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Effect of Amitriptyline on Behavioural and Physical Activity in Albino Wistar Rats

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KEYWORDS

Neurotransmitters, Elevated plus-maze test**,** Amitriptyline, Morris Water Maze test, behavioural activity

ABSTRACT:

Research Aim: This research aims to examine the effects of the tricyclic antidepressant Amitriptyline on the behavioural and physical activities of rats. The study carried out some physical tests and evaluated the results.

Methods: We conducted a comparative studybetween control group and study group (Amitriptyline-injected) rats of age 16 months. The rats' behavior was evaluated using the Elevated Plus-maze, the Morris Water Maze, and the locomotor test.Plasma corticosterone levels were monitored using blood sampling and performed microtiter plate scintillation proximity assay. The changes in the activity among rats were observed.

Results: Rats with a single dose of 10 mg kg-1 of Amitriptyline showed no change in locomotor activity, but repeated doses caused hyperactivity. Imobility was significantly reduced with each dosage of Amitriptyline [F (7, 14) = 10,38, $p<0.001$. The results were similar across all doses.The total escape delay in the elderly rats was not substantially changed by Amitriptyline when compared to the aged controls "($p=0.2$). The two groups of treated elderly rats had similar average swim speeds (19 ± 0.5 cm/sec for controls and 19 ± 0.8 cm/sec for Amitriptyline), while the young control rats swam much faster (24 ± 0.6 cm/sec; $p<0.001$ compared to aged controls). In comparison to the aged controls, Amitriptyline significantly lowered anxiety-associated behaviours in the old rats ($p<0.01$), decreased the amount of time spent in the closed arm, and raised the percentage of time spent in the open arms ($p<0.05$)". Age and the percentage of time spent in the open arms did not correlate ($p=0.13$ compared to the younger control group). [8].

Conclusion: Amitriptyline may not be able to induce locomotor activity when administered alone in a single dosage regimen, according to the study's results. Amitriptyline showed overall escape latency and anxiety-associated" behaviors. As a tricyclic antidepressant, Amitriptyline shows decreased ability in both the physical and behavioural parameters in rats.

1. Introduction

The chemical messengers in your body are called neurotransmitters. Signal transmission between cells in the nervous system, muscles, or glands is carried out via neurotransmitters. The transmission of these signals allows you to move your limbs, perceive feelings, maintain your heart rate, and process and react to all the data received from your surroundings and other internal organs [1]. In most cases, precursor molecules are present in high concentrations in the cell and are used as building blocks for neurotransmitters, which are primarily produced in neurons. Endorphins, adrenaline, serotonin, glutamate, dopamine, and norepinephrine are among the neurotransmitters often present in the body and brain. A variety of chemical messengers called neurotransmitters include amino acids, peptides, and monoamines. A monoamine may be synthesised by altering only a single amino acid [2]. It is common practice to treat a range of behavioural disorders using medications that influence neurotransmitters, which impact mood [3]. Neurotransmitter receptors allow certain cells to accept chemical messengers sent by neurons, which trigger the body to respond appropriately [4].

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In addition to serotonin and acetylcholine, the brain makes use of a variety of neurotransmitters for various purposes. The most important of these are glutamate and GABA, followed by glycine, dopamine, norepinephrine, and norepinephrine.

Role of different types of neurotransmitters

Glycine and gamma-aminobutyric acid (GABA) are the primary inhibitory neurotransmitters, on the other hand. For instance, about 40% of the inhibitory processing in the brain is mediated by GABA. While serotonin is important in learning and motor control, dopamine is involved in reward, emotion, and executive functions, among many others. There may be a role for dopamine in neurological and psychiatric disorders as well [6]. The neurotransmitter serotonin regulates a wide variety of mental processes and neural activities; hence, many psychiatric and neurological medications aim to influence this neurotransmitter. Created by the CNS and sympathetic nerves, norepinephrine is a monoamine. The release of norepinephrine in the brain influences several processes, including inflammation, sleep, attention, and focus. Additionally, it helps regulate the autonomic nervous system's reactions. Another neurotransmitter that regulates motivational behaviour, alters eating behaviour, increases wakefulness, and mediates homeostatic processes in the body is histamine [7]. Amitriptyline and other antidepressants have anti-inflammatory effects, which several studies have shown to be a key component in their effectiveness.T ricyclic antidepressants like Amitriptyline [Figure 1] have many medical applications, including the treatment of depression and neuropathic pain. It shows the neuroprotective benefits of this mixed-type serotoninergic and noradrenergic uptake inhibitor, which also has substantial anticholinergic and antihistaminergic actions [8, 9]. This research study explores to find out the effect of Amitriptyline on the physical and behavioural activity of rat's using various methods.In our study, we found that Amitriptyline shows significantly decreased activity in rats.

