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Acute and Chronic Arecoline: Effects on a Scopolamine-Induced Deficit in Complex Maze Learning

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BRATT, A. M., M. E. KELLY, A. M. DOMENEY, R. J. NAYLOR AND B. COSTALL. *Acute and chronic arecoline: Effects on a scopolamine-induced deficit in complex maze learning.* PHARMACOL BIOCHEM BEHAV **53**(3) 713–721, 1996.—These studies tested the effect of arecoline, a nonselective muscarinic agonist, administered either acutely or by chronic peripheral infusion via osmotic minipumps, on a scopolamine-induced deficit in a Stone (14 unit) T-maze task in rats. Scopolamine alone (0.125–1.0 mg/kg, IP) dose-dependently impaired maze acquisition, increasing maze run-times and to a lesser extent, the number of errors committed. Neither acute administration of arecoline (5.0 and 10.0 mg/kg, IP), when tested against a deficit induced by scopolamine (0.25 mg/kg, IP), nor chronic arecoline administration (30 and 50 mg/kg per 24 h), when tested against a deficit induced by scopolamine (0.5 mg/kg), were able to ameliorate the decrements in maze performance. In fact, the higher dose of arecoline (50 mg/kg per 24 h) infused over 10 days potentiated the scopolamine-induced deficit, with respect to latency. These data indicate that dose selection is of great importance when employing arecoline in tests of learning and memory and that the influence of the method of administration of arecoline on the behavioural outcome warrants further study.

Stone maze Arecoline Scopolamine Memory and learning Cholinergic system Continuous drug infusion Osmotic minipumps

IT IS NOW well accepted that a disruption of the central cholinergic system plays a pivotal role in memory dysfunction (3). Several lines of evidence have led to this conclusion. For example, in Alzheimer's disease (AD), the most common of the dementias, a degeneration of the basal forebrain nuclei develops insidiously and leads to loss of cholinergic neurones (65), cholinergic dysfunction in the neocortex, and cognitive impairment (18, 44). Early postmortem studies investigating tissue taken from Alzheimer patients have also highlighted age-related reductions in the cholinergic presynaptic marker enzyme, choline acetyltransferase (ChAT), in areas associated with cognitive function such as the cerebral cortex (7) and hippocampus (41). Other markers of cholinergic function, such as acetylcholine synthesis and high affinity choline uptake, were also found to be reduced in these brain areas (53).

In addition to lesion studies that have employed destruction of the basal forebrain to induce cognitive impairments in animals (25,39), the use of antimuscarinic drugs such as scopolamine in preclinical and clinical studies has added further strength to the cholinergic hypothesis of memory dysfunction. Scopolamine has been found to produce memory deficits in both man (10,49,50) and in animals in a range of tests (11; for a review, see 56). For example, in the rodent, muscarinic blockade produces a deficit in performance of the T-maze reinforced alternation paradigm (1), the radial maze (12), the water maze (32,63) and the Stone maze (55).

Strategies aimed at enhancing cholinergic neurotransmission and, thus, correcting the cholinergic deficit have previously been forwarded, and these include the administration of precursors (35), acetylcholinesterase (AchE) inhibitors (5),

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and cholinergic agonists (16,31). All of these strategies aim to increase synaptic levels of acetylcholine in brain areas involved in mnemonic processing.

Cholinergic agonists, such as arecoline, oxotremorine, RS-86, and bethanechol, have been tested in the treatment of AD in attempts to tonically increase the functioning of muscarinic systems (23,24). There have been mixed reports of efficacy in AD patients, demonstrating either improvement in cognitive performance with multiple dose arecoline infusions (62), or little or no effect with intracranial infusions of bethanechol (34).

In several experiments, cholinergic agonists have been used in rodent memory paradigms. However, few of these have produced consistent or, indeed, promising outcomes (52,48). More success has been found with the anticholinesterase inhibitor drugs, such as physostigmine, in enhancing cognition under deficit conditions (52). One possible explanation for the reported variability of muscarinic agonists in improving impaired cognition may relate to their short duration of action. In the present experiment, it was, therefore, considered important to test the effects of maintaining constant blood levels of arecoline by continuously infusing this muscarinic agonist over 10 days and testing its effect upon scopolamine-induced cognitive deficits in rats, the aim being to persistently stimulate muscarinic receptors located postsynaptically.

The purpose of the present study was to establish a dose-response effect of scopolamine on the ability of rats to complete a highly complex maze task involving the use of a mixture of spatial working, reference, cue, and taxon learning skills. An evaluation was then made of the ability of arecoline, a muscarinic agonist, to influence the scopolamine-induced maze learning deficit when administered peripherally in either an acute fashion, or infused chronically via osmotic minipumps implanted intraperitoneally.

METHODS

Subjects

A total of 145 male Hooded Lister rats, Bradford bred, of age 12 weeks and weight 250–300 g, were used in these four experiments. For all experiments, rats were housed in groups of 5 in perspex cages lined with wood shavings in a large colony room that was illuminated between 0730–1930 h. In all experiments, rats were tested in the Stone maze. The rats were maintained at approximately 85% of their free-feeding weight by food presented during each Stone maze trial and by post-session supplementary feeding. Water was available ad lib, except during the Stone maze trials.

