Pharmacology Biochemistry & Behavior, Vol. 35, pp. 955–962. © Pergamon Press plc, 1990. Printed in the U.S.A. 0091-3057/90 $3.00 + .00

The Effects of Ondansetron, a 5-HT_3 Receptor Antagonist, on Cognition in Rodents and Primates

J. M. BARNES, B. COSTALL, J. COUGHLAN, A. M. DOMENEY, P. A. GERRARD, M. E. KELLY, R. J. NAYLOR, E. S. ONAIVI, D. M. TOMKINS AND M. B. TYERS

Postgraduate Studies in Pharmacology, University of Bradford, Bradford BD7 1DP and Neuropharmacology Department, Glaxo Group Research Ltd. Ware, Hertfordshire SH12 0DJ

Received 5 December 1989

BARNES, J. M., B. COSTALL, J. COUGHLAN, A. M. DOMENEY, P. A. GERRARD, M. E. KELLY, R. J. NAYLOR, E. S. ONAIVI, D. M. TOMKINS AND M. B. TYERS. *The effects of ondansetron, a 5-HT_3 receptor antagonist, on cognition in rodents and primates*. PHARMACOL BIOCHEM BEHAV 35(4) 955–962, 1990.—The selective 5-HT_3 receptor antagonist, ondansetron, has been assessed in three tests of cognition in the mouse, rat and marmoset. In a habituation test in the mouse, ondansetron facilitated performance in young adult and aged animals, and inhibited an impairment in habituation induced by scopolamine, electrolesions or ibotenic acid lesions of the nucleus basalis magnocellularis. Arecoline failed to improve basal performance in young adult mice but inhibited the impairment caused by scopolamine and lesions of the nucleus basalis magnocellularis. In the T-maze reinforced alternation task in rats, ondansetron and arecoline antagonised a scopolamine-induced impairment. In an object discrimination and reversal learning task in the marmoset, assessed using a Wisconsin General Test Apparatus, ondansetron improved performance in a reversal learning task. We conclude that ondansetron potently improves basal performance in rodent and primate tests of cognition and inhibits the impairments in performance caused by cholinergic deficits.

Ondansetron Cognition 5-HT_3 receptor

A decline in cholinergic neurotransmission may be a major factor in age-related CNS deterioration of cognitive abilities. The cholinergic hypothesis is based on specific dysfunctions in cholinergic markers in the brains of patients suffering from age-related memory loss, the induction of behavioural impairments in animals following lesion of central cholinergic systems, and changes in cognitive performance in animals and man by pharmacological manipulations of the cholinergic system (7, 11, 25, 43). The hypothesis suggests that cholinomimetic drugs should improve cognitive performance, and administration of precursors (lecithin and choline) acetylcholinesterase inhibitors (physostigmine, tetrahydroaminoacridine) and cholinergic agonists (arecoline, oxotremorine) to animals or man has, in many instances, caused small improvements in performance [see reviews by Bartus *et al.* (17); Heise (19)]. However, the duration of action of muscarinic agonist drugs is often short and the range of effective doses is severely limited by side effects. At the present time there is no safe and reliable way to enhance central cholinergic neurotransmission.

An alternative approach is to investigate the role of other neurotransmitters in regulating acetylcholine release. For example, there is considerable evidence that acetylcholine release is under an inhibitory 5-hydroxytryptaminergic tone. Thus, systemically administered 5-HT agonists, quipazine and 5-methoxy-N,N-dimethyltryptamine, increase striatal acetylcholine levels (14), suggesting reduced release, and in in vitro experiments 5-HT agonists reduce acetylcholine release from striatal slices (18,21). Conversely, 5-HT synthesis inhibition or destruction of 5-HT cells in the dorsal raphe nucleus can potentiate acetylcholine release and turnover in the striatum, cortex and hippocampus (35,40). The effects in the cortex and hippocampus may be particularly relevant to an understanding of changes in cognitive performance, and it has been concluded that the inhibitory action of a 5-HT pathway on hippocampal cholinergic activity may be relevant to memory (15). Therefore, it could be hypothesised that the actions of 5-HT to reduce acetylcholine release may afford a novel site of drug action to influence cholinergic function and cognition. To test the hypothesis we have pursued both a behavioural and biochemical approach which has been enabled by the development of agents having selective actions on the 5-HT receptor subtypes [see Bradley *et al.* (9)]. We have already reported that 5-HT_3 receptors mediate the inhibitory effects of 5-HT on acetylcholine release (5), and in the present study we investigate the actions of the 5-HT_3 receptor antagonist, ondansetron (10) in rodent and primate tests of cognition.

METHOD

Animals

Male albino BKW mice, 25–30 g, 6 to 8 weeks old (i.e.,

"young adults") and 33–38 g, 8 to 10 months old (i.e., "aged" animals; the mouse life span is approximately 22 months) were housed in conditions of constant temperature (21 ± 1°C) in groups of 10 and given free access to food and water. Mice were kept on a 12-hr light/dark cycle with lights off at 07.00 hr.

Male Lister Hooded rats 250–300 g, 11 to 15 weeks old (i.e., "young adults," the rat life span is approximately 3 years) were housed in groups of 5 and given free access to food and water ad lib or until the start of behavioural testing (see below). Rats were kept on a 12-hr light/dark cycle with lights off at 09.00 hr. The temperature was maintained at 21 ± 1°C.

Common marmosets (*Callithrix jacchus*), body weights 315 ± 20 g, 16 months to 4 years old (the marmoset life span is approximately 15 years) of either sex were housed as single sex pairs. They were allowed food (Mazuri primate diet, SDS Ltd, Essex) and water ad lib. Additionally, marmosets received an assortment of fruit, brown bread or malt loaf daily and a vitamin supplement (Duphasol B/602; Duphar Veterinary Ltd., Southampton) weekly in fruit juice. Holding rooms were maintained at 25 ± 1°C at a humidity of 55%. Rooms were illuminated for 12 hr with 12-hr dark cycle, with lights on between 07.00 and 19.00 hr. Simulated dawn and twilight periods were programmed to occur 0.5 hr before and after the main lights came on or went off respectively. During the 12-hr dark period a single 60-W red bulb was illuminated to avoid complete darkness.