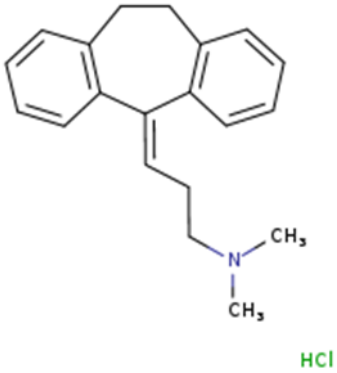

Figure 1: Structure of Amitriptyline

MATERIALS AND METHODS

Animals:

We used 16-month-old rats and split them into two groups: control and study (Figure 2). The study collection was treated orally with Amitriptyline drug (26.8±0.3 ml/d) solution dissolved in sterile water of dose for around 6 months. Similarly control group rats were treated with saline (23±0.6 ml/d). The weight of the animals and how much medication they were given were tracked weekly [10].

Figure 2: Albino Wistar Rats

Reagents and Medications:

Sigma-Aldrich Quimica of Madrid, Spain, supplied the Amitriptyline hydrochloride, which was diluted in a saline solution containing 0.9% NaCl. A variety of

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substances, including saline, “Amitriptyline (7.5 mg/kg, 15 mg/kg, or 30 mg/kg)”, ethanol, saline solution, Ethylenediamine tetraacetic acid (EDTA), and others, were injected intraperitoneally into the rats [11].

Apparatus:

The Elevated Plus Maze (EPM) Apparatus [12], photoresistor actometers, Morris water maze apparatus, were used in the study [Figure 3, 4].

Figure 3: Elevated Plus Maze (EPM) Apparatus

Figure 4: Morris water maze apparatus

Methods:

Locomotor test:

Rats were acclimated for 30 minutes in separate photoresistor actometers with two light beams before being administered intracerebrally with a medication to monitor their Locomotor activity. If the patient had to have recurrent treatment, the (5 µg/per side) dosage of (+)-Amitriptyline was given either 2 or 72 hours after the first or last dose of the antidepressant or saline, respectively. We started measuring Locomotor activity 60 minutes after injecting (+)-Amitriptyline.

Morris Water Maze (MWM) Test:

Animals in this experiment will attempt to flee from a big pool of water, which represents a stressful environment or stimuli. A little platform, either floating on top of the water or slightly below it, is present in the pool. The animals may get off the water and stand up on this little platform, which is simple to handle thanks to its mesh or grooved material design [13].A 26°C water-filled, latex-opacified open-field water labyrinth with a diameter of 1.8 m was used to teach rats. During the course of the trial, prominent visual signals outside of the labyrinth stayed there. As part of the behavioral testing, the animals had to seek out a hidden submerged platform that was 1.5 cm below the surface and 10 cm in diameter. Although the animals were tested against one another, the platform remained in the same location for every experiment. North, south, east, and west were the four starting points we used as we worked our way around the pool. For four days, the animals completed four trials each day [14].

The rats were put in the pool with their backs to the wall at the beginning of the trials. The trials would stop when the rats reached the platform; if they didn't in 120 seconds, they would be manually led there. Next experiment, the rat was promptly returned to the water at a new starting location after 30 seconds on the platform. Using a ceiling-mounted video camera and a computerised tracking system, which included an HVS image analyzer and an Acorn Archimedes computer, researchers were able to observe the rats' latency and swim patterns. After five days, the rats were tested for retention using a probe. This was accomplished by removing the platform and recording the swim route and duration in the platform ("training") quadrant for a duration of 60 seconds [15].

Ascending plus-maze examination:

A black plastic cross-shaped platform, the raised plus-maze was designed by Panlab of Barcelona, Spain. The device had two arms that were open and facing each other, measuring “50Ꝑ10 cm, and two arms that were the same size but were encased in walls that were 40 cm high”. Each of the four limbs was joined by a centre 10 cm2 region. The labyrinth was 64 centimetres above the ground. One of the open arms was the starting point for the rat's unfettered exploration of the labyrinth, which began with its placement in the centre of the room. “Counts of open-arm and closed-arm entries, as well as the amount of time spent on each, were recorded during

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this period". In the time between rats, ethanol was used to wipe the equipment clean [16].

