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ANXIOLYTIC EFFECT OF CLONIDINE IN MICE

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ABSTRACT

Anxiety disorders are the most common psychiatric disorders in the world among both children and adults. Benzodiazepines are more commonly used anxiolytic drugs. Since it is found that anxiety disorder patients have poorly regulated noradrenergic system, we have studied the anxiolytic effect of Clonidine a α_2 agonist which reduces the sympathetic outflow. We evaluated the anxiolytic effect of Clonidine using pentylene tetrazole to induce anxiety. Pentylene tetrazole is a CNS stimulant which produces anxiety at lower doses and convulsions at higher doses. Anxiety is evidenced by increased grooming activity in mice. 18 naïve male adult mice were divided into three groups of 6 animals each, namely control, standard and test groups. 30 minutes prior to administration of PTZ control group received water for injection, standard group received injection diazepam and test group received Clonidine intraperitoneally. The test group of animals showed significant reduction in the duration and frequency of grooming activity and thus this study proved the anxiolytic effect of Clonidine.

KEYWORDS: Anxiety, Clonidine, Grooming activity, Pentylene tetrazole.

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INTRODUCTION

Anxiety disorders are the most common psychiatric disorders in the world among both children and adults. Much of the emotional and economic burden caused by these disorders could be alleviated by improving the diagnosis and treatment.[1] In most cases, women are more likely to have anxiety disorders than men, a phenomenon that still begs for an adequate explanation. Of particular interest in the finding that social phobia is more common in women than men. Recent studies also suggest that chronic anxiety disorder may increase the rate of cardiovascular related mortality.[2] Everyone experiences anxiety. It is characterized most commonly as a diffuse, unpleasant, vague sense of apprehension often accompanied by autonomic symptoms such as head ache, perspiration, palpitations, tightness in the chest, mild stomach discomfort and restlessness, indicated by an inability to sit or stand still for long. [3] The disability and health costs caused by anxiety are high and comparable with those of other common medical conditions such as diabetes, arthritis or hypertension. People with anxiety disorders experience impaired physical and role functioning, more work-days lost due to illness.[4] A variety of animal studies are now routinely used to study fear and anxiety. Converging evidence now indicates that the amygdala plays a crucial role in the development and expression of conditioned fear and certain effects caused by stress. The central nucleus of amygdala has direct projections to a variety of anatomical areas that might mediate many of the symptoms of fear and anxiety.[5] At least three neurotransmitter systems appear to modulate the symptoms of many anxiety disorders. These are the norepinephrine, serotonin, GABAergic systems. The locus ceruleus hypothesis of panic disorder is one of the more solidly supported neurobiological hypotheses. It posits that increased efferent activity from the locus ceruleus predisposes to panic attacks through the sympathetic nervous system. Since α receptor regulates the activity of locus ceruleus through negative feedback, it is a reasonable candidate gene for panic disorder. Anxiety disorder patients have poorly regulated noradrenergic system that has occasional burst of locus ceruleus. Stimulation of locus ceruleus produces a fear response in animals and ablation of that area inhibits the ability of the animals to form a fear response. Human studies have found that, in patients with panic disorder, α adrenergic agonists and α_2 adrenergic antagonists can produce frequent and severe panic attacks. Conversely Clonidine, a α_2 adrenergic agonist reduces anxiety symptoms in some experimental and therapeutic situations.[6] Anxiety states and sleep disorders are common problems and hence sedative hypnotics and anxiolytics are among the most widely prescribed drugs today. Benzodiazepines are the most commonly prescribed group of anxiolytics and sedatives. . They were primarily introduced as anxiolytics and hypnotics. There is concern regarding their use because of their potential for dependence and abuse as well as negative effects on cognition and memory. One area of concern regarding the use of benzodiazepines in the treatment of anxiety is the potential for habituation, dependence and abuse .[7] Hence there is always a search for drugs with lesser side effects. 'Clonidine', a α_2 agonist known for its antihypertensive effect also possesses sedative, hypnotic, anxiolytic, and analgesic properties.[8] Hence the present study is undertaken to find out the anxiolytic effect of clonidine in comparison with the standard drug diazepam in mice.

MATERIALS AND METHODS

This study was carried out in the Institute of Pharmacology and Central animal house, Madurai Medical College, Madurai and was conducted for a period of eight months from 15-09-2009 to 11-05-2010, after obtaining ethical clearance from the Institutional Animal

Ethical Committee, Madurai Medical College, Madurai.

