

# INTERACTION OF VERAPAMIL AND LITHIUM AT THE NEUROMUSCULAR JUNCTION ON RAT ISOLATED MUSCLE-HEMIDIAPHRAGM

H. R. Sadeghipour<sup>1</sup>, M. Mesbahian<sup>1</sup> and A.R. Dehpour<sup>2</sup>

(1) Department of physiology, Faculty of Medicine, Tehran university of Medical Sciences, Tehran, Iran

(2) Department of pharmacology, Faculty of Medicine, Tehran University of Medical Sciences, Tehran, Iran

**Abstract** - It has been reported that either lithium or verapamil can potentiate the neuromuscular blocking activity of certain neuromuscular blockers. In the present investigation, possible interaction of verapamil with lithium has been described. The dose - response effects of verapamil and lithium on diaphragmatic contractility were assessed *in vitro*. Mechanical responses of the muscle to indirect (nerve) and direct (muscle) electrical stimulation were recorded. Verapamil depressed rat diaphragm twitch tensions induced by nerve stimulation in a dose - dependent manner with the 50 percent depression of the original twitch tensions ( $IC_{50}$ ) by  $5.6 \times 10^{-6}$  mmol/l.

The  $IC_{50}$  of verapamil for direct stimulation of the muscle was  $1.1 \times 10^{-5}$  mmol/l. Partial replacement of sodium chloride by lithium chloride (0.5, 1.5 and 5 mmol/l) in the medium did not change the depressant effect of verapamil on muscle twitches induced by direct (muscle) or indirect (nerve) electrical stimulation.

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**Key words:** Neuromuscular junction, skeletal muscle, verapamil, lithium

## INTRODUCTION

Verapamil is a calcium antagonist used widely in the treatment of cardiovascular disorders (1 - 4). The mammalian skeletal muscle blocking properties of verapamil have been well documented since the first paper by Kenneth and Schwartz (5). The effect of verapamil on the indirect as well as direct electrical stimulation inducing tension in skeletal muscle has been reported by several investigators (6-10). In skeletal muscle verapamil by some intracellular mechanisms induced calcium release from the sarcoplasmic reticulum (SR) and increased calcium sensitivity of contractile proteins (6). However in neuromuscular junction (NMJ), the drug interacts directly (independent of calcium channels) with the end - plate receptor to cause a shortening of open channel lifetime and probably also blockade of closed channels

(11).

Lithium is used extensively in the control of affective disorders despite the fact that its mode of action is still unknown (12). It has been reported that lithium reduces the supply of inositol, the key substrate for the phosphoinositide cascade, by inhibiting some of the enzymes which hydrolyse the inositol phosphates (13). The interactions of verapamil and lithium with other neuromuscular blocking agents, have been studied by other workers (14 - 18) who concluded that verapamil or lithium augmented twitch depression. We have previously reported that lithium can inhibit the neuromuscular blockade induced by aminoglycoside antibiotics in normal and diabetic rats (19). These findings prompted us to study the possible interactions between lithium and verapamil at neuromuscular junction.

## MATERIALS AND METHODS

All experiments were performed on the phrenic nerve hemidiaphragm preparation of the rat. Male albino rats weighing 150 - 200g were used. After decapitation, a triangular - shaped section of hemidiaphragm was dissected with its phrenic nerve and mounted on palmer H 95 perspex electrode and transferred within 15 min to a 50 ml bath containing mammalian krebs solution, at 37°C. Isolated muscle-hemidiaphragm was prepared as above. The nutrient medium had the following composition (expressed as the ratio of mmol/l of deionized water): NaCl (Merck) = 113; KCl (Merck) = 4.7; CaCl<sub>2</sub> (BDH) = 2.5; MgSO<sub>4</sub> (Riedel of Haen AG.) = 1.2

### Interaction of Verapamil and Lithium at NMJ

NaHCO<sub>3</sub> (May and Baker) = 25; NaH<sub>2</sub>PO<sub>4</sub> (Merck) = 2.5, Glucose (Merck) = 11.5. The medium was aerated with oxygen containing carbon dioxide (5%) throughout the experiments. The pH of medium was checked several times during the experiments and was found to be 7.2. The resting tension of the muscle was adjusted at 5 g. The preparations were supramaximally stimulated via the phrenic nerve with square wave stimuli at a repetitive rate of 0.2 HZ; 120 V supramaximal voltage (120 V) and 2 ms pulse duration, the muscle was directly stimulated at a repetitive rate of 0.2 HZ with supramaximal stimuli (120 V) and 20 ms pulse duration delivered by a Grass S88 stimulator. The resulting twitch tensions which quantitated by F - 60 force displacement transducer, were recorded on a polygraph (Narco Bio - System). Verapamil was obtained from Knoll AG. Germany and lithium chloride was obtained from Merck. Finally figures in the text are representative of at least 6 similar experiments which were highly reproducible. All values are expressed as mean  $\pm$  SE. Student's t-test was used to determine the statistical significance between the mean values (unpaired data analysis). P-value of 0.05 or less was considered statistically significant.

