Pergamon

Pharmacology Biochemistry and Behavior, Vol. 48, No. 3, pp. 613–619, 1994

0091-3057/94 $6.00 + .00

0091-3057(93)E0073-D

Levemopamil Injection After Cerebral Oligemia Reduces Spatial Memory Deficits in Rats

CHRISTINE HEIM,*[1] MARIA SIEKLUCKA† AND KARL-HEINZ SONTAG‡

*Department of Psychiatry, University of Göttingen, v.Siebold-Str. 5, D-37075 Göttingen, Germany
†Department of Pharmacology, Medical Academy, Lublin, Poland
‡Department of Pharmacology, Max-Planck-Institute for Experimental Medicine, Hermann-Rein-Str. 3, D-37075 Göttingen, Germany

Received 3 May 1993

HEIM, C., M. SIEKLUCKA AND K.-H. SONTAG. *Levemopamil injection after cerebral oligemia reduces spatial memory deficits in rats.* PHARMACOL BIOCHEM BEHAV **48**(3) 613–619, 1994.—Transient reduction of cerebral blood flow to oligemic levels as produced by bilateral clamping of carotid arteries (BCCA) in pentobarbital anesthetized Wistar rats leads to spatial orientation deficiencies in a water maze test 8–10 days after surgery. These deficiencies are more pronounced in 4-month-old than in 6-week-old animals. Levemopamil [(2S)-2-isopropyl-5-(methylphenethylamino)-2-phenylvaleronitrile hydrochloride], a Ca^{2+} channel blocker and 5-HT_2 antagonist, prevents the deficiencies in groups of animals of both ages, even when administered 24 h after the transient vessel occlusion. Levemopamil did not influence the maze performance of sham-operated control rats. Levemopamil, therefore, appears to modulate mechanisms that are altered specifically either by, or as a consequence of, the BCCA procedure. Levemopamil did not influence the altered GABA or ACh content in different vulnerable brain structures following BCCA, showing that the substance acts via additional mechanisms affected by the BCCA procedure.

Oligemia Oxygen deficit Spatial memory Learning Water maze Cerebral protection
Ca^{2+} antagonist 5-HT_2 antagonist GABA Acetylcholine

BILATERAL clamping of the carotid arteries of pentobarbital anesthetized rats for 24 min results in spatial orientation deficiencies when analyzed by the Morris water maze test (21). These deficiencies are similar to those observed in rats with hippocampal damage caused by an ischemic insult due to four-vessel occlusion (4-VO) (21). Damage to the pyramidal CA1 neurons as well as spatial memory deficiencies in animals after 4-VO can be ameliorated by preinjection of 30 mg/kg of levemopamil (3), a Ca^{2+}- and 5-HT_2-antagonist (18). It has been suggested that the observed protection is due to a vasodilatatory effect, both 5-HT_2- and Ca^{2+}-antagonistic effects being implicated, preventing deleterious calcium overload in ischematized neurons (3).

BCCA does not cause hippocampal damage (16,21,29), but reduces the cerebral blood flow (4,5). A recent study demonstrated that levemopamil applied 30 min before BCCA improves the spatial learning capacity of animals in a water maze (5). As levemopamil was reported to have protective effects in ischemia even when administered after the insult (15,24,28), experiments were undertaken to examine whether posttreatment with the drug also influences the functional deficits of BCCA animals. To this end, rats subjected to BCCA for 24 min or to sham operations were administered with levemopamil 24 h after surgery. Neurochemical studies were undertaken to see whether levemopamil affected the GABA or ACh content, as long-lasting changes in these two neurotransmitters occur after BCCA treatment (16,32).

METHOD

Male Han-Wistar rats (own breeding) aged 6 weeks (200–220 g) or 4 months (350–450 g) were used. The animals were housed in groups of eight or five and exposed to a 12 L : 12 D cycle (room temperature of 21 °C, humidity of 55%) and given food and water ad lib.

Surgical Procedure

The animals were anesthetized with pentobarbital (Nembutal, Sanofi, 60 mg/kg IP) and the common carotid arteries were clearly exposed and clamped with thread (Bilateral

[1] Requests for reprints should be addressed to Christine Heim at her current address: Department of Psychiatry, University of Göttingen, von-Siebold-Str.5, D-37075 Göttingen, Germany.

Clamping of the Carotid Arteries: BCCA) for 24 min. After removing the threads, the restoration of the blood flow was visually inspected and the surrounding skin sutured. Sham operated animals had their carotids exposed but not clamped.

One series of experiments was conducted using 6-week-old animals: one set of animals was sham operated and injected with 0.9% NaCl (n = 6), or levemopamil [(2S)-2-isopropyl-5-(methylphenethylamino)-2-phenyl-valeronitrile hydrochloride] 30 mg/kg IP (n = 6) 24 h later; another set of BCCA animals received 0.9% NaCl (n = 9) or was injected with 30 mg/kg levemopamil (n = 8) 24 h later.

