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The Behavioral Effects of Heptyl Physostigmine, a New Cholinesterase Inhibitor, in Tests of Long-Term and Working Memory in Rodents

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DAWSON, G. R., G. BENTLEY, F. DRAPER, W. RYCROFT, S. D. IVERSEN AND P. G. PAGELLA. *The behavioral effects of heptyl physostigmine, a new cholinesterase inhibitor, in tests of long-term and working memory in rodents.* PHARMACOL BIOCHEM BEHAV **39**(4) 865–871, 1991.—We assessed the effects of heptyl physostigmine, a new cholinesterase inhibitor, in a mouse tail-flick (TF) test, a mouse and rat passive avoidance test, a rat conditioned suppression-of-drinking (CSD) test, a rat Random Interval (RI) response rate test and a rat delayed matching-to-position (DMTP) test. In the TF test, a dose of 8.0 mg/kg of heptyl induced a significant antinociceptive effect that was in excess of 75% of the maximum possible effect 300 minutes after administration. In the mouse passive avoidance test, a dose of 3.0 mg/kg of heptyl fully reversed, and a dose of 1.0 mg/kg partially reversed, a scopolamine-induced (0.2 mg/kg) deficit. In the rat passive avoidance test, a dose of 8.0 mg/kg fully reversed a scopolamine-induced (0.2 mg/kg) deficit, while a dose of 4.0 mg/kg of heptyl was without effect. In the same experiment, a dose of 0.6 mg/kg of physostigmine partially reversed the scopolamine-induced deficit. In the CSD test, a dose of 8.0 mg/kg of heptyl fully reversed, and doses of 1.0 and 4.0 mg/kg of heptyl partially reversed, the deficit induced by scopolamine (0.4 mg/kg). In the RI response rate test, doses of 8.0 mg/kg and 0.6 mg/kg of physostigmine fully suppressed lever pressing for food rewards. Doses of 4.0 mg/kg of heptyl and below had no effect on lever-pressing rates. In the working memory test (DMTP), 4.0 mg/kg heptyl partially reversed the scopolamine-induced deficit (0.2 mg/kg) in the number of correct choices made, but did not affect the scopolamine-induced deficit in the number of trials completed. These results suggest that heptyl is a long-lasting cholinesterase inhibitor that produces reversals of scopolamine-induced deficits in the rodent at doses that do not induce behavioral toxicity. It would appear that heptyl is a cholinesterase inhibitor that will permit a thorough assessment of the cholinergic hypothesis of dementia of the Alzheimer's type.

Heptyl physostigmine Dementia Cholinesterase inhibitors Cognition Rats Mice

SINCE the discovery in the early 1970s that choline acetyltransferase (ChAT) is markedly reduced in the brains of patients with Alzheimer's disease (AD) compared with those of age-matched controls (1), much research has focused on the development of cholinomimetic replacement therapy as a potential treatment for the disease. Early studies indicated that treatment with the cholinesterase inhibitor physostigmine alone (5) and in combination with lecithin (12) may be an effective treatment for AD. However, the therapeutic usefulness of physostigmine is limited by its short half-life, poor bioavailability and narrow therapeutic window (6).

The interest in cholinesterase inhibitors was revived in the mid 1980's with a report by Summers et al. (14) of the effects of oral 9-amino-1,2,3,4-tetrahydroaminoacridine (THA) on the memory deficit observed in patients diagnosed as suffering from AD. In a double-blind, placebo-controlled, cross-over study, 17 patients were treated for three weeks with THA. The results were dramatic: THA therapy improved performance on all four of the assessment tests used. Unfortunately, the relatively high doses of THA used in this study are also known to cause hepatocelluar injury (9), which limits its long-term therapeutic utility. Since then, the development of novel cholinesterase inhibitors which are free from such side effects has progressed rapidly.

A recent development has been the discovery of a carbamate derivative of physostigmine, heptyl physostigmine (heptyl). Heptyl has recently been shown to have a greater duration of action and a greater margin of safety than physostigmine (2,8). It was also reported that a 5.0-mg/kg dose of heptyl (IM) produced a peak inhibition of rat brain cholinesterase (75–80%) 60 minutes after administration, while a 0.3-mg/kg dose of physostigmine produced maximal inhibition (50–55%) after just 15 minutes. However, while inhibition of cholinesterase by physostigmine returned to baseline levels after 120 minutes, the inhibition induced by heptyl was still 70% after 360 minutes (8). Moreover, it was found that the LD_{50} of heptyl was 35.0 mg/kg compared to 0.6 mg/kg for physostigmine (8).

