

NEOSTIGMINE AMELIORATE HISTOLOGICAL DEFICIT ON AMYGDALA OF KETAMINE-INDUCED SCHIZOPHRENIA IN WISTAR RAT

Elizabeth Finbarrs-Bello^{1,2*}, Eni Ogonnia Egwu², Oluwatomilayo Patience Ojo³

^{1,2} Enugu State University of Science and Technology, [Nigeria], Department of Anatomy, Faculty of Basic Medical Sciences, Parklane, Enugu State.

² Ebonyi State University, [Nigeria], Department of Anatomy, Faculty of Basic Medical Sciences, Ebonyi State

³ University of Ilorin, [Nigeria], Department of Anatomy, Faculty of Basic Medical Sciences, Kwara State

*elizabeth.finbarrs-bello@esut.edu.ng

Abstract

Ketamine is an N-methyl-D-aspartate (NMDA) antagonist that blocks the production of glutamate, thereby reducing the level of glutamate in the brain which is capable of inducing schizophrenic like cognitive deficit. Neostigmine is a cholinesterase inhibitor that enhances memory and improves cognition. We investigate the histological effect of Neostigmine on the amygdale of Ketamine- induced schizophrenia in Wistar rat. Twenty (20) Wistar rats were divided into four groups (n=5). Group A received 0.1ml saline; Group B, C and D received 25mg/kg Ketamine for 7days. On the day 8, Group B was sacrificed while Group C was treated with 0.5mg/kg Neostigmine and Group D was treated with 0.1ml saline for 21days. Thereafter, the rats were anesthetized with 50mg/kg thiopental sodium, and aortic perfusion was performed with 4% paraformaldehyde. The amygdale was stained with H& E, Cresyl fast violet and Golgi silver stains. Neostigmine reversed histological changes in all the parameters investigated. Neostigmine appear to be a promising agent in preventing or reversing ketamine effects on neurons in this model, which suggests build up of acetylcholine transmitter.

Keywords: *Schizophrenia, Neostigmine, Anticholinesterase, Glutamate*

Introduction

Schizophrenia is a mental illness that causes psychosis and is characterized by a wide range of neurological, biochemical and structural defects. One of its glaring feature is a form of cognitive impairment. Preclinical researches and clinical patient evaluations have identified causative culprits like genetic factor, previous illness, lack of sleep, and environmental or chemical factors including substance abuse, alcohol, stress and not excluding head injuries [1,2,3]. Schizophrenia has been modeled in rodents using various substances such as cocaine, amphetamine and phencyclidine like Ketamine to understand its pattern, pathophysiology, pharmacotherapy and neurobehavioral interventions [3,4].

Ketamine schizophrenia model exhibits similar symptoms in rodents and humans. Ketamine blocks the production of glutamate, thereby reducing the level of glutamate in the brain which is capable of inducing schizophrenic like cognitive symptoms amongst others in rodents [5]. The treatment choices often resolve the symptoms but failed to provide effective cure or alleviate the cognitive deficit and also exhibits side effects that are unbearable for patients. Thus, there is need for new therapeutic approach which may require combination or a follow up of antipsychotic with an adjunct that is known to possess cholinergic effect. Since the cognitive deficit is produced via the acetylcholine pathway drugs such as physostigmine and Neostigmine might be useful.

Neostigmine is a drug mostly used in the treatment of myasthenia gravis and an effective reversible competitive acetylcholinesterase inhibitor (AChEI) that inhibits the cholinesterase enzyme and prevents the breakdown of acetylcholine neurotransmitter [6]. Reversible inhibitors of AChEI transiently increase the ACh levels and are found effective in disease and therapeutic interventions, where an increase in ACh level is desired. These therapeutic mechanisms increase both the level and duration of action of acetylcholine, enhance memory and improve cognition in brain regions like the hippocampus and amygdala [7,8]

Acetylcholine is an important modulator that drives changes in neuronal and glial activity. Understanding the direct structural action of drugs that improve cognitive functions is key to improve the pathway for therapeutic development, intervention, provide effective care and management of disorders of cognition with cognitive enhances.

