

## IDENTIFICATION OF VOLTAGE-SENSITIVE CALCIUM CHANNELS IN *SETARIA CERVI* (NEMATODA: FILARIOIDEA) AND THEIR ROLE IN REGULATION OF SPONTANEOUS MOTILITY

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**Abstract:** Whole worm of *Setaria cervi* and its nerve muscle preparation exhibit rhythmical movements when suspended in modified Ringer's solution in an isolated organ bath. Deprivation of calcium from the bathing fluid results in gradual reduction in the amplitude and rate of contraction till the movements ceased completely. Similar results were obtained by adding EDTA (5 µg/ml) to the bath. The effect was concentration related and was evident early with higher concentration of EDTA. Acetylcholine which increases spontaneous movements of the whole worm as well as N.M. preparation failed to do so when the movements were inhibited either in calcium free solution or in the presence of EDTA. Addition of calcium channel blocker, Nifedipine to the bathing fluid also resulted in concentration related reduction of movements of the preparation of *Setaria*. Further, the stimulant response of Ach was also reduced by Nifedipine and this too was related to the concentration of calcium channel blocker in the bath. The evidence clearly indicates that presence of calcium is essential for the stimulant response of Ach on *S. cervi*, which like mammalian tissues contain calcium channels that can be blocked by specific blocking agents.

The abbreviations for Acetylcholine (Ach), Calcium channel blocker (CCB), Nerve muscle preparation (n.m. preparation).

**Key words:** calcium channel nifedipine acetylcholine movement

### INTRODUCTION

*Setaria cervi*, cosmopolitan nematode parasite of cattle resembles closely to human filarial worms in its response to drugs and can therefore be used for the screening of potential antifilarial agents (1, 2).

*Setaria* exhibits vigorous rhythmical movements which can be recorded on a kymograph by suspending the worm in an isolated organ bath. Also a nerve-muscle preparation of the worm exhibits similar movements (3).

However, the sensitivity of the nerve-muscle preparation to drugs is very high as compared to the whole worm (3).

Acetylcholine causes stimulation of the movements

of whole worm as well as n.m. preparation while 5-HT and GABA decreases the movements. Further the presence of Ach and Cholinesterase has also been shown in the parasite (4).

Modified Ringer's solution in which worms were brought from the slaughter house to the laboratory which is also used as bathing fluid in isolated organ bath contains calcium. Deprivation of calcium from modified Ringers solution results in inhibition of movements and consequently reduction survival time of *Setaria*. This prompted the investigations to find out whether *S. cervi*, like mammalian tissues contain calcium channels, and these channels, if present, can be blocked by known calcium channel blocking agents. Further an attempt has been made to find out correlation

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between the stimulant effect of excitatory neurohumor acetylcholine with the availability of calcium fluid.

### METHODS

Adult *S. cervi* were obtained from peritoneal cavity of the freshly slaughtered cattle (*Bubalis bubalus* Linn.) and brought to the laboratory in modified Ringer's solution maintained at 37°C. In the laboratory the worms were repeatedly washed with the same solution to free them from any extraneous material.

**Nerve-Muscle preparation:** A worm was placed in a petridish containing modified Ringer's solution. Two dissecting needles were inserted at one end of the worm, the cuticle was split longitudinally apart in one stroke. The anterior ½" of the worm was cut off to eliminate the influence of the nerve ring and the cephalic ganglia. The remaining constituted essentially the nerve-muscle preparation which was tied at both ends and suspended in the isolated organ bath

containing modified Ringer's solution (without calcium chloride). Aeration was not required as it does not improve the motility of the worm. The anterior end was attached to the frontal writing lever. After about 15 min when the preparation was stabilized to elicit spontaneous uniform movements, the drugs were added to the organ bath so as to act directly on N.M. complex.

### RESULTS

The N.M. preparation of *S. cervi* when suspended in isolated organ bath containing modified Ringer's solution exhibits spontaneous movements which continues for more than 6 hrs. Further, the preparation responds to the addition of Ach to the bathing fluid by stimulation characterised by increase in tone and amplitude of contractions.