Another series of experiments used 4-month-old rats and consisted of a sham-operated group of animals that was injected with 0.9% NaCl 24 h after surgery (n = 9), a group of animals treated with 30 mg/kg levemopamil 24 h after BCCA (n = 8), and a group of BCCA animals that received 0.9% NaCl 24 h later (n = 7).

A second series of 4-month-old rats (sham n = 8 or BCCA n = 8, respectively) was operated as described above and confronted with a visible platform in the same water maze set.

An additional set consisting of 12 sham-operated rats (half injected with 0.9% NaCl and the other 6 with 30 mg/kg of levemopamil IP 24 h later) and 12 BCCA rats (6 receiving 0.9% NaCl and the other 6 30 mg/kg levemopamil IP 24 h later) was killed 9 days after surgery and specific brain areas were analyzed for GABA and ACh content.

Behavioral Tests

Navigation task with an invisible platform. Spatial memory was tested in a water maze [(26); see also (21)] 8–10 days after surgery. Care was taken to ensure that the overall configuration of the room remained unchanged during all experiments. After one habituation trial of 1 min on the first day, during which no platform was present, the animals were tested during the next 3 days for their ability to escape from the water (26°C) by climbing on a platform that was mounted 1 cm below the water level and, therefore, not visible to the rats when swimming. Each experiment consisted of 20 escape trials spread out over 3 successive days. Each rat received one block of four escape trials on the first experimental day and two blocks of four trials on each of the following 2 days. During each trial, a rat was placed into the water, facing the wall, at one of four starting positions around the pool, randomly assigned. For each rat, the location of the hidden platform remained unchanged during the whole experiment. There were no intramaze cues signaling the location of the platform; thus, rats had to navigate with the help of extramaze spatial information. The latency to find the platform was measured on each trial. If the animals could not find the platform within 120 s they were placed on it by hand. After each trial 20 s was allowed for resting on the platform and for inspecting the surrounding and processing spatial information. Immediately after the final escape trial, spatial bias was tested by removing the platform and counting the number of crossings of the original platform position during a probe trial lasting 1 min. As all trials were recorded on video tape, it was possible to record the swim path for each rat on escape trials 13–20 on a map of the pool and to determine the distance swum. The average swimming speed could be calculated from these data.

Navigation task with a visible platform. An additional experiment was carried out by offering a visible platform to a separate group of animals to exclude performance deficits due to the BCCA treatment. The procedure used was as for the experiments with the invisible platform, except during the last 16 trials (experimental day 2 and 3) where a white platform above the water level was presented to the animals.

Determination of Regional GABA Content

Rats were decapitated 9 days after BCCA. The heads were immediately placed in liquid nitrogen for 6 s (42). The brains were removed and the different structures, viz. hippocampus (HPC), frontal cortex (FCX), substantia nigra (SN), amygdala and piriform cortex (AMY), striatum (STRIA), hypothalamus (HYP), thalamus (THAL), superior colliculus (CS), cerebellum (CER), and pons, dissected on an ice plate. GABA content was measured according to the method of Lowe et al. (25) and modified by Sutton and Simmonds (35), as described by Kleinrok and Turski (23). In a previous study it was shown that during the decapitation and dissection, no increase of GABA in the tissue could be observed (31). The GABA content was calculated in μmol/g of wet tissue.

Determination of Regional ACh Content

Animals were decapitated and their heads plunged immediately in liquid nitrogen for about 6 s. HPC and FCX were then dissected and stored frozen in liquid nitrogen. Tissues were subsequently homogenized in 5 vol of ice-cold 0.1 M perchloric acid, adjusted to pH 4.0 with 1 M CH_3COOK. After 10 min in ice, the samples were centrifuged at 10,000 $\times$ g for 10 min and stored at -50°C. Samples were then thawed, centrifuged at 5,000 $\times$ g for 5 min and 20 μl of the supernatant was subjected to HPLC analysis (33). The HPLC procedure involved the use of an enzyme reactor containing acetylcholineesterase and choline oxidase immobilized onto a sepharose column combined with an electrochemical detector equipped with a platinum working electrode and Ag/AgCl reference electrode (Biometra, Göttingen, Germany). Standard curves of freshly prepared ACh were produced daily. The ACh content was expressed as nmol/g of fresh tissue.

Statistics

An unpaired Student's t-test was used to calculate differences in the GABA and ACh content. Analysis of variance for unequal groups (ANOVA) followed by Gabriel's multiple comparison of means (13) were used for calculation of differences in escape latency, distance swum, and swimming speed. Differences between groups of sham-operated and BCCA-treated animals after posttreatment with either NaCl or levemopamil, respectively, were considered to be significant at $p < 0.05$. Differences in the number of crossings of the original platform location were calculated using the Mann–Whitney U-test.

RESULTS

Behavioral Studies

Navigation task with an invisible platform. Young animals at the age of 6 weeks as well as animals at the age of 4 months learned to locate the platform and improved their capacity to escape from the water. Sham-operated animals of both groups reached a very high level of performance by climbing the platform within a few seconds (mean: 6.11 ± 1.30 or 11.7 ± 3.74, for 6-week or 4-month-old rats, respectively, for the last four escape trials) (Fig. 1A,B).

