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Active Avoidance Behavior in Guinea Pigs: Effects of Physostigmine and Scopolamine

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PHILIPPENS, I. H. C. H. M., B. P. C. MELCHERS AND O. L. WOLTHUIS. *Active avoidance behavior in guinea pigs: Effects of physostigmine and scopolamine.* PHARMACOL BIOCHEM BEHAV **42**(2) 285–289, 1992.—Behavioral training of guinea pigs by conventional methods, such as used for rats and mice, appears difficult. Hence, only a few behavioral experiments with guinea pigs have been described in the literature. An active avoidance technique in an automated two-way shuttlebox is described using sound as a conditioned (CS) and a tactile stimulus (a stream of air ruffling their fur) as an unconditioned (UCS) stimulus. Acquisition is fairly rapid and reproducible. Doses of physostigmine that caused moderate blood acetylcholinesterase inhibition induced dose-dependent performance decrements. These decrements were counteracted by a sign-free dose of scopolamine.

Active avoidance Behavior Shuttlebox Guinea pig Physostigmine Scopolamine

ADMINISTRATION of drugs or exposure to neurotoxic agents may disturb behavior. For the detection of such behavioral effects, usually rats or mice are employed. However, for experiments on the pretreatment and therapy of intoxications with organophosphate cholinesterase (ChE) inhibitors these species may have some drawbacks, in particular for in vivo studies. In contrast to human blood, the blood of rats and mice contains large amounts of carboxylesterases, which act as scavengers for ChE inhibitors. This difference may hamper extrapolation to man of data obtained with these rodent species. Hence, several authors investigating ChE inhibitors prefer the use of guinea pigs, animals that have only small concentrations of carboxylesterase present in their blood.

In some cases, it is desirable to measure the behavioral effects of ChE inhibitors, for example, when testing carbamates for their potential use as pretreatment agents against organophosphate poisoning (8). Unfortunately, reports on behavioral experiments in guinea pigs are rare. Upon a quick computer search, only nine papers were found: two on psychophysical experiments for auditory thresholds (13,14), one on open-field behavior (15), one on motor performance in swimming test (10), one on water maze (12), and four on foot-shock-motivated shuttlebox acquisition and performance (1,3,4,11), three of which originate from the same laboratory. Pilot experiments in our own laboratory resulted in erratic behavior in open-field tests and lack of reproducible performance and freezing of the animals in foot-shock-motivated shuttlebox avoidance conditioning, even at low scrambled foot-shock levels of 100–200 μA (constant-current principle). In an investigation of different stimuli, it was observed that a stream of air ruffling the fur of the guinea pig might be used as an unconditioned (UCS) stimulus in a shuttlebox test. The present results show that this is indeed the case and further demonstrate that the effects of physostigmine alone or in combination with scopolamine can be measured in a sensitive and reproducible fashion.

METHOD

Animals

Dunkin-Hartley albino guinea pigs CrL:(HA)BR (Charles River) with a starting body weight of 275–325 g were used. All animals were male and experimentally naive.

Apparatus and Procedures

The two-way shuttlebox consists of two equal compartments (23 × 23 × 23 cm) with a linoleum floor connected by a gate, guarded on each side by an infrared beam, through which the animal may cross from one compartment to the

