Pharmacology Biochemistry and Behavior, Vol. 56, No. 4, pp. 737–745, 1997

0091-3057/97 $17.00 + .00

PII S0091-3057(96)00517-5

Is Chlormethiazole Neuroprotective in Experimental Global Cerebral Ischemia? A Microdialysis and Behavioral Study

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Received 23 February 1996; Revised 2 August 1996; Accepted 2 August 1996

THAMINY, S., J.-M. REYMANN, N. HERESBACH, H. ALLAIN, P. LECHAT AND D. BENTUÉ-FERRER. *Is chlormethiazole neuroprotective in experimental global cerebral ischemia? A microdialysis and behavioral study.* PHARMACOL BIOCHEM BEHAV **56**(4) 737–745, 1997.—Chlormethiazole, an anticonvulsive agent, has been shown to have a possible neuroprotective effect against cerebral ischemia. In addition, chlormethiazole inhibits methamphetamine-induced release of dopamine, protecting against this neurotransmitter's neurotoxicity. The aim of this work was to ascertain whether, in experimental cerebral ischemia, chlormethiazole administration attenuated the ischemia-induced rise of the extracellular concentration of aminergic neurotransmitters and whether it reduces ischemia-induced deficits in memory and learning. Histology for assessment of ischemic damage was also included. The four-vessel occlusion rat model was used to induce global cerebral ischemia. Aminergic neurotransmitters and their metabolites in the striatal extracellular fluid obtained by microdialysis were assayed by high-performance liquid chromatography–electrochemical detection. The drug was administered either IP (50 mg·kg^{-1}) or directly through the dialysis probe (30 μM) 80 min before ischemia. For the behavioral test and histology, the drug was given IP (100 mg·kg^{-1}) 1 h postischemia. The results obtained did not demonstrate any statistically significant evidence that chlormethiazole has an effect on the ischemia-induced rise in extracellular dopamine and serotonin levels. There was also no variation in metabolite levels. Behavioral measures (learning, recall) were not changed appreciably by the treatment. We observed no significant cell protection in the hippocampus (CA_1, CA_4), striatum, and entorhinal cortex in animals treated with chlormethiazole. We conclude that, under our experimental conditions, chlormethiazole has little or no effect on the neurochemical, neurobehavioral, and histological consequences of global cerebral ischemia. © 1997 Elsevier Science Inc.

Chlormethiazole Aminergic neurotransmitters Cerebral ischemia Learning Memory Neuroprotection

CHLORMETHIAZOLE (INN: clomethiazole) was initially recognized as an anticonvulsive agent (9) with sedative and hypnotic effects (10). It has long been used in the treatment of alcohol withdrawal (40). Chlormethiazole interacts with the gamma-aminobutyric acid (GABA) receptor complex in rat brain (17,31,43,47,63). Its action does not involve presynaptic mechanisms, and it is not a direct agonist at the $GABA_A$-receptor. Chlormethiazole appears to act on the Cl^- channel of the receptor. Its effect on dopaminergic transmission appears to be secondary to GABA-mediated mechanisms. It does not interact with the *N*-methyl-D-aspartate (NMDA) receptor complex at any of the known sites modulating function (16).

Recently, it has been shown that chlormethiazole protects against hippocampal neurodegeneration following transient forebrain ischemia in the gerbil (14,15) and that it reduces the area of infarcted cortical tissue after focal cerebral ischemia in the rat (58,61). It also reduces edema of the cortex after a photochemically induced infarct (26). Other anticonvulsive agents (barbiturate, phenytoin, etomidate) that also modulate the GABA receptor have been found to have neuroprotective effects against cerebral ischemia (60). Similarly, it has been

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demonstrated in rats that chlormethiazole protects against methamphetamine-induced neurotoxicity of both dopamine in the striatum and serotonin in the cortex and the hippocampus (28). Chlormethiazole also attenuates the dopamine release that occurs in animals given methamphetamine; this inhibition could explain the protective action observed in rats treated with neurotoxic doses of the compound (2,67).

The major increase in extracellular levels of neurotransmitters occurring during experimental cerebral ischemia [see (45) for review] has been well documented with the in vivo microdialysis technique, which shows excitatory amino acid release (6,30) as well as release of catecholamines and indolamines (7,22,53). The role played by these monoamines in the development of neurotoxicity subsequent to cerebral ischemia has been recognized (24,33). Evidence of cerebral ischemia-induced neurological sequelae is found in the cognitive deficiency demonstrated by tests exploring memory and learning (64,65,68).

The aim of this work was to determine whether or not the described protective effect of chlormethiazole against cerebral ischemia could be related to lower dopamine and/or serotonin release, as has been observed for the neurotoxic effect of methamphetamine, and whether histological evidence for reduced brain damage translates into reduced impairments in learning and memory.

