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RESEARCH ARTICLE

Regulatory role of Calcium Channel Blockers on spontaneous muscular activity of *Gastrothylax crumenifer*, a rumen amphistome

PAWAN KUMAR VERMA, DINESH KUMAR, S. K. TANDAN, ANIL KUMAR SRIVASTAVA and RAJINDER RAINA

For author affiliations, see end of text.

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ABSTRACT

Major proportion of intracellular calcium (Ca^{2+}) is achieved through opening of calcium channels present in the plasma membrane which play an important role in regulating neuromuscular coordination and release of neurotransmitters from nerve terminals. Blockade of these calcium channels adversely affects contractile process and release of neurotransmitters in majority of the neuromuscular preparations in vitro. In present study, the cumulative addition of verapamil (10^{-7}-10^{-3} M) caused marked excitation in amplitude, baseline tension and frequency of spontaneous muscular activity of *Gastrothylax crumenifer* a rumen amphistome. Diltiazem (10^{-6}-10^{-3} M) caused a significant and concentration-dependent increase in amplitude and frequency of spontaneous muscular activity of isometrically mounted rumen amphistome. It also caused significant rise in baseline tension at 10^{-5} to 10^{-3} M concentrations. Addition of nifedipine (10^{-7}-10^{-3} M) elicited significant and concentration-dependent rise in amplitude and baseline tension, as compared to control values without significantly effecting frequency of spontaneous contraction.

Keywords: *comma separated keywords*

Gastrothylax crumenifer is a common rumen amphistome belonging to class trematode. The infestation of this parasite in ruminants results in decreased growth, production and reproductive performance of productive animals besides decreasing the quality and quantity of animal products [1]. Synthetic anthelminthics currently in use have long been considered the only effective way of controlling these parasitic infections. Injudicious and frequent use of these anthelminthics has resulted in the development of resistance. Furthermore, residual toxicity and adverse reactions in animals have been associated with the available synthetic anthelminthics [2,3]. Therefore, there is a need to develop specific drug(s) targeting various macro-molecular components of these parasites.

Neuromuscular system of helminthes is an important area for target identification and drug development. Acetylcholine, a major inhibitory neurotransmitter of trematodes has been demonstrated immunocytochemically to be present in peripheral and central nervous system [4]. Calcium ions (Ca^{++}) play an important role in neurotransmitter release from the nerve terminals and neuromuscular coordination [5]. Bathing medium free of Ca^{++} ions reduces the spontaneous muscular activity of *Schistoma mansoni* [6]. Similarly increasing external Ca^{++} ions concentration in the medium mimics the inhibitory effect of ACh on spontaneous muscular activity of split preparation of adult *Fasciola hepatica* and *Hymenolepis diminuta* [7,8]. However, calcium channels blockers, diltiazem and verapamil, cause marked stimulation followed by paralysis of *Schistoma mansoni* and *Fasciola gigantica*, respectively. [9,10]. Contractions induced by calcium-dependent depolarization have been observed in dispersed muscle fibres of *Schistoma mansoni* [11] whereas nicardipine, a calcium channel blocker, blocks these contractions [12].

Calcium currents have been recorded from muscle fibers of *Bdelloura candida*. However, Ca^{++} currents could not be recorded from muscle fibers of *S. mansoni* [13] and *F. gigantica* [14]. Recently, a number of components vital for Ca^{++} storage and release involving Ryanodine receptors (RyR) present in sarcoplasmic reticulum have been demonstrated in the genera of *Schistosomes* [15,16].

Table 1. Effects of verapamil, diltiazem, nifedipine, and on amplitude (g) baseline tension (g) and frequency (per 5 min) of spontaneous muscular activity of *Gastrothylax crumenifer*

