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# Pentoxifylline Improves Learning and Memory in Glutamate-Lesioned Rats

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CUNHA, G. M. A., P. J. P. BEZERRA, M. D. D. SALDANHA, M. C. CAVALCANTE, V. M. S. DE BRUIN AND G. S. B. VIANA. *Pentoxifylline improves learning and memory in glutamate-lesioned rats.* PHARMACOL BIOCHEM BEHAV **66**(4) 687–694, 2000.—The present work shows the effects of pentoxifylline (ptx), on learning and memory in rats with hippocampal lesions induced by glutamate (glu). Immediately after stereotaxic procedures and in the absence or presence of glu lesions, animals were treated with ptx (50, 100, or 200 mg/kg, IP) for 6 days. Twenty-four hours after the last injection, behavior and memory tests were performed, animals were sacrificed, and hippocampi dissected for cAMP determination or histopathological studies. Results from the T-maze task showed a less learning ability in the glu-lesioned group compared to other ones. Ptx alone or associated with glu significantly improved memory acquisition, but not memory consolidation compared to glu-lesioned rats. Except for the increased locomotor activity observed in the ptx100+glu-treated group compared to saline, no other difference was detected in the open-field test. A significant impairment in avoidance performance was observed in glu-lesioned group as compared to saline or to other groups in the short as well as in the late phase of memory. All groups showed an improved water-maze performance over time with similar performances on the final day of acquisition. The impairment in memory retention observed in glu-lesioned rats was reversed by the pretreatment with ptx200. Glu induced hippocampal lesion and reduced cAMP levels. Both effects were blocked by ptx, suggesting that its action may be the result of increased cAMP levels and/or inhibition of adenosine A1 receptors. © 2000 Elsevier Science Inc.

Pentoxifylline Memory cAMP Glutamate

PENTOXIFYLLINE (ptx) is a dimethylxanthine derivative that may induce physiologic and pharmacological effects by several mechanisms including translocation of extracellular calcium, increase in cAMP and cGMP caused by inhibition of phosphodiesterases, and blockade of adenosine receptors. This drug has been used in cases of chronic occlusive arterial disease, and its mechanism of action is still poorly defined. Other therapeutic indications for ptx are under investigation, considering the fact that it has inhibitory effects on various inflammatory mechanisms, including complement cascade, neutrophil adherence, and cytokine production (4,13).

The inhibition of specific phosphodiesterase (PDE) isoforms, notably PDE4, and consequent elevation of intracellular cAMP levels have been shown to inhibit cytokine release, for example, TNF-alpha (34). Some PDE inhibitors are thought to possess a cerebral vasodilating effect (9,19) and/or a cerebral activating effect (26), and have been used for the treatment of cognitive disfunction (16).

The A1 adenosine receptor is linked to the inhibition of adenylyl cyclase activation and, in the nervous system, is thought to mediate the inhibition of transmitter release and the reduction in neuronal activity. In 1996, Katchman and Hershkowitz (21) showed that the adenosine A1 antagonism increases specific synaptic forms of glutamate release during anoxia. It has been shown (17) that the chronic treatment with the adenosine receptor antagonist caffeine evokes an up-regulation of A1 adenosine receptors, and increases coupling of the receptor to G proteins in rat brain membranes.

Excitotoxicity is a process whereby excessive stimulation of neurons, usually induced by glu, triggers neuronal death. It

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is known that *N*-methyl-D-aspartate (NMDA)-glutamate receptors and other calcium conducting ion channels play a key role in excitoxicity. Also, metabotropic glutamate receptors may mediate long-lasting electrical signals in the brain, which are closely related to long-term potentiation and memory functions.

In the present work, we wanted to investigate the role of glutamatergic receptors in the water-maze test acquisition. All groups were tested in an open-field apparatus prior to water-maze training, which provides a spatial experience, and might function as a NMDA channel activator in the hippocampus. Besides, passive avoidance and T-maze tests were also performed to measure memory acquisition and retention. Thus, the objectives of the present work were to study the effects of ptx on the impairment of learning and memory functions induced by glutamate lesions in rat hippocampus, evaluated through three different learning tasks. Besides, effects of ptx on glutamate excitotoxicity, and rat locomotor activity were also evaluated. Finally, the effects of pentoxifillyne and/or glutamate were demonstrated on hippocampus cAMP levels.

