วารสารเภสัชวิทยา ปีที่ 9, 2530
Thai J Pharmacol Vol 9, 1987

The Effect of Verapamil on Skeletal Muscle Contractile Responses in Rats

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Abstract

Verapamil at the concentrations ranging from 0.01-0.08 mM produced a dose-related twitch depression in isolated rat phrenic nerve-hemidiaphragm preparations. The maximum effective concentration was 0.05 mM which produced 76 percent depression. Neurally evoked twitch was affected more significantly than directly evoked one. Verapamil at a concentration of 0.04 mM significantly synergized the in vitro neuromuscular blocking effect of pancuronium (0.001 mM) and of succinylcholine (0.001 mM). Verapamil (0.05 mM) inhibited twitch potentiation produced by 2.6 mM caffeine in curarized rat diaphragm preparations. This drug also abolished acetylcholine-induced contracture in denervated rat gastrocnemius preparations. It was also found that calcium exerts only a partial antagonistic effect on the neuromuscular blockade produced by verapamil in vitro. The effects of verapamil on skeletal muscle contractile responses in rats and its possible sites of action are discussed.

Introduction

The effect of verapamil on neuromuscular transmission has not been well established. Chiarandini and Bentley (1) suggested that verapamil has a neuromuscular blocking effect. In 1982, Standaert suggested that Ca^{++} enters motor nerve terminal via special proteins forming channels through the nerve membrane (2). The twitch depression of verapamil on the twitch height of gastrocnemius muscle in indirect stimulation in dog was reported by Lawson, Kraynack, and Gintautas (3). Pang and Sperelakis (4) had found that the slow action potential disappears in the muscle fiber that T-tube system has been destroyed, this indicates that calcium channel is located in the T-tubule system. They also found that verapamil readily entered and accumulated inside myocardial cell, smooth muscle cell, and skeletal muscle.

Verapamil synergized the neuromuscular blocking effect produced by vecuronium in patient under general anesthesia. In 1985, Silinsky (5) suggested that the depolarization of the nerve terminal opens calcium channel at localized regions in the nerve ending. In this study, neuromuscular effect of verapamil will be investigated in both qualitative and quantitative analysis.

The purpose of this study is to elucidate the pharmacological activities of verapamil on

myoneural junction, motor nerve, and skeletal muscle. The interaction of verapamil with standard neuromuscular blocking agents and some related compounds will be investigated in order to evaluate its effects on contractile responses and its possible mechanisms.

Methods

The experimental animals used in this investigation were adult albino rats of Sprague-Dawley strain, weighing about 150-250 grams of both sexes. The animals were supplied by the animal center of the Faculty of Medicine, Chiang Mai University.

The isolated rat phrenic nerve-hemidiaphragm preparation was set up to study the effect of verapamil on contractile responses, interaction with some neuromuscular blocking drugs and related compounds.

The rat sciatic nerve gastrocnemius muscle was set up to record the contractile response in situ for the study of the interaction of verapamil and acetylcholine (ACh) contracture in denervated gastrocnemius muscle preparation, and the effect of verapamil on the neuromuscular blocking effect of drugs.

All of the techniques used in this study were modified by the author (6).

Results

The Effect of Verapamil on Neurally and Directly Evoked Twitches

Verapamil in the doses of 0.01-1 mg/kg was injected intra-arterially into albino rats. A slight depressive effect on muscle contractile responses was observed. In isolated rat phrenic nerve-hemidiaphragm preparation, verapamil (0.01-0.08 mM) produced a dose-related twitch depression. The maximum effective concentration was 0.05 mM which produced 76 percent twitch depression. The percent twitch depression produced by verapamil on both neurally and directly evoked twitches was compared. It was found that the twitch depression of neurally evoked twitch was significantly greater than that of directly evoked twitch ($p<0.05$) as shown in Table 1.

Table 1 Comparison of the percent twitch depression of verapamil in neurally and directly evoked twitches in rat phrenic nerve-hemidiaphragm preparations

Drug	Concentration (mM)	Percent twitch depression* neurally evoked	directly evoked
Verapamil	0.04	39.4 ± 8.0	14.4 ± 4
	0.05	76.6 ± 7.1**	24.7 ±7
	0.06	74.5 ± 13.8	38.0 ± 9
	0.08	64.8 ± 5.0	54.1 ± 12

*mean ± SEM ; **$\bar{p}<0.001$

The Interaction of Verapamil and Neuromuscular Blocking Agents

Pancuronium was selected as a representative of the non-depolarized neuromuscular blocking agents. In this study, verapamil 0.04 mM followed by pancuronium 0.001 mM were added into the tissue bath, and the amplitude of contractile response was observed. It was found that pancuronium did not show any definite depressive effect on neurally evoked twitch. Verapamil (0.04 mM) produced only 39 percent twitch depression. In presence of pancuronium, the same concentration of verapamil caused significant decrease in twitch contractile responses ($p<0.005$) as shown in Table 2.