Amygdala is a complex structure in the medial temporal lobe and connects to the brain region involved in wide variety of behavioral, emotional and cognition functions [9]. The amygdala is involved in fear conditioning, reward processing, emotional memory, regulation and modulation of variety of cognitive functions. The flow of information through the amygdala circuit is modulated by various neurotransmitter systems including the glutamate, GABA, dopamine and acetylcholine. The amygdala can also directly modulate functions of cortical areas particularly, the hypothalamus and hippocampus of the limbic system. In an early study we showed that Neostigmine improved cognitive deficits in the hippocampus of same model [10]. We assessed if an injection of Neostigmine would enhance or improve histological changes in rats model of schizophrenia

Methods

Drugs

Neostigmine injection was used as standard anticholinesterase inhibitor, Ketamine hydrochloride injection (Rotex Medica, Trittau Germany) was used to induce schizophrenia in the rats. The drugs were purchased from registered pharmacy store in Enugu Nigeria and the doses of the drug were selected based on data from literature and drug information leaflet.

Animals

A total of Twenty (20) Wistar rats of both sexes, 8 weeks old, average weight of 180g were obtained from animal laboratory of the Department of Physiology, College of Medicine, University of Nigeria Enugu Campus and housed at the Animal facility of Anatomy Department, Ebonyi State University. The rats were housed individually in a temperature-controlled (20 - 22°C) room under 12 h : h light/dark cycles (light on at 7am) with free access

to standard rodent chow food pellets and tap water for two weeks. After this acclimation period, at the age of 10 weeks, the rats were randomized into two and weight-matched groups: a control group (controls, n= 4) and treatment group (cases, n = 16) by generating random numbers using standard. All conditions and handling of the animals and protocols used in this study were approved by the Departmental ethics Committee of Ebonyi State University and conducted according to the institutional guideline.

Experimental design

The control received 0.1ml of normal saline, the treatment group was divided into 3 sub-groups (B, C, D) each group consists of five rats (n=5). Schizophrenia was induced in the 3 groups (B, C, D) which received 25mg/kg Ketamine hydrochloride body weight interperitonally (I.P), for 7 days. Thereafter, C and D were then treated with 0.5mg /kg of Neostigmine and 0.1 ml normal saline intraperitoneally respectively for 21days. The symptoms observed were side to head rocking and continuous staggering locomotion.

Histology

Once the experiment was completed on the 8th day, groups A and B were anesthetized with Na-thiopentone (50mg/kg/bw, i.p) and sacrificed, groups C and D were sacrificed on day 22 via transcadiac perfusion- fixation with 4% paraformaldehyde via the heart was performed on the rats. The brains were isolated from the anesthetized rats as per the study design and stored in 4% formaldehyde solution for 72 hours fixation. These were then processed for paraffin section (dehydration, embedding, paraffin block, section cutting). The deparafinized sections were stained using H and E, Cresyl Fast Violet (Nissls substance) and Golgi Silver Stain and evaluated. Photomicrographs were captured using research photographic microscope in the Biotechnology center Ebonyi State University, Nigeria.

Results

In this study section of amygdale of the control negative control given physiological saline (vehicle) revealed numerous normal neurons with centrally localized nuclei in cytoplasm. The untreated schizophrenic model which recieved only Ketamine

showed neurons with eccentric nuclei and features that characterised cytoplasmic vacuolations. Following 7days administration of Neostigmine (treated model), several neurons exhibited feactures of normal neuronal cells. The treatment control (saline) revealed neurons that appeared relatively normal without cytoplasmic vacuolation (fig 1). Golgi stain sections of the control (saline) revealed normal neurons stained brownish, untreated model and treatment control revealed severe and mild depletion of neuron respectively but, Neostigmine treated increased neuronal population (fig 2). Cresyl fast violet was used to stain nissls substances, The control (saline) normal Nissls substances, Ketamine untreated section showed qualitative decrease presences of Nissls substance,while Neostigmine treated section revealed and Saline treated both increased Nissls substance fig 3 Saline treated .

Discussion

Several studies have shown that the amygdale is involved in cognitive and emotional behavioral functions [11,12]. Overt structural and functional changes in the amygdale occur from enormous arrays of pharmaceutical and environmental chemicals which can lead to depression and schizophrenia [8, 9, 13]. Ketamine, an N-methyl-D-aspartate (NMDA) antagonist which blocks the production of glutamate and reduces the level of glutamate in the brain induces schizophrenic-like cognitive deficit in rodents [3]. The cognitive impairment is ameliorated by the administration of cholinesterase inhibitors such as with Neostigmine.

