Journal of Medical Sciences

ISSN 1682-4474

science alert

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Research Paper

JMS (ISSN 1682-4474) is an International, peer-reviewed scientific journal that publishes original article in experimental & clinical medicine and related disciplines such as molecular biology, biochemistry, genetics, biophysics, bio-and medical technology. JMS is issued six times per year on paper and in electronic format.

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J. Med. Sci., 6 (6): 1021-1024
November-December, 2006

Anticonvulsant and Anxiolytic Effects of Calcium Channel Blockers in Mice

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This study reports on the anticonvulsant and anxiolytic effects of two well-known calcium channel antagonists, verapamil and nifedipine in mice. The anticonvulsant effect of these drugs was screened utilizing strychnine animal seizure model. The anxiolytic effect of these compounds was assessed using elevated plus maze paradigm. The results of the study showed that verapamil (5-20 mg kg^{-1}, i.p) and nifedipine (5-10 mg kg^{-1}, i.p) exhibited anticonvulsant activity as evidenced by their ability to prolong the onset of seizures produced by strychnine (1 mg kg^{-1}, i.p) in mice. Verapamil (20 mg kg^{-1}) offered 100% protection against convulsions or death induced by strychnine. Similar effects were observed in animals pretreated with 10 mg kg^{-1} of nifedipine. In the anxiolytic test, verapamil, but not nifedipine significantly ($p<0.05$) modified the number of entries in a similar manner to diazepam in the elevated plus maze paradigm. Taken together, these findings suggest that verapamil possess both anticonvulsant and anxiolytic effects, whilst nifedipine only exhibited anticonvulsant activity in mice.

Key words: Verapamil, nifedipine, anticonvulsant, anxiolytic, effects

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INTRODUCTION

Calcium channel blockers are currently employed in the treatment of cardiovascular diseases such as hypertension, angina and arrhythmia (Gogfraind *et al*., 1986). The usefulness of these drugs in these disorders is due to the inhibition of transmembrane influx of calcium into the cardiac and smooth muscles (Gogfraind *et al*., 1986).

The recognition of the functional roles of calcium in the genesis of epileptic seizures and anxiety states has raised the hope that calcium channel blockers might offer beneficial effects in these conditions (Cortes *et al*., 1984; Pucilowski, 1992). Indeed, studies have shown that some calcium channel blockers offered beneficial effects in the treatment of anxiety associated with alcohol or benzodiazepine withdrawal (Pucilowski, 1992).

The potential utility of calcium blockers in the treatment of convulsive seizures has also been reported in previous studies (Meyer *et al*., 1990; Desarro *et al*., 1988). The efficacy of calcium antagonists in this neurological disorder might be due to the blockade of voltage-gated calcium channel in the brain (Desarro *et al*., 1988; Rogawski and Porter, 1990).

It is well reported in literature that drugs such as ethosuximide, trimethadione and valproic acid exert their anti-seizure effects in part through the blockade of voltage-regulated calcium ion channels (McNamara, 2001; Rogawski and Porter, 1990). However, compounds with specific calcium channel blocking property might demonstrate a more potent anti-seizure activity and with minimal side effects than these drugs. In this study, we evaluate the anticonvulsant and anxiolytic properties of two well-known calcium channel blockers, verapamil and nifedipine in mice.

MATERIALS AND METHODS

Laboratory animals: Albino mice of either sex (18-20 g) obtained from the Laboratory animal center, College of Medicine, University of Lagos, Nigeria were used in the study. They were kept in a well-ventilated environment and had free access to food and water *ad libitum*.

Drugs: Verapamil, nifedipnie, diazepam, phenobarbitone, strychnine and sodium chloride were used in the study.

Anticonvulsant test: The animals (6 mice/group) were pretreated with verapamil (5-20 mg kg^{-1}, i.p), nifedipnie (2.5-10 mg kg^{-1}, i.p), saline (10 mL kg^{-1}) or phenobarbitone (40 mg kg^{-1}, i.p) 30 min before induction of convulsions with i.p injection of strychnine (1 mg kg^{-1}). Each animal was observed for the onset of convulsions or death for a period of 30 min after strychnine administration.

Anxiolytic test: The elevated plus maze paradigm was used in this study. The apparatus consisted of an elevated maze with two intercepting arms, two of which are opened and two closed. Anxiolytic test was carried out by measuring the proportion (number) of entries into the open and close arms (Barrett, 1991). Anxiolytic effect is indicated when the test drug increases the number of entries into the open arm of the elevated plus maze. The animals (6 mice/group) were pretreated with verapamil (5-20 mg kg^{-1}, i.p), nifedipnie (5-10 mg kg^{-1}, i.p), saline (10 mL kg^{-1}, i.p) or diazepam (5 mg kg^{-1}, i.p). Thirty minutes later, each mouse was placed in the center of the elevated plus maze, facing one of the enclosed arms. The number of entries into the open and closed arms for a period of 10 min was recorded.

Statistical analysis: Data obtained are expressed as mean±SEM. Statistical analysis was carried out using ANOVA. p-values less than 0.05 were considered statistically significant.