Observations	Concentrations					
	Control	10^{-7} M	10^{-6} M	10^{-5} M	10^{-4} M	10^{-3} M
Diltiazem						
Amplitude (g)	0.49±0.03	0.51±0.03	0.56±0.04**	0.61±0.06**	0.64±0.06**	0.68±0.05***
Baseline tension (g)	0.20±0.02	0.21±0.02	0.23±0.03	0.24±0.02**	0.27±0.03**	0.29±0.02***
Frequency/5min.	43±4.09	47.5±6.14	49.5±5.20*	50±5.42*	54±4.11*	55.5±5.85*
Verapamil						
Amplitude (g)	0.44±0.05	0.45±0.05	0.47±0.04	0.55±0.03	0.58±0.03*	0.75±0.08***
Baseline tension (g)	0.15±0.01	0.17±0.02	0.19±0.02*	0.22±0.02**	0.24±0.02***	0.27±0.03***
Frequency/5min.	46.5±3.94	48.5±5.41	49.5±3.18	50±2.65	51±5.38**	53.5±4.69*
Nifedipine						
Amplitude (g)	0.58±0.05	0.67±0.04*	0.73±0.08*	0.75±0.06***	0.77±0.09***	0.80±0.09***
Baseline tension (g)	0.22±0.02	0.24±0.02	0.25±0.02	0.26±0.03*	0.29±0.03**	0.30±0.03***
Frequency/5min.	54±4.39	58.5±3.54	61±5.19	60±4.53	57±3.47	55±4.44

*= p<0.05; **=p<0.01; ***=p<0.001; as compared to the controls

The present study was planned to investigate the role of different groups of voltage sensitive calcium channel blockers on spontaneous muscular activity of isometrically mounted parasitic rumen amphistome, *Gastrothylax crumenifer*.

Material and Methods

Collection of rumen amphistomes

Mature and healthy *Gastrothylax crumenifer* were collected from the rumen of freshly slaughtered goats at local abattoir in warm (38±1°C) Hank's Balanced Salt Solution (HBSS) in an insulated container and brought to the laboratory. They were kept in the BOD incubator at 38±1°C until further use. The amphistomes (*G. crumenifer*) were identified before experimentation.

Tissue preparation and mechanical recording

The spontaneously active whole mature amphistome was mounted isometrically in HBS solution at 38±1°C as per the method described for *Gigantocotyle explanatum* [17]. In short, the amphistome was mounted with the help of two fine hooks. One hook was inserted 1-2 mm caudal to anterior sucker and fixed to the tip of aeration tube and another hook was pierced through the surface of acetabulum and connected to the isometric force transducer. The spontaneous muscular activity of isometrically mounted amphistome was recorded in Chart Window 4 Software programme. (Powerlab, AD Instruments, Australia).

Experimental protocol

Graded molar concentrations (10^{-7}- 10^{-3} M) of different groups of calcium channel blockers; verapamil (Phenylalkylamine derivative) (Sigma, USA), diltiazem (benzothiazepine derivative) (Sigma, USA) and nifedipine (dihydropyridine derivative) (Sigma, USA) were applied after equilibration of the fluke to examine their effects on spontaneous muscular activity of *G. crumenifer*.

Data collection and statistical analysis

Three attributes, viz., the amplitude (average of all peaks per five minutes), baseline tension (average of all minimum levels of contractions used for measuring amplitude) and frequency (total number of contractions in 5 min) of spontaneous muscular contractions were utilized to evaluate the effect of different concentrations of different groups of calcium channel blockers. Experiment with each drug was repeated at least six times on fresh isometrically mounted *G. crumenifer.*

The results are presented as mean ± standard error of mean and paired'*t*' test was applied to measure the level of significance [18].

Results

The isometrically mounted amphistomes exhibited rhythmic phasic contractile activity continuously for hours. The mean amplitude, baseline tension and frequency of the rhythmicity recorded every 15 min after applying the tension of 200 mg, were 0.42 ± 0.03 g (n=6), 0.13 ± 0.01 g (n=6) and 45 ± 2.90 contractions/5 min time period (n=6), respectively. The isometrically mounted amphistomes exhibited apparently uniform pattern of spontaneous muscular activity for a period of 2 h. There was no significant difference in amplitude (0.38 ± 0.02 g; n=6), baseline tension (0.11 ± 0.02 g; n=6) and frequency (41 ± 3.44/ 5 min; n=6) of spontaneous contractions recorded 2 h after mounting as compared with those recorded 15 min after applying the tension to the amphistome. The representative recording is given in Fig. 1.

Effect of calcium channel blockers on spontaneous muscular activity of G. Crumenifer

Diltiazem, a benzothiazepine derivative causes a significant and concentration-dependent (10^{-6} to 10^{-3} M) increase in amplitude and frequency of spontaneous muscular activity as compared with control amplitude (0.49 ± 0.03 g) and frequency (43.0 ± 4.09/ 5 min). It also caused marked rise in baseline tension at 10^{-5} to 10^{-3} M concentrations in a concentration-dependent manner as shown in Table 1 and Fig 2a and 2b.

