

## REGULATION OF CHOLINOMIMETIC ACTION OF PYRANTEL PAMOATE BY CALCIUM CHANNELS IN *SETARIA CERVI*

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( Received on November 21, 1995 )

**Abstract :** Nerve muscle preparation of *Setaria cervi* (Nematoda : Filarioidea) exhibits spontaneous rhythmical movements when suspended in isolated organ bath containing modified Ringer's solution. Pyrantel pamoate (50 ng/ml) when applied caused initial short lasting stimulation followed by irreversible paralysis. When suspended in calcium free bathing fluid the movements of n.m. preparation showed a gradual decrease both in amplitude and rate of contraction till the movements ceased completely. The effect was similar when EDTA was added to the bath fluid.

The stimulant effect of Pyrantel pamoate was blocked in calcium free solution and in bath applied EDTA. Calcium channel blocker Nifedipine in a concentration of 500 ng/ml blocked the effect of Pyrantel pamoate (50 ng/ml). Neither stimulation nor depression of movements was evident with higher concentration of PP (250 mg/ml) the stimulant effect of Pyrantel pamoate was blocked while the depressant effect characterized by decrease in amplitude of calcium is essential for the stimulant effect of Pyrantel pamoate and its response on n.m. preparation is similar to Acetylcholine.

**Key words :** pyrantel pamoate      calcium channel      nifedipine  
nematode      filariasis

**Abbreviation :** Nerve muscle preparation (n.m. Preparation on)  
Acetylcholine (Ach), Pyrantel Pamoate (PP).

### INTRODUCTION

*Setaria cervi*, a filarial nematode parasite of cattle and its nerve-muscle preparation exhibit rhythmic movements when suspended in an isolated organ bath (1, 2). These movements are regulated by a primitive nervous system consisting of circum-oesophageal ganglia which act as the central nervous system of the helminth and four longitudinally running nerves. The four parallel running muscle bands make contacts with longitudinal nerve fibres at intervals (3). Acetylenoline (Ach) has been

identified as excitatory neurotransmitter in *S. cervi*. Besides *S. cervi* has a well developed mechanism for the synthesis of Ach and also has cholinesterase for the destruction of excess amount of Ach at the neuromuscular junction (4).

The action of Ach is depended upon the presence of  $Ca^{++}$  in the absence of which Ach fails to elicit stimulations both in the whole worm and nerve-muscle preparations of *Setaria* (5). Pyrantel pamoate, a commonly used anthelmintic causes initial stimulation followed

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by paralysis, an action similar to that elicited by the addition of Ach to the bath fluid. It has been demonstrated that primary site of action of Pyrantel pamoate in *Ascaris* is on Ach receptors. The understanding of Ach receptors and the knowledge that Pyrantel pamoate mimic its action is an important development in the understanding of the mechanism of action of anthelmintics.

To elucidate that the effect of Pyrantel pamoate like Ach is also dependent upon the presence of  $Ca^{++}$  ions and ionic channels are involved in the regulation of excitatory response, the present study was designed.

### METHODS

Adult *S. cervi* were obtained from the peritoneal cavity of freshly slaughtered cattle and immediately kept in vacuum flask containing modified Ringer's solution (glucose 0.25 gm/L) at 37°C. In the laboratory, the worms were given repeated wash with the same solution to free them from any extraneous material. Dale's organ bath of 20 ml capacity, fitted with a frontal writing lever was set up for recording the worm motility. The bath was filled with freshly prepared modified Ringer's solution. The temperature of the bath was maintained at 37°C. Aeration was not required as it did not improve the motility of the worm. Movements of the worm were recorded on slow moving drum.

**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 half inch of the worm was cut off to eliminate the influence of the nerve ring and the cephalic ganglia. The remaining nerve-muscle preparation which was tied at both ends was suspended in the isolated organ bath containing modified Ringer's solution. After 15 min when the preparation was stabilized to elicit spontaneous movements, the drugs were added to the organ bath so as to act directly on nerve-muscle complex.

### RESULTS

**Pyrantel pamoate on nerve-muscle preparation of *S. cervi*:** The nerve-muscle preparation of *S. cervi* contracted spontaneously and was allowed for some time to become stable. When Pyrantel pamoate was added to the solution bathing the worm, there was immediate stimulation characterised by increase in tone and amplitude followed by paralysis at a concentration of 50 ng/ml (Fig.1). Recovery did not occur after repeated washings.

