

Study on Neuromuscular Blockade Action of Verapamil in Albino Rats

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ABSTRACT

Background: Calcium Channel Blockers (CCBs) are now widely employed in the treatment of cardiovascular diseases and peri-operative hypertension. It has been reported that calcium channel blockers inhibit neuromuscular transmission. They have been shown to increase the neuromuscular blockade produced by neuromuscular blocking agents in in-vitro muscle nerve preparations. The present study is undertaken to demonstrate the effect of calcium channel blocker, verapamil on neuromuscular transmission in albino rats.

Objectives: To study the neuromuscular blockade action of verapamil in albino rats.

Methods: Twenty four albino rats of either sex weigh 150-250gms are selected and are randomly divided into 4 equal groups. The experimental rats are divided into four groups of 6 rats each and they are given the following treatment.

Group 1(Control) - Normal saline (1ml/ kg),

Group 2 (Standard) - Pancuronium (0.04 mg/kg)

Group 3-Verapamil (2.5mg/kg),

Group 4-given Verapamil (10mg/kg).

The time of onset of hind limb paralysis and total duration of recovery are noted using inclined screen method.

Results: Analysis of the results of group 3 that was received 2.5mg/kg of Verapamil, there was no onset of paralysis, in group 4 that received injection Verapamil 10mg/kg, showed neuromuscular blockade activity. The mean onset of hind limb paralysis was delayed compared to standard group and the mean duration of hind limb paralysis was shorter than standard group. It was statistically significant ($P \leq 0.05$).

Interpretation and conclusion: It is generally held that external calcium is not necessary for the contraction of mammalian skeletal muscle, the demonstration of inward calcium currents that can be abolished by CCBs in these muscles prompted to re-examine the effect of Verapamil on the neuromuscular transmission. The present study allows us to determine the neuromuscular blockade activity of Verapamil.

Key words: Calcium channel blockers, Neuromuscular blocking agents, Hind limb Paralysis, Verapamil, and Pancuronium

INTRODUCTION

The neuromuscular blocking drugs have revolutionized the practice of anesthesia. Skeletal muscle relaxation and paralysis can occur interruption of function at several different sites, that includes the central nervous system, myelinated somatic nerves, unmyelinated motor nerve terminals, nicotinic acetylcholine receptors, the motor end plate and the muscle membrane or intra-cellular contractile apparatus itself [1,2].

Calcium Channel Blockers (CCBs) are chemically heterogeneous group of drugs that inhibit the ionic current carried through the slow calcium channel in cardiac and vascular smooth muscle. This action is the basis of their usefulness in the treatment of cardiovascular disorders [3].

The excitation – contraction coupling in skeletal muscle is thought to depend mainly on intra-cellular stores of calcium. The calcium entry blockers might not be expected to exert any significant effects on voluntary muscle contraction. However, recent studies have suggested that the calcium entry blocker Verapamil, can inhibit neuromuscular transmission in anaesthetized cats and dogs [4].

Anesthesiologists may have to deal with the interaction of calcium entry blockers with muscle relaxants in patients who are on prolonged calcium antagonist therapy before surgery or others giving one or two doses of verapamil IV for treatment of supraventricular paroxysmal tachycardia or reduction of ventricular rate associated with atrial fibrillation or flutter during anesthesia [5].

There are many studies showing potentiation or interaction of Neuromuscular Blockers with CCBs but, very few in vivo studies to demonstrate the neuromuscular blockade action of CCBs alone.

In the light of the aforementioned developments, this study has been undertaken to evaluate the neuromuscular blockade action of verapamil.

MATERIAL & METHODS

The present study was conducted in the Department of Pharmacology, JJM Medical College, Davangere, India, with the approval of institutional ethical committee.

Wistar albino rats of either sex weighing 150-250g were selected. The animals were housed under standard conditions with free access to water and food. The animals were screened for normal motor activity before initiating the procedure.

While studying the new property of drug it had to be compared with the existing standard reference drug already in use. Pancuronium is the ideal non-depolarizing blocker which is commonly used taken as standard drug to compare the neuromuscular blockade action of verapamil.