Effects of verapamil, a phenylalkylamine derivative on control amplitude, baseline tension and frequency of spontaneous contractions of isometrically mounted rumen fluke is shown in Table 1, and Fig 3a and 3b. Verapamil in cumulative concentrations at an increment of 1 log unit produced significant excitation in the amplitude (at 10^{-4} and 10^{-3} M), baseline tension (10^{-6} to 10^{-3} M) and frequency (at 10^{-4} and 10^{-3} M) of spontaneous muscular activity of the rumen fluke.

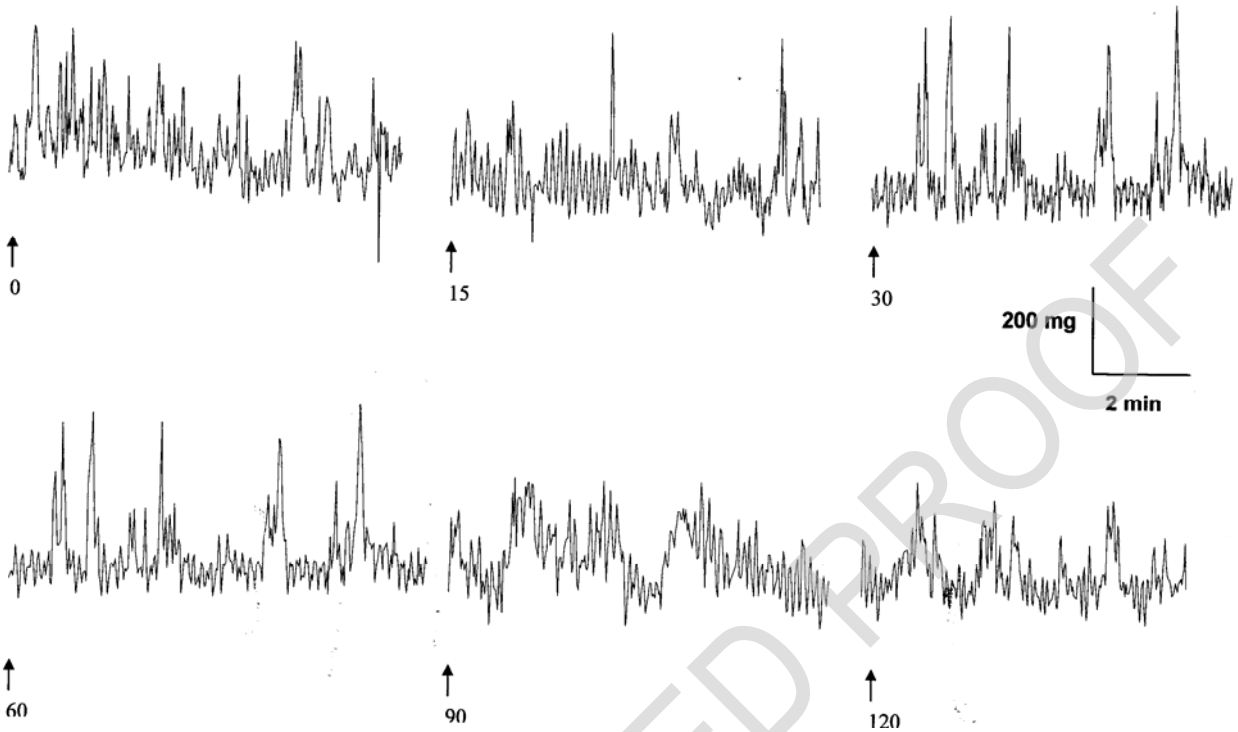

Fig. 1. Time-dependent control recording of spontaneous muscular activity of *G. crumenifer* (0 to 120 min)

