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The 5-HT_2 Receptor Antagonist Ketanserine Prevents Electroconvulsive Shock- and Clonidine-Induced Amnesia

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GENKOVA-PAPAZOVA, M., M. LAZAROVA-BAKAROVA AND V. D. PETKOV. *The 5-HT_2 receptor antagonist ketanserine prevents electroconvulsive shock- and clonidine-induced amnesia.* PHARMACOL BIOCHEM BEHAV **49**(4) 849–852, 1994. – The 5-HT_2-selective antagonist ketanserine was examined for its ability to prevent electroconvulsive shock (ECS)- or clonidine-induced performance deficit in the passive avoidance situation (step-down) in rats. The posttrain intraperitoneal injection of ketanserine at doses of 3 and 10 mg/kg prevented the ECS- or clonidine-provoked amnesia upon retention tests given 3 h, 24 h, and 7 days after training. The present data favor the view that the selective modification of 5-HT_2 receptors after training can prevent the performance deficit in step-down-trained rats and suggest a role of the 5-HTergic neurotransmitter system in memory. The results of this study further suggest that 5-HTergic receptor antagonists might be useful in treatment of cognitive disorders.

Amnesia Electroconvulsive shock Clonidine Memory Passive avoidance Serotonin Ketanserine Rats

THE ROLE of the brain serotonergic neurotransmitter system in cognition is a subject of increasing research interest. Most of the authors state that stimulation of serotonergic neurotransmission impairs learning and memory (3,10,11,32,34,36, whereas its inhibition improves memory (1,29,35,37). However, there are data to suggest that the inhibition of serotonergic neurotransmission leads to an impairment of learning and memory (41,48,49). Our previous data have also shown that the serotonergic antagonists metergoline, methysergide, and ritanserine administered before training impair passive avoidance retention in step-down-trained rats (41). In recent years, experimental data have accrued to demonstrate that the effects of some serotonergic antagonists [i.e., 5-HT_2-selective (ketanserine, pirenperone) and nonselective (metergoline, methysergide)] on the one-trial passive avoidance retention in mice are time dependent. Administration of the antagonist before training dose-dependently impairs retention, whereas posttrain administration dose-dependently improves retention (1,2). Posttrain injection of the 5-HT_2-selective receptor antagonist ketanserine attenuates the age-related deficit in middle aged (12 months) and aged (22 months) rats and significantly increases the step-through latencies (35). Posttrain administration of ketanserine or mianserine protects against the hypoxia-induced deficit of a passive avoidance response in rats (45).

All these data stimulated us to study the influence of the posttrain administration of ketanserine on the amnesia provoked by electroconvulsive shock (ECS) or by the alpha$_2$-adrenoceptor agonist clonidine in step-down-trained rats.

METHOD

Subjects

The experiments were carried out on 105 male Wistar rats weighing 180–200 g. They were housed in plastic cages 80 × 40 × 10, 15 animals per cage, in a room maintained at constant temperature and 12 L : 12 D cycle. Food and water were provided ad lib except during training and testing.

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Apparatus and Procedures

Passive avoidance with negative reinforcement (step-down) was used. Step-down training was performed in a apparatus consisting of a chamber with metal grid floor for punishment: electrical stimulation (50 Hz; 0.4–0.6 mA alternating current for 10 s) and a plastic mobile platform fixed in the middle of the floor. The rat was placed on the platform and as soon as it moved off the platform with all four legs the foot shock was applied. The negative reinforcement creates a conditioned reflex, and on each subsequent trial the animal tended to remain on the platform for a longer time to escape the electric foot shock. Six successive training sessions were carried out to reach the learning criterion (remaining on the platform for at least 60 s). Retention tests were given 3 h, 24 h, and 7 days after training. Immediately after reaching the learning criterion (the first correct response), electroconvulsive shock or alpha$_2$-adrenoceptor agonist clonidine was applied to provoke memory deficit.

Electroshock stimulation (monophase rectangular pulses with current intensity of 50 mA, single-phase duration of 1 ms, stimulation frequency of 50 Hz, and trial duration of 0.5 s) by silver corneal electrodes inducing clonic-tonic seizures (27) was applied immediately after the first correct response to impair memory. A sham electroshock (SECS) was applied to the controls.

Clonidine was injected intraperitoneally (IP) at a dose of 0.1 mg/kg immediately after the first correct response.

Ketanserine was injected IP at doses of 3 and 10 mg/kg immediately after the rats had reached the learning criterion, but before application of the amnestic agent. Controls were injected in the same manner with saline.

Drugs

Ketanserine tartarate (Janssen, Belgium) and clonidine (Bohringer) were used. The drugs were dissolved in saline.

