EFFECTS OF 5, 7-DIHYDROXYTRYPTAMINE-INDUCED DEPLETION OF BRAIN SEROTONIN ON RADIAL ARM-MAZE TASK IN RATS

VASILE P.HEFCO*[1], ANDREEA-IOANA HEFCO[2], SORIN STRATULAT[2], LUCIAN HRITCU[1]

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Abstract: Adult rats pretreated with desipramine (25 mg/kg i.p.30 min before anesthesia) in order to protect noradrenergic system, were subjected to intracerebroventriculare injection of 5, 7 –dihydroxytryptamine (5, 7-DHT, 150 µg, 4.5 µl/ventricle), a chronic neurotoxin of the central serotonergic function. After 1.5 months later, we assessed the working memory and reference memory in radial 8 arm-mazes. Serotonergic depletion impaired more significantly short-term memory tested by means of the average working memory errors, entries to repeat and average time taken to consume all five baits during 12 days training. Long-term memory, explored by means of reference memory errors, was less impaired. It is concluded that serotonin, among other neurotransmitters, play one important role in cognitive functions, including learning and memory.

INTRODUCTION

Studies directed at understanding the mechanism for action of serotonin (5-HT) are based on evidence that 5-HT may be involved in the modulation of brain function such as appetite, thermoregulation, aggression and sexual behavior (Carlsoon 1987; Sandyk 1992). 5-HT is also known to be associated with various pathophysiological processes, including depressive disorders, schizophrenia and dementia (Sandyk, 1992). 5HT also appears to facilitate synapse formation and maintenance, and thereby to modulate the net number of synapse in the developing and mature brain (Okado et al. 1993; Chen et.al.1994, Matsukawa et al. 1997).

The serotonergic neural system derives mainly from neurons in the dorsal and ventral raphe nuclei with projection to virtually every brain region that subserves cognition. There is neurochemical and pathological evidence of a decline in the function of the serotonergic system of Alzheimer’s disease (AD) patients (Cross, 1990; Greenamyre and Maragos, 1993; Reinikainen et al. 1990;, 1988; Young and Penney, 1994). It has been proposed that the abnormalities in the serotonergic system in AD may be related to behavioral disturbance such as depression, aggressive behavior and anxiety rather to cognitive dysfunction (Palmer et al. 1988).However, studies with experimental animals, have provided evidence for the serotonergic system being involved in cognitive processes (see for review Cassel and Jeltsch, 1995; Steckler and Sahgal, 1995). For example, lesions of raphe nuclei, induced by 5, 7, DHT, impaired delayed spatial alternation in a T-maze at a delay (60 sec) but not at shorter delays (5 and 30 sec) (Wenk et al.1987), such indicating an impairment in working memory. However, there are also studies which have shown that manipulations at the serotonetgic system do not alone cause changes in cognitive function per se (Jakala et al. 1993; Richter-Lewin and Segal, 1989; Riekkinen et al. 1991;Sahgal and Keith 1993; Sakurai and Wenk, 1990,).Thus, the role of serotonergic system in cognition is rather controversial.

The majority of behavioral paradigms used in order to elucidate the role of serotonergic function were based on punishment or aversive situations (passive/active avoidance, water maze).Although these experiments are valid in their own right, it would also be of importance to examine serotonergic function in other learning tasks which should hopefully allow a clearer statement. The aim of the present study was to investigate whether the serotonergic system is involved in working and reference memory as tested in radial arm- maze where the behavior of rats is food motivated. Working memory is an essential part of cognition and its deficit is one of the earliest cognitive problems seen in the AD (Bartus and Dean, 1988). Working memory consists of information which is useful in the immediate future and working memory also permits the manipulation of this information. Working memory is also linked to retrieval of stored knowledge (Carlson, 1994; Goldman-Rakic, 1995). The prefrontal cortex is an important area for working memory processing. Reference memory is a long-term memory. In the present study we used the radial 8 arm-maze task in order to measure the working and reference memory. Depletion of serotonin in rat brain was attained by 5, 7 –DHT, a chronic neurotoxin of the central serotonergic function, which induced neural degeneration acting on the neuronal cell bodies.

