance of abnormal behaviors, such as seizure and hyperirritability, which were scored as 2 points or higher. Immediately after confirming the occurrence of stroke, 1% NaCl solution was replaced by tap water.

The animals that died by stroke before the task and those that were given 1% NaCl and yet developed no strokes for 55 days were omitted.

Apparatus. Active avoidance response was examined with a two-way shuttle box (RSC-001, Muromachi Kikai, 46 × 19.5 × 20 cm), consisting of two identical compartments. A buzz (2.8 kHz, 85–90 dB) of 5-s duration as a conditioned stimulus (CS) was given with a buzzer placed on the ceiling in the center of the box. If the animal did not move to the other compartment during the CS period, a 0.6 mA electric stimulus was applied for a maximum of 5 s as an unconditioned stimulus (UCS) through the grid floor made of 3 mm wide stainless steel rods, spaced 11 mm apart, connected to the shock generator/scrambler (SGS-001, Muromachi Kikai). The intertrial interval (ITI) was varied in a range of 20–60 s (variable interval). The conditioned avoidance response was considered to be positive when the animal moved to the other compartment by CS alone and, thus, avoided the electric shock (UCS). The shuttle box was enclosed in a ventilated sound-attenuating chamber (MC-050, Muromachi Kikai, 80 × 60 × 60 cm), and control of the test schedule, measurement of avoidance responses, and mathematical procedures were performed with an automatic data processing system (DAC-104, Muromachi Kikai).

Experimental design. From the day of stroke occurrence, the SHRSP were orally treated with clentiazem or nimodipine once a day. From the fourth day after the occurrence of stroke, the active avoidance learning task was started and continued for 20 successive days. Each animal was given one session (15 trials) a day 1 h after the drug administration.

All experimental data were collected under opened conditions.

Experiment 2: Passive Avoidance Task in Mice

Animals. Male mice (Japan SLC: ddY, 27–29 g, 5 weeks old, n = 24–41) were used. They were housed in groups of 25 in plastic cages (42 × 26 × 15 cm) before use and were transferred to stainless steel five-compartment wire mesh cages (75 × 25 × 14 cm) in groups of six per each compartment prior to the experimental period (for 2 days). Animals were kept under the same controlled conditions described for rats.

Apparatus. A conventional step-through type passive avoidance training box, divided into two compartments (a light room, 9 × 7 × 14 cm, and a dark room, 14 × 14 × 14 cm), was prepared on the basis of the light-dark box of Bammer and Chesher (1). A guillotine door opening (3 × 3 cm) was made on the floor in the center of the partition between the two compartments. The light room had a flat floor and was illuminated by a 15 W white fluorescent rod light located 25 cm above the box floor. The dark room had a grid floor, which was made of 3 mm wide stainless steel rods spaced 7 mm apart and used to give a foot shock delivered by a solid-state shocker/scrambler (model 111-33, Leihigh Valley Electronics). The box was enclosed in a ventilated sound attenuating chamber (Bio Medica LTD, 100 × 60 × 60 cm) with white noise for masking.

Experimental design. The guillotine door on the path was opened in advance, and a mouse was gently placed in the light room with its head away from the path. When the mouse had

TABLE 1

SCORING SYSTEM FOR NEUROLOGICAL SYMPTOMS IN SHRSP WITH STROKE

Score	Neurological Symptoms
0	Normal
1	Slight decrease in motor activity, or slight excitement
2	Marked decrease in motor activity, or hyperirritability
3	No walking
4	Cannot stand without support, paralysis of hind limbs

put all four limbs in the dark room, the guillotine door was closed and a 0.5 mA foot shock was applied immediately for 3 s (acquisition trial).

In a CO_2 gas exposure experiment, the mouse was immediately transferred to an air-tight glass container (700 cc) supplied with 100% CO_2 gas at a flow rate of 3000 ml/min and kept anoxic for 20 s. The mouse was taken out of the container and then ventilated artificially by massaging on the chest until spontaneous motion was provoked by touch stimulation after restoration of the righting reflex. After that, the mouse was exposed to CO_2 gas once again for 15 s under the same conditions as above. Twenty-four hours after the acquisition trial, the mouse was placed again in the light room in the same manner as the day before, and the time until their four limbs completely entered the dark room (latency of response) was measured (retention trial). The latency was measured to a maximum of 300 s, and that exceeding this level was regarded as 300 s.