A preliminary report has indicated that heptyl is active in passive avoidance, a behavioral test of cognition (2). The purpose of the present study was to replicate and extend these results. However, before investigating the effects of heptyl in cognitive tests, it would be useful to establish the onset and duration of brain cholinesterase inhibition in vivo. It is well estab-

lished that cholinomimetic compounds induce antinociception. We have found that the mouse tail-flick test of antinociception provides a useful in vivo model for establishing the onset and duration of the central action of cholinomimetics in conscious animals. Thus we began our series of experiments with this procedure before attempting a replication of the mouse passive avoidance results described previously. In subsequent tests, we evaluated the effectiveness of heptyl in long-term and working memory models of AD in the rat.

METHOD

Animals

Male albino BTKO and CD1 mice (25–30 g, 6–8 weeks old) housed in groups of five were used. Male hooded Lister rats and Sprague-Dawley rats (300–450 g, 3–4 months old) housed in groups of four were also used. All the animals were maintained on a 12/12-hour light/dark cycle (with the light component coinciding with normal daylight hours) in temperature- and humidity-controlled rooms. Unless stated to the contrary, all animals had free access to food and water during the experiment.

Drugs

Scopolamine hydrobromide and physostigmine were supplied by Sigma Chemical Company, St. Louis, MO. Heptyl physostigmine was supplied by Mediolanum Farmaceutici, Italy. All compounds were freshly prepared in 0.9% saline before each test.

Statistical Analysis

All statistical analyses were carried out using programs from the Biomedical Data Program (BMDP) statistical library. If the ANOVA (BMDP 2V) reached statistical significance, post hoc tests were calculated according to Tukey's Studentised Range method (supplied by BMDP 7D). When it was appropriate, preplanned orthogonal contrasts (BMDP 4V) were used instead of post hoc tests.

Apparatus and Procedures

Mouse antinociception test. Fifty male BTKO mice served as subjects in this test. Antinociception was assessed using a Scorel tail-flick meter (model number DS 20) using a method previously described by Harris et al. (4). The mouse was held in the gloved hand of the experimenter with its head pointing toward the wrist. Its tail was placed over a light-sensitive switch directly under an electric bulb that provided both heat and light. When the bulb was switched on, a timer was activated, and the heat and light was focused on the animal's tail by a reflector placed just above the bulb. When the mouse flicked its tail to avoid the heat stimulus, the light-sensitive switch was activated, turning off both the bulb and the timer. The intensity of the heat source was adjusted to give a mean tail-flick latency (TFL) between 1.5 and 5.0 s. If the mouse had not flicked its tail by 8.0 s, it was removed and the TFL was recorded as 8.0 s. This cutoff point was employed to prevent damage to the tail of the mouse. In the baseline phase of the experiment, each mouse was tested three times. If the average TFL fell between 1.5 and 5.0 s, the animal was included in the drug phase of the experiment; if not, it was excluded from further testing.

In the drug phase of the experiment, the 50 mice were randomly assigned to one of five groups (n = 10): *Group 1*, vehicle (veh); *Group 2*, morphine (16.0 mg/kg); *Group 3*, 1.0 mg/kg heptyl; *Group 4*, 3.0 mg/kg heptyl; *Group 5*, 8.0 mg/kg heptyl. The morphine group was included as a positive control. All of the treatments were administered IP. Up to 120 minutes postinjection, TFLs were recorded every 15 minutes, then every 20 minutes up to 180 minutes postinjection. The final three readings were taken at 225, 255, and 300 minutes postinjection. Results for this phase of the experiment are expressed as the percentage of the maximum possible antinociceptive effect (% MPE) and are given by the equation:

$$\%MPE = \frac{T_1 - B_1}{8 - B_1} \times 100$$

where T_1 = test latency, B_1 = the mean baseline latency and 8 is the cutoff time (seconds) (11).

Mouse passive avoidance test. Fifty male CD1 mice served as subjects in this test. The mice were tested in a two-compartment chamber, one with a ceiling and the other without, of external dimensions of 20 × 23 × 35 cm. The floor of the chamber consisted of 3-mm steel bars spaced 3 mm apart through which a 2-s, 0.4-mA scrambled electric shock (supplied by a Coulbourn grid floor shocker, model number E13-08) was administered. The floor of the open compartment was covered by a closely fitting white Perspex insert and was brightly lit by a 240-V 100-W lamp placed 30 cm directly above the Perspex floor. The mouse could gain access to the dark side of the chamber through an aperture measuring 7.0 × 5.0 cm in the wall separating the two compartments. A white noise generator provided background masking noise.

On the first day of the experiment, the mice were placed in the dimly lit experimental room and allowed to habituate for two hours. At the beginning of the experiment, each mouse was assigned to one of five experimental groups: *Group 1*, veh/veh; *Group 2*, veh/scopolamine (scop) (0.2 mg/kg); *Group 3*, 1.0 mg/kg heptyl/scop; *Group 4*, 3.0 mg/kg heptyl/scop; *Group 5*, 8.0 mg/kg heptyl/scop. Heptyl physostigmine or vehicle was administered SC 80 minutes before, and scopolamine or vehicle (SC) 20 minutes before the beginning of the training trial.