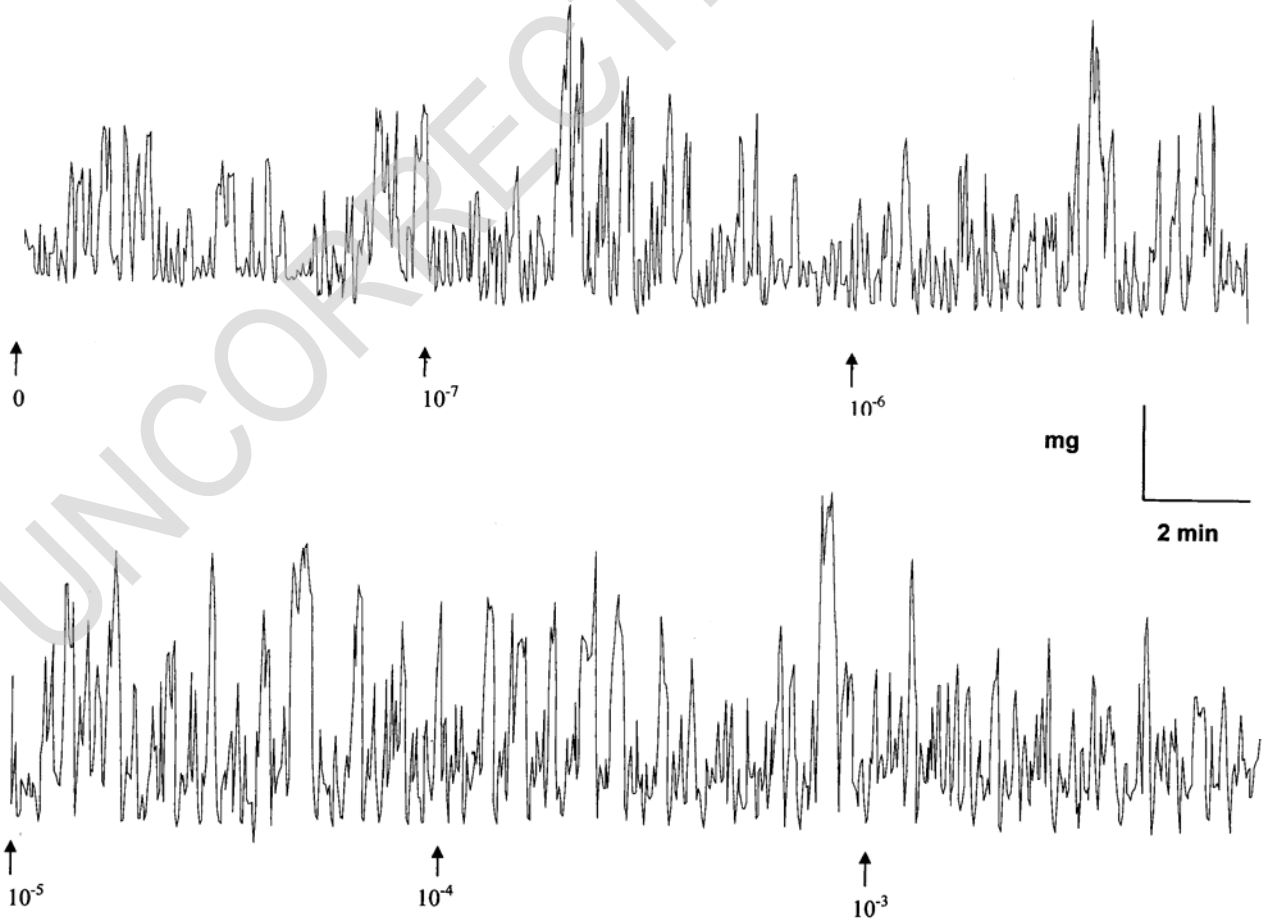

Fig. 2a. Effects of different concentrations (10^{-7}M to 10^{-3}M) of Diltiazem on spontaneous muscular activity of *G. crumenifer*