In an ECS treatment experiment, immediately after the acquisition trial, the mouse received an electroshock (AC 20 mA, 0.5 s; ECS) delivered by electroshocker (USA-200, Unique Medical), through the electrodes (3 mm in diameter, 3 mm in length) inserted in both ears with 0.85% NaCl solution. After restoration of the righting reflex, the mouse was treated with ECS under the same condition as above. The other procedures were the same as above.

Drugs were orally administered 1 h before the acquisition trial or 1 h before the retention trial.

Drugs

Clentiazem (Lot No. 503010, 603010) and nicardipine (Lot No. 0478170B) used were synthesized at the Organic Chemistry Research Laboratory of Tanabe Seiyaku Co. Ltd. (Saitama, Japan). Nimodipine was purchased from Sigma Chemical.

Clentiazem and nicardipine were dissolved or suspended in distilled water. Nimodipine was suspended in distilled water with the use of two to three drops of Tween 80 (Katayama Chemical). The drugs were orally administered to SHRSP in a volume of 2 ml/kg and to mice in a volume of 10 ml/kg.

Statistical Analysis

All data were expressed as the mean ± SE. Statistical analysis was carried out by Mann–Whitney's *U*-test and Duncan's multiple comparison test followed by Kruskal–Wallis's *H*-test. The differences were considered to be significant when $p < 0.05$.

RESULTS

Experiment 1: Active Avoidance Task in SHRSP

SHRSP began to show the typical seizure (score 2) of the development of stroke, such as repetitive unilateral lifting of a paw, repetitive upper-body twisting, or tremor-like generalized convulsion, within 2 weeks after first receiving 1% NaCl solution. Out of 117 animals, three animals that developed no strokes for 55 days and 16 animals that died of stroke before the task were omitted from the study.

The mean body weight of nonstroke SHRSP slightly increased (about 3.1%) during the experimental period. On the other hand, the mean body weight of poststroke SHRSP decreased transiently for a few days after the onset of stroke. Thereafter, the body weight increased gradually to the prestroke level within 8–9 days after the development of stroke (Fig. 2).

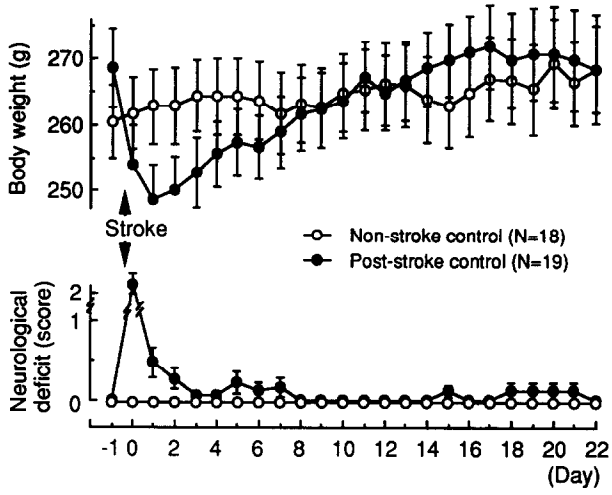

FIG. 2. Body weight and neurologic deficit in nonstroke and poststroke SHRSP.

The mean neurologic score of each group during the first 4 days from the development of stroke is shown in Table 2. The mean neurologic score was 2.16–2.46 on the day of the development of stroke, but it decreased immediately to 0.46–1.09 on the next day (Table 2). During the rest of the experimental period, most poststroke animals did not show neurological deficits or convulsion (Fig. 2). In addition, responsiveness of the poststroke SHRSP to the buzzer or foot shock was almost the same as that of the nonstroke SHRSP.

