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Effect of DAU 6215, a Novel 5-HT_3 Receptor Antagonist, on Scopolamine-Induced Amnesia in the Rat in a Spatial Learning Task

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PITSIKAS, N., A. BRAMBILLA AND F. BORSINI. *Effect of DAU 6215, a novel 5-HT_3 receptor antagonist, on scopolamine-induced amnesia in the rat in a spatial learning task.* PHARMACOL BIOCHEM BEHAV **47**(1) 95-99, 1994.— The effects of different doses (1, 10, 30, and 100 μg/kg, IP) of a new 5-hydroxytryptamine$_3$ (5-HT_3) receptor antagonist, 3-α-tropanyl)1*H*-benzimidazolone-3-carboxamide chloride (DAU 6215), on memory and performance deficits induced by SC 0.2 mg/kg scopolamine were assessed in the Morris water maze task. No effect was observed on the performance of rats treated with DAU 6215 alone. The doses of 10 and 30 μg/kg DAU 6215 attenuated these scopolamine-induced behavioral deficits.

DAU 6215 5-HT_3 receptor Scopolamine Spatial learning Morris water maze

SEROTONIN [5-hydroxytryptamine (5-HT)] seems to play a role in learning and memory processes (1,18). It is still unclear, however, the exact role of 5-HT in modulating cognition (1,18). In fact, both 5-HT facilitation or inhibition of learning and memory has been reported depending on the behavioral procedure and on the timing of pharmacological manipulation (1). In addition, depletion of 5-HT by lesions results in impairment (2,18) facilitation (1) or no effect on learning and memory processes (1,2,8). Nevertheless, several lines of evidence suggest that the functionality of the serotonergic system may be seriously compromised in pathologies where cognition is affected, such as in Alzheimer's disease (6,11,20).

Multiple 5-HT receptor subtypes are identified in the CNS (25). Recently, the involvement of 5-HT_3 receptors in learning and memory was reported. In a working memory procedure assessed in a T-maze, the 5-HT_3 receptor-selective antagonist, ondansetron, prevented scopolamine-induced impairment of rats' performance (4). In the habituation test in mice, ondansetron facilitated performance of aged animals and inhibited impairment induced by scopolamine or by lesions of the nucleus basalis (4). In the marmoset, ondansetron improved reversal learning and prevented scopolamine-induced amnesia in an object discrimination task (4,7,12).

Recently, a novel 5-HT_3 receptor antagonist, 3-α-tropanyl)1*H*-benzimidazolone-3-carboxamide chloride (DAU 6215), has been synthesized and characterized (26). The aim of the present study was to evaluate the effects of DAU 6215 on cognition abilities in the rat. The efficacy of this compound to antagonize scopolamine-induced amnesia was determined in the Morris water maze (19), a complex spatial learning task. This behavioral procedure assesses spatial reference rather than working memory as evaluated in a previously mentioned study (4). Spatial navigation tasks are valid models for studying animal cognition that may resemble human spatial navigation deficits (13,14).

METHOD

Animals

Male CD-COBS rats (Charles River, Calco, Italy) weighing 200-220 g were housed in Makrolon cages (35 × 45 × 20 cm), five per cage, and maintained on a 12 L : 12 D cycle (light 0700 h) with free access to food and water. Animals were randomly divided into four experimental groups based upon their pharmacological treatment: control-saline (SAL; n = 10); DAU 6215 (n = 10); scopolamine (SCOP; n = 10); and DAU 6215 + SCOP (n = 10). This experimental design was replicated with naive rats for each dose of DAU 6215 (1, 10, 30, and 100 μg/kg). Experiments were carried out in the room where only these animals were housed and took place between 0900 and 1300 h.