Experiments in the Mouse

Habituation test. Testing was carried out daily between 08.30 and 12.30 hr. Mice were taken from a dark home environment in a dark container to the experimental room maintained in low red lighting, and placed into the centre of the white section of a white and black test box. The box (45 × 27 × 27 cm high) was divided. Forty percent of the area was painted black and illuminated under a red light (60 W, 0 lux) and the other painted white and brightly illuminated with a white light (1 × 60 W, 400 lux) located 17 cm above the box. Access between the two areas was enabled by a 7.5 × 7.5 cm opening located at floor level in the centre of the partition. Behaviour was assessed via remote video-recording and the latency to move from the white to the black section was measured. The brightly lit area of the black and white test box has aversive properties, mice normally distributing their behaviour preferentially in the black compartment [see (12)]. On repeated daily testing mice habituate to the test system with a reduced latency in movement from the white to the black section.

Stereotaxic techniques. Mice were anaesthetised with chloral hydrate (150 mg/kg SC) and placed in a Kopf stereotaxic frame. Using standard stereotaxic techniques, lesions of the nucleus basalis magnocellularis were induced using either electrolytic lesions or injections of ibotenic acid located ant. 2.3 mm (to the zero of the Kopf frame); vert. 4.5 mm (below the skull surface) and lat. ±2.1 mm from the midline. Electrolesions of the nucleus basalis magnocellularis were induced by use of a 0.3 mm stainless steel electrode insulated except at the tip and passing a current of 1 mA for 10 sec. Ibotenic acid was prepared in phosphate buffer to pH 7.0 and lesions produced by injecting 2 μg in 0.25 μl over 5 sec (a further 4 min was allowed for deposition) from Hamilton syringes attached via polythene tubing to 0.3 mm stainless steel injection units.

Biochemistry. At the end of the experiments, mice with lesions were killed for determination of the levels of cholineacetyltransferase in the septum, frontal cortex, hippocampus and striatum using the radioenzymatic technique of Fonnum (16), with a modified incubation period of 10 min. [^{14}C]Acetylcoenzyme A (54 m Ci mmol, Amersham, UK) was used at a final concentration of 0.75 mmol l.

Experiments in the Rat

T-maze reinforced alternation task. Animals were trained on a food reinforced alternation task using a modification of the protocol of Salamone *et al.* (36). Food was withdrawn 2 days prior to testing and animals were deprived of food for 23 hr per day. Water was available ad lib and body weight was maintained at 85%. Animals were taken from the holding room to the dimly lit test room 30 min before testing. Experiments were carried out between 08.00 and 15.00 hr using an elevated T-maze. The start arm measured 80 × 10 cm and the side arms were 60 × 10 cm with food wells 3 cm deep at each end. The T-maze was elevated 30 cm above the ground.

On day 1 each rat was allowed 10-min habituation to the maze. Both food wells were baited with banana flavoured pellets and pellets were also scattered along the approach arm. The rats were then subjected to a period of reinforced alternation training, days 2–5 being designated "pretraining" days with days 6–9 "training" days. All reinforced alternation training consisted of paired trials (each pair consisting of a "run"). The first trial was the "forced" trial in that one arm was blocked whilst the other arm was baited. The second trial of the pair was a "choice" trial in which reward pellets were placed in the arm opposite to that reinforced in the first trial of the pair. A correct choice was when the rat entered the arm and passed a point 20 cm along the arm containing the food in the choice trial. In addition to correct/incorrect choice, latency to reward was recorded for both forced and choice trials.

Four runs per day were carried out on pretraining days (intertrial interval 0 sec, interrun interval 30 sec) and 6 runs per day during training (intertrial interval 30 sec; interrun interval 60 sec). The number of lefts and rights was random (following Gellerman Schedule) and was balanced across the test groups.

Experiments in the Marmoset

Object discrimination and reversal learning tasks were assessed using a Wisconsin General Test Apparatus. Behavioural testing was carried out between 10.00 hr and 15.30 hr in a room where temperature and lighting conditions were identical to those of the holding rooms. Following the initial training of an object discrimination task to 90% correct performance, the task set for the marmosets was to select between the two stimuli (junk objects) covering two food wells, one of which contained a food reward. The task was to select the food-rewarded stimulus presented to the animal on a pseudorandom Gellerman schedule. On completing 6 consecutive correct responses on the first food rewarded object (initial discrimination task) the reward paradigm was changed so that the marmoset was required to select the second, initially unrewarded object, to the same criterion (reversal task). Objects remained constant throughout the 5-day test periods: the last object stimulus of one day was always the first stimulus of the following day. Marmosets received ondansetron or vehicle 40 min prior to testing on each day of a 5-day test period. After each test week, animals continued on trial for a further 5 days without drug treatment. During the treatment week dosing was carried out according to a blind, randomised cross-over design. The mean (± SE) differences between drug and vehicle controls for the number of trials to criterion for all marmosets within a dose group on all days were calculated.

Statistical Analysis

Behavioural results were analysed using two-way analysis of variance (repeated measure analysis) followed by Dunnett's test (rodent tests) and a paired *t*-test (marmoset tests).

Drugs

Ondansetron (GR38032F); (1,2,3,9-tetrahydro-9-methyl-3-

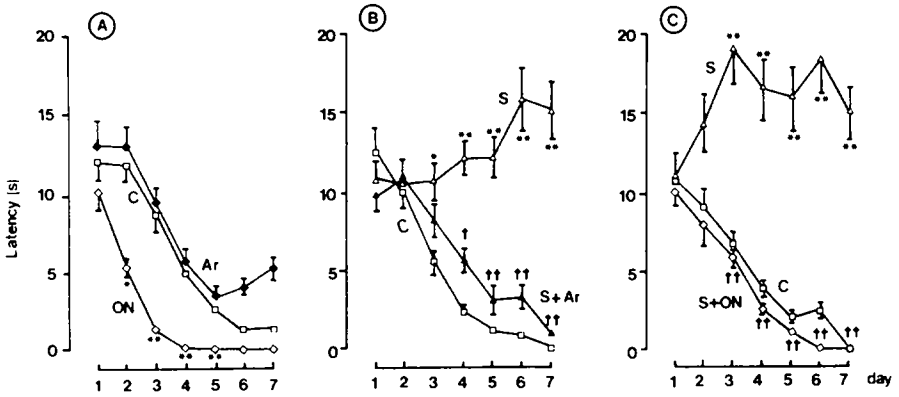

FIG. 1. Influence of arecoline and ondansetron on the habituation profile of young adult mice in the white and black test box and scopolamine impairment. (A) Indicates the response of vehicle (C, □—□), arecoline (Ar, ◆—◆ 50 mg/kg/day by intraperitoneal infusion from an osmotic minipump) and ondanestron (ON, ◇—◇ 10 ng/kg IP b.i.d.), (B and C) Indicate the effect of scopolamine (S, △—△ 0.25 mg/kg IP b.i.d.) and scopolamine plus arecoline (S + Ar) and scopolamine plus ondansetron (S + ON). n = 10, vertical bars indicate S.E. of means. Significant differences in the latency of the first movement from the white to the black area compared to vehicle controls are indicated $*p<0.05$, $**p<0.001$, a significant antagonism of scopolamine action by arecoline and ondansetron indicated $\dagger p<0.01$ and $\dagger\dagger p<0.001$ (Dunnett's t-test).