Sampling of Blood:

We drew 300 µl of blood at either the beginning of the day (8:00 A.M., morning sample) or the end of the day (8:00 P.M., evening sample) to find the baseline plasma corticosterone levels. Thirty seconds after a tail nick, the samples were collected. We centrifuged blood samples that had been collected into Eppendorf tubes coated with EDTA, set them on ice, and then chilled them to -20°C. A previously published radioimmunoassay was adjusted for use in a microtiter plate scintillation proximity assay to assess plasma corticosterone levels. The coefficients of variance within each experiment were 9.4% and between each experiment they were 9.2% [17].

Consequences

Experimental animals' actions:

The rats in the control group seemed to make three distinct sorts of escape attempts while dangling from the recording device:(1) forward or backward running;(2) body twisting in an effort to grasp the bond; and(3) body jerks. The rats froze and remained still after many tries.

Effect of drugs on immobility:

Imobility was significantly reduced with each dosage of Amitriptyline [F(7,14)= 10,38, p<0.001. The results were similar across all doses. For Locomotor activity, sedative antidepressants like Amitriptyline reduce immobility at dosages that have been demonstrated to be sedative in the past [18]. Table 1 shows the influence of Amitriptyline on the duration of immobility. Effect of Amitriptyline on immobility length (number of seconds during the 6-minute test) (Table 1) [19]

Effect of Amitriptyline Locomotor activity:

Although a single dosage of 10 mg kg-1 of Amitriptyline had no effect on the Locomotor activity of rats, the hyperactivity generated by the drug was amplified with subsequent administrations (Table 2) [20].

Results of Morris Water Maze test:

The adult rats were tested in the water maze for the first time at the age of 18 months following two months of amitriptyline medication. After eight months of medication, they were evaluated again at 24 months of age. Figure 3 demonstrates that all rats, regardless of age (young, F(11,36)=12.0; elderly controls, F(22,69)=49.4; aged Amitriptyline-treated, F(10,33)=37.0; all p<0.001), acquired the hidden-platform task with skill.

On the first day of training, younger rats" (six months old) had a shorter average escape latency than older rats (p<0.05), but there was no difference on subsequent training days; after four days of training, both groups reached the same escape nadir (<20 sec) (Figure 3). There was no change in mean escape latency after two months of Amitriptyline therapy in 18-month-old rats. "Amphetylline enhances spatial memory retention in young rats, as shown by the probe test (the proportion of time spent in the training quadrant)", but has no effect on acquisition, as shown by escape latency. The probe test timings in rats that were 18 months old after being treated with Amitriptyline for 2 months were comparable to those in rats that were either treated with water or young controls "(41.6±4% for the 6-month-old control group, 42.9±2.0% for the 18-month-old control group, and 46.2±2.6% for the 18-month-old Amitriptyline group). After 8 months of treatment with either water alone or amitriptyline, the rats were retested in the water maze with the young controls at 24 months of age. The aged rats showed continuing learning over the first two days of training, as evidenced by a decrease in mean escape latency when compared to the water controls (F(34,105)=6.57, p<0.001; F(28,87)=14, p<0.001). The mean escape latency of the trainees on the final training day did not differ statistically significantly from that of the young controls". Figure 3 shows that Amitriptyline had no statistically significant effect on the total escape delay between the ageing rats and the aged controls (p=0.2). Table 3 shows that both groups of elderly rats treated with Amitriptyline and controls had similar average swim speeds "(19±0.5 cm/sec and 19±0.8 cm/sec, respectively), but the young rats used as controls swam much faster (24±0.6 cm/sec, p<0.001)" than the older rats.

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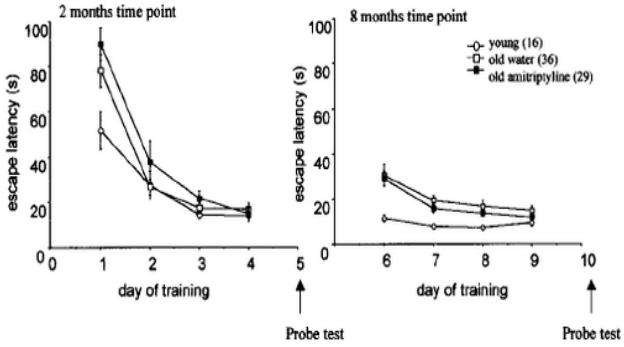

Figure 5: The acquisition stage, which takes place after two months of Amitriptyline medication and again after eight months, involves participants navigating water mazes for many days in an effort to find a concealed platform. Test subjects were administered probe tests on testing days 5 and 10. For the sake of comparison, individuals who were 6 months old served as controls. The average ± standard error of the mean escape delay from four distinct trials is displayed for each day. Rat counts are displayed in brackets.