Statistics

Percentage of rats remaining on the platform for at least 60 s was recorded. The data were assessed for significance of difference by χ^2 criterion (13).

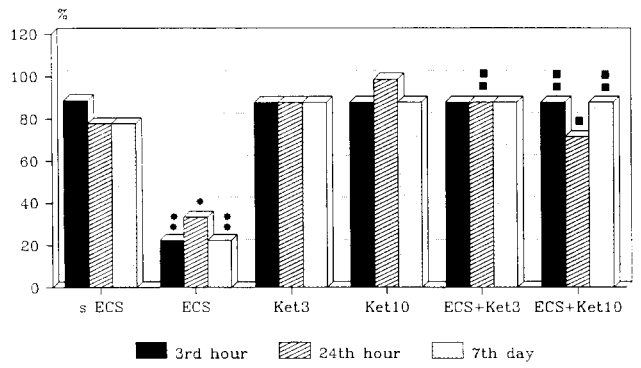

FIG. 1. Effect of ketanserine (3 and 10 mg/kg) on ECS-induced memory disturbances in step-down passive avoidance in rats. On the ordinate, percentage of rats attaining the learning criterion; on the abscissa, (C), controls; (sECS), sham ECS; (Ket3), ketanserine (3 mg/kg); (Ket10), ketanserine (10 mg/kg). *Statistical significance vs. sECS-group ($p < 0.01$); **statistical significance vs. sECS-group ($p < 0.001$); ■statistical significance vs. ECS-treated rats ($p < 0.01$); ■■statistical significance vs. ECS-treated rats ($p < 0.001$).

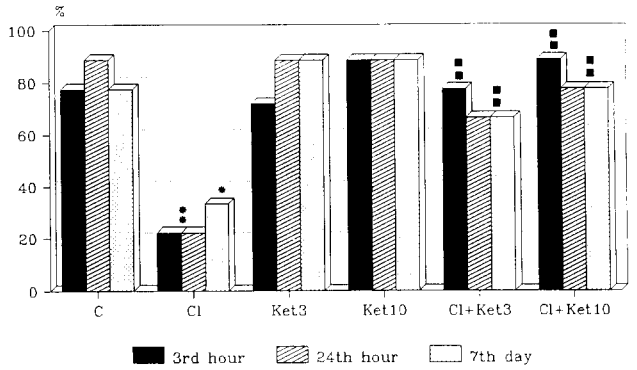

FIG. 2. Effect of ketanserine (3 and 10 mg/kg) on clonidine-induced memory disturbances in step-down passive avoidance in rats. On the ordinate, percentage of rats attaining the learning criterion; on the abscissa, (C), controls; (Cl), clonidine (0.1 mg/kg); (Ket3), ketanserine (3 mg/kg); (Ket10), ketanserine (10 mg/kg). *Statistical significance vs. controls ($p < 0.01$); **statistical significance vs. controls ($p < 0.001$); ■■statistical significance vs. clonidine-treated rats ($p < 0.001$).

RESULTS

Posttrain administration of the selective 5-HT$_2$ antagonist ketanserine abolished the electroconvulsive shock- and clonidine-induced amnesia.

The corneal ECS applied immediately after training provoked a retrograde amnesia. The percentage of rats reaching the learning criterion upon retention testing at 3 h, 24 h, and 7 days after training was significantly lower in ECS-treated rats than in SECS-treated controls. Ketanserine at both doses used completely abolished the ECS-induced amnesia. The percentage of rats reaching the learning criterion upon the three retention tests was considerably higher than that in ECS-treated rats and did not differ from the percentage in the SECS group (Fig. 1).

Posttrain injection of the alpha$_2$-adrenoceptor agonist clonidine provoked a retrograde amnesia. The percentage of rats that had acquired the task upon retention testing 3 h, 24 h, and 7 days after training was markedly decreased in clonidine-treated rats compared to controls. Ketanserine at doses of 3 and 10 mg/kg antagonized the memory-impairing effect of clonidine. Thus, the percentage of rats reaching the learning criterion upon the three retention tests was considerably higher than that in the clonidine-treated group and did not differ from the percentage in the controls (Fig. 2).

DISCUSSION

The present study showed that the 5-HT$_2$-selective antagonist ketanserine completely abolished the amnesia induced by electroconvulsive shock or by the alpha$_2$-adrenoceptor agonist clonidine in the passive avoidance situation, supporting the view about the retention-improving effects of posttrain administration of 5-HT$_2$ antagonists. Posttrain administration of ketanserine abolishes the hypoxia-induced deficit of a passive avoidance response in rats (45) and improves retention in middle-aged and aged rats (35). Methysergide, administered before training, prevents the ECS-induced retrograde amnesia

in a passive avoidance situation (34). Ritanserine and mianserine administered after step-down training abolishes the *p*-chloroamphetamine-induced amnesia in mice (30). Our results favor the view of the differential role of serotoninergic transmission during different phases of learning and memory processes. Thus, pretrain administration of cyproheptadine impairs retention in mice trained for passive avoidance (6), whereas posttrain administration with mianserine improves retention in Y-maze-trained mice (50).