Morris Water Maze Task

The apparatus has been described elsewhere (23). Each trial involved placing the rat in the pool, close to and facing the wall in one of the four equally spaced quadrants in which the

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pool was ideally divided. Animals were allowed to swim freely until they found the escape platform. If a rat failed to find the landmark within 60 s, it was placed on it by the experimenter. The intertrial interval was 30 s, during which time the rat remained on the platform. Each rat performed four trials for 4 consecutive days. During this training period, the escape platform was located in a fixed position in the middle of quadrant 2. The time to reach the platform (latency) was recorded. This parameter was averaged for each block of trials and for each rat, whose daily performance was thus characterized. On day 5, rats performed a spatial probe trial. This trial consisted of removing the platform from the pool and allowing the rat to swim for 60 s in search of it. The distance swam in each of the four quadrants of the tank was calculated as a percentage over 60 s. If the rat demonstrated a persistent preference during the trial to navigate in the pool quadrant where the escape platform had previously been placed, this was taken to indicate that the animal had acquired the spatial task and remembered it (15).

Drug Administration

Drugs were freshly prepared daily. Animals were injected each day before undertaking the behavioral procedure. Control rats were treated with vehicle (NaCl, 0.9%) IP 45 min before starting the test. Scopolamine HBr (Sigma Chemical Co., St. Louis, MO) was dissolved in saline and injected SC at the dose of 0.2 mg/kg 30 min before beginning the behavioral training. DAU 6215 Cl (Boehringer Ingelheim Italia S.p.A., Milan, Italy) was dissolved in saline and administered IP at the doses of 1, 10, 30, and 100 μg/kg 15 min before scopolamine treatment or 45 min before starting the experiment. Doses of compounds are expressed as bases.

Statistical Analysis

The effects of drugs on daily performance (latency) during task acquisition were calculated by an analysis of variance (ANOVA) with a split-plot design (between-within subjects) (17). Spatial probe trial data were analyzed by the nonparametric test of Friedman followed by the Newman-Keuls test (24). For comparing the animal's persistence of swimming on the previously reinforced quadrant, a Kruskal-Wallis test was used (17).

RESULTS

Escape Latencies

In all four replications of the experimental design, SAL- and DAU 6215-treated animals reached asymptotic on day 3 of testing. A per se memory effect of DAU 6215 was not observed. Treatment with SCOP produced a severe deficit in rats' performance as compared to that of SAL-treated animals, $F(1, 18) = 29$, $p < 0.01$ (Fig. 1A), $F(1, 18) = 89.8$, $p < 0.01$ (Fig. 2A), $F(1, 18) = 52.7$, $p < 0.01$ (Fig. 3A), and $F(1, 18) = 68$, $p < 0.01$ (Fig. 4A).

Treatment with 1 or 100 μg/kg DAU 6215 did not reverse the scopolamine-induced amnesia. Animals' performance was impaired as compared to that of their controls, $F(1, 18) = 15.8$, $p < 0.01$, $F(1, 18) = 36.1$, $p < 0.01$, and was not different from that of SCOP-treated rats (Figs. 1A and 4A, respectively). SCOP rats treated with 10 or 30 μg/kg DAU 6215 displayed an overall performance significantly better than that of SCOP-treated rats, $F(1, 18) = 21.3$, $p < 0.01$; $F(1, 18) = 7$, $p < 0.05$, respectively (Figs. 2A and 3A), but less efficient as compared to that of SAL-treated animals, $F(1, 18) = 42$, $p < 0.01$; $F(1, 18) = 6$, $p < 0.05$, respectively. In all four experimental replications, the escape latencies expressed by all DAU 6215 + SCOP rats were poorer than those displayed by SAL-treated rats (Figs. 1A, 2A, 3A, and 4A).

Spatial Probe Trial

Animals treated either with SAL or DAU 6215 swam preferably in pool quadrant 2, where the escape platform was located during the training trials: a) In Figs. 1B, 2B, and 3B, quadrant 2 vs. the three remaining quadrants' p was < 0.01); b) in Fig. 4B, quadrant 2 vs. quadrant 1 and 3 p was < 0.01 and vs. quadrant 4 p was < 0.05.

SCOP-treated rats did not show preference for any pool quadrant. The percentage of distance swam by these rats in the previously reinforced quadrant was significantly lower than that of SAL- and DAU 6215-treated rats ($p < 0.01$) (Figs. 1B, 2B, 3B, and 4B).