[(2 - methyl - 1H - imidazol -l-yl) -methyl]-4H-carbazol -4-one,HCl ·2H$_2$O) (Glaxo), arecoline·HBr (Sigma) and scopolamine·HBr (Sigma) were prepared in saline. Ibotenic acid (Sigma) for intracerebral injection was prepared in phosphate buffer neutralised to pH 7.0. Doses are expressed as the base and were administered intraperitoneally in a volume of 1 ml/100 g in the mouse and 1 ml/kg in the rat and marmoset.

RESULTS

Selection of Dosage Regimes

Preliminary studies in the mouse and rat were required to establish dose regimes of scopolamine and arecoline that would not unnecessarily modify peripheral cholinergic function. The use of acute treatments with arecoline (5 to 50 mg/kg) revealed a brevity of action and the development of severe changes in gastrointestinal function. Therefore, arecoline was administered continuously via an Alzet osmotic minipump located in the peritoneal cavity in doses of 10, 30, 50 and 75 mg/kg/day. In rats, the 50 mg/kg/day dose was associated with diarrhoea, tremor and prostrate appearance; such effects were absent using 30 mg/kg/day which was selected for further use. However, in the mouse a dose of 50 mg/kg/day was selected as the maximal dose failing to induce autonomic dysfunction.

The ability of scopolamine to disrupt peripheral cholinergic function was assessed by changes in pupil diameter. In rats the dose response curve to scopolamine was found to be steep, 0.1 mg/kg IP failing to alter pupil diameter, whereas 0.5 mg/kg caused a maximal 206% increase. A dose of 0.25 mg/kg scopolamine was selected for future studies as a threshold dose causing a smaller (55%) yet significant increase in pupil diameter. A dose of 0.25 mg/kg IP was also selected for use in young adult mice. Higher doses increased pupil diameter by some 270% and were associated with the development of a "jerky" motor behaviour. Aged mice were particularly susceptible to the effects of scopolamine, a dose of 0.25 mg/kg IP causing death in some mice; a dose of 0.1 mg/kg IP was selected for the studies using aged animals.

Ondansetron does not directly influence the autonomic nervous system and causes no overt behavioural changes in normal animals. However, ondansetron is highly effective in reducing aversive responding in rodent and primate models of anxiety (24) and care was taken to use "subanxiolytic" doses in the rodent and primate tests of cognition.

Habituation Test in Mice

On repeated exposure to the black/white test box young adult mice habituate by moving more rapidly from the white to the black area. Generally, for young adult mice the habituation occurs over a 4- to 6-day period, with a reduction in latency of movement from 10 to 12 sec to 1 to 4 sec by the 5th or 6th day of test (Fig. 1). Treatment with arecoline, 50 mg/kg/day by IP infusion, failed to modify the habituation profile. In contrast, mice treated with ondansetron, 10 ng/kg IP b.i.d. showed a reduced latency in moving from the white to the black area (Fig. 1A). Treatment with scopolamine (0.25 mg/kg IP b.i.d.) impaired the ability of mice to habituate to the test box (Fig. 1B), although the motor behaviour remained normal and mice located the opening to allow entry into the black area in the same way as untreated animals. The dose of scopolamine was critical; a lower dose of 0.125 mg/kg IP b.i.d. caused inconsistent changes and higher doses induced a "jerky" behaviour about the white area, the mice showing an apparent failure to find the opening in the partition. The habituation profile was not modified by treatment with N-methyl-scopolamine 0.25 mg/kg IP b.i.d. The inhibitory action of scopolamine (0.25 mg/kg IP b.i.d.) on habituation was prevented by arecoline (50 mg/kg/day by IP infusion) or ondansetron (10 ng/kg IP b.i.d.) (Fig. 1B and C).

Both ibotenic acid lesions and electrolesions of the nucleus basalis magnocellularis disrupted habituation to the black/white test box. Both lesions were shown to reduce ChAT activity in the frontal cortex (by 34–57%) without significant influence on ChAT activity in the hippocampus, septum or striatum (Table 1). The impairment in habituation by the ibotenic acid lesion and electrolesion of the nucleus basalis was inhibited by a continued treatment with arecoline (50 mg/kg/day IP infusion) or ondansetron (10 ng/kg IP b.i.d.) (Fig. 2).

In contrast to findings with young adult mice, in aged mice the slight reduction in latency of movement into the black area failed

TABLE 1

MODIFICATIONS OF FOREBRAIN CHOLINEACETYLTRANSFERASE (ChAT) ACTIVITY IN THE MOUSE 9 DAYS AFTER TREATMENT WITH ELECTROLESIONS AND IBOTENIC ACID LESIONS OF THE NUCLEUS BASALIS MAGNOCELLULARIS

		ChAT (pmoles/min/mg protein)			
Treatments	n	Frontal Cortex	Hippocampus	Septum	Striatum
No treatment	5	2516 ± 137	2993 ± 205	2510 ± 117	6368 ± 496
Sham lesion	4	3099 ± 162	2674 ± 176	2420 ± 184	5932 ± 471
Electrolesion	5	1350 ± 158†	2562 ± 155	2452 ± 103	5221 ± 455
Ibotenic acid lesion	4	2051 ± 112*	3356 ± 180	2519 ± 159	5956 ± 442

Values are mean ± S.E.M., *$p<0.05$ and †$p<0.01$ (compared with sham lesion value, Student's *t*-test).

to achieve significance. However, from the first day of treatment with ondansetron (10 ng/kg IP b.i.d.), aged mice habituated rapidly and latency to move to the black area was reduced throughout the 5-day test period (Fig. 3A). On the 6th day of treatment with ondansetron or vehicle, aged mice received an injection of scopolamine (0.1 mg/kg IP) and were tested after 45 min. Scopolamine impaired performance in aged mice receiving a vehicle treatment, but no impairment in the habituation response was observed in the mice treated with ondansetron (Fig. 3B).