The mean avoidance rate of active avoidance response in the nonstroke control group promptly increased to about 90% during the first 10 sessions, and remained at the same level (89–97%) thereafter (Fig. 3). However, the avoidance rate of the poststroke control group rose only gradually (below 40% compared with the nonstroke control group during the first 10 sessions), and did not exceed 53% during the second 10 sessions. Administration of clentiazem (1, 3, and 10 mg/kg/day) dose dependently increased the mean avoidance rate (Fig. 4). Especially, that of the 10 mg/kg group reached the level of 60% or more during the first 10 sessions and kept 60–80% levels thereafter until the end of the experiment. The remaining two groups treated with the lower doses of clentiazem also showed avoidance rates higher than the poststroke control, although the differences of statistical significance were sporadic. In the groups administered nimodipine (1 and 10 mg/kg/day), higher avoidance rates were observed at the dose of 1 mg/kg/day rather than at 10 mg/kg, but statistical significance was observed only at the second session (Fig. 5).

Experiment 2: Passive Avoidance Task in Mice

CO_2 gas exposure-induced amnesia. The mean latency in the retention trial was shortened to one-half or less in the anoxia control group exposed to CO_2 gas immediately after the acquisition trial as compared with the nonanoxia group. When clentiazem (3, 10, and 30 mg/kg) was administered 60 min before the acquisition or 60 min before the retention trial, the mean latency was not significantly different from that in the anoxia control group (Table 3).

ECS-induced amnesia. The mean latency in the retention trial was shortened to about one-third in the control group treated with ECS immediately after the acquisition trial or to

TABLE 2

NEUROLOGIC SCORES FROM THE ONSET OF THE STROKE TO THE FIRST DAY OF SHUTTLE AVOIDANCE TASK IN EACH POSTSTROKE GROUP

Drugs	Dose (mg/kg)	n	Neurologic Score (Mean ± SE) Day 0	Day 1	Day 2	Day 3
	–	19	2.16 ± 0.13	0.47 ± 0.19	0.26 ± 0.13	0.05 ± 0.05
Clentiazem	1	12	2.31 ± 0.11	0.46 ± 0.22	0.31 ± 0.21	0.23 ± 0.17
	3	16	2.25 ± 0.10	1.00 ± 0.25	0.29 ± 0.19	0.00 ± 0.00
	10	12	2.42 ± 0.17	0.67 ± 0.26	0.25 ± 0.13	0.08 ± 0.08
Nimodipine	1	12	2.46 ± 0.13	0.91 ± 0.28	0.16 ± 0.11	0.00 ± 0.00
	10	12	2.42 ± 0.14	1.09 ± 0.30	0.64 ± 0.23	0.39 ± 0.18

Day 0 = development of stroke.

about one-sixth in the group treated before the retention trial as compared with the untreated group. Clentiazem (3, 10, and 30 mg/kg), when administered 60 min either before the acquisition trial or 60 min before the retention trial, caused no change in the mean latency. Likewise, administration of either nimodipine (1 mg/kg) or nicardipine (10 mg/kg) before the acquisition trial produced no significant change in the latency (Table 4).

DISCUSSION

Results of the active avoidance task might possibly be influenced by not only changes in learning ability but also by changes in gross behavior such as the locomotor ability of experimental animals. Therefore, when the avoidance rate of the poststroke SHRSP does not increase as rapidly as that of the nonstroke SHRSP, it is necessary to consider the possibility of not only the decrease in learning ability but also the decreases in hearing ability for a buzzer as the conditioned stimulus, sensitivity against the foot shock, and locomotor ability for avoidance. When the poststroke SHRSP continued to be given the NaCl-containing diet, their gross conditions such as locomotor function worsened and their diet intake and body weight both decreased, and they died in the early stage after the development of stroke (13,25). Probably for these reasons there have been few reports of learning experiments using poststroke SHRSP. In this study, however, 1% NaCl drinking water was changed to normal tap water immediately after the development of stroke, and then redevelopment of stroke and death of poststroke SHRSP were minimized. In the present study, the mean body weight of the poststroke SHRSP decreased only transiently after the development of stroke, but soon started to increase on the second day after the stroke, and caught up with that of the nonstroke SHRSP on the 10th day after the development of stroke. Although the mean neurological score was high (2.16–2.46) on the day of the onset of stroke, it came down to the normal level after the second day, and the response to the buzzer and foot shock of poststroke SHRSP on the third day onward was almost the same as that of the nonstroke SHRSP. Therefore, the slow rise of the avoidance rate of the poststroke SHRSP was thought to be not due to locomotor dysfunction but due to some kind of dysfunction in learning ability. It has been already reported

