2. Neuropharmacological screening techniques for pharmaceuticals: A review

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Summary. This review presents various models to screen various traditional drugs and modern drugs for neurological 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. This screening model can be use to study of memory enhancing, Anxiolytic, Antidepressant and anticonvulsant activity.

Introduction

The Central nervous system (CNS) comprising of the brain and spinal cord process information with the help of chemical messenger viz. neurotransmitter, neuromodulators, neuroregulators, neuromediators and neurotropic factor which act via specific mechanism to mediate neurotransmission, neurotransmitter viz., nor adrenaline, adrenaline, dopamine, Gamma Amino Butyric Acid (GABA), glutamate, acetylcholine, 5-hydroxytryptamine (5-HT), peptides viz. endorphins, serotonin, glycogen and vasoactive intestinal polypeptides (VIP) etc and neuromodulator viz. prostaglandins (PGs), purines and neuropeptides interact with their recognition sites i.e. receptor and regulate the function of CNS (1).
According to the world health report (WHO 2001) approximately 450 million people suffer from a mental or behavioral disorder, yet only a small minority of them receive even the most basic treatment this amount to 12.3% of the global burden of disease and will rise to 15% by 2020 (2).

Drug acting in the central nervous system were among the first to be discovered by the primitive human and are still the most widely used group of pharmacological agents. The CNS acting drugs are invaluable therapeutically, because they can produce specific physiological and psychological effects from the vast array of material medica of the indigenous system so many plants have been reported to have activity against CNS disorders and thus act as very useful remedies for the alleviation of human suffering (3).

In the search of new therapeutic product for the treatment of neurological disorder medicinal plant research worldwide has progressed constantly, demonstrating the pharmacological effectiveness of different plant species in a variety of animal models.

Many classical animal models of preliminary pharmacological tests of activities on CNS which provide information about action upon psychomotor performance, motor behaviour and neurotoxicity. The depression activity gives an indication of the level of the excitability of the CNS and this decrease may be related to sedation resulting from depression of CNS (4).

This review presents various models to screen various traditional drugs and modern drugs for neurological 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.

**Neuropharmacological screening techniques**

**1. Memory enhancing activity**

Dementia is a mental disorder characterized by loss of intellectual ability sufficiently severe as to interfere with one's occupational or social activities. Dementia is of several types and it invariably involves impairment of memory. The most common cause of dementia is Alzheimer's disease, which is a progressive neurodegenerative disorder associated with loss of neurons in distinct brain areas. The central cholinergic pathways play a prominent role in learning and memory processes (5). Centrally acting antimuscarinic drugs (e.g. scopolamine) impair learning and memory both in animals (6) and human beings (7). Epidemiological studies of Indian population reveal that dementia is largely a hidden problem (8). Prevalence rates for dementia increase exponentially with advancing age (9 &10). Since allopathic system
of medicine is yet to provide a radical cure, it is worthwhile to look for new directions, which would minimize the memory loss seen in elderly patients. Various laboratory models for testing learning and memory are

1. Scopolamine induced amnesia (Interoceptive Behaviour Model).
2. Diazepam induced amnesia (Interoceptive Behaviour Model).
3. Elevated plus maze (Exteroceptive Behaviour Model).
4. Shuttle box avoidance (Two-way shuttle box).
5. Passive avoidance paradigm (Exteroceptive behavior models).

### 1.1. Scopolamine-induced amnesia in mice

#### Principle

The administration of the antimuscarinic agent scopolamine to young human volunteers produces transient memory deficits (11). Analogously, scopolamine has been shown to impair memory retention when given to mice shortly before training in a dark avoidance task (12, 13, & 14). 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 (15).

#### Procedure

The antimuscarinic agent scopolamine is administrated to young human volunteers produces transient memory deficits. The scopolamine has been shown to impair memory retention when given to mice shortly before training in a dark avoidance task.