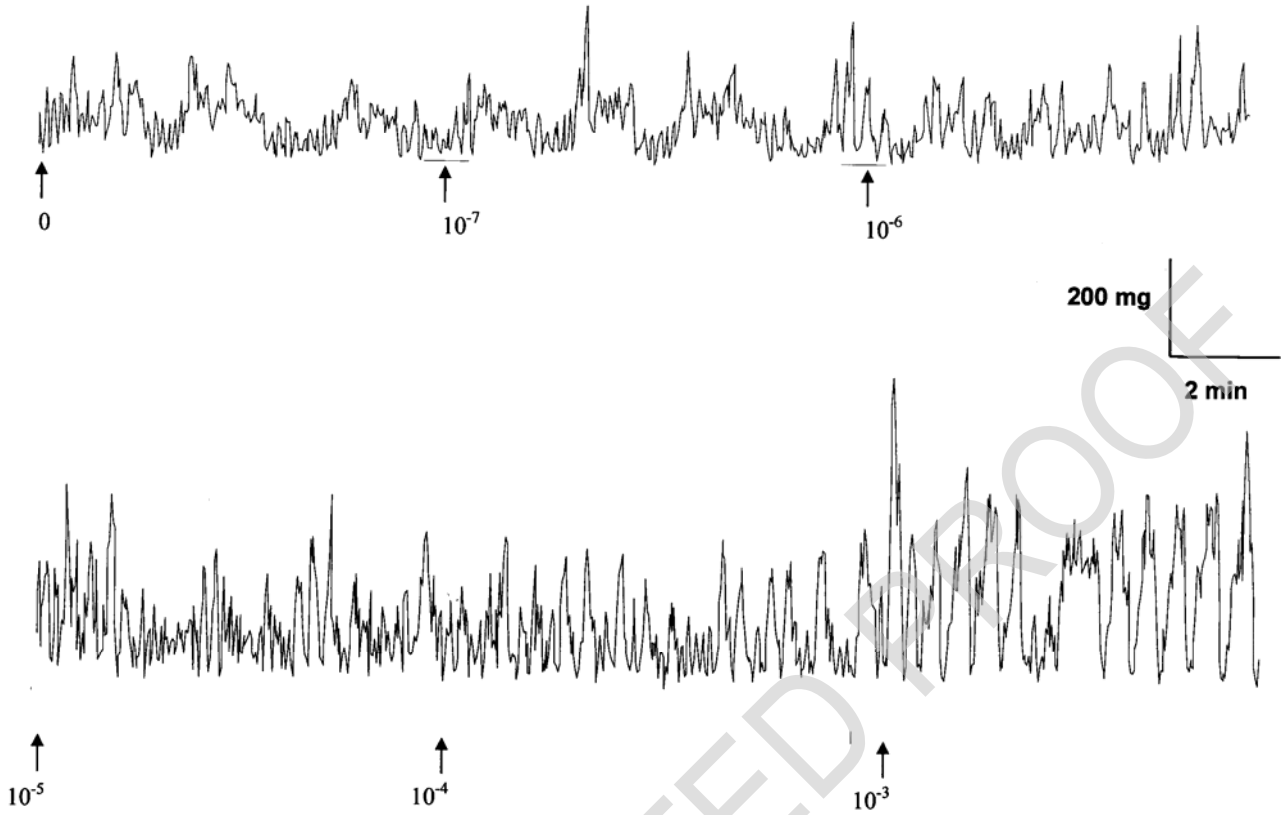

Fig. 3a. Effect of different concentrations (10^{-7}M to 10^{-3}M) of Verapamil on spontaneous muscular activity of *G. crumenifer*