## METHOD

### *Experimental Protocol*

*Stereotaxic procedures.* Male Wistar rats (200–250 g), from the Animal House of the Federal University of Ceará, were housed individually in plastic cages in a 12 L:12 D cycle with free access to water and food. Animals were anesthetized with thiopental (30 mg/kg, IP) and chloral hydrate (150 mg/kg, IP), and placed in a stereotaxic instrument (Stoelting Co., Avordale, IL). Five holes were opened on each side of the skull with an electrical drill. Saline or glutamate (glu) in a volume of 0.2 μl were applied in different sites to the right and left hippocampus, using a 5-μl Hamilton microsyringe. In the case of glu, each side of the hippocampus received a total dose of 48 μg. The final concentration of glu in both sides was 96 μg, which was sufficient to produce lesions in the CA1 and CA3 hippocampal areas, according to histological monitoring of HE-stainned slices performed in a separated group of animals. The same procedure was followed with saline (sham-operated controls). Coordinates for the injections from bregma (27) were CA3 = AP: −2.7, L: ± 2.0, V: 5.4; AP: −4.2, L: ± 3.2, V: 5.7; AP: −5.8; L: ± 4.5; V: 4.8; CA1 = AP: −3.0, L: ± 1.0, V: 4.5; AP: −4.5, L: ± 2.8, V: 4.6; AP: −5.8, L: ± 4.5, V: 6.0. Immediately after surgery, and in the presence or absence of glu-induced hippocampal lesions, animals were treated with ptx (50, 100, or 200 mg/kg, IP) for 6 days. Twenty-four hours after the last ptx injection, behavior and memory tests were performed. Tests sequence was the following: first day, open-field and water-maze tests for memory acquisition measurements were performed. On the second day, water-maze followed by the T-maze test also for memory acquisition measurements, named avoidances 1 and 2, were carried out. On the third day, only the passive-avoidance test was performed for short memory evaluation followed in the next day by the water-maze test for memory-retention measurements and the passive-avoidance test for late memory evaluation. In the last day, the T-maze test was carried out again for working memory measurements. Twenty-four hours after the last test, animals were sacrificed, and the hippocampi dissected for cAMP determination or histological analyses. Procedures were in compliance with the National Institutes of Health Guide for Care and Use of laboratory animals.

### *Open Field*

The open-field test (6) was used for testing the behavioral responses of rats (9 to 16 animals per group) to a novel environment. The round floor was made of white wood (150 cm diameter) and divided in 19 squares. The animal was placed in the center of the arena and left free to explore the environment for 1 min. After this time, the number of squares crossed was recorded for 3 min.

### *Passive Avoidance (12)*

A two-compartment apparatus (48 × 22 × 22 cm) from Ugo Basil, Italy, was used. In the acquisition trial, each rat was placed individually in the light compartment. When the animal entered the dark compartment a foot shock of 0.5 mA was delivered through the grid floor until the animal returned to the light compartment. The latency time to enter the dark compartment was measured up to a cutoff time of 300 s (baseline). The animal was removed from the apparatus, and the trial repeated 15 min later, even with those that reached the cut off time (short memory). The retrieval trial was perfomed in the same manner, 24 h later, but now no animal was shocked (late memory). The number of animals ranged from 8 to 12.

### *Water Maze (24)*

A modified Morris water maze was used to assess visual-spatial learning and memory in rats. A circular swimming pool made of black plastic (132 cm in diameter, 40 cm high) was filled up to 10 cm of the surface with tap water at 25°C. Powdered milk was added to the pool to make the water opaque and prevent visualization of the platform. Four points were marked in the rim of the pool and designated as North, South, East, and West. On days 1–2, rats were trained (six trails per day) to locate and escape onto a plastic platform (15 × 15 × 19 cm) placed on the Northwest point, 2 cm beneath the water surface. The ceiling used in the first exposure (cutoff point) and all other trainings was 54 s, and all animals that did not find the platform in this period of time were placed again on the platform for a further 10 s. Forty-eight hours after the 2 training days, all animals were tested for their memory of platform location in the water maze (retention). The platform was removed from the maze, and each subject was started in the maze from the east side and observed in the maze for 60 s. The latency to reach original platform location and the number of time each animal crossed were recorded. The number of animals ranged from 8 to 12.

### *Elevated T-Maze (35)*

The elevated T-maze was made of wood, and had three arms of equal dimensions (50 × 10 cm). One arm, enclosed by 40-cm high walls, was perpendicular to two opposed open arms. The apparatus was elevated 50 cm above the floor, and the experiments were performed with the observer inside the room. Before the experiment, animals (9–21 rats per group) were gently handled for 5 min. Each rat was placed at the end of the enclosed arm of the T-maze, and the time taken to withdraw from this arm with the four paws was recorded (baseline latency). The same measurement was repeated in two subsequent trials (avoidance 1 and avoidance 2, memory acquisition) at 30-s intervals. Three days later avoidance latency was measured again (avoidance 3, memory retention).

*cAMP Assay*

Hippocampi were rapidly dissected out and homogenized in a 50 mM Tris/HCl buffer, pH 7.5, containing 4 mM EDTA. The 20% homogenate (w/v) was heated for 5 min in a water bath at 100°C, and centrifuged at 12,500 rpm for 10 min. The cyclic AMP assay (Amersham's cyclic AMP [$^3$H] assay system) was based on the competition between an unlabeled and a fixed quantity of the tritium-labeled cAMP for binding to a protein, which has a high specificity and affinity for cyclic AMP. Briefly, 50 μl of each sample (in duplicate) together with 50 μl of the labeled cyclic AMP, and followed by 100 μl of the binding protein were added to the assay tubes. The tubes were placed into a refrigerator at 2–8°C for 2 h, and then 100 μl of the charcoal suspension were added. After centrifugation for 5 min, 200 μl of the supernatant were removed and placed in scintillation vials for counting. Hippocampal cAMP levels were determined by comparison with a standard curve, and results expressed as pmol/mg of protein.

