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Evaluation of Cilnidipine Effects on Rodent Locomotor Activity Using Actophotometer

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Abstract

Cilnidipine, a dual L/N-type calcium channel blocker, is commonly used in the management of hypertension. Beyond its cardiovascular effects, the influence of cilnidipine on central nervous system activities, such as locomotor function, remains underexplored. This study aims to evaluate the impact of cilnidipine on locomotor activity in rodents, utilizing an actophotometer for precise measurement. In this experiment, adult male wistar rats were divided into four groups: control, vehicle, low-dose cilnidipine (5 mg/kg), and high-dose cilnidipine (10 mg/kg). The drugs were administered intraperitoneally once daily for 21 consecutive days. Locomotor activity was assessed using an actophotometer, which quantifies the movement of animals based on interruptions of light beams in a closed chamber. Measurements were taken on 1st day, 14th day and 21st day 30 minutes after drug administration.The results indicated that cilnidipine administration did not lead to any significant changes in locomotor activity compared to the control and vehicle groups. Both the low-dose and high-dose cilnidipine groups exhibited locomotor activity levels similar to those observed in the control group, with no significant differences in the total distance moved or the number of beam breaks. These findings suggest that cilnidipine does not exert central nervous system depressant effects at the doses tested, indicating that its action is predominantly peripheral with minimal impact on motor function.In conclusion, this study provides evidence that cilnidipine, while effective as a peripheral vasodilator, does not significantly alter locomotor activity in rodents. The lack of change in movement highlights that cilnidipine's effects are primarily limited to its intended cardiovascular targets, with negligible influence on the central nervous system at therapeutic doses. These findings contribute to the broader understanding of cilnidipine's pharmacological profile and its safety in terms of motor function. Future studies should continue to explore the broader neuropharmacological implications of cilnidipine, ensuring comprehensive safety profiles for patients.

Keywords: Cilnidipine, Locomotor activity, Actophotometer, Calcium channel blocker

Introduction

Stress is defined as all the body's responses that upset the usual balance of chemicals in the body, creating a state of threatening homeostasis, and putting a person's life in danger. Stress triggers many changes in the body, such as changes in behaviour, bodily processes, and the hyper-activation of the hypothalamo-pituitary-adrenal (HPA) axis.[1] Multiple studies have shown that long-term stress is a contributing factor to the onset of neurological disorders, skin diseases, gastrointestinal diseases, and cardiovascular diseases.[2–7]Calcium serves as a multifunctional cellular messenger and plays a crucial role in regulating important neuronal activities such as synaptic transmission, gene expression, and synaptic plasticity, which are all involved in the processes of learning and memory.[8]

Calcium channel blockers, such as cilnidipine, are known to inhibit the influx of calcium ions into cells, particularly in the cardiovascular system. However, recent studies have suggested that these drugs may also have effects on the CNS. Calcium signalling plays a crucial role in various neuronal processes, including neurotransmitter release, synaptic plasticity, and neuronal excitability. Disruption of calcium homeostasis in the CNS has been implicated in various neurological disorders, such as stress-related disorders, anxiety, and depression.[9] Research has shown the extensive distribution of L-type and N-type calcium channels in several regions of the brain, such as the hippocampus, thalamus, and cortex.[10,11]

The actophotometer is a useful tool for assessing locomotor activity in rodents, which can serve as an indicator of CNS function. Locomotor activity is regulated by various neurotransmitter systems and brain regions, and changes in activity can reflect alterations in CNS function. By measuring locomotor activity before and after the administration of cilnidipine, researchers can evaluate the potential effects of the drug on the CNS. Voltage gated calcium channels are mainly responsible for the entry of calcium ions from outside the cell. The voltage activated calcium channels, L- and N-type, are essentially differentiated by the pore forming α1 subunits (from three big families Cav1, Cav2, Cav3) that give them unique electrophysiological characteristics. The Cav1 family is responsible for encoding L-type calcium channels, which consist of four distinct members (Cav1.1–Cav1.4). On the other hand, Cav2.2 is responsible for encoding N-type calcium channels.[9]

Research has shown that N-type calcium channels have a significant impact on neurotransmission, synaptic plasticity, and brain development. N-type calcium channels are situated in the pre-synaptic region and control the release of neurotransmitters. Verapamil and nifedipine, which are L-type calcium channel blockers, have been shown to inhibit the development of stomach ulcers caused by cold/restraint stress and the activation of the renin-angiotensin-aldosterone system.[12–14]

In addition, studies have shown that L-type calcium channel blockers may eliminate the neuroadaptive response caused by restraint stress, which includes the development of sensitivity to amphetamine.[15]