The scopolamine test is performed in groups of 12 male NMRI mice weighing 26-32g in a one-trial, passive avoidance paradigm. After five minutes, administration of 3 mg/kg i. p. scopolamine hydrobromide, each mouse is individually placed in the bright part of a two-chambered (bright and dark) apparatus for training.

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 -see 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. The 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 see, 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 latencies 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.

1.2. Elevated plus-maze (exteroceptive behaviour model)

Principle

Out of many possibilities to modify maze tests e.g. water maze (16), the Y-mace, the radial maze (17), and the elevated plus maze (18 & 19) have found acceptance in many laboratories. The test has been proposed for selective identification of anxiolytic and anxiogenic drugs. Anxiolytic compounds, by decreasing anxiety, increase the open arm exploration time; anxiogenic compounds have the opposite effect.

Procedure

Elevated plus maze used to evaluate learning and memory in mice. The procedure, technique and end point for testing learning and memory is followed as per parameters described by the investigators working in the area of neuropsychopharmacology.

The apparatus consists of two open arms (16cm x 5cm) and two closed arms (16cm x 5cm x12cm). The arms extended from a central plat from (5cm x5cm) and the maze is elevated to a height of 25 cm of the floor. On the first day, such mouse is placed at the end of an open arm, facing way from the central platform. Transfer latency (TL) is taken by mouse with all its four legs to move into one of enclosed arms. TL is recorded on the first day. If the animal did not entered arms within 90 sec. It is gently pushed into one of two enclosed arms and TL is recorded at 90 s. then three consecutive mice are allowed to explore the maze for another 10s and then returned to its home cage. Retention of this learned –task is examined 24 H after the first day trial. Another laboratory model viz. passive avoidance apparatus was employed to
substantiate the findings and overcome the limitations of the elevated plus-maze.

1.3. 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 (20).

Procedure

Rats of both sex are used and maintained under standard conditions. The apparatus 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 cm$^2$) into two 25 x 15 cm$^2$ 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 factor.
1.4. Passive avoidance paradigm (exteroceptive behavior model)

Principle

One of the most common animal tests in memory research is the inhibition to imitate activities or learned habits. The term “passive avoidance” is usually employed to describe experiments in which the animal learns to avoid a noxious event by suppressing a particular behaviour.

Procedure

Passive avoidance behavior based on the negative reinforcement used to examine the long term memory (21 & 22). The apparatus considered of a box (27cm x 27cm x 27cm) having three vales of wood and one wall of Plexiglas, featuring a grid floor (3mm stainless steel rods set 8mm apart), with a wood platform(10cm x 7cm x 1.7cm), in center of grid floor. The box is illuminated with 15 W bulbs during experimental period. Electric shock (15V AC) is delivered to the grid floor. Training is carried out in two similar sessions. Each mouse gently placed on wood platform set in center grid floor. When the mouse stepped down place all its paws on the grid floor, shock is delivered to 15 sec and step-down latency (SDL) is recorded. SDL is defined as time taken by mouse to sleep down from wood platform to grid floor all its paws on grid floor. Animal showing the SDL in rang (2-15 sec) during first test are used for the second session and the retention test. The second session carried out at 90 min after the first test. When animal stepped down for period of 60sec retention is tested after 24 h in the similar manner, except electric shock is not applied to grid floor. Each mouse again place on the platform and the SDL is recorded, with the upper cut of time of 300 sec.

Olfactory learning

Principle

Odour sensitativity of Rodents are provide learning information on the environment and the learning of successive olfactory discrimination problems in rats is closely related to the acquisition rules of higher primates. Odor-reward associations are learned in few trials as odors exert more discriminative control over other sensory modalities like tones or lights (23). Animals have to learn to discriminate an arbitrary designated positive odor or negative one.
**Procedure**