Treatment of rats with 1 or 100 μg/kg DAU 6215 did not counteract scopolamine-induced spatial bias impairment. Moreover, these animals swam less in the "correct" quadrant as compared to SAL- and DAU 6215-treated rats ($p < 0.01$) (Figs. 1B and 4B). SCOP rats treated with 10 or 30 μg/kg DAU 6215 swam significantly more in the previously reinforced quadrant (quadrant 2) ($p < 0.01$, vs. quadrant 1 and 3, but not vs. quadrant 4). The percentage of the distance covered in quadrant 2 by these rats did not differ from that of the SAL- and DAU-treated animals (Figs. 2B and 3B).

DISCUSSION

In agreement with previous studies, scopolamine disrupts acquisition of a spatial navigation task in the rat (16,22,27). Animals treated with scopolamine displayed poor escape latencies and were unlikely to discriminate the previous reinforced pool quadrant during the spatial probe trial, as compared to the control population. SCOP animals, which received the doses of 10 and 30 μg/kg DAU 6215, improved their daily performance over trials and demonstrated a higher preference for the platform quadrant during the spatial probe trial as compared to scopolamine-treated animals. However, this improvement did not reach the level of performance of controls. The facilitatory effect of this 5-HT_3 receptor antagonist on learning was not seen at the "side" doses of 1 and 100 μg/kg and revealing a U-shaped dose-effect curve.

Our results are in line with previous findings in which restoring effects of 5-HT_3 receptor antagonists on cognition have been reported (4,7). These effects have been observed in different behavioral paradigms performed in young and aged rodents, and in primates, in which amnesia had been established by pharmacological treatment or lesion (4,7).

The present results provide additional evidence that the blockade of 5-HT_3 receptors may attenuate cognitive deficits when spatial reference memory is assessed. Both facilitatory or inhibitory per se memory effects of 5-HT_3 receptors antagonists have also been proposed (9,12,21).

An electrophysiological study demonstrated that DAU 6215 antagonizes the 5-HT blocking effect on long-term potentiation induction in the rat hippocampus (10). This suggests that the described cognition-enhancing activity of 5-HT_3 antagonists may reside in the (partial) antagonism, during theta activity, of inhibition mediated by 5-HT released by raphe projections in the hippocampus (10).

