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# SHORT COMMUNICATIONS

## EFFECTS OF IONOPHORE-MEDIATED TRANSPORT ON THE CARDIAC RESTING POTENTIAL

BY MOHAMMAD FAHIM*, ALLEN MANGEL† AND BERTON C. PRESSMAN

*Department of Pharmacology, University of Miami School of Medicine, P.O. Box 016189, Miami, FL 33101 U.S.A.*

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The monovalent carboxylic ionophores form lipid-soluble complexes with alkali cations which transport these ions across membranes. In a biological setting, they promote an electrically neutral exchange of intracellular potassium for extracellular sodium (Pressman, 1976). The ionophore monensin, which has a complexation preference for sodium (Pressman, 1968), has very little capability for complexing or transporting calcium or catecholamines (Pressman, Painter & Fahim, 1980). However, monensin produces a strong positive inotropic effect (Pressman, Harris, Jagger & Johnson, 1967; Sutko *et al.* 1977; Shlafer, Somani, Pressman & Palmer, 1978; Saini, Hester, Somani & Pressman, 1979) which is attenuated, but not abolished, by β-adrenergic blockade (Sutko *et al.* 1977; Shlafer *et al.* 1978; Saini *et al.* 1979). This indicates that the inotropic effect is partially mediated through an indirect release of catecholamines and, also, through a more direct mechanism, presumably by an alteration of transcellular cation gradients. Studies with cardiac Purkinje fibres show that monensin produces a shortening of the action potential, without an accompanying alteration in the resting potential (Sutko *et al.* 1977; Shlafer *et al.* 1978). Inasmuch as monensin should decrease the transcellular gradients of both sodium and potassium, one would expect that not only the action potential, dominated by the sodium diffusion potential, but also the resting potential, dominated by the potassium diffusion potential, should decrease. The present investigation was directed toward resolving the paradox of why monensin fails to alter the resting potential of cardiac muscle segments under the same conditions which alter the action potential. Our results show that depolarization of the resting potential, induced by monensin, is normally masked by the activity of the electrogenic sodium pump, and if the pump is inhibited by ouabain, then the expected depolarization is observed.

Adult New Zealand albino rabbits were killed by cervical dislocation. The heart was quickly removed and placed in saline of the following composition (in mM); NaCl, 140; KCl, 4·7; $CaCl_2$, 1·5; $MgCl_2$, 1·0; D-glucose, 10·0; *N*-2 hydroxyethyl piperazine-*N*-2 ethanesulphonic acid (Hepes), 5·0. The saline was pH adjusted to 7·2. A 2 $cm^2$ section of the left ventricular septum was dissected from the heart and pinned

* Present address: V.P. Chest Institute, University of Delhi, P.O. Box 2101, Delhi 110007, India.
† Present address: Department of Pharmacology, W26-208, Merck Institute for Therapeutic Research, West Point, PA 19486, U.S.A.

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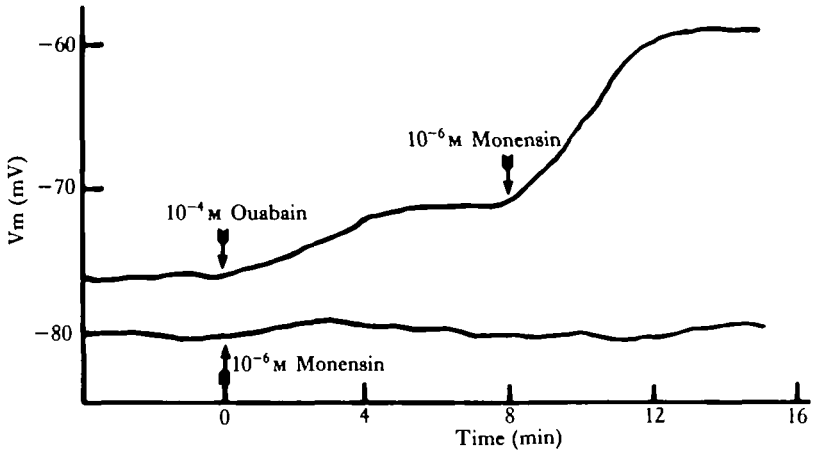


Fig. 1. Effects of $10^{-6}$ M monensin on the rabbit ventricular muscle membrane potential with and without ouabain pretreatment.

on a Sylgard-based chamber. Preparations were perfused with oxygenated (100% $O_2$) saline and maintained at 37 °C. Following equilibration, membrane potentials were recorded intracellularly with glass microelectrodes filled with 3 M-KCl. Electrode tip resistances ranged from 20–50 MΩ. Signals were fed to a high input impedance amplifier (WPI) and displayed on a curvilinear polygraph (Grass Instr.). Results are expressed as mean ±S.E. Probabilities were calculated by students *t*-test.

We found that the ventricular muscle membrane potential (−72·4 ± 5·4 mV) was not significantly changed ($P > 0{\cdot}05$) after the addition of $10^{-6}$ M monensin. A statistically significant depolarization was, however, observed following application of $10^{-4}$ M ouabain. With ouabain, the membrane potential stabilized within 5 min to −67·8 ± 5·3 mV ($N = 7$) (Fig. 1). Ouabain also induced a train of rhythmic action potentials in those preparations which were previously quiescent. If $10^{-6}$ M monensin was added after ouabain, then a further 12 mV depolarization, to −55·4 ± 4·3 mV ($N = 7$), was observed (Fig. 1). Lower doses of monensin ($10^{-7}$ M) produced a smaller depolarization, of approximately 5 mV ($N = 4$), in ouabain-treated preparations.

The effects of the potassium selective ionophore, valinomycin, on the ventricular muscle resting potential, were also examined. As shown in Fig. 2, after addition of $10^{-6}$ M valinomycin, the membrane potential hyperpolarized by approximately 7 mV, to −80 mV. A similar effect was observed by Hinkle & Van der Kloot (1973) with striated muscle, but not by Vogel & Speralakis (1978) with guinea pig heart. In preparations showing spontaneous electrical activity, valinomycin initially slowed and eventually terminated action potentials (Fig. 2).

The observed ouabain-induced depolarization can be attributed to elimination of the contribution of the electrogenic sodium pump from the resting potential. The subsequent depolarization by monensin suggests that stimulation of the electrogenic sodium pump may normally obscure the effects of monensin on the resting potential. In other words, following inhibition of the sodium pump, the effects of monensin are unmasked. Increases in sodium pump activity, after monensin, may result from changes in the level of intracellular sodium or may represent a direct response intended