Inclusion Criteria:

- Animals weighing >150 and <250g
- More than 21 days of prior use of any experimental purpose.
- Age < 4 months
- Healthy with normal behavior

Exclusion Criteria:

- Animals weighing < 150g and > 250g.
- Within 21 days of prior use for any experimental purpose.
- Age > 4 months
- All visible diseases

A total number of 24 rats of either sex were divided into four

groups of six rats each.

Group 1 (Control): Normal saline 1ml/ kg

Group 2 (Standard): Pancuronium 0.04 mg/kg

Group 3 (Test Group-1): Verapamil 2.5mg/kg

Group 4 (Test Group-2): Verapamil 10mg/kg

Drugs were injected by a 24-25 gauge needle attached to a 1ml tuberculin syringe into the intraperitoneal cavity of rats. After injecting the drug, animals, the neuromuscular blockade activity was observed over a period of 1 hour using inclined screen method.

Inclined screen method

An inclined screen was used for testing curare-like agents for testing compounds for muscle relaxant activity [6]. The rats were placed at the upper part of the inclined screen and were given 30 seconds to hang on or to fall off. The following parameters were observed.

1. Time of Onset of hind limb paralysis (in minutes)
2. Total duration of paralysis (in minutes)

RESULTS

The study includes total number of 24 rats divided into four groups of six rats each and received the drugs accordingly. The time of onset of hind limb paralysis & total duration of paralysis recorded

in minutes in each group has been provided in [Table/Fig-1 and 2]. There was no hind limb paralysis seen with Group 3 Verapamil 2.5 mg/kg [Table/Fig-1 and 3]. Neuromuscular blockade action was seen with Group 4 Verapamil 10mg/kg., but the onset of action was delayed (26.3 min ±4.3 min) compared to Group 2 Standard treated with Pancuronium (5.5 min ± 1.5 min). Group 4 Verapamil 10mg/kg showed shorter duration of hind limb paralysis. (25.8 min ± 19.4 min) compared to Group 2 Standard (37.3 min ± 6.8 min) [Table/Fig-2 and 4).The results of intra and inter group comparisons were statistical significant (p≤0.05).

STATISTICAL ANALYSIS

Results were presented as Mean ± SD. One way ANOVA was used for multiple comparisons followed by Bonferroni's post-hoc test for comparison between groups. For all the tests a 'p' value of 0.05 or less was considered for statistical significance

DISCUSSION

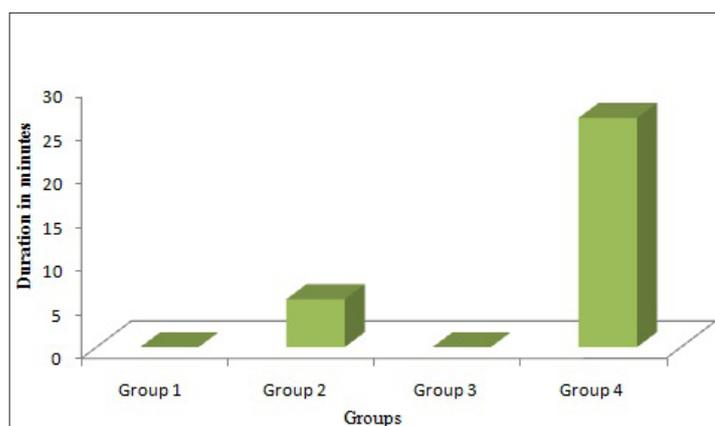
The effects of calcium channel blockers have been extensively investigated in the cardiac muscle, smooth vascular, respiratory and intestinal, there are few studies on the action of these drugs on neuromuscular activity. The skeletal muscle calcium channel contains slow type, similar to that observed in cardiac and vascular smooth muscles, with special binding sites for calcium channel blockers. These channels in cardiac and vascular smooth