*Histological Analysis*

A group of control, ptx, and glu-treated animals were killed by decapitation and used for histological analyses. Brains were removed and fixed in 10% formalin for 3 days. After an initial coronal section at the level of the optic nerve, 1 mm slices were sequentially obtained in anterior and posterior directions. Slices were stained with hematoxillin and eosin for light microscopy studies. Histopathological analyses were done in a single blinded way, and structures were assessed according to Paxinos and Watson (27). The degree of brain damage was based on the presence of gliosis, picnosis, and vacuolization. The severity of lesion was defined along a percentage scale from 0 (none) to 100 (severe) within each structure examined by light microscopy (100×) and previously defined to be reliable for morphological analysis (7,28). Results are expressed as such: 0 or absent—there is no involvement of the structure; mild or 25%—there is more than 25% and less than 50% involvement of the structure; moderate or 50%—there is more than 50% and less than 75% involvement of the structure; severe—more than 75% involvement of the structure. Animals were defined as having brain damage if one or more structures showed at least 50% involvement.

*Drugs*

L-Glutamic acid (Sigma, St. Louis, MO) was dissolved in PBS. Pentoxofylline 400 mg/ml, ampoules (Trental-Hoescht, Brazil), sodium thiopental (Abbott, Brazil), and chloral hydrate (Sigma) were used. All other drugs and reagents were of analytical grade.

*Statistical Analyses*

The Fischer test was used for comparison among treatments in the open-field test. Because a cutoff time of 300 s was established for avoidance latency, nonparametric analysis was used in the case of T-maze data. In this model, for comparison within group along trials, was used Friedmann's test, whereas one-way ANOVA followed by the test of Bonferroni was used to detect significant differences among treatments within the same trial. Significance level was set at $p < 0.05$. Latencies to reach the platform during water maze acquisition were also analyzed by the test of Friedmann. If animals did not reach the platform in 54 s, they were given a score of 54. For the retention test, one-way ANOVA was used to evaluated latency and crossing differences. The *t*-test was used to access the origin of group differences. In the passive-avoidance test, one-way ANOVA followed by Bonferroni test were used to compare both short and late phase memory within each group. The cAMP assay was also assessed by one-way ANOVA followed by the Tukey test. The histological studies were analyzed by Kruskal–Wallis and Mann–Whitney tests.

## RESULTS

*Effects of Pentoxifylline and Glutamate on the Elevated T-Maze*

As illustrated in Fig. 1, glu affected the performance of inhibitory avoidance during acquisition (avoidance 1), but not in avoidance 2, or when memory was tested 3 days later (avoidance 3) compared to controls. The test of Friedmann showed significant changes in inhibitory avoidance latency (avoidances 1 and 2 compared to baselines) in saline, $\chi^2(3) = 22.411$, $p < 0.0001$, glu, $\chi^2(3) = 18.483$, $p < 0.0003$, ptx 100, $\chi^2(3) = 7.300$, $p < 0.0629$, ptx100+glu, $\chi^2(3) = 9.485$, $p < 0.0235$, and ptx200 + glu, $\chi^2(3) = 7.754$, $p < 0.0514$-treated groups. This indicates good avoidance acquisition and memory retention. However, the performance of the glu-treated group was not as efficient (avoidance 2). There were significant differences (one-way ANOVA) among groups at avoidance 2 $F(5, 81) = 3.013$, $p < 0.015$, meaning that memory was improved by the drug treatment at these trials. Although memory consolidation was well maintained in ptx100 group compared to other groups, no difference was detected among treatments in avoidance 3. Bonferroni test showed significant differences ($p < 0.05$) for ptx100 (300.0 ± 0.00 s) compared to glu (99.43 ± 30.46 s) (avoidance 1), for ptx100 (300.0 ± 0.00 s), and ptx200+glu (272.15 ± 17.533 s) compared to glu