Table 2 The synergistic effect of verapamil (0.04 mM) on the neurally evoked twitch depression produced by pancuronium (0.001 mM) in vitro

Number of experiment	Percent twitch depression produced by		
	Pancuronium	Verapamil	Pancuronium + Verapamil
1	3.2	3.2	100
2	0	0	100
3	0	36.6	100
4	0	48.0	100
5	0	63.0	100
mean ± SEM	0.6 ± 0.6	30.2 ± 12.4	100 ± 0

Note : Verapamil significantly synergized the neuromuscular blocking effect of pancuronium in vitro ($p < 0.005$).

Table 3 The synergistic effect of verapamil (0.04 mM) on the neurally evoked twitch depression produced by succinylcholine (0.001 mM) in vitro

Number of experiment	Percent twitch depression produced by		
	Succinylcholine	Verapamil	Succinylcholine + Verapamil
1	0	3.2	100
2	33	0	100
3	0	44.0	65
4	0	71.5	100
5	0	48.0	100
mean ± SEM	6.6 ± 6.6	33.3 ± 13.8	93 ± 7.0

The depolarized neuromuscular blocking drug used in this experiment was succinylcholine. Succinylcholine (0.001 mM) produced a slight decrease in muscle twitch. In the presence of verapamil (0.04 mM) the percent twitch depression was increased to 93 percent as demonstrated in Table 3.

From this study, it was found that verapamil significantly ($p < 0.05$) synergized the twitch depression produced by both neuromuscular blocking agents, pancuronium and succinylcholine.

Verapamil also produced a synergistic effect with pancuronium and succinylcholine in rat sciatic nerve gastrocnemius preparations in situ. Besides this, verapamil showed synergistic effect with hemicholinium in isolated rat phrenic nerve-hemidiaphragm preparation.

The Influence of Calcium on the Effect of Verapamil on Muscle Contractile Response

Calcium plays an important role in the release of ACh from motor nerve ending. In this study, calcium chloride could antagonize neuromuscular blockade produced by calcium