Materials required for the study

Animals

18 inbred male adult albino mice each weighing 18 to 25 grams, obtained from Central animal house, Madurai Medical College was utilized in this study. Animals were allowed standard diet and tap water ad libitum.

Standard drug

Injection Diazepam is mixed with normal saline to obtain a solution of concentration 0.01 mg/ml and is administered intraperitoneally at the dose of 1.5 mg/kg.[9]

Test drug

Tablet Clonidine was dissolved in water for injection and administered intraperitoneally at graded doses of 0.05 mg/kg and 0.1 mg/kg. [10]

Pentylenetetrazole

Pentylenetetrazole was dissolved in cold saline and administered intraperitoneally at the dose of 5 mg/kg to induce anxiety.[11]

Water for injection

Water for injection was administered intraperitoneally to control group of animals.

Normal saline

Normal saline was used as a vehicle for diazepam and pentylenetetrazole.

METHODOLOGY

Pentylenetetrazole induced Anxiety

18 adult male naive mice weighing 18-25 grams were divided into three groups of 6 animals in each group namely control, standard, and test groups. 30 minutes prior to administration of pentylenetetrazole 0.5 mg/kg intraperitoneally, Control group of animals received water for injection intraperitoneally, Standard group of animals received injection Diazepam 1.5 mg/kg intraperitoneally and Test group of animals received 0.1 mg/kg of aqueous preparation of Clonidine intraperitoneally After pentylenetetrazole administration, the animals were placed in isolation and observed individually for grooming activity. The number of bouts of grooming and the duration of grooming activity were observed for 10 minutes for each animal. The results were tabulated and analyzed statistically using Student's t-test.

RESULTS

Anxiolytic effect

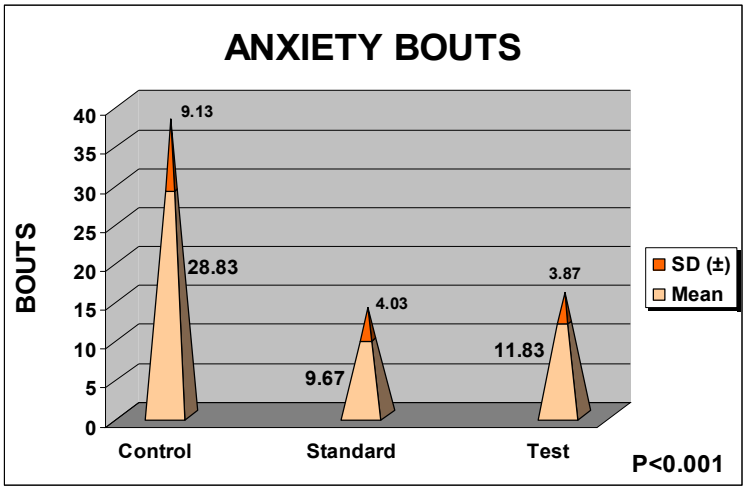
The animals were observed individually for grooming activity after pentylenetetrazole administration. Anxiolytic effect was measured by a reduction in the number of bouts and duration of grooming induced by pentylenetetrazole. Both the standard and the test group of animals showed reduction in the number of grooming bouts as well as duration of grooming. The average number and duration of bouts in the control group of mice is 28.83 ± 9.13 and 51.33 ± 7.39 seconds respectively. The average number and duration of bouts in the standard group of mice is 9.67 ± 4.03 and 24.67 ± 6.5 seconds respectively. The average number and duration of bouts in the test group of mice is 11.83 ± 3.87 and 21 ± 2.83 seconds respectively. The results were tabulated in Table I and analyzed statistically using unpaired student's t-test. The results were highly significant ($P < 0.001$) for both test and standard groups in comparison with control group statistically.