### RESULTS

After the twitch tension had become stable for at least 20 min, cumulative amounts of verapamil were added to the bath with 10 min intervals. Depression of the twitch heights were quantitated at the end of each interval and the IC<sub>50</sub> values (concentration resulting in 50 percent depression of the original twitch tension) of verapamil were calculated from logarithmic dose response curves and found to be  $5.6 \times 10^{-6}$  mol / l for nerve stimulation and  $1.1 \times 10^{-5}$  mol / l for direct stimulation. Also depression of twitch tensions of rat phrenic nerve and skeletal muscle hemidiaphragm were compared with constant concentration of verapamil (Fig. 1).

In order to study the interaction between verapamil and lithium at neuromuscular junction and skeletal muscles, the preparation was incubated by 0.5, 1.5 and 5 mmol / l of lithium chloride 30 min before the use of

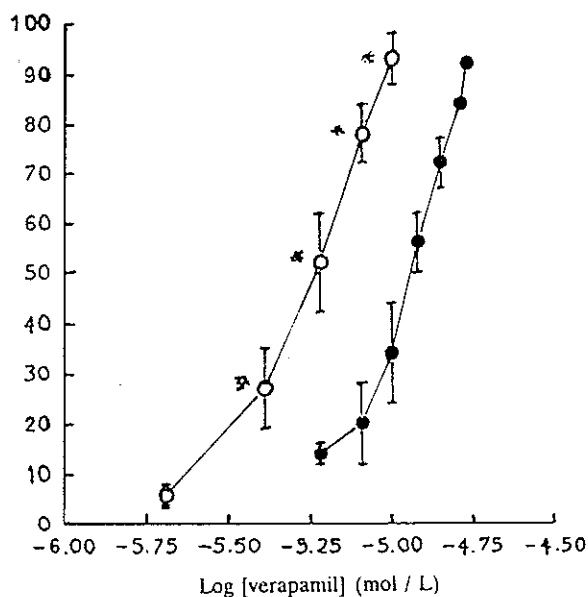


Fig. 1. Logarithmic dose - response curves of neuromuscular junction (O) and skeletal muscle (●) blocking activity of verapamil on rat isolated hemidiaphragm. Transmural field stimulus is indirectly (0.2Hz; 2ms duration; 120 v supramaximal voltage) and directly (O. 2H; 20ms duration ; 120v supramaximal voltage). Values are means  $\pm$  S.E. of six observations, \* P<0.05.

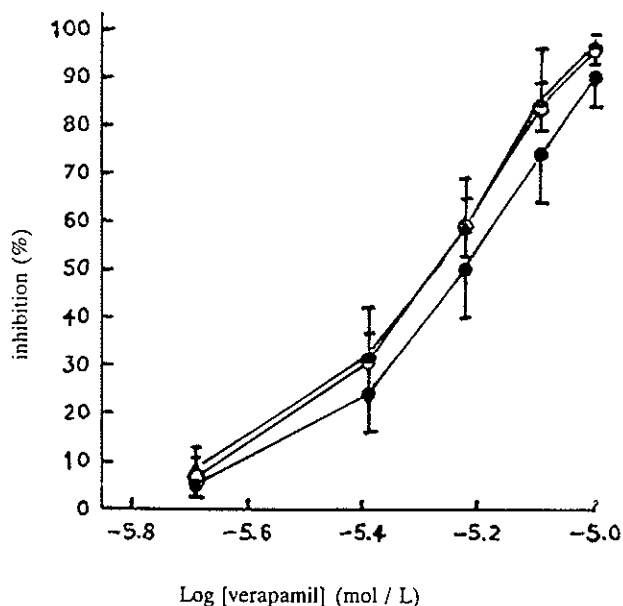


Fig. 2. The effect of replacement of sodium chloride by 0.5 mmol / l (●), 1.5 mmol / l (O), 5 mmol / l (Δ) of lithium chloride on action of verapamil in skeletal muscle. Values are means  $\pm$  SE of six observations. All are non - significant.