Six-week-old animals, after 24 min of BCCA treatment, showed an increased escape latency compared to their sham-operated controls [group differences: $F(1,13) = 12.45$, $p <$

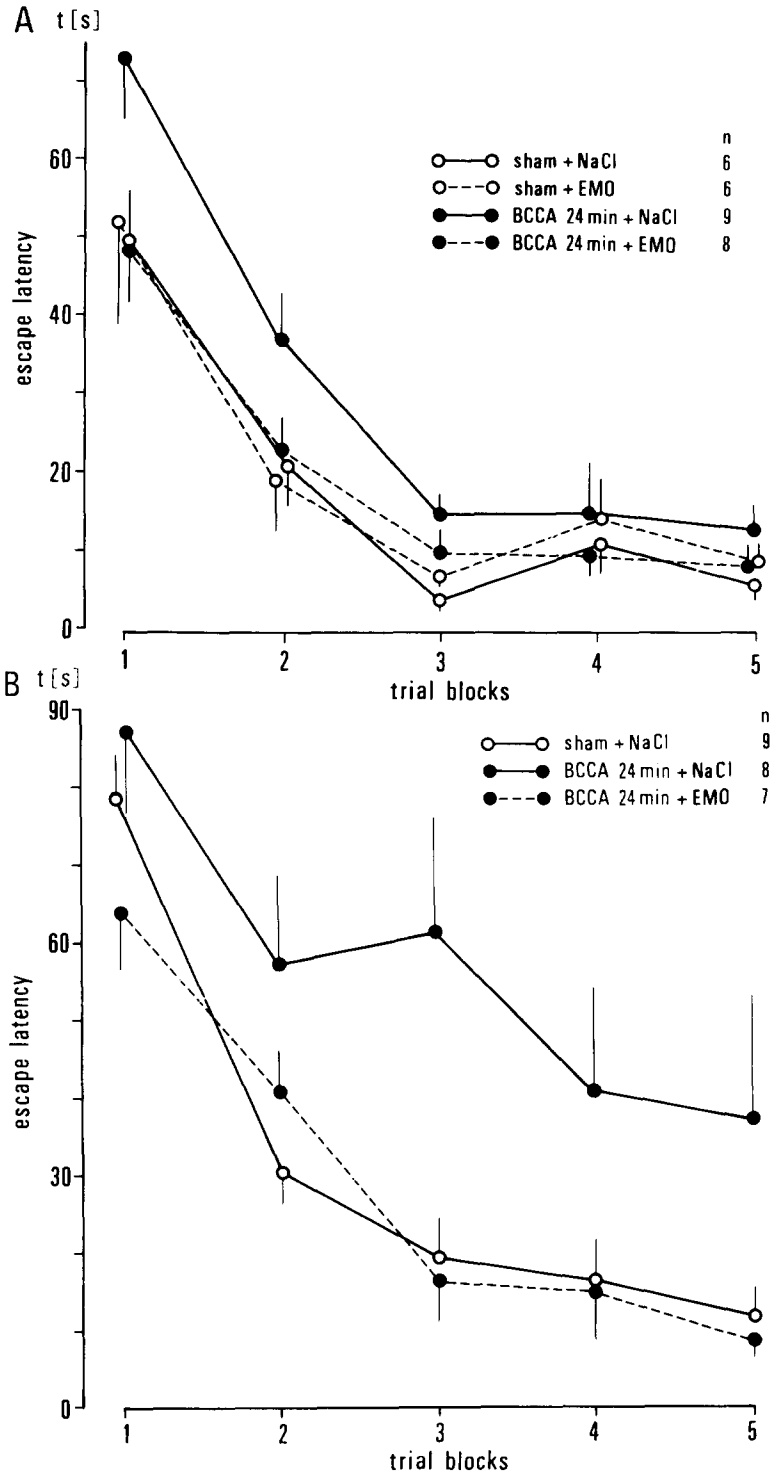

FIG. 1. Escape latency to reach the hidden platform during five trial blocks. (A) Group of 6-week-old animals; $*p < 0.05$: BCCA + NaCl vs. sham + NaCl, BCCA + NaCl vs. BCCA + levemopamil (EMO), BCCA + NaCl vs. sham + EMO. (B) Group of 4-month-old rats; $*p < 0.05$: BCCA + NaCl vs. sham + NaCl, BCCA + NaCl vs. BCCA + EMO. Statistics by analysis of variance for unequal groups (ANOVA) and Gabriel's multiple comparison of means. One trial block consisted of four escape trials, each starting from one of the start locations SW, NW, NO, SO, according to the points of the compass, semirandomly assigned. Rats were tested 8–10 days after sham operation or BCCA, respectively. EMO or NaCl was injected 24 h after surgery.

