Screening models for Anti-Amnesic activity evaluation: A Review

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ABSTRACT
Alzheimer’s disease is the most common form of dementia. The disease gets worse as it develops - it is a progressive disease. There is no current cure for Alzheimer's, although there are ways of slowing down its advance and helping patients with some of the symptoms. Alzheimer's is also a terminal disease; it is incurable and causes death. Evidence suggests that compounds especially from natural sources are capable of providing protection against dementia. It is necessary to Screen out medicinal plants for their anti-amnesic potential. Therefore an attempt has been made to review different models for estimating anti amnesic properties of natural products from medicinal plants.

Keywords: Alzheimer’s disease, Dementia, medicinal plant.

INTRODUCTION
Alzheimer’s disease (AD) is a neurodegenerative disease causing memory loss and dementia, which mostly affects the elderly population. The pathophysiology of AD is complex including defective beta-amyloid protein metabolism, abnormalities of glutaminergic, adrenergic, serotonergic and dopaminergic neurotransmission, and the potential involvement of inflammatory and oxidative pathways. According to World Health Organization (WHO), AD affects 22 million people worldwide, out of which; over 3 million are in India. Its prevalence rises sharply from about 5% at the age of 95 years. AD has become a major problem, particularly in developed countries due to increasing old age population with a high life quality. Based on clinical and experimental evidences, Acetylcholine is considered as the most important neurotransmitter involved in the regulation of cognitive function. Besides the neuro pathological hallmarks of Alzheimer’s disease, neurofibillary tangles and neurotic plaques, Alzheimer’s disease are characterized by a consistent deficit in cholinergic neurotransmission particularly in basal forebrain. Currently, there’s no cure for Alzheimer’s disease. Sometimes doctor prescribed, drugs to improve symptoms that often accompany Alzheimer’s, including sleeplessness, wandering, anxiety, agitation, and depression. The drugs currently used are Tacrine hydrochloride (Cognex) and Donepezil hydrochloride (Aricept), Rivastigmine (Exelon) and Galantamine (Reminyl). They bolster the efficiency of nerve cells most affected by Alzheimer disease. However, the effects are short lived and don’t cure the disease. This review presents various models to screen various traditional drugs and modern drugs for anti-amnesic effects. This brings a compilation make easy to go through the various models at same time and make it easy to screen the drugs. It reveals principle, apparatus and procedure of these models.
SCREENING TECHNIQUES

Various laboratory models for testing learning and memory are,

- Scopolamine induced amnesia (Interoceptive Behavior Model).
- Memory impairment by basal forebrain lesion in rats (Interoceptive Behavior Model).
- Ischemia-induced amnesia in gerbils (Interoceptive Behavior Model).
- Elevated plus maze (Exteroceptive Behavior Model).
- Passive avoidance paradigm (Exteroceptive behavior models).
- Morris water maze (Exteroceptive behavior models).
- Shuttle box avoidance (Two-way shuttle box).

SCOPOLAMINE INDUCED AMNESIA IN MICE

Principle

The administration of the anti muscarinic agent scopolamine to young human volunteers produces transient memory deficits. Analogously, scopolamine has been shown to impair memory retention when given to mice shortly before training in a dark avoidance task. The ability of a range of different cholinergic agonist drugs to reverse the amnesic effects of scopolamine is now well documented in animals and human volunteers. However, the neuropathology of dementia of the Alzheimer type is not confined to the cholinergic system.

Procedure

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After a brief orientation period, the mouse enters the second darker chamber. Once inside the second chamber door is closed which prevents the mouse from escaping, and a 1 mA, 1 -sec foot shock is applied through the grid floor. The mouse is then returned to the home cage. Twenty-four hours later, testing is performed by placing the animal again in the bright chamber. Latency in entering the second darker chamber within a 5 min. test session is measured electronically. Whereas untreated control animals enter the darker chamber in the second trial with latency about of 250 sec, treatment with scopolamine reduces the latency to 50 sec. The test compounds are administered 90 min before training. A prolonged latency indicates that the animal remembers that it has been punished and, therefore, does avoid the darker chamber. Using various doses after treatment with test compounds are expressed as percentage of latencies in mice treated with scopolamine only. In some cases straight doses-response curves can be established whereas with other drugs inverse U-shaped dose responses are observed.