Stone Maze Testing

The Stone maze (59) consisted of a large square (144 × 144 cm) perspex maze having clear inner panels (width 6 mm) forming the alleys of the labyrinth and opaque sides 42 cm high. Figure 1 depicts a cross sectional view of the maze. The entire maze was supported on a 50-cm high table with a video camera positioned 2 m above the maze top. The maze comprised a series of alleys, 12 cm in width, with 14 blind ending error points separating the start box from the food reward in the goal box. The maze had only one correct route; this was equally divided into 4 maze sections by 5 transparent perspex guillotine doors that could be raised or lowered to prevent backtracking. Doors were operated using a system of fine wires from an observation point 2 m distant from the maze.

A food reward consisting of sweetened banana-flavoured wet mash (Bioserve banana pellets, Labsure rat chow, sucrose and water) was placed in the goal box at the end of the maze. Rats received one 5-min trial in the maze per day. At the start of each trial, the rat was placed in the start box and the guillotine door raised. A trial commenced when the rat had moved both forelimbs, snout, and upper body out of the start box. The trial ended when the rat reached the goal box and made contact with the food reward or after 5 minutes had elapsed. The rat was allowed to remain on the maze for 30 s at the end of the trial to take the reward before being replaced in the home cage. Any rats not reaching the goal within the maximal time of 300 s were placed near the food reward for 30 s before being removed from the maze.

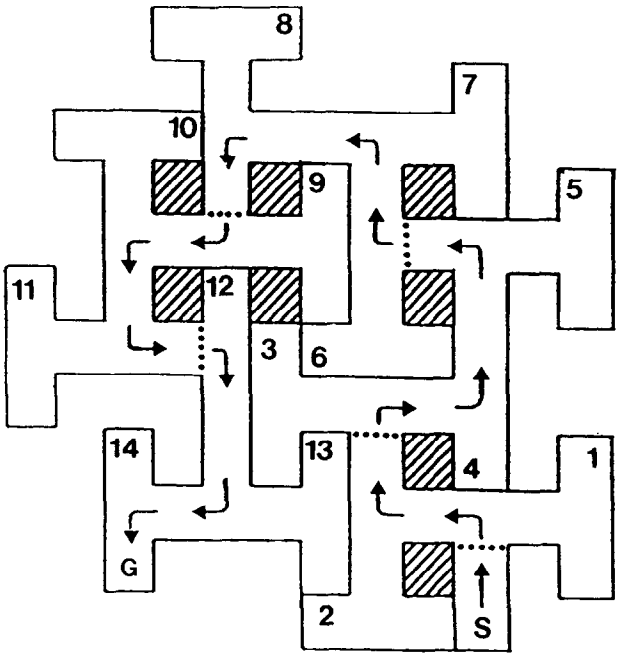

FIG. 1. A schematic cross sectional diagram of the Stone maze. Numbers 1–14 refer to the blind ending error points. The arrows demonstrate the single correct maze path between the start and goal boxes. Lines of black circles represent perspex guillotine-style doors.

To obviate the effect of olfactory cues upon the rat's performance of the maze, the walls and floor of the maze were washed with a pheromone masking solution (Hibitane, ICI 5% in water) following each trial. All experiments were video recorded and kept on tape for subsequent analysis.

Experiment 1

Effects of scopolamine in the stone maze. The purpose of this experiment was to establish dose–response effects of peripherally administered scopolamine. Animals (n = 50) were assigned to the following drug treatment groups; saline treated (n = 10), and (n = 8) for the scopolamine (0.125, 0.25, 0.5, or 1.0 mg/kg) and methylscopolamine (0.5 mg/kg) treated groups. Rats received one trial in the maze each consecutive day for a period of 15 days.

Experiment 2

Effects of acute arecoline on scopolamine-induced deficits in the stone maze. In this experiment, rats received one

trial in the maze on consecutive days for a period of 7 days to determine if acutely administered arecoline could ameliorate the scopolamine-induced deficits. Rats were divided into 5 treatment groups (n = 8/group) that received either saline, 0.25 mg/kg scopolamine, arecoline 10.0 mg/kg, arecoline 5.0 mg/kg + scopolamine 0.25 mg/kg, or arecoline 10.0 mg/kg + scopolamine 0.25 mg/kg.

Experiments 3 and 4

Effects of chronic infusion of arecoline on scopolamine-induced deficits in the stone maze. In these experiments, rats were divided into 4 groups (Experiment 3: n = 6/group, Experiment 4: n = 8/group) that were implanted with osmotic minipumps containing either saline or doses of arecoline (Experiment 3: 30 mg/kg per 24 h, Experiment 4: 50 mg/kg per 24 h). Additionally, half of the rats in both experiments received injections of scopolamine (0.5 mg/kg) 30 min prior to testing in the Stone maze over 10 consecutive days.

