INTRODUCTION

In the context of safety pharmacology it is important to verify that CNS drugs are devoid of detrimental effects on cognitive function. The following procedures provide an index of a drug's ability to impair cognitive function relevant to safety assessment. In the passive avoidance test (PA), rats receiving an electric shock in a recognizable environment on a later occasion an avoidance of the same environment. In the Morris Water Maze (MWM) test, rats placed into a circular tank containing water learn to find a fixed and invisible escape platform using extra-maze visual cues. In the Delayed Alternation (DA) test, rats placed in a Skinner box learn to alternate their lever-presses between two retractable levers to obtain food rewards. Standard pharmacological reference agents were used to confirm robust cognitive impairments in each of these assays as a means to provide a potential future benchmark to novel CNS drugs.

MATERIAL & METHODS

Animals:
- Male rats (Wistar-Han, 2-month old, Elevage Janvier, France) were used. They were housed in groups of 2, 5 or 6 in macrolon cages with wood litter (41 x 25 x 18 cm). They had free access to water and food, except in DA (15g/rat/day).

Experimental procedure:
- Passive Avoidance: A rat is placed individually into the light compartment (30 x 30 x 30 cm) of a two-compartment box. After 30 seconds, the door to the dark compartment (20 x 20 x 12.5 cm) is opened. When the rat has entered the dark compartment, the door is closed and the rat immediately receives a 0.8 mA foot-shock (Coulbourn Shock Generator) for 2 seconds. The animal is removed immediately after the shock and is replaced in its home cage (Session 1). 48 hours later the rat is placed again in the light compartment with the door closed (Session 2). The door is opened after 30 seconds and the rat's latency to cross to the dark compartment is recorded (cut-off time = 180 seconds).
- Morris Water Maze: The Morris Maze consists of a circular water tank (Diameter = 15 cm) filled with water maintained at 26-28°C and with an escape platform (Diameter = 1.5 cm) always in the same position 1.5 cm beneath the surface of the water. The water is made opaque by addition of milk powder rendering the platform invisible. The animals are given 4 training sessions over 4 consecutive days. Each training session consists of 4 consecutive trials in the Morris Maze, each separated by 60 seconds. For each trial the animal is placed in the maze at one of two starting points equidistant from the escape compartment and allowed to find the escape compartment. The animal is left on the escape platform for 60 seconds before starting a new trial. If the animal does not find the platform within 120 seconds, the experimenter removes it from the water and places it on the platform. During the 4 trials the animals start the maze twice from each starting point in a randomly determined order per animal. A probe test is performed on Day 5 without the platform and the animal is allowed to swim freely in the maze for 60 seconds.
- Delaysed Alternation: Scopolamine was administered p.o. 30 minutes before a single acquisition session. During each acquisition session the animals were given 36 trials separated by a 10 second inter-trial interval. Each trial commences with the presentation of a single lever (left or right). A lever-press results in the delivery of a food pellet and the retraction of the lever. 5 seconds later, 2 levers are presented and the animals have to press the lever opposite to that previously presented to obtain a food pellet (delayed alternation i.e. non-matching to sample). If the animal does not respond to a one, or two-lever presentation within 20 seconds, the lever(s) are withdrawn without food reinforcement and the next trial starts 10 seconds later. Sessions terminate after the animal has completed 36 trials, or after 30 minutes have elapsed.
- Treatments:
  - Passive Avoidance: Scopolamine and MK-801 were administered s.c. whereas diazepam was administered i.p. 30 minutes before Session 1.
  - Morris Water Maze: Scopolamine and MK-801 were administered s.c. 30 minutes before each of the four acquisition sessions (with no administration before the probe test). Chlordiazepoxide (CDP) was administered p.o. 60 minutes before a single acquisition session.
  - Delayed Alternation: Scopolamine was administered i.p. 30 minutes whereas diazepam was administered p.o. 60 minutes before each of the ten acquisition sessions and once daily during the weekend between the 2 test weeks.

RESULTS

Passive Avoidance

Morris Water Maze

Delayed Alternation

In the PA test, control animals showed a clear increase in the step-through latency at Session 2, as compared with Session 1, indicating that the animals had remembered the shock received at Session 1 (passive avoidance behavior). Scopolamine (0.125-1 mg/kg s.c.), MK-801 (0.025-0.1 mg/kg s.c.) or Diazepam (0.5-2 mg/kg i.p.) significantly (p's < 0.05) decreased the step-through latency at Session 2 in a dose-dependent manner without affecting the step-through latency at Session 1, suggesting amnesia.

In the MWM, control animals showed a clear decrease in escape latency and in distance swum (data not shown) over the acquisition period and spent about 40% of their time or the total distance swum (data not shown) in the platform quadrant during the probe trial, indicating clear learning and retention/memory of the location of the hidden platform. Scopolamine (0.5 mg/kg s.c.) or MK-801 (0.05-0.1 mg/kg s.c.) significantly increased escape latencies (p's < 0.05) and distance swum (data not shown) during acquisition sessions and decreased the % time (p's < 0.05) or distance swum (data not shown) in the target quadrant during the probe test, suggesting learning and memory impairment. CDP (10 mg/kg p.o) clearly attenuated the decrease in escape latency and distance swum from Trial to Trial over a single acquisition session (global mean scores over the last 3 trials as compared with the first trial, p's <0.01), suggesting impairing effects on learning during the acquisition of a Morris Maze task.

In the DA test, scopolamine (0.5 mg/kg i.p.) and diazepam (5 mg/kg p.o) significantly decreased the number of correct responses (p's < 0.05), indicating working memory deficits. Scopolamine also increased simple (p < 0.01) and choice (p < 0.001) reaction times, indicating deficits on attention [simple reaction times] and information processing speed [choice reaction time].

CONCLUSION

These results confirm that anti-cholinergic agents, NMDA antagonists and benzodiazepines induce robust cognitive deficits in each of these selected tests and which can be used to benchmark novel CNS compounds against. These cognitive assays may provide utility in determining potential undesirable effects or discharging perceived risks with novel CNS drugs under development.

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