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Behavioral changes after acetylcholinesterase inhibition with physostigmine in mice

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Abstract

The effect of the central and peripheral acetylcholinesterase (AChE) inhibitor, physostigmine (PHY), was examined on spatial memory using a water maze, motor activity as well as acoustic startle response (ASR) and prepulse inhibition (PPI) in C57BL/6J mice. PHY was administered intraperitoneally (IP) at doses of 0.0, 0.01, 0.03, 0.1 and 0.3 mg/kg and the mice were tested 30 min after injection. Administration of PHY reduced motor activity in the open field in a dose-dependent fashion, with notable decreases in activity observed at 0.1 and 0.3 mg/kg. The results also showed that animals receiving 0.1 mg/kg spent more total time in the peripheral zone than in the central zone. The water maze data showed impairment of acquisition and performance of the task, accompanied by a reduced swimming time and enhanced thigmotaxis at a dose of 0.1 mg/kg. We also found that the ASR was significantly decreased after 0.03 and 0.1 mg/kg with no change in PPI. These results indicate that central plus peripheral cholinesterase inhibition (ChEI) decreased ASR, which is contrary to our previous experiments with the peripheral ChEI pyridostigmine bromide (PB), suggesting different involvement of cholinergic systems in modulating ASR in mice.
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Keywords: Acoustic startle response; Locomotor activity; Water maze; Physostigmine; Mice; Cholinesterase

1. Introduction

Cholinesterase inhibitors (ChEI) of carbamate, organophosphorus and aminoacridine structures are widely used and intensively studied agents. Prior work from this laboratory examined the behavioral effects of the peripherally active acetylcholinesterase (AChE) inhibitor pyridostigmine bromide (PB). The present study is focused on the evaluation of behavioral changes after central and peripheral AChE inhibition by physostigmine (PHY). The rationale for this study was to determine whether differences exist between ChEI with combined peripheral/central and peripheral only AChE action on different types of behavior. The results of these experiments will be useful in comparing the data from studies employing chemical warfare agents.

Physostigmine (PHY) is an old drug, isolated from Calabar beans by Jobst and Hesse in 1864. The first therapeutic use of this drug was in 1877 by Laquer, in the treatment of glaucoma, and is one of its clinical uses today (Hardman et al., 1995). PHY has recently regained prominence due to its use in the clinical trials of Alzheimer's disease and to its potential as a potent prophylactic antidote to chemical warfare agent poisoning, providing protection for both central and peripheral cholinesterase (ChE; Leadbeater et al., 1985). PHY is a reversible cholinesterase inhibitor and has a short duration of action. Since it is a tertiary amine, PHY is lipid soluble and hence crosses the blood–brain barrier (BBB) to produce central actions (Somani et al., 2001).

The measurement of movement and locomotion is central to obtaining qualitative and quantitative information about an animal's general behavior. Changes in an animal's spatial

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location and/or changes in the spatial relationship of the animal's limbs and body have consequences for the expression and measurement of all aspects of behavior. PHY increased rat exploratory activity after intracerebroventricular injection of a limited dose range (Sienkiewicz-Jarosz et al., 2003). PHY administered subcutaneously decreased the distance traveled in open field in a dose-dependent fashion (Wang and Fowler, 2001). The dose of 0.5 mg/kg PHY administered intraperitoneally (IP) decreased rat locomotor activity as well (Sienkiewicz-Jarosz et al., 2000); similarly in C57BL/6J mice, PHY administered IP decreased locomotor activity (Retz et al., 1987).