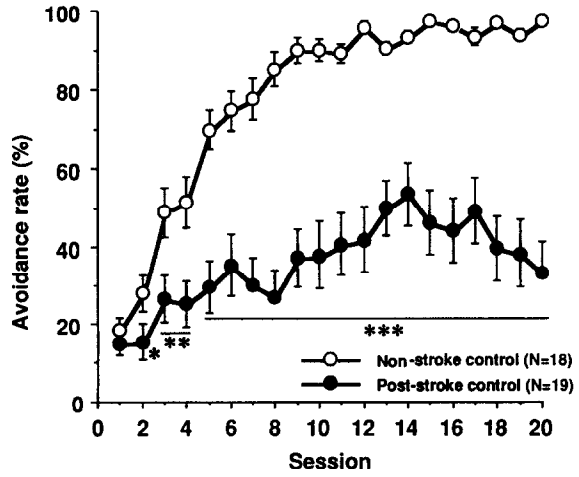

FIG. 3. Shuttle avoidance learning ability in non- and poststroke SHRSP. Distilled water was orally administered 60 min before the start of each session. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$ compared with nonstroke control (Mann–Whitney U-test).

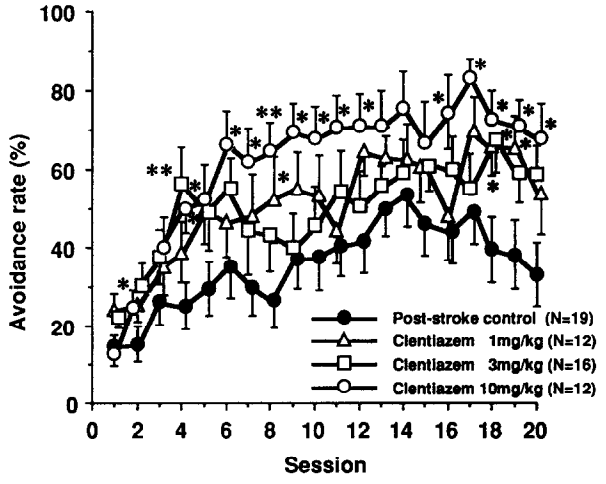

FIG. 4. Effect of clentiazem on shuttle avoidance learning ability in poststroke SHRSP. The avoidance rate of the poststroke control group plotted was the same in Fig. 3. The drug was administered orally 60 min before the start of each session. Distilled water was administered orally to the control. $*p < 0.05$ compared with poststroke control (Duncan's multiple comparison test).

TABLE 3

ABSENCE OF THE AMELIORATING EFFECT OF CLENTIAZEM ON THE LATENCY OF RETENTION TRIAL IN ANOXIC MICE

Treatment				
Drug	Dose (mg/kg/)	CO_2 Exposure	No. of Mice	Latency (s, mean ± SE)
1) Preacquisition trial				
Control	–	–[a]	27	166.2 ± 17.2]*
	–	+	26	83.0 ± 14.8]*
Clentiazem	3	+	27	76.4 ± 12.6 NS[b]
	10	+	26	77.8 ± 11.6 NS
	30	+	24	60.6 ± 9.5 NS
2) Preretention trial				
Control	–	–	29	150.5 ± 18.6]*
	–	+	29	54.0 ± 6.8]*
Clentiazem	3	+	29	38.0 ± 8.2 NS
	10	+	29	47.9 ± 10.4 NS
	30	+	29	35.9 ± 5.2 NS

Clentiazem was administered orally to mice 60 min before the acquisition trial 1) or retention trial 2). Foot shock (0.5 mA, 3 s).

[a]Without CO_2 exposure.

[b]NS; not significantly different from the anoxic control group (Duncan's multiple comparison test).

*$p < 0.01$, compared with the respective control group with CO_2 exposure (Mann-Whitney *U*-test).