Number of open ($F(2,28)=2.7$; $p=0.08$) and closed ($F(2,28)=0.6$; $p=0.6$) arm entries ” on the elevated plus-maze did not vary with age or Amitriptyline medication while measuring locomotion.The use of Amitriptyline considerably diminished "anxiety-associated" behaviours in older rats, as evidenced by an increase of 103% in the open arm time, a decrease of 51% in the closed arm time, and an increase of 82% in the percentage of open arm time compared to the aged control group ($F(2,28)= 3.9$; $p<0.05$).

Discussion

According to current research, Amitriptyline, an antidepressant, might be a good way to treat cognitive deficits, physical activity, and behavioural problems in rats [21]. A single dosage of 10 mg/kg of Amitriptyline had no effect on the Locomotor hyperactivity in this investigation, but the effect was amplified with subsequent administrations of the drug. After the final dosage of antidepressant, you will see this impact [22].It was shown that Amitriptyline significantly reduced immobility in relation to dosage [$F (7,14)=10, 38$, $p<0.001$. Amitriptyline and other sedative antidepressants reduce immobility at levels that have been found to be sedative for Locomotor activity in the past [23]. After two months of therapy with Amitriptyline, the Morris Water Maze test was given to the aged rats twice: at 18 and 24 months of age. When compared to the aged controls, Amitriptyline had no significant effect on the overall escape latency in the older rats ($p=0.2$). Older rats in both the control and Amitriptyline groups swam at comparable average speeds (19±0.5 cm/sec for controls and 19±0.8 cm/sec for Amitriptyline), in contrast to the much quicker swimming of the younger rats (24±0.6 cm/sec; $p <0.001$) in the control group.

Age and the number of open arm entries ($F(2,28)=0.6$; $p=0.6$) or Amitriptyline therapy and increased plus-maze locomotion ($F(2,28)=2.7$; $p=0.08$) did not correlate. The quantity of time spent on the open arms was not significantly affected by age ($p=0.13$ when compared to young controls), but amitriptyline did reduce "anxiety-associated" behaviors in the older rats. Amitriptyline treatment dramatically reduced plasma corticosterone levels and anxiety-related behaviors in older rats, suggesting a more systemic alteration in stress hormone levels and behavior. Blood samples in rats show that chronic amitriptyline treatment decreases hypothalamic-pituitary-adrenal (HPA) function. [24].

Conclusion

In conclusion, we found that Amitriptyline did not show any Locomotor hyperactivity when given in single dose, but continuous administration results in Locomotor hyperactivity whereas Amitriptyline decreases immobility at different doses. At first, the total rat escape latency was reduced, but the control rats' speeds were much quicker when they were younger than when they were older. Age did not affect the amount of time spent in the open arms, but Amitriptyline significantly decreased the older rats' "anxiety-associated" behaviors. Finally we found that Amitriptyline shows decreased ability in both the physical and behavioural parameters in rats, as it shows its effect as tricyclic antidepressants.

References

1. Neurotransmitters. Cleveland Clinic; 2022. (https://my.clevelandclinic.org/health/articles/22513-neurotransmitters)
2. Neurotransmitter. Wikipedia; 2022. (https://en.wikipedia.org/wiki/Neurotransmitter)
3. Pradeep Kumar Samal, Shani Sharaf, N.R. Beck, J.S. Dangi. Antiepileptic Effects of Sapindus

Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(6), 1510-1516 | ISSN:2251-6727

mukorossi in rats. Research J. Pharm. and Tech. 5(11): Nov. 2012; Page 1417-1420.