TABLE 1

DISTANCE SWUM (cm) TO REACH THE HIDDEN PLATFORM AS MEASURED DURING THE LAST EIGHT ESCAPE TRIALS (MEAN OVER EIGHT TRIALS)

	A	
BCCA + NaCl	vs. Sham + NaCl : 123.84 ± 16.27	NS
198.46 ± 19.00	vs. BCCA + EMO : 163.32 ± 19.35	NS
Sham + EMO	vs. BCCA + EMO : 163.32 ± 19.35	NS
184.40 ± 26.52	vs. Sham + NaCl : 123.48 ± 16.27	NS
	B	
Sham	vs. BCCA + NaCl : 369.30 ± 87.08	NS
260.90 ± 25.43	vs. BCCA + EMO : 199.82 ± 30.11	NS
BCCA + NaCl	vs. BCCA + EMO : 199.82 ± 30.11	*
369.30 ± 87.08		

Values are presented in cm as mean ± SEM. (A) Group of 6-week-old animals. (B) Group of 4-month-old animals. Group differences by analysis of variance for unequal groups (ANOVA) and Gabriel's multiple comparison of means: *$p < 0.05$ vs. BCCA + NaCl.

TABLE 3

SPATIAL BIAS

	n	No. of Crossings	Range
	A		
Sham	12	4	3-8
BCCA + NaCl	9	3*	2-5
BCCA + EMO	8	4‡	2-7
	B		
Sham	9	4	2-9
BCCA + NaCl	8	2.5†	0-4
BCCA + EMO	7	4‡	2-11

Number of crossings of the former platform location (No. of crossings) as median and range during the final probe trial where no platform was present. (A) For the group of 6-week-old rats. Since there was no difference between sham animals with and without posttreatment, both groups were taken together for calculation. (B) For the group of 4-month-old animals.

Statistics by Mann-Whitney U-test: *$p < 0.01$; †$p < 0.05$ vs. sham; ‡$p < 0.05$ vs. BCCA + NaCl.

0.0008, ANOVA; Fig. 1A]. However, on the last eight escape trials, both the distance swum as well as the speed of NaCl-treated BCCA animals showed insignificant differences compared to saline-treated sham-operated controls (Tables 1A, 2A).

As in 6-week-old animals, the escape latency of 4-month-old NaCl-treated BCCA rats was significantly increased [group differences: $F(1,14) = 16.85$, $p < 0.0001$, ANOVA; Fig. 1B]. However, in this group the swimming speed measured during the last eight escape trials was significantly reduced compared with equivalent sham-operated control rats (Table 2B), although the distance swum was not significantly altered (Table 1B). Observation of the NaCl-treated BCCA animals suggests that this decreased swimming speed was due to repeated horizontal rotations, or scrabbling at the edge of the pool before switching their behavior and swimming towards the platform position.

TABLE 2

SWIMMING SPEED (cm/s) AS MEASURED DURING THE LAST EIGHT ESCAPE TRIALS (MEAN OVER EIGHT TRIALS)

	A	
BCCA + NaCl	vs. Sham + NaCl : 20.84 ± 1.68	NS
16.63 ± 0.70	vs. BCCA + EMO : 22.26 ± 1.29	†
SHAM + EMO	vs. BCCA + EMO : 22.26 ± 1.29	NS
19.20 ± 0.65	vs. Sham + NaCl : 20.84 ± 1.68	NS
	B	
Sham	vs. BCCA + NaCl : 13.78 ± 0.52	*
23.48 ± 0.80	vs. BCCA + EMO : 23.13 ± 0.86	NS
BCCA + NaCl	vs. BCCA + EMO : 23.13 ± 0.86	†
13.78 ± 0.52		

Values in cm/s as mean ± SEM. (A) Group of 6-week-old animals. (B) Group of animals at an age of 4 months. Group differences were calculated by analysis of variance for unequal groups (ANOVA) and Gabriel's multiple comparison of means: *$p < 0.05$ vs. sham, †$p < 0.05$ vs. BCCA + NaCl.

Although the increase in time to reach the platform was greater for 4-month-old BCCA animals (Fig. 1B) than for 6-week-old BCCA animals (Fig. 1A), 24 h posttreatment with levemopamil prevented the increase in escape latency in both groups [group differences: $F(3,25) = 6.27$, $p < 0.0005$ for 6-week-old rats; $F(2,21) = 14.30$, $p < 0.0001$ for 4-month-old animals; Fig. 1A and B].

By measuring the distance swum, it could be shown that the reduction in escape latency was due to a shorter swim path in the group of 4-month-old rats only [group differences: $F(2, 21) = 5.65$, $p < 0.0042$, for BCCA+EMO vs. BCCA+ NaCl; Table 1B]. On the other hand, the calculation of swimming speed showed a significant increase for levemopamil posttreated BCCA animals of both groups compared to saline treated BCCA rats [group differences: $F(3,25) = 4.22$, $p < 0.0064$ for 6-week-old rats, Table 2A; $F(2,21) = 32.63$, $p = 0.0001$ for 4-month-old rats, Table 2B]. In the probe trial, a significant decrease in the crossing of the original platform location could be observed in both BCCA groups (Table 3A,B). This decrease could also be prevented by posttreatment with levemopamil (Table 3A,B).

In sham-operated animals, the application of levemopamil did not lead to a further improvement in the maze performance (Fig. 1A, Tables 1A, 2A). The swimming speed and the distance swum were not significantly altered (Tables 1A, 2A).