T-Maze Reinforced Alternation Task in Rats

Subchronic treatment of rats with scopolamine (0.25 mg/kg IP b.i.d.) both during the pretraining and training days significantly reduced the number of correct responses made, $F(3,21)=4.87$, $p<0.01$. Concurrent treatment with ondansetron (10 ng/kg IP b.i.d.) significantly attenuated the effect produced by scopolamine on choice performance (see Fig. 4A). The performance of all treatment groups improved over the 9-day test period, $F(7,49)=5.4$, $p<0.01$ (two-way ANOVA). Scopolamine treatment also delayed the forced, $F(3,12)=61.9$, $p<0.01$, and choice, $F(3,12)=56.9$, $p<0.01$, latencies (two-way ANOVA). These measurements were antagonised by ondansetron (Fig. 4A and 4B). Ondansetron, when administered alone, did not improve the normal performance of the task compared to control, vehicle-treated animals, $F(1,4)=0.73$, $p>0.05$ (as indicated by no increase in % correct responses compared to control).

The scopolamine-induced reduction in % correct responses was also inhibited by arecoline (30 mg/kg/day IP infusion) during the first three pretraining days and prevented during the training days (Fig. 5A). The scopolamine-induced delay in forced and choice latencies was also inhibited by arecoline (Fig. 5B and C). Arecoline, when administered alone, did not improve the normal performance of the task compared to control, vehicle-treated animals, $F(3,12)=1.93$, $p>0.05$.

Object Discrimination and Reversal Learning Tasks in Marmosets

Treatment with ondansetron (1 and 10 ng/kg SC b.i.d.) throughout a 5-day test period significantly decreased the number of trials to criterion in both the object discrimination and reversal learning task. The object reversal task was more difficult for marmosets to perform and therefore more trials were required before reaching criterion. Ondansetron produced greater improve-

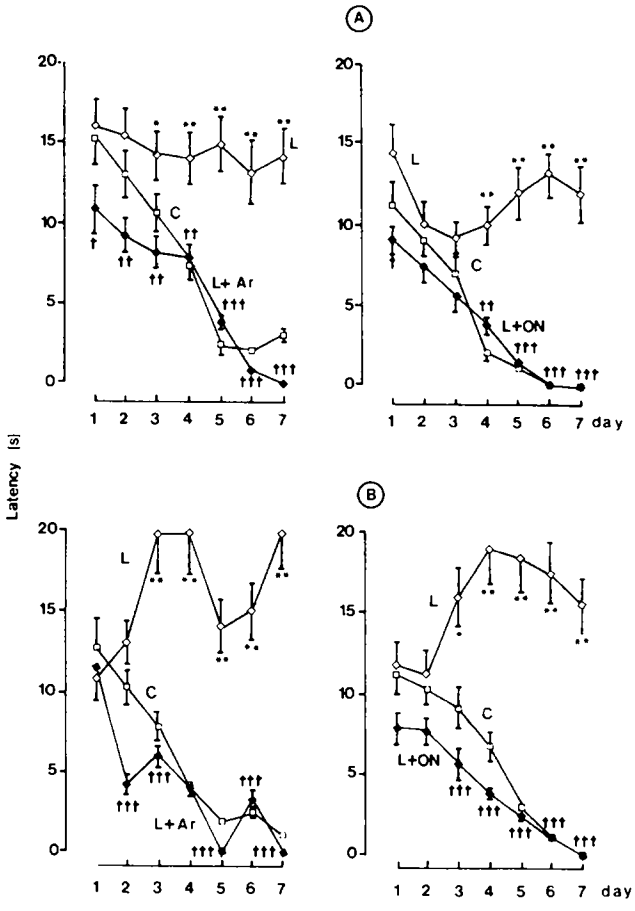

FIG. 2. Effects in young adult mice of (A) electrolesions and (B) ibotenic acid lesions of the nucleus basalis magnocellularis and influence of arecoline and ondansetron on the habituation profile in the black and white test box. Nonlesioned mice received vehicle (C, □—□) and lesioned mice received vehicle (L, ◇—◇) or arecoline (L + Ar, ◆—◆ 50 mg/kg/day by IP infusion via osmotic minipumps) or ondansetron (L + ON ◆—◆, 10 ng/kg IP b.i.d.). n = 10, vertical bars indicate S.E. of means. A significant increase in the latency of movement from the white to the black section of the test box is indicated *$p<0.05$, **$p<0.001$; a significant attenuation of the effects of the lesions is indicated †$p<0.05$, ††$p<0.01$, †††$p<0.001$ (Dunnett's *t*-test).

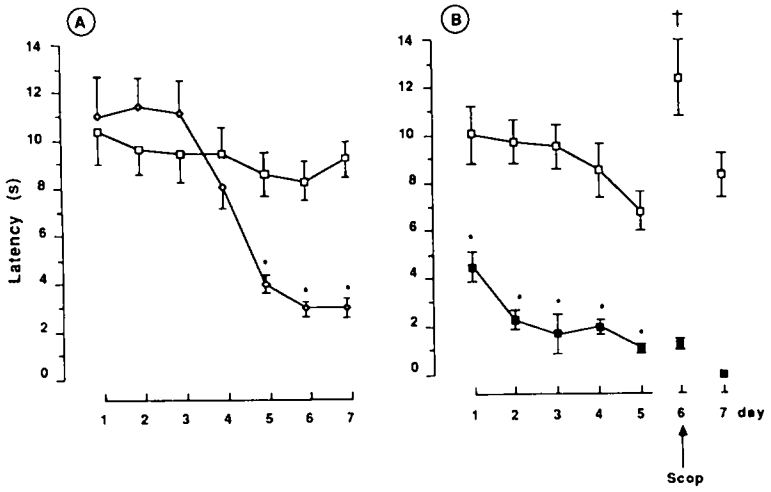

FIG. 3. The habituation profile of young adult and aged mice in the black and white test box; influence of ondansetron on basal learning and scopolamine impairment. (A) Young adult (◇—◇) and aged (□—□) mice received vehicle injections, (B) aged mice received daily injections of vehicle (□—□) or ondansetron (■—■ 10 ng/kg IP b.i.d.) and on the 6th day (↑) received an additional treatment of scopolamine (Scop) (0.1 mg/kg IP). (A) n = 15, (B) vertical bars indicate S.E. of means. Significant differences in the latency of the initial movement from the white to the black area between (A) aged and young adult mice and (B) treatment with ondansetron and vehicle values are indicated *$p<0.001$ (Dunnett's t-test); a significant increase in the latency of movement induced by scopolamine relative to vehicle controls is indicated †$p<0.05$ (Dunnett's t-test).