Rats (Sprague-Dawley or Long Evans) are generally used. In procedures described in the literature (24), animals are deprived of water for 48 h before the training and during the test they receive *ad libitum* water for only 30 min. The olfactory apparatus is a rectangular box (30 × 30 × 55 cm) with a photosensitive cell mounted on top of the water spout/odour outlet. Rats are trained to approach the water spout and to brake the light beam. Responses to the positive odor are rewarded with water while responses to the negative odour results in the presentation of a light flash. The intertrial interval before the presentation of a new odor is usually 15 s. and the sessions last 30 min. per day. Sessions are terminated when the rat makes 90% correct choices or after 400 trials. The animals are rewarded with 0.05 ml of water when it brakes the beam to the positive odour or when it does not respond to the negative odour. Incorrect responses (“no go” to the positive odour or “go” to the negative odour are followed by a flash and a longer intertrial interval). Results are reported as the % correct responses or as a logit transformation of the % correct/incorrect response ratio.

**Water maze test**

**Principle**

Water maze test is a behavioral procedure widely used in behavioral neuroscience to study spatial learning and memory. It was developed by neuroscientist Richard G. Morris. Who used it to show that lesions of the hippocampus impaired spatial learning. Spatial learning of rats can be tested in a water maze as described by Morris (25).

**Procedure**

The water maze consists of a circular tank with 100 cm diameter and a wall 20 cm above the water level. A circular platform (9 cm diameter, covered with white linen material for grip) is hidden 2 cm below the water level. The water is made opaque using titanium dioxide suspension and is kept at about 23 °C during the experiment. Training takes place on three consecutive days, with the rats receiving 4 consecutive trials per day with an inter-trial interval of 6–10 min. Each trial is started from one of four assigned polar positions with a different sequence each day. The latency to find the platform is measured as the time of placement of the rat in the water to the time it finds the platform. If the animal fails to find the platform in any trial within 3 min it is placed on it for 10 s. On day four a probe test is performed. The platform is removed and the time spent in
the target quadrant and the number of annulus crossings (across the actual location where the platform has been located) in the first 60 s of exposure are measured. The time to the first annulus crossing is also taken as a measure of performance on the 13th (i.e. probe) trial.

Anxiolytic activity

Principle

The animal model is considered one of the most widely validated tests of assaying sedative and anxiolytic substances such as benzodiazepines. The test drug induced anxiolytic effect beginning at lower doses employed. An increase of most important variables of EPM test was found as follows: the percentage of time of mice spend on the open arms as well as the percentage of entries in the dark arms. The anxiolytic effect is also evidenced through light and dark test. As with the EPM test, this model is useful for modeling of anxiety. The low dose dependent effect could be attributed to biological variability, as well as chemical complexity of the test drug. Various model of anti anxiolytic testing are (26 & 27).

1. Elevated plus-maze model (EPM).
2. Forced swimming test (FST).
3. Light–dark test (LDT)
4. Open–field test (OFT)
5. Cork gnawing test in the rat
6. Mcpp induced anxiety in rats

1. Elevated plus-maze (EPM)

This is widely validated to measure anxiety to rodents. This apparatus is made of Plexiglas and consisted to two open arms (30 cm x 5 cm) with 30 cm walls. The arms extended from the central platform (5 cm x 5 cm). This wall is elevated 38.5 cm from the room floor. The each animal is placed at the center of maze, facing one of the enclosed arms. Number of entries and time spend in enclosed and open is recorded for 5 min test. Entry an arm is defined as animal placing all four paws onto the arm. All tests are tapped by using a video camera. After each test, the maze is carefully cleaned up with wet tissue paper (10% ethanol solution).

2. Forcing swimming test (FST)

The FST is most widely used pharmacological in vivo model assessing anti depressant activity. The development of immobility when mice are
placed in inescapable cylinder fill with water reflects cessation of persistent escape directed behavior. The apparatus consist of clear Plexiglas cylinder (20cm height x 12 cm diameter) filled to a 15cm depth within the water (25°C) in the pre-test session, every animal is placed individually into cylinder for 15 min. 24h prior to the 5min swimming test the test drug and distilled water are administered three time, immediately after 15 min pre-test, 18 and 1h prior to the swimming test. During the test session a trained observer registered the immobility time, considered to be when the mouse made no further attempts to escape, apart from the movements necessary to keep its head above the water. It is suggested that the immobility reflected a state of lowered mood in which the animals had given up hope of finding an exit and had resigned themselves to the experiments situation.