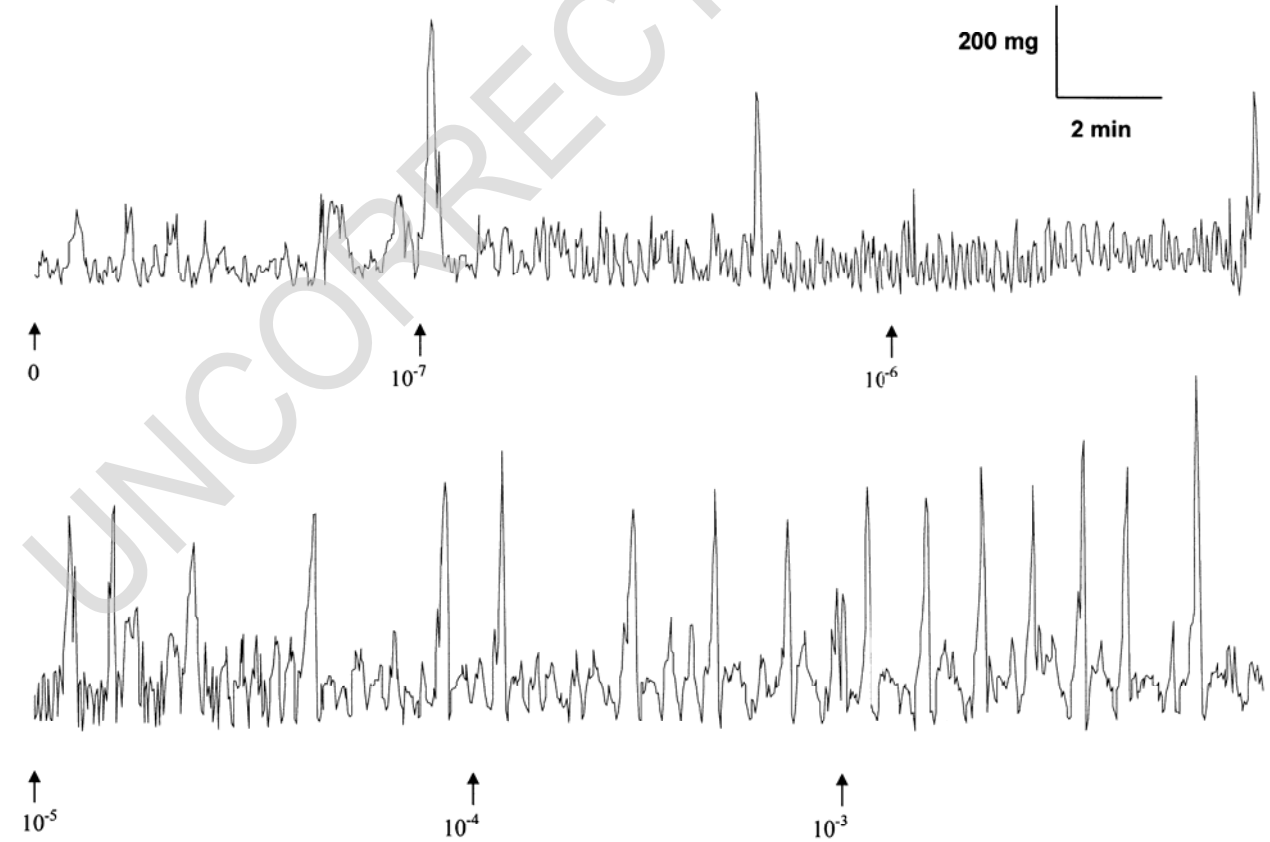

Fig. 4a. Effects of different concentrations (10^{-7}M to 10^{-3}M) of Nifedipine on spontaneous muscular activity of *G. crumenifer*

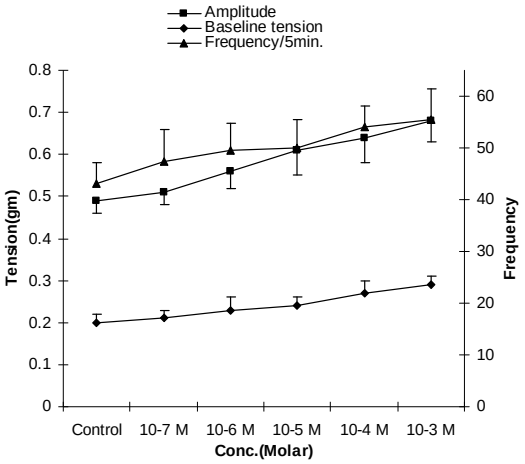

Fig. 2b. Effect of different concentrations (10^{-7} M to 10^{-3} M) of Diltiazem on amplitude (g) baseline tension (g) and frequency (per 5 min) of spontaneous muscular activity of *G. crumenifer*

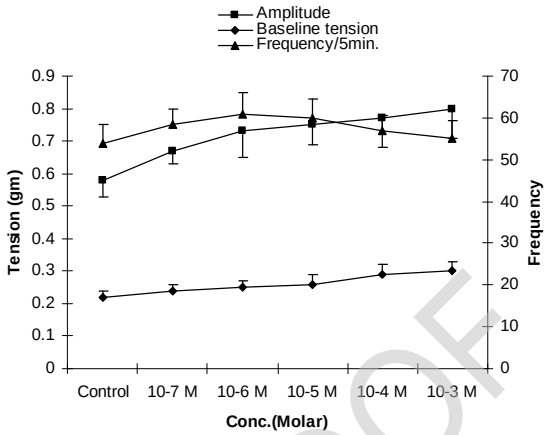

Fig. 4b. Effect of different concentrations (10^{-7} M to 10^{-3} M) of Nifedipine on amplitude (g) baseline tension (g) and frequency (per 5 min) of spontaneous muscular activity of *G. crumenifer*