The acoustic startle response (ASR) is a behavioral reflex evoked by abrupt, intense sound that is readily elicited in a wide variety of animal species, including humans (Landis and Hunt, 1939). In rodents, it is a sensitive method for determining how different neurotransmitter systems modulate sensorimotor activity (Davis, 1980). Despite its relatively simple, reflex-like appearance, the startle response magnitude can be modulated by a variety of external and internal variables. Under appropriate experimental conditions, startle has a nonzero baseline and can be enhanced or attenuated (Koch, 1999). The ASR magnitude is reduced if a distinctive nonstartling tactile (Pinckney, 1976), visual (Campeau and Davis, 1995), or acoustic (Hoffman and Ison, 1980) stimulus is presented 30–500 ms prior the startling stimulus. This phenomenon is termed prepulse inhibition (PPI) and is used as an operational measure for sensorimotor gating mechanisms (Hoffman and Ison, 1980). Studies using PHY report different results in rats and guinea pigs (Jones and Shannon, 2000; Philippens et al., 1997). While the influence of PHY on ASR and PPI in mice has not been reported, we found in our previous experiments that pyridostigmine bromide (PB) increased ASR and reduced PPI in mice (Lucot et al., 2002).

The aim of this study was to determine the effect of PHY on locomotor activity, spatial memory and ASR and PPI in C57BL/6 mice in order to compare its central plus peripheral action with that of peripherally active PB, and to establish normative data in mice for ongoing studies with chemical warfare agents.

2. Materials and methods

2.1. Animals

Male C57BL/6J mice (HARLAN, body weight 26–28 g, aged 3 months) were individually housed in plastic cages with wood shaving bedding in a temperature-controlled room (T=70°F) with 12:12-h light/dark cycle (lights off from 1700 h). Standard pellet diet and tap water were provided ad libitum. After 10 days of acclimatization, the mice were subjected to 3 days of handling before behavioral testing. All experiments were in compliance with the National Institutes of Health Guide for Care and Use of Laboratory Animals and the protocol was approved by the Institutional Laboratory Animal Care and Use Committee.

2.2. Treatment

Physostigmine (eserine sulfate, control no. 6864, Nutritional Biochemicals, Cleveland, OH) was dissolved in 0.9% sodium chloride (saline) and administered intraperitoneally (IP) 30 min before testing (10 ml/kg).

2.3. Behavioral protocols

2.3.1. Water maze protocol

The water maze apparatus (Videomex-V, Columbus Instruments Int., Columbus, OH) consisted of a circular pool 110 cm in diameter, which was filled with water to a depth of 50 cm and had a circular plastic platform 8 cm in diameter placed 0.5 cm below the surface of the water. The water temperature was 75°F and colored with white tempera paint to hide the platform. The pool was placed in a room surrounded with fixed spatial cues (cabinet, table, computer, pictures, camera, sink, etc.). For descriptive data collection, the pool was subdivided into four equal quadrants formed, which intersect in the center of the pool at right angles and into two concentric circles subdividing the pool into peripheral and central (60 cm in diameter) zones. The protocol for the water maze was previously described in Dell'Anna et al. (1997). Testing consisted of placing the mouse in one of the randomly chosen quadrants facing the pool wall and permitting it to swim for 2 min. If it failed to find the platform, it was gently guided to the platform and stayed there for 20 s before the next trial. If it found the platform, it was permitted to remain for 20 s before the next trial. All mice received four consecutive trials, each randomly started in a different quadrant. The water was cleared of any debris with a scoop. The mouse was patted dry, placed in a home cage, allowed to rest on a heating pad until dry and then returned to the colony. The mice (n=18) were acclimated to the facility for 10 days and then handled 3 min per day 3 days before testing. Prior to the test, each mouse was placed in the water without the platform and observed for 60 s to verify that it was able to swim. Mice were injected IP with either saline or 0.1 mg/kg physostigmine 30 min before testing. On Monday through Friday, the platform was hidden in the SE quadrant and the latency time to find the platform was recorded (Place Navigation—phase I of spatial memory acquisition). The following Monday to Wednesday, the platform was moved from SE to NW quadrant, 1 cm above the water surface and the mice tested using the same protocol as the first week (Cue Navigation). On Thursday and Friday, the platform was hidden in the NW quadrant and testing was conducted (Place Navigation—phase II of spatial memory acquisition). The initial dose 0.1 mg/kg of PHY was selected based on Symons et al. (1988), who reported that this dose improved spatial learning in C57BL/10J mice.