3. Light dark test (LDT)

The apparatus consists of a Plexiglas box with two compartments (20 x 20 cm) one of which is illuminated with a white light while the other remained dark. Each animal is placed at the centered of the illuminated compartments; facing one of the dark places, as well as the number of entries in each space is recorded for 5 min.

4. Open field test (OFT)

Open field test area is made of acrylic transparent walls and black floor (30 cm x 30 cm x 15 cm) divided into nine square of equal area. The open field is used to evaluate the exploratory activity of animal (28). The observed parameters are the number of square crossed (with the four paws) and number of rearing.

5. Cork gnawing test in the rat

Principle

Cork gnawing behavior in the rat has been proposed for screening method for buspirone-like anxiolytics (29).

Procedure

Adult male rats are used in experiment. They are housed 4 per cage on a regular light/dark cycle with free access to food and water except for the period between injection and the end of a test session. For the test session one animal is placed in a stainless steel cage with wire mesh bottom. A session
consists of placing the subject in the test cage with a cork stopper weighing between 2–3 gm for 30 min. Initially, the amount gnawed is relatively high and variable within and between subjects. After 30 training sessions, the Ultrasounds are recorded with suitable detectors with 42 kHz as the center of a 10 kHz recording range. The output of the detectors is fed into pen recorders. The total number of ultrasonic cries in the two sessions of hand holding and the two sessions of tail holding are calculated and used as the control activity of each pup. Any pup producing a total of less than 50 ultrasounds when held by the tail is excluded from the drug study. The pups are kept in the home cage in the test laboratory until the afternoon. Three to four hours after the first test the pups are randomly allocated to several equally seized groups, weighed, marked, and dosed intraperitoneally either with the vehicle or drug and placed back in the home cage. Thirty min after dosing, each pup is subjected to the same handling stress as that used in the morning session, and the total number of sounds produced is calculated in the same way. The afternoon response to tail holding is expressed as a factor of the morning response. The mean factor for the saline-treated animals is taken to be 100% in calculations of percentage changes in ultrasound emission by drugs.

6. mCPP induced anxiety in rats

Principle

The metabolite of the antidepressant drug trazodone 1- (3-chlorphenyl) piperazine (= mCPP), classified as 5- HT1C agonist (30). The compound induces hypophagia (31) and hypolocomotion (32).

Procedure

Male Sprague Dawley rats (220–250 g) are housed in groups of 6 under a 12 h light/dark cycle with free access to food and water.

mCPP - induced locomotion

Rats are placed in a room adjacent top the experimental room on the day of the procedure. They are dosed either orally 1 h, or i.p. 30 min before the locomotion test with test compound or vehicle, and injected 20 min before the test with 7 mg/kg mCPP i.p. or saline in groups of four. Rats are returned to their home cages after dosing. At 0 h they are each placed in automated locomotor activity cages made of black Perspex with a clear Perspex lid and sawdust covered floor under red light for 10 min. During this time, locomotion is recorded by means of alternately breaking two photocell beams traversing opposite ends of the box 3.9 cm above floor level.
**MCPP-induced hypophagia**

Rats are individually housed on day 1 and on day 3 they are deprived of food. Twenty-three hours later, they are orally treated with the test drug or vehicle and returned to their home cages. Forty min later, they are given 5 mg/kg mCPP or saline i.p. and again returned to their home cages. After a further 20 min, weighted amount for their normal food pellets are placed in their food hoppers and the amount remaining after 1 h are measured.

The effect of the test compound on mCPP-induced hypolocomotion is determined by one-way ANOVA and Newman-Keuls test. The dose producing 50% disinhibition of mCPP is also estimated.