MATERIALS AND METHODS

Animals and Ischemia

This study was conducted with Sprague–Dawley (Charles River) adult male rats with a mean weight of 300–320 g; food and water were provided ad lib. Animals were housed in an approved animal house, and the care provided for them before, during, and after the protocol was in compliance with ethics and good laboratory practices (authorization number A 35006 of the Veterinary Department of the French Health Ministry). Ischemia was induced by the four-vessel occlusion (4-VO) technique initially proposed by Pulsinelli and Brierley (54). The animals were anaesthetized with 350 $mg \cdot kg^{-1}$ chloral hydrate injected intraperitoneally. The vertebral arteries on both sides were exposed through a dorsal cervical skin incision and permanently electrocoagulated with a coagulasem. Both common carotid arteries were isolated with surgical thread, and the next day they were clamped for 20 min. Body temperature of the animals were maintained at 37.5°C with a heating pad, a rectal probe, and a CMA/150 temperature controller (Carnegie Medicine). Only those animals that completely lost their righting reflex and showed no convulsive signs were retained for further experimentation [rats with postischemic seizures show a very different pattern of aminergic neurotransmitter release (4)].

Microdialysis

While the animals were still under anaesthesia, just after the electrocoagulation of the vertebral arteries, they were transferred to a stereotaxic frame (Kopf) and a probe guide cannula (CMA 10 Carnegie Medicine) was implanted above the right striatum according to stereotaxic coordinates for Paxinos and Watson's atlas (51) and fixed with dental cement. Three hours before the beginning of ischemia, a 4-mm-long microdialysis probe (CMA 10 Carnegie Medicine) was positioned into the probe guide while the animal was awake. The dialysis probe was perfused with Ringer's solution (NaCl 147 mM, KCl 4 mM, $CaCl_2$ 1.2 mM) at a flow rate of 1 μl/min. Dialysates were collected for each 20-min period (80 min before onset of ischemia, during ischemia, and 180 min after) in microtubes with 5 μl of a 0.5 M perchloric acid solution containing 4-dihydroxyhydrocinnamic acid (internal standard), then the samples were frozen and stored at −75°C until analysis. Recovery from the probe was measured by dialysing a standard solution in vitro at the end of each microdialysis session, in order to check the quality of the probe, but results were not corrected for recovery. Only the last four values obtained before ischemia were retained for analysis. The median intrastriatal position of the probe was checked anatomically at the end of each experiment.

Protocol Design and Drug Treatment

Chlormethiazole was administered as ethane disulfonate.

Neurochemistry study. The animals were divided into four groups according to drug administration. In group I ($n = 9$), chlormethiazole was administered intracerebrally directly via the dialysis probe at a concentration of 30 μM diluted in Ringer's solution. Perfusion was switched to the solution containing the test compound 80 min before the beginning of the ischemia and maintained until the end of the dialysis session. Group II ($n = 13$) was the control group for intrastriatal administration; these animals were perfused with Ringer's solution alone. In group III ($n = 7$), chlormethiazole was administered IP at a dose of 50 $mg \cdot kg^{-1}$ in isotonic saline (refer to the concentration of the base); the injection was done 80 min before the beginning of the ischemia. Finally, group IV ($n = 7$) was the control group for IP injection: only isotonic saline was injected.

Behavioral study. The Control Group consisted of 10 animals with neither ischemia nor treatment. In the Coagulation Group ($n = 10$), the vertebral arteries were coagulated but no ischemia was induced, as the carotid arteries remained free. In the Ischemia Group ($n = 8$), ischemia was induced and the animals were treated with saline solution. Finally, in the Ischemia–Treatment Group ($n = 8$), the animals were given an intraperitoneal injection of chlormethiazole (100 $mg \cdot kg^{-1}$) 1 h after ischemia.

Histological study. Two different groups were differentiated: the Ischemia Group ($n = 7$) and the Ischemia–Treatment Group ($n = 11$), as described above.

Assay of Neurotransmitters and Their Metabolites

Dopamine (DA), serotonin (5-HT), and their respective metabolites—dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), and 5-hydroxyindol acetic acid (5-HIAA)—were assayed on an automated high-performance liquid chromatography processor (SP 8810 isocratic pump, SP 8880 refrigerated autosampler, SP 4290 integrator, Spectra-Physics) fitted with a Coulochem II electrochemical detector (E.S.A., analytical cell model 5011). The analytic column used was a Spherisorb cartridge, 230 × 4.6 mm, ODS2, 5 μm. The mobile phase was composed of a 0.05 M citrate/0.06 M acetate buffer, 0.5% counterions (Pic B8, Waters), 17% methanol, and 200 mg EGTA. Each sample (25 μl) was totally injected without preparation.