At the beginning of the training trial, the mouse was placed in the open compartment with its nose pointing into one of the corners opposite the dividing wall. When it stepped through to the dark side, the step-through latency (STL) was recorded, the aperture was closed and the electric shock was delivered. The mouse was then immediately removed from the dark side of the chamber and returned to its home cage. At the end of the experiment, the mice were returned to their normal holding room.

Twenty-two hours later, the mice were returned to the experimental room and allowed to habituate for two hours. At the end of this period, each mouse was placed in the bright open compartment, and the STL to the dark compartment was recorded. If the mouse did not step through to the dark side within 300 s, it was removed and the STL was recorded as 300 s.

Rat passive avoidance test. The apparatus for this procedure was a scaled-up version of the two-compartment chamber described above. Its external dimensions were 40 × 40 × 50 cm, and the grid floor consisted of 6-mm steel bars spaced 6 mm apart. The rat could gain access to the dark side of the apparatus through an aperture measuring 14.5 × 10 cm in the dividing wall.

At the beginning of the experiment, 60 Sprague-Dawley rats were placed in the experimental room and allowed to habituate for two hours before they were assigned to one of five groups: *Group 1*, veh/veh; *Group 2*, veh/scop (0.2 mg/kg); *Group 3*, 4.0 mg/kg heptyl/scop; *Group 4*, 8.0 mg/kg heptyl/scop; *Group 5*, scop/0.6 mg/kg physostigmine (phy). The procedure for this experiment was identical to that described for the mouse passive

avoidance test with the exception that two training trials were given. The second training trial was conducted one hour after the first. If the rat failed to cross to the dark side of the chamber on the second training trial, it was removed after 300 s and its STL was recorded as 300 s.

The conditioned suppression-of-drinking test. This test, the Random Interval response rate test and the delayed matching-to-position (DMTP) test were conducted in standard operant chambers (Coulbourn Instruments, model number E10-10) that were interfaced to Euro-Beeb microcomputers running the online control language Spider (Paul Fray Ltd.). The chambers were housed in sound- and light-resistant shells fitted with fans that provide both ventilation and background masking noise. The floor of each chamber consisted of 6-mm steel bars spaced 11 mm apart through which electric shocks could be delivered. Each chamber was fitted with a food magazine (30 × 40 mm) located in the centre of the front wall 10 mm above the grid floor and into which food pellets (45 mg dustless pellets, Bioserv, Sandown Scientific, Esher, UK) could be delivered.

For the CSD test, a sonic noise generator (Coulbourn Instruments, model number E12-02) fitted with a 1000-ohms in-line resistor was fitted to the top right-hand corner of the instruments panel. The sonic noise generator provided an 80-dB, 2.8-KHz tone which served as an auditory stimulus. Through the food magazine, the animal could drink a sweet solution (3.0% glucose + 1% saccharin) by licking a metal drinking spout. The rate at which the animal licked the tube was monitored by a Coulbourn Lickometer (model number E24-01).

The CSD test was conducted over five days. For two days before and throughout the experiment, the 60 Sprague-Dawley rats were deprived of water for 22.5 hours in each 24-hour period. On the first, second and fourth days, the animals were placed in the testing chamber and allowed free access to the sweet solution for 20 minutes.

On the third day, the animals were randomly assigned to one of five treatment groups (n = 10): *Group 1*, veh/veh; *Group 2*, veh/scop (0.4 mg/kg); *Group 3*, 1.0 mg/kg heptyl/scop; *Group 4*, 4.0 mg/kg heptyl/scop; *Group 5*, 8.0 mg/kg heptyl/scop. Scopolamine was administered SC 20 minutes before and heptyl or vehicle was given SC 80 minutes before the beginning of a conditioning session. During the conditioning session, the drinking tube was removed from the operant chamber. Approximately five minutes after the beginning of the session, the sonic noise generator was switched on, and 29 s later, a 0.4-mA shock was delivered to the rat's feet. One second later, the tone and shock terminated together. This tone-shock pairing was repeated a further three times with at least five minutes between each pairing.

On Day 5, the test day, the animals were placed in the operant chambers and allowed to drink freely. When each had completed 150 licks on the tube, the tone was activated and the latency to complete the next 50 licks was recorded. If an animal had not resumed licking within 300 s of the onset of the tone, the tone was switched off and the lick latency was recorded as 300 s. At the end of this session, the animals were returned to their home cages and given free access to water.