MATERIALS AND METHODS

Animals

The experiments were carried out on male Wistar rats weighing 225-250g at the start of the experiment. They were fed and allowed to drink water at libitum. Rats were treated in accordance with institutional guidelines.

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 (45 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.

Radial arm-maze task

The radial arm-maze used in the present study consisted of 8 arms, numbered from 1 to 8 (48 x 12 cm), extending radially from a central area (32 cm in diameter). The apparatus was placed 40 cm above the floor, and surrounded by various extra maze cues placed at the same position during the study. At the end of each arm there was a food cup that had a single 50 mg food pellet. Prior to the performance of the maze task, the animals were kept on restricted diet and body weight was maintained of 85% of their free-feeding weight over a week period, with water being available ad libitum.

Before the actual training began, the animals were shaped for 4 days to run to the end of the arms and consume the bait. The bait was initially available throughout the maze, but gradually was restricted to the food cup. Briefly, each animal was placed individually in the center of the maze and subjected to working and reference memory tasks, in which same 5 arms (no. 1, 2, 4, 5, and 7), were baited for each daily training trial. The other 3 arms (no. 3, 6, 8) were never baited. The training trial continued until all 5 baits had been consumed or until 5 minutes had elapsed. An arm entry was counted when all four limbs of the rat where within an arm. Measures was made of the number of working memory errors (entering an arm containing food, but previously entered), and reference memory errors (entering an arm that was not baited). The time taken to consume all five baits was also recorded. Reference memory is regarded as a long-term memory for information that remains constant over repeated trials (memory for the positions of baited arms), whereas working memory is considered a short-time memory in which the information to be remembered changes in every trial (memory for the positions of arms that had already been visited in each trial) (Durkin, 1994; Olton et al., 1979). Each animal was subjected to one trial each day.

Entries to repeat, choice accuracy was measured by entries to repeat, which was the number of arms entered until a repeat entry was made in the same arm in working or reference type memory, respectively.

Statistical analysis

Results were expressed as mean ± S.E.M. The results were analyzed statistically by means of the Student’s “t” test. $p<0.05$ was taken as the criterion for significance.

RESULTS AND DISCUSSIONS

1. Effects of 5, 7-DHT lesion on memory performance in rats.

Experimental data were registered 1.5 months after 5, 7-DHT lesions. Serotonin depletion impaired more significantly short-term memory, tested by means of the numbers of working memory errors and average working memory errors (Fig 1), average time taken to consume all five baits (Fig.2) and entries to repeat (Fig. 3) during 12 days training.

Long-term memory, explored by means of number of reference memory errors (Fig 4), average reference memory errors (Fig. 4) and entries to repeat (Fig 3), was less impaired during 12 days training of rats with 5, 7-DHT lesions.

Chronic (5, 7-DHT) disruption of the central serotonergic function is not sufficient to produce reliable effects on learning and memory processes. In rats with chronic serotonergic dysfunctions, a significant impairment, but of less amplitude, undergoes only working memory.

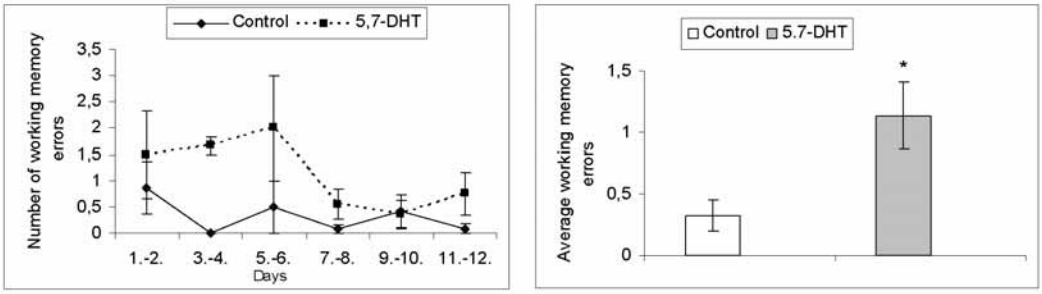

Fig. 1 Effects of chronic serotonin depletion on numbers of working memory errors and average working memory errors during 12 days training. Values are means ±SEM.*p<0.05 vs. control group

