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Effects of the muscarinic agonist, 5-methylfurmethiodide, on contraction and electrophysiology of *Ascaris suum* muscle

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Abstract

Contraction and electrophysiological effects of 5-methylfurmethiodide (MFI), a selective muscarinic agonist in mammals, were tested on *Ascaris suum* muscle strips. In a contraction assay, MFI produced weak contraction and was less potent than levamisole and acetylcholine. Atropine (3 μM) a non-selective muscarinic antagonist in mammalian preparations, did not affect contractions produced by MFI. Mecamylamine (3 μM) a nicotinic antagonist in *A. suum* preparations, blocked the MFI contractions indicating that MFI had weak nicotinic agonist actions. In two-micropipette current-clamp experiments MFI, at concentrations greater than 10 μM, produced concentration-dependent depolarizations and small increases in membrane conductance. The depolarizing effects were not abolished by perfusing the preparation in a calcium-free *Ascaris* Ringer solution to block synaptic transmission, suggesting that MFI effects were mediated by receptors on the muscle and were calcium-independent. A high concentration of mecamylamine, 30 μM, only reduced the depolarizing responses by 42%, indicating that MFI also had effects on non-nicotinic receptors. Three non-nicotinic effects in the presence of 30 μM mecamylamine were identified using voltage-clamp techniques: (i) MFI produced opening of mecamylamine-resistant non-selective-cation channel currents; (ii) MFI inhibited opening of voltage-activated potassium currents; and (iii) MFI increased the threshold of voltage-activated calcium currents. We suggest that a drug that is more selective for voltage-activated potassium currents, without effects on other channels like MFI, may be exploited pharmacologically as a novel anthelmintic or as an agent to potentiate the action of levamisole. In a larval migration assay we demonstrated that 4-aminopyridine (4-AP: a potassium channel blocker) potentiated the effects of levamisole but MFI did not.

Keywords: Methylfurmethiodide; *Ascaris suum*; Electrophysiology; Muscarinic receptors; Nicotinic receptors; Potassium channels; Calcium channels levamisole; 4-Aminopyridine

1. Introduction

The development of novel anthelmintics has been limited, so it is important to identify novel therapeutic lead compounds as well as methods for enhancing the potency of existing compounds that might counter drug resistance. The methods may include pharmacological agents that increase responses to existing anthelmintics. Previously our studies have focused on levamisole and related drugs. Levamisole belongs to the ionotropic cholinergic agonist group of anthelmintics that includes pyrantel, and that selectively produces muscle cell depolarization and spastic paralysis in parasitic nematodes (Aceves et al., 1970; Aubry et al., 1970). We have shown, using current-clamp, voltage-clamp and patch-clamp, that electrophysiological responses to these anthelmintics can be observed in body muscle cells of the nematode parasites *Ascaris suum* and *Oesophagostomum dentatum* (Martin, 1982; Pennington and Martin, 1990; Robertson and Martin, 1993; Robertson et al., 1994, 2002; Dale and Martin, 1995; Evans and Mar-

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tin, 1996; Martin et al., 2002; Trailovic et al., 2002). These studies have identified nematode nicotinic acetylcholine gated receptor channels (nAChRs) over the nematode muscle cell surface that are selectively and directly gated by these anthelmintics. These studies have described, down to the single-channel level, the agonist action and channel-blocking action of the anthelmintics.

If it were possible to find a drug that could stimulate nematode metabotropic cholinergic (G-protein coupled, muscarinic-like) receptors, it might be developed as a stand alone anthelmintic, or it might be used with ionotropic cholinergic anthelmintics to increase their potency. There is evidence in *A. suum* of metabotropic cholinergic receptors on muscle cells. Colquhoun et al. (1991) have tested a range of cholinergic agonists on the electrophysiology of *A. suum* muscle cells and found furtrethonium to be one of the more potent muscarinic agonists. Segerberg and Stretton (1993) have also found evidence of muscarinic-like receptors on *A. suum* muscle mediating depolarization and contraction; and Martin and Valkanov (1996) have described effects of acetylcholine potentiating the opening of mecamylamine-resistant non-selective cation channel currents (I_{bcat}) in isolated *A. suum* muscle bags. In *Caenorhabditis elegans* three G-protein coupled acetylcholine receptors (GAR-1, GAR-2 and GAR-3) have been reported (Hwang et al., 1999; Lee et al., 2000). GAR-3 is pharmacologically similar to mammalian muscarinic receptors. GAR-1 and GAR-2 are pharmacologically unlike mammalian muscarinic receptors and are not antagonized by usual concentrations of muscarinic antagonists.