Alzet® osmotic minipumps model 2MLI (5.1 cm long × 1.4 cm diameter), implanted intraperitoneally, were used in these experiments. These comprised a flexible reservoir of volume 2.11 ± 0.08 ml, capable of delivering drug continuously at a nominal pumping rate of 5.3 ± 0.26 μl/hr (217 μl/24 h), for 14 days. The osmotic minipump system consisted of the body of the pump, plus the flow moderator complete with a plastic dome-shaped cap to prevent damage to the tissues at the site of implantation. The flow moderator was designed to minimise diffusion of the drug away from the site of administration. Solutions of arecoline hydrochloride for infusion were prepared in 0.9% saline and loaded into the pumps via a sterile syringe under aseptic conditions to reduce the risk of infection following implantation, Once loaded, pumps were placed into a beaker of sterile 0.9% saline that was placed into a water bath at 37°C for 12 h to allow priming of the pumps. The priming procedure was carried out so that the pumps would commence pumping prior to implantation. This procedure obviated the risk of a delayed pumping startup time, that has previously been found to be a source of experimental inaccuracy in the rat (29).

Surgical implantation of minipumps. Rats were anaesthetised with isofluorane delivered in carrier gases O_2 1 l/min and N_2O 1 l/min using a Boyle's inhalation anaesthetic apparatus. The skin of the abdominal region was shaved and swabbed with absolute alcohol. A small midline lower abdominal skin incision was made followed by a fine incision into the muscle layer of the abdominal wall. The tissue was blunt dissected apart and then one osmotic minipump was inserted, delivery portal first, into the intraperitoneal cavity. Care was taken to prevent the entry of pelt hairs into the abdomen to minimise the risk of infection. The whole pump was then moved gently to be positioned on one side of the intraperitoneal cavity; after which, the wound was dusted with antibiotic powder and the muscle layers and the skin sutured. Rats received benzylpenicillin (50 mg/kg, IM) postoperatively to prevent infection. Recovery from the operative procedure was rapid, and this allowed behavioural testing to commence 24 h following surgical procedures.

In experiments 3 and 4, rats received one trial in the Stone maze each consecutive day for a period of 10 days. At the conclusion of these experiments, the rats were killed and the osmotic minipumps removed to check for patency. This was achieved by cracking open the individual pumps to examine the state of the internal drug reservoir. Normal pumping was assumed to have occurred when the flexible drug reservoir appeared totally collapsed.

Dependent Measures and Statistics

Two indices were used to quantify the acquisition of the Stone maze task. The maze run time, or latency (s) from initially moving out of the start box to contacting the food reward, and the number of errors were measured. In judging an error, a strict criterion was employed to minimise subjective bias. The whole head, both forelimbs, and the upper body (up to the level of the diaphragm) of the rat had to pass into the T-portion of any blind-ending alley designated as an error point (Fig. 1 shows the error points numbered 1–14). When this occurred, a single error was counted. If a rat either reentered the previously incorrect alley, or entered another blind alley, an additional error was counted. Thus, cumulative error scores were composed of both initial, as well as perseverative, entries into blind alleys.

Stone maze latency and error data were first subjected to the Bartlett test (66) for homogeneity of variances. Because variances failed to show homogeneity, a $\log_{10}$ and a square root transformation were employed for latency and number of errors, respectively, prior to running an analysis of variance (ANOVA). Transformed data were analysed by means of an ANOVA for repeated measures with treatment as the within subjects variable, and trial as the between subjects variable. When applicable, ANOVA was followed by pairwise treatment comparisons using Dunnett's t-test.

Drugs

Scopolamine hydrobromide, N-methylscopolamine and arecoline hydrochloride were purchased form Sigma Chemical Company and were made up in a vehicle of 0.9% saline. Scopolamine and methylscopolamine were administered IP by acute injection given as 30-min pretreatments. The drugs were coded so that the experimentor was blind to the treatments employed and drugs were administered randomly throughout any experimental population. Arecoline was either administered acutely as once daily intraperitoneal injections for 7 days given as a 10-min pretreatment prior to maze testing, or by chronic intraperitoneal infusion over 10 days via osmotic minipumps.

RESULTS

Experiment 1

Effects of scopolamine in the stone maze. Figure 2A depicts the effect of scopolamine upon the $\log_{10}$ latency to complete the Stone maze and Fig. 2B shows the square root of the number of errors made in the task. As shown in these figures, rats given daily injections of saline clearly learned the task over the 15 test trials, showing reductions in both the latency to complete the maze and decreases in the number of errors committed. Similarly, rats in all other groups showed significant improvements in maze performance over trials ($\log_{10}$ latency, $F_{14,602} = 77.9$, $p < 0.001$; SQRT errors, $F_{14,602} = 43.65$, $p < 0.001$). However, injections of scopolamine (0.125–1.0 mg/kg, IP) produced a dose-related deficit in maze learning, as indicated by an increase in the latency to complete the task as compared to saline-treated rats ($\log_{10}$ latency, $F_{5,43} = 17.82$, $p < 0.001$). Scopolamine (0.25–1.0 mg/kg, IP b.i.d.) also significantly increased the number of errors committed (square root of the errors, $F_{5,43} = 7.61$, $p < 0.001$). Methylscopolamine (0.5 mg/kg, IP), in comparison, did not significantly modify the performance of rats in the Stone maze throughout the 15 trials. It should be noted that because scopolamine at doses of 0.25 and 0.5 mg/kg was found to pro-