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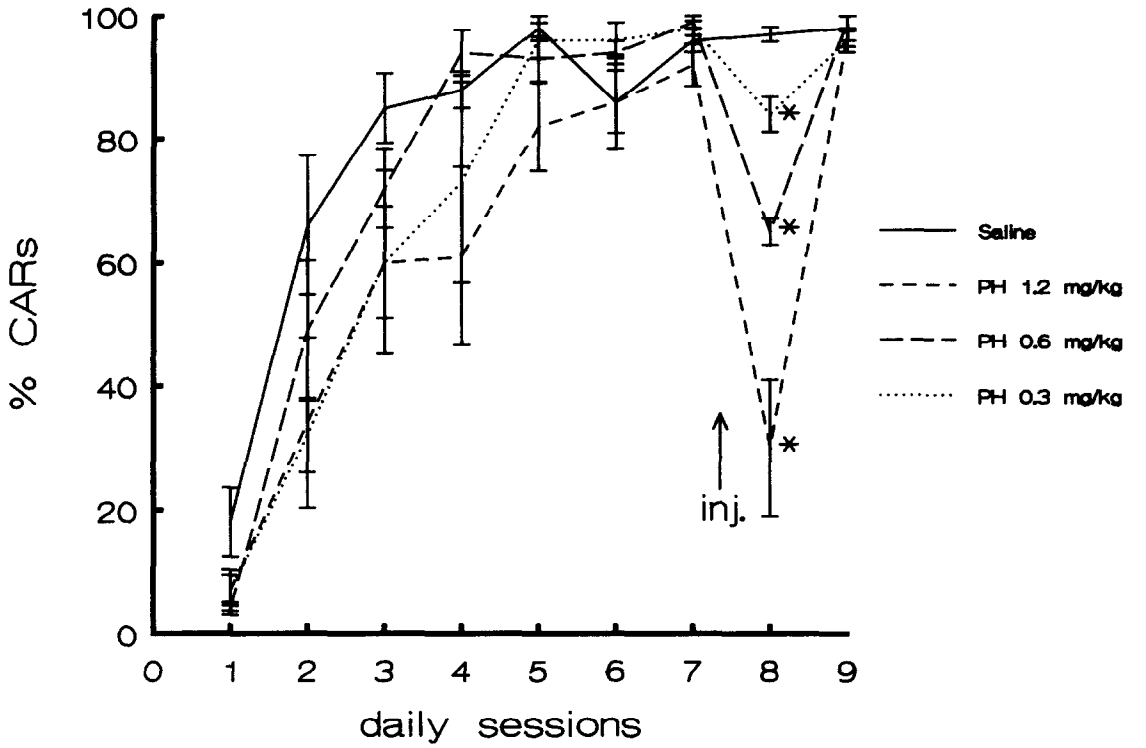

FIG. 1. Mean (±SEM) acquisition rates and performance of guinea pigs. Four groups of guinea pigs (n = 5/group) were trained in a two-way shuttlebox (CS, sound; UCS, air stream; 1 session/day, 20 trials/session). On day 8, different doses of physostigmine (PH) were SC injected and 30 min later another session started. It can be seen that acquisition rates of the four groups is very similar. Performance levels at day 7, judged by the % correct avoidance responses (CARs), are >90%. Different doses of physostigmine cause dose-dependent performance decrements. After 24 h performance returned to preinjection values. *Significantly different from each other and from controls. inj., injection.

other. Per day, each animal received one session of 20 trials, during which the animal had to learn to avoid the UCS by moving into the other compartment within 10 s after the sound signal [conditioned stimulus (CS)] had been turned on. The sound signal consists of white noise passing through a band-pass filter with a center frequency of 10 kHz and a slope of 12 db/oct at a level of 65 dB measured in the middle of each cage. The sound signal stops when the guinea pig has passed through the gate. When the animal fails to avoid, a stream of air (6,250 cm^3/s, air tube diameter 1 cm) directed into the compartment in which the animal is present is turned on and stops when the animal has escaped into the other compartment. The intertrial interval is 25 s (±20% random). When the guinea pig fails to escape, the air stream is turned off after 20 s. After animals had reached their criterion, which was 80% or more correct avoidance responses, drugs were injected SC. Two experiments were carried out. In the first, animals were injected with saline (1 ml/kg) or physostigmine in different doses (0.3, 0.6, or 1.2 mg/kg) to investigate the sensitivity of this test for a centrally active carbamate. In the second experiment, the two highest doses of physostigmine were injected alone or in combination with scopolamine (100 μg/kg). Control animals received saline (1 ml/kg) in combination with scopolamine (100 μg/kg). In both experiments, animals were tested 0.5 and 24 h after injection. Avoidances, escapes, and intertrial responses were detected by the sequential interruption of the infrared lightbeams and were processed by a Hewlett-Packard Vectra personal computer, programmed in Pascal. In a separate group of unanesthesized animals, blood samples (5 μl) were drained from their ear veins immediately before and 10, 30, and 60 min after injection of the same physostigmine doses used in Experiment 1, that is, 0.3, 0.6, or 1.2 mg/kg SC. Acetylcholinesterase (AChE) activity was determined as follows: Blood samples (5 μl) were immediately mixed with 1% saponin (BDH, Poole, England) and frozen in liquid N_2. After appropriate dilution, samples were assayed for AChE activity using a radiometric method (7); the acetylcholine (ACh) end concentration used was 12 μM; [^{3}H]ACh iodide (NEN, Dreieich, Germany) was diluted to a specific activity of 600 MBq · $mmol^{-1}$. To limit decarbamoylation of physostigmine-inhibited AChE, samples were kept at 0°C until they were incubated with [^{3}H]ACh for 15 min at 20°C. Ethopropazine (2.5 μM; Sigma Chemical Co., St. Louis, MO) was used as specific inhibitor of butylcholinesterase (BuChE). AChE from electric eel was used as a reference.