Navigation task with a visible platform. The mean escape latency during the last eight escape trials was the same for BCCA animals and sham-operated controls (3.95 ± 0.44 s for sham or 3.94 ± 0.43 s for BCCA, respectively). With respect to the distance swum (mean ± SEM: 101.369 ± 13.42 cm for sham or 98.58 ± 9.03 cm for BCCA, respectively) and the swimming speed (mean ± SEM: 27.02 ± 0.95 cm/s for sham or 27.85 ± 1.52 cm/s for BCCA, respec-

TABLE 4

GABA CONTENT IN DIFFERENT BRAIN STRUCTURES AS MEASURED 9 DAYS AFTER 24 MIN OF BCCA

	SN	HPC	FCX
Sham + NaCl	3.62 ± 0.14	2.40 ± 0.08	2.34 ± 0.07
Sham + EMO	3.62 ± 0.17	2.48 ± 0.07	2.40 ± 0.11
BCCA + NaCl	4.37 ± 0.19*	3.07 ± 0.07†	2.84 ± 0.11‡
BCCA + EMO	4.33 ± 0.18¶	3.09 ± 0.05#	2.89 ± 0.13¶

Values are presented in μmol/g of fresh tissue. SN = substantia nigra; HPC = hippocampus; FCX = frontal cortex; EMO = levemopamil. NaCl or levemopamil in a dose of 30 mg/kg, respectively, were injected IP 24 h after surgery.

Significances: *¶$p < 0.05$ vs. sham + NaCl or EMO, respectively; ‡$p < 0.01$ vs. sham + NaCl or EMO, respectively; †#$p < 0.001$ vs. sham + NaCl or EMO, respectively; Student's t-test.

tively), no significant difference between the groups could be determined.

Determination of Regional GABA or ACh Content

Neurochemical studies revealed a significant increase in the GABA content in the HPC, FCX, SN (not in AMY, STRIA, HYP, THAL, CS, CER, pons; not shown) and a significant decrease in the ACh content in the HPC (not in frontal cortex) 9 days after 24 min of BCCA (Tables 4 and 5). Levemopamil treatment did not have an effect on GABA or ACh content in either BCCA or sham-operated control rats (Tables 4 and 5).

DISCUSSION

As reported previously (5,16,21), BCCA treatment for 24 min resulted in an increased latency to escape from the pool by finding the hidden platform. This deficiency in spatial orientation as shown here is more pronounced in 4-month-old animals than in 6-week-old rats [see also (38)]. It is also shown that a single application of levemopamil in a dose of 30 mg/kg 24 h after the carotid occlusion prevents the spatial memory deficiencies in young and matured rats. In sham-operated animals, levemopamil had no clear effect, though there was a tendency for the swimming speed and path to be reduced and increased, respectively, suggesting that the substance has a different effect on BCCA-treated rats from that of sham-operated controls.

TABLE 5

ACh CONTENT AS MEASURED 9 DAYS AFTER SURGERY

	HPC	FCX
Sham + NaCl	19.85 ± 0.89	11.91 ± 0.92
Sham + EMO	20.64 ± 0.85	12.32 ± 1.20
BCCA + NaCl	14.92 ± 1.05*	11.46 ± 0.67
BCCA + EMO	14.07 ± 1.07†	11.45 ± 0.76

Values are presented in nmol/g of fresh tissue. HPC: hippocampus; FCX: frontal cortex. NaCl or levemopamil (EMO), 30 mg/kg, respectively, were injected IP 24 h after either BCCA or sham operation.

Significances: *$p < 0.01$ vs. sham + NaCl or EMO, respectively; †$p < 0.001$ vs. sham + NaCl or EMO, respectively; Student's t-test.

The present results show that the swimming speed of BCCA animals is decreased, but this is only significant in the older animals. According to Whishaw et al. (41), swim speed decreases with age and is associated with the age-related decline of the cholinergic system (14). The decreased swimming speed in BCCA animals, however, is probably due to difficulties in using distant spatial cues rather than to defects of motor activation per se, as in the water maze test with the visible platform BCCA animals showed no deficiencies in reaching the platform. These findings are consistent with the idea that motor performance is not affected by the BCCA treatment. This is supported by the open-field behavioral studies that have revealed that, 1 week after 24 min of BCCA, rats are more active over a 24-h observation period as measured by their distance traveled and rearing movements (16,39) compared to sham-operated controls. Neither in the chimney test nor on the rota rod did BCCA animals manifest motor deficits (16).

The improvement in escape latency brought about by levemopamil seems to be caused by an increased swimming speed, a change which was also observed in 4-VO animals pretreated with levemopamil (3). This conclusion is supported by the analysis of the distance swum where differences between treated and untreated BCCA groups were barely measurable. Only in the older animals, aged 4 months, was the improvement of the maze performance achieved by a decrease in the distance swum combined with a drastic increase in the swimming speed; however—in contrast to results obtained in 4-VO animals (3)—this increase did not surpass sham control values.