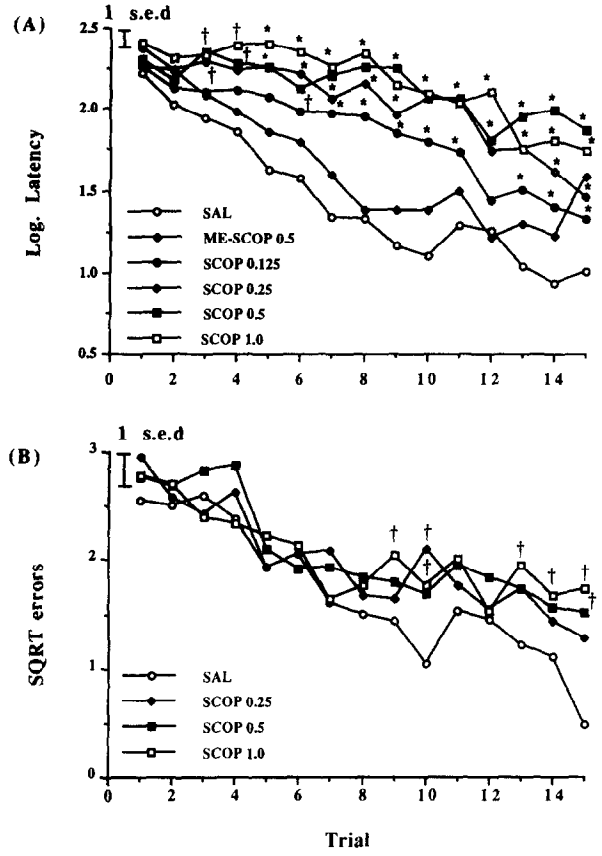

FIG. 2. The effect of scopolamine (SCOP, 0.125–1.0 mg/kg, n = 8) methylscopolamine (ME-SCOP, 0.5 mg/kg, n = 8) and saline (SAL, 1.0 ml/kg, n = 10) on (A) the $\log_{10}$ latency (s) to perform the Stone maze and (B) on the square root of the number of errors made in the task. It should be noted that for clarity, error scores of the groups that received either ME-SCOP 0.5 mg/kg or SCOP 0.125 mg/kg are not presented because initial ANOVAs indicated that the number of errors committed by rats in these groups were not significantly different from those of control animals on any test day. The bar displaced to the right of the y-axis presents one standard error of the difference (SED) between group means (1 SED). Significant differences between groups, as compared to saline vehicle treated rats, are indicated as: †$p < 0.05$, *$p < 0.01$, ANOVA followed by Dunnett's t-test.

duce robust deficits in maze learning, these doses were chosen for use in subsequent experiments to investigate the possible cognitive-enhancing effects of arecoline.

Experiment 2

Effects of acute arecoline on scopolamine-induced deficits in the stone maze. Figure 3 depicts the effect of acute peripheral injection of arecoline (5 and 10 mg/kg) upon the scopolamine-induced (0.25 mg/kg) deficit in the acquisition of the Stone maze task over a period of 7 days of testing. Arecoline failed to influence maze learning when administered acutely alone, and failed to modulate the scopolamine-induced maze deficit. ANOVA revealed a significant group effect with respect to $\log_{10}$ latency ($F_{4,32} = 27.33$, $p < 0.001$) and the SQRT of the number of errors, ($F_{4,32} = 9.95$, $p < 0.001$). This was observed because scopolamine (0.25 mg/kg, IP)-treated rats performed the maze with higher latencies and numbers of errors as compared to the saline-treated rats, and these deficits were not ameliorated by acutely administered arecoline.

This method of acute bolus intraperitoneal injection of arecoline produced some emergence of cholinergic peripheral side effects indicated by the display of slight flattened body posture, piloerection, and intermittant salivation and purposeless chewing. These overt side effects were slightly more pronounced in the rats receiving the higher dose of arecoline. It is possible that the appearance of such behaviours may have influenced the efficient execution of the maze task and, as a result of this, the experiment was terminated at the end of 7 days.

Experiments 3 and 4

Effects of chronic infusion of arecoline on scopolamine-induced deficits in the stone maze. Figures 4 and 5 depict the effects of chronic peripheral infusion of arecoline (30 mg/kg

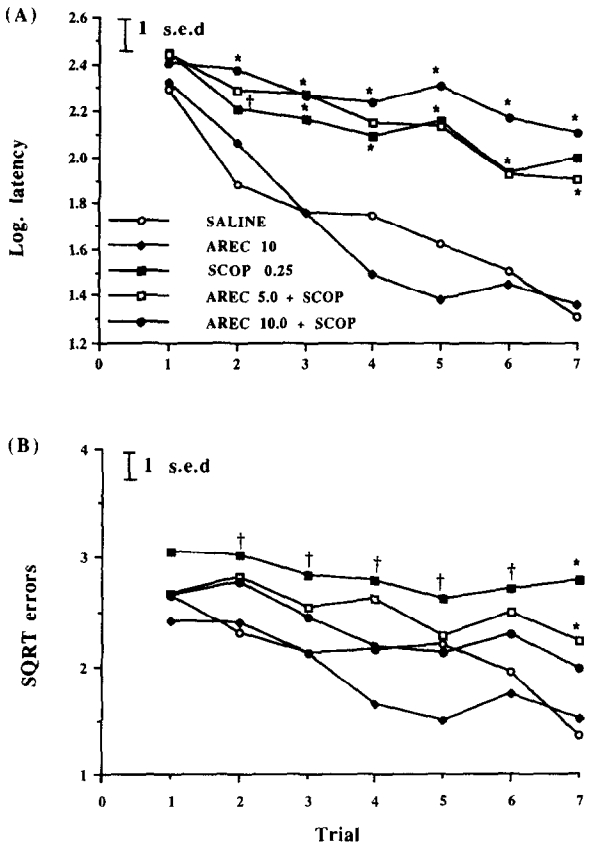

FIG. 3. The effect of the peripheral administration of scopolamine (0.25 mg/kg, IP) alone (SCOP 0.25, n = 8), acute peripheral injection of arecoline (10.0 mg/kg, IP) alone (AREC 10, n = 7), arecoline (5 mg/kg, IP) plus scopolamine (0.25 mg/kg, IP) (AREC 5 + SCOP, n = 8), arecoline (10 mg/kg, IP) plus scopolamine (0.25 mg/kg, IP) (AREC 10 + SCOP, n = 8), and saline (1.0 ml/kg, IP) (SAL, n = 6) on the performance of the Stone maze task over 7 days in the rat. (A) depicts the $\log_{10}$ transform of the latency (s) to complete the maze and (B) depicts the square root (SQRT) of the number of errors made. The bar displaced to the right of the y-axis depicts one standard error of the difference (SED) of the group means (1 SED). Significant differences, as compared to saline vehicle treated rats, are represented as: †$p < 0.05$, *$p < 0.01$, ANOVA followed by Dunnett's t-test.

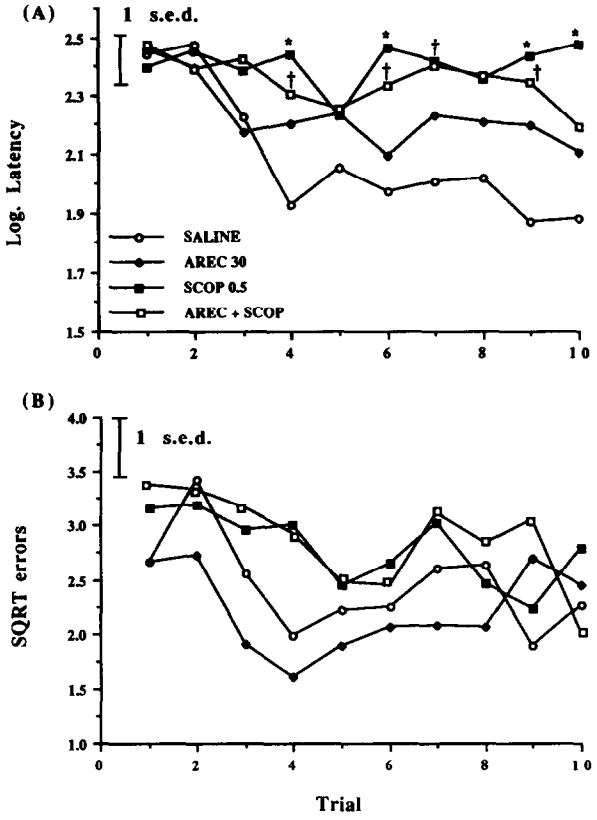

FIG. 4. The effect of arecoline (30 mg/kg per 24 h, IP) (AREC 30, n = 6) infused chronically by the intraperitoneal route over 10 days via Alzet osmotic minipumps on the deficit in Stone maze performance induced by scopolamine (0.5 mg/kg, IP) (SCOP 0.5, n = 6). Mean data over the 10 trials are represented as (A) $\log_{10}$ latency to complete the maze and (B). Square root (SQRT) of the number of errors made. The scale bar displaced to the right of the y-axis in each graph depicts one standard error of the difference (SED) of the group means (1 SED). Values significantly different from those of saline vehicle treated rats are represented as; †$p < 0.05$, *$p < 0.01$, ANOVA followed by Dunnett's t-test.

per 24 h and 50 mg/kg per 24 h), respectively, over 10 days on the deficit in Stone maze performance induced by scopolamine (0.5 mg/kg, IP). Regardless of the treatment, the performance of all groups improved over time in experiment 3 ($\log_{10}$ latency, $F_{9,171} = 7.04$, $p < 0.01$ and SQRT errors, $F_{9,171} = 4.07$, $p < 0.001$) and experiment 4 ($\log_{10}$ latency, $F_{9,252} = 17.95$, $p < 0.001$ and SQRT errors, $F_{9,252} = 2.81$, $p < 0.004$). However, peripheral injections of scopolamine (0.5 mg/kg) retarded the acquisition of maze learning in both experiments, acting to increase latencies to complete the maze, ($\log_{10}$ latency; Experiment 3; $F_{3,19} = 3.32$, $p < 0.042$; Experiment 4: $F_{3,28} = 15.39$, $p < 0.001$), and to produce increases in the number of maze errors that approached significance (SQRT errors; Experiment 3: $F_{3,19} = 2.57$, $p < 0.085$; Experiment 4: $F_{3,28} = 2.59$, $p < 0.072$). It must be noted here that scopolamine (0.5 mg/kg, IP) produced a more pronounced deficit, with respect to increased maze run-times in experiment 3, as compared to that found in experiment 4 using the same dose. This interexperiment variability in the observed effects of scopolamine may have occurred as a consequence of lowered group sizes.

The lower dose of arecoline (30 mg/kg per 24 h), when infused alone, failed to influence maze performance over the 10-day infusion period (experiment 3). Conversely, the higher dose of arecoline, (experiment 4), induced a significant deficit in maze performance *per se*, comparable to that produced by scopolamine. Rats receiving the higher dose of arecoline (50 mg/kg per 24 h) performed with significantly higher maze latencies on trial days 6, 7, 8, 9, and 10 ($p < 0.05$, ANOVA followed by Dunnett's t-test) and increased numbers of errors on trial days 7 and 9 ($p < 0.05$) when compared to rats receiving vehicle.

When combined with scopolamine, both doses of arecoline were found to be ineffective in ameliorating the deficit in maze performance. In fact, the higher dose of arecoline (50 mg/kg per 24 h, experiment 4), resulted in a significant potentiation of the scopolamine-induced deficit with respect to latency on trial days 6, 9, and 10 ($p < 0.05$).

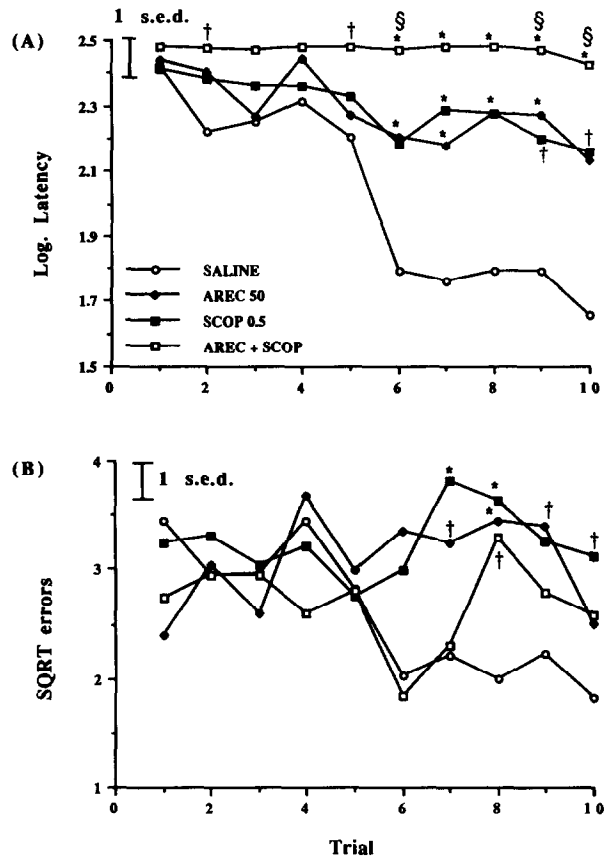

FIG. 5. The effect of arecoline (50 mg/kg per 24 h, IP) (AREC 50, n = 9) infused chronically by the intraperitoneal route over 10 days via Alzet osmotic minipumps on the deficit in Stone maze performance induced by scopolamine (0.5 mg/kg, IP) (SCOP 0.5, n = 8). Mean data over the 10 trials are represented as; (A) $\log_{10}$ of the latency to perform the Stone maze and (B) the square root (SQRT) of the number of errors made. The scale bar displaced to the right of the y-axis depicts one standard error of the difference (SED) between the group means (1 SED). Significant differences are indicated as † $p < 0.05$, *$p < 0.01$, as compared to saline vehicle treated rats and §$p < 0.05$, as compared to scopolamine treated rats (n = 8); ANOVA followed by Dunnett's t-test.

DISCUSSION

The present studies support the cholinergic hypothesis of memory dysfunction (3) in that treatment of young rats with a muscarinic antagonist produced a deficit in the acquisition of a complex maze task. Scopolamine treatment impaired Stone maze performance, according with the work of other groups (27,55), including our own (9); increasing the maze run times (latency) and, to a much lesser extent, the number of errors, in a dose-dependent manner. It proved impossible, however, to reverse such deficits with the doses of the muscarinic agonist arecoline given in these experiments either by conventional acute peripheral administration or by chronic intraperitoneal infusion.

It should be noted that increases in the latency to complete the maze were not completely mirrored by parallel increases in the number of errors committed by rats treated with scopolamine. This lack of correspondence between latencies and errors that occurred provide some additional information about the nature of the scopolamine-induced deficits in maze acquisition. Scopolamine treated rats often spent longer periods of time hesitating in sections of the maze in their attempts to locate the correct route. This pattern of high latency scores with a concomitant low number of errors appeared to be characteristic of the behavioural profile of action for lower doses of scopolamine on the acquisition of this maze task. Therefore, it was considered that latency constituted the most stable dependent variable reflecting true drug effects upon memory. However, the highest dose of scopolamine (1.0 mg/kg, IP) significantly increased both the number of errors committed and the maze latencies; this may have been due to the increased propensity for scopolamine-treated rats to perseverate at error points.

The effects of scopolamine appeared to be centrally mediated because the peripherally acting muscarinic antagonist, *N*-methylscopolamine, which does not cross the blood-brain barrier at low doses, had no significant impact on Stone maze performance. These data indicated that blockade of central muscarinic receptors by scopolamine, which is known to be a muscarinic subtype nonselective antagonist (13), disrupts cognitive performance by pharmacological perturbation of central cholinergic transmission.

Previous reports have suggested that scopolamine primarily affects encoding processes in this maze because no significant deficit was observed when scopolamine was administered to rats following acquisition (55), and it has also been shown to have little effect upon storage and retrieval processes in the Stone maze task (26). The scopolamine-induced behavioural deficit observed in the current studies resembled that measured in two populations of aged rats (12 and 20 months) using the Stone maze (8). Rats receiving scopolamine appeared to utilise an alternation strategy in solving the task, as do aged rats, and it has been suggested that this may occur due to a detrimental effect of scopolamine on spatial working memory. This refers to the short-term memory required to process information, within individual trials, pertaining to the animal's position in the maze environment in relation to other distant fixed objects surrounding it in space (22).

Scopolamine-treated rats learned the maze with extended training; however, in later trials their performances continued to be inferior to those achieved by control rats. It is possible that the retardation in maze acquisition may have been attributable to a nonspecific effect of scopolamine on performance, causing the rats to perform the task more slowly. On the other hand, rats recieving the highest dose of scopolamine (1.0 mg/kg, IP) investigated the maze sufficiently well to score significantly more errors than control animals, suggesting that the drug induced little motoric impairment. It is also unlikely that peripherally induced effects, such as deficits in visual acuity, contributed to the maze deficit because *N*-methylscopolamine, the peripherally acting muscarinic antagonist, dilated the pupils maximally, while leaving maze performance unimpaired (unpublished findings).

The specific nature of the scopolamine deficit in the performance of spatial learning tasks remains unclear, with some reports stating that scopolamine degrades spatial working memory, leaving reference memory (memory used to remember fixed information between trials) intact (4). Other reports indicate that both processes are impaired (38). However, it is known that the ability to use distal cues in efficient place-learning is degraded by scopolamine treatment and the use of proximal cues, or direct cues close to the body of the animal, remains intact (64). Indeed, it is beyond the scope of the current study to make definitive conclusions concerning this point because the Stone maze constitutes an extremely complex maze task, for the solution of which rats are required to call into practice a range of cognitive abilities including spatial working and reference memory, cue, and taxon (information obtained from body positioning) processes. Additionally, studies have indicated that scopolamine degrades processes of attention (14,21,50) and because a high level of attention is required during each Stone maze trial, due to the complexity of the task, this effect, indeed, may have had some influence on performance.

Behavioural studies have indicated that cholinergic neurotransmission in the hippocampus plays an essential role in cognition. From this, it may be assumed that the Stone maze constitutes, in part, a spatial task because an intact septohippocampal system has been implicated in efficient maze acquisition. Lesions of the fimbria-fornix (FIFO) have been shown to disrupt maze acquisition, with FIFO-lesioned rats demonstrating a deficit similar to that of rats receiving scopolamine (28). Intrahippocampal injections of muscarinic antagonists have been reported to disrupt spatial learning of a swimming pool task (6), and a 3-panel runway task by blockade of M_1 muscarinic receptors (37). The M_1 receptor subtype predominates in the hippocampus, constituting 80% of the muscarinic receptor population (42). It is, therefore, possible that the effects of scopolamine and arecoline to disrupt and augment cognitive ability, respectively, may involve an interaction with distinct populations of muscarinic receptors; for example, within the hippocampus, where arecoline has been shown to increase the frequency of theta rhythm EEG activity (2).

Arecoline differentially modulated the scopolamine-induced deficits in this complex maze task according to the dose and method of administration employed. Acute bolus dosing of arecoline failed to attenuate the scopolamine-induced deficit and failed to modify performance when administered alone. This method of administration, apart from failing to reverse the cognitive deficit, was considered unacceptable due to side effects observed that were typical of the cholinergic syndrome, including body tremor, hypothermia, salivation, and lacrimation (40), presumably due to stimulation of peripheral cholinoceptors.

Previous pharmacokinetic data has indicated that arecoline has a very short half-life of approximately 6 min in the body (23,30) and reaches peak brain concentrations within 3 min (54); therefore, it was considered that some slow release method of administration may prove advantageous in maintaining constant blood levels of the drug. Continuous chronic

peripheral infusion of arecoline via osmotic minipumps was employed, to increase the duration of tonic central M_1 muscarinic receptor stimulation (51), with the aim of thereby increasing any putative cognitive-enhancing action. It was found that arecoline, when administered in this manner, differentially modified the scopolamine deficit, either having no effect or potentiating this deficit, depending upon the dose employed. Such a differential profile of action is characteristic of the muscarinic agonists, because several animal and clinical studies have demonstrated an inverted U-shaped dose-response effect with these drugs, suggestive of a very narrow range of doses over which they are effective in improving cognitive performance (3,57). This may be a factor contributing to the range of previously published contradictory data obtained from behavioural studies in rats and primates (47, 48,52).

An additional factor possibly leading to the negative data observed with arecoline probably relates to the drug's mechanism of action at muscarinic receptors. Arecoline is reported to be nonselective, stimulating both M_1 and M_2 muscarinic cholinoceptors (57), with some agonistic effect at nicotinic receptors at high doses (23). Autoradiographic analyses of agonist binding to muscarinic receptor subtypes have demonstrated that arecoline binds with higher affinity to the M_2 subtype (34), but also binds to the M_1 subtype, which is found to predominate in the basal ganglia, hippocampus, and the cortex (61).

Biochemical and behavioural data have shown M_2 receptors to exist as autoreceptors in the cholinergic system (26), which are located both pre and postsynaptically (62). Nordstrom and Bartfai (36) produced in vitro evidence of a role for M_2 muscarinic autoreceptors in regulating the release of acetylcholine in the brain. It may be assumed from this data that the cholinergic agonist arecoline may have dual actions in modulating cholinergic tone: it may have a direct action of stimulating M_1 muscarinic receptors and it may stimulate M_2 autoreceptors to decrease acetylcholine release, thereby producing a deleterious effect.

In the present studies, a high dose of arecoline, when continuously infused over 10 days, potentiated the deficit in Stone maze performance produced by scopolamine, suggesting that the combined action of these drugs can produce an additive effect to impair cognition. Both in vitro (20) and in vivo (17) techniques employed in the rat have demonstrated that muscarinic blockade increases the release of acetylcholine from key brain regions implicated in cognitive processing. It is feasible that the increase in release of acetylcholine induced in this way could contribute to the autoreceptor M_2 agonistic action of arecoline to further depress the overall amount of acetylcholine released, so that the M_1 muscarinic blockade by scopolamine is not reversed.

Some clinical studies have reported that cholinomimetic therapies improve the cognitive performance in AD patients when administered continuously over extended treatment periods. For example, long-term continuous infusion of arecoline was found to improve verbal memory in AD patients (46, 60). Also, chronic administration of physostigmine, either by intravenous infusion (15) or as a result of long-term oral treatment (58) has been shown to be effective in the treatment of the disease. Additionally, arecoline and physostigmine have also been shown to reverse scopolamine deficits in a battery of human cognitive tests in disease-free subjects (43).

More contradictory findings have been reported on the effects of cholinomimetic therapies in animal models of dementia. For example, some workers have reported that arecoline fails to attenuate the effects of scopolamine in primates on a test of visual recognition memory (48), whereas others report an ameliorative effect on deficits induced in performance of the same task by lesions of the nucleus basalis of Meynert (47). Also, in a spatial alternation paradigm, arecoline was found to be ineffective, but physostigmine possessed some efficacy in reversing a scopolamine-induced performance deficit (52). Considering the animal behavioural data in total, it could be possible that such discrepant results may be dose- or task-related.

Although it has been acknowledged that multitransmitter systems may contribute to memory disorder (19), there continues to be much clinical interest shown in developing specific cholinergic therapy for AD and related dementias (16,23). Muscarinic receptors of the postsynaptic M_1 subtype appear to be relatively spared in AD (33,45), and this adds potential scope for use of muscarinic agonists in the treatment of this disease. Furthermore, because arecoline has previously been shown to produce a similar physiological response in young and aged rats, demonstrating no muscarinic receptor function impairment with age (54), such a treatment strategy could have beneficial effects in age-related cognitive decline.

Despite the apparent ineffectiveness of chronically administered arecoline for ameliorating a scopolamine-induced deficit in maze learning in these investigations, it is suggested that studies employing a more extensive dose range of the cholinergic agonist may enhance the literature on this topic. Such preclinical studies may elucidate whether or not the chronic administration of muscarinic agonists holds potential in the treatment of human cognitive dysfunction.

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