#### EFFECT OF PYRANTAL PAMOATE ON NERVE-MUSCLE PREPARATION

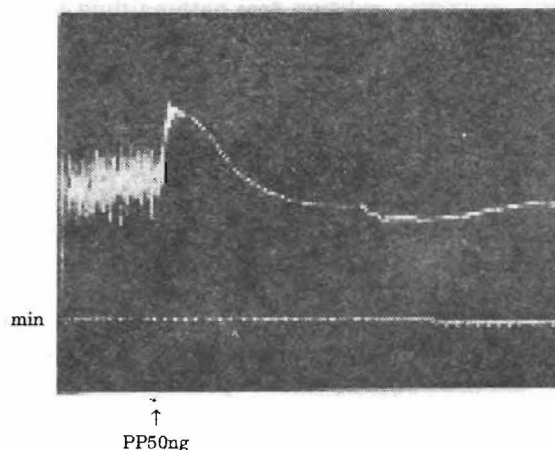


Fig. 1 : Effect of Pyrantel pamoate on spontaneous movement of nerve muscle preparation of *S. cervi*. A concentration of 50 ng/ml of Pyrantel pamoate caused stimulation followed by spastic paralysis of preparation.

Norton and deBeer (6) noted that the sensitivity to piperazine was much greater in the open *Ascaris* preparation where the cuticle was removed than in intact *Ascaris*. Although the filarial cuticle is quite different from *Ascaris* cuticle it may still act as a permeability barrier.

**Pyrantel pamoate in  $Ca^{++}$  free solution:** When the nerve-muscle preparation was suspended in  $Ca^{++}$  free modified Ringer's solution, the rhythmicity of the spontaneous movement was reducing gradually. Pyrantel

pamoate (10 µg/ml), when added in the bath fluid caused stimulation followed by paralysis of the worm. But here the stimulation was characterised by increase in rate and amplitude only but not in tone (Fig. 2). In second set of experiment, Ca<sup>++</sup> was not deleted from modified Ringer's solution whereby EDTA at a concentration of 10 µg/ml was added in the bathing fluid. This resulted in a gradual reduction in amplitude of contraction of the worm. On addition of Pyrantel pamoate (10 µg/ml), no stimulation of movement of nerve-muscle preparation was observed. There was irreversible paralysis (Plate 2).

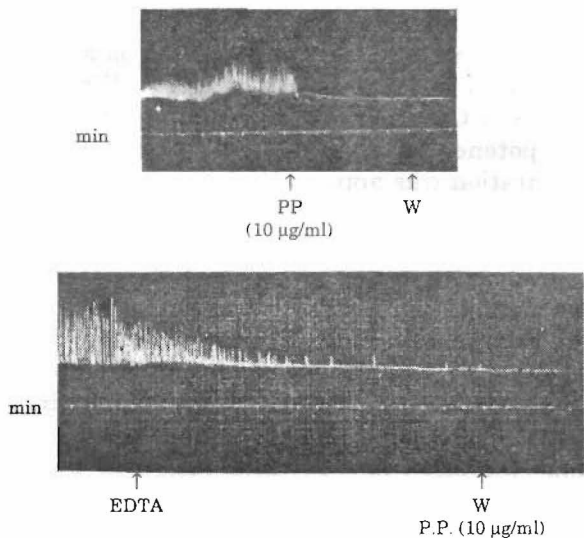


Fig. 2 : Upper panel: Effect of Pyrantel pamoate (10 µg/ml) on spontaneous movement of nerve muscle preparation of *S. cervi* in calcium free modified Ringer's solution. The amplitude and rate of contractions decreased immediately. Repeated washing failed to restore movements of the preparation after the addition of drug. The initial stimulant response was not observed.

Lower panel: Effect of EDTA on spontaneous movement of nerve muscle preparation of *S. cervi*. Arrow indicate the time of addition of EDTA to the bath. The amplitude showed gradual reduction. The response to Pyrantel pamoate (10 µg/ml) was blocked.

*Nifedipine and pyrantel pamoate:* In a fresh set of experiments, normal contraction of preparation was recorded. Nifedipine was added at a concentration (500 ng/ml) lower than that

which brings about paralysis of the worm. On addition of Pyrantel pamoate (50 ng/ml) without washing in between, the stimulatory effect of Pyrantel pamoate was inhibited. The preparation was washed, nifedipine (500 ng/ml) was added, followed by addition of higher concentration (250 ng/ml) of Pyrantel pamoate. It was observed that there was no stimulation of movement of preparation. Instead of stimulation followed by depression and paralysis, there was a gradual reduction in amplitude and rate of rhythmical movement of the preparation leading to irreversible paralysis (Fig.3).