ments in performance on the reversal task than against the initial discrimination task over the same dose ranges (Fig. 6). Peak effects on both discrimination and reverse learning performance for ondansetron were obtained with the low dose of 1 ng/kg SC b.i.d. although significant reductions ($p<0.01$) in trials to criterion were obtained at the 10 ng/kg dose level (Fig. 6). Within 2 days following cessation of ondansetron treatment the performance of marmosets returned to predrug levels for both discrimination and reversal learning. There were no significant differences between the mean performance values for pre- and posttreatment

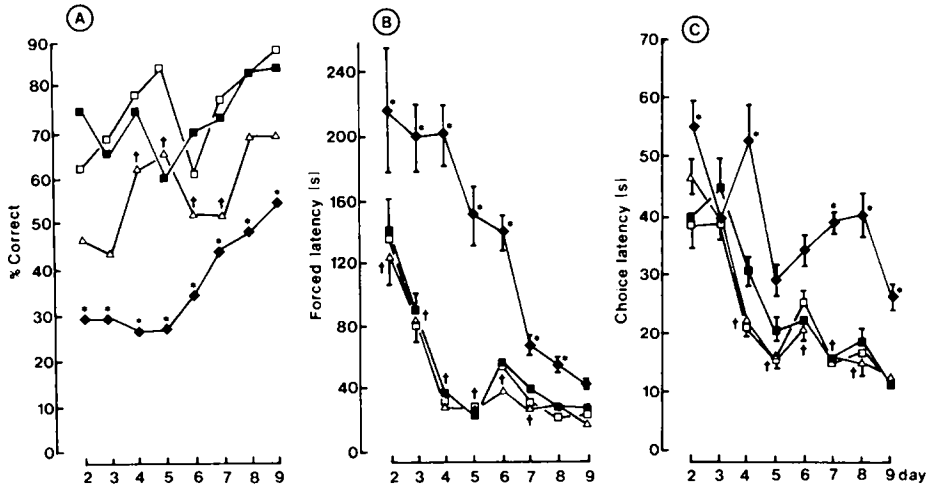

FIG. 4. Effects of ondansetron and scopolamine in a food-reinforced alternation task in the rat using an elevated T-maze. The effect of scopolamine 0.25 mg/kg IP b.i.d. ◆—◆ (n = 8), scopolamine 0.25 mg/kg IP b.i.d. plus ondansetron 10 ng/kg IP b.i.d. △—△ (n = 8), ondansetron 10 ng/kg IP b.i.d. ■—■ (n = 8) and saline 1 ml/kg IP b.i.d. □—□ (n = 8) on performance measured as (A) % correct responses, with additional measures of (B) forced and (C) choice latencies. Significant differences between the treatment groups are indicated as *$p<0.05$ compared to control and †$p<0.05$ compared to scopolamine (two-way ANOVA followed by Dunnett's t-test).

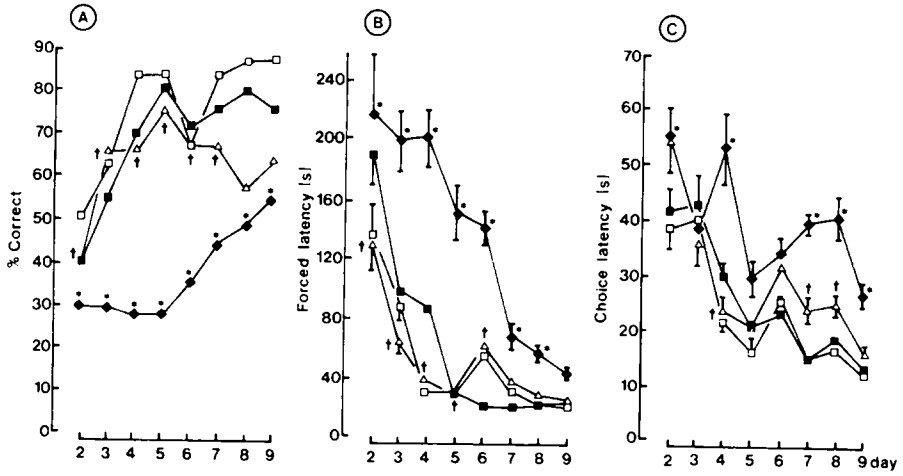

FIG. 5. Effects of arecoline and scopolamine in a food-reinforced alternation task in the rat using an elevated T-maze. The effect of scopolamine 0.25 mg/kg IP b.i.d. ◆—◆ (n = 5), scopolamine 0.25 mg/kg IP b.i.d. plus arecoline 30 mg/kg/day IP △—△ (n = 5), arecoline 30 mg/kg/day IP ■—■ (n = 5) and saline 1 ml/kg IP b.i.d. □—□ on performance measured as (A) % correct responses, with additional measures of (B) forced and (C) choice latencies. Significant differences between the treatment groups are indicated as $*p<0.05$ compared to saline-treated controls and $\dagger p<0.05$ compared to scopolamine treatment group (two-way ANOVA followed by Dunnett's *t*-test).

periods. Ondansetron was ineffective at a dose of 0.01 ng/kg SC b.i.d.

DISCUSSION

The present results provide evidence that the selective 5-HT_3 receptor antagonist, ondansetron, improves performance in rodent and primate tests of cognition. In the mouse habituation test, on daily testing mice "learn" to move more rapidly from a light aversive environment to a dark area. In doses which, in themselves had no effect to reduce aversive responding, ondansetron improved performance in young adult and, more particularly, in aged mice, which normally failed to habituate. The experiments in "aged" mice indicate the advantage of using a low basal level of responding to demonstrate an improvement in performance. There is considerable evidence that brain cholinergic systems are linked with behavioural functions of learning, memory and information processing (see introduction). That scopolamine treatments and lesions of the nucleus basalis magnocellularis, a major source of neocortical cholinergic input (22, 23, 27, 37), produced marked impairment in the mouse habituation test is consistent with a central cholinergic involvement in processes such as stimulus detection, attention and other cognitive events relevant to habituation. Age-related decreases in performance in many behaviours have also been connected to a cholinergic deficit [see review by Bartus *et al.* (7)], and such deficits may partly explain the decreased performance of aged mice in the habituation test.