**Antidepressant activity**

Antidepressant activity is indicated the mood elevating due to various mechanisms of the antidepressant drugs, such as inhibition of the enzyme of monoamine oxidase, inhibition of reuptake bioamines and enhancement of the concentration of 5-HT e) ct. Later on, inhibition of reuptake of bioamines was found to be main mechanism of action to down regulation of β receptor (33). Several lines of preclinical and clinical evidence indicates that enhancement of 5-HT mediated neurotransmission might underline the therapeutic effect of most of the antidepressant. This behavioral effect very similar to that found by other author after treating mice with classical antidepressant drugs as IMI (34).

Various models for antidepressant activities are as follows.

1. Despair Swim Test
2. Learned helplessness test
3. Muricide behaviour in rats
4. Tail suspension test in mice

**1. Despair swim test**

**Principle**

Behavioural despair was proposed as a model to test for antidepressant activity. It was suggested that mice or rats forced to swim in a restricted space from which they cannot escape are induced to a characteristic behaviour of immobility. This behaviour reflects a state of despair which can reduce by several agents which are therapeutically effective inhuman depression (35 & 36).
**Procedure**

Male Sprague-Dawley rats weighing 160–180 g are used. They are brought to the laboratory at least one day before the experiment and are housed separately in Makrolon® cages with free access to food and water. Naive rats are individually forced to swim inside a vertical, Plexiglas cylinder (height: 40 cm; diameter: 18 cm, containing 15 cm of water maintained at 25°C). Rats placed in the cylinders for the first time are initially highly active, vigorously swimming in circles, trying to climb the wall or diving to the bottom. After 2–3 min activity begins to subside and to be interspersed with phases of immobility or floating of increasing length. After 5–6 min immobility reaches a plateau where the rats remain immobile for approximately 80% of the time. After 15 min in the water the rats are removed and allowed to dry in a heated enclosure (32 °C) before being returned to their home cages. They are again placed in the cylinder 24 h later and the total duration of immobility is measured during a 5 min test. Floating behaviour during this 5 min period has been found to be reproducible in different groups of rats. An animal is judged to be immobile whenever it remains floating passively in the water in a slightly hunched but upright position, its nose just above the surface. Test drugs or standard are administered one hour prior to testing. Since experiments with the standard drug (imipramine) showed that injections 1, 5 and 24 h prior to the test gave the most stable results in reducing floating these times are chosen for the experiment.

**2. Learned helplessness in rats**

**Principle**

Animals exposed to inescapable and unavoidable electric in one situation later fail to escape shock in a different situation when escape is possible (37). This phenomenon was evaluated as a potential animal model of depression (38 & 39). On day 19th of the investigation rats are subjected to foot shock (60 scrambled shocks, 15s duration, 0.8 mA, every min) in a two compartment jumping box with the escape door to the unelectrified adjoining compartment closed. The exercise continued for one h. On day 21, 48 hour afterwards the rats are subjected to avoidance training using the same apparatus but keeping the escape route to the un-electrified chamber open. During this avoidance training, the rats are placed in the electrified chamber allowed to acclimatise for 5 min before being subjected to 30 avoidance trials with an intertrial interval of 30s. During the first 3 s of trials a buzzer
stimulus is presented followed by electro shock through the grid floor for the next 3s. The avoidance response, characterized by escape to the adjoining “safe” chamber during conditioned stimulus, is noted. Failure to escape during unconditioned stimulus within 15 s is assessed as “escape failure”.

3. Muricide behaviour in rats

Principle

Horovitz et al. described a selective inhibition of mouse-killing behaviour in rats by antidepressants (40). The test can be used to evaluate antidepressants such as tri-cyclic and MAO inhibitors.