The results obtained with test procedures involving a visible or invisible platform support the suggestion that prior knowledge of the platform position increases the swimming speed and fastest escape from the water (in the shortest time). A drug that will increase the speed to fulfil the navigation task may, therefore, be helpful for a successful navigation capacity during a given time.

The improvement of memory impairment caused by post-administration of levemopamil 24 h after BCCA suggests that subsequent to reperfusion an induction of memory deficiencies takes place as a result of different mechanisms, such as transmitter imbalances, viz. long-lasting GABA increase and/or ACh decrease (16,32).

Deficits in the cholinergic system are supposed to be related to memory impairment during aging and dementia, e.g., Alzheimer's disease (AD) (1,9,12,30). Selective decrease in cholinergic activity in the hippocampus and cortex causes re-

markable impairments in spatial memory tasks (40), and intrahippocampal cholinergic transplants can ameliorate the deficiencies (19,37). Moreover, an altered GABA neurotransmission was reported to be connected to memory deficits (6,7,20) and to be involved in induction mechanisms of long-term potentiation (LTP) (8,10,34) or long-term depression (LTD) (2). However, neither the increase in GABA content, which occurs 12 h after the vessel occlusion (17), nor the decrease in ACh content after the BCCA treatment could be abolished by posttreatment of levemopamil. Two alternative interpretations of these findings are: a) neither transmitter alteration might be connected with the observed behavioral deficiencies, and b) the ameliorating effects are achieved via compensatory mechanisms and/or direct action on learning and memory processes.

One possible explanation for the observed effects of levemopamil could be a direct action on learning and memory processes, i.e., via its Ca^{2+}-channel-blocking property. Hypoxia/ischaemia induces glutamate/aspartate neurotransmitter release, lactate accumulation, and reduced glucose supply, leading to disturbances of the intracellular Ca^{2+} homeostasis and Ca^{2+} sequestration. Elevation in cytosolic Ca^{2+} could gradually lead to activation of catalytic enzymes which may be directly responsible for cell dysfunction and cell injury. Other catabolic activities could include the activation of phospholipases that can degrade cell membranes, thus initiating a cascade of destructive reactions, such as release of arachidonic acid, production of oxygen free radicals, and peroxidative degradation of lipid membranes (22). In recent studies on the BCCA model, lactate accumulation in striatum, hippocampus, and frontal cortex (4) could be measured during the occlusion, although 14 days later an elicited lipid peroxidation was evident in the frontal cortex and striatum of rats (27).

Difficulties in reducing the Ca^{2+}-induced afterhyperpolarization (AHP) that occur in aging hippocampal neurons might be responsible for behavioral learning deficits (11). Administration of nimodipine, a high-threshold calcium (L-type) blocking substance, could reduce AHP—a process that was consistent with behavioral facilitation. This capacity to facilitate hippocampally dependent learning tasks was observed especially in aging or affected animals (11,22).

We suggest, therefore, that similar mechanisms in respect to Ca^{2+} homoeostasis may act in BCCA, ischemia, and aging, and that the pharmacological intervention with a Ca^{2+} antagonist 24 h after carotid occlusion may interrupt further consequences of disturbed Ca^{2+} homoeostasis. The vasodilatatory effect (36) and the possibly increased glucose supply may contribute to repairing functional deficits.

In summary levemopamil, a Ca^{2+} and 5-HT_2 antagonist, prevents the spatial orientation deficiencies of rats elicited by 24 min of BCCA, when administered 24 h after surgery. This effect was achieved by a reduction in the distance swum and, even more, by an elevation of the reduced swimming speed back to control levels. Levemopamil did not influence the GABA or ACh content in BCCA or sham-operated control rats, although the transmitter contents were altered by the BCCA procedure itself, suggesting a specific action of the substance on learning and memory processes per se, possibly working by an interruption of membrane specific disturbances of Ca^{2+} homeostasis. In nonaffected animals, no improvement of the maze performance was observable after levemopamil treatment. Thus, levemopamil seems to be of good therapeutic value for the posttreatment of spatial memory impairments occurring after transient cerebral oligemic events.

ACKNOWLEDGEMENTS

The skillful technical assistance of H. Ropte and M. Schindler is gratefully acknowledged. Levemopamil was kindly provided by Knoll AG, Germany. The authors are indebted to Dr. H. Stadler (Biometra, Göttingen, Germany) for the use of his technical equipment for ACh measurement. We thank Dr. N. N. Osborne for his helpful comments while preparing the manuscript and Dr. R. Morris for his valuable criticism. This study was supported by Deutsche Forschungs-gemeinschaft, and BMFT 01KL9101/0 Sonderforschungsbereich 330 "Organprotektion."