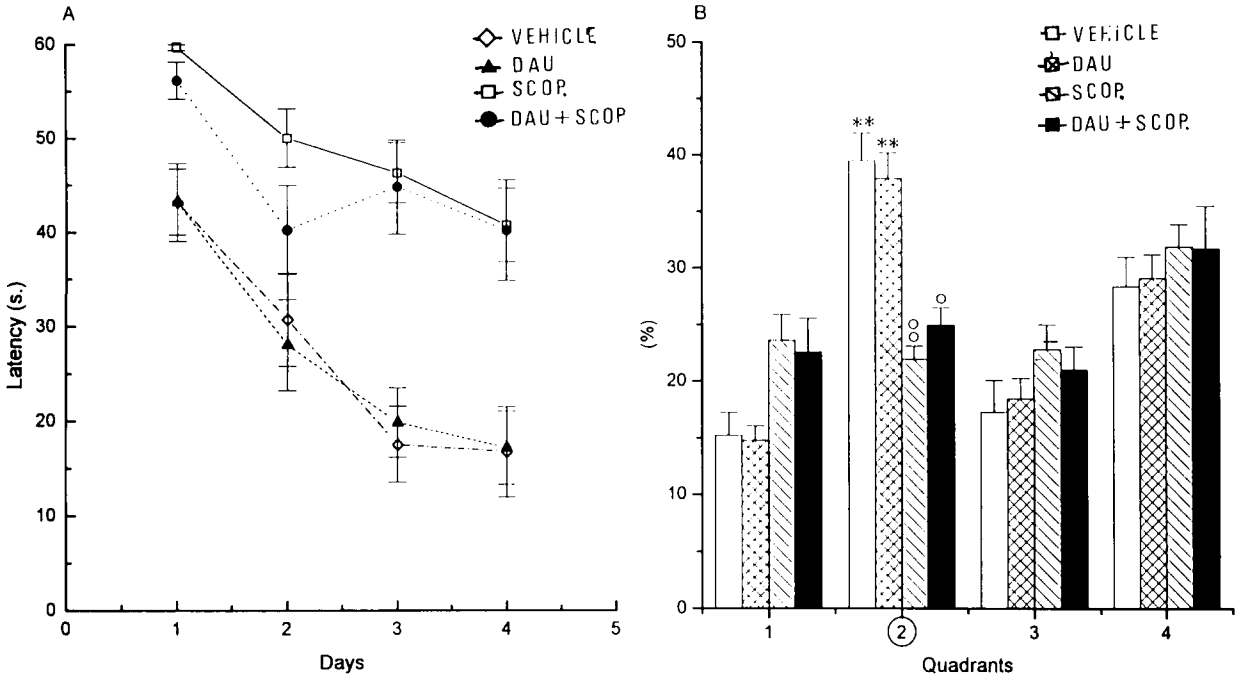

FIG. 1. DAU 6215 (1 μg/kg, IP), scopolamine (0.2 mg/kg, SC), or vehicle (0.9% NaCl, IP) were injected prior to each day's training session. (A). Mean latency to find the escape platform. (B). Mean percentage of total distance swum in each pool quadrant. During the training period, the platform was located in the center of quadrant 2 (solid ring). $^{**}p < 0.01$ vs. first, third, and fourth quadrants. $^{\circ\circ}p < 0.01$ vs. the respective control groups on the same quadrant.