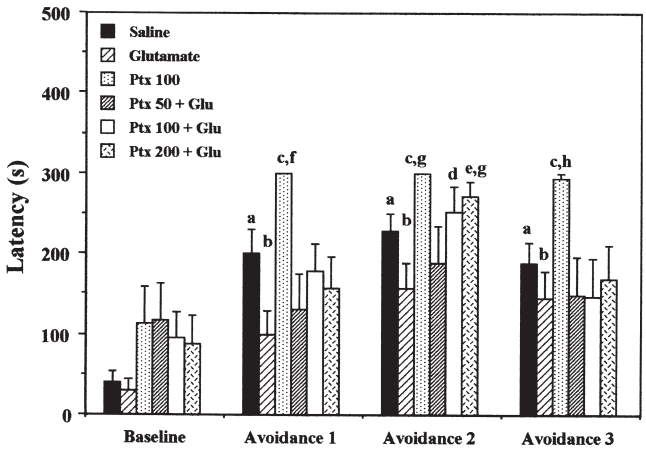


FIG. 1. Elevated T-maze test in rats without (saline or ptx, 100 mg/kg IP administered for 6 days) and with glutamate-induced hippocampal lesions (glu and ptx+glu groups). Ptx (50, 100, or 200 mg/kg IP) was administered daily for 6 days. Vertical bars represent means ± SEM. Number of animals per group ranged from 6 to 21. (a) vs. saline (baseline), (b) vs. glu (baseline), (c) vs. ptx 100 (baseline), (d) vs. ptx100+glu (baseline), (e) vs. ptx 200+glu (baseline), (f) vs. glu (avoidance 1), (g) vs. glu (avoidance 2), (h) vs. glu (avoidance 3). Baselines: Friedmann test; avoidances: ANOVA and post hoc Bonferroni test.

(156.95 ± 32.200 s) (avoidance 2), and for ptx100 (293.55 ± 5.760 s) compared to glu (144.76 ± 33.740 s) (avoidance 3).

*Effects on Passive Avoidance*

Figure 2 shows that controls (saline-treated group) presented an improved performance in the early phase of memory as well as in the late phase of memory consolidation (short- and long-term memories) compared to its baseline, $F(2, 30) = 6.088$, $p < 0.006$. On the other hand, glutamate-lesioned group (glu-group) did not present any alteration in latencies in the passive task compared to its baseline, which indicates an impairment of memory acquisition and retention, $F(2, 33) = 1.143$, $p < 0.33$. An improved performance was detected in the ptx100, $F(2,24) = 15.804$, $p < 0.0001$, ptx100+glu, $F(2, 45) = 3.024$, $p < 0.05$, and ptx200+glu, $F(2, 27) = 3.915$, $p < 0.03$, compared to its baseline. Differences in performances were observed among saline, glu, ptx100, ptx50+glu, ptx100+glu, and ptx200+glu-treated groups in the short memory, $F(5, 60) = 3.039$, $p < 0.0164$. In this condition, a significant impairment was observed in glu-lesioned rats compared to saline, $t(17) = 2.363$, $p < 0.03$, and un improvement with ptx50+glu group compared to glu-lesioned rats, $t(19) = 4.207$, $p < 0.0005$. In the late phase of memory, a significant difference was detected among groups, $F(5, 60) = 12.629$, $p < 0.0001$), and in glu-lesioned rats a significant impairment in performance was observed compared to all other groups at $p < 0.001$ (saline = 252.57 ± 31.70 s; glu = 29.92 ± 14.54 s; ptx100 = 300.00 ± 0.00 s; ptx50+glu = 300 ± 0.00 s ptx100+glu = 165.68 ± 33.93 s; ptx200+glu = 221.19 ± 35.41 s).

*Effects on Locomotor Activity*

No difference in locomotor activity was detected in the groups treated with saline, glu, ptx100, or ptx50+glu. However, an increased locomotor activity was observed in the ptx100+glu-treated group (24.81 ± 4.00, $p < 0.02$) and ptx200+glu (25.3 ± 4.98) compared to the saline-treated one (13.43 ± 2.981) (Fig. 3). An increased open-field activity is consistent with a hypothesis of hippocampal dysfunction, and in the present work, this effect was increased in the presence of pentoxifylline.

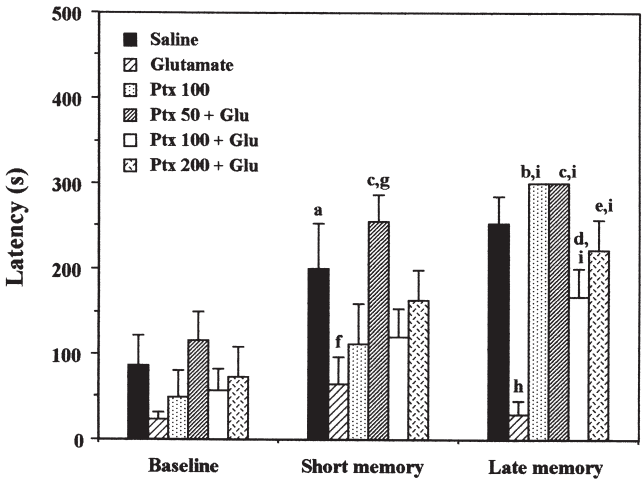


FIG. 2. Inhibitory passive avoidance test in rats without (saline or ptx, 100 mg/kg IP administered for 6 days) and with glutamate-induced hippocampal lesions (glu and ptx+groups). Ptx (50, 100 or 200 mg/kg IP) was administered immediately after glutamate-induced lesion, for 6 days. $p < 0.05$ (a) vs. saline (baseline), (b) ptx100 (baseline), (c) vs. ptx50+glu (baseline), (d) vs. ptx100+glu (baseline), (e) vs. ptx200+glu (baseline, Friedman test, (f) vs. saline (short memory), (g) vs. glu (short memory), (h) vs. saline (late memory), (i) vs. glu (late memory). Baselines: Friedmann test; short and late memory: ANOVA and post hoc Bonferroni test.