Procedure

Male Sprague-Dawley rats (300–350 g) are isolated for 6 weeks in individual cages. They have access to food and water ad libitum. One mouse is placed into the rat’s cage. About 10 to 30% of rats kill the mouse by biting the animal through the cervical cord. Only rats consistently killing mice within 5 min after presentation are used for the test. The mice are removed 15 to 45 s after they have been killed in order to prevent the rats from eating them. Drugs are injected i.p. to the rats before the test. Mice are presented 30, 60 and 120 min after drug administration.

4. Tail suspension test in mice

Principle

The “tail suspension test” was described by (41) as a facile means of evaluating potential antidepressants. The immobility displayed by rodents when subjected to an unavoidable and inescapable stress has been hypothesized to reflect behavioral despair which in turn may reflect depressive disorders in humans.

Procedure

Male mice weighing 20–25 g are used preferentially. They are housed in plastic cages for at least 10 days prior to testing in a 12 h light cycle with food and water freely available. Animals are transported from the housing room to the testing area in their own cages and allowed to adapt to the new environment for 1 h before testing. Groups of 12 animals are treated with the test compounds or the vehicle by intraperitoneal injection 30 min prior to
testing. For the test the mice are suspended on the edge of a shelf 58 cm above a table top by adhesive tape placed approximately 1 cm from the tip of the tail. The duration of immobility is recorded for a period of 5 min. Mice is considered immobile when they hang passively and completely motionless for at least 1 min. The percentage of animals showing the passive behavior is counted and compared with vehicle treated controls. Using various doses, ED50 values can be calculated.

**Anti-Convulsant activity**

Different type of epilepsies that is grand mal, petit mal or psychomotor type can be studied in laboratory animals. Various model of Ant convulsion test are (42).

1. Pentylenetetrazole (PTZ) Seizer Test
2. Electro Shock (MES) –Induced Convulsions
3. Strychnine-induced convulsions
4. Picrotoxin-induced convulsions
5. Other Test
   a. Toxicity profile
   b. Effect on pentobarbital –induced sleeping time
   c. Motility test
   d. Amphetamine toxicity test

1. **Pentylenetetrazole (PTZ) seizer test**

**Principle**

This assay has been used primarily to evaluate antiepileptic drugs. However, it has been shown that most anxiolytic agents are also able to prevent or antagonize Metrazol-induced convulsions.

**Procedure**

Mice of either sex with a body weight between 18 and 22 g are used. The test compound or the reference drug is injected sc. or i.p. or given orally to groups of 10 mice. Another group of 10 mice serves as control. Fifteen min after sc.-injection, 30 min after i.p.-injection, or 60 min after oral administration 60 mg/kg MTZ (Metrazol) are injected subcutaneously. Each animal is placed into an individual plastic cage for observation lasting 1 h. Seizures and tonic-clonic convulsions are recorded. At least 80% of the animals in the control group have to show convulsions.
2. Electro shock (MES) induced convulsions

Principle

The electroshock assay in mice is used primarily as an indication for compounds which are effective in grand mal epilepsy. Tonic hind limb extensions are evoked by electric stimuli which are suppressed by antiepileptics but also by other centrally active drugs (43).

Procedure

The maximum electrical shock (MES) induced convulsion in animals represented grand mal type of epilepsy. These are type of procedures use to studies convulsions and to test to anticonvulsant drugs in laboratory animals. In MES convulsions electric shock is applied through the corneal electrode, through optic stimulation cortical excitation are produced. The MES – convulsion are divided into five phase such as Tonic flexion, Tonic extensor, Clonic convulsions, stuper, recovery or death. A substance is known to possess anticonvulsant property if it reduces or abolished the extensor phase of MES convulsions. This procedure may be used to produce convulsions both in rat and in mice.

In this method place corneal electrodes on the cornea and apply the prescribed current and different stages of conclusions are noted as described in previous paragraph. Note the time (sec) spent by the animal in each phase of the conclusions. Inject phenytoin i.p. in rats. Wait for 30 min and subject the animals to electro-convulsions as described. Note the reduction in time or abolition of tonic extensor phase of MES convulsions.