Instrumental learning tests. For the Random Interval response rate test and the DMTP test, the operant chambers were fitted with two retractable levers, one on each side of the food magazine, and a house light was placed 21 cm above the food magazine. In the Random Interval test, 60 male hooded Lister rats, and in the DMTP test, 40 male Sprague-Dawley rats (maintained at 85% of their free-feeding weight by postsessional feeding) were trained on three successive days to collect food pellets from the magazine. The onset and offset of the house light indicated the beginning and the end of the session. During the session, a 24-V, 2.8-W magazine light illuminated the food magazine for five seconds each time a pellet was delivered. Following this training, the left lever was inserted into the box and every lever press resulted in the delivery of a food pellet. These sessions ended after one hour or after the rat had pressed the lever 30 times, whichever was sooner. Three daily sessions of this training were given before the animals were transferred to their respective training sessions proper.

Random interval training. Following lever press training, the animals were exposed over a period of four days to progressively increasing Random Interval (RI) schedules of RI 7 s, RI 15 s, RI 30 s and, finally, a schedule of RI 60s. Each session lasted for 30 minutes, at the end of which the rats were returned to their home cages and fed immediately. The animals remained on the RI 60-s schedule for 15 days, during which time response rates stabilised. On the 16th day, the animals were randomly assigned to one of six drug groups (n = 8): *Group 1*, veh; *Group 2*, 0.1 mg/kg heptyl; *Group 3*, 1.0 mg/kg heptyl; *Group 4*, 4.0 mg/kg heptyl; *Group 5*, 8.0 mg/kg heptyl; Group 6, 0.6 mg/kg physostigmine. Each group, with the exception of Group 6, was administered the appropriate treatment SC 80 minutes before the beginning of the session. Group 6 was given physostigmine five minutes before the beginning of the session. On the 17th day, the animals were again trained on the baseline RI 60-s schedule. The RI schedule was used because it maintains a constant lever-pressing rate throughout the session (7).

Delayed matching-to-position test. When reliable lever pressing had been established, a discrete-trial procedure was introduced: At the beginning of the session, the left or the right lever was randomly selected and inserted into the chamber. When the lever was pressed, it was retracted, the house light was extinguished, the magazine light was illuminated and a pellet was delivered. The first nose poke response following delivery of the food pellet extinguished the magazine light and five seconds later initiated the next trial. As on the first trial, the left or right lever was randomly selected, inserted into the chamber and initiated the sequence as described above.

When lever-pressing rates had stabilised within the discrete-trial procedure, clear Perspex dividers (155 × 255 mm) were placed midway between each lever and the food magazine. As a consequence of the positioning of the dividers, in order to collect a food pellet the animal had to turn or back away from the lever that it had pressed and enter the area in front of the magazine between the Perspex dividers. At this stage, the lever press continued to retract the lever, extinguish the house light and illuminate the magazine light, but the pellet was not delivered until the next nose poke. When lever pressing had again stabilised, the penultimate training stage began. During this stage, each trial began as before with the random selection and insertion of one of the levers. When this "sample" lever was pressed, it was withdrawn, the house light was extinguished, and the magazine light was illuminated. The next nose poke response, instead of causing the delivery of a food pellet, resulted in the insertion of both levers into the chamber, the extinguishing of the magazine light and the illumination of the house light. In this "choice" stage, the animal had to press the sample lever again to produce a food pellet. If it pressed the sample lever, the choice was recorded as correct, the levers were retracted, the house light was extinguished, the magazine light was illuminated and a pellet was delivered. The next nose poke response extinguished the magazine light and, after a delay of approximately five seconds, initiated the next trial. If the animal pressed the alternative to the sample lever, both levers were retracted, the house light was extinguished and a five-second dark timeout was imposed. Thus, in combination with the Perspex walls, the nose poke response requirement prevented the animals from solving the problem using a mediating strategy such as standing in front of the sample

lever until it reappeared at the choice stage.

This training continued for at least 30 daily sessions, five days per week (depending on individual differences in acquisition rate), before the final stage of training was introduced. During this stage, one of five delays (0, 2, 4, 8, and 16 s) was introduced between the sample stage and the choice stage. The contingencies were identical to those imposed during the previous stage of training except that the selected delay began when the sample lever was retracted. The first nose poke response after the delay had ended reintroduced the choice levers into the chamber. This training continued until the animals performed with greater than 95% accuracy at each of the five delays. The animals achieved this criterion performance between 30 and 40 sessions of training.

On each Friday of the following five weeks of daily training, the rats were given a 0.2-mg/kg dose of scopolamine. On the Friday of the sixth week, the animals were randomly assigned to one of four groups (n = 10): *Group 1*, veh/veh; *Group 2*, veh/scop (0.2 mg/kg); *Group 3*, 1.0 mg/kg heptyl/scop; *Group 4*, 4.0 mg/kg heptyl/scop. Heptyl and vehicle were administered SC 80 minutes before, and scopolamine SC five minutes before the beginning of the session.