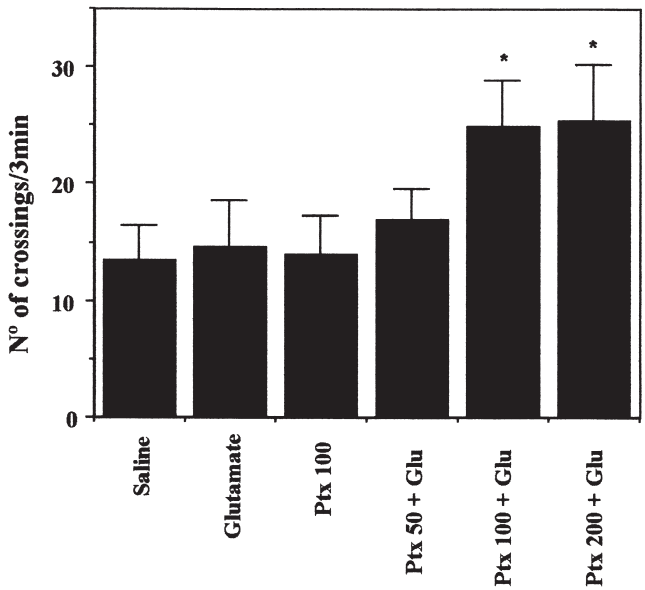


FIG. 3. Open-field test in rats without (saline or ptx 100 mg/kg IP administered daily for 6 days) and with glutamate-induced hippocampal lesion (glu and ptx+glu groups). Ptx (50, 100, or 200 mg/kg IP) was administered daily for 6 days. Number of squares crossed by each animal was recorded for 3 min. Number of animals per group ranged from 6 to 16. *$p < 0.05$, vs. saline (Fischer test).

*Effects on Water Maze*

Figure 4 showed that all groups improved their own water-maze acquisition performance (i.e., latency to find the platform) along trials as evidenced by a significant decrease in daily latency [saline, $\chi^2(11) = 67.737$, $p < 0.0001$; glu, $\chi^2(11) = 47.138$, $p < 0.0001$; ptx100, $\chi^2(11) = 30.795$, $p < 0.0012$; ptx100+glu, $\chi^2(11) = 37.5$, $p < 0.0001$, and ptx200+glu, $\chi^2(11) = 44.088$, $p < 0.0001$]. All groups presented a similar performance on the final day of acquisition (trial 12, $p > 0.05$). Mean times to reach the platform location (retention), 48 h after the last training day, were significantly different between groups, $F(4, 55) = 3.057$, $p < 0.0240$. The mean latency of the glu, ptx100, and ptx100+glu groups were 26.8 ± 3.9; 24.9 ± 6.9; 28.1 ± 6.7 s, respectively, while controls and ptx200+glu reached the platform location in 10.9 ± 3.5 and 11.8 ± 1.4 s, respectively (Fig. 5). These results showed that the impairment in memory retention observed in glu-lesioned rats, $t(31) = 2.875$, $p < 0.0072$ vs. saline, was reversed by the high pentoxifylline dose, ptx200+glu-treated group, $t(25) = 2.407$, $p < 0.0238$ vs. glu. No differences were detected in the number of crossings in either group.

*Histological Analysis*

Glu (24, 48, 96, and 192 μg)-induced brain lesions in a dose-dependent manner. We used a 96 μg dose that produced

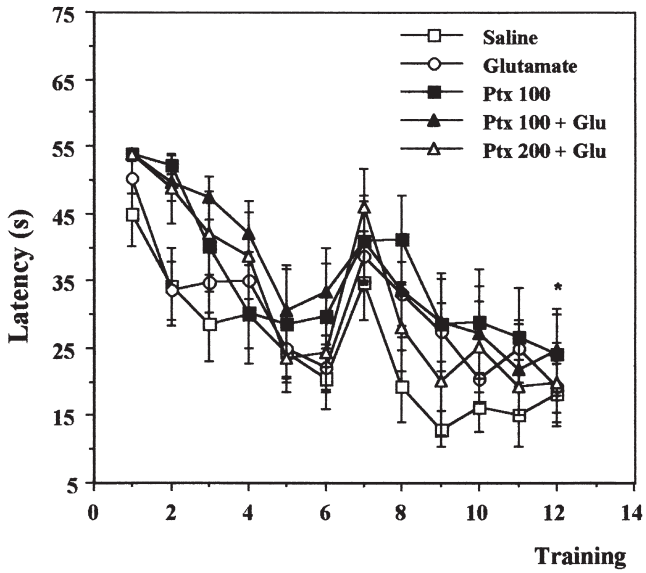


FIG. 4. Mean time to reach the platform on each of six acquisition trials. With the exception of controls and ptx (100 mg/kg, IP, for 6 days), all other groups were submitted during the stereotaxic procedures to glutamate-induced hippocampal lesions followed by the administration of ptx 100 or 200 mg/kg IP for 6 days. Vertical bars indicate means ± SEM. Number of animals per group ranged from 8 to 19. $< 0.05$ *Friedmann test (comparing first and last trials).