4. Edokwe Chinelo, Obidoa Onyechi, Joshua Parker Elijah. Biochemical Effects of Persea americana (Avocado) and Dacryodes edulis (African Pear) Extracts on Liver Function Test of Albino Wistar Rats. Research J. Science and Tech. 2010; 2(2): 34-37.
5. Sheffler ZM, Reddy V, Pillarisetty LS. Physiology, neurotransmitters. In Stat Pearls [Internet] 2022 May 8. StatPearls Publishing.
6. Hwang J, Zheng LT, Ock J, et.al;. Inhibition of glial inflammatory activation and neurotoxicity by tricyclic antidepressants. Neuropharmacology. 2008 Oct 1;55(5):826-34.
7. Amitriptyline. Drugbank.com; 2022 (https://go.drugbank.com/drugs/DB00321)
8. Yau JL, Noble J, Hibberd C, et.al;. Chronic treatment with the antidepressant Amitriptyline prevents impairments in water maze learning in aging rats. Journal of Neuroscience. 2002 Feb 15; 22(4):1436-42.
9. Parra A, Everss E, Monleon S, et.al; Effects of acute Amitriptyline administration on memory, anxiety and activity in male and female mice. Neuroscience Research Communications. 2002 Nov; 31(3):135-44.
10. Elevated Plus Maze. Behavioural and functional neuroscience https://med.stanford.edu/sbfnl/services/bm/ep/bml-plusmaze.html
11. Richard G.M. Morris. Morris water maze. Scholarpedia 2008; 3: 6315. http://www.scholarpedia.org/article/Morris_water_maze;
12. Mfem CC, Seriki SA. Impact of Amitriptyline on learning and memory. Insights on the Depression and Anxiety. 2021 Apr 14;5(1):009-15.
13. Maj J, Wedzony K. Repeated treatment with imipramine or Amitriptyline increases the Locomotor response of rats to (+)-amphetamine given into the nucleus accumbens. Journal of pharmacy and pharmacology. 1985 May;37(5):362-4.
14. Porsolt RD (1981) Behavioral despair. In: Enna SJ, Malick JB, Richelson E (eds) Antidepressants: Neurochemical, behavioral and clinical perspectives. Raven Press New York, pp 121-139
15. Steru L, Chermat R, Thierry B, Simon P. The tail suspension test: a new method for screening antidepressants in mice. Psychopharmacology. 1985 Mar;85(3):367-70.
16. Monago, C.C, Gozie G.C., Joshua P.E.. Antidiabetic and Antilipidemic Effects of Alkaloidal Extract of Emilia sonchifolia in Rat. Research J. Science and Tech. 2010; 2(3): 51-56
17. M. Shakeera Banu, K. Sujatha, W. Sherly Beena, N. Divya. Antihyperglycemic and Antioxidant potentials Psidium guajava leaf extract and its isolated fraction in alloxan-induced diabetic rats. Research J. Pharm. and Tech. 5(4): April 2012; Page 541-546.
18. NimbekarT.P., Katolkar P.P., Patil A.T. Effects of Manilkara hexandra on blood glucose levels of normal and Alloxan induced diabetic rats. Research J. Pharm. and Tech. 5(3): March 2012; Page 367-368.
19. Nur Samsu, Setyawati Soeharto, Muhaimin Rifai, Achmad Rudijanto. The Superiority of Rosmarinic Acid over Vitamin E in Preventing Development and Inhibiting Progression of Diabetic Nephropathy in Rats. Asian J. Pharm. Res. 2020; 10(3):131-139.
20. Naveen K L, Ananya Bhattacharjee, Karunakar Hegde. A Study to Evaluate the Neuroprotective property of Aqueous Extract of Mentha piperita Leaves on Haloperidol Induced Parkinsonism in Experimental rats. Asian Journal of Pharmaceutical Research. 2023; 13(3):139-4.
21. P. Kumaravel, AK. Prabhakaran, G. Melchias, A. Edward, P. Natarajan, S. Sathishkum. Monosodium glutamate exposure alters histopathological and biochemical variables in rats under constant light. Asian J. Res. Pharm. Sci. 4(1): Jan.-Mar. 2014; Page 01-04.
22. D. Santhosha. Pharmacognostic Study, Phytochemical Screening and Neuroprotective Activity of Methanolic extract of Gynandropsis gynandra against Transient Global Ischemia induced brain damage in rats. Asian J. Res. Pharm. Sci. 2019; 9(3):156-162.
23. Nitin M, Prasad K, Dastapur A, Suryawanshi S. Influence of Coenzyme Q10 on Phenothiazine Induced Extrapyramidal Symptoms in Rats. Research J. Pharmacology and Pharmacodynamics. 2010; 2(3): 248-251.

24. Arun Kumar Jaiswal. Neurobehavioural effects of prenatal sodium valproate exposure in rat offspring. Res. J. Pharmacology & Pharmacodynamics.2016; 8(3): 127-133.