RESULTS

The Antinociceptive Effect of Heptyl Physostigmine

Fifteen minutes postinjection, the mice treated with morphine had a pronounced antinociceptive response which declined over the next 125 minutes until, at 140 minutes postinjection, they no longer differed significantly from vehicle controls (Fig. 1). Doses of 1.0 mg/kg and 3.0 mg/kg of heptyl failed to induce an antinociceptive response at any of the time intervals sampled. However, 8.0 mg/kg of heptyl induced an antinociceptive response that was first detected 30 minutes postinjection ($p<0.05$). The %MPE increased over the next 90 minutes until it reached a maximum at 120 minutes postinjection (mean = 91.15, s.e. = 8.85) and declined slowly to reach a level of approximately 77.0% 300 minutes postinjection. At this point, testing was terminated to prevent damage to the tails of the mice.

The Effect of Heptyl Physostigmine on the Mouse Passive Avoidance Test

Figure 2 shows the mean STLs of the veh/veh, veh/scop, 1.0 mg/kg heptyl/scop and 3.0 mg/kg heptyl/scop groups before, and 24 h after, the administration of the electric shock. On the training day, one mouse in the 1.0 mg/kg heptyl/scop group and one in the 3.0 mg/kg heptyl/scop group failed to cross over to the dark chamber and were therefore excluded from the experiment. Although the group treated with the vehicle control stepped through rapidly from the brightly lit chamber to the dark chamber before the training trial, their latency to do so again 24 hours later was significantly increased ($p<0.05$).

By contrast, the mice treated with scopolamine alone before the training trial stepped through to the dark chamber on the test trial almost as rapidly as they had on the training trial. This scopolamine-induced amnesia was partially reversed by a 1.0-mg/kg dose, and fully reversed by the 3.0-mg/kg dose of heptyl ($p<0.05$). The mice treated with 8.0 mg/kg heptyl and scopolamine failed to cross from the brightly lit chamber to the dark chamber on the training trial and, as a consequence, do not appear in Fig. 2.

The Effect of Heptyl Physostigmine on the Rat Passive Avoidance Test

The mean STLs before, one hour after and 24 h after electric shock administration are shown for each group in Fig. 3. As in the mice passive avoidance test, scopolamine induced a significant amnesia in the rat that is evident from the short STLs one hour and 24 hours after the first training trial. Post hoc tests revealed that only the STLs of vehicle group and the 8.0 mg/kg heptyl/scop group were significantly longer than those of the veh/scop group, both one hour and 24 h after the first training trial ($p<0.05$).

The STLs of the group given phy/scop were also significantly longer than those of the veh/scop group 24 hours after the first training trial, but not one hour after ($p<0.05$). The STLs of

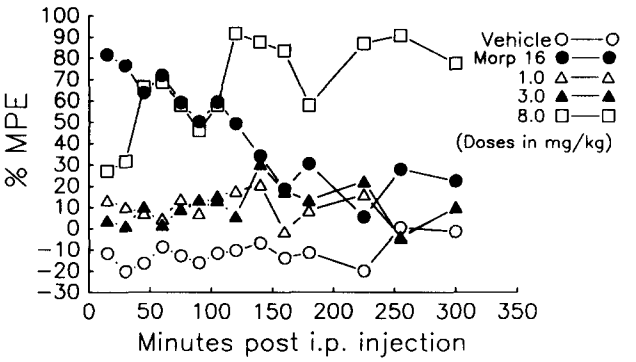

FIG. 1. The %MPE scores are shown for each of the treatment groups (n = 10) in the tail-flick antinociception test. All compounds were administered IP following the evaluation of the mean baseline tail-flick latency. A two-way analysis of variance with factors TREATMENT by TIME revealed a significant effect of each factor and an interaction between them. It is clear from the figure that the interaction is due to the different times of onset of morphine and the 8.0-mg/kg dose of heptyl physostigmine. Group comparisons at individual time points were made using post hoc Tukey Studentized range tests.

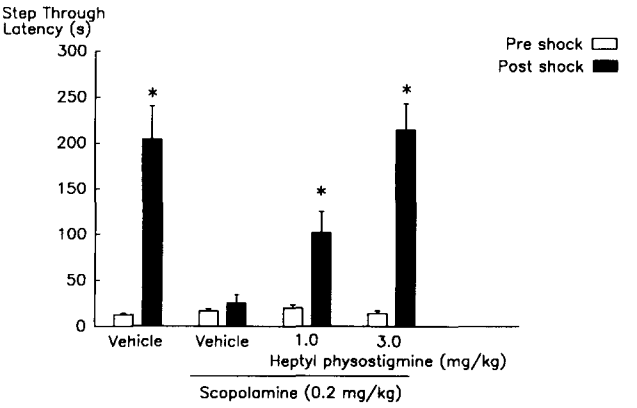

FIG. 2. Mean step-through latencies are shown for each of the treatment groups (n = 10) before the administration of an electric shock (open bars) and 24 hours after administration (filled bars). The fine line on each bar represents the standard error of the mean step-through latency. No scores are shown for the group that was administered 8.0 mg/kg of heptyl physostigmine and scopolamine, as they failed to cross over to the dark chamber during the training trial. Heptyl physostigmine was administered 80 minutes before, and scopolamine (0.2 mg/kg) and vehicle 20 minutes before, the first training trial. Step-through latencies were log transformed before being subject to an analysis of variance followed by post hoc Tukey Studentized range tests. *$p<0.05$ compared to the scopolamine/vehicle group.