The study investigates the histological effects of neostigmine on the amygdale of ketamine- induced schizophrenia. The ketamine induction of schizophrenia was positive, which affirms that subanesthetic dose of katamine induces reliable changes in brain behavior and parameters of dopaminergic, glutermatergic and serotonergic neurotransmissions which is the basic for an animal model in schizophrenia research [3,14,15,16]. Similarly, ketamine causes neurotoxic effect that was characterized by cytoplasmic vacuolations in the histological sections of the amygdale. This finding has been consistently observed with ketamine neurotoxicity in most areas of the brain and neurofunctional impairments [17]. Ketamine

effect varies based on the dose, frequency of exposure and intensity of the noxious stimuli, however, repeated ketamine usage is a known neurotoxicant.[18].

Exposure to clinically relevant concentration of ketamine such as its subanesthetic dose employed in this study elevates NMDA receptor level which has been associated with enhanced damage to neurons [19]. This rise in NMDA receptor expression is a compensatory up-regulation that allows for a high or toxic influx of calcium into neurons. Consequently, removal of ketamine from the system provokes neuronal cell death which is largely due to an elevated reactive oxygen species generated [19].

Ketamine produces a dose dependent neurotoxicity in most animal species including humans; It could increase expression of astrocytes in the same model of behavioral and cognitive deficits [3]. In this study the depletion of neurons in the Golgi silver stained section suggests degeneration of neurons. But, positive and increased neuronal cell affinity in the Cresyl fast violet stained sections indicate presence of Nissl substance. This possibly suggests enhanced neurotransmitter synthesis or release under the influence of ketamine. Glutamate and GABA are the two neurotransmitters mostly affected by ketamine dosing. While both have opposing actions of excitation and inhibition of neurons, the balance is optimal for brain function. Glutamate hypothesis of schizophrenia implicates glutamergic dysfunction in the mechanism of the underlying cognitive and other dysfunction in schizophrenia.

Neostigmine acted as treatment which ameliorates the effect of ketamine. In our previous studies we observed the neostigmine administration acted as treatment to the Ketamine-induced psychotic cognitive deficit rats by reversing its effect. The results demonstrate that cholinesterase inhibitors improve structural changes in this model. The effect could be attributed to the fact that ketamine-induced model has an altered cholinergic state. Such model also exhibits cognitive impairment in behavioral test like the T maze in addition to the structural changes [10]. In addition, theories also support the fact that there is an optimal range of cholinergic turnover for optimal behavioral and hippocampus or limbic system functions. This

provides further support for the role of cholinergic system dysfunction and cognitive impairment in the model used. The neural damage can also be ameliorated by cholinesterase inhibitor. Cholinergic enhancing drugs also facilitated neurogenesis and pharmacological augmentation of cholinergic activity enhanced learning and memory performance in cognitively impaired animals [20]. Intrahippocampal physostigmine increased available amount of acetylcholine which increased the activity of the extended limbic system that was dysfunctional [20]. In conclusion, therapeutic dose of Neostigmine administered had a correcting effect on the amygdala of ketamine-challenged models of schizophrenia by reversal of the histological deficits observed in the amygdala.

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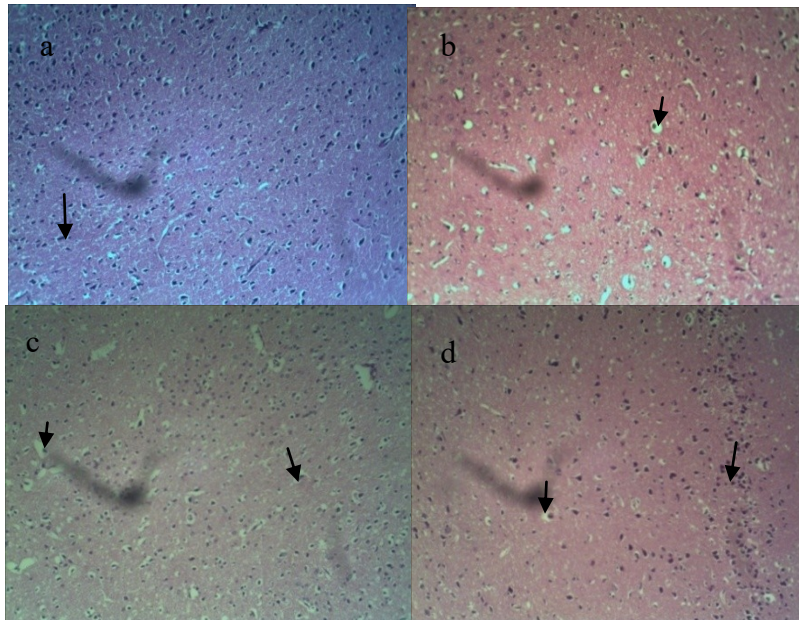


Figure 1: Photomicrograph of amygdala of Wistar rat control (saline), normal neuronal cells cytoplasm with centric nuclei (arrow) (b) Ketamine untreated, cytoplasmic vacuolations (arrow) (c) Neostigmine treated, relative normal neuronal cells (arrow) (d) Saline treated relative normal cells. H&E. x 200.

