Voltage activated calcium channels in somatic muscle of filarial nematode *Setaria cervi*

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Acetylcholine (Ach), levamisole and pyrantel pamoate all cause stimulation of spontaneous rhythmic movements of whole worm and nerve muscle preparation of filarial nematode *Setaria cervi*. These stimulant effects are manifested only in the presence of available Ca²⁺ or extracellular Ca²⁺. Electrical stimulation of nerve muscle preparation of *Setaria cervi* elicited depolarization and increase in amplitude and tone of contractions. Electrical current stimulates Ca²⁺ entry leading to depolarization and during the phase of depolarization addition of any of the three stimulants viz. Ach, levamisole or pyrantel pamoate fails to elicit any response on nerve muscle preparation. The findings indicate that electrical stimulation, excitatory neurotransmitter Ach and stimulant anthelmintics levamisole and pyrantel pamoate all produce their stimulant effect by triggering entry of Ca²⁺ into the muscle cell. Further, blocking the calcium channels by nifedipine and thereby the entry of Ca²⁺ into the cells blocks the stimulant effect of Ach levamisole and pyrantel pamoate.

Ca²⁺ is the primary regulator of contraction in *S. cervi*. The contractile response of preparation of *S. cervi* are very dependent on extracellular Ca²⁺. In the absence of available Ca²⁺ or addition of EDTA to the bathing fluid, a calcium chelating agent or of calcium channel blocking agents nifedipine, verapamil and diltiazem results in inhibition of spontaneous rhythmic movements of the worm preparation. The rhythm of contraction and relaxation in muscle of *Setaria* like other smooth muscles may be associated with a tidal movement of calcium from the sites at the cell membrane to other sites inside the cell and such a flow of calcium ions could be associated with a flow of electric charge.

The present study was designed to study the effect of application of electric current to n.m. preparation of *S. cervi* and its effect on the stimulant effects on Ca²⁺ mediated stimulant effect of levamisole and pyrantel pamoate and that calcium channel blocking agent nifedipine.

**Materials and Methods**

Adult *Setaria cervi* were picked up from the peritoneal cavity of freshly slaughtered water buffalo (*Bubalis bubalis* Linn.) and were brought to the laboratory in vacuum flask containing modified Ringer’s solution. The nerve-muscle preparation was made as per method described earlier and tied at both ends by threads. The tail end was tied with a hook at the bottom of isolated organ bath. For electrical stimulation a silver wire connected to the anode of a stimulator (Grass model S48 Grass instrument Co-Quincy MA, USA) was inserted into the tail end of the n.m. preparation tied at the bottom of the isolated organ bath. The silver wire in turn was connected to the electric stimulator through a flexible insulated wire. Another wire of similar type connected to the other terminal (cathode) of the stimulator and was immersed in the bathing fluid of the isolated organ bath. Care was taken that this wire does not come in the contact with the worm preparation. Stimulus current was given 5 volt/sec. Each experiment was repeated at least six times at 15 min intervals.

Application of electric current causes stimulation of the worm. The size of contraction and extent of upward shift of the baseline following application of electric stimulation varied with the size of the worm and the height of prestimulation contractions. The response to electrical stimulation could be elicited again after some time when the n.m. preparation had regained normal rhythmic spontaneous movements.

The n.m. preparation shows spontaneous rhythmic movements with alternate contraction and relaxation,
which were recorded on a slow moving kymograph. All the contractions are not necessarily of the same size and reflect the normal movements of the worm required for locomotion for locating themselves in the enviroiment and in search for food.

The resting tone of the worm attains a constant level as is reflected by a stable baseline. The preparation takes nearly 15 min to stabilize itself.

Result

Modification of response of pyrantel pamoate by electrical stimulation—Addition of Pyrantel pamoate in a concentration of 50 ng/ml of bath fluid caused immediate increase in the tone of the n.m. preparation. The rate was not visibly affected but the amplitude was reduced. After about five min the tone started decreasing and attained predrug level in about 15-20 min. No recovery was however seen with amplitude and rate of contractions (Fig. 1).

In another experiment the n.m. preparation was stimulated by a direct repetitive current which caused increase in tone and amplitude of contractions. When the stimulation was stopped the tone was restored to pre stimulation level but the amplitude of contractions was reduced as compared to the control and these continued to remain so thereafter. Pyrantel pamoate added to the bath from 50 ng to 10 mg/ml failed to elicit the stimulant response, which is typical of the drug. However, in a concentration of 25 mg/ml (Five hundred times higher see Fig. 1) pyrantel pamoate caused stimulation characterized by increase in tone only (Fig. 2 upper panel). Which was followed by complete paralysis of the worm movements. During paralysis application of electric stimulus of the same strength or addition of Ach (5 ng/ml) failed to modify the movements of the n.m. preparation (Fig. 2 lower left panel). Repeated changes of bath fluid also failed to restore the movements (Fig. 2 lower right panel).

