

(7- NITROINDAZOLE), A SPECIFIC NEURONAL NITRIC OXIDE SYNTHASE INHIBITOR AND ECS-INDUCED RETEROGRADE AMNESIA

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ABSTRACT

Electro Convulsion Therapy (ECT) has been used for more than 300 years in an attempt to treat several mental illness. Electroconvulsive therapy is the electrical induction of a generalized tonic – clonic convulsions with the aim of treating an abnormal mental state or neurological disorders. The salutary finding of a complementary review of patients perspectives on ECT found that at least one-third reported significant memory loss after treatment. Electrical stimulation has left out brain with cognitive impairments. We designed to study the effects of the 7- Nitro Indazole in electro convulsive induced amnesia in mice.

Keywords: Amnesia, Seizures, Convulsions, Nitric oxide.

INTRODUCTION

Artificially induced convulsions have been used for more than 300 years in attempts to treat severe mental illnesses. Cerletti and Bini first used electricity as the means of induction in Rome in 1938, and this turned out to be a much more reliable method than, for example, the administration of camphor. Electroconvulsive therapy (ECT) is the electrical induction of a generalized tonic-clonic convulsion with the aim of treating an abnormal mental state or neurological disorder; efficacious treatment requires a series of inductions given two or three times a week (Andrade, 1992).

Indications for ECT

A comprehensive systematic review concluded that real ECT was substantially more efficacious than sham ECT (standardized effect size 0.91) and more efficacious than pharmacotherapy (standardized effect size 0.80) in the short-term treatment of depressive illness. [5]. The salutary finding of a complementary review of patients' perspectives on ECT found that at least one-third reported significant memory loss after treatment[6].

This was the major reason that National Institute for Clinical Excellence (NICE) later recommended that ECT be used only to achieve rapid and short-term improvement of severe symptoms after an adequate trial of other treatment options has proved ineffective and/or when the condition is considered to be potentially life-threatening[7]. This guidance is not consistent with the extant recommendations from the Royal College of Psychiatrists, which identified wider indications for ECT (Andrade, 1992).

Nitric oxide synthases

The nitric oxide synthases NO is produced by a group of enzymes denominated nitric oxide synthases (NOS). There are four members of the NOS family:

- neuronal NOS (nNOS),
- endothelial NOS (eNOS),
- inducible NOS (iNOS) and
- Mitochondrial NOS (mtNOS).

The last one is an isoform of nNOS present in the inner mitochondrial membrane. All the NOSs share between 50 and 60% sequence homology. nNOS and eNOS are Ca²⁺-calmodulin-dependent enzymes

constitutively expressed in mammalian cells that generate increments of NO lasting a few minutes. In contrast, iNOS is Ca²⁺-calmodulin independent and its regulation depends on de novo synthesis. iNOS is expressed following immunological or inflammatory stimulation in macrophages, astrocytes, microglia and other cells producing high amounts of NO lasting hours or days

All the NOS isoforms have four prosthetic groups: flavin adenine dinucleotide (FAD), flavin adenine mononucleotide (FMN), iron protoporphyrin IX (heme) and tetrahydrobiopterin (BH₄). FAD, FMN and heme are involved in the redox reactions leading to the synthesis of NO. Heme and BH₄ comprise the scaffold that maintains the substrate channel. For this reason BH₄ is absolutely necessary for NOS activity. NOS structure shows two biodomains working independently. The first one consists of a C-terminal reductase domain containing sites to bind NADPH, FAD, FMN and Ca²⁺-calmodulin. The binding of Ca²⁺-calmodulin triggers the activation of the enzyme opening a gate for the electron flux into the active center of the NOS. The N-terminal domain has oxygenase activity containing sites to bind BH₄, heme and L-arginine (L-Arg). L-Arg is used by NOS to produce NO and citrulline in a process requiring NADPH and O₂. L-Arg is a semi-essential amino acid since it can be synthesized from glutamate or produced by recycling citrulline in the citrulline-NO cycle with argininosuccinate synthetase (AS) and argininosuccinate lyase (AL). L-Arg can be taken through a cationic pH- and sodium-independent carrier.

A constitutive isoform of NOS called neuronal NOS (nNOS or NOS I) is found in neurons. It is expressed in populations of developing (Bredt and Snyder, and mature neurons. nNOS has also been found in rat astrocytes, the adventitia of a subset of rat brain blood vessels, rat cardiac myocytes, rat skeletal myocytes, human lung epithelial cells, rat macula densa, human testis, rat penile corpora cavernosa, urethra and prostate and human skin. nNOS is Ca²⁺-dependent like eNOS, and is also regulated

through reversible Ca²⁺-calmodulin binding.