For dopamine, the sensitivity threshold was 0.5 nM. An automatic attenuation (detector + integrator) change before elution of serotonin allowed evaluation of its baseline release under acceptable conditions. Sensitivity was never a limiting factor for any of the metabolic assays. The sensitivity of the

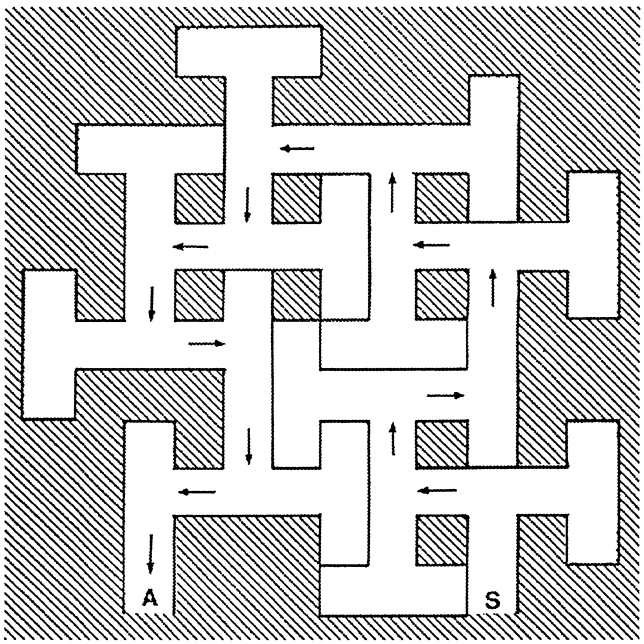

FIG. 1. Diagram of the 14-T maze.

detector and the attenuation of the integrator were chosen at values that would avoid signal saturation at high values.

Behavioral Tests

Apparatus. A modified Stone 14-unit spatial T-maze was used (Fig. 1). The configuration of this maze has been described previously (25).

Procedures. Two weeks before beginning the behavioral tests, the amount of feed was reduced enough to lower the animals' weights to 80% of their initial weights. Six days before beginning the tests, the animals were accustomed twice a day to concentrated milk feedings, the food reward used in the learning test. The learning test began in the 14-unit T-maze with one test per day, run between 0900 h and 1400 h, for 13 days. The rats were positioned in the start chamber (S) and gently introduced into the maze; the door was then closed. A timer was started when the rat had completely left the start chamber and stopped when the milk reward was reached. If the animal did not reach the arrival chamber (A) within 5 min, it was gently shown the way and given the reward for 10 s. These tests began 75 days after inducing ischemia. An error was counted each time the rat entered a branch of the maze that did not directly lead to the reward.

After the first 13 test days, when the learning process was achieved in the control animals, as shown by their constant scores, the direction of the maze was inverted: the animals were placed in the arrival chamber (A) and had to reach the previous starting chamber (S) to get their reward. One run per day was then done for eight test days. For each animal and for each run, the number of errors and the run time to reach the reward were counted.

Histological Evaluation

Six days after carotid artery occlusion, the animals were decapitated. The brains were removed and placed in 10% formaldehyde for 24 h, processed, and embedded in paraffin. Coronal sections were cut at a thickness of 5 μm at the level of the anterior hippocampus and stained with hematoxylin-eosin-safran (HES) and cresyl violet (CV). Quantification of neuronal damage was performed by direct visual counting of neurons using a light microscope at a magnification of 40×. The normal and damaged cells were counted and the percentage of necrotic neurons was calculated for CA_1 and CA_4 regions of the hippocampus. The presence of pyknosis and hyperchromasia in neurons was used to confirm ischemic cell changes. Cell counts were also performed in the striatum and the entorhinal cortex. For each of these regions, the cell counts were averaged across the right and left hemispheres to generate a mean cell count at each level for each rat. Results are expressed as the percentage of neurons damaged.

Statistical Analysis

Significance was set at 0.05 for all analyses.

Neurotransmitter and metabolite levels. For the four groups of animals, results are presented as the time course of the mean (±SEM) values for each group for the four samples drawn before ischemia, one during ischemia, and nine during recirculation. For each neurotransmitter or metabolite, the concentration was compared using variance analysis: ANOVA for repeated measurements for the pre- and postischemic periods and one-way ANOVA for the ischemic period.

Behavioral tests. The number of errors and run time are presented as daily means for each group. The scores were compared using ANOVA for repeated measurements followed by the Newman–Keuls rank test.

Histological measurement. The two groups were compared using the unpaired Student's *t*-test.

RESULTS

Effect of Chlormethiazole on Dialysate Neurotransmitter and Metabolite Concentrations

The results are given in Figs. 2 (DA and metabolites) and 3 (5-HT and metabolite).

For dopamine, there was no group effect during the period prior to ischemia [$F(3, 31) = 2.32$, $p = 0.095$], but there was a time effect [$F(3, 93) = 4.06$, $p = 0.009$]. During the ischemia period, there was a major rise in extracellular levels in both control groups: a 400-fold increase in group II (Ringer's solution in dialysis probe) and a 300-fold increase in group IV (saline IP) compared with the last pre-ischemic level. This rise was highly significant [$F(1, 32) = 200, p < 0.001$]. Even though the DA levels in the animals treated with chlormethiazole were lower than those in control animals, the comparison between the concentrations obtained for the four groups during the 20-min sample period of ischemia did not demonstrate any significant difference [$F(3, 32) = 1.81$, $p = 0.164$].