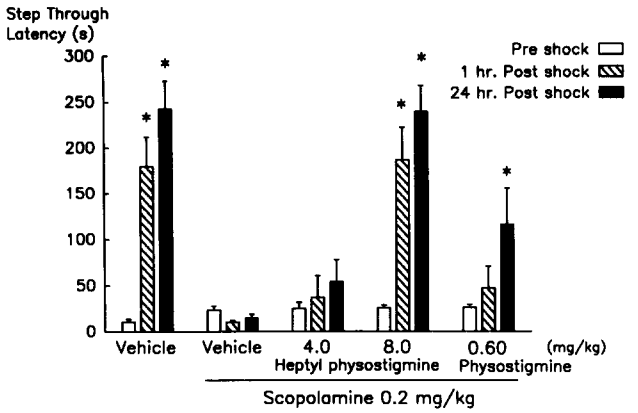

FIG. 3. Mean step-through latencies are shown for each of the treatment groups (n = 12) before the administration of an electric shock (open bars), one hour after administration (hatched bars) and 24 hours after administration (filled bars). The fine line on each bar represents the standard error of the mean step-through latency. Heptyl physostigmine was administered 80 minutes before, scopolamine and vehicle 20 minutes before and physostigmine (SC) five minutes before the first training trial. Step-through latencies were log transformed before being subjected to an analysis of variance followed by post hoc Tukey Studentized range tests. *$p<0.05$ compared to the scopolamine/vehicle group.

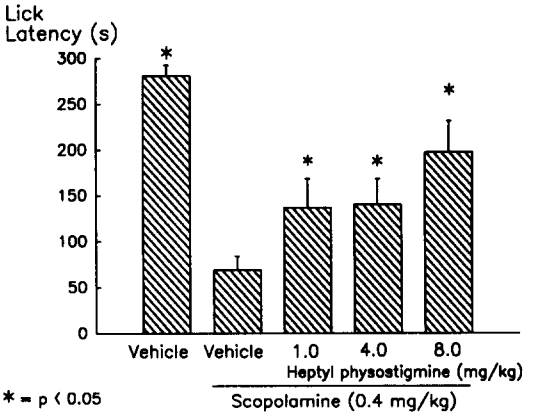

FIG. 4. Mean latencies to complete 50 licks after the onset of a tone that 48 hours earlier had predicted the application of an electric shock are shown for each treatment group (n = 12). The fine line on each bar represents the standard error of the mean lick latency. Heptyl physostigmine was administered 80 minutes before, and scopolamine (0.4 mg/kg) and vehicle 20 minutes before, the conditioning session. Preplanned orthogonal contrasts provided by BMDP 4V were used to compare individual treatment groups to the scopolamine/vehicle group. *$p<0.05$.

veh/phy group were also significantly shorter than those of the vehicle control group, confirming what inspection of Fig. 3 suggests: that this was only a partial reversal of the scopolamine-induced amnesia.

The Effect of Heptyl Physostigmine on the Rat Conditioned Suppression-of-Drinking Test

Figure 4 illustrates the mean latency for each of the treatment groups on Day 5 to make 50 licks after the onset of a tone, which 48 h earlier had predicted the onset of an electric shock. One animal in the veh/scop group and one in the 1 mg/kg heptyl/scop group failed to initiate licking on Day 4 and, as a consequence, were excluded from the experiment. As heptyl reliably reversed the scopolamine-induced performance deficits in the mouse and rat passive avoidance tests, preplanned contrasts using BMDP 4V were used to make group comparisons in this test.

As Fig. 4 illustrates, the rats in the veh/scop group initiated licking significantly sooner after the onset of the tone than the animals in the vehicle control group. The preplanned tests confirmed that this scopolamine-induced performance deficit was partially reversed by 1.0 and 4.0 mg/kg, and fully reversed by 8.0 mg/kg of heptyl ($p<0.05$).

The Effect of Heptyl Physostigmine on Random Interval Lever-Pressing Rates

As the variance of the lever-pressing rates tends to increase with the mean, the raw rates were subjected to a square root transformation before being subjected to an analysis of variance. Figure 5 shows the square root of the lever-pressing rates for each treatment group on the day before, the day of, and the day after the administration of treatment. Figure 5 shows that doses of 0.6 mg/kg of physostigmine and 8.0 mg/kg of heptyl completely suppressed lever pressing for food rewards. There is some suggestion in Fig. 5 that a dose of 4.0 mg/kg of heptyl also reduced lever-pressing rates below those observed on both the pre- and postdrug days; however, these differences failed to reach statistical significance ($p>0.05$).