It was observed that n.m. preparation of *S. cervi* when suspended in an isolated organ bath containing

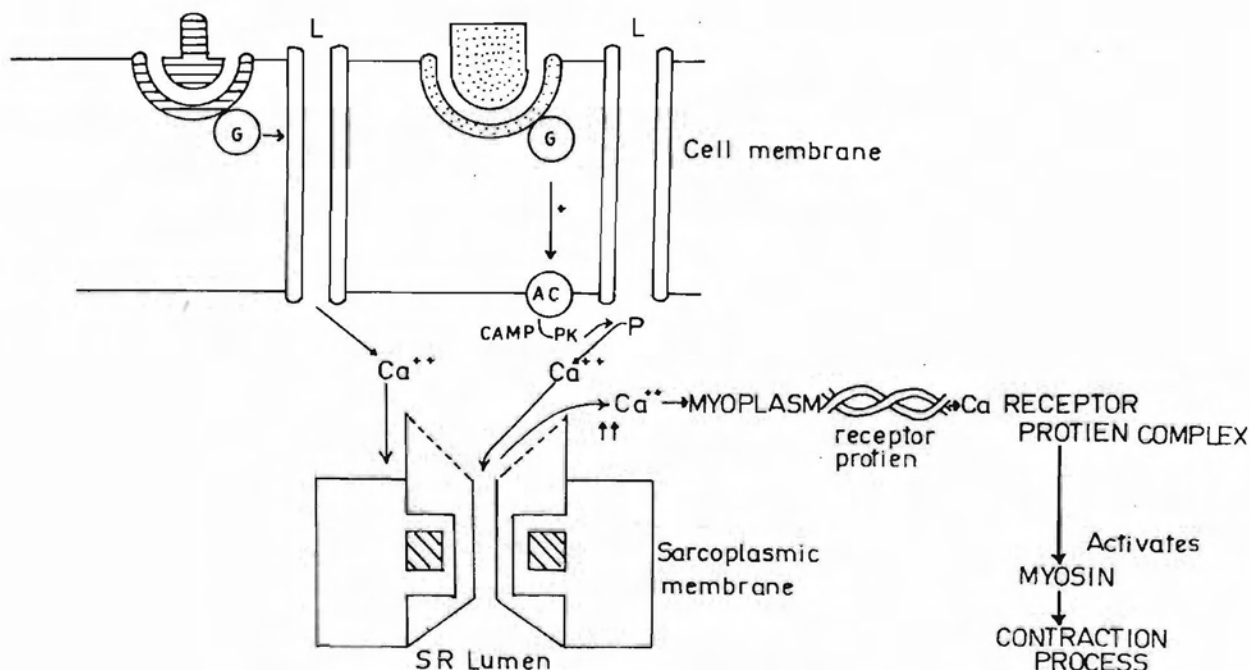


Fig. 1 : Model of calcium channels in cell membrane and sarcoplasmic reticulum of *Setaria cervi* with release of calcium for contraction process.

calcium free modified Ringer's solution showed a gradual reduction in spontaneous motility. This reduction in spontaneous motility. This reduction in movements was reversed by addition of calcium chloride to the bath fluid. The n.m. preparation bathed in calcium free modified Ringer's solution fails to respond to not only usual concentration of Ach ( $10 \mu\text{g}/\text{ml}$ ) but to very high concentrations as well ( $1000 \mu\text{g}/\text{ml}$ ) (Fig. 2).

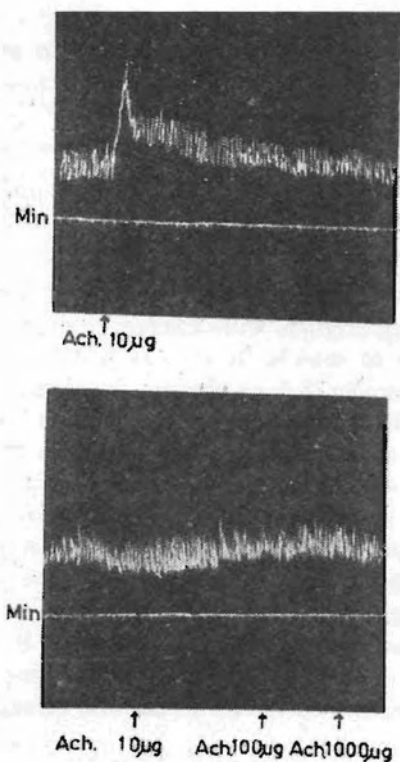


Fig. 2: Effect of Acetylcholine on spontaneous motility of nerve muscle preparation of *S. cervi* (above).

Effect of Acetylcholine on nerve muscle preparation in a calcium free solution on nerve muscle preparation of *S. cervi* (below).