For the two DA metabolites, DOPAC and HVA, there were no significant difference between the groups before ischemia [$F(3, 32) = 0.96$, $p = 0.424$ and $F(3, 32) = 1.09$, $p = 0.366$, respectively], but there was a significant time effect [$F(3, 96) = 45$, $p < 0.001$ and $F(3, 96) = 40$, $p < 0.001$, respectively], demonstrating that the levels were not still stable. The significant drop-off from the pre-ischemic levels to lower levels observed at the time of ischemia was not different between the groups [$F(3, 32) = 0.56$, $p = 0.642$ and $F(3, 32) = 0.19$, $p = 0.670$, respectively], and there was no significant difference between the groups during the postischemic

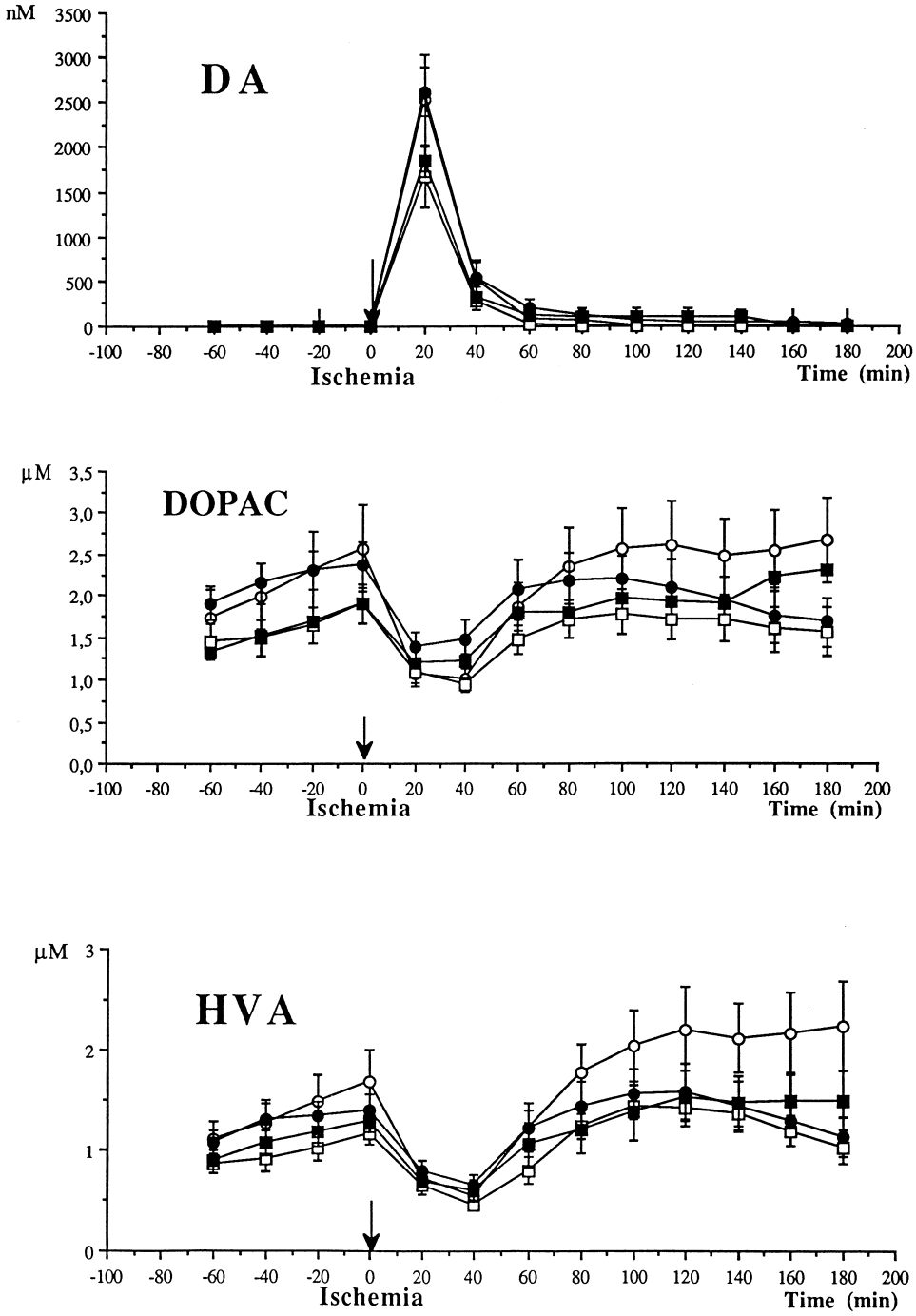

FIG. 2. Time course of dopamine (DA) and acidic metabolite (DOPAC and HVA) levels (mean ± SEM) before, during, and after a 20-min episode of cerebral ischemia. (■) Group I (chlormethiazole in dialysis probe); (●) Group II (Ringer's solution in dialysis probe); (□) Group III (chlormethiazole IP); (○) Group IV (saline IP).

period [$F(3, 29) = 0.71$, $p = 0.553$ and $F(3, 29) = 1.23$, $p = 0.315$].

