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COMPARATIVE BEHAVIORAL TOXICITY OF PHYSOSTIGMINE IN FERRETS AND RATS

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Abstract

Marshall-bred ferrets (n = 10) and Sprague-Dawley derived rats (N = 12) were monitored in a photocell cage for 20 minutes following an IP injection of physostigmine salicylate or vehicle. Doses ranged from 0.0125 to 0.4 mg/kg for ferrets and 0.05 to 0.8 mg/kg for rats in equal logarithmic intervals. A repeated measures Latin-square design was used with a minimum of 72 hours between tests. Vertical activity (rearing), horizontal activity (ambulation), and movement time were recorded. Both ferrets and rats showed a reduction in rearing at doses of 0.1 mg/kg or greater.

Ambulation was significantly reduced from vehicle control levels at doses of 0.05 mg/kg or greater for ferrets and 0.1 rng/kg or greater for rats. Ferrets exhibited a significant decrease in movement time at doses of 0.025 mg/kg or greater, whereas rats did not show this reduction until doses of 0.1 mg/kg or larger were administered. It thus appears that, using these behavioral measures, ferrets are more responsive to the effects of the cholinesterase inhibitor, physostigmine, than are rats.

Key words: Physostigmine salicyclate, toxicity, ferrets rats

INTRODUCTION

Preclinical safety evaluation for most therapeutic agents requires that testing be performed in both rodent (primarily rats and mice) and nonrodent species. Dogs and nonhuman primates are the predominantly used nonrodent species for toxicologic studies. The expense, difficulty in obtaining adequate supplies, and the recent rise in public concern have made it increasingly difficult to use these animals for research and testing purposes. The ferret, like the dog, is a member of the order Carnivora and has been the subject of many toxicologic and behavioral studies. These nocturnal animals are relatively small and inexpensive in comparison to dogs and monkeys. They can be readily obtained from local suppliers and can be housed easily under laboratory conditions.

Various differences exist in behavior, anatomy, and physiology as well as in the chemical disposition of drugs in ferrets and rats. The purpose of the present study was to directly compare the effects of physostigmine, a carbamate cholinesterase inhibitor, on the motor behavior of both ferrets and rats in order to provide information that would assist in the selection of a test species for toxicologic evaluation.

MATERIALS AND METHODS

Animals

Ten adult, sexually mature male fitch ferrets (*Mustela putorius furo*), approximately 13 months of age and weighing between 1550 and 1800 g, were obtained from a local supplier. They were individually housed in modified monkey cages (64X46X70 cm), provided with Purina cat food and water at all times (except during testing), and maintained on a 12:12 light-dark cycle with lights on at 0700.

Twelve mature adult male albino Sprague-Dawley derived rats (*Rattus norvegicus domesticus*) were approximately 11 weeks of age at the onset of the experiment and weighed between 250 and 295 g. They were individually housed in stainless steel cages (20.5 X 17.5 X 25.0 cm), with Purina rat chow and tap water freely available, and maintained under the same light-dark regimen as the ferrets but in a separate room.

Drugs

Physostigmine salicyclate was obtained in 2 ml ampules at a concentration of 1 mg/ml and dissolved in a vehicle composed of 0.1 % sodium bisulfite, 2.0% benzyl alcohol, and 97.9% sterile water. Physostigmine dilutions were prepared fresh daily, using the same vehicle formulation. The drug was administered IP in a volume of 1ml/kg bodyweight for both ferrets and rats.

Apparatus

Motor behavior was automatically recorded by a computerized Digiscan animal activity monitor using infrared light beam sensors. The apparatus was used to test both ferrets and rats. The size of the test arena and the position of the infrared photodetectors were adjusted according to the species being examined. For ferrets, the acrylic arena was 1 X 1 m, and the horizontal and vertical photodetectors were positioned 5.5 cm and 23.0 cm, respectively, above the floor of the apparatus. When rats were tested, two 0.5 X 0.5 m acrylic cages were placed diagonally into the arena used to monitor the ferrets, thereby allowing two rats to be tested simultaneously. Horizontal activity sensors were positioned 5.5 cm and vertical activity sensors (rearing) 16 cm above the floor of the cage. The three measures recorded were: (1) vertical activity (rearing), which corresponded to the number of times the vertical beams were broken by the animal, (2) horizontal activity (ambulation), which was expressed as the distance traversed in meters based on information computed from the horizontal

photodetectors, and (3) movement time, which was the time the animals spent moving during the 20-minute test sessions.

Procedure

Ferrets and rats were administered physostigmine or vehicle and placed immediately into the activity monitor for 20 minutes. Each animal was given a complete dose series of physostigmine, one dose every 3-4 days according to a modified Latin-square design. In this manner each animal served as its own control. Based on pilot studies, ferrets were administered vehicle, 0.0125, 0.025, 0.05, 0.1, 0.2, and 0.4 mg/kg physostigmine, and rats were given vehicle, 0.05, 0.1, 0.2, 0.4 and 0.8 mg/kg of the drug. The activity monitor was cleansed with a 70% alcohol solution between trials and allowed to air dry. All testing was performed during the light portion of the light-dark cycle.

Statistical Analysis

Rearing, ambulation, and movement time were totaled for the 20-minute test period, and each parameter was subjected to a one-way repeated measures analysis of variance. Posthoc comparisons were made using Dunnett's test.

RESULTS

In order that absolute values can be determined, means ± SEM of the vehicle control levels have been included in the results. Administration of physostigmine to both ferrets and rats resulted in a dose-dependent decrease in rearing. The number of vertical movements differed significantly from vehicle control levels at 0.05 mg/kg physostigmine or greater for ferrets and 0.1 mg/kg or greater for rats (vehicle means: ferret=158 ± 31, rats= 131 ± 17). Ambulation in meters, an index of horizontal activity, decreased with increased doses of physostigmine in both ferrets and rats. Ambulation was significantly reduced from vehicle levels at 0.05 mg/kg or greater for ferrets (vehicle = 125 ± 9m) and at 0.1 mg/kg or greater for rats (vehicle = 25 ± 3 m).

DISCUSSION

The results of this study clearly indicate that administration of physostigmine salicyclate disrupts rearing, ambulation and movement time in a dose-dependent fashion in both ferrets and rats. When compared to the rat, ferrets exhibited nearly a threefold increase in movement time and a fivefold increase in ambulation scores under vehicle conditions. It is possible that these high baseline levels of activity were responsible for the increased sensitivity of the ferret. We feel that this is unlikely. The ferret

continued to exhibit increased responsiveness to physostigmine even when baseline vertical activity levels were similar for both species. The results of the present study are in agreement with other studies reporting decreases in motor behavior following administration of physostigmine to rodents. Although analysis of motor behaviors following injection of physostigmine to ferrets has not been reported, cholinomimetics, such as arecoline, have been shown to disrupt motor coordination in this species. Considered together, these results indicate that the ferret is more responsive to chemical substances, such as physostigmine and soman, than the rat. Moreover, the ferret is similar to the dog in its behavioral response to cisplatin and metabolism of compounds, such as bifluranol. These investigations suggest that the ferret should be actively considered as an alternative non-rodent species for toxicologic evaluation.

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