GROUPS	SERIAL NO.OF RATS						MEAN	SD
	1	2	3	4	5	6		
Group 1 Control (Normal saline)	--	--	--	--	--	--	--	--*
Group 2 Standard (Pancuronium)	7	5	5	7	3	6	5.5	1.5
Group 3 Verapamil 2.5mg/kg	--	--	--	--	--	--	--	--*
Group 4 Verapamil 10mg/kg	20	30	27	25	32	24	26.3	4.3

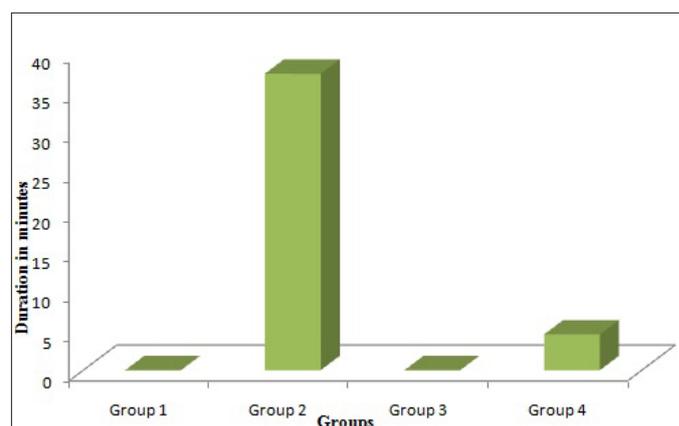
[Table/Fig-1]: Time taken for onset of hind limb paralysis following injection (in minutes). (* No hind limb paralysis)

GROUPS	SERIAL NO.OF RATS						MEAN	SD
	1	2	3	4	5	6		
Group 1 Control (Normal saline)	--	--	--	--	--	--	--	--*
Group 2 Standard (Pancuronium)	45	30	28	40	42	39	37.3	6.8
Group 3 Verapamil 2.5mg/kg	--	--	--	--	--	--	--	--*
Group 4 Verapamil 10mg/kg	15	20	65	18	15	22	25.8	19.4

[Table/Fig-2]: Total duration of hind limb paralysis (in minutes). (* No hind limb paralysis)



[Table/Fig-3]: Bar diagram showing mean duration of onset of hind limb paralysis



[Table/Fig-4]: Bar diagram showing mean duration of hind limb paralysis

muscles are very sensitive to CCBs, where as skeletal muscles are less sensitive to CCBs [7]. Because of these peculiarities in some physiological processes in the muscle or neuromuscular junction may be affected by these drugs.

The results of the present study demonstrate that Verapamil showed neuromuscular blockade activity at 10mg/kg. In a previous study done by Patel BG et al., demonstrating the interaction of verapamil with neuromuscular blocker gallamine in rats, three graded doses of verapamil 2.5mg/kg, 5mg/kg & 10 mg/kg potentiated the neuromuscular blockade action of gallamine [8]. As compared to the earlier study, where 2.5 mg/kg can potentiate the action but in this study, at 2.5 mg/kg Verapamil could not show the neuromuscular blockade property alone.

Although it is not designed primarily to investigate the underlying neuromuscular depressant action of calcium channel blocker verapamil, several mechanisms have been proposed. Calcium is essential for the release of acetylcholine at neuromuscular junction. It has been postulated that verapamil inhibits conductance of the presynaptic membrane to calcium. Some studies suggest that verapamil affects transmitter release or may interfere with neuromuscular transmission by blocking the action of acetylcholine [9].

CONCLUSION

In the present study, verapamil demonstrated the neuromuscular blockade activity. In order to specify the mechanisms involved in neuromuscular blockade activity of verapamil more extensive studies are required. Whatever the actual mechanism(s) involves, the present data suggests that the clinicians should be aware of

such potential neuromuscular blockade action in patients receiving long term therapy with calcium channel blockers

LIMITATIONS OF STUDY

The study has several limitations. It has been carried out only in one species of animals like rats and needs to be extended to other animals as well and experiment was conducted using only one screening method. The comparison other CCBs on neuromuscular transmission could not be done as there are very in vivo studies.

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