moderate and severe lesions (Fig. 6b) in opposition to controls injected with saline (Fig. 6a). The pretreatment of animals with ptx100, partially reversed glu-induced hippocampal lesions (Fig. 6c), and this group presented only 25% of mod-

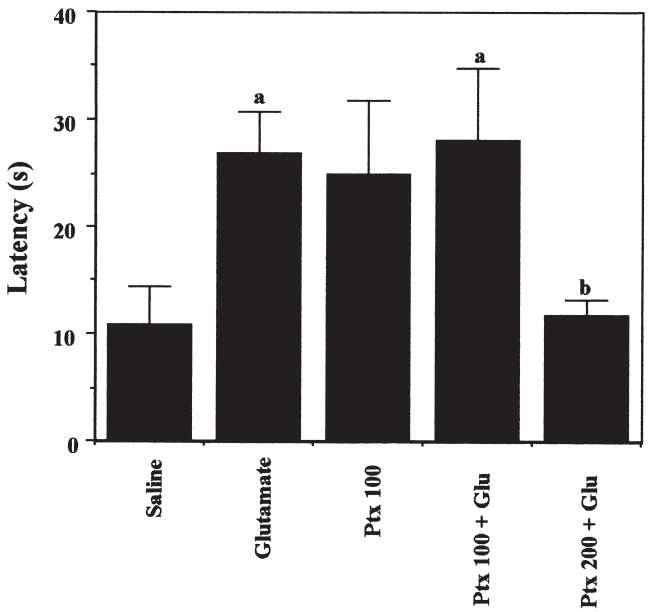


FIG. 5. Water-maze task in rats without (saline or saline plus ptx, 100 mg/kg IP, administered for 6 days) or without glutamate-induced hippocampal lesions (glu and ptx+glu groups). Ptx (100 or 200 mg/kg IP) was administered daily for 6 days. Bars represent means ± SEM. Number of animals per group ranged from 8 to 19. $p < 0.05$, (a) vs. saline, (b) glu (ANOVA and *t*-test).

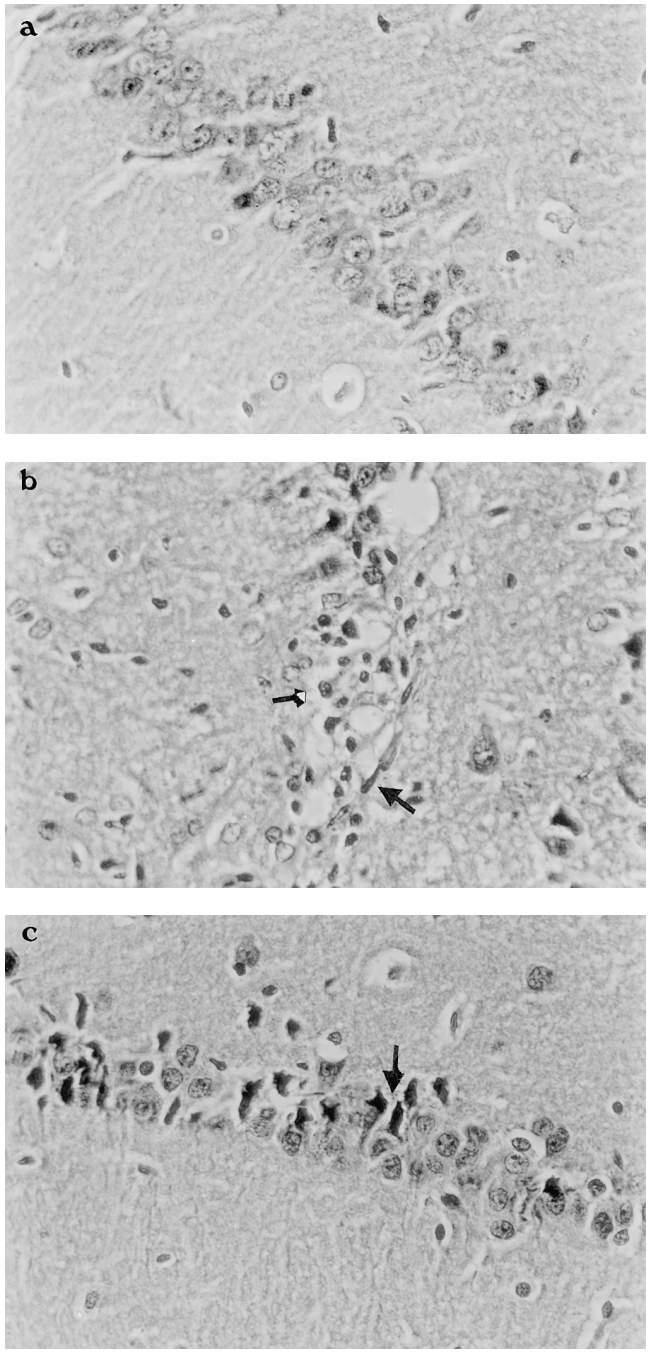


FIG. 6. (a) Light microscopy of hippocampus (CA1 subfield) of rats stereotaxically treated with saline; (b) glutamate (96 μg) showing picnosis (arrows), neuronal death with destruction of neuronal pallisade, (c) neuronal pallisade of hippocampus of rats treated with pentoxifylline and glutamate, showing rare picnotic cells (arrows). HE × 100.