2.3.2. Open-field test

Locomotor activity was evaluated in an automated open-field system with infrared photo-beams (Motor Monitor, Version 3.11, 2000, Hamilton Kinder, Poway, CA). The open field was 16×16 in. (40.6×40.6 cm), and it was divided into central (12×12 in.) and peripheral zones. The mice (n=18) were placed in the center of the open-field arena and the following variables of motor activity were recorded: locomotor activity, fine movement and rearing. Moreover, distance traveled, total time, rest time, number of entries and head pokes in individual zones were also recorded. As in the case of the water maze experiment, all animals were regularly handled before individual tests in order to minimize handling-related stress. The animals were assigned to the groups (Control vs. PHY group) according to their basal locomotor activity which was evaluated before any injections. The mice received saline or PHY injections weekly, beginning with the lowest dose. One day before every administration, we observed motor activity in open field to screen out possible effects of habituation or cumulative drug effects. Thirty minutes after IP administration of PHY at 0.0, 0.01, 0.03, 0.1 or 0.3 mg/kg, mice were placed in the open field for 15-min sessions. After the session, the number of fecal pellets (defecation) was noted for assessment of emotional reactivity and the open-field arena was cleaned with 70% alcohol solution and allowed to dry.

2.3.3. Acoustic startle response and prepulse inhibition

Mice were tested in the SM100 Startle Monitor System Version 4.0 (Hamilton Kinder, 2001) for acoustic startle response (ASR) and prepulse inhibition (PPI). The system was programmed for six types of white-noise burst stimulus trials: no stimulus (background, 60 dB), prepulse (70 dB), pulse (100 or 120 dB), prepulse plus pulse (70+100 dB or 70+120 dB). Each trial type was presented 10 times in 10 blocks. Stimuli were presented in random order to avoid order effects and habituation. The intertrial interval varied from 9 to 16 s. All animals were regularly handled before individual tests in order to minimize handling-related stress. Mice were pair matched according to baseline values into the experimental groups using the average response to 100 and 120 dB. In the chamber, mice were loosely restrained in holders that were placed on a sensing plate transforming movements of the body (jerks) into an analog signal through an interface. Finally, percentage prepulse inhibition measures were calculated as follows: the difference between the pulse alone and the prepulse+pulse trials, divided by the pulse alone and multiplied by 100. Percentage scores are typically used to minimize the effect of individual variation of startle amplitude on prepulse inhibition. Behavioral tests were conducted 30 min after IP administration of PHY at 0.0, 0.01, 0.03 or 0.1 mg/kg. The values in the figures are presented as a peak startle amplitude [Newtons] during a 250-ms recording window.

2.4. Blood collection

For basal ChE activity measurements prior to treatment, blood was collected (~50 μl) in heparinized cap tubes from cut tails and stored on ice in 1.5 ml microfuge tubes. The mice (n=24; 8 for each dose group) were sacrificed 30 min after PHY administration (0.0, 0.01 or 0.1 mg/kg) by decapitation and trunk blood was collected in 1.3 ml microfuge tubes with heparinized beads (Sarstedt, Newton, NC).

2.5. Blood cholinesterase activity

Cholinesterase (ChE) activity was determined by a modified version of the colorimetric assay of Ellman et al. (1961) using a Packard Fusion™ Microplate Analyzer. Fresh whole blood was diluted 1:100 with 0.1 M $NaPO_4$ pH 7.4 buffer. Total ChE activity was measured in diluted whole blood samples. Blood acetylcholinesterase (AChE) activity was determined at 77 °F by inhibiting butyrylcholinesterase (BChE) with 100 μM *iso*-OMPA (tetraisopropylpyrophosphoramide). BChE activity was then calculated by subtracting AChE activity from total ChE activity.

2.6. Statistical analysis

The data were analyzed by means of one-way or two-way analyses of variance (ANOVAs) followed by a Duncan post hoc test to assess statistical significance. For the water maze data, ANOVA for repeated measures was conducted with the drug (PHY vs. Control) as the between-subject factor, and day (repeated measure: average value per trial per day) as the within-subject factor. These tests were run using the statistics program, STATISTICA 6.1 (StatSoft, Tulsa, OK). The results are presented as means±S.E.M. The confidence limit of $p<0.05$ was considered as statistically significant.

