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Effects of Piracetam on Retention and Biogenic Amine Turnover in Albino Rats

K. NALINI,*[1] K. S. KARANTH,† A. RAO‡ AND A. R. AROOR*

**Department of Clinical Biochemistry and Nutrition, †Department of Pharmacology, and ‡Department of Biochemistry, Kasturba Medical College, Manipal 576119, Karnataka, India*

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NALINI, K., K. S. KARANTH, A. RAO AND A. R. AROOR. *Effects of piracetam on retention and biogenic amine turnover in albino rats.* PHARMACOL BIOCHEM BEHAV **42**(4) 859–864, 1992. – The chronic effects of orally administered 2-pyrrolidone acetamide (piracetam) on one-trial, passive avoidance task were studied in albino rats. The effects on the contents of norepinephrine (NE), dopamine (DA), and serotonin (5-HT) in the brain and on the levels of their metabolites both in the brain and urine were also assessed. Significant improvement was observed in the retention ability compared with saline-administered controls. The contents of NE, DA, and 5-HT and their metabolites in the brain were significantly decreased after piracetam administration. The urinary metabolite levels were also significantly decreased except total 3-methoxy-4-hydroxyphenyl glycol (MHPG). These data indicate that piracetam causes an overall decrease in the turnover of central monoamines. Thus, the results of this study implicate the involvement of NE, DA, and 5-HT systems in learning and memory processes. Piracetam did not exert any GABAergic effect as shown by the absence of change in the brain GABA levels.

Piracetam Passive avoidance Norepinephrine Dopamine Serotonin GABA

2-PYRROLIDONE acetamide (piracetam), a cyclic derivative of GABA and the forerunner of the pyrrolidones, has been recognized as a nootropic agent and is gaining importance in the treatment of cognitive disorders (18). The beneficial effect of piracetam on cognitive function has been shown in various experimental models and clinical trials. In experimental animals, piracetam has been claimed to facilitate acquisition of learning, augment memory consolidation, and resist memory impairment induced by aversive stimuli (17). Administration of piracetam (600 mg/kg) orally for 5 days completely abolished the memory-impairing effect of diethyl dithio carbamate and clonidine in step-down passive avoidance (16) and in two-way active avoidance tests after clonidine in albino rats (28). Combination of piracetam with lecithin has shown therapeutic efficacy in Alzheimer's patients (39,45) and in demented patients (14). Piracetam produced improvement in performance in dyslexics (8,43), in some adult males with alcoholic psychosis (44), and improved the mental performance of normal individuals (11).

The effects of acute and chronic administration of piracetam on different brain neurotransmitter systems have also been studied. Although a cyclic derivative of GABA, piracetam did not alter either synthesis, release, or uptake of GABA. Acute treatment with piracetam (IP) increased the dihydroxyphenylacetic acid (DOPAC) levels in the striatum, whereas chronic administration increased normetanephrine in the cerebral cortex. No changes were observed in homovanillic acid (HVA), dopamine (DA) or 5-hydroxyindoleacetic acid (5-HIAA) levels in the rat striatum (31). Nyback et al. (34) reported elevated levels of metabolites of DA and norepinephrine (NE) in the rat brain after intraperitoneal administration of piracetam.Another study (4) showed that the lower dose (20 mg/kg, IP) produced a decrease in 5-hydroxytryptamine (5-HT) levels and increase in NE levels. The higher dose of piracetam (100 mg/kg) produced the opposite effect. No significant changes were observed in DA levels. Thus, all these results indicate that piracetam exerts some effect on monoaminergic neurotransmitter systems but the results are ambiguous.

Hence, the present study was carried out to assess the simultaneous alterations in the brain contents of biogenic amines, namely, NE, DA, and 5-HT, and their metabolites following chronic treatment with the clinically used dose of piracetam computed for rats. Simultaneous changes in the urinary levels of these metabolites were also determined to see if the urine metabolite levels reflect the changes in the turnover of the biogenic amines in the brain. GABA levels in the brain were also estimated. Because monoamines have been reported to be involved in learning and memory processes (2,30,47),

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

this study attempts to correlate the changes in the neurotransmitters with the cognitive effect of piracetam.

METHOD

Selection of Animals

Male, albino rats aged 60–90 days (inbred), Wistar strain, weighing 140–180 g were housed in groups of two with food and water ad lib. Rats were maintained in a reversed 12 D:12 L cycle.