MATERIALS AND METHODS

Aim and Objective

Electrical stimulation to the brain has the adverse effects of cognitive impairment. Ketamine is a drug an NMDA receptor antagonist that prevents the cognitive impairment by preventing the saturation of the glutamate release during the long term potentiation. Here the aim of the study is to determine the effects of the 7-NITROINDAZOLE in electro convulsive induced amnesia in mice

Treatment

Group 1 : Control

Group 2 : Receive only electrical stimulation treatment

Group 3 : Receive 7 NI (50mg/kg) + electrical stimulation treatment

Work plan

GROUP 1 : (day -3 to -1), MWT , day (0 – 5) no electrical stimulation, day (6) MWT

GROUP 2 : (day -3 to -1), MWT , day (0 – 5) electrical stimulation, day (6) MWT

GROUP 3 : (day -3 to -1), MWT , day (0 – 5) electrical stimulation + 7NI, day (6) MWT

Experimental procedure

Animals

Swiss albino mice obtained from the animal house of the Saastra College of Pharmacy, Varigonda, weighing 30 – 35 g of mice were used. The animals were kept under standard conditions for light and dark cycle with food and water ad libitum and cages with soft bedding. All the experiments were carried out between 09:00 and 15:00 h. All procedures were reviewed and approved by the I.A.E.C

Drug treatment

7-Nitroindazole (7-NI), were purchased from Alpha Aesar dissolved in 0.9% saline. 7-Nitroindazole was dissolved in saline using a few drops of Tween-80 (Volke et al., 2003). All drugs were prepared immediately prior to use and given intraperitoneally (i.p).

Morris water maze test

Pre-training for the water maze

- The main component of the water maze set up should be a round pool, about 6 feet in diameter and about 3 feet deep. If you are recording the task with a video camera, make sure all sides of the maze are within the camera's field of view.
 - Fill up the water maze with tap water, which should be close to 26°C. This may take several hours, so should be done well in advance. Periodically check the water temperature so that it is within one degree of 26°C.
 - Place the escape platform in the center of the pool. During training, it must be exposed, one inch above the water. This teaches the rat that there is a platform, and that it is the way to get out of the water. Later, after the animal is trained and ready for testing, the escape platform will be just below the surface of the water, and will not be visible because the water will be made opaque with milk or non-toxic paint. Now, the water maze is ready for training the animals.
- 3.1. For the water maze training, the platform should be in the center of the pool and exposed one inch above the surface, so the animal knows that it's there. The water should be within one degree of 26°C.
 - 3.2. Each animal will undergo three consecutive trials. First, place the animal on the platform for twenty seconds
 - 3.3. The water maze has 4 starting positions: north, south, east, or west. Take the animal to one of these positions. Lower the animal into the water by supporting it with your hand and bringing it down gently into the water tail-end first. Do not stress the animal out by dunking it in head first.
 - 3.4. Let the animal swim/search for the platform for a maximum of

60 seconds. At first, the animal may swim around the edge of the pool looking for a way out. Eventually, the animal will learn to search for the platform and climb up.

- 3.5. Once the rodent reaches the platform, stop the timer, and record the time. If it doesn't find the platform in 60 seconds, then record the time for this trial as one minute. Do not pick up the animal if it fails to reach the platform. Teach the animal that it must swim to the platform. Therefore, gently guide the animal to the platform with your hand. Let the animal sit on the platform for 15 seconds. If it falls or jumps off, gently guide it back. This will train the animal that it must stay on the platform to be rescued from the pool.
- 3.6. Repeat the same procedure for two more trials, starting at a different direction for each trial.
- 3.7. Once the animal has completed all three trials, dry it off with a towel. Repeat the three-trial training process for all the animals consecutively. Keep the directions the same for all of them, and record their times.
- 3.8. Now that the animals are trained, they are ready to perform the water maze test.