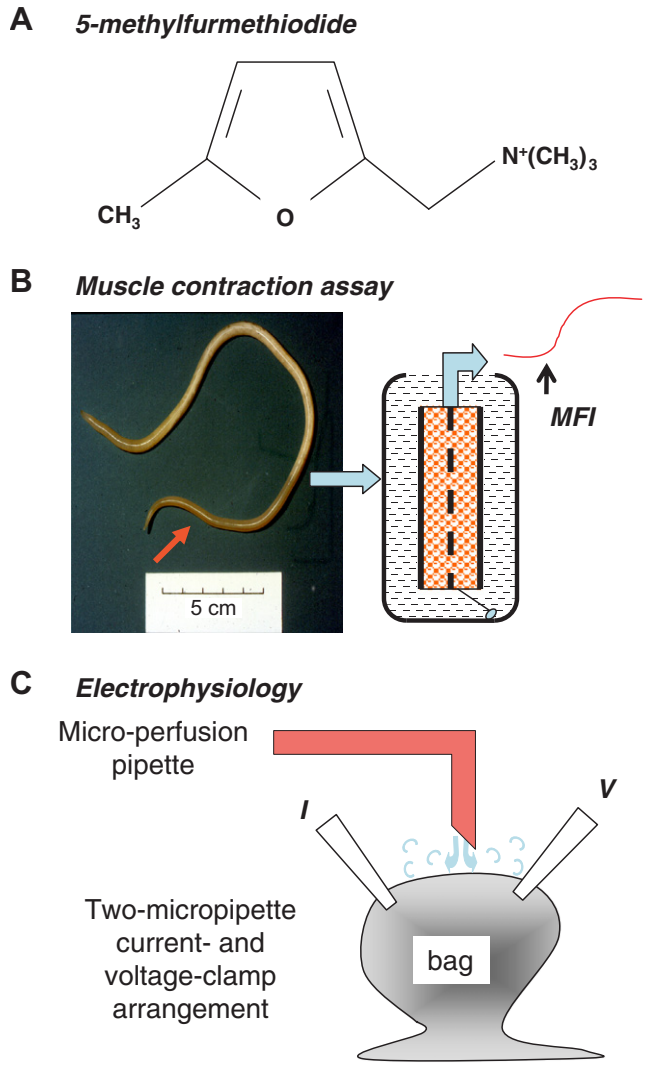

Fig. 1. Different methods were used to investigate the pharmacology of 5-methylfurmethiodide (MFI). (A) The chemical structure of MFI. (B) *Ascaris suum* muscle strip preparations are dissected from the anterior region of the worm and mounted in a water bath for the contraction assay. (C) Diagram of the two micropipette current-clamp and voltage-clamp recording from the bag region of the *A. suum* muscle cell and the application of solutions by microperfusion. The current-injecting micropipette (*I*) and voltage-sensing micropipette (*V*) are illustrated.

Colquhoun et al. (1991) tested effects on membrane potential of *A. suum*, using a number of mammalian cholinergic agonists including furtrethonium, muscarine, arecoline, pilocarpine, McN A343, bethanocol and oxtremorine. They found that furtrethonium was the most potent. We therefore selected 5-methylfurmethiodide (MFI, Fig. 1A) which is a potent mammalian muscarinic agonist (Newberry and Priestley, 1987) for further testing. In this paper we show that MFI in *A. suum* has four effects: (i) it is a weak nicotinic agonist; (ii) it opens mecamylamine-resistant non-selective cation channels; (iii) it inhibits its opening of voltage-activated potassium channels; and (iv) it increases the threshold for activation of calcium channels. We discuss the significance of mecamylamine-resistant channels and receptor operated channels (ROCs: ROCs are part of a group of channels also known as TRPs). We also illustrate the use of inhibition of voltage-sensitive potassium channels as a method for potentiation of cholinergic anthelmintics.

2. Materials and methods

2.1. Collection of worms

Adult *A. suum* (Fig. 1B) were obtained weekly from the Tyson pork packing plant at Storm Lake, IA. Worms were maintained in Locke's solution [NaCl (155 mM), KCl (5 mM), $CaCl_2$ (2 mM), $NaHCO_3$ (1.5 mM) and glucose (5 mM)] at 32 °C. The Locke's solution was changed daily.

2.2. Muscle contraction assay

Ascaris suum were used for the contraction studies within 72 h of collection, since the ability to contract vigorously to cholinergic agonists declined after this period. Two 1-cm body-flap preparations, one dorsal and one ventral, were made from each *A. suum* female from the region anterior to the genital pore (Fig. 1B). Each flap was monitored isometrically by attaching a force transducer in an experimental bath maintained at 37 °C containing 10 ml *Ascaris* Ringer solution: NaCl (23 mM), Na-acetate