Ischemia led to a significant increase in 5-HT [$F(1, 26) = 82.5$, $p < 0.001$] and a significant fall in 5-HIAA [$F(1, 26) = 45.8$, $p < 0.001$] compared with the pre-ischemic levels, but there was no significant difference among the four groups for the metabolite either before, during, or after ischemia [before: $F(3, 24) = 0.66$, $p = 0.582$ and $F(3, 32) = 2.56$, $p = 0.072$; during: $F(3, 26) = 1.34$, $p = 0.284$) and $F(3, 32) = 1.97$, $p = 0.138$; after: $F(3, 17) = 1.01$, $p = 0.411$ and $F(3, 29) = 2.66$, $p = 0.067$].

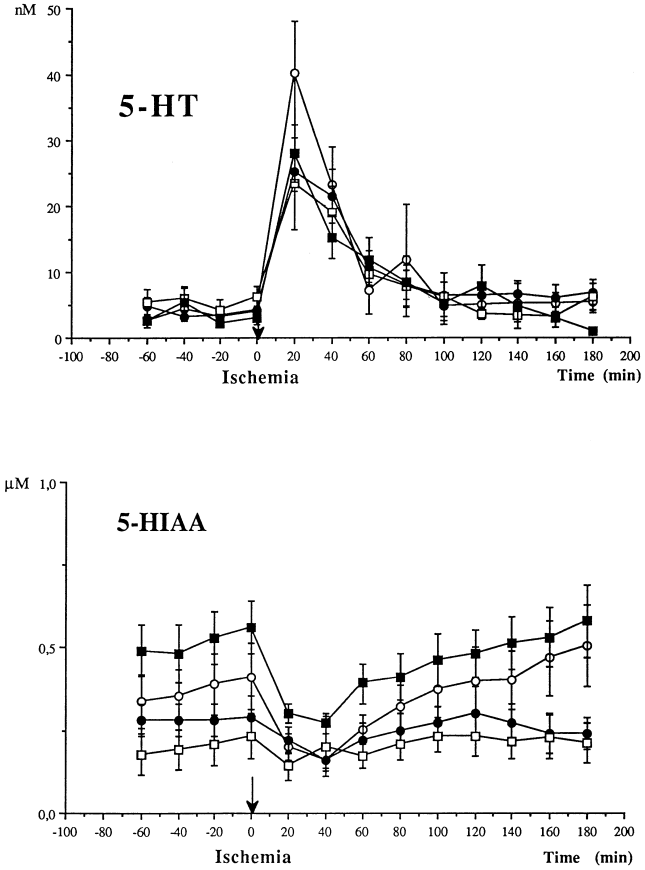

FIG. 3. Time course of serotonin (5-HT) and 5-HIAA levels (mean ± SEM) before, during, and after a 20-min episode of cerebral ischemia. (■) Group I (chlormethiazole in dialysis probe); (●) Group II (Ringer's solution in dialysis probe); (□) Group III (chlormethiazole IP); (○) Group IV (saline IP).

Effects of Chlormethiazole on Ischemia-induced Behavioral Deficits

The results are shown in Figs. 4 (mean number of errors) and 5 (mean run times).

First Learning Phase

Errors. The number of errors made by the animals declined significantly during the learning period [$F(12, 384) = 21.99, p < 0.001$], but differently for the different groups [$F(3, 32) = 5.94, p = 0.002$]. There was no difference between the animals in the Control or Coagulation Groups, but these two groups were significantly different from the Ischemia Group without treatment. The rats in the Ischemia–Treatment Group did less well than the control animals, and though they were not different from the animals in the Ischemia Group without treatment, they also showed no significant difference from the animals in which the vertebral arteries were coagulated, a condition that led to slightly poorer performance. The animals in the Ischemia Group without treatment were statistically different from the Control and Coagulation Groups. There was also a trial × group interaction [$F(36, 384) = 1.64, p = 0.013$]. During the first 4 days of the test, there was no difference among the four groups.

Run times. Run times changed significantly during the test periods [$F(12, 384) = 58.39, p < 0.001$] and significantly differently for the four groups [$F(3, 32) = 3.18, p = 0.037$]. There was a trial × group interaction [$F(36, 384) = 1.54, p = 0.027$]. The differences between the groups were significant at day 4 for Control versus Ischemia rats and Coagulation versus Ischemia rats and also at day 13 for Control versus Ischemia rats and Control versus Ischemia–Treated rats.