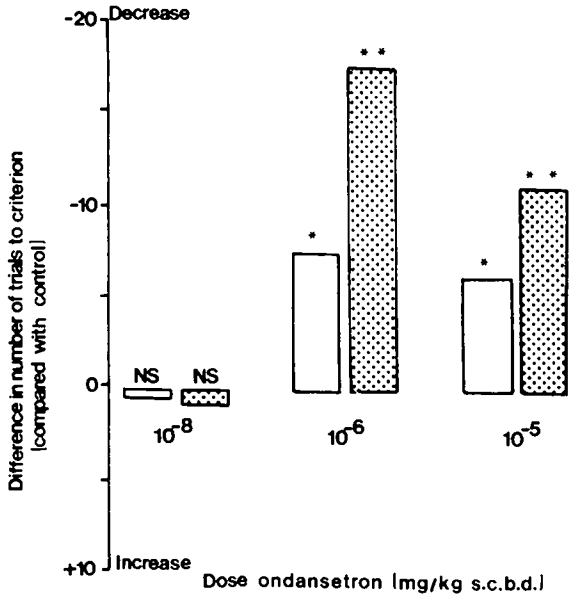

FIG. 6. Improvements induced by ondansetron in marmoset performance in an object discrimination (open histograms) and reversal learning task (closed histograms) using a Wisconsin General Test Apparatus. Marmosets received ondansetron 0.01, 1.0 or 10 ng/kg SC b.i.d. 40 min prior to testing on each of the 5 test days. After each test week, animals continued on trial for a further 5 days without drug treatment. Differences in the mean number of trials to criterion for 5 days in comparison with vehicle-treated control animals were calculated. S.E. means were 4.7–11.1%. A decrease in the number of trials to criterion indicates an improvement in performance. $*p<0.05$, $**p<0.005$ (paired *t*-test comparing each treatment group with corresponding pretreatment control values).

The impairments caused by scopolamine and lesions of the nucleus basalis were inhibited by ondansetron. The two effects of ondansetron to improve basal performance and attenuate an impairment caused by a cholinergic deficit may be related, and reflect the ability of 5-HT_3 receptor antagonists to prevent the inhibitory effect of 5-HT on acetylcholine release (5). If this hypothesis is correct, the results of the lesion studies indicate that the residual cholinergic input to the frontal cortex is sufficient to mediate an improvement in performance. Alternatively, since cortical cholinergic afferents appear to demonstrate plasticity after nucleus basalis lesions (33,44), an action of ondansetron on the nonlesioned cholinergic input from the medial septal area (29) to the hippocampus and associated structures may be sufficient to compensate for the cholinergic deficit. However, caution remains in interpreting the effects of nucleus basalis lesions (and the action of ondansetron) solely in terms of cholinergic effects since the behavioural effects of nucleus basalis lesions are not correlated to a cholinergic loss in some behavioural tests (39).

The primary pharmacological evidence supporting a cholin-

ergic involvement with cognition are the deficits which occur to scopolamine and the reversal by cholinergic agents such as physostigmine, tetrahydroaminoacridine and arecoline [see reviews by Bartus *et al.* (7); Candy *et al.* (11); Swaab and Fliers (38); Giacobini (17)]. In the present work arecoline inhibited the impairment of mouse habituation caused by scopolamine and nucleus basalis lesions, but the well known difficulties in the use of the cholinergic agents were readily apparent. The use of arecoline necessitated a careful dose titration and continuous administration to avoid severe autonomic side effects. Furthermore, arecoline failed to increase basal performance of mice in the habituation test, and this may partly reflect an inability to administer an adequate dose, limited by the development of incapacitating peripheral effects. The use of arecoline is in marked contrast to the use of ondansetron, which was capable of increasing basal performance and preventing the impairment induced by a cholinergic deficit, in the complete absence of autonomic effects. It remains possible that ondansetron may induce a more effective stimulation of the cholinergic system than can be achieved by the cholinomimetic actions of arecoline on postsynaptic receptor sites.

In the rat, spontaneous alternation in a T-maze is strongly influenced by spatial cues and spatial memory is highly susceptible to anticholinergic drugs and hippocampal lesions (32, 36, 42). In the present study, using reinforced alternation, both ondansetron and arecoline inhibited scopolamine-induced disruption of T-maze performance in the young adult rat. The use of young adult animals was necessary to demonstrate the scopolamine-induced impairment; aged animals are already impaired. In this test ondansetron also increased basal performance in the less demanding training period when only one arm of the T-maze was open. However, in the more difficult T-maze alternation task, basal performance assessed by the choice latency and percentage correct responses was not improved by either ondansetron or arecoline. This may relate to a higher basal level of performance which is difficult to improve upon.

The marmoset was used as a primate model of object discrimination and reversal learning, known to be sensitive to changes in cholinergic function (34). After the initial training period ondansetron produced a significant improvement in performance in the reversal learning task. As observed in the rodent models, ondansetron was highly potent, being effective in doses as low as 1 ng/kg and such effects were achieved in the absence of sedation or any overt changes in autonomic functioning. It is also of note that such changes were observed in young adult animals. Long-term studies are in progress to determine whether the efficacy of ondansetron is even more apparent in aged populations.

The consistent and highly potent action of ondansetron to enhance performance in rodent and primate tests of cognition would indicate that 5-HT may normally exert an inhibitory effect, and there is evidence to support this hypothesis. Thus, in an early study, Woolley (46) reported that mice showed a reduced maze learning ability when brain 5-HT was increased and enhanced learning ability with decreased brain 5-HT. Evidence that amnesic agents or events leading to amnesia can modify forebrain 5-HT is reviewed by Essman (13), and 5-HT itself has been shown to interfere with the acquisition or retention of a conditioned or passive avoidance response [see Essman (13)]. However, the early studies focussed on avoidance behaviour and there is contradictory evidence for the role of 5-HT in cognition [see Ogren (30); Ogren *et al.* (31)]. Thus, 5-HT receptor antagonists such as methysergide and mianserin have been found to facilitate, impair or have no effect on the acquisition and retention of "memory" in animals (1, 4, 8) and similar results are reported following the depletion of forebrain 5-HT (13,30). In tests with an important spatial component, e.g., the radial arm maze and Morris water maze, 5-HT and 5-HT$_2$ receptor antagonists methysergide and ketanserin are reported to have no effect on performance (8,20). In contrast, lesions of the median raphe nucleus are reported to impair acquisition or performance in an 8-arm radial maze and discrimination tasks (3,45), although Asin and Fibiger (2) have questioned the involvement of serotonergic neurones in such effects.