3. Strychnine-induced convulsions

Principle

Strychnine is induced convolution action due to interference with postsynaptic inhibition mediated by glycine. It is an important inhibitory transmitter to nerve conduction in the spinal cord, and strychnine is blocked selective and competitive antagonism. The inhibitory effects of glycine at all glycine receptors. Strychnine-sensitive postsynaptic inhibition in higher centers of the CNS is also mediated by glycine.

Procedure

Groups of 12 mice of either sex with a weight between 20 and 25 g are used. They are treated orally with the test compound or the standard (e.g. diazepam
5 mg/kg). One hour later the mice are injected with 2 mg/kg strychnine nitrate i.p. The time until occurrence of tonic extensor convulsions and death is noted during a 1 h period. With this dose of strychnine convulsions are observed in 80% of the controls. *ED$_{50}$*-values are calculated using various doses taking the percentage of the controls as 100%. For time-response curves the interval between treatment and strychnine injection varies from 30 to 120 min.

4. Picrotoxin-induced convulsions

**Principle**

Picrotoxin is GABA$_{a}$-antagonist results chloride ion channel blocked due to GABA$_{a}$ receptor complex causes Picrotoxin induced convulsions which is used further evaluate CNS-active compounds.

**Procedure**

Groups of 12 mice of either sex with a weight between 20 and 25 g are treated either orally or i.p. with the test compound or the standard (e.g. 10 mg/kg diazepam i.p.). Thirty min after i.p. treatment or 60 min after oral administration the animals are injected with 3.5 mg/kg s.c. picrotoxin and are observed for the following symptoms during the next 30 min: clonic seizures, tonic seizures, death. Times of onset of seizures and time to death are recorded. For time-response curves the animals receive the drug 30, 60 or 120 min prior to picrotoxin. Protection is expressed as percent inhibition relative to vehicle control. The time period with the greatest percent inhibition is said to be the peak time of drug activity. *ED$_{50}$*- values are calculated taking the percentage of seizures in the control group as 100%.

5. Other methods

1. Effect on pentobarbital-induced sleeping time

Rats were divided into groups [n=6] the group received dose of anticonvulsants intraperitonealy, while the control group received an equal volume of vehicle. After 10 min al animal received 50mg/kg (i.p.) of pentobarbital. The time that elapsed between loss of recovery of right reflex was taken as the seeping time and was recorded both are control and pretreated animal.

2. Motility test

For locomotor activity studies, mice receiving anticonvulsant drug were placed in group of five in the rectangular case of activity meter (U. Basil,
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Milano). Two group of five mass each where are each dose of anticonvulsant drug 127.5 and 255mg/kg and similar vehicle treated group are used as control activity count are recorded at 10 min.internals for period of two hr after treatment (44) results of the test substances with that of control at each time interval and expressed as activity count of the test substances from that of control.

3. Amphetamine toxicity test

Male albino mice weighing 25-30 g are divided into four groups of 10 each. The amphetamine toxicity test is carried out as described (45). Briefly, control animals received intraperitonealy injection of the vehicle (saline) while the test animals were injected with extract. Both control and experimental animals received 5 mg/kg amphetamine 30 min later and all mice are aggregated into cubic cages with wire mesh sides. These cages are placed in noise-controlled room at 30 °C temperature for 5 hour. At the end of this period, the number of mortality was noted and recorded.

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References

17. Di Ciccod D, Anatal S, Ammassan Teule M. Prenatal to expose of gamma/neutran irradiation; and sensory motor alternation and peridical effect on learning, teratology, 1991; 43:61-70.
Neuropharmacological screening techniques for pharmaceuticals: A review


35. Jeffrey MG, Linda CK. Litwin, and Jeffrey BM. Behavioral evidence for fl-adrenoceptor subsensitivity after subacute antidepressant α2-adrenoceptor antagonist Naunyn-Schmiedeberg’s Arch Pharmacol 1985; 329: 355- 358.