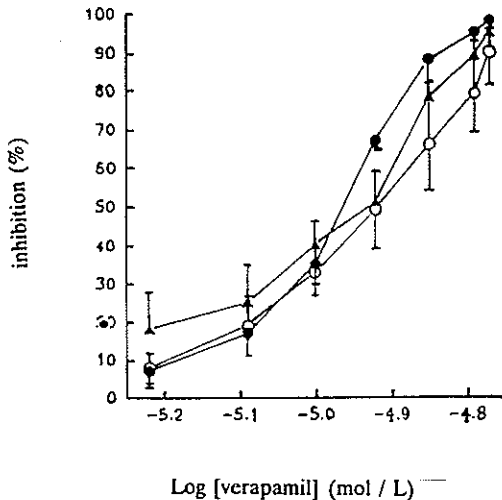


Fig. 3. The effect of replacement of sodium by 0.5 mmol / l (●) 1.5 mmol / l (○), 5 mmol (Δ) of lithium chloride on action of verapamil in neuromuscular junction. Values are means  $\pm$  SE of six observations. All are non - significant.

verapamil. Partial replacement of sodium by lithium in the medium did not change the depressant effect of verapamil on muscle twitches induced by direct (muscle) or indirect (nerve) electrical stimulation (Fig. 2,3). Furthermore, lithium per se did not have any effect on the twitch heights in the same condition (19). In all experiments, the inhibitory effect of verapamil on prejunctional (nerve) stimulation was greater ( $p < 0.05$ ) than direct skeletal stimulation (Fig. 1-3).

## DISCUSSION

The role of calcium in the contraction of skeletal muscle is well known, although the coupling mechanism between the electrical signal in the transverse-tubule and the release of calcium from the sarcoplasmic reticulum (SR) is not fully understood (20-22). Pretreatment of frog muscles with calcium channel blockers such as verapamil or methoxyverapamil (D600) abolished or markedly depressed contractures elicited by  $K^+$  and twitches evoked by electrical

stimulation (23,24). Reports about the effects of verapamil on twitch tension in mammalian skeletal muscles are contradictory. Varagic and Kentera have reported a transient increment of the twitch in rat diaphragm muscle followed by a depression on the tension (25). Skirboll has reported an increment in the contractile force of cat muscles after intra-arterial injection of verapamil (26).

On the other hand, it has been shown that twitch and tetanic force in isolated mammalian muscles were depressed by verapamil or D600 (27, 28). Also Lee and Tsien (29) have demonstrated that the depression of the twitch observed by verapamil is more manifest when the muscle is repetitively stimulated and this frequency - dependent effect is characteristic of several calcium channel blockers. These observations led to conclude that the effects of organic calcium antagonists on mammalian skeletal muscles are not from blockade of calcium entry and most likely result from action(s) on other processes. In the present study, verapamil depressed the twitches of the muscle induced by direct or indirect stimulation. The  $IC_{50}$  for the verapamil induced inhibition of twitch tension induced by motor nerve stimulation was  $5.6 \times 10^{-6}$  mol / l. The calcium antagonist verpamil was also capable of blocking the twitch tension induced by direct stimulation of the muscle ( $IC_{50} = 1.1 \times 10^{-5}$ ) which is approximately 2 - fold higher than dose required to block twitch tension induced by nerve stimulation. This is consistent with previous reports investigating the effect of verpamil (11).

Single channel data has confirmed the specific interaction of verapamil with the nicotinic acetylcholine receptors showing closed channel blockade at low concentration, and at higher levels the shortening of open channel lifetime. It is suggested that both forms of blockade may be involved in neuromuscular depressant activities of verapamil (11). This is in contrast to binding studies of Kraynack (9) which showed that verapamil had a preferential effect on indirectly elicited twitch tension versus, directly elicited twitch tension in cats.

Replacement of sodium by lithium has been shown to have profound effects on transmission at a number

### Interaction of Verapamil and Lithium at NMJ

of junction sites (17). It has been reported by Brainisteanu and Volle (17) that replacement of sodium by lithium resulted in a progressively developing increase in the amplitude and quantal content of endplate potentials of the frog neuromuscular junction. In addition, lithium caused an increase in the probability of transmitter release in this preparation. These investigators have claimed that the effect of lithium on transmitter release can be attributed to the accumulation by the nerve terminals of lithium, resulting in an increased level of intracellular calcium. Furthermore, lithium has been reported by Vizi (30) to decrease acetylcholine synthesis in rat brain cortex as well as reduced acetylcholine release from nerve terminals in strips of guinea pig ileum. In our experiments partial replacement of sodium by lithium in the medium does not change the twitch heights significantly (19). The effect observed here is contradictory to the results previously noted by Vizi, Brainisteanu and Volle. This may be due to the relatively low concentrations of lithium which were used in our experiments. Previous works suggested that lithium potentiated the blockade of certain muscle relaxant drugs. However, in this study we were not able to show that lithium potentiated the neuromuscular blockade induced by verapamil. It seems that this may be due to interference of lithium with acetylcholine release from nerve terminal which counteracts the verapamil depressant effects.

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