3. Results

3.1. Morris water maze

Administration of 0.1 mg/kg PHY increased latencies to locate both the hidden and the visible platform during testing [$F(1,16)=5.57$, $p<0.03$; Fig. 1]. We found an influence of drug administration on swimming speed [$F(1,16)=10.67$, $p<0.005$], which was probably caused by decreased locomotor activity after PHY administration (see below). Circle zone analysis of the water maze revealed a preference for the periphery over the center circle ($PHY_{periphery}$ 90.6±1.4% vs. $Control_{periphery}$ 75.5±1.6%) [$F(1,16)=9.13$, $p<0.008$], which we confirmed using zone analysis in the open field (below). The dose of 0.03 mg/kg physostigmine did not change spatial learning and there were no significant

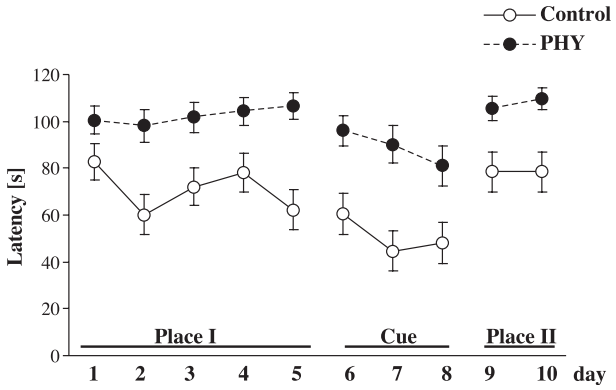

Fig. 1. Latency to reach the platform in Morris water maze exhibited by saline- or physostigmine (PHY—0.1 mg/kg)-treated mice. Main effect of drug administration ($p<0.03$). Place I phase represents latency to locate hidden platform in the SE quadrant, Cue phase represents latency to locate visible platform in the NW quadrant and Place II phase represents latency to locate hidden platform in the NW quadrant. Values are presented as means±S.E.M.

differences in latency time to locate the platform [$F(1,12)=0.42$; $p=0.53$; data not shown]. The average speed and time spent in the peripheral circle in both groups were equal (data not shown).

3.2. Open field

Administration of physostigmine affected open-field motor activity in a dose-dependent fashion. The two-way ANOVA demonstrated a significant main effect of treatment [saline vs. PHY; $F(1,59)=228.3$, $p<0.001$] and dose [$F(3,59)=29.84$, $p<0.001$] on the basic movements. There was a significant effect of treatment×dose interaction and a post hoc test revealed a significant decrease in activity at 0.03, 0.1, 0.3 mg/kg ($p<0.001$; Fig. 2). Rearing behavior was also affected significantly. There was a main effect of treatment [$F(1,59)=63.21$, $p<0.001$] and dose [$F(3,59)=4.73$, $p<0.005$] and post hoc testing revealed an impairment

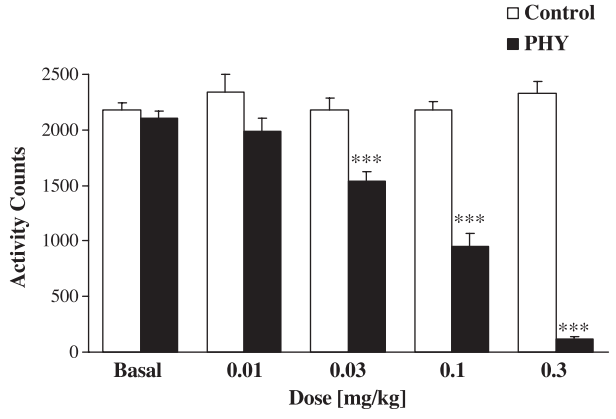

Fig. 2. Locomotor activity (basic movements) in the open field observed for saline-treated (Control) or physostigmine (PHY)-treated mice at the various doses. Data are presented as means±S.E.M. ($n=9$ for each dose; ***$p<0.001$—significant difference compared to Control).