In another set of experiments, addition of EDTA ( $5 \mu\text{g}/\text{ml}$ ) to the bath fluid resulted in gradual reduction in the motility of spontaneous movements of n.m. preparation of *S. cervi*. This response was similar to that observed when the n.m. preparation was suspended in calcium free modified Ringer's solution. However, the response with EDTA was delayed in onset. The response to EDTA was concentration related. Further

addition of higher concentration EDTA ( $30 \mu\text{g}/\text{ml}$ ) resulted in complete cessation of spontaneous movements which was evident within 5 min of its addition to the bathing fluid. The inhibition brought about by addition of EDTA was irreversible as repeated washings with the bathing fluid failed to restore the motility.

Addition of KCl to the bathing fluid when the movements were still inhibited resulted in short-lasting restoration of the spontaneous movements of n.m. preparation characterised by increased in tone, amplitude and rate of contractions (Fig.3).

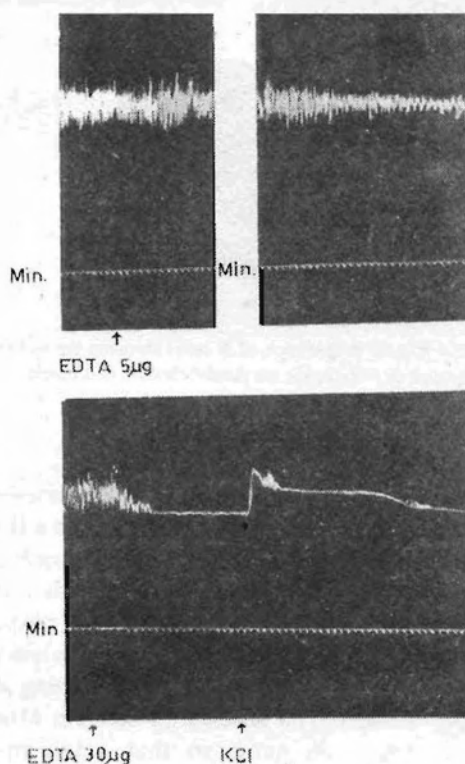


Fig.3: Effect of EDTA and addition of Potassium Chloride in the calcium free solution on nerve muscle preparation of *S. cervi*.

*Effect of calcium blocker, Nifedipine on the response of acetylcholine:* Addition of Acetylcholine to the bathing fluid causes increase in the spontaneous motility of the nerve-muscle preparation of *Setaria cervi*. The stimulation is characterised by increase in

tone, rate and amplitude (Fig. 2). Prior addition of Nifedipine 10  $\mu\text{g}$  to the bath inhibited the stimulant effect (Fig. 4). With further increase in the concentration of Nifedipine (15  $\mu\text{g}$ ), the effect was more marked and thus was concentration related (Fig. 4). When the concentration of Nifedipine was further increased to 20  $\mu\text{g}/\text{ml}$ , the stimulant effect of Acetylcholine was completely blocked (Fig. 4).

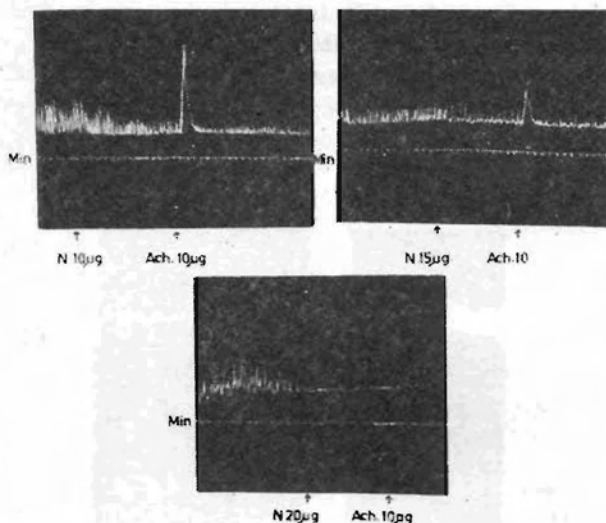


Fig. 4: Nerve muscle preparation of *S. cervi* showing the inhibitory response of Nifedipine on Acetylcholine in a concentration related effect.

## DISCUSSION

Experimental evidence obtained from varieties of tissues has established the role of calcium as a link in "Stimulus secretion coupling". The physiological contraction of skeletal and smooth muscles is elicited by calcium released from the sarcoplasmic reticulum (5) or by the presence of extrinsic calcium when such evidence is sought from a tissue in bathing fluid containing calcium. The absence of calcium exert an inhibitory effect on actin so that actin myosin interaction is prevented (6).