Main study

1. To begin experimental trials with the water maze, fill up the tank so the platform is one inch below the surface of the water. Use non-fat dry milk, or 125 milliliters of non-toxic white tempera paint, to make the water opaque.
2. The lighting and water temperature should be the same as in the training process. Each animal will undergo 12 trials, which will be 3 trials for each starting direction. Each trial will last 60 seconds. Before beginning, choose the order of the starting directions. Do not use

- the same start direction twice in a row, and also do not repeat the same order for any of the directions.
3. Facing the wall of the pool, the animal handler will place the animal in the water, and will then step back from the pool and sit in a designated spot while the animal performs the maze task.
 4. Monitor the animal until it reaches the platform, and record the time it took. If the animal doesn't reach the platform in 60 seconds, the handler will guide it to the platform, as in training. Either way, let the animal sit for 10 seconds, and then dry it off and return the animal to a holding cage.
 5. Continue with each animal for all 12 trials, with the animal handler returning to the same designated spot during each trial. The order of testing should be: trial 1 for all animals, trial 2 for all animals, trial 3 for all animals, etc. There should be an inter-trial interval of at least 2 minutes. Periodically, clean out the pool, make sure the platform is in place, and check that the water temperature is the same.
 6. After all animals have completed 12 trials; they will each perform one probe trial, in which the platform is removed from the pool. The probe trial is performed to verify the animal understands of the platform location, and observe the strategy that the animal follows when it the platform is not there. The handler will release the animal starting from the north. Record the number of times the animal crosses discovers the center of the pool during the 30 seconds.
 7. When all the probe trials are complete, dry off the animals and drain the pool (Morris, 1984).

Administration of ECS

Electroconvulsive shock was applied via bilateral clip electrodes. The stimulus parameters were 150V, 60 Hz, sine wave, during 2 s. Each stimulation elicited tonic - clonic seizures. We have chosen these procedures due to its resemblance to ECT treatment in clinical practice, be it in the number of shocks applied or in the intensity of the stimulus (Andrade et al., 2002).

RESULTS AND DISCUSSION

Table1: Morris Water Maze Test

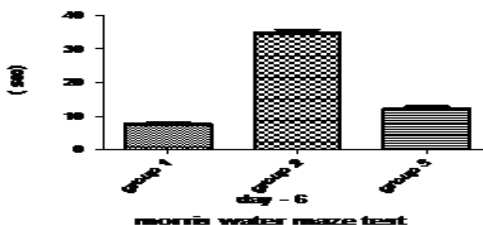
| Treatment | Time taken to reach the hidden platform (sec) | | | |
|-----------|--|----------------------------|----------------------------|--------------|
| | ITL day -3 | 1 st RTL day -2 | 2 nd RTL day -1 | Day 6 |
| Group 1 | 44 ± 3.34 | 10.6 ± 1.96 | 5.5 1.784 | 7.5 ± 2.34 |
| Group 2 | 36.5 ± 1.99 | 18.66 ± 5.5 | 3.3 ± 4.08 | 32 ± 4.43*** |
| Group 3 | 56.83 ± 3.54 | 18.5 ± 7 | 4.66± 4.102 | 10 ± 3.32*** |

*** -p<0.001, ** -p<0.01, * -p<0.05, ns – non significant

Data were expressed as mean ± S.E.M (n =6, animals in each group).

The groups compared here was

- Group 1 and Group 2
- Group 2 and Group 3



From the results we came to know that there is an extremely significant difference between the group 1 and group 2 shows that all the animals in group 2 has taken more time to find the hidden platform in Morris water maze test. This shows that all the animals in groups 2 have cognitive impairment after receiving the Electroconvulsive shock.

When comparing the results of group 2 and group 3, there is an extremely significant difference between them. This shows that the group 3 receiving the 7-nitroindazole prevents the Electroconvulsive therapy induced cognitive impairment in mice.

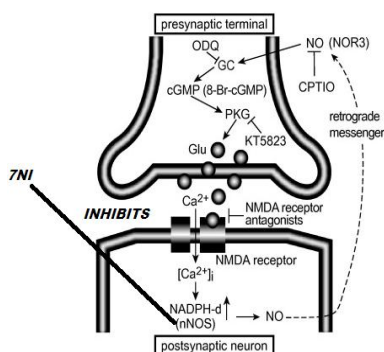


Fig. 1:

There is an accumulating evidence that nitric oxide acts as a retrograde messenger by increasing the glutamate in the presynaptic terminal. Also glutamate is the neurotransmitter involved in the long term potentiation. While ECS there is an increase in the three nitric oxide synthase in different regions of the rodent's brain. From the results the 7 NI receiving group decreases the time for the animals to reach the hidden platform when compared to the group 2 receiving only the ECS. This shows that the saturation of the glutamate release during the ECS is prevented by 7 NI by preventing the synthesis of nNOS in brain.

CONCLUSION

ECT has gaining importance again for several psychiatric disorders. The major adverse effects of the ECT are retrograde amnesia. 7 nitroindazole a specific neuronal

nitric oxide inhibitor prevent the cognitive impairment associated with ECS. This shows that 7 nitroindazole is a promising therapeutic agent for the retrograde amnesia during the ECT in future.

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