TABLE 4

ABSENCE OF THE AMELIORATING EFFECT OF Ca ANTAGONISTS ON THE LATENCY OF RETENTION TRIAL IN ELECTROCONVULSIVE SHOCK (ECS)-TREATED MICE

Treatment				
Drug	Dose (mg/kg)	ECS	No. of Mice	Latency (s, mean ± SE)
1) Preacquisition trial				
Control	–	–[a]	30	173.2 ± 18.1]*
	–	+	28	51.4 ± 6.2]*
Clentiazem	3	+	23	44.4 ± 5.7 NS[b]
	10	+	31	48.3 ± 8.6 NS
	30	+	33	52.0 ± 10.0 NS
Control	–	–	40	192.4 ± 15.5]*
	–	+	41	58.7 ± 9.5]*
Nimodipine	1	+	30	61.6 ± 2.6 NS
Nicardipine	10	+	40	72.9 ± 10.7 NS
2) Preretention trial				
Control	–	–	32	196.7 ± 15.1]*
	–	+	32	29.5 ± 6.5]*
Clentiazem	3	+	32	23.1 ± 2.8 NS
	10	+	32	23.9 ± 4.9 NS
	30	+	32	26.1 ± 4.1 NS

Clentiazem was administered orally to mice 60 min before the acquisition trial 1) or retention trial 2). Foot shock (0.5 mA, 3 s).

[a]Without ECS.

[b]NS; not significantly different from the ECS-treated control group (Duncan's multiple comparison test).

*$p < 0.01$, compared with the respective control group with ECS (Mann-Whitney *U*-test).

that the learning ability of nonstroke SHRSP using the maze task is the same or rather a little better than that of normotensive Wistar-Kyoto (WKY) rats, the origin of SHRSP (18-21). Then, it could be suggested that the only one episode of stroke could produce serious damage to the learning ability of the animals.

It was clearly shown that chronic oral administration of clentiazem (1, 3, and 10 mg/kg/day) ameliorated the impairment of learning ability in the poststroke SHRSP on the active avoidance task. The doses of clentiazem adopted in this study are known to exert no influence on the spontaneous motor activity in rats (12). Accordingly, the increase in the avoidance rate in the clentiazem-treated groups was not due to the change in spontaneous movement caused by the drug but due to an ameliorating effect of the drug against the dysfunction of learning ability. Nimodipine, at the low dose (1 mg/kg/day) rather than at the high dose (10 mg/kg/day), showed an ameliorating effect, although the effect was obviously less potent than that of clentiazem. Nimodipine is known to show the same level of amelioration at the oral doses of 1 and 3 mg/kg against the learning dysfunction induced by removal of the cerebral cortex in rats (17), and it also affects the learning ability with an inverted U-shaped dose-response curve in chicks on the visual discrimination task (4). Although the real reason for the reversion of efficacy of nimodipine at the higher dose is unclear, the dose of 10 mg/kg of nimodipine was apparently an overdose.

It may be difficult to imagine that clentiazem acts as a Ca antagonist directly on one of the learning and memory processes such as acquisition, consolidation, retention, and recall processes. However, clentiazem is thought to have a brain protective action because it inhibits Ca influx into cultured hippocampal neurons from rat embryos (2) and prevents the KCN-induced neuronal death of cultured hippocampal neurons from neonatal rats in a dose-dependent manner (34). From these reports, it is conceivable that clentiazem may influence the learning and memory disorders induced by neuronal damage through its anti-ischemic, neuroprotective action. If so, clentiazem is expected to show an ameliorating effect on experimental amnesia through the same action. Therefore, the action of clentiazem was tested against the experimental amnesia induced by anoxia or ECS treatment. In these models, the memory impairments are considered to be due to a disturbance of the processes after the consolidation process of memory, because CO_2 gas exposure or ECS treatment was performed immediately after the acquisition trial. The memory impairments induced by physical (brain injury, electroconvulsive shock) or chemical (drugs, anoxic treatment) means in the mouse on the one-trial passive avoidance task are improved by various drugs including cholinergic drugs (physostigmine, oxotremorine) (28,31) and nootropic drugs (piracetum, oxiracetum) (29,31). In the present study, however, clentiazem administered orally 60 min before the acquisition or retention trial did not cause a recovery from the amnesia in these models. Neither nimodipine nor nicardipine administered 60 min before the acquisition trial caused recovery from the amnesia induced by ECS treatment. As to the reason why clentiazem did not influence the amnesia induced by CO_2 gas exposure or ECS treatment, the doses used in this study may have been too low. Namely, the maximal intracerebral concentration of clentiazem administered orally at the dose of 10 mg/kg is thought to be only 0.3 μM (6). On the other hand, the concentration of clentiazem to show the protective action against KCN-induced cell death in cultured neuronal cells is known to be 3-10 μM (34). Then, an ameliorating effect on memory processes based on its direct neuroprotective action could not have been expected. Therefore, in the poststroke experiment, the ameliorating effect of clentiazem was not thought to be due to its direct neuroprotective action.