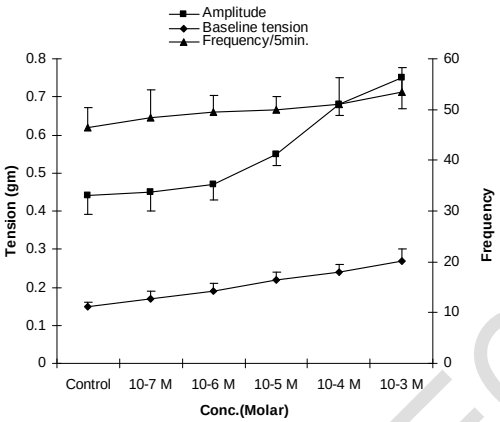

Fig. 3b. Effect of different concentrations (10^{-7} M to 10^{-3} M) of Verapamil on amplitude (g) baseline tension (g) and frequency (per 5 min) of spontaneous muscular activity of *G. crumenifer*

Nifedipine, a dihydropyridine derivative at 10^{-7} to 10^{-3} M concentrations caused significant and concentration-dependent rise in amplitude compared with that of control. At 10-5 to 10-3 M concentrations it also caused concentration-dependent and significant increase in baseline tension. Nifedipine did not show any concentration-dependent and significant effect on frequency of the fluke (Table 1 and fig 3a and 3b).

DISCUSSION

Intracellular calcium (Ca^{2+}) is responsible for the muscular contraction and release of neurotransmitters from nerve terminals in mammals. Removal of extracellular Ca^{2+} and /or blockade of calcium channels adversely affect contractile process and release of neurotransmitters in majority of the neuromuscular preparations *in vitro*. In the present study, however, calcium channel blockers from different groups elicited an excitatory response in amplitude and baseline tension of muscular activity of *G. crumenifer*. The effect of the calcium channel blockers on amplitude and baseline tension in all groups show similar excitatory patterns with a variation in the intensity. Out of these different groups dihydropyridine derivatives are producing pronounced contractile responses as compared to other blockers. The results are in agreement with the earlier reports on *S. mansoni* [9] and *F. gigantica* [10]. However, it has been shown that Ca^{2+}-free bathing medium reduced the spontaneous muscular activity of *S. mansoni* [6], while increasing external calcium ion concentration, mimicked the inhibitory effects of ACh on spontaneous muscular activity of split-preparation of adult *F. hepatica* [7]. The probable mechanism may be that these calcium channel blockers inhibit the release of inhibitory neurotransmitters at the nerve terminals as it is well documented that the release of neurotransmitter at nerve terminal requires Ca^{2+} [5] or these calcium channel blockers may be producing a direct stimulatory effect on trematode neuromuscular system. Verapamil and diltiazem have significant stimulatory effects on muscular contraction whereas Nifedipine did not have significant effect on the frequency of the activity. This may be due to reversal of action of sodium-calcium exchanger proteins resulting from blocking of calcium cannels [19]. It is likely that similar mechanism operates in muscular tissue of *G. crumenifer* and may be responsible for Nifedipine not to significantly increase the frequency of spontaneous muscular activity in *G. crumenifer*. It will be interesting to study the exact mechanism for this excitatory effect on spontaneous muscular activity with calcium channel blockers in trematodes at molecular level.

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Current Author Addresses

Pawan Kumar Verma, Junior ScientistDivision of Pharmacology and ToxicologyF.V.Sc. & A.H. R.S.Pura, Jammu, J&K , India. E-mail: drpawankv@yahoo.co.in (Corresponding author)

Dinesh Kumar, Senior Scientist, Division of Pharmacology and Toxicology, Indian Veterinary Research Institute, Izatnagar, Bareilly - 243 122 (UP), India.

S. K. Tandan, Senior Scientist, Division of Pharmacology and Toxicology, Indian Veterinary Research Institute, Izatnagar, Bareilly - 243 122 (UP), India.

Anil Kumar Srivastava, Dean, Faculty of Veterinary Sciences and Animal Husbandry, SKUAST-J, R.S. Pura, Jammu (J&K) India.

Rajinder Raina, Head, Division of Pharmacology and Toxicology, F.V.Sc. & A.H., SKUAST-J, R.S. Pura, Jammu (J&K), India.