REFERENCES

1. Bartus, R. T.; Dean, R. L.; Beer, B.; Lippa, A. S. The cholinergic hypothesis of geriatric memory dysfunction. Science 217:408–417; 1982.
2. Bashir, Z. I.; Collingride, G. L. NMDA receptor-dependent transient homo- and heterosynaptic depression in picrotoxin-treated hippocampal slices. Eur. J. Neurosci. 4:485–490; 1992.
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. Block, F.; Sieklucka, M.; Schmidt-Kastner, R.; Heim, C.; Sontag, K.-H. Metabolic changes during and after transient clamping of carotid arteries in normotensive rats. Brain Res. Bull. 31:91–96; 1993.
5. Block, F.; Szabo, L.; Jaspers, R. M. A.; Heim, C.; Sontag, K.-H. Levemopamil reduces spatial learning deficits following transient occlusion of common carotid arteries in normotensive rats. Acta Neurol. Scand. 88:35–40; 1993.
6. Castellano, C.; Brioni, J. D.; McGaugh, J. L. GABAergic modulation of memory. In: Squire, L. R.; Lindenlaub, E., eds. The biology of memory. Symposia Medica Hoechst 23. Stuttgart: F. K. Schattauer Verlag; 1989:361–378.
7. Chrobak, J. J.; Napier, T. C. Delayed nonmatch-to-sample performance in the radial arm maze: Effects of dopaminergic and gabaergic agents. Psychopharmacology (Berlin) 108:72–78; 1992.
8. Collingridge, G. L.; Bashir, Z. I.; Blake, J. F.; Davies, C. H.; Davies, S. N.; Irving, A. J.; Randall, A. D.; Schofield, J. G. Amino acid receptors and LTP in area CA_1 of the hippocampus. In: Squire, L. R.; Lindenlaub, E., eds. The biology of memory. Symposia Medica Hoechst 23. Stuttgart: F. K. Schattauer Verlag; 1989:233–250.
9. Coyle, J. T.; Price, D. L.; DeLong, M. R. Alzheimer's disease: A disorder of cortical cholinergic innervation. Science 219:1184–1190; 1983.
10. Davies, C. H.; Collingridge, G. L. Physiological regulation of hippocampal synaptic transmission by $GABA_B$ receptors. Pharmacol. Commun. 2:98–103; 1992.
11. Disterhoft, J. F.; Black, J.; Moyer, J. R., Jr.; Thompson, L. T. Calcium mediated changes in hippocampal neurons and learning. Brain Res. Rev. 16:196–198; 1991.
12. Fibiger, H. C. Cholinergic mechanisms in learning, memory and dementia: A review of recent evidence. Trends Neurosci. 14:220–223; 1991.
13. Gabriel, K. R. A simple method of multiple comparison of means. J. Am. Stat. Assoc. 73:364–366; 1978.
14. Gage, F. H.; Björklund, A.; Stenevi, U.; Dunnett, S. B.; Kelly, P. A. Intrahippocampal septal grafts ameliorate learning impairments in aged rats. Science 225:533–536; 1984.
15. Ginsberg, M. D.; Lin, B.; Morikawa, E.; Dietrich, W. D.; Busto, R.; Globus, M.Y.-T. Calcium antagonists in the treatment of experimental ischemia. Arzneimittelforschung/Drug Res. 41:334–337; 1991.
16. Heim, C.; Sieklucka, M.; Block, F.; Schmidt-Kastner, R.;