The availability of compounds with a selective action on different subtypes of 5-HT receptors may allow better definition of the role of 5-HT in cognition, and the present data would indicate an important involvement of 5-HT$_3$ receptors. 5-HT$_3$ receptors have been located in different cortical and limbic systems (6, 26, 41) and in the entorhinal cortex have been shown to mediate an inhibitory effect of 5-HT to reduce acetylcholine release (5). An action of 5-HT$_3$ receptor antagonists at such sites would facilitate cholinergic function and contribute to the improved performance in tests of cognition.

In summary, the present results provide the beginnings of an understanding of the role of 5-HT$_3$ receptors in cognition. The ability of ondansetron to improve performance in tests of cognition in three species, with a complete absence of cholinergic side effects, provides the rationale for a more detailed analysis of the potential to modify memory, attention, reaction time, acquisition, retrieval and other components of cognition.

REFERENCES

1. Altman, H. J., Normile, H. J. Enhancement of the memory of a previously learned aversive habit following pre-test administration of a variety of serotonergic antagonists in mice. Psychopharmacology (Berlin) 90:24–27; 1986.
2. Asin, K. E., Fibiger, H. C. Spontaneous and delayed spatial alternation following damage to specific neuronal elements within the nucleus medianus raphe. Behav. Brain Res. 13:241–250; 1984.
3. Asin, K. E.; Wirtschafter, D.; Kent, E. W. Discrimination learning and reversal following electrolytic median raphe lesions. Soc. Neurosci. Abstr. 5:269–270; 1979.
4. Bammer, G. Pharmacological investigations on neurotransmitter involvement in passive avoidance responding: A review and some new results. Neurosci. Biobehav. Rev. 6:247–296; 1962.
5. Barnes, J. M.; Barnes, N. M.; Costall, B.; Naylor, R. J.; Tyers, M. B. 5-HT$_3$ receptors mediate inhibition of acetylcholine release in cortical tissue. Nature 338:762–763; 1989.
6. Barnes, N. M.; Costall, B.; Naylor, R. J. [^{3}H]zacopride: ligand for the identification of 5-HT$_3$ recognition sites. J. Pharm. Pharmacol. 40:548–551; 1988.
7. Bartus, R. T.; Dean, R. L.; Beer, B.; Lippa, A. S. The cholinergic hypothesis of geriatric memory dysfunction. Science 217:408–417; 1982.
8. Beatty, W. W.; Rush, J. R. Spatial working memory in rats: Effects of monoaminergic antagonists. Pharmacol. Biochem. Behav. 18: 7–12; 1983.
9. Bradley, P. B.; Engel, G.; Feniuk, W.; Fozard, J. R.; Humphrey, P. P. A.; Middlemiss, D. N.; Mylecharane, E. J.; Richardson, B. P.; Saxena, P. R. Proposals for the classification and nomenclature of functional receptors for 5-hydroxytryptamine. Neuropharmacology 25:563–576; 1986.
10. Butler, A.; Hill, J. M.; Ireland, S. J.; Jordan, C. C.; Tyers, M. B. Pharmacological properties of GR38032F, a novel antagonist at 5-HT$_3$ receptors. Br. J. Pharmacol. 94:397–412; 1988.
11. Candy, J. M.; Perry, E. K.; Perry, R. H.; Court, J. A.; Oakley, A. E.; Edwardson, J. A. The current status of the cortical cholinergic system in Alzheimer's disease and Parkinson's disease. In: Swaab, D. F.; Fliers, E.; Mirmirman, M.; Van Gool, W. A; Haaren, F., eds. Ageing of the brain and Alzheimer's disease. Progress in brain research, vol.