Chemicals

All chemicals were obtained commercially. Solutions were freshly prepared before use.

Statistics

The multiple t-test of Welch was used. When the term significant is used, this means $p < 0.05$ (two tailed).

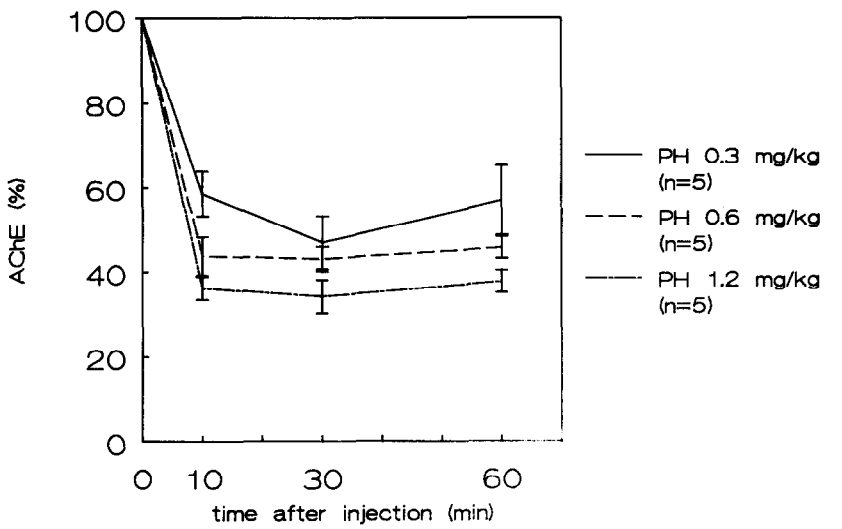

FIG. 2. Effect of single SC injections with physostigmine (PH) in doses of 0.3, 0.6, or 1.2 mg/kg on the mean (±SEM) AChE activity in blood from unanesthesized guinea pigs. Values (mean ± SEM) are expressed as percentages of preinjection values.

RESULTS

General

Upon leaving the tube supplying the air stream, a hissing sound is produced that might act as an UCS. In a pilot experiment, by aiming the air tube in another direction it could be established that this hissing sound did not act as an UCS. During the experiments, performance of animals was continuously monitored by color TV. In contrast with earlier experiences with foot-shock, freezing did not occur.

Intertrial responses (ITRs), usually taken as a measure of the activity levels of animals, increased during training and varied largely between animals. For example, for all animals in Experiment 1 ranges of ITRs were 0–>5 in Session 1, 0–>15 before injection in Session 7 (with one exception making 40 ITRs), and 0–>17 after injection during Session 8, again with one exception making 27 ITRs (not the same animal that made 40 ITRs during Session 7). There were no significant differences between the averaged ITRs of the groups either before or after the injections.

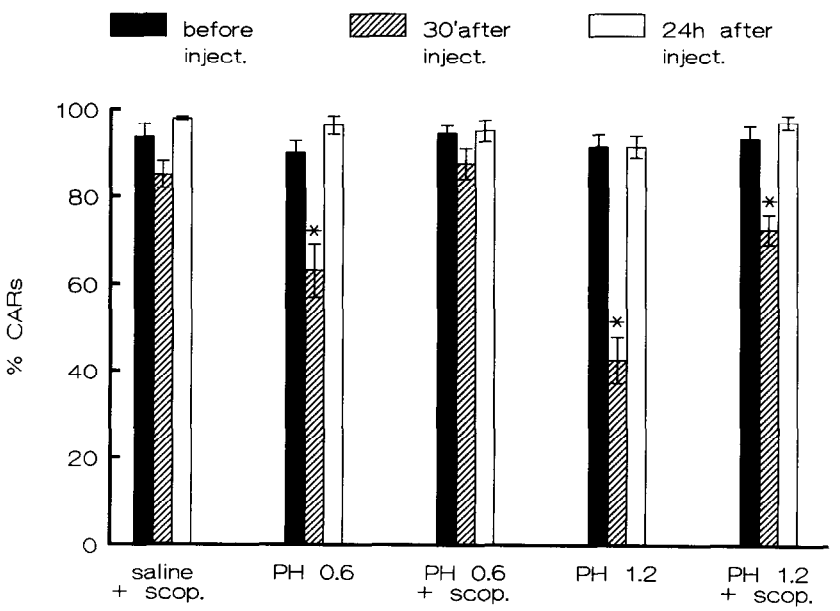

FIG. 3. Shuttlebox performance (mean ± SEM) of guinea pigs 24 h before, 30 min after, and 24 h after SC injection of either 0.6 or 1.2 mg/kg physostigmine (PH) alone or in combination with 100 μg/kg scopolamine SC. It can been seen that this dose of scopolamine has no significant effect, whereas it effectively counteracts the behavioral decrements caused by physostigmine. *Significantly different from performance 24 h before and 24 h after injection.

