ROLE OF THE SEROTONIN IN MEMORY PROCESSES IN THE RAT

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Abstract: Chronic 5, 7-dihydroxytryptamine (5, 7-DHT, 150 µg,i.c.v.) disruption of the central serotonergic function, is able to interfere with learning and memory processes in the rat. Serotonin depletion significantly diminished spontaneous alternation % in Y-maze task, which suggest the impairment of short-term memory. Long-term memory does not undergo significant changes. Parachlorophenylalanine (200µg i.c.v. x 3 days) a semichronic serotonin neurotoxin, do not impaired long-term memory. This effect of serotonin depletion was not produced at the level of organism motricity that, in turn, would allow an enhancing efficiency of another neurotransmitters contribution to memory processes, as number of arm entries was not affected by serotonin depletion. It is concluded that learning and memory processes is a multitransmitter system function, in which serotonin play an important role.

INTRODUCTION

There are several reason for the suggestion that cholinergic neurotransmission mediate cognitive processes: cholinergic drugs alter cognitive performance in humans (Drachman and Leavitt,1974) and animals (Hefco et. 2003); cholinergic neurons undergo degeneration in dementing disorders (Davies and Maloney, 1976), and cognitive deficits in demented patients have been correlated with cholinergic dysphunction (Perry et al. 1978), suggesting that at least some of the cognitive impairments in dementia are due to cholinergic damage; lesions of cholinergic systems induce cognitive deficits in animals (Flicker et al. `1983).

Other neurotransmitter systems have also been implicated in these processes and serotonin (5-HT) is though to be involved. Drugs acting at serotonergic system influence human (Crook 1991) as well as animal (Steckler, Sahgal, 1995) cognition; serotonergic neurons degenerate in disorders associated with dementia (Steckler and Sahgal, 1995) although the literature concerning the role of 5-HT in cognition is more controversial than the literature covering the function of cholinergic systems in mediating these behavioral processes.

The majority of serotonergic neurons are restricted to clusters of cell lying within or near the raphe regions of the pons and medulla. The dorsal raphe nucleus (DR) containing approximately half of all serotonergic neurons in the brain (Steckler and Sahgal, 1995) and the medial raphe nucleus (MR) have received most attention in the literature. Ascending projections of these two nuclei travel through the medial forebrain bundle and projections from the DR reach hypothalamic nuclei, thalamic nuclei, basal ganglia, septal area, cerebral cortex and hippocampus. The MR projects predominantly to hippocampus and septum. Further, there are serotonergic neurons located outside these areas, for example, within the hypothalamus, the interpeduncular complex and reticular formation of the brain.

The aim of the present work was to study the effects of lesions of all these different serotonergic neurons by means of i.c.v. administration of 5, 7 –DHT, a chronic serotonergic neurotoxin, acting on the neuronal cell bodies, and parachlorophenilalanine (PCPA), a semichronic serotonergic neurotoxin, which blocks the synthesis of serotonin, on learning and memory processes evidenced by means of Y-maze task and multi-trial passive avoidance task. Our data suggest that serotonin play one important role on learning and memory processes.

MATERIAL AND METHODS

Animals

The experiments were carried out on male Wistar rats weighing 225-250g at the start of the experiment. The rats were treated in accordance with the Guidelines for Animal Experiments of the “Al.I.Cuza” University and of the U.S. National Institute of the Health Guide for the Care and Use of Laboratory Animals. The rats were housed three per cage with free access to food and water under controlled laboratory conditions (a 12-h light/dark cycle with light on at 8:00 a.m., 22±0.5 0 C).

Surgery

All surgical procedures were conducted under aseptic conditions, under sodium pentobarbital (45 mg /kg b.w. i.p.) anesthesia. Injections into the lateral ventricle were performed stereotaxically through a 10µl Hamilton syringe at the following coordinates (in mm): A-0.5mm (from bregma), L±1.3 (from midline), V- 4.3 (from bregma), with the incisor bar set at 3.3 mm beneath the level of the interaural line (Paxinos and Watson 1986).After each injection, the needle was left in situ for 5 min, retracted 2 mm, and a second delay of 4 min was allowed before complete retraction.

All rats sustaining a 5, 7-DHT lesion were pretreated with desipramine (25 mg/kg,i.p. in saline, Sigma) 20 min before anesthesia in order to protect noradrenergic system. Rats were injected (4.5 µl/ ventricle) with 150 µg/free base) of 5,7-DHT (creatine sulfat salt; 338µg dissolved in 20 µl of physiological saline containing 0.2 mg/ml ascorbic acid, Sigma).Control rats were treated exactly as the rats subjected to the 5,7-DHT lesions except that no 5,7-DHT was present in the saline solution

Parachlophenylalanine (PCPA), which blocks the synthesis of serotonin, was administered i.c.v. through a plastic (silastic) cannula (0.9 mm O.D), implanted stereotaxically in the left cerebral ventricle at the following coordinates (in mm): A-0.5 (from bregma), L 1.3, V-4.3 (from bregma) (Paxinos and Watson, 1986).The cannula was positioned with acrylic dental cement and secured by one stainless steel screw. After surgery the rats were isolated in separate cage and protected with large spectrum antibiotic (Manolidis et al. 2004). Five to seven days after surgery, the cannula was connected to a Hamilton micro syringe and 200µg PCPA/ 5µl for three consecutive days, was administered at a rate of 1µl/min in desipramine pretreated rats. Before withdrawal the syringe was left in place for an additional 3 min to minimize dragging the injected solution. Learning and memory test was started 48 h after the last PCPA administration.