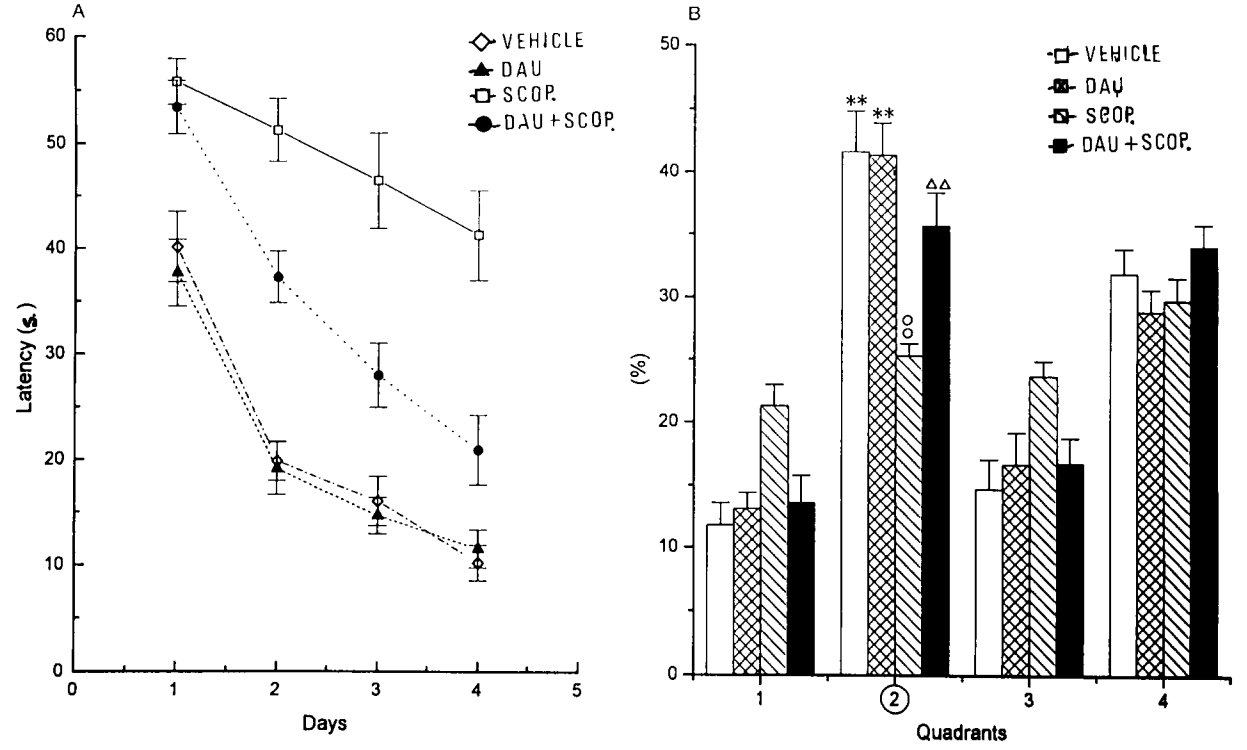

FIG. 2. DAU 6215 (10 μg/kg, IP), scopolamine (0.2 mg/kg, SC), or vehicle (0.9% NaCl, IP) were injected prior to each day's training session. (A). Mean latency to find the escape platform. (B). Mean percentage of total distance swum in each pool quadrant. During the training period, the escape platform was located in the center of quadrant 2 (solid ring). $^{**}p < 0.01$ vs. first, third, and fourth quadrants. $^{\triangle\triangle}p < 0.01$ vs. first and third quadrants. $^{\circ\circ}p < 0.01$ vs. the respective control groups on the same quadrant.

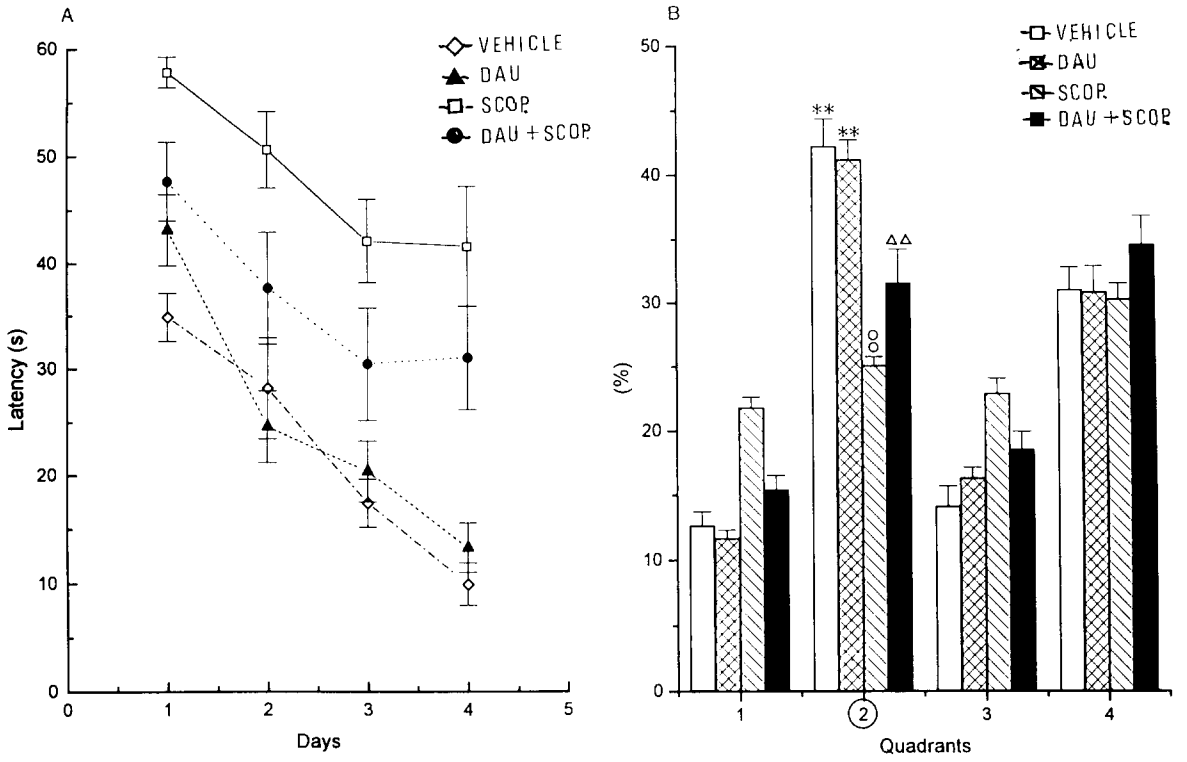

FIG. 3. DAU 6215 (30 μg/kg, IP), scopolamine (0.2 mg/kg, SC), or vehicle (0.9% NaCl, IP) were injected prior to each day's training session. (A). Mean latency to find the escape platform. (B). Mean percentage of total distance swum in each pool quadrant. During the training period, the escape platform was located in the center of quadrant 2 (solid ring). $^{**}p < 0.01$ vs. first, third, and fourth quadrants. $^{\Delta\Delta}p < 0.01$ vs. first and third quadrants. $^{\circ\circ}p < 0.01$ vs.the respective control groups on the same quadrant.