erate lesions and no severe lesions compared to the group treated only with glu (96 μg), which showed 29.5% of severe and 52.9% of moderate lesions (Table 1).

*cAMP Assay*

Glu drastically reduced ($p < 0.0001$) cAMP levels in the hippocampus (0.1867 pmol/mg protein), and the effect was re-

TABLE 1

HISTOPATHOLOGICAL ALTERATIONS INDUCED BY GLUTAMATE IN THE RAT HIPPOCAMPUS, AND THEIR BLOCKADE BY PENTOXIFYLLINE

| Groups | Lesion (%) | | | | Number of animals |
|---|---|---|---|---|---|
| | Absent | Mild | Moderate | Severe | |
| Saline | 100.0 | — | — | — | 07 |
| Glutamate (μg) | | | | | |
| 24 | 75.0 | 25.0 | — | — | 04 |
| 48 | 57.2 | 42.8 | — | — | 07 |
| 96 | — | 17.6 | 52.9 | 29.5 | 17† |
| 192 | — | — | 33.3 | 66.7 | 06 |
| Ptx100* + Glu96 | — | 75.0 | 25.0 | — | 12‡ |

Results are expressed as mean levels of a percentage scale such as: 0 or absent—there is no involvement of the structure; mild or 25%—there is more than 25% and less than 50% involvement of the structure; moderate or 50%—there is more than 50% and less than 75% involvement of the structure; severe—more than 75% involvement of the structure.

*Ptx100: pentoxifylline 100 mg/kg, IP, administered for 6 days. Sections stained with HE.

†vs. saline, ‡vs. glutamate ($p < 0.001$, Kruskal–Wallis and Mann–Whitney tests.

versed by ptx (100 and 200 mg/kg) in a dose-dependent way (0.3767 and 1.060 pmol/mg protein for ptx 100 and 200 mg/kg respectively.) At the higher dose of ptx (200 mg/kg), cAMP levels were similar to those observed in control and ptx 100 alone (0.968 and 1.170 pmol/mg protein respectively) (Table 2).

## DISCUSSION

Activation of excitatory amino acid receptors in the mammalian brain has been postulated as a cytotoxic mechanism involved in neurodegenerative diseases (11). The excitotoxic mechanism is thought to begin with a pathological process that triggers increased glu activity and excessive opening of calcium channels, poisoning the cell and resulting in the production of free radicals that overwhelm the cell with toxic actions on its membrane and organelles, and ultimately killing it (3).

TABLE 2

cAMP CONCENTRATIONS IN RAT HIPPOCAMPI WITHOUT (SALINE AND PENTOXIFYLLINE) AND WITH GLUTAMATE-INDUCED LESIONS

| Group | Dose | cAMP (pmol/mg Protein) |
|---|---|---|
| Saline | — | 0.968 ± 0.19 |
| Glutamate | 96 μg | 0.186 ± 0.09† |
| Pentoxifylline | 100 mg/kg | 1.170 ± 0.17 |
| Ptx + Glu | 100 mg/kg + 96 μg | 0.376 ± 0.08 |
| | 200 mg/kg + 96 μg | 1.060 ± 0.19‡ |
| | 100 mg/kg + 96 μg* | 0.387 ± 0.11 |

Pentoxifylline was administered intraperitoneally in rats for 6 days, immediatly after hippocampal glutamate-induced lesions.

*Animals received a single injection of pentoxifylline.

†vs. saline, ‡vs. glutamate ($p < 0.01$, one-way ANOVA, Tukey–Kramer test).

The present work showed that glu impaired short-term memory as well as long-term memory, as measured by the inhibitory avoidance foot shock, and these effects were completely reversed by ptx. Ptx-treated group was effective in this learning acquisition task at the earlier measurements (short-term memory), and presented a better performance as related to the saline-treated group on long-term memory, meaning that the drug action is time related.

The latency to withdraw from the enclosed arm in the T-maze task after glu administration was shorter at avoidance 1 compared to control, but increased at avoidance 2. This means that despite the presence of brain lesions, animals were still able to learn, although less efficiently then in the ptx-treated group. This result was observed along trials suggesting that a progressive learning process occurred in the glu-treated group. Ptx, as expected, significantly increased latency times along trials up to 300 s (considered as the cutoff time) and, at the two higher doses, completely reversed the impairment in memory consolidation caused by glu. Unlike the Morris water task, that measures spatial memory impairments, the T-maze test is more useful for measurements of drug effects on working memory, which is probably less affected by glu lesions.