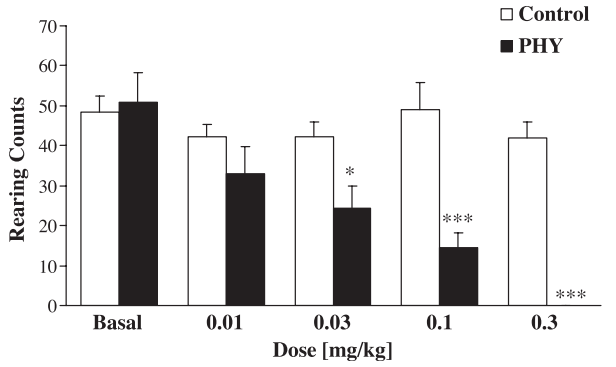

Fig. 3. Exploratory behavior (rearing) in the open field observed for saline-treated (Control) or physostigmine (PHY)-treated mice at the various doses. Data are presented as means±S.E.M. ($n=9$ for each dose; *$p<0.05$; ***$p<0.001$—significant difference compared to Control).

at 0.03 ($p<0.05$), 0.1 and 0.3 mg/kg ($p<0.001$; Fig. 3). The doses of 0.1 and 0.3 mg/kg increased the total time in the peripheral zone and decreased it in the central zone [$F(1,59)=12.21$, $p<0.001$; Table 1].

3.3. Acoustic startle response and prepulse inhibition

We found that the ASR elicited by the 100-dB stimulus was significantly decreased at 0.03 mg/kg PHY [$F(1,13)=7.17$, $p<0.019$] and 0.1 mg/kg [$F(1,13)=4.75$, $p<0.048$], as was the ASR elicited by the 120-dB stimulus at 0.03 mg/kg [$F(1,13)=5.29$, $p<0.039$] and 0.1 mg/kg [$F(1,13)=8.12$, $p<0.014$; Figs. 4 and 5]. The PHY dose 0.01 mg/kg was ineffective. There were no significant effects on the PPI (data not shown).

3.4. Blood cholinesterase activity

There was a significant drug effect on blood total ChE and AChE activity after PHY administration [$F_{ChE}(1,21)=5.95$, $p<0.009$; $F_{AChE}(1,21)=8.16$, $p<0.002$]. The post hoc test revealed a significant decrease in activity at 0.1 mg/kg ($p<0.05$; Fig. 6). The total ChE and AChE activity were

Table 1
Zone preference in the open field observed for saline-treated (Control) or physostigmine (PHY)-treated mice at the various doses

Dose	Peripheral zone		Central zone	
	Control (n=9)	PHY (n=9)	Control (n=9)	PHY (n=9)
Basal	429.7±30.5	474.3±32.2	470.3±30.5	425.7±32.2
0.01 mg/kg	574.7±12.1	658.9±11.8	325.3±34.3	241.1±31.2
0.03 mg/kg	623.2±13.1	672.3±9.4	276.8±37.1	227.7±26.6
0.1 mg/kg	523.2±9.8	714.7±15.9*	376.8±29.3	185.3±47.6*
0.3 mg/kg	613.0±11.7	769.5±31.1*	287.0±35.2	130.4±93.2*

Data are presented as means of time spent in the zone±S.E.M.
* $p<0.05$—significant difference compared to Control.