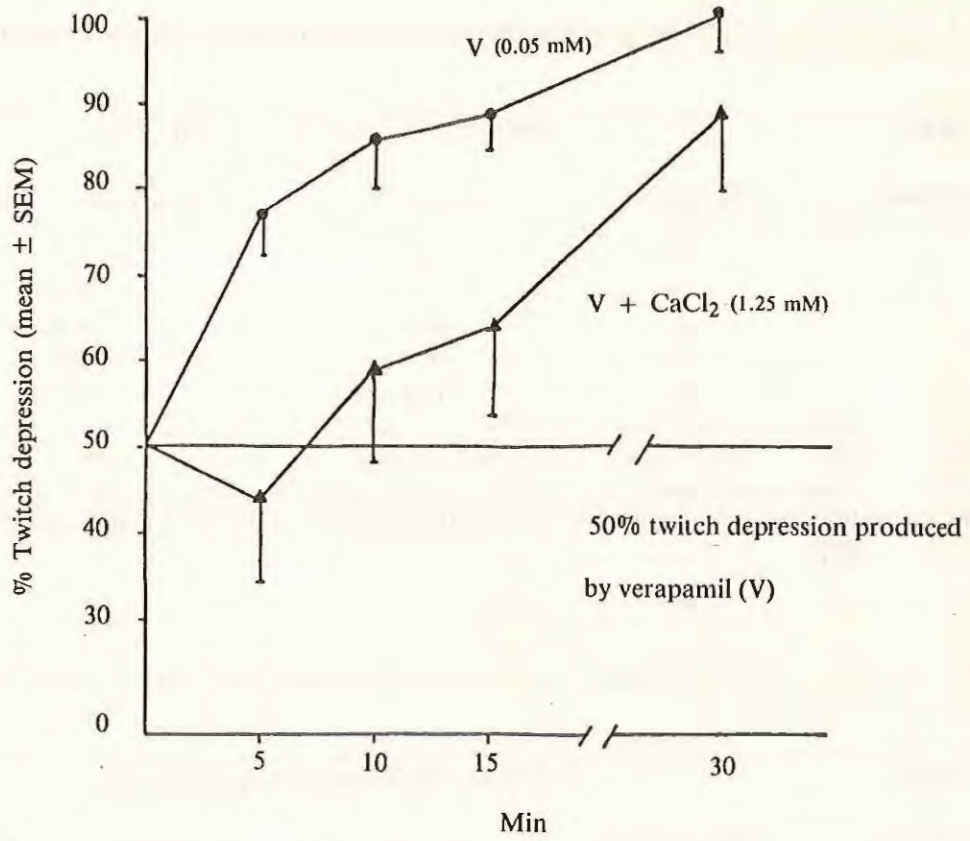

Figure 1 The time-action relationship of calcium chloride on the 50% twitch depression produced by verapamil (0.05 mM) in rat phrenic nerve-hemidiaphragm preparations.

deficiency condition, and at the same concentration it could antagonize 50 percent twitch depression produced by verapamil as illustrated in Figure 1. Calcium exerts only a partial antagonistic effect on the neuromuscular blockade produced by verapamil in in vitro study.

The Influence of Verapamil on the Effect of Caffeine

In 1954, Huidobro and Amenbar (7) reported that caffeine increased the twitch tension of indirectly stimulated muscle in cats. In this study, caffeine at the concentration of 2.6 mM produced a slight twitch potentiation in both neurally and directly evoked contractile reponses. The percent twitch potentiation produced by caffeine in neurally evoked twitch was not significantly different from that of directly evoked twitch in in vitro nerve-muscle preparations (unpublished observations). At 50 percent twitch depression produced by 0.05 mM verapamil, caffeine could not increase the twitch amplitude of the neurally evoked twitch.

In directly curarized nerve-muscle preparations, caffeine produced twitch potentiation. The peak potentiation was 33 percent as shown in Table 4. The peak potentiation produced by caffeine in presence of verapamil was significantly less than that of caffeine alone ($p<0.001$). This result showed the inhibition of verapamil on twitch potentiation produced by caffeine. It was proposed that verapamil directly suppressed the skeletal muscle contractile response.

The Effect of Verapamil on Acetylcholine-Contracture in Denervated Rat Gastrocnemius Preparations

Acetylcholine-contracture produced in chronically denervated rat gastrocnemius

Table 4 The effect of verapamil (0.05 mM) on the twitch potentiation produced by caffeine (2.6 mM) in curarized rat hemidiaphragm preparations

Number of Experiment	Percent of peak potentiation produced by	
	Caffeine	Caffeine + Verapamil
1	36.6	23.8
2	38.5	0
3	46.2	15.4
4	25.0	17.2
5	20.0	11.5
mean ± SEM	33.2 ± 4.7	13.6 ± 3.9*

*$p<0.01$ compared with caffeine alone.

Table 5 The effect of verapamil (0.5 mg/kg) on acetylcholine-contracture in denervated rat gastrocnemius preparations

Number of experiment	Tension in gram of ACh - contracture		Percent decreased from control
	Control	15 min after verapamil	
1	1.6	0.6	62.5
2	2.8	0.4	85.7
3	2.4	0	100.0
4	1.4	0.4	71.4
5	1.3	0.4	69.2
mean ± SEM	1.9 ± 0.2	0.4 ± 0.05*	

*$p<0.005$ compared with control.

preparation is the sensitive method to determine whether the site of neuromuscular blocking action is postsynaptic or motor endplates (8). Acetylcholine was injected intra-arterially into denervated rats at a dose of 0.6 mg/kg, and the contracture was obviously observed. It was found that the tension (in gram) of ACh-contracture at 15 minutes after verapamil (0.5 mg/kg) was significantly less than that of ACh alone ($p<0.005$). Thus verapamil abolished the amplitude of ACh-contracture as shown in Table 5.

From this study, verapamil could produce a complete neuromuscular blockade in rat phrenic nerve-hemidiaphragm preparation. The drug suppressed ACh-contracture, and also blocked both neurally and directly curarized PTP (unpublished observations). Verapamil could inhibit the twitch potentiation of caffeine in curarized rat diaphragm preparation. Thus, verapamil may possibly have two main sites of action : neuromuscular junction and skeletal muscle.

Discussion

Verapamil produced a dose-related depression on neurally and directly evoked twitches in rat phrenic nerve-hemidiaphragm preparations. The twitch amplitude of neurally evoked twitch was significantly depressed more than that of directly evoked twitch. It seemed that the site of depressive effect of verapamil was myoneural junction, and probably at the muscle itself.