Drug Administration

Piracetam was administered orally for a duration of 15 days at the dose of 50 mg/kg body weight, while control rats received saline orally for 15 days. Drug was administered everyday at 8:00 a.m.

Passive Avoidance Test

Ten controls and 10 piracetam-treated rats were included in the study. The effect of piracetam on retention performance was assessed using a two-compartment step-through passive avoidance test (7). The test was done on the fourteenth day of treatment 3 h after drug administration. Rats were allowed to explore the two-compartment apparatus for 3 min individually. The time when the animal entered the dark compartment or left it was noted. The learning session was followed immediately. The glass door between the two compartments was closed and the rat was confined to the dark compartment wherein three inescapable electric foot-shocks of 0.8 mA were delivered for 1 s each at an interval of 20 s. Rats were then returned to their home cages. Twenty-four hours after the shock, animals were tested for retention performance. Piracetam and saline were given to the respective groups in their home cages before carrying out the retention test to exclude state dependency. Each rat was allowed to explore the two-compartment apparatus similar to the first exploration. The time of its entry into and exit from the dark compartment was noted for a duration of 3 min. The increase in latency to enter the dark compartment and the decrease in the time spent inside the dark compartment during the second exploration, that is, 24 h after the inescapable shock, was interpreted as good retention performance.

Bright and Dark Arena Test

Bright and dark arena test (10) was carried out to examine if piracetam caused any freezing effect on rats in the bright side. Controls as well as drug-treated rats were allowed free run in a two-chambered arena where two thirds of the area was illuminated and one third was darkened. The time spent by rats in the bright and dark arenas was noted over a period of 5 min.

Reaction Time on Hot Plate

The hot plate test (50) was carried out to see if piracetam had any effect on pain threshold of rats. The plate was maintained at 55°C and the time taken by rats to jump out of the plate was measured.

Biochemical Study

A different set of controls and piracetam-treated rats were used for biochemical study ($n = 16$).

Urine Collection and Analysis

On the fourteenth day of drug administration, rats were kept in separate metabolic cages for 24-h urine collection. Urine was collected in containers containing 0.5 ml 6 N HCl and few drops of liquid paraffin. After 24 h, the urine samples were transferred to labeled tubes and centrifuged to get a clear specimen. The volume of each urine sample was noted and stored at −20°C until further analysis for HVA, VMA, total 3-methoxy-4-hydroxyphenyl glycol (MHPG), and 5-HIAA.

Colorimetric reactions following various extraction steps were employed in the estimations of HVA (24), Vanil mandelic acid (VMA) (42), and 5-HIAA (41). MHPG in urine was estimated by the spectrophotometric method of Bigelow et al (5). Urinary creatinine was estimated using Jaffe's reaction (6). The values of these metabolites were expressed in μg/mg creatinine.

Preparation and Analysis of Brain Tissue Sample

After urine collection, rats were killed by swift decapitation. Brains were rapidly removed, excluding the cerebellum and olfactory bulbs, and transferred to ice-cold saline. The weight of the individual brain was noted and immediately homogenized in 5 ml 0.4 N ice-cold perchloric acid containing 0.1% EDTA disodium salt and 0.05% sodium metabisulphite and internal standard dihydroxy benzylamine (DHBA) using a Teflon–glass homogenizer (Thomas Scientific, Philadelphia, PA). The homogenates were centrifuged at 12,000 rpm for 10 min at 4°C. The supernatants were removed and stored at −20°C until assayed.

High-Performance Liquid Chromatography

Biogenic amines and their metabolites, namely, NE, DA, 5-HT, MHPG (unconjugated), HVA, and 5-HIAA, in the brain homogenate were estimated using high-performance liquid chromatography (HPLC) with electrochemical detector. The amines and their metabolites were isolated on a C18 reverse-phase column of particle size 5 μm and 25 cm length using the isocratic elution method. The mobile phase was composed of 70 mM sodium acetate buffer, pH 4.5, containing 1 ml 10% EDTA and 0.05 M hexane sulfonic acid in 1 l buffer. The buffer was filtered through a 0.45-μm millipore membrane filter and then mixed with methanol in 87:13 (v/v) ratio before use. The mobile phase was degassed using an on-line degasser before passing through the column. The flow rate was kept at 1.5 ml/min. The voltammetric detector with a glassy carbon electrode was used for electrochemical detection of the amines and metabolites. The detector potential was set at 0.8 V vs. an Ag/AgCl reference electrode with a sensitivity of 1 nA. Twenty-five microliters of the filtered homogenate was injected without prior processing. Elution pattern of the peaks are as shown in Fig. 1. The actual concentration of the amines was calculated by comparing the recovery of each standard amine from the crude homogenate with that of the internal standard. The amount of the amines and their metabolites were expressed in ng/gm weight of the brain.