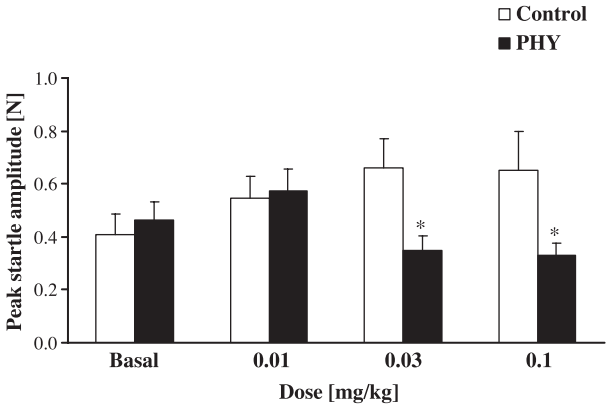

Fig. 4. Acoustic startle response (ASR) after 100 dB stimulus observed for saline-treated (Control) or physostigmine (PHY)-treated mice at the various doses. Data are presented as means±S.E.M. (*p<0.05—significant difference compared to Control).

also significantly decreased compared to the dose of 0.01 mg/kg (p<0.05).

4. Discussion

Our results show that IP administration of PHY did not improve spatial memory in mice. The water maze data initially appeared to indicate that the dose of 0.03 mg/kg of physostigmine had no effect on spatial learning while the dose of 0.1 mg/kg impaired acquisition and performance of the task, but it also reduced swimming time and enhanced thigmotaxis. Addition of the locomotor activity dose–response data confirm that the dose of 0.1 mg/kg reduces activity and enhances thigmotaxis, two factors which could interfere with finding the platform independent of any effect on learning, thus offering an alternative explanation for the failure to acquire and perform the learning task. Furthermore, PHY impaired locomotor activity in a dose-dependent fashion with a robust decrease at the dose of 0.3 mg/kg, and significantly decreased ASR to the 100- and 120-dB stimuli at the doses 0.03 and 0.1 mg/kg. The cholinesterase assay

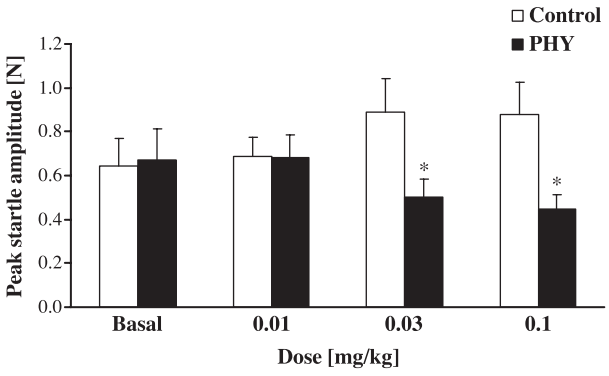

Fig. 5. Acoustic startle response (ASR) after 120 dB stimulus observed for saline-treated (Control) or physostigmine (PHY)-treated mice at the various doses. Data are presented as means±S.E.M. (*p<0.05—significant difference compared to Control).

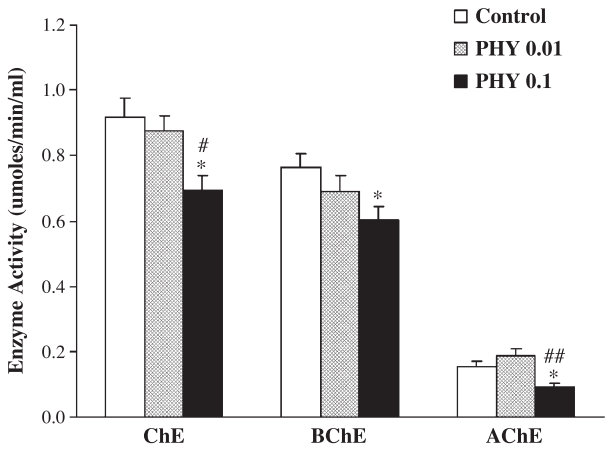

Fig. 6. Blood cholinesterase activity observed for saline-treated (Control) or physostigmine (PHY)-treated mice at the doses of 0.01 and 0.1 mg/kg (ChE—cholinesterase, BChE—butyrylcholinesterase, AChE—Acetylcholinesterase; *p<0.05—significant difference compared to Control; $^{\#}p$<0.05; $^{\#\#}p$<0.01—significant difference compared to the dose 0.01 mg/kg).

results showed a significant decrease in enzyme activity at the dose 0.1 mg/kg but not at 0.01 mg/kg.