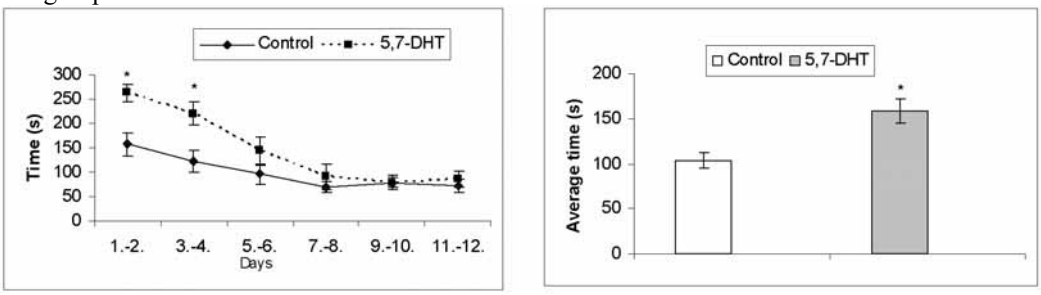

Fig.2. Effects of chronic serotonin depletion on time taken to consume all five baits during 12 days training. and average time during this period. Values are means ±SEM.*p<0.05 v.s. control groups

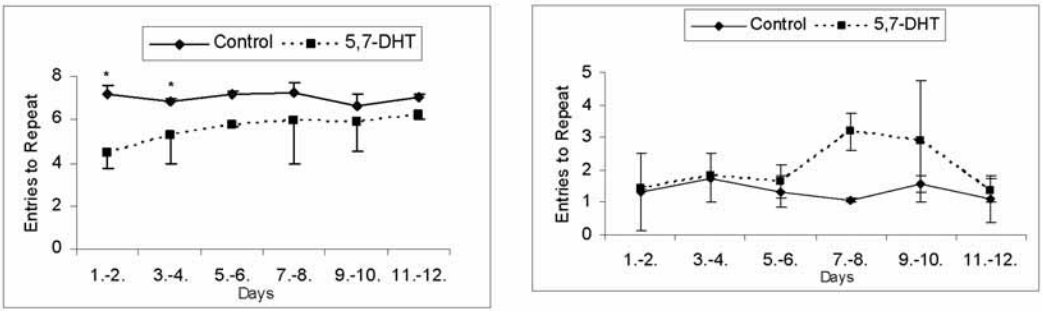

Fig.3.Effects of 5,7-DHT lesion on entries to repeat in working memory (left) and reference memory (right).Values are means ±E.S.M for two successive days.*p< 0.05 v.s. control groups

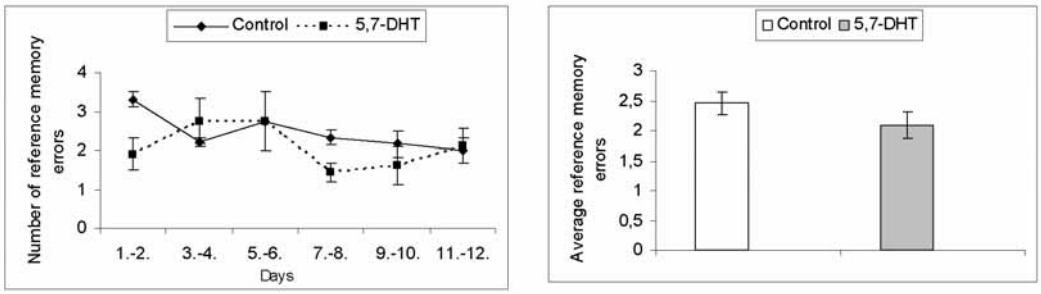

Fig.4. Effects of chronic serotonin depletion on numbers of reference memory errors and average reference memory errors during 12 days training. Values are means ±SEM.