Brain GABA concentration was determined by ninhydrin reaction after ion exchange separation (40).

Statistical Analysis

The data are expressed as mean ± SD and Student's *t*-test was used for comparison of the data of the two groups.

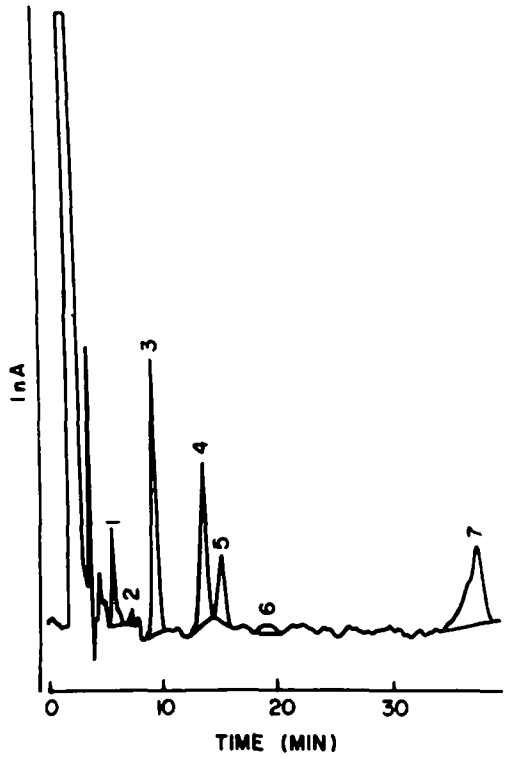

FIG. 1. Chromatogram of the catechols and 5-OH indoles from rat brain (10 μl of the homogenate injected). Peaks: (1), NE; (2), MHPG; (3) DHBA; (4), DA; (5) 5-HIAA; (6), HVA; (7), 5-HT.

RESULTS

Two-Compartment Passive Avoidance

The results of the passive avoidance performance comprising latency and time spent in the dark compartment are shown in Fig. 2. Piracetam-treated rats exhibited a significantly higher latency (154.9 s, $p < 0.01$) in the second exploration period than control rats (71.3 s), whereas they spent significantly less time in the dark compartment compared to control rats. The mean time spent by the piracetam group was 3.5 s ($p < 0.001$) whereas it was 62.3 s in the controls during the second exploration period. These results show that treatment with piracetam for 15 days improved retention and rats did not develop tolerance toward piracetam.

There was no difference in the rearing and exploratory activity between control and piracetam-treated rats as observed in the bright and dark arena test. All rats displayed a preference for the dark arena and there was no difference between the two groups in the amount of time spent in the dark arena as well as in time spent in the bright arena. The time spent by control rats in the dark arena was 207 ± 10.7 s ($n = 5$) whereas it was 209 ± 22.8 s ($n = 5$) for drug-treated rats. The time spent in the bright arena by controls and drug-treated rats was 35 ± 8.6 and 36.8 ± 9.5 s, respectively.

There was no difference in the time taken by rats of the two groups to jump out of the hot plate. The mean time taken by control and piracetam-treated rats was 2.4 and 2.2 s, respectively.

Rats of the two groups did not show any difference in their appetitive behavior and their weight gain was comparable.

Brain Monoamines and Their Metabolites

The data on the levels of brain amines, namely NE, DA, and 5-HT, and their metabolites, namely, MHPG, HVA, and 5-HIAA, in control and piracetam-treated rats are given in Table 1. Piracetam treatment caused a significant reduction in the concentration of all three amines and their metabolites in the brain. The mean decrease in DA content was 47.5% ($p < 0.001$) and the amount of its metabolite, HVA, decreased by 53.5% ($p < 0.001$) compared to control levels. The amount of NE and its metabolite, MHPG (unconjugated), also decreased significantly ($p < 0.001$), the mean decrease in the values being 58.6% for NE and 46.2% for MHPG. Similarly, the 5-HT and 5-HIAA levels in brains decreased by 46.2% ($p < 0.001$) and 25% ($p < 0.001$) of control values, respectively.