PHY works by inhibiting the chemical destruction of acetylcholine (ACh) in the synapses, causing increased ACh transmission. Being a tertiary ammonium, PHY effectively crosses the blood–brain barrier (Smith and Swash, 1979) and alters central cholinergic pathways. Central cholinergic systems are implicated in many brain functions, such as learning and memory, habituation, locomotor activity, drug dependence and emotional reactions. Moser (1995) discovered that the profiles of the acute effects of cholinesterase inhibitors (aldicarb, carbaryl, parathion, DFP, chlorpyrifos, fenthion and diazinon) were similar.

It is well known that the central cholinergic systems play an important role in learning and memory and it is generally assumed that in the adult organism, the hippocampus interacts with the neocortex during memory “consolidation” so as to enable information to be permanently stored in cortical sites. Symons et al. (1988) suggest that the hippocampus plays an important role in the acquisition and performance of spatial memory tasks, such as the water maze. Morphological differences in hippocampal cell count and volume exist in selectively inbred strains of mice (Wimer et al., 1980; Abusaad et al., 1999), providing the opportunity to study hippocampal involvement in learning and memory in intact mice. Symons et al. (1988) found that the acquisition deficit of C57BL/10J mice, caused by a low level and volume of the hippocampal cells, can be overcome by increasing cholinergic function with PHY at the dose of 0.1 mg/kg. However, we observed no improvement in acquisition. The dose of 0.1 mg/kg of PHY impaired acquisition and performance of the task, reduced swimming speed and enhanced thigmotaxis while the dose of 0.03 mg/kg had no effect. Although both C57BL/6J and C57BL/10J mice have a similarly low hippocampal cell number and volume with respect to the genetically defined divisions of the pyramidal

layer (Wimer et al., 1980; Abusaad et al., 1999), increased stimulation of the cholinergic system by administration of PHY did not enhance spatial memory in C57BL/6J as it does in C57BL/10J (Symons et al., 1988). Drug-induced improvements in learning tasks are generally seen when the subject is impaired by an experimental manipulation or genetic abnormality. The dose of 0.1 mg/kg produced extensive locomotor inhibitory effect, which may interfere with learning.

The largest magnitude of change produced by ChEI occurs in autonomic (cholinergic stimulation), neuromuscular (weakness) functions and motor activity. Loizzo et al. (1996) observed significant decreases in motor activity in C57BL/6 mice 15 min after 0.1 mg/kg IP of PHY, but not after 0.025 and 0.05 mg/kg, whereas we also observed changes at 0.03 mg/kg after 30 min. One possible explanation for this apparent discrepancy is that we measured responses 30 min after injection rather than 15 min. Xia et al. (1981) reported that ChE inhibition with PHY in vivo reaches peak levels at 30 min. In the rat, PHY increased exploratory activity after intracerebroventricular injection of a limited dose range (Sienkiewicz-Jarosz et al., 2003) while a dose of 0.5 mg/kg administered IP decreased locomotor activity (Sienkiewicz-Jarosz et al., 2000). PHY administered subcutaneously decreased the distance traveled in the open field in a dose-dependent fashion (Wang and Fowler, 2001). These data suggest that PHY has a similar locomotor activity decreasing effect in both rats and mice.