The electroconvulsive shock is a widely used experimental method for inducing retrograde amnesia. It impairs consolidation and retrieval of memory traces (44). The ECS induces changes in beta- and alpha$_2$-adrenoceptor binding [for review see (18,28)] and increases the 5-HT$_2$ receptor number (19). The memory deficit caused by ECS is similar to that provoked by posttraining injection of beta-endorphin. The ECS stimulates the release of brain enkephalins and causes naloxone-reversible retrograde amnesia (22).

The observed amnestic effect of posttrain administration of the alpha$_2$-adrenoceptor agonist clonidine confirms our earlier results showing memory deficit after clonidine in active and passive avoidance situations (17,23). There is evidence that clonidine provokes memory disturbances in shuttle box-trained rats [e.g., (20,21,23)] and in water maze-trained rats (39). The alpha$_2$-adrenoceptor agonist guanphazine also has an amnestic effect that is completely antagonized by the selective alpha$_2$-adrenoceptor antagonist atipasole (43). There are fragmentary data about the memory-improving effect of clonidine and guanphazine in old monkeys (4,5). Interesting are the clinical data about deteriorated associative learning and retention in hypertensive patients continuously treated with clonidine (15).

It should be also noted that both ECS (postictally) and clonidine promote the occurrence of increased slow-wave activity, which may contribute to the amnestic effect that these agents have (13) because the occurrence of high-amplitude, slow-wave activity in the neocortex impairs the consolidation of the memory trace in humans (24).

Recently, a large body of research has demonstrated the role of multiple neurochemical deficits in patients with Alzheimer's disease, senile dementia, etc. The experimental and clinical data have shown age-related combined deficits of catecholaminergic and acetylcholinergic neurotransmission [for review see (31,51)]. Baskys et al. (8) and Brunello et al. (9) suggested 5-HTergic transmitter changes in the rat brain with age: an increased turnover of 5-HT, a decreased high-affinity uptake of 5-HT, and decreased postsynaptic action of 5-HT on the hippocampal granule cells. Radioligand binding studies in rats (9,40) and in human brains (33) demonstrated an age-related decrease in the populations of 5-HT receptors. A partial denervation of 5-HTergic neurones (loss of 5-HT$_1$ and 5-HT$_2$ receptors and decreased presynaptic serotonergic activity) was also observed (12,38). However, the role of 5-HT deficit in cognition in the case of Alzheimer's disease is still unknown; there are data in the literature showing the stimulation of brain 5-HT activity in the brain impairs, whereas the inhibition of this activity facilitates, learning and memory (31).

When interpreting the data about the protective effect of ketanserine against clonidine-induced amnesia, one should consider the role of the interactions between serotonergic and noradrenergic neurotransmitter systems for cognition. Some studies suggest functional reciprocal relationships between the two systems. Anatomical relationships are indicated by the identification of 5-HT nerve terminals in locus coeruleus [for review see (32)]. Clonidine at doses of 0.1–1 mg/kg increases the firing of 5-HT cells in the dorsal raphe nucleus by activating a facilitatory adrenergic influence on the 5-HT neurons (46). Such a facilitatory adrenergic influence has also been reported by Gallager and Aghajanian (16) and by Baraban and Aghajanian (7). Based on these results, we suggest that not only the clonidine-inhibited noradrenergic neurotransmission but also the clonidine-stimulated 5-HTergic neurotransmission underlies the amnestic effect of the drug. In the case of clonidine-induced amnesia, the balance between the noradrenergic and the 5-HTergic system is changed and the 5-HTergic activity predominates. Such a suggestion is consistent with the view that the age-related memory deficit is connected with the age-related imbalance in the central neurotransmitters with a possible development of regional "serotonergic dominance" (47). 5-HTergic neurons in the dorsal raphe nucleus are involved in the anticonvulsive effect of clonidine on pentylenetetrazol-induced seizures in rats (26). In both clonidine- and ECS-induced amnesia, the balance between the neurotransmitter systems is disturbed and there is a predominance of the inhibitory influences on learning and memory. It is very likely that ketanserine exerts its antiamnestic effect through decreasing the dominance of the 5-HTergic activity.

The present results suggest that ketanserine might be useful for combatting the amnestic effects of drugs in human patients.

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