Second Learning Phase After Inversion of the Maze

Errors. The number of errors made by the animals differed significantly during the period of adaptation to the new task [$F(7, 224) = 89.5, p < 0.001$], but the scores were different among the groups [$F(3, 32) = 7.90, p < 0.001$]. There was no difference between the two Control Groups nor between the two Ischemia Groups, but the Control and Coagulation Groups differed from the Ischemia and Ischemia–Treated animals. There was a trial × group interaction [$F(21, 224) = 3.92, p < 0.001$]. During the last two days of the test, there was no significant difference between the four groups because of wide intragroup variations.

Run times. There was a significant difference in run times [$F(7, 224) = 100.34, p < 0.001$], and the scores were different among groups [$F(3, 32) = 6.73, p < 0.001$]. Similarly, there was no difference between the two groups of Control animals nor between the two groups of Ischemia animals, but the Control and Coagulation animals differed from the Ischemia and Ischemia–Treated animals.

When the maze was inverted, the animals made four times more errors on the first run than they had made on the first day of the first phase, and 80–100% of the animals, depending on the group, did not reach the reward within 5 min. The Control animals and the Coagulation animals improved their performance very rapidly, but after 8 days, the Ischemia animals and the Ischemia–Treated animals made the same number or more errors than they had on the first day of the first phase and still had long run times.

Effects of Chlormethiazole on Neuropathological Assessment

The results are expressed as mean ± SEM of the percentage of damaged neurons (Fig. 6). The results obtained in nontreated animals of the Ischemia Group show a clearcut increase in the percentage of neuronal loss, with a maximum in the hippocampus and more particularly within the CA_1 area. In the group of animals subjected to ischemia and treated with chlormethiazole (Ischemia–Treatment Group), the percentage of damaged neurons in the four studied brain structures was systematically lower than in the Ischemia Group, but the difference never reached statistical significance (hippocampus zone CA_1: $p = 0.152$; hippocampus zone zone CA_4: $p = 0.148$; striatum: $p = 0.088$; entorhinal cortex: $p = 0.168$).

DISCUSSION

Transient global forebrain ischemia causes energy failure, depolarization, and a massive release of neurotransmitters; then starts a cascade of events triggering calcium and free radical-mediated toxicity, leading finally to neuronal cell death (5,32,37,41). Release of excitatory amino acids plays a predominant role in triggering cell death (11,62), but release of ami-

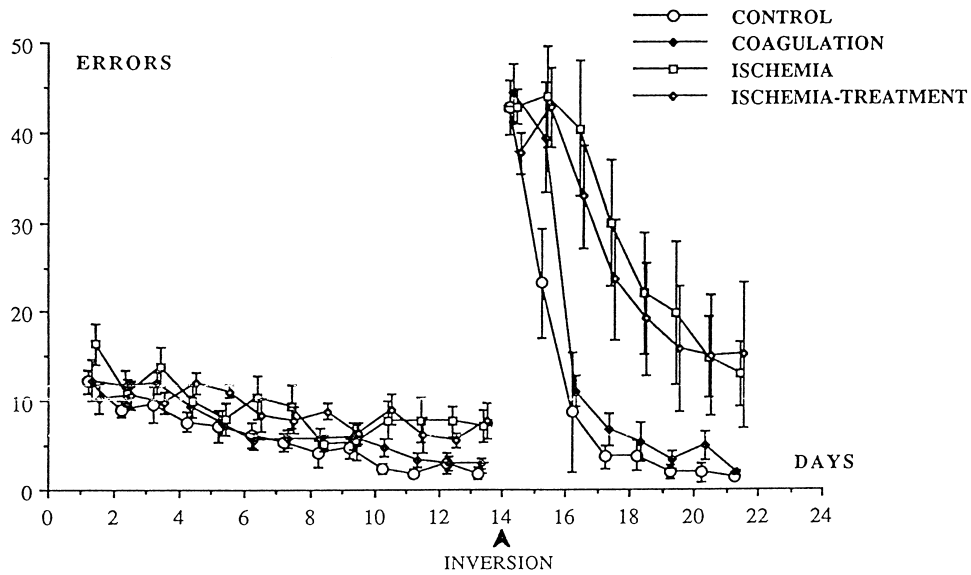

FIG. 4. Number of errors (mean ± SEM) made in the 14-T maze by control rats, coagulation rats, and rats exposed to a 20-min episode of transient forebrain ischemia alone and in combination administration of chlormethiazole (100 mg·kg^{-1} IP) 1 h postischemia. Performance in the test was assessed starting on the 75th day after ischemia.

nergic neurotransmitters also takes part in this process. The direct neurotoxic effect of high levels of DA is known (20). Several teams have proven that the destruction of dopaminergic cell bodies leads to a decline in the deleterious consequences of cerebral ischemia (12,23). Likewise, 5-HT is neurotoxic (36) and there is a link between the degree of 5-HT release and neuron destruction in the CA_1 zone of the hippocampus in ischemia (24). Therefore, as well as NMDA receptor antagonism (1,38,42,52), antagonists of monoaminergic receptors have been used as pharmacological approaches to neuroprotection (29). Additionally, inhibitors of monoamine release have also been investigated as neuroprotectant agents (56).