The correct placement of the cannula was controlled at the end of experiment using a methylene blue dye injected through the implanted cannula.

Learning and memory tasks

Step-through passive avoidance task

In brief, a step-through type passive avoidance apparatus consisting of two compartments (25 X 15 X 15 high), one illuminated and one dark, both equipped with a grid floor, was used. The two compartments were separated by a guillotine door. In the acquisition trials, each rat was placed in the illuminated compartment; when the animal entered the dark compartment, the door was closed and an inescapable foot shock (0.3 mA, 5 s) was delivered through the grid floor. The rat was removed after receiving the foot shock and was placed back into the light compartment. The door was again opened 30 s later to start the next trial. The training continued until the rat stayed in the light compartment for a 120-s period on a single trial. After 24 h, each rat was placed in the compartment and the step-through latency was recorded until 300 s had elapsed (retention trial). The step-through latency in the retention trial was used as the index of retention of the training experience (Yamada et al., 1996). Longer retention latencies were interpreted as indicating better retention of the training experience.

Y-maze task

Short-term memory was assessed by spontaneous alternation behavior in the Y-maze task. The Y-maze used in the present study consisted of three arms (35 cm long, 25 cm high and 10 cm wide) and an equilateral triangular central area. The rat was placed at the end of one arm and allowed to move freely through the maze for 8 min. An arm entry was counted when the hind paws of the rat were completely within the arm. Spontaneous alternation behavior was defined as entry into all three arms on consecutive choices. The number of maximum spontaneous alternation behaviors was then the total number of arms entered minus 2 and percent spontaneous alternation was calculated as (actual alternations/maximum alternations) X 100 (Yamada et al., 1996). Spontaneous alternation behavior is considered to reflect spatial working memory, which is a form of short-term memory.

Statistical analysis

The results are expressed as means ±S.E.M. The results were analyzed statistically using Student’s t-test. Values of $P < 0.05$ were regarded as significant.

RESULTS AND DISCUSSIONS

1. Effects of the 5, 7 –DHT serotonergic lesion on memory.

1.5 months after 5, 7-DHT serotonergic lesion, short-term memory, explored by means of Y-maze task, undergo a significant impairment indicated by a decrease of spontaneous alternation %. This decrease can not be attributed to a change of locomotors activity, because the number of arms entries is not significantly changed (Fig.1).

Chronic serotonin depletion with 5, 7-DHT does not produce a significant change in long term memory as measure with multi-trial passive avoidance test (Fig.2).

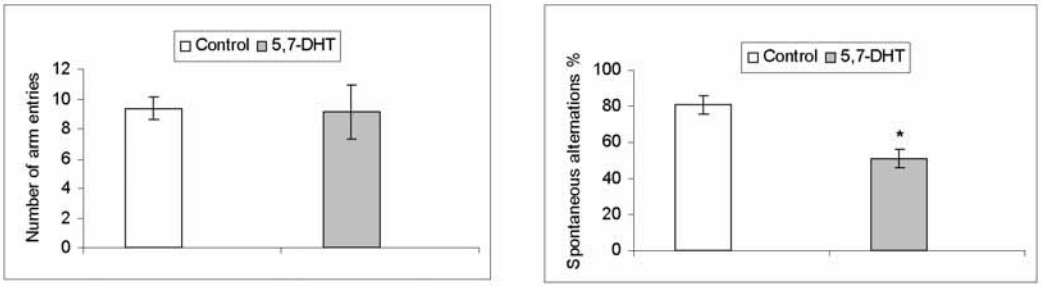

Fig.1. Alterations of number of arm entries and spontaneous alternation % in Y-maze task induced by serotonergic depletion with 5, 7 –DHT. Values are means ± S.E.M. * P< 0.05 vs. control group.

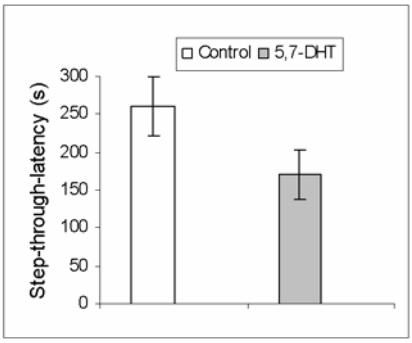

Fig.2.Alterations of step-through latency induced by serotonergic system lesion with 5, 7 – DHT at 24 h after acquisition training in multi-trial passive avoidance test. Values are means ± S.E.M.