In preliminary experiments, it was found (not shown) that acquisition was not faster when two sessions (each of 20 trials, one in the morning, one in the afternoon) were given instead of only one session of 20 trials per day.

Experiment 1

In Fig. 1, it can be seen that even with small groups of guinea pigs (n = 5/group) comparable acquisition rates may be obtained. Upon reaching a high level of performance, dose-dependent decrements of performance were found 30 min after SC injection of different doses of physostigmine. Twenty-four hours later, animals had returned to their preinjection performance level. The changes in blood AChE activity following these doses of physostigmine were determined in parallel groups of animals; the results (Fig. 2) show that there was a weak dose-dependent inhibition of blood AChE. The average values of all ranged between 41–66% inhibition of AChE.

Experiment 2

In Fig. 3, the combined results are shown of two independent, procedurally identical experiments. Both experiments were performed with four animals/treatment group; the results were virtually identical and therefore combined. In essence, part of the experiment, that is, groups treated with 0.6 or 1.2 mg/kg physostigmine, are a repetition of Experiment 1, providing very similar results. It can also be seen in Fig. 2 that scopolamine (100 μg/kg, SC) by itself causes no significant effect. It counteracts the performance decrement caused by physostigmine 0.6 mg/kg to practically preinjection values and improves the performance decrement caused by 1.2 mg/kg physostigmine significantly. In all cases, performance was back to preinjection levels after 24 h.

DISCUSSION

The levels of carboxylesterases in blood of guinea pigs are lower than in mice and rats and much closer to those in human and nonhuman primates. These blood carboxylesterases act as scavengers for organophosphates (OPs) and their blood levels may be the most important determinant for the large differences in OP toxicity across species [(9); see also (2)]. Hence, the guinea pig is a suitable experimental animal to investigate prophylactic, pretreatment, and therapeutic measures against OP intoxication, particularly for in vivo studies.

An essential step in the evaluation of the efficacy of these measures is the assessment of the behavioral incapacitation that may be induced by a prophylaxis or pretreatment or the incapacitation that remains after therapy. However, as mentioned earlier, reports on the assessment of guinea pig behavior are scarce and the results of several attempts in our laboratory left much to be desired. After investigating different stimuli, an air stream was subsequently applied as a UCS in a two-way shuttlebox design. The results are shown in Fig. 1: Acquisition is fairly rapid and reproducible between four groups of animals, variability in performance between animals is acceptable, and performance levels reached in 6–7 days was close to 100% avoidances. The administration of different low doses of physostigmine demonstrates that dose–response effects can be reliably obtained. It is not likely that this was due to deterioration of the condition of animals; upon close observation, no overt behavioral changes were detected, whereas their level of activity, judged by the number of ITRs, was not significantly changed.

A carbamate was chosen because carbamate pretreatment protects against OP intoxication (6); pyridostigmine pretreatment has now been adopted by several nations. However, pyridostigmine as a quaternary compound does not readily penetrate the CNS, and does not protect against postintoxication behavioral incapacitation. The tertiary carbamate physostigmine also protects against OP intoxication and since it passes the blood–brain barrier it should offer some protection of the CNS against an OP intoxication and thereby counteract the postintoxication incapacitation. However, at the dose levels of physostigmine required to provide protection against OP intoxication (causing 40–70% carbamoylation of blood AChE) physostigmine itself may induce behaviorally incapacitating effects, presumably due to the fact that it penetrates the brain. It will be clear that incapacitation induced by a pretreatment is unacceptable. Hence, Leadbeater et al. [for a full discussion of these problems, see (8)] attempted to counteract these behaviorally incapacitating effects of physostigmine pretreatment by combining physostigmine with a small dose of the cholinolytic scopolamine [see also (5)]. This combination was effective in a swim test. However, this test measures gross motor performance and it is obvious that other behavioral tests should be applied to investigate the efficacy of the combination of these two drugs. It was for this reason that the second experiment was carried out. As seen in Fig. 3, the addition of a sign-free dose of scopolamine indeed counteracts the performance decrements caused by physostigmine. The results obtained so far indicate that for these and other purposes the behavioral method presented here may be worthwhile considering. The results of the AChE measurements in blood (Fig. 2) demonstrate that the method is sensitive enough to measure the behavioral effects of protective doses of physostigmine that cause a 41–66% inhibition of blood AChE.

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