MEMORY IMPAIRMENT BY BASAL FOREBRAIN LESIONS IN RATS

Principle

Memory impairment can be produced by lesions caused by bilateral injections of ibotenic acid into the basal forebrain of rats. Water maze tasks, habituation tasks, passive avoidance tasks with a light/dark compartment apparatus, and the inhibition of the decrease of choline acetyl transferase activity in the cortex can be used to evaluate the effect of drugs.

Procedure

Male Wistar rats weighing 270–310 g are anesthetized with sodium pentobarbital (45 mg/kg i.p.) and placed in a stereotaxic apparatus. Neurotoxic lesions of the basal forebrain are produced by injection of ibotenic acid. An injection needle connected to a 5-μl micro syringe is inserted into the basal forebrain, identified according to the Paxinos and Watson (1986) atlas of rat brain (1.5 mm posterior, 2.8 mm bilateral to the bregma, 7.3 mm below the dura). Ibotenic acid is dissolved in 50 mM Na phosphate buffer at a concentration of 12 μg/ml.
and then 0.5 ml (6 μg per side) is infused for 5 min. The injection needle is left in place for an additional 5 min to allow the toxin to diffuse away from the needle tip. One week later, the contra lateral side is treated in the same manner. The same procedure is used to administer microinjections of 50 mM Na phosphate buffer into the basal fore brain of sham-operated rats. The lesion sites are mainly distributed in the ventromedial globus pallidus.

Three to 5 weeks after the first lesion, the animals are tested on the acquisition of a task in a Morris watermaze (Morris 1981), on a habituation task in a novel situation, and in a passive avoidance task with light and dark compartments. The rats are treated once a day during the experiment.

After the behavioral experiments, the animals are sacrificed for determination of choline acetyl transferase activity in the brain according to Fonnum (1975). The tissue is homogenized (4% w/v) in cold 50 mM Na phosphate buffer (pH 7.4), and Triton X-100 (0.55, v/v) is added to homogenates to ensure enzyme release. To 75 μl of enzyme solution, 125 μl of substrate mixture (0.4 mM [14C]acetyl-Co A (50.6 mCi/mmol), 300 mM NaCl, 50 mM Na phosphate buffer (pH 7.4), 8 mM choline chloride, 20 mM EDTA-2Na, and 0.1 mM physostigmine) is added in a scintillation vial and the mixture is incubated at 37 °C for 30 min. After the incubation, 0.8 ml of cold 50 mM phosphate buffer, 0.5 ml of acetonitrile containing 2.5 mg of tetraphenyl borate and 2.0 ml toluene are added to the scintillation vial. The vials are shaken lightly and allowed to stand overnight before radioactivity is determined.

ISCHEMIA-INDUCED AMNESIA IN GERBILS

Principle
Impairment of cerebral metabolism induced by reduced blood supply is known to induce cognitive deficit. Because of the absence of posterior communicating arteries in the brain of Mongolian gerbils, complete forebrain ischemia can be produced by occluding both common carotid arteries resulting in amnesia.

Procedure
Male Mongolian gerbils (strain Hoe:Gerk) weighing 50–70 g are anesthetized by i.p. pentobarbital injection. Both common carotid arteries are exposed through a ventral neck incision and occluded for 5 or 10 min with miniature aneurysm clips. In sham-operated controls, the common carotid arteries are exposed but not occluded. Twenty-four hours after occlusion, each animal is placed in the bright part of a light/dark-chambered apparatus for training. After a brief orientation period, the gerbil enters the second, dark chamber. Once inside the second chamber, the door is closed which prevents the animal from escaping, and a 100 V, 2-s foot shock is applied through the grid floor. The gerbil is then returned to the home cage. Twenty-four hours later, testing is performed by placing the animal again in the bright chamber. The latency in entering the second dark chamber within a 5 min test session is measured electronically. The latency compared with sham-operated controls is decreased depending on the duration of ischemia. After drug treatment, an increase of latency in entering the dark compartment indicates good acquisition.

ELEVATED PLUS-MAZE

Principle
Elevated plus-maze served as the exteroceptive behavioral model to evaluate memory in mice.

Procedure
The elevated plus-maze for mice consisted of two open arms (16cm×5cm) and two covered arms (16cm×5cm×12cm) extend from a central platform (5cm×5cm), and the maze was elevated to a height of 25cm from the floor. On the first day (i.e. eighth day of drug treatment), each mouse was placed at the end of open arm, facing away from the central platform. Transfer latency (TL) was defined as the time (in seconds) taken by the animal to move from the open arm into one of the covered arm with all its four legs. TL was recorded on the first day (training session) for each animal. The mouse was allowed to explore the maze for another 2 minutes and then returned to its home cage. Retention of this learned task (memory) was examined 24 hours after the first day trial (i.e. ninth day, 24 hours after last dose). Significant reduction in the TL value of retention indicated improvement in memory.