In our study, the amplitudes of the changes in the levels of both DA and 5-HT neurotransmitters and of their metabolites during an episode of ischemia were in close agreement with

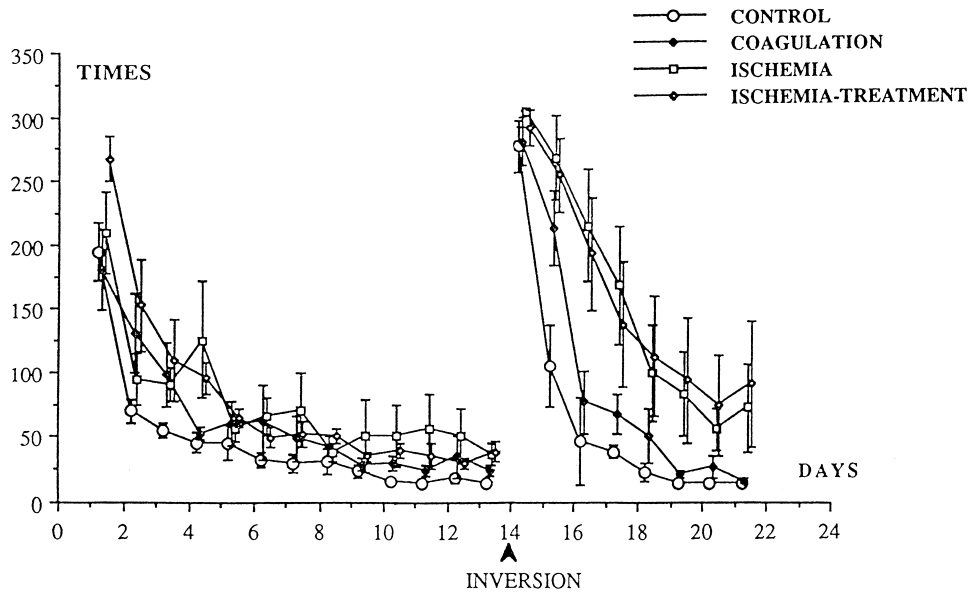

FIG. 5. Run times (mean ± SEM) in the 14-T maze by control rats, coagulation rats, and rats exposed to a 20-min episode of transient forebrain ischemia alone and in combination with administration of chlormethiazole (100 mg·kg^{-1} IP) 1 h postischemia. Performance in the test was assessed starting on the 75th day after ischemia.

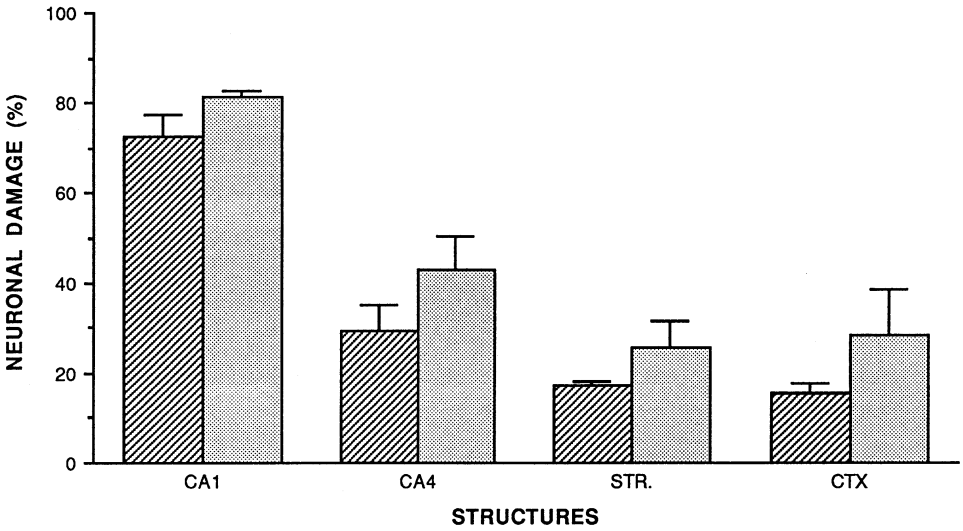

FIG. 6. Effect of chlormethiazole on neuronal degeneration. Chlormethiazole (100 mg·kg^{-1} IP) was administered 1 h postischemia; ischemic controls received saline. Columns represent mean ± SEM. Histological assessment was performed 6 days after carotid artery occlusion. (▨) Ischemia–Treated Group; (□) Ischemia Group.

those reported earlier [(18,46), for example]. Under our experimental conditions, we demonstrated that when administered locally or via a systemic route, chlormethiazole (at the dose used by Baldwin et al. (2) to lower methamphetamine-induced DA release) lowered dopamine release by only a very small amount, which was not statistically significant. We did not find any effect on the level of dopamine metabolites or on the serotoninergic system.