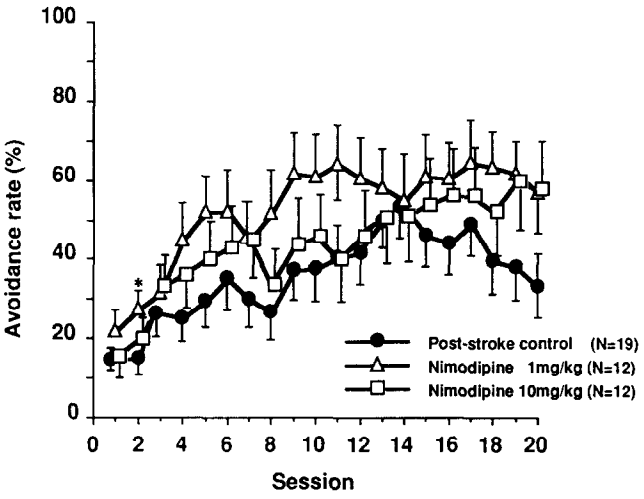

FIG. 5. Effect of nimodipine on shuttle avoidance learning ability in poststroke SHRSP. The avoidance rate of the poststroke control group plotted was the same in Fig. 3. The drug was administered orally 60 min before the start of each session. Distilled water was administered orally to the control. *$p < 0.05$ compared with poststroke control (Duncan's multiple comparison test).

It has been reported that clentiazem exhibits its hypotensive action by single oral administration (3 mg/kg), single intravenous administration (0.3 and 0.6 mg/kg) or chronic administration (10 mg/kg/day × 5 weeks) in SHRSP or SHR (8,10,26). Moreover, clentiazem has a high selectivity for the cerebral arteries (13), and this drug certainly prevented the angiographic narrowing and vasospasm after subarachinoid hemorrage and reduced the infarct size after the middle cerebral artery occlusion in rabbits (11,32). In addition, clentiazem shows a prophylactic effect on the development of stroke (13) and therapeutic effects on the neurological symptoms, and prevent histological damage of the brain (14). Nimodipine has been reported to increase cerebral blood flow in both normal animals and cerebral artery impairment models (16) and to ameliorate the decrease in the learning ability of SHR on the light-dark discrimination task (27). Taken together with these findings, the ameliorating effects of clentiazem and nimodipine may probably be due to their improving effects on the impairment of cerebral circulation after the stroke. These actions are thought to stimulate recovery from the histological damage of brain in SHRSP, and protect from redevelopment of stroke.

In conclusion, clentiazem clearly showed an ameliorating effect on the impaired learning ability of SHRSP after the development of stroke. This ameliorating action is considered to be not due to its direct action on the acquisition or retention process of memory but probably due to an amelioration of the general condition produced by its improving action on the cerebral circulation after stroke.

ACKNOWLEDGEMENTS

The authors would like to thank Dr. R. Ishida for helpful suggestions throughout the study and Dr. S. Takeyama for reading the manuscript.