It is known that lesions of the hippocampus by a variety of experimental procedures effectively disrupts acquisition of spacial learning tasks, such as the Morris water task (25,33,36). In the present work, glu did impair spatial learning acquisition. Except for the association of ptx 200 mg/kg plus glu, which was not different from controls, meaning that ptx at the highest dose reversed glu effects, all other groups showed learning impairment.

Our results also showed increased locomotor activity in the glu-lesioned group pretreated with ptx, but no alteration in locomotor activity was observed with glu or ptx alone. As the open-field test was performed 24 h after the last injection of ptx, it is possible that the lower plasma level of ptx was not sufficient to produce the expected xanthine's stimulant effects. However, it is not clear why the ptx pretreatment of the glu-lesioned group intensified locomotor activity.

It is known, that xanthines present significant behavioral effects on measures of locomotor activity, schedule-controlled behavior, drug-self administration, and learning and memory. The behavioral-stimulant effects of xanthines appear to be mediated mainly by their adenosine-antagonist actions, and may be limited by their phosphodiesterase (PDE) inhibition (18). Recent studies (29) indicated that both adenosine $A_1$ and $A_{2A}$ receptors play a functional role in the control of motor activity, and therefore, the blockade of both receptor subtypes is involved in the motor stimulating properties of methylxanthines.

It has been demonstrated (23), that glu or *N*-methyl-D-aspartate (NMDA) could induced the generation of oxygen-derived free radicals. On the other hand, it has been shown that nitric oxide (NO) can evoke a concentration-dependent release of adenosine from rat hippocampus (15), and such NO-stimulated adenosine release may contribute to some of the reported effects of NO donors in the nervous system. Recently (8), it was demonstrated that kainate also evoked release of adenosine from rat hippocampus, which is mediated by activation of non-NMDA receptors, and may involve propagation of action potentials and production of free radicals.

By producing free radicals including NO, glu could also indirectly increase adenosine release and further contribute to

the increased production of free radicals causing cell death. Ptx antagonizes adenosine receptors which, as in the case of $A_1$ receptors, act by inhibiting cAMP production, cAMP has been considered to play a crucial role in the learning and memory functions. cGMP and cAMP have been proposed to participate in the early and late stages of long-term potentiation (LTP) respectively (2). These results indicate that cGMP-regulated processes in the hippocampus play an important role in the early stages of memory consolidation, and that cAMP signalling pathways are involved in the late post-training memory processing of inhibitory avoidance learning. According to Bernabeu et al. (1), the late phase of memory consolidation of an inhibitory avoidance is modulated by cAMP/protein kinase A, PKA signaling pathways, and by dopamine $D_1/D_5$ receptors in the hippocampus. It has been shown (1) that adenylyl cyclase activators such as forskolin, $D_1/D_5$ receptors agonists such as SKF38393, and 8 Br cAMP ameliorate memory processes. In fact, cAMP injection into the lateral ventricle is effective against the experimental amnesia in mice (10).

Recent evidence suggests that alpha-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) and metabotropic receptors may contribute significantly to neuronal injury. Although the activation of both *N*-methyl-D-aspartate (NMDA) and non-NMDA inotropic glutamate receptors can trigger formation of oxygen species (3), it appears that excitotoxicity mediated via the non-NMDA glutamate receptors is more susceptible to inhibition by antioxidant compounds (8). On the other hand, metobotropic receptors group III are linked to inhibition of adenylate cyclase and reduced cAMP production. It has been shown (5) that DCGIV, an agonist at NMDA receptors, by activating NMDA receptors, leads to a release of adenosine. It has also been shown that rolipram, also a phosphodiesterase inhibitor, increases brain cAMP levels in vitro (14) and in vivo (30) studies. Results by Imanishi et al. (20) indicated that rolipram ameliorates impairments of learning and memory in rats and mice, and suggest that these effects are the result of elevating cAMP levels caused this compound. Other phosphodiesterase inhibitors have been shown to enhance learning and memory functions, and to ameliorates experimentally induced amnesia or cognitive dysfunction in both animals and humans (12,32). In addition, it has been demonstrated (22) neuroprotective effects of elevated cyclic nucleotide levels against lipid peroxidation and lipid peroxidation products-mediated neural toxicity.

Our work also showed that ptx and glu respectively significantly increased and decreased hippocampal cAMP levels compared to controls, and ptx reversed the inhibitory effects observed in the hippocampus of glu-lesioned rats. Recent data (31) showed that ptx reduced cerebral injury and preserved neurologic function in transient global ischemia in rats. All together, our data suggest that the effect of ptx on glu cytotoxicity may be the result of increased cAMP levels produced by ptx and/or inhibition of adenosine $A_1$ receptors. As a matter of fact, our work showed that ptx totally reversed the decrease in cAMP levels produced by glu, bringing hippocampal cAMP levels close to those of controls. However, the fact of ptx having antiinflammatory properties may also contribute to this action.

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