Modification of response of levamisole by electrical stimulation—On the same lines as for pyrantel pamoate the experiments were done with levamisole. The preparation of Setaria was stimulated by direct repeated electrical stimulus and the activity was recorded. When the movements were stabilized in post stimulation phase, levamisole (5 ng/ml) was added to the bath fluid. Levamisole caused stimulation of the movements characterized by increase in tone only followed by paralysis (Upper panel of Fig. 3). Application of electrical stimulus
Fig. 4—Application of electric current (ES) caused stimulation of n.m. preparation of S. cervi. In post stimulation phase nifedipine was added to the bath fluid (2 μg/ml). Further electric stimulation (ES) of n.m. preparation failed to evoke any response, again during the phase of paralysis failed to evoke any response. Acetylcholine (5 μg/ml) added to the bath fluid also did not cause any increase in the motility of the n.m. preparation (Fig. 3 lower left panel).

Repeated changes of the bath fluid too was not able to restore the movements of the n.m. preparation (Fig. 3 lower right panel).

Modification of response to electrical stimulation by nifedipine—Stimulant effect of electric current on the n.m. preparation was recorded. During the post stimulation phase when the preparation had stabilized nifedipine (2 μg/ml) a calcium channel blocking agent (CCB) was added to the bath fluid. Following addition of nifedipine, the n.m. preparation was electrically stimulated. The electrical stimulus failed to evoke any response (Fig. 4).

Discussion
The main conclusion of the present study are that application of electric current to the n.m. preparation of S. cervi leads to its stimulation. Single stimulus gives one spike. When the preparation is stimulated repeatedly the post stimulation spontaneous contractions are smaller in amplitude as compared to prestimulation contractions. Pharmacological agents like levamisole and pyrantel pamoate both used as anthelmintics and nifedipine a calcium channel blocking agent blocks the stimulant response of electrical stimuli and that of Ach, an excitatory neurotransmitter of S. cervi.

The main source of activator Ca^{2+} is intracellular and not extracellular Ca^{2+}. The sarcoplamic reticulum is the intracellular source of activator Ca^{2+} and can store sufficient Ca^{2+} for activating maximal contractions. The major site of Ca^{2+} action is thought to be at the level of calmoduline dependent activation of the enzyme myosin light chain kinase. Application of direct current to the complex of Setaria elicited stimulation. The repetitive stimuli brings about increase in force of contractions evidenced by high amplitude each time when the current is applied. The spontaneous rhythmic contractions are reestablished when the application of current is stopped. However, the amplitude in the post electrical stimulation phase is smaller than the prestimulation phase. This may be attributed to reduction in intracellular source of activator Ca^{2+}.

In calcium free medium due to absence of available Ca^{2+} at the cell membrane, cations other than Ca^{2+} can only move inside the cell, thus producing scarcity of Ca^{2+} ions inside the cell. The Ca^{2+} operated calmodulin dependent activation of the enzyme myosin light chain kinase does not take place and the spontaneous movements of the muscle cease altogether. Studies with the voltage activated current in somatic muscle of Ascaris suum has observed that low Ca^{2+} solution in the bath fluid was likely to abolish currents activated as a result of calcium entry and has inferred that Ca^{2+} activated channel currents could be present and be involved in the production of slow waves and modulation waves. Similar situation is provided when a calcium channel blocking agent is applied to the bath fluid. Although Ca^{2+} is present in bathing solution but due to blockade of ionic channels meant for the transfer of Ca^{2+} from cell membrane to inside the cell, the transfer of Ca^{2+} does not take place, the intracellular concentration of Ca^{2+} required to trigger action potential is not achieved even after application of a stimulus. On nifedipine treated n.m. preparation of S. cervi application of electric stimulation or addition of Ach to the bath fluid does not modify the movements. This is reasonable to
assume that both electric stimuli and Ach acts by facilitating transfer of Ca$^{2+}$ from cell membrane to inside cell.

Anthelmintic mode of action have suggested that primary site of action of pyrantel and levamisole on *Ascaris* muscle is Ach receptors$^7$. Ganguly *et al.*$^8$ has shown that depolarising effect of pyrantel pamoate is elicited only in the presence of Ca$^{2+}$ and the effect was blocked in the presence of nifedipine. Patch clamp studies with cell attached and inside-out patches have shown that levamisole applied extra cellularily activated voltage sensitive cation selective channels. The channel open and burst time increased with hyperpolarization. At higher concentration of levamisole long closed-time separating clusters of burst were observed at both hyperpolarized and depolarized membrane potentials and this was interpreted as desensitization$^9$.

The findings of the present study indicate that stimulation of the n.m. of *Setaria* elicited by electric current or executed through the agency of Ach, as is done by levamisole and pyrantel pamoate depends upon the availability of Ca$^{2+}$. In the absence of which or when the calcium channels are blocked these stimuli fails to elicit a response.

**References**