REFERENCES

1. Bammer, G.; Chesher, G. B. An analysis of some effects of ethanol on performance in a passive avoidance task. Psychopharmacology (Berlin) 77:66–73; 1982.
2. Bleakman, D.; Chard, P. S.; Foucart, S.; Miller, R. J. Block of neuronal Ca^{++} influx by the anti-ischemic agent TA3090. J. Pharmacol. Exp. Ther. 259:430–439; 1991.
3. Block, F.; Jaspers, R. M. A.; Heim, C.; Sontag, K.-H. S-Emopamil ameliorates ischemic brain damage in rats: Histological and behavioural approaches. Life Sci. 47:1511–1518; 1990.
4. Deyo, R. A.; Panksepp, J.; Conner, R. L. Nimodipine alters acquisition of a visual discrimination task. Behav. Neural Biol. 53:149–152; 1990.
5. Edmonds, H. L., Jr.; Wauquier, A.; Melis, W.; van den Broeck, W. A. E.; van Loon, J.; Janssen, P. A. J. Improved short-term neurological recovery with flunarizine in a canine model of cardiac arrest. Am. J. Emerg. Med. 3:150–155; 1985.
6. Fukushima, T.; Nakamura, S.; Ito, Y.; Ohashi, M.; Takaichi, O. Metabolic fate of clentiazem in rats after consecutive oral administration: Plasma and tissue distribution and metabolites in plasma and brain. Xenobiol. Metab. Disp. 7:833–843; 1992.
7. Grotta, J. C.; Pettigrew, L. C.; Rosenbaum, D.; Reid, C.; Rhoades, H.; McCandless, D. Efficacy and mechanism of action of a calcium channel blocker after global cerebral ischemia in rats. Stroke 19:447–454; 1988.
8. Isshiki, T.; Pegram, B. L.; Frohlich, E. D. Immediate and prolonged hemodynamic effects of TA-3090 on spontaneously hypertensive (SHR) and normal Wistar-Kyoto (WKY) rats. Cardiovas. Drugs Ther. 2:539–547; 1988.
9. Isshiki, T.; Pegram, B. L.; Frohlich, E. D. Hemodynamic comparison of diltiazem and TA-3090 in spontaneously hypertensive and normal Wistar-Kyoto rats. Am. J. Cardiol. 62:79–84; 1988.
10. Iwatsuki, N,; Ono, K.; Koga, Y.; Amaha, K. Prevention of postischemic hypoperfusion after canine cardiac arrest by nicardipine. Crit. Care Med. 15:313–317; 1987.
11. Kaminow, L.; Bevan, J. Clentiazem reduced infarct size in rabbit middle cerebral artery occlusion. Stroke 22:242–246; 1991.
12. Katayama, T.; Yamamura, M.; Kudo, Y., Matsuoka, Y.; Ishida, R. General pharmacology of Ca antagonist clentiazem (TA-3090): Effects on central nervous system. Jpn. Pharmacol. Ther. 20: 2857–2866; 1992.
13. Kikkawa, K.; Murata, S.; Iwasaki, H.; Toriumi, W.; Banno, K.; Nagao, T. Prophylactic effects of a new 1,5-benzothiazepine calcium antagonist on stroke in salt-loaded stroke-prone spontaneously hypertensive rats. Arzneimittelforschung/Drug Res. 42: 781–786; 1992.
14. Kikkawa, K.; Murata, S.; Kurosawa, H.; Toriumi, W.; Iwasaki, H.; Nagao, T. Effect of clentiazem (TA-3090) with posttreatment on neurologic and histologic disorders of stroke-prone spontaneously hypertensive rats with history of stroke. J. Cardiovas. Pharmacol. 23:166–174; 1994.
15. Kikkawa, K.; Murata, S.; Nagao, T. Calcium antagonistic and spasmolytic activities of a new 1,5-benzothiazepine derivative in isolated canine and monkey arteries. Arzneimittelforschung/ Drug Res. 38:526–531; 1988.
16. Langley, M. S.; Sorkin, E. M. Nimodipine; A review of its pharmacodynamic and pharmacokinetic properties, and therapeutic potential in cerebrovascular disease. Drugs 37:669–699; 1989.
17. LeVere, T. E.; Burgler, T.; Sandin, M.; Gray-Silvia, S. Recovery of function after brain damage: Facilitation by the calcium entry blocker nimodipine. Behav. Neurosci. 103:561–565; 1989.