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PASSIVE SHOCK AVOIDANCE PARADIGM

Principle
Passive avoidance, based on negative reinforcement, was recorded to examine the long term memory.

Procedure
The apparatus consisted of a box (27cm×27cm×27cm) having three walls of wood and one wall of Plexiglas, featuring a grid floor (made up of 3mm stainless-steel rods set 8mm apart), with a wooden platform (10cm×7cm×1.7cm) in the center of the grid floor. The box was illuminated with a 15W bulb during the experimental period. Electric shock was delivered to the grid floor. The rats were initially trained as follows: each rat was placed on a wooden platform set in the center of the grid floor. When the rat stepped down and placed its paw on the grid floor, shock (foot shock: 50Hz; 1.5mA; 1s) was delivered and step down latency (SDL) was recorded. SDL is defined as the time taken by the rat to step down and place all four paws on the grid floor. Rats showing SDL in the range of 2-15s during the training session were taken for the acquisition and the retention task. The acquisition task was carried out 90min after the training session. During the acquisition test, animals were removed from the shock free zone if they did not step down for a period of 60s. Retention was tested after 24h in a similar manner, except with an upper cut-off time of 180s.
MORRIS WATER MAZE

Principle
A task was developed where rats learn to swim in a water tank to find an escape platform hidden under the water (Morris 1984). As there are no proximal cues to mark the position of the platform, the ability to locate it efficiently will depend on the use of a configuration of the cues outside the tank. Learning is reflected on the shorter latencies to escape and the decrease on the length of the path to find the platform. Although rodents can find the platform by using non-spatial strategies, the use of a spatial strategy is the most efficient way to escape and young animals develop the spatial strategy after a small number of trials.

Procedure
Different strains of rats are generally used (Long Evans, Wistar, Sprague-Dawley). The apparatus is a circular water tank filled to a depth of 20 cm with 25 °C water (Brioni et al. 1990; Morris 1984). Four points equally distributed along the perimeter of the tank serve as starting locations. The tank is divided in four equal quadrants and a small platform (19 cm height) is located in the centre of one of the quadrants. The platform remains in the same position during the training days (reference memory procedure). The rat is released into the water and allowed 60–90 s. to find the platform. Animals usually receive 2–4 trials per day for 4–5 days until they escape onto the platform. Well trained rats escape in less than 10s.

SHUTTLE BOX AVOIDANCE (Two way shuttle box)

Principle
Compared to runway avoidance, shuttle box avoidance (two-way shuttle-box) is a more difficult task. Since the animal is not handled between trials, the shuttle box can be easily automated.

Procedure
Compared to runway avoidance, shuttle box avoidance (two-way shuttle-box) is a more difficult task. The apparatus used consist of a rectangular box 50 x15 cm 2 with 40 cm high metal walls, and an electrifiable grid floor. The box is divided by a wall with a manually or solenoid operated guillotine door (10 x 10 cm2) into two 25 x 15 cm2 compartments. Each compartments can be illuminated by a 20 W bulb mounted in the hinged Plexiglas lids. A fixed resistance shock source with an automatic switch (0.5 sec on 1.5 sec off) is used. Simple programming equipment provides for automatic delivery of the command stimulus (CS) and the unconditioned stimulus (US). The apparatus is placed in a dimly lit room with a masking noise background (whit noise) of 60 dB. The animal is allowed to explore the apparatus for 5 minutes with the connecting door open and the compartment lights switched off. The guillotine door is then closed.20 sec the light is switched on in the compartments containing the animal and the door is opened. A tone (CS) is presented and 5 sec later the floor shock is
applied in this illuminated compartment and continued until the animal escapes to the dark side of the compartment; the connecting door is close and the shock discontinued. After in variable inter trial (ITI; 30-90Sec) the light is switched on in previous dark compartment, the door is opened and animal is required to cross to another side. The training is continued until animal reaches critical of 9 avoidances in 10 consecutive trials. Retention is tested at different interval after the original training by the retaining the animal to same criterion again. The animal need to reach the safe on both days is measured. In addition the number error (not reaching the safe area) is recorded. The task is rather difficult due to lack of permanent safe area ‘lack of simple instrumental response, presences of variable aversive gradient and increased weight of emotion factor6.

**Fig: 4 Shuttle Box Avoidance**

**REFERENCES**


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