2. Effect of parachlorophenylalanine serotonergic lesion on memory processes.

48 hours after last PCPA lesion of the serotonergic system, long-term memory, explored by means of multi-trial passive avoidance test, do not suffer significant impairment (Fig.3).

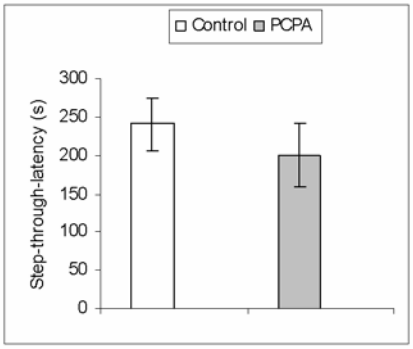

Fig.3. Effects of parachlorophenylalanine lesion of serotonergic system on step-through-latency at 24 h after acquisition training in multi-trial passive avoidance test. Values are means ± S.E.M.

Chronic serotonin depletion by means of 5, 7 –DHT, impaired significantly short-term memory, in Y- maze test, without affecting significantly long-term memory and locomotion.

PCPA, semichronic serotonin synthesis inhibition (Routhsalainen et al. 1998), do not induce a significant impairment of long-term memory performance in the rats.

As seen from our present data as well as data obtained in serotonin-depleted rats (5, 7 –DHT) in radial arm-maze performance (Hefco et al.,2005), the normal concentration of serotonin is more important for short-term memory than for long-term memory storage, the last form of memory being not affected in chronic (5, 7,-DHT) or semichronic (PCPA) serotonin-depleted rats.

Long term-memory ensures the consolidation of information for long-term retrieval or recognition, and short-term memory allows the maintenance of information during short periods of time to execute a particular action or sequential actions (Baddeley, 1995). The different effect of serotonin on short-term and long-term memory can be attributed to the fact that different brain regions are involved in storage and retrieval in these two categories of memory. In addition, the molecular mechanisms that underlie short- and long-term memory are different (DeZazzo and Tully, 1995).

Knowledge of the mechanism by which serotonin contributes to learning and memory requires an in-depth investigation of the role played by specific types or subtypes of 5-HT receptors, especially those localized in particular cerebral structures underlying defined cognitive functions. The hippocampus is conceived as a key structure involved in long-term memory (O’Keefe and Nadel, 1978), but also in working (short-term) memory (Olton et al. 1979), the prefrontal cortex is also considered as a key structure to complete working memory tasks (Fuster, 1989; Tulving, 1991).To mediate the actions of 5-HT, at least 15 distinct 5-HT receptors have been identified which are divided into seven main families (For ref. Roth et al. 2004)

5-HT may exert neurotrophic effect on cholinergic neurons mediated by at least 5-HT1A receptors (Riad et al. 1994; Whitaker-Azmitia et al, 1990, Buhot et al. 2003).

5-HT2A receptors are also found on the cell bodies of dopaminergic neurons in the ventral tegmental area, where they may modulate dopamine neuronal activity (Nocjar et al. 2002, Roth et al. 2004).

Also there are studies which suggest an intimate association between NMDA and 5-HT2A and imply that drugs with potent 5-HT2A antagonistic actions may prove beneficial at improving cognition in schizophrenia, perhaps by normalizing NMDA receptor functioning (Varty et al. 1999).

5-HT4 receptors modulate the release of acetylcholine, dopamine, GABA and serotonin (Barnes and Sharp, 1999).

5-HT6 receptors exert a tonic inhibitory control over acetylcholine release in cortex and hippocampus.

Parachlorophenilalanine (PCPA) may induce catecholaminergic alterations in most regions of the brain. Also, it does not affect all functional markers of the serotonergic innervation (i.e. 5-HT uptake sites are preserved, (Cassel and Jeltsch, 1995; Dewar et al. 1992).

The correlation between the decrease in cholinergic activity and cognitive impairment has lead to attempts to develop cholinergic replacement therapy (acetylcholine precursors, acetylcholinesterase inhibitors, muscarinic receptor agonist) (Davis et al. 1993, Iversen, 1993,; Patel, 1995). However, this replacement therapy has produced only a slight improvement in cognitive decline of Alzheimer’s disease (AD) patients (Patel, 1995). Thus, it could be hypothesized that cognitive impairment of AD patients is not entirely owing to the cholinergic

degeneration, but it could be a result of combined degeneration of cholinergic and other ascending pathways, e.g. serotonergic projections. The clinical effectiveness of cholinergic replacement therapy in AD could perhaps be augmented by an amelioration of the serotonergic neurotransmissions. This combined therapy could lead to an improvement in learning and memory.

CONCLUSIONS

Chronic (1.5 months postoperatively) serotonin depletion (5, 7 –DHT) significantly impaired short-term memory as evidenced in Y-maze task by significantly diminished spontaneous alternation %.Locomotion was not affected.

In the same experimental condition, long-term memory is not significantly impaired.

Parachloophenylalanine, a semichronic serotonin neurotoxin, do not impaired long-term memory.

The effects of serotonin on memory processes can be attributed to its own cognitive effects and its interactions with other neurotransmitter systems.

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