18. Matsuo, T.; Kihara, T.; Tanimura, T. Learning ability of SHR and SHRSP in the single water T-maze. Med. J. Kinki Univ. 12: 473–482; 1987.
19. Matsuo, T.; Kihara, T.; Tanimura, T. Learning ability of SHR and SHRSP in the under water T-maze. Med. J. Kinki Univ. 13: 1–6; 1988.
20. Matsuo, T.; Kihara, T.; Tanimura, T. Learning ability of SHR and SHRSP in the water E maze. Med. J. Kinki Univ. 14:1–7; 1989.
21. Matsuo, T.; Kihara, T.; Tanimura, T. Learning ability of SHR and SHRSP in the Morris water maze. Med. J. Kinki Univ. 15:1–4; 1990.
22. Meyer, F. B.; Anderson, R. E.; Yaksh, T. L.; Sundt, T. M., Jr. Effect of nimodipine on intracellular brain pH, cortical blood flow and EEG in experimental focal cerebral ischemia. J. Neurosurg. 64:617–626; 1986.
23. Murata, S.; Kikkawa, K.; Nagao, T. Cerebral vasodilating and spasmolytic actions of a new Ca-antagonist, clentiazem (TA-3090), in anesthetized animals. J. Cardiovas. Pharmacol. 19:790–797; 1992.
24. Murata, S.; Kikkawa, H.; Yabana, H.; Nagao, T. Cardiovascular effects of a new 1,5-benzothiazepine calcium antagonist in anesthetized dogs. Arzneimittelforschung/Drug Res. 38:521–525; 1988.
25. Nagaoka, A.; Kakihana, M.; Fujiwara, K. Effects of idebenone (CV-2619) on neurological deficits following cerebrovascular lesions in stroke-prone spontaneously hypertensive rats. Jpn. Pharmacol. Ther. 12:5345–5352; 1984.
26. Narita, H.; Murata, S.; Yabana, H.; Kikkawa, K.; Sugawara, Y.; Akimoto, Y.; Nagao, T. Long-lasting hypotensive and antihypertensive effects of a new 1,5-benzothiazepine calcium antagonist in hypertensive rats and renal hypertensive dogs. Arzneimittleforschung/Drug Res. 38:515–520; 1988.
27. Nomura, M. Effect of nimodipine on brightness discrimination learning test in Wistar-Kyoto and spontaneously hypertensive rats. Arzneimittleforschung/Drug Res. 38:1282–1286; 1988.
28. Santucci, A. C.; Kanof, P. D.; Haroutunian, V. Effect of physostigmine on memory consolidation and retrieval processes in intact and nucleus basalis-lesioned rats. Psychopharmacology (Berlin) 99:70–74; 1989.
29. Spignoli, G.; Pepeu, G. Oxiracetum prevents electroshock-induced decrease in brain acetylcholine and amnesia. Eur. J. Pharmacol. 126:253–257; 1986.
30. Steen, P. A.; Newberg, L. A.; Milde, J. H.; Miclenfelder, J. D. Nimodipine improves cerebral blood flow and neurologic recovery after complete cerebral ischemia in the dog. J. Cereb. Blood Flow Metab. 3:38–43; 1983.
31. Verlous, R.; Scotto, A.-M.; Gobert, J.; Welfert, E. Effects of nootropic drugs in a scopolamine-induced amnesia model in mice. Psychopharmacology (Berlin) 95:226–230; 1988.
32. Vorkapic, P.; Bevan, J. A.; Bevan, R. D. Clentiazem protects against chronic cerebral vasospasm in basilar artery. Stroke 22: 1409–1413; 1991.
33. Wauquier, A.; Edmonds, H. L., Jr.; van den Broeck, W. A. E.; Melis, W.; van Loon, J. Effects of flunarizine on recovery of cerebral function following cardiac arrest. Neuropsychobiology 13:147–151; 1985.
34. Yamauchi, R.; Kobayashi, T.; Murata, S. Effects of clentiazem (TA-3090) on hippocampal neuronal death by anoxia. Jpn. J. Pharmacol. 55(Suppl. I):406; 1991.
35. Young, W. L.; Josovitz, K.; Morales, O.; Chien, S. The effect of nimodipine on postischemic cerebral glucose utilization and blood flow in the rat. Anesthesiology 67:54–59; 1987.