EFFECT OF NIFEDIPINE AND PYRANTAL PAMOATE IN NERVE-MUSCLE PREPARATION

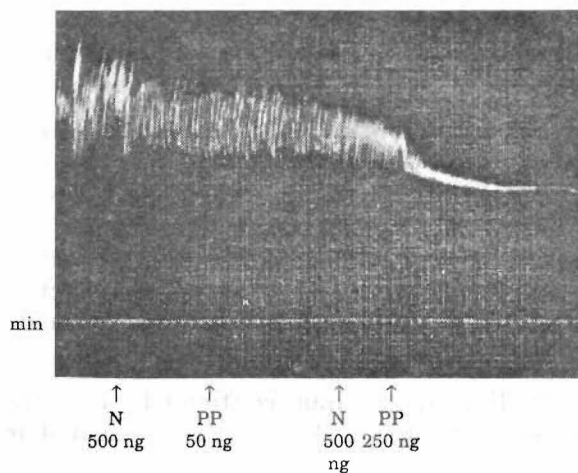


Fig. 3 : Effect of Nifedipine on the response of Pyrantel pamoate in the nerve muscle preparation of *S. cervi*. Arrows indicate the time of addition of drugs. Prior addition of Nifedipine (500 ng) blocked the response of Pyrantel pamoate (PP) (250 ng). A higher concentration of 250 ng of Pyrantel pamoate caused only paralysis of the preparation. Initial stimulation was not observed.

### DISCUSSION

These experiments demonstrate that the recording of contractions from filarid *S. cervi* is a convenient and reliable method of observing the effect of drugs. The long thread-like fragile parasite can be suspended in isolated organ bath and with the nerve-muscle complex directly exposed to the bath fluid containing drug and nerve ring removed during surgery, the

preparation becomes highly sensitive to the drug as compared to the intact preparations. Minimum concentration of Ach required to elicit response in nerve-muscle preparations is 25000 times less than that required for a similar response in the whole worm preparation. The nerve muscle preparation was found more useful for studying the basic physiology, pharmacological response and mechanism of drug action; because substances are not required to cross a barrier like cuticle and can directly reach the site of action.

The main conclusion of the present study is that *S.cervi* has voltage sensitive  $Ca^{++}$  channels. In the absence of available extracellular calcium, Ach as well as Pyrantele pamoate fails to elicit stimulation of nerve-muscle preparation of *Setaria*.

Electrophysiological techniques have demonstrated that Pyrantele is more potent agonist at *Ascaris* Ach receptors than at vertebrate nicotinic receptors where it has also weak nicotinic action (7, 8). The selective action of this drug allows it to be used as an effective anthelmintic killing the nematode parasite without harming the host.

Earlier study has confirmed that the excitatory nature of Ach *S.cervi* is elicited in presence of  $Ca^{++}$  channels are present and is

involved in production of slow waves. Anthelmintic mode of action studies have identified sites of action for piperazine, dihydroavermectin, pyrantele and levamisole (9). The primary site of action of pyrantele and levamisole in *Ascaris* is Ach receptors (7). Body wall musculature, contractility-myogenic in origin, excitatory neurotransmitter, Ach-like process in *Ascaris* are in many ways similar to filarid model. Thus an understanding of the relationship between Ach receptor over Pyrantele pamoate and the function of  $Ca^{++}$  at the receptor site is necessary for determination for which the present study is highlighted.

In the present study also, it is observed that the depolarising effect of Pyrantele pamoate is elicited only in presence of  $Ca^{++}$ . When we employed  $Ca^{++}$  free medium to study the effect, the potency of Pyrantele on *Setaria* muscle preparation was apparently lower in absence of  $Ca^{++}$ . When investigated further with calcium channel blocker, depolarising effect was nil. Thus it is clear that the presence of co-operatively interacting  $Ca^{++}$ , either through its entry by voltage-gated channels or release from organelle, is necessary for excitatory process by agents acting through Ach receptors-be it Ach itself or other agents acting through the same reception like Pyrantele-insight by this study.

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