Table 1
Anxiolytic effect

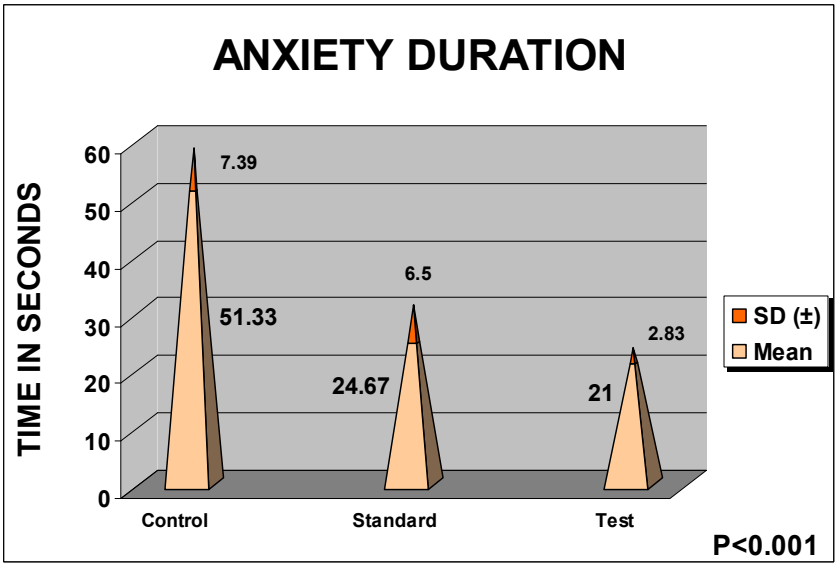
GROUP	TREATMENT	NUMBER OF BOUTS (MEAN ±	DURATION OF BOUTS (MEAN ± SD)
CONTROL	Water for Injection + Pentylenetetrazole	28.83 ± 9.13	51.33 ± 7.39
STANDARD	Diazepam + Pentylenetetrazole	9.67 ± 4.03 ***	24.67 ± 6.5 ***
TEST	Clonidine + Pentylenetetrazole	11.83 ± 3.87 ***	21 ± 2.83 ***

*Bouts : Control Vs Standard & Test - *** P<0.001*
*Duration : Control Vs Standard & Test - *** P<0.001*

Graph 1
Anxiety bouts



Graph 2
Anxiety duration



DISCUSSION

Anxiety is a normal reaction to stress, but when it becomes excessive it may fall under the classification of an anxiety disorder.[12] It is a feeling of apprehension, uncertainty or tension stemming from the anticipation of an imagined or unreal threat. Approximately 4-6% of the population suffers from anxiety. It is so severe that it disrupts routine life functions. Hence anxiolytic drugs are extensively prescribed. [10] Anxiety is a cardinal symptom of many psychiatric disorders and an almost inevitable component of many medical and surgical conditions. Indeed it is a universal human emotion, closely allied with appropriate fear and presumably serving psychobiologically adaptive purposes.[7] The locus ceruleus noradrenergic and dopaminergic systems are believed to increase autonomic arousal and vigilance in response to threat. Overstimulation of such adaptive mechanisms by repeated or chronic stressors as well as deficits in function of components of these systems could theoretically lead to pathological responses observed in anxiety disorders.[2] Clonidine is a presynaptic α_{2}-adrenergic receptor agonist that is approved for use as an antihypertensive agent. Stimulation of α_{2}-adrenergic receptors reduces the firing rate of noradrenergic neurons and reduces the plasma concentrations of norepinephrine. Because of widespread actions of the noradrenergic system, Clonidine has also been adopted for use as a psychopharmacological agent. It is also used as a pharmacological probe to assess central α_{2}-receptor sensitivity in psychiatric disorders.[13] In this study Anxiolytic effect was evaluated by observing the grooming effect induced by pentylenetetrazole in the dose of 5 mg/kg intraperitoneally. Self grooming is an important part of animal behavioral repertoire, which in rodents has long been considered as a complex ethologically “rich” response, particularly sensitive to various endogenous or exogenous factors. Grooming normally proceeds in a cephalocaudal direction and consists of licking the paws, washing movements over head, fur licking and tail/genitals cleaning. Pentylenetetrazole is a powerful CNS stimulant which causes convulsions in high doses and produces anxiety in low doses. Anxiety induces increased grooming activity in rodents.[14] In this study there was a highly significant reduction in number and duration of grooming bouts with clonidine in comparison with the control statistically. In the present study clonidine showed significant anxiolytic effect. Several other studies have demonstrated the sedative, hypnotic effects of clonidine which is due to its actions on α_{2} adrenoceptors of dorsal horn.[8]

CONCLUSION

Sleep and anxiety disorders are common nowadays and hence several classes of sedative, hypnotic and anxiolytics are widely prescribed. Benzodiazepines are the most commonly used sedative, hypnotic and anxiolytic drugs. Benzodiazepines can cause several unwanted side effects like tolerance, dependence and withdrawal symptoms. Acute overdose may result in respiratory depression.α_{2} adrenoceptor agonist, clonidine has been used for 30 years as centrally acting antihypertensive agent. It is found to be useful in psychiatric disorders by its central action. Clonidine possesses sedative and hypnotic effects also. The present study has proved that clonidine significantly reduces the anxiety induced by pentylene tetrazole. Further clinical studies to evaluate the safety, will render clonidine as a popular anxiolytic agent, especially in situations associated with sympathetic release.

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