The mechanism of ACh release from motor nerve terminal is calcium-dependent process (2). An increase in extracellular calcium concentrations should increase the amounts of ACh released per stimulation (5). In this investigation, the increase in extracellular calcium could partially antagonize 50 percent twitch depression produced by verapamil. It might be expected that verapamil somehow exerts its effect on the release of ACh.

In this study, the increase in extracellular calcium could not antagonize the twitch depression of verapamil. It seemed likely that the depressive effect of verapamil could not be antagonized by the increasing amounts of ACh at myoneural junction. Duran and his coworkers (9) suggested that verapamil could exert its effect on this synapse by observing the synergistic effect of pancuronium or succinylcholine on verapamil. In this study, it was found that verapamil synergized the blocking effect produced by succinylcholine, pancuronium, and hemicholinium in both in vitro and in vivo preparations.

The neural post-tetanic potentiation (PTP) is a presynaptic phenomenon which have been stated for its mechanisms as the increase of ACh released per stimulation immediately after the termination of tetanic shock applying to the motor nerve (10). In this study, verapamil abolished neural PTP. Kraynack (11) proposed that the possible actions of verapamil at presynaptic site of neuromuscular junction were probably due to the reduction of calcium conductance at the presynaptic membrane, changing cyclic-AMP level or inhibiting membrane calcium pump into motor nerve terminal. These actions may interfere the mobilization of ACh or its actual release.

The postsynaptic evidence of neuromuscular blocking effect caused by verapamil was investigated in chronically denervated rat muscle preparations. Axelsson and Thesleff (8) proposed the sensitive method to determine postsynaptic neuromuscular blocking action by studying the effect of drug on muscle contraction produced by closed intra-arterial injection of ACh. They also reported that there was a supersensitivity of the motor endplate in chronically denervated muscle which was due to spreading of the area where cholinergic receptor existed.

In this study, verapamil significantly abolished the amplitude of ACh-produced contracture in chronically denervated rat muscle. This indicated that the drug possibly possesses postsynaptic action. The mechanism of action would be that the drug altered the sensitivity of the endplate membrane to exogenous ACh, but whether this drug alters the receptor is still unknown.

From this study, it would be proposed that verapamil possibly exerted its depressive effect on neuromuscular transmission by influencing Ca^{++} mobilization at motor nerve terminal. Another consideration that the postsynaptic effect of verapamil probably decreased endplate sensitivity to ACh should not be overlooked.

Verapamil was found to decrease the amplitude of directly evoked twitch in curarized nerve-muscle preparations. This indicated the direct action of verapamil on skeletal muscle. It is known that the xanthine derivative, caffeine, affects skeletal muscle contractile response (12). Sato and colleagues (13) postulated that caffeine produced twitch potentiation on skeletal muscle by presynaptic and intracellular actions. In this investigation, caffeine caused an increase in amplitude of both directly and neurally evoked contractile responses. The mechanism of action of caffeine on skeletal muscle cells has been proposed to stimulate sarcoplasmic reticulum, and probably the coupling mechanism between the transverse tubular system and terminal cystern

is activated leading to the release of a large amount of calcium into myoplasm as reported by Sato et al. (13). Caffeine could also induce contracture by releasing calcium from storage site. Goldberg and Singer (14) proposed that caffeine increased the twitch tension in neurally evoked muscle by increasing ACh release from motor nerve terminal.

In this investigation, verapamil could inhibit twitch potentiation produced by caffeine in directly evoked curarized diaphragm preparations. The drug also suppressed the direct-stimulated muscle PTP in the nerve-muscle preparation. In this aspect, it would be proposed that the drug could inhibit intracellular calcium ion binding sites such as troponin C or interfere calcium release from storage site.

It could be proposed that verapamil inhibited some amount of calcium released from sarcoplasmic reticulum during stimulation the process of removal residual calcium from myoplasm to longitudinal or terminal cystern in skeletal muscle.

It would be concluded that verapamil inhibit myoneural junction probably by affecting 1) motor nerve terminal 2) motor endplate 3) skeletal muscle. The primary site of action was proposed to be postsynaptic or endplate. However, the presynaptic action of verapamil in interfering with calcium mobilization into motor nerve terminal should not be disregarded. The proposed mechanisms of action of verapamil at skeletal muscle were 1) reduction of motor endplate sensitivity to ACh and 2) interfering with calcium-dependent excitation-contraction coupling in releasing process.

Acknowledgement

The authors would like to thank Knoll AG. Ludwig Hafen, West Germany and Pacific Investment (Thailand) Ltd. for the supply of verapamil in this study.

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