Scientists have endeavoured to examine the impacts of N-type and L-type voltage dependent calcium channels on several brain-related behavioural activities.However, it has been shown that the participation of spinal N-type calcium channels, rather than L-type channels, is responsible for the analgesic effects caused by cold water swimming stress.[16]

Several studies have investigated the effects of cilnidipine and other calcium channel blockers on stress-related behaviors and CNS function using animal models. For example, a study by Anjaneyulu and Chopra (2003) [17]found that cilnidipine and nimodipine, another calcium channel blocker, produced beneficial effects in restoring immobilization stress-induced behavioral alterations in mice. Another study by Anjaneyulu et al. (2003) [18]demonstrated that cilnidipine and nimodipine had anti-stress effects in immobilization-subjected mice, as evidenced by improved behavioral parameters and reduced corticosterone levels.

The use of an actophotometer in these studies allows for the quantitative assessment of locomotor activity, which can provide insights into the CNS effects of cilnidipine. By comparing the locomotor activity of mice or rats treated with cilnidipine to those receiving a control treatment, researchers can determine if the drug has a stimulant or depressant effect on the CNS. This information can contribute to our understanding of the potential therapeutic applications of cilnidipine in stress-related disorders and other neurological conditions.

Cilnidipine, a cerebro-selective calcium channel blocker that targets both L-type and N-type calcium channels, is hypothesized to mitigate the physiological and behavioral impacts of stress-induced immobilization in animals. Due to its selectivity for the brain, cilnidipine may effectively reduce stress-related biomarkers and alleviate anxiety and depressive-like behaviors caused by immobilization. This study aims to explore cilnidipine's potential therapeutic role in managing stress-related disorders and enhancing mental health outcomes in animal models.

Material and method

Animals

Swiss albino mice weighing 25± 5 g were employed in present study. Animals were fed on standard laboratory feed and water was given ad libitum. Animals were housed in the Institutional animal house and were exposed to natural cycles of light and dark. The experimental protocol was approved by Institutional Animal Ethics Committee (IAEC) and care of the animals was carried out as per the guidelines of the Committee for Control and Supervision of Experimental Animals (CCSEA).

Drugs and chemicals

Cilnidipine and Fluoxetine were used in the present study. Chemicals and reagents used were purchased from Hospital Pharmacy of Teaching institute.

In behavioural investigations, it is essential to familiarise the animals with the test equipment. This is done to prevent any inaccurate effects caused by the novelty of the apparatus, which might in turn decrease the variability in the experimental results. Afterwards, the mice were exposed to Actophotometer then underwent a series of test on day 1, 14 and 21. The tests included evaluation of motor activity using actophotometer.

Actophotometer test

The locomotor activity is considered as an index of alertness and was assessed by keeping the mice in the actophotometer individually. The locomotor activity was assessed in terms of counts per 10 min.[19] In an actophotometer, the movement of the animal interrupts the beam of light falling on the photocell and a count is recorded digitally. Therefore, the number of counts is directly related to movement of the animal inside the actophotometer chamber.

Experimental protocol

Four groups, each comprising of six Swiss albino mice, were employed in the present study. Group A was treated with normal saline, Group B was treated with standard drug Fluoxetine, whereas Group C and D were given Cilnidipine 5mg and 10mg respectively. Actophotometer was done 30 minutes after intraperitoneal injection of drug.

Statistical analysis

The results were expressed as mean± standard deviation. Different groups were analysed using one-way ANOVA followed by post-hoc analysis using Bonferroni Multiple Comparison Test. The value of $p<0.05$ was statistically significant.

Results

Locomotor activity on Day 1

The locomotor activity was assessed for different groups over 10 minutes. The locomotor activity in terms of counts in the control group was 21.83±2.9 while in the Fluoxetine group was 19.66±2.4, Cilnidipine 5mg/kg dose: 22±2.8, Cilnidipine 10mg/kg dose: 21.6±4.6.

Locomotor activity on Day 14

The locomotor activity was assessed for different groups over 10 minutes. The locomotor activity in terms of counts in the control group was 22.6±3.1while in the Fluoxetine group was 20±3.7, Cilnidipine 5mg/kg dose: 23.8±3.7, Cilnidipine 10mg/kg dose: 20±3.7.

Locomotor activity on Day 21

The locomotor activity was assessed for different groups over 10 minutes. The locomotor activity in terms of counts in the control group was 20±3.3 while in the Fluoxetine group was 18.8±3.4, Cilnidipine 5mg/kg dose: 19.8±4.2, Cilnidipine 10mg/kg dose: 20±5.8.