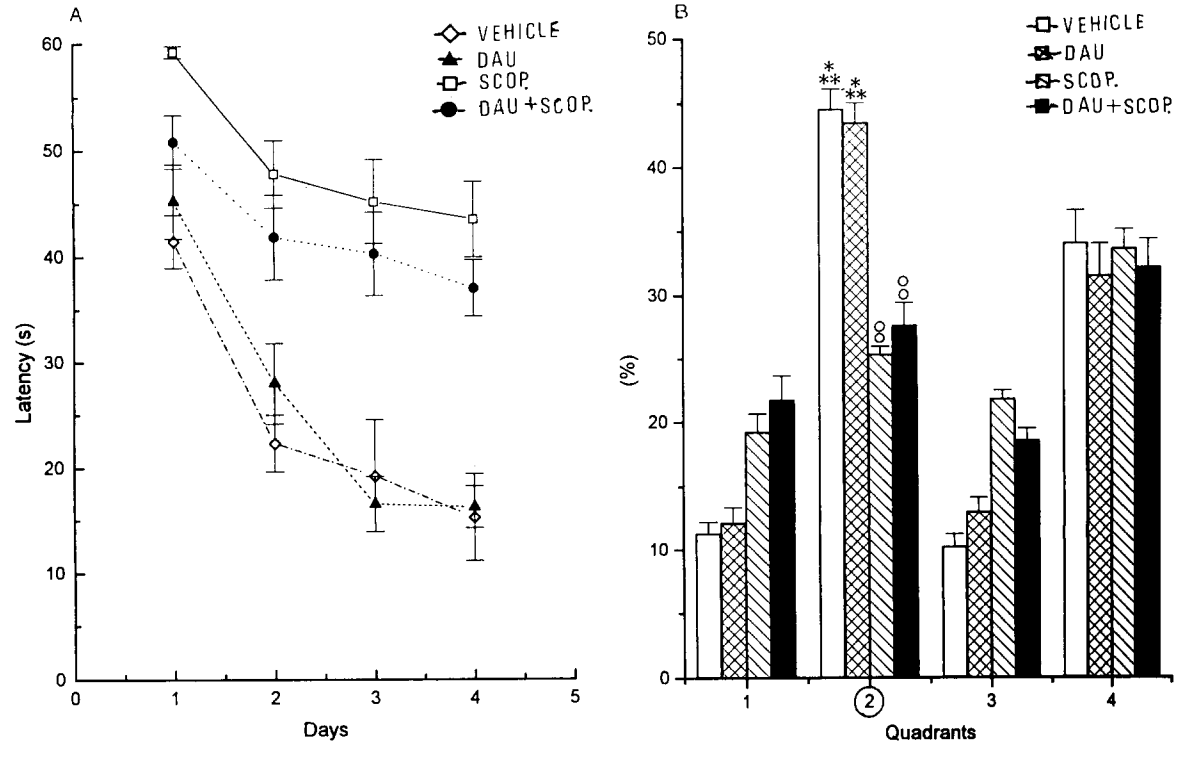

FIG. 4. DAU 6215 (100 μg/kg, IP), scopolamine (0.2 mg/kg, SC), or vehicle (0.9% NaCl, IP) were injected prior to each day's training session. (A). Mean latency to find the escape platform. (B). Mean percentage of total distance swum in each pool quadrant. During the training period, the escape platform was located in the center of quadrant 2 (solid ring). $^{*}p < 0.05$ vs. fourth quadrant. $^{**}p < 0.01$ vs. first and third quadrants. $^{\circ\circ}p < 0.01$ vs. the respective control groups on the same quadrant.

Similar to other 5-HT_3 receptor antagonists (3,5), DAU 6215 antagonizes the inhibitory effect of 2-CH_3-5-HT on reducing acetylcholine release in the cortex (Consolo, personal communication).

In conclusion, DAU 6215, a 5-HT_3 receptor antagonist, attenuates scopolamine-induced cognitive impairment in the rat as assessed in the Morris water maze task. This attenuation occurs in a non-dose-dependent manner.

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