The somatic longitudinal musculature of *S. cervi*, the subject of this experiment is also innervated by processes and possess synapse and muscle end plate, though not identical with other animals (Lee and Atkinson). *S. cervi* exhibit spontaneous rhythmical contractions when kept in modified Ringer's solution at 37°C (2).

This solution contains calcium chloride. But when calcium was depleted from the solution, the movements were affected prominently i.e. gradual reduction of amplitude and rate of contraction. This indicates the necessity of presence and role of calcium in contraction process. It is generally accepted that calcium in the cell serve two functions viz. i) carry charge resulting in depolarization and ii) calcium influx can serve as an intracellular messenger. So this depression in contraction may be due to absence of extracellular calcium. This point can be clarified more emphatically by adding EDTA (7) in the presence of  $\text{CaCl}_2$  in modified Ringer's solution. Suspended in modified Ringer's solution from which calcium was not deleted, nerve-muscle preparation of *S. cervi* continued to exhibit rhythmical spontaneous motility, whereby addition of EDTA reduced the spontaneous movement leading to complete reversible paralysis.

Next comes the question of potassium, whether it has got any relation with calcium with regards to contraction of muscle. It is seen that after adding excess potassium (8.4 mg approx.) in the calcium free solution, the contraction of n.m. preparation of *S. cervi* were slowly restored, although for a short while. Potassium ions appear to have a potentiating action on transmitter release in addition to its depolarizing action (8). Here potassium with its own depolarizing action initiated the restoration or it opened the calcium channels, a few, through which very small amount of intracellular calcium came into action. It can be explained in alternate way that acetylcholine release from nervemuscle preparations can also be augmented by excess potassium (7).

Initial stimulation of the worm characterised by increase in tone and amplitude followed by depression is the biphasic response of acetylcholine in n.m. preparation of *S. cervi* (9).

This typical response was not observed when the bathing solution was deprived of  $\text{Ca}^{2+}$ . This highlights the involvements of calcium in the mechanism of release of transmitter substance, Ach. It is also seen that on prior addition of Ach, i.e. there was no stimulatory effect of acetylcholine on nerve muscle preparation which was previously blocked by Nifedipine.

It is generally accepted that  $Ca^{2+}$  is the primary regulator of contraction in smooth as in striated muscle. Various studies suggested that calcium had a presynaptic action on transmitter release. Miniature end plate potentials represent basic units (quanta) of transmitter activity and the end plate potentials consists of summation of such quanta (10, 11).

Calcium is capable of increasing the release of quanta of acetylcholine during depolarization by nerve impulse. Calcium deprivation depress miniature end plate potential, thus it appear that calcium dependent mechanism governs acetylcholine action at nerve terminals. The somatic longitudinal musculature of these nematodes are also innervated by process though not typical as in other animals (Lee & Atkinson). It is in this part of *S. cervi* i.e. muscle end plate where the interaction of calcium and Ach take place. Thus from this analysis we can explain that absence of calcium will reduce the release of Ach. Regarding intrinsic Ach, it can be explained in a similar fashion when calcium was deleted from the suspending fluid or on addition of EDTA or CCB, no extra calcium ions were available for extrinsic acetylcholine. The movement which went on initially was only due to extrinsic calcium. Therefore after the addition of acetylcholine, there was no extrinsic calcium ion which could have interacted with acetylcholine for its action to take place.

The evidence presented clearly indicate that like mammalian tissues, filarial nematode parasite of cattle *S. cervi* possesses calcium channels and presence of calcium is essential for maintaining its activity. Further these calcium channels behave in a manner similar to those present in mammalian tissues and their blocking agents are also same.

On the basis of the experimental evidence, a model of calcium channel in cell membrane and sarcoplasmic reticulum of *Setaria Cervi* has been proposed (Fig.1). The cell membrane contains voltage regulated L type of calcium channels as they are blocked by Nifedipine and opened up transiently by high concentration of  $K^+$ . The calcium ion enters through gated channels and stimulates the valve of stored  $Ca^{++}$  from sarcoplasmic reticulum. The  $Ca^{++}$  combines with receptor protein and forms a complex which causes contraction of myosin. Such a phenomenon which exists in large number of vertebrate and invertebrate tissues may also operate in *Setaria*, and other nematodes. Similar evidence gathered in future may strengthen this hypothesis.

This new insight in the mechanism of control of spontaneous movements of *Setaria* and the relationship of calcium ion with the excitatory neurotransmitter acetylcholine will be helpful in elucidating mechanism of action of antifilarial agents and designing new strategy for killing and containing the parasite.

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