The startle response is composed of a constellation of reflexes elicited by sudden, relatively intense stimuli. It offers many advantages as a behavioral measure of central nervous system (CNS) activity and can be measured in numerous species, including humans. Anxiogenic drugs, such as yohimbine (Morgan et al., 1993; Fendt et al., 1994), and drugs that reduce inhibitory neurotransmission in the CNS, such as the glycine receptor antagonist strychnine (Kehne and Davis, 1984; Koch and Friauf, 1995), enhance the ASR, whereas drugs that reduce overall excitability of the CNS, such as ethanol or diazepam, attenuate the ASR (Berg and Davis, 1984; Grillon et al., 1994). Although the ASR is a sensitive method for examining modulation of CNS activity in rodents, there are still different interpretations as to how cholinergic systems influence the startle response and PPI in different rodent species. In guinea pigs, PHY dose-dependently enhanced the startle reflex with the maximally effective dose around 0.3 mg/kg, which produced AChE inhibition comparable to our results at the dose of 0.1 mg/kg (Philippens et al., 1996, 1997; Fig. 6). In rats, doses of PHY from 0.01 to 0.1 mg/kg administered subcutaneously had no effect on the ASR or PPI (Jones and Shannon, 2000). However, tacrine (10 mg/kg) significantly decreased startle amplitude (Jones and Shannon, 2000). In our study with mice, PHY caused a significant decrease in the ASR at doses of 0.03 and 0.1 mg/kg, in contrast to what was observed in other species. These discrepancies confirm the finding of Davis (1980), that the role of the cholinergic system in modulating ASR is unclear. Possible explanations for the species differences are differences in body weights and different direct or indirect interactions of PHY with other neurotransmitter systems. In the case of cholinesterase inhibition by organophosphorus agents, differences in carboxylesterase activity among the species also need to be considered (Maxwell et al., 1987).

The caudal pontine reticular nucleus (PnC) is one of the key elements of the primary ASR circuit; it mediates the ASR and is also the recipient of ASR-modulating inputs from a variety of other brain regions that enhance the ASR by aversive states or which reduce the ASR by prepulses (Koch, 1999). The attenuating effect of acoustic prepulses on the ASR probably affects the primary ASR pathway at the level of the PnC (Lingenhöhl and Friauf, 1994; Willott et al., 1994; Carlson and Willott, 1998) by activation of inhibitory cholinergic projections from the pedunculopontine tegmental nucleus (PPTg) to the PnC (Koch et al., 1993; Swerdlow and Geyer, 1993). Systemic administration of muscarinic agonists and microinfusion of the muscarinic/nicotinic agonist carbachol into the PnC decreases the ASR in rats demonstrating that ACh is inhibitory in this brain region. Microinfusion of scopolamine produces an increase in the ASR (Fendt and Koch, 1999), suggesting that this inhibitory circuit is tonically active. Thus, it is reasonable that PHY would decrease the ASR as it would augment tonic inhibitory action. While nicotine produces a small increase in the ASR of C57BL/6J mice (Lewis and Gould, 2003), the muscarinic effects of the excess ACh made available by PHY were of greater magnitude than that of its nicotinic effects. The decrease in ASR produced by PHY may arise from its action on the same neural pathway that underlies PPI. In this case, the PHY may have maximally activated this modulatory input, precluding any other effect from the prepulse itself.

In a previous study, we focused on the effect of chronic administration of pyridostigmine bromide (PB) on ASR, PPI and locomotor activity (Lucot et al., 2002). These results showed that chronic exposure of mice to PB (10 mg/kg/day) resulted in an exaggerated ASR, reduced PPI and a nonsignificant trend to decreased locomotor activity. These behavioral changes were apparent only during exposure to PB and returned to control values when the minipumps ran out of drug. These results indicate an opposite effect of peripheral cholinergic activation on ASR than we observed with PHY, which is a central ChEI as well. However, because PB is a quaternary ammonium carbamate, the increase in the ASR could be due to a direct action on the skeletal neuromuscular junction, since there is a direct action of neostigmine and other quaternary ammonium anti-ChE agents on skeletal muscle. For instance, the intraarterial

injection of neostigmine into chronically denervated muscle, or into normally innervated muscle in which essentially all the AChE has been inactivated by prior administration of diisopropyl fluorophosphate, evokes an immediate contraction, whereas PHY does not (Hardman et al., 1995).

In our study, we found that administration of PHY decreased the locomotor activity in a dose–response manner, suggesting that the influence of combined central plus peripheral AChE inhibition is the same as peripheral only inhibition. However, peripheral cholinesterase inhibition alone increased ASR whereas central plus peripheral cholinergic enzyme inhibition decreased the ASR in mice. While studies with other rodent's species showed similar effect of PHY on the locomotor activity, the effects of PHY on the ASR varied, suggesting species differences in cholinergic modulation of that response.

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