Jaspers, R.; Sontag, K.-H. Transient occlusion of carotid arteries leads to disturbed spatial learning and memory in the rat. In: Krieglstein, J.; Oberpichler, H., eds. Pharmacology of cerebral ischemia 1990. Stuttgart: Wissenschaftliche Verlagsgesellschaft mbH; 1990:53–61.
17. Heim, C.; Sieklucka, M.; Sontag, K. H. Transient clamping of carotid arteries (BCCA) increases, four-vessel-occlusion (4-VO), however, decreases GABA content in most vulnerable brain structures. Effect on spatial learning in the water maze? Abstracts of the 4th International Symposium on Pharmacology of Cerebral Ischemia, Marburg; 1992:80.
18. Hofmann, H. P.; Raschak, M.; Unger, L. (S)-Emopamil, a novel calcium and serotonin antagonist for the treatment of cerebrovascular disorder. 1st communication: Pharmacological profile. Arzneimittelforschung/Drug Res. 39:304–308; 1989.
19. Ikegami, S.; Nihonmatsu, I.; Kawamura, H. Transplantation of ventral forebrain cholinergic neurons to the hippocampus ameliorates impairment of radial-arm maze learning in rats with AF64A treatment. Brain Res. 548:187–195; 1991.
20. Izquierdo, I. Dopamine receptors in the caudate nucleus and memory processes. Trends Pharmacol. Sci. 13:7–8; 1992.
21. Jaspers, R. M. A.; Block, F.; Heim, C.; Sontag, K.-H. Spatial learning is affected by transient occlusion of common carotid arteries (2VO): Comparison of behavioural and histopathological changes after '2VO' and 'four-vessel-occlusion' in rats. Neurosci. Lett. 117:149–153; 1990.
22. Khachaturian, Z. S. The role of calcium regulation in brain aging: Reexamination of a hypothesis. Aging 1:17–34; 1989.
23. Kleinrok, Z.; Turski, L. Kainic acid induced wet-dog shakes in rats. The relation to central neurotransmitters. Naunyn Schmiedeberg's Arch. Pharmacol. 314:37–46; 1980.
24. Kunkel, M.; Block, F.; Sontag, K.-H. S-Emopamil reduces ischemic brain damage in rats: Functional and morphological approaches. Wiss. Zeitschrift Humboldt-Universität Berlin, R. Medizin 41(1):105–108; 1992.
25. Lowe, J. P.; Robinson, E.; Eyerman, G. S. The fluorimetric measurements of glutamic decarboxylase and its distribution in brain. J. Neurochem. 3:8–18; 1958.
26. Morris, R. Developments of a water-maze procedure for studying spatial learning in the rat. J. Neurosci. Methods 11:47–60; 1984.
27. Melzacka, M.; Weiner, N.; Heim, C.; Schmidt-Kastner, R.; Sieklucka, M.; Sontag, K.-H.; Wesemann, W. Effect of transient reduction of cerebral blood flow on membrane anisotropy and lipid peroxidation in different rat brain areas. Neurochem. Int. 1994 (in press).
28. Nakayama, H.; Ginsberg, M. D.; Dietrich, W. D. (S)-Emopamil, a novel calcium channel blocker and serotonin S2 antagonist, markedly reduces infarct size following middle cerebral artery occlusion in the rat. Neurology 38:1667–1673; 1988.
29. Schmidt-Kastner, R.; Heim, C; Sontag, K.-H. Comparison of histological changes produced in rats by cerebral ischemia (4-vessel occlusion) or cerebral oligemia (2-vessel occlusion). In: Elsner, N.; Barth, F. G., eds. Sense organs – Interfaces between environment and behaviour (Proceedings of the 16th Göttingen Neurobiology Conference). Stuttgart: Georg Thieme Verlag; 1988:323.
30. Shimohama, S.; Taniguchi, T.; Fujiwara, M.; Kameyama, M. Changes in nicotinic and muscarinic cholinergic receptors in Alzheimer-type dementia. J. Neurochem. 46:288–293; 1986.
31. Sieklucka, M.; Bortolotto, Z.; Heim, C.; Block, F.; Sontag, K.-H. Decreased susceptibility to seizures induced by bicuculline after transient bilateral clamping of the carotid arteries in rats. J. Neural Transm. Gen. Sect. 83:127–137; 1991.
32. Sieklucka, M.; Heim, C.; Block, F.; Sontag, K.-H. Transient reduction of cerebral blood flow leads to longlasting increase in GABA content in vulnerable structures and decreased susceptibility to bicuculline seizures. J. Neural Transm. Gen. Sect. 88:87–94; 1992.
33. Stadler, H.; Nesselhut, T. Simple and rapid measurement of acetylcholine and choline by HPLC and enzymatic-electrochemical detection. Neurochem. Int. 9:127–129; 1986.
34. Stevens, D. R.; Cotman, C. W. Long-term potentiation of excitatory and slow inhibitory potentials in the hippocampal-septal projection of the rat. Brain Res. 558:120–122; 1991.
35. Sutton, J.; Simmonds, M. Effects of acute and chronic pentobarbitone on the γ-aminobutyric acid system in rat brain. Biochem. Pharmacol. 23:1801–1808; 1974.
36. Szabo, L.; Hofman, H. P. (S)-Emopamil, a novel calcium and serotonin antagonist for the treatment of cerebrovascular disorders. 3rd communication: Effect on postischemic cerebral blood flow and metabolism, and ischemic neuronal cell death. Arznimittelforschung/Drug Res. 39:314–319; 1989.
37. Tarricone, B. J.; Keim, S. R.; Simon, J. R.; Low, W. C. Intrahippocampal transplants of septal cholinergic neurons: High-affinity choline uptake and spatial memory function. Brain Res. 548:55–62; 1991.
38. de la Torre, J. C.; Fortin, T.; Park, G. A. S.; Butler, K. S.; Kozlowski, P.; Pappas, B. A.; de Socarraz, H.; Saunders, J. K.; Richard, M. T. Chronic cerebrovascular insufficiency induces dementia-like deficits in aged rats. Brain Res. 582:186–195; 1992.
39. Ulrich, F.; Bortolotto, Z.; Heim, C.; Block, F.; Sontag, K.-H. Disturbances of REM-sleep after transient reduction of cerebral blood flow. Abstracts of the 14th International Symposium on Pharmacology of Cerebral Ischemia, Marburg; 1992:30.
40. Walsh, T. J.; Tilson, H. A.; DeHaven, D. L.; Mailman, R. B.; Fisher, A.; Hanin, I. AF64A, a cholinergic neurotoxin, selectively depletes acetylcholine in hippocampus and cortex, and produces long-term passive avoidance and radial arm maze deficits in the rat. Brain Res. 321:91–102; 1984.
41. Whishaw, I. Q.; O'Connor, W. T.; Dunnett, S. B. Disruption of central cholinergic systems in the rat by basal forebrain lesions or atropine: Effects on feeding, sensorimotor behaviour, locomotor activity and spatial navigation. Behav. Brain Res. 17:103–115; 1985.
42. Wood, J. D.; Watson, W. J.; Ducker, A. J. The effect of hypoxia on brain γ-aminobutyric acid levels. J. Neurochem. 15: 603–608; 1968.