The results of the analysis of the amine metabolites in urine are given in Table 2. The levels of the metabolites excreted in the urine, except for MHPG, were significantly low in the piracetam-treated group. The mean reduction observed was 21.6% ($p < 0.001$) for HVA, 11.5% ($p < 0.05$) for VMA, and 22.4% ($p < 0.05$) for 5-HIAA.

Brain GABA levels were not altered by piracetam treatment (Table 1).

DISCUSSION

The present study examined the effect of piracetam on retention in passive avoidance and on the metabolism of biogenic amines in the brain. Consistent with the results of the previous studies (16,17), chronic piracetam treatment enhanced retention in a passive avoidance task.

The results of the bright and dark arena test, as well as the hot plate test, rule out the possibility of freezing effect in the bright chamber and sensitizing effect of piracetam on rats. The freezing effect on rats due to the shock received during the acquisition is also ruled out as controls receive the same shock.

Piracetam treatment resulted in a significant decrease in the levels of all three amines and their metabolites in the brain

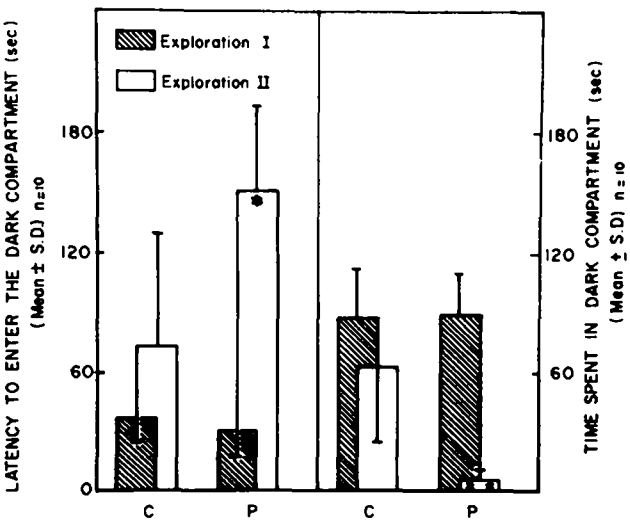

FIG. 2. Two-compartment passive avoidance test. Latencies to enter the dark compartment (left) and time spent in the dark compartment (right) in explorations I and II in control (C) and piracetam-treated (P) groups. $^{*}p < 0.01$ and $^{**}p < 0.001$ compared to the control group.

TABLE 1

LEVELS OF BIOGENIC AMINES AND THEIR METABOLITES (ng/g BRAIN WEIGHT) AND GABA (mg/g BRAIN WEIGHT) IN THE BRAIN OF CONTROL AND PIRACETAM-TREATED RATS

Group	DA	HVA	NE	MHPG	5-HT	5-HIAA	GABA
Controls (n = 16)	840 ± 110	66.4 ± 11.1	285 ± 66	39.3 ± 7.1	522 ± 87	408 ± 80	1.41 ± 0.29
Piracetam (n = 16)	441 ± 89*	30.9 ± 10.9*	118 ± 22*	21.2 ± 3.9*	281 ± 47*	306 ± 50*	1.52 ± 0.26

Values are expressed as mean ± SD.
*$p < 0.001$ vs. control group.

in the present study. This is in contrast to earlier findings where an increase in the metabolite levels were observed (31,34). No change in DA levels was observed in another study (4). The discrepancies may be attributed to the different dosages, duration of treatment, or analytical methods used in these studies.

The decrease observed in the amine levels in our study can be correlated to the memory-enhancing ability of piracetam. The exact role played by DA in mediating learning and memory function is not yet clear. Quite a few reports are available suggesting a positive role for DA in cognitive function. It has been reported that animals fail to acquire avoidance responses if trained after an intracisternal or intranigral injection of 6-hydroxydopamine (6-OHDA) (3,9). On the other hand, intraventricular injection of 0.1 μg DA improved the retention of the passive avoidance step-through task (23). However, the findings of the present study suggest that improved retention is associated with low levels of DA. There are several reports supporting this finding. Different studies using DA agonists and electrical stimulation suggested that heightened activity in the dopaminergic system during learning and memory might lead to retention failure. Fernandez-Tome et al. (13) found that apomorphine administered before or immediately after training on a passive avoidance step-through task produced marked amnesia when tested for 24-h retention. Similar results were observed with the electrical stimulation of the substantia nigra (38). However, the impairments observed in active and passive avoidance performances on treatment with DA antagonists such as haloperidol (21) and pimozide (22) or due to 6-OHDA lesions (15) were attributed to the deficit produced in motor response rather than the learning process.