70. Amsterdam: Elsevier; 1986:106–132.
12. Costall, B.; Jones, B. J.; Kelly, M. E.; Naylor, R. J.; Tomkins, D. M. Exploration of mice in black and white test box: Validation as a model of anxiety. Pharmacol. Biochem. Behav. 32:777–785; 1989.
13. Essman, W. B. Serotonin in learning and memory. In: Essman, W. B., ed. Serotonin in health and disease. vol. III. The central nervous system. New York: Spectrum Publications; 1978:69–143.
14. Euvrard, C.; Javoy, F.; Herbert, A.; Glowinski, J. Effect of quipazine, a serotonin-like drug, on striatal cholinergic interneurones. Eur. J. Pharmacol. 41:281–289; 1987.
15. Fibiger, H. C.; Lepiane, F. G.; Phillips, A. G. Disruption of memory produced by stimulation of the dorsal raphe nucleus: mediation by serotonin. Brain Res. 155:380–886; 1978.
16. Fonnum, F. A rapid radiochemical method for the determination of choline acetyltransferase. J. Neurochem. 24:407–409; 1975.
17. Giacobini, E. Models and strategies of cholinomimetic therapy of Alzheimer disease. In: Dowdall, M. J.; Hawthorne, J. N., eds. Cellular and molecular basis of cholinergic function. Chichester: Horwood; 1987:882–901.
18. Gillett, G.; Ammor, S.; Fillion, G. Serotonin inhibits acetylcholine release from rat striatum slices: Evidence for a presynaptic receptor-mediated effect. J. Neurochem. 45:1687–1691; 1985.
19. Heise, G. A. Facilitation of memory and cognition by drugs. Trends Pharmacol. Sci. 8:65–68; 1987.
20. Hunter, A. J.; Roberts, F. F. The effects of 8-hydroxy-2-(di-n-propylamino) tetralin (8-OHDPTA) on spatial learning in the Morris water maze task. In: Dourish, C. T.; Ahlenius, S.; Hutson, P. H., eds. 5-HT_{1A} receptors. Chichester, England: Ellis Horwood Ltd.; 1987: 278–285.
21. Jackson, D.; Bruno, J. P.; Stachowiak, M. K.; Zigmond, M. J. Inhibition of striatal acetylcholine release by endogenous serotonin. Brain Res. 457:259–266; 1988.
22. Johnston, M. V.; McKinney, M.; Coyle, J. T. Evidence for a cholinergic projection to neocortex from neurons in the basal forebrain. Proc. Natl. Acad. Sci. USA 76:5392–5396; 1979.
23. Johnston, M. V.; McKinney, M.; Coyle, J. T. Neocortical cholinergic innervation in the rat. Exp. Brain Res. 43:159–172; 1981.
24. Jones, B. J., Costall, B.; Domeney, A. M.; Kelly, M. E.; Naylor, R. J.; Oakley, N. R.; Tyers, M. B. The potential anxiolytic activity of GR38032F, a 5-HT_3-receptor antagonist. Br. J. Pharmacol. 93: 985–993; 1988.
25. Kesner, R. P.; Adelstein, T.; Crutcher, K. A. Rats with nucleus basalis magnocellularis lesions mimic mnemonic symptomatology observed in patients with dementia of the Alzheimer's type. Behav. Neurosci. 101:451–456; 1987.
26. Kilpatrick, G. J.; Jones, B. J.; Tyers, M. B. Identification and distribution of 5-HT_3 receptors in rat brain using radioligand binding. Nature 330:746–748; 1987.
27. Lehmann, J.; Nagy, J. I.; Atmadja, S.; Fibiger, H. C. The nucleus basalis magnocellularis: the origin of a cholinergic projection to the neocortex of the rat. J. Neurosci. 5:1161–1174; 1980.
28. Moos, W. H.; Davis, R. E.; Schwarcz, R. D.; Gamzu, E. R. Cognition activators. Med. Res. Rev. 8:353–391; 1988.
29. Odenfeld-Nowak, B.; Narkiewicz, O.; Bialowas, J.; Dabrowska, J.; Wierscko, A.; Gradkowska, M. The influence of septal nuclei lesions on activity of acetylcholinesterase and choline acetyltransferase in the hippocampus of the rat. Acta Neurobiol. Exp. (Warsz.) 34:583–601; 1974.
30. Ogren, S. O. Central serotonin neurones and learning in the rat. In: Osborne, N. N., ed. Biology of serotonergic transmission. Chichester: Wiley; 1982:317–334.
31. Ogren, S. O.; Fuxe, K.; Archer, T.; Hall, H.; Holm, A-C.; Kohler, C. Studies on the role of central 5-HT neurons in avoidance learning: a behavioural and biochemical analysis. In: Haber, B.; Gabay, S.; Issorides, M. R.; Alivisatos, S. R., eds. Serotonin current aspects of neurochemistry and function. Advances in experimental medicine and biology. vol. 133. New York: Plenum Press; 1981:681–704.
32. Olton, D. S.; Papas, B. C. Spatial memory and hippocampal function. Neuropsychologia 17:669–682: 1979.
33. Pedata, F.; Lo Conte, G.; Sorbi, S.; Marconcini-Pepeu, I.; Pepeu, G. Changes in high affinity choline uptake in rat cortex following lesions of the magnocellular forebrain nuclei. Brain Res. 233:359–367; 1982.
34. Ridley, R. M.; Bowes, P. M.; Baker, H. G.; Crow, T. J. An involvement of acetylcholine in object discrimination learning and memory in the marmoset. Neuropsychologia 22:253–263; 1984.
35. Robinson, S. E. Effect of specific serotonergic lesions on cholinergic neurons in the hippocampus, crotex and striatum. Life Sci. 32: 345–353; 1983.
36. Salamone, J. D.; Channell, S. L.; Welner, S. A.; Gill, R.; Robbins, T. W.; Iversen, S. D. Nucleus basalis lesions and anticholinergic drugs impair spatial memory and visual discrimination performance in the rat. In: Dowdall, M. J.; Hawthorne, J. N., eds. Cellular and molecular basis of cholinergic function. Chichester, England: Ellis Horwood Ltd.; 1987:834–840.
37. Saper, C. B. Organisation of cerebral cortical afferent systems in the rat. I. Magnocellular basal nucleus. J. Comp. Neurol. 222:313–342; 1984.
38. Swaab, D. F.; Fliers, E. Clinical strategies in the treatment of Alzheimer's disease. In: Swaab, D. F.; Fliers, E.; Mirmirman, M.; Van Gool, W. A.; Haaren, W. A., eds. Ageing of the brain and Alzheimer's disease. Progress in brain research. vol. 70. Amsterdam: Elsevier; 1986:413–428.
39. Thompson, R.; Gibbs, R. B.; Ristic, G. A.; Cotman, C. W.; Yu, J. Lack of correlation between cortical levels of choline acetyltransferase and learning scores in rats with globus pallidus lesions. Brain Res. 367:402–404; 1986.
40. Vizi, E. S.; Harsin, L. G.; Zsilla, G. Evidence of the modulatory role of serotonin in acetylcholine release from striatal interneurones. Brain Res. 212:89–99; 1981.
41. Watling, K. J.; Aspley, S.; Swain, C. J.; Saunders, J. [^{3}H]quaternised ICS 205-930 labels 5-HT_3 receptor binding sites in rat brain. Eur. J. Pharmacol. 149:397–398; 1988.
42. Watts, J.; Stevens, R.; Robinson, C. Effects of scopolamine on radial maze performance in rats. Physiol. Behav. 26:845–851; 1981.
43. Wenk, G.; Hepler, D.; Olton, D. Behaviour alters the uptake of [^{3}H]choline into acetylcholinergic neurons of the nucleus basalis magnocellularis and medial septal area. Behav. Brain Res. 13: 129–138; 1984.
44. Wenk, G.; Olton, D. S. Recovery of neocortical choline acetyltransferase activity following ibotenic acid injection into the nucleus basalis of Meynert in rats. Brain Res. 293:184–186; 1984.
45. Wirtshafter, E.; Asin, K. E. Impaired acquisition and performance of an 8-arm radial maze task following electrolytic lesions of the median nucleus of the raphe. Exp. Neurol. 79:412–421; 1983.
46. Wooley, D. W. A method for demonstration of the effects of serotonin on learning ability. In: Ya Mikhel'son, M.; Longo, V. G., eds. Pharmacology of conditioning, learning and retention. Oxford: Pergamon Press; 1965:231–236.