I.c.v. injections of 5, 7 –DHT, depleted cortical 5-HT by about 50% and produced a nearly complete lesion of the serotonergic projections to the hippocampus (95% depletion of 5-HT) (Steckler and Sahgal. 1995).Moreover i.c.v. 5, 7-DHT do not affect norepinephrine and dopamine release at the prefrontal cortex (Temel et al. 2003) or hippocampal acetylcholine release (Nilsson et al. 1992).In rat hippocampal slices, 5, 7-DHT lesions enhance the evoked overflow of acetylcholine (Birthelmer et al., 2002).

Cholinergic systems have been linked to cognitive processes such as attention, learning and mnemonic function. However, other neurotransmitters system, such as serotonergic one, which may have only minor effects on cognitive functions on their own, interact with cholinergic function and their combined effects may have marked behavioral actions. Serotonergic-cholinergic interactions could be of importance in the mediation of learning processes and working memory. It was hypothesized that it is primarily the hippocampus where serotonergic and cholinergic systems interact in the mediation of working memory (Steckler and Sahgal 1995). As concerned long-term memory, our data do not allow an unambiguous conclusion about the role of these interactive processes in the mediation of long-term reference memory.

To mediate the actions of 5-HT, at least 15 distinct 5-HT receptors have been identified which are divided into seven receptor main families (Roth et al. 2004; Barnes and Sharp,1999) on the basis of their structural, functional and to some extent pharmacological characteristics (Barnes and Sharp 1999)..In rats, the activation of 5-HT1A or 5-HT1B receptors as well as the inhibition of 5-HT2 receptors was found to exacerbate the deficit due to central muscarinic blockade or to cholinergic lesions (Cassel and Jeltsch 1995). Conversely, the blockade of 5-HT3 receptors was found to attenuate the cognitive deficits due to central cholinergic disruption.

In vivo and in vitro, 5-HT1B agonists attenuate the release of acetylcholine in at least the hippocampus. Thus, the cognitive perturbations induced by such agonist might, in some respects, be explained by their inhibitory effects on central cholinergic function. The cognitive alteration induced by serotonergic depletion cannot be reduced to a simple and direct modulatory influence that the drug might exert on central cholinergic function.

5-HT appears closely involved in the modulation of neuronal functions in a diverse region of the brain by changing activities of the glutamatergic system and long term depletion of serotonin leads to selective changes in glutamate receptor subunits (Shutoh et al. 2000). In addition to the effects of serotonin on cholinergic and glutamatergic function, one has to consider the possibility that serotonin has its own cognitive actions, for instance in modulating functions that may be essential for mnesic processes to occur efficiently (e.g., anxiety, arousal, attention etc). In view to

decipher the mechanisms involved in serotonin action on learning and memory in rats, future research is required.

CONCLUSIONS

On the basis of our results obtained by chronic (5, 7 –DHT) disruption of the central serotonergic function, we can conclude that in the rats, chronic serotonin depletion affect more obvious short-term memory. Long-term memory, explored by means of reference memory in radial-arm maze, was less affected by chronic (1.5 month postoperatively) serotonin depletion. The effects of serotonin depletion can be attributed to the interaction between serotoninergic system with other neurotransmitters systems or to its own cognitive actions.

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1) “Al.I.Cuza “ University of Iasi, B-dul Carol I, Nr. 11, 700506, Iasi-Romania;
2) “Gr.T.Popa” University of Medicine and Pharmacy of Iasi, 700506 Iasi, Romania
* vhefco@uaic.ro