RESULTS

Effects on Strychnine-induced seizures: Verapamil (25 mg kg^{-1}, i.p) or nifedipnie (10 mg kg^{-1}, i.p) offered 100% protection against convulsive episodes or death induced by strychnine (1 mg kg^{-1}, i.p) in mice. However, at 12 mg kg^{-1}, verapamil could only delay the onset of seizures and reduced the number of death in strychnine-treated mice. Similar effects were produced by 4 mg kg^{-1} dose of nifedipine (Table 1).

Anxiolytic effect: Diazepam (5 mg kg^{-1}) produced an increase in the number of entries into the open arm of the elevated-plus maze. In similar a manner, verapamil

Table 1: Effect of verapamil and nifedipine on strychnine-induced convulsions in mice

Treatments	Dose (mg kg^{-1})	Onset of seizures (min)	Death (%)	Protection (%)
Saline	-	8.0±2.8	100	-
Verapamil	5.0	8.4±1.5	60	40
Verapamil	10.0	10.8±0.2*	40	60
Verapamil	20.0	-	0	100
Nifedipine	2.0	13.2±1.2	100	0
Nifedipine	4.0	15.0±0.4	60	40
Nifedipine	10.0	-	0	100
Phenobarbitone	40.0	-	0	100

Each value represents the mean±SEM for 6 mice per group. Each animal was pretreated with saline or each drug 30 min before induction of convulsions with i.p injection of strychnine (1.0 mg kg^{-1}, 1.p). $p<0.05$ when compared with saline-control group (ANOVA)

Table 2: Anxiolytic property of verapamil and nifedipine in mice

Treatment	Dose (mg kg^{-1})	No. of entries	
		Open arm	Closed arm
Saline	-	1.3±0.34	4.50±0.24
Verapamil	5	2.2±0.15	3.90±0.33
Verapamil	10	2.1±0.29	2.84±0.29
Verapamil	20	3.5±0.50	2.10±0.40
Nifedipine	2.5	1.2±0.4	4.10±0.20
Nifedipine	5.0	1.1±0.26	4.50±0.40
Nifedipine	10	1.3±0.20	4.40±0.34
Diazepam	5	2.6±0.21	3.00±0.25

Each value represents the mean±SEM for 6 mice per group. $p<0.05$ when compared with saline-control group (ANOVA)

(5-20 mg kg^{-1}) increased the number of open arm entries in a dose-related manner (Table 2). However, nifedipine (5-10 mg kg^{-1}) did not significantly after the number of open arm entries in this study (Table 2).

DISCUSSION

The results of the study showed that verapamil and nifedipine possess anticonvulsant activity in mice. This is evidenced by their ability to offer significant protection against convulsions and death induced by strychnine in mice. In the anxiolytic test, verapamil, a phenylalkylamine type of calcium antagonists significantly modified the number of entries into the open arm of the elevated plus maze in a similar manner to diazepam. However, nifedipine, a dihydropyridine type of calcium blockers did not significantly alter the number of entries in the elevated pus maze.

It is well established in literature that the neuronal pathway that underlies strychnine convulsions is related to the blockade of glycine-mediated inhibitory neurotransmission (Taylor and Insel, 1990). Previous studies have shown that calcium channel antagonists possess membrane-stabilizing properties and therefore could reduce seizure episodes in an epileptic brain (Meyer *et al*., 1990). Furthermore, calcium channel antagonists have been found to block the inflow of sodium into detonated neurone as well as inhibit the release of glutamate, an excitatory neurotransmitter from such neurones (Meyer *et al*., 1990; McNamara, 2001; Rogawski and Porter, 1990).

Recently, glutamate has been implicated in the pathogenesis of convulsive seizures (McNamara, 2001). The protective effect demonstrated by verapamil and nifedipine against strychnine-induced seizures in this study further supports the results of previous investigations (Desarro *et al*., 1988). Meyer *et al*. (1990) also reported that nimodipine, a dihydropyridine calcium blocker, offered significant protection against electroshock seizures in similar manner to phenytoin. However, further studies are necessary to establish the precise mode by which calcium antagonists inhibit convulsive seizures.

The elevated plus maze is the most widely used paradigm for assessing novel compounds with anxiolytic properties (Barrett, 1991; Pellow *et al*., 1985). Under non-drug conditions, rodents enter the closed arm of the elevated plus maze (Barrett, 1991). The anxiolytic effect is envisaged when the test compound increases the number of entries into the open arm of elevated plus maze (Barrett, 1991; Pellow *et al*., 1985). It is relevant to note that anxiety is related to the release of anxiogenic substances like glutamate, noradrenaline, adrenaline, serotonin and steroids in response to a variety of stimuli (Insel *et al*., 1991).

Verapamil has been shown to interact with receptors of various neurotransmitters that play crucial roles in the modulation of mood and behaviour (Pucilowski, 1992). In addition, verapamil has been found to inhibit the release of steroids and also blocked sodium channel (Smith *et al*., 1996; Boullin and Grahma-smith, 1986). It is suggestive that these actions of verapamil apart from inhibition of calcium channel might be playing a significant role in its anxiolytic effect observed in this study.

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