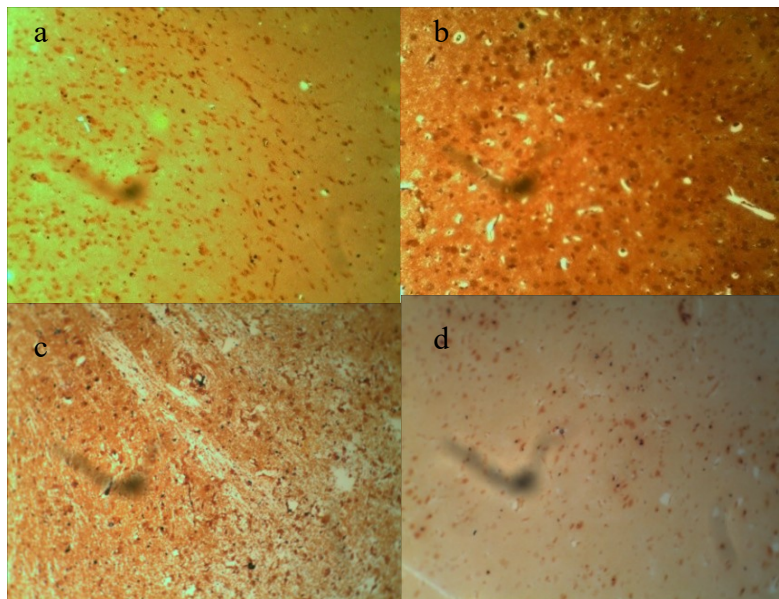


Figure 2: Photomicrograph of amygdala of Wistar rat control (saline) normal neurons brownish (b) Ketamine untreated depletion of neurons (c) Neostigmine treated increase neurons (d) Saline mild depletion of neurons. Golgi silver stain. x 200.

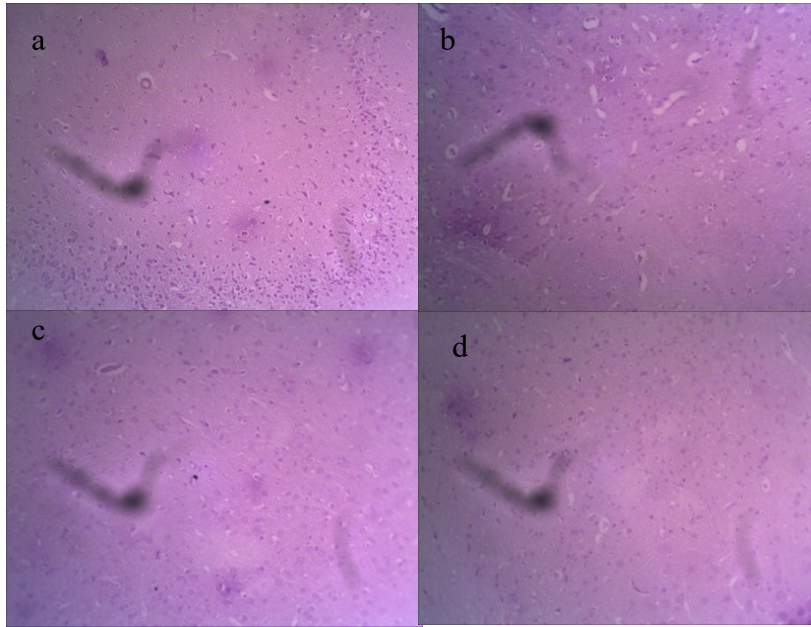


Figure 3: Photomicrograph of amygdale (a) control (saline) normal Nissls substances (b) Ketamine untreated decrease Nissls substance (c) Neostigmine treated increased Nissls substance (d) Saline treated mild increase in Nissls substance. Cresyl Fast Violet. x200.