On the 21st day, the Fluoxetine group showed a notable difference in the average locomotor activity compared to control group. On days1,14, and21, the treatment group's mean locomotor activity was not notably distinct from that of the control and standard groups. (Table 1, Graph 9-12)

Table 1

Effect of various drugs on Locomotor activity in Actophotometer.

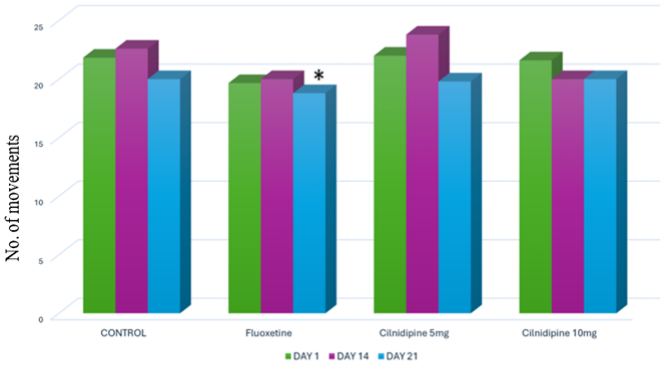
Day of study	Effect on locomotor activity			
	Control	Fluoxetine	Cilnidipine 5mg	Cilnidipine 10mg
1	21.83±2.9	19.66±2.4	22±2.8	21.6±4.6
14	22.6±3.1	20±3.7	23.8±3.7	20±3.7
21	20±3.3	18.8±3.4 *	19.8±4.2	20±5.8

Note:

1. Data expressed as mean ± SD.
2. ANOVA followed by Bonferroni's multiple comparison test.
3. *p<0.05 in comparison to control group.

Graph 1

Overall effect of various Drugs on locomotor activity in Actophotometer



Note:

1. Data expressed as mean ± SD.
2. ANOVA followed by Bonferroni's multiple comparison test.
3. *p<0.05 in comparison to control group.

The line crossings are taken as an indicator of motor activity. In control group, the number of line crossings was decreased significantly. Administration of cilnidipine (10 mg/kg, IP) and Fluoxetine (10 mg/kg, IP) 30 min prior resulted in significant attenuation of immobilization stress-induced decrease in line crossings.

However, administration with cilnidipine (5 mg/kg, IP) did not modulate the frequency of line crossings.

Discussion

In the present investigation, immobilization-induced acute stress resulted in significant decrease in locomotor activity (decrease in frequency of counts in the actophotometer test). Our earlier studies have shown that stress produces behavioral alterations including decrease in locomotor activity, exploratory behavior and social behavior.[19,20]Immobilisation is a multifaceted stressor that encompasses both physical and psychological aspects. The immobilization-induced stress paradigm is often used to study stress because it is intense enough to affect several biological systems involved in stress response, including the HPA axis.

In the present study, as a consequence of immobilization might result in rise in the serum corticosterone levels due to hyperactivity of the HPA-axis. Stress associated memory impairment may also be linked to an increased level of the corticosterone as previous studies have shown that longer period of stress or high dose of the corticosterone produces memory impairment.[21]

In contrary to this, short period of stress or low dose/release of the corticosterone in the body enhances the memory.[22]In the present study, pretreatment with cilnidipine (L and N-type calcium channel blocker) significantly attenuated decrease in mobility. Prior research has shown that immobilisation stress is linked to elevated calcium levels in brain synaptosomes and an increase in the number of calcium channels in several brain areas, including the hippocampus.[23]The current study findings, which indicate a positive effect on behaviour when using L-type calcium blockers, align with a recent report that highlights the significant involvement of CaV1.3 L-type calcium channels in the manifestation of depression-like behaviour. This is supported by evidence showing that mice lacking CaV1.3 exhibit antidepressant and anxiolytic-like characteristics.[24]

The earlier investigations have established the existence of L-type calcium channels on the HPA-axis and important involvement of calcium in the corticosterone production is also well recognised. The investigations have also reported the reduced corticosterone release in presence of calcium channel blockers. On the other hand, investigations have also suggested that the corticosterone potentiates the calcium influx indicating the positive feedback mechanism in the corticosterone release. Therefore, it is conceivable to argue that blocking of L-type calcium channels on the HPA axis may be responsible for reduced corticosterone release.[18,25]

Conclusion

Cilnidipine produce beneficial effects in restoring acute immobilization stress-induced decreased mobility that may be possibly linked to attenuation of the corticosterone release.

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