The loss of striatal neurons observed after injecting methamphetamine might also result from the high dopamine release induced (57). Using this model, Baldwin et al. (2) showed that chlormethiazole (50 mg·kg^{-1} IP) has a histological protective action and attenuates the enhanced dopamine release that occurs in animals given methamphetamine. Our results are quite different, but the model is also different. Amphetamine induces release of cytosolic dopamine, a Ca^{2+}-independent mechanism not requiring nerve impulse flow (39). The major rise in the extracellular level of the neurotransmitters triggered by ischemia results from a mechanism still incompletely understood. Ca^{2+}-dependent exocytosis would, at least partly, be implicated, because this rise is abolished when the perfusion is calcium free but supplemented with cobalt (19) or partially inhibited by the adjunction of ω-conotoxin (50). However, the relative contributions of exocytosis, deficient reuptake, altered oxidative metabolism, leakage, and inversion of the transporter implicated in reuptake of the increased extracellular neurotransmitter during ischemia remain to be determined. Nevertheless, several studies have demonstrated that it is possible to have a pharmacological effect on this rise (8,49).

It should also be noted that in Baldwin's study (2) the action of chlormethiazole on dopamine release was not seen at the first administration of methamphetamine, but only after the second or third injections. In addition, chlormethiazole (100–1000 μM) does not alter methamphetamine- or K^+-evoked release of endogenous dopamine from striatal prisms in vitro. In vitro release of adenosine from hippocampal slices stimulated by hypoxia/hypoglycemia is not statistically decreased at any of the three concentrations (30, 100, 300 μM) of chlormethiazole studied (66).

We did not find any evidence of a chlormethiazole effect on the serotoninergic system but, as has been shown earlier, this compound does not have any antagonist effect on the neurotoxicity caused in mice by 5-7-dihydroxytryptamine on serotoninergic transmission (59), nor on serotoninergic neuronal cell loss produced by *p*-chloroamphetamine or fenfluramine (13). Conversely, it has a protective effect against neurodegeneration triggered by 3,4-methylenedioxymethamphetamine (MDMA, or "Ecstasy") (13,34).

In the behavior part of our study, we found the classically described cognition deficiency induced by global ischemia (64,65). The ischemic rats had a learning deficit and adapted incompletely, and after a long period, to a new task (inversion of the maze). The hippocampus is an important brain region responsible for controlling spatial learning and memory behavior (3,48). Administered IP 1 h after the end of the transient ischemia at a dose of 100 mg·kg^{-1}, chlormethiazole had little or no protective effect on ischemia-induced deficits. Our test required flexibility in spatial learning, since the animal had to abandon a learned strategy and produce an errorless "mirror strategy" to reach its reward. What was observed in the ischemic animals treated with chlormethiazole was an improvement in error score solely in the first phase of learning. The ischemic animals can learn the new version but at a much slower rate and reach a plateau at a higher rate of error than the controls.

When considering the histological assessment, the extent and the degree of the lesions observed in the animals with untreated ischemia and in the different observed brain areas are in agreement with results published for the present animal model as well as for occlusion duration (21,55). The lack of statistically significant protection, specifically within the hippocampus, after drug treatment, is in parallel with the observed absence of memory performance maintenance; in this respect, however, no correlation has yet been described between CA_1

cell loss in ischemic rats and indices of spatial ability (44). These findings are less optimistic than those reported by Cross et al. (14), who showed that following 5 min of transient forebrain ischemia in the gerbil, a single dose of chlormethiazole (100 $mg \cdot kg^{-1}$ IP, a dose and administration route similar to our protocol) prevented neurodegeneration of the hippocampal pyramidal neurons even when given up to 3 h after the ischemic episode. However, a 5-min episode of transient ischemia in the gerbil is a less drastic model than a 20-min occlusion of the four cerebral arteries in the rat, and the difference of sensitivity between both those experimental models of brain ischemia may explain the discrepancy between our results and previously published data. The duration of the carotid clamping in the present study may be considered to be too long, but Iwasaki et al. (35) showed that 5 or 10 min of ischemia, in the same model, did not affect task acquisition in a test of spatial cognition.

In conclusion, we found that administration of chlormethiazole in the rat induced a weak, nonsignificant, improvement trend of the three main evaluation criteria, namely decreased cerebral ischemia-induced dopamine release, minimal improvement in memory performance, and nonsignificant neuronal protection. Thus, the mechanism that might account for a neuroprotective effect of chlormethiazole in cerebral ischemia [see (27) for review], a neuroprotection that we were unable to clearly demonstrate, is not likely to involve inhibition of aminergic neurotransmitter release.

ACKNOWLEDGEMENTS

We thank Mrs. M. Daubémont, J. Monlibert, and A. Denais for their excellent technical assistance.

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