The results of the studies on the role of the NE system in learning and memory are equivocal. Depletion of NE using synthesis inhibitors like diethyl dithiocarbamate (DDC) and FLA 63 impaired the performance of many behavioral tasks (46,51). The amnesia caused by DDC could be reversed by intraventricular injections of NE (48). Posttraining peripheral administration (IP) of adrenaline enhanced retention at low to moderate doses and impaired retention at higher doses (20,32). But, contradicting reports have come from other studies. The peripheral as well as central administration of epinephrine, NE, and their agonists were found to suppress avoidance behaviour (25,33,49). In addition, reduction in brain DA and NE levels was observed after learning in active and passive avoidance tasks with improved 24-h retention (19,37). The reduced concentrations of NE in the present study correlate well with the above observations. The role of 5-HT in learning and memory is found to be inhibitory in various experimental studies. 5-HTP administration, systemic or intracerebral, has been shown to impair passive avoidance performance (12,35). Serotonin antagonists (1) and depletion of brain 5-HT were found to facilitate learning and enhanced the retention process in avoidance tasks (29). The destruction of 5-HT neurons or the use of the 5-HT synthesis inhibitor *p*-chlorophenylalanine (PCPA) resulted in blocking the impairment of active and passive avoidance caused by *p*-chloroamphetamine (PCA) administration (36). The decreased levels of 5-HT in brain of the piracetam-treated group favors the inhibitory role of 5-HT in learning and memory.

Moreover, the decrease in the levels of the amines is accompanied by a decrease in their respective metabolites in all three amine systems. These results indicate that piracetam causes an overall decrease in the turnover of central monoamines.

The results of the estimation of the metabolites in the urine show that the urinary HVA levels decreased significantly after piracetam treatment. This decrease in urinary HVA may be considered an index of decreased central DA activity as there is simultaneous reduction in DA and HVA levels in the brain. This hypothesis is further supported by the earlier report that 50% of urinary HVA represents DA metabolism of central origin (26).

Although VMA, the major metabolite of NE, levels in urine were reduced significantly, the data is not sufficient to suggest any relationship between the central NE metabolism and either urinary VMA or MHPG levels or both. VMA in urine is of both central as well as peripheral origin, the latter being the predominant source. Moreover, no change was observed in the total MHPG levels in urine of these animals.

TABLE 2

LEVELS OF URINARY BIOGENIC AMINE METABOLITES (μg/mg CREATININE) IN CONTROL AND PIRACETAM-TREATED RATS

Group	HVA	VMA	MHPG	5-HIAA
Controls (n = 16)	11.1 ± 2.2	15.6 ± 2.5	9.4 ± 2.8	4.9 ± 1.5
Piracetam (n = 16)	8.7 ± 2.7*	13.8 ± 2.0†	9.1 ± 2.8	3.8 ± 1.2†

Values are expressed as mean ± SD.
*$p < 0.01$, †$p < 0.05$ vs. control group.

Even though MHPG is the major metabolite of NE in the brain, it is not so in the urine. Of the total urinary MHPG, only 20% is from brain NE (27). However, the present study is limited by the fact that unconjugated MHPG was estimated in the brain rather than total MHPG.

Similarly, the lowered excretion of 5-HIAA may reflect the decreased central 5-HT metabolism as it is associated with the reduced concentrations of both 5-HIAA and its precursor 5-HT in the brain.

The absence of any change in brain GABA levels rules out the possibility of GABA-mimetic action of piracetam in mediating its effects (4).

In conclusion, the alterations produced by piracetam in the concentrations of DA, NE, 5-HT, and their metabolites in the brain suggest the involvement of all three monoaminergic systems in learning and memory. Probably, a balanced interaction between the different neurotransmitter systems is essential for optimal learning and memory process rather than the functioning of any single system on its own. The urinary metabolite levels may reflect the central aminergic activity and their estimations may be useful under certain conditions, especially in clinical studies. However, further study on the biogenic amine levels and their receptors in different regions of the brain is needed to elucidate the exact mechanism of action of piracetam.

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