The Effects of Heptyl Physostigmine on the Scopolamine-Induced Deficit in the Delayed Matching-to-Position Test

The mean number of the percent correct choices made by each treatment group at each of the five delays is shown in Fig. 6. Two rats in the 1.0 mg/kg heptyl/scop group failed to complete more than 20 trials and were therefore excluded from the statistical analysis. In this test, scopolamine (0.2 mg/kg) induced a significant decrease in choice accuracy at each of the five delays ($p<0.05$ vs. vehicle controls). An analysis of variance revealed a treatment-by-delay interaction. This interaction was due

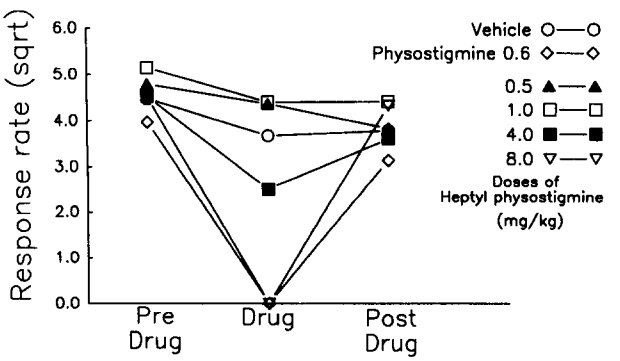

FIG. 5. The mean of the square root response rates on a Random Interval schedule is shown for each treatment group (n = 8). Doses of heptyl physostigmine and vehicle were administered (SC) 80 minutes before and physostigmine 5 minutes before the beginning of the Drug Day session. The square root lever-pressing rates were subjected to an analysis of variance, and group comparisons were made using post hoc Tukey Studentized range tests. *$p<0.05$ compared to the vehicle group.

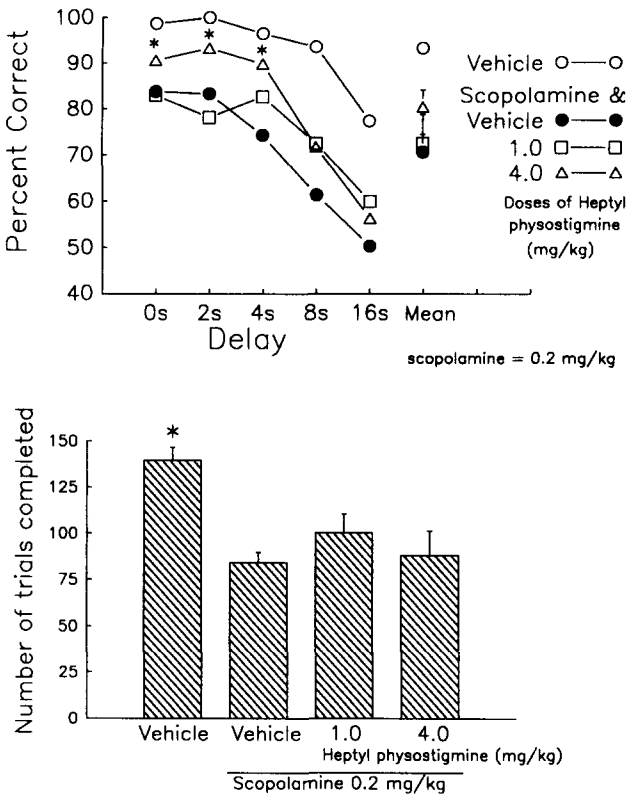

FIG. 6. Mean percentage correct scores (top panel) and the square root of the mean number of trials completed (bottom panel) on the DMTP test are shown for each treatment group (n = 10,8). Numerals indicate the dose of the heptyl physostigmine administered (SC) 80 minutes before the beginning of the test session. Scopolamine (0.15 mg/kg, SC) was administered five minutes before the beginning of the session. Preplanned orthogonal contrasts provided by BMDP 4V were used to compare the percent correct scores and the number of trials completed by the individual treatment groups to the scopolamine/vehicle group. *$p<0.05$.

to the steeper decline in the percent correct measure with increasing delay between sample and choice in the veh/scop group compared to the vehicle control group. The 4.0-mg/kg dose of heptyl reversed the scopolamine-induced deficit at the 2, 4, and 8-s delays ($p<0.05$, one-tailed, preplanned contrasts); the 1.0-mg/kg dose was without effect at all delays.

The lower panel shows the effects of the various treatments on the number of trials completed regardless of whether a choice was correct or not. Scopolamine reduced the number of trials completed, a deficit which was not reversed by either of the doses of heptyl administered. It appears that scopolamine has adverse effects on motor or motivational aspects of this task which are not restored by the administration of heptyl.

DISCUSSION

Heptyl physostigmine appears to be a relatively long-lasting, well-tolerated cholinesterase inhibitor that reverses the amnesic effects of the centrally acting muscarinic receptor antagonist scopolamine in a range of cognitive tasks. In the mouse tail-flick test, an 8.0-mg/kg dose of heptyl appeared to induce a strong and long-lasting antinociceptive effect. Although the duration of onset was relatively slow compared to that of 16.0 mg/kg of morphine, the duration of action was significantly longer. It has been suggested that the antinociception induced by cholinomimetics, both cholinesterase inhibitors and muscarinic agonists, is mediated superspinally (10); if so, this result suggests that heptyl inhibits brain cholinesterase relatively slowly, but once it does, it has a very durable effect.

However, in the mouse passive avoidance test, the mice given a combination of 8.0 mg/kg of heptyl and 0.2 mg/kg of scopolamine were completely ataxic and were unable to step through from the bright compartment to the dark compartment during the training trial. The ataxia induced by this drug combination suggests that the apparent antinociceptive effect observed in Experiment 1 was due in whole, or part, to ataxia rather than an antinociceptive action of the heptyl. Visual observations of the BKTO mice during Experiment 1 did not reveal a drug-induced ataxia, suggesting that the immobility observed in the CD1 mice during Experiment 2 was due either to the combination of heptyl physostigmine and scopolamine or a hitherto unobserved greater sensitivity to cholinesterase inhibitors in this strain of mice. Nonetheless, it remains that, in vivo, heptyl physostigmine's onset of action is slow, but long lasting.

There was some indication that the efficacy of heptyl in the rat was test dependent. In the passive avoidance test, a dose of 8.0 mg/kg heptyl was required to fully reverse the scopolamine-induced (0.2 mg/kg) deficit in the passive avoidance test, but 4.0 mg/kg was without effect. By contrast, although the 8.0-mg/kg dose fully reversed the scopolamine-induced deficit in the conditioned suppression-of-drinking (CSD) test, doses of 1.0 and 4.0 mg/kg of heptyl partially reversed the performance deficit. This efficacy/task interaction was consistently observed in a number of replications of both experiments.

The complexity of task/drug interactions was also underlined in the DMTP test. In order to obtain food rewards in the DMTP test, the rats were required to complete a complex behavioral chain requiring both intact cognitive processing and good motor coordination. In the PA and CSD tests, training takes place in the presence of compounds while testing takes place in their absence. That is, the amnesic effect of scopolamine (and any reversal by target compounds) is assessed while the animal is in a drug-free state. In contrast, training in the DMTP task is conducted in the absence of scopolamine and the compound of interest, while testing is carried out in their presence. Thus the compromising effect that scopolamine has on motor coordination, motivational or cognitive processes can be assessed directly. (A deficit in motor coordination or motivation is usually indicated by a reduction in the number of trials completed, and an effect on cognitive processing is indicated by a reduction in the number of correct choices made once the behavioral chain has been completed.)

Since the RI response-sensitivity test showed that an 8.0-mg/kg dose of heptyl completely abolishes lever pressing for food rewards, whereas a dose of 4.0 mg/kg or lower has little or no effect, only doses of 1.0 and 4.0 mg/kg of heptyl were assessed for their ability to reverse a scopolamine-induced deficit in the DMTP test. Although the 4.0-mg/kg dose of heptyl partially reversed the scopolamine-induced (0.2 mg/kg) deficit in the number of correct choices made, 1.0 mg/kg had no effect. In contrast, neither dose had any effect upon the number of trials successfully completed. These findings suggest that the effects that scopolamine has upon motor coordination or motivation are not reversed by heptyl, while, more importantly, the effects of scopolamine on cognitive processing are reversed.

It is clear from the data obtained from the RI response rate test that compounds of this type have side effects that are not directly observable. Although De Sarno et al. (8) have shown the ED_{50} of heptyl to be 35.0 mg/kg, the compound clearly compromises behavior at much lower doses. The rats treated

with 8.0 mg/kg of heptyl in the RI test were not visibly distressed by the side effects usually associated with cholinomimetic compounds, e.g., salivation and diarrhoea (3,13). Nonetheless, lever pressing for food rewards was abolished by this dose, suggesting that heptyl caused some internal malaise. It is not yet clear what level of cholinesterase inhibition will be necessary to enhance cognition in patients with AD; 5.0 mg/kg of heptyl physostigmine inhibits up to 80% of brain cholinesterase in the rat, but clearly high levels of cholinesterase inhibition do have adverse effects which may limit the therapeutic usefulness of the compound.

In conclusion, heptyl reverses the deficit induced by scopolamine in a range of rodent behavioral tests of long-term and working memory. More importantly, these reversals are achieved at doses that do not induce behavioral toxicity. It would appear that heptyl physostigmine finally provides a realistic opportunity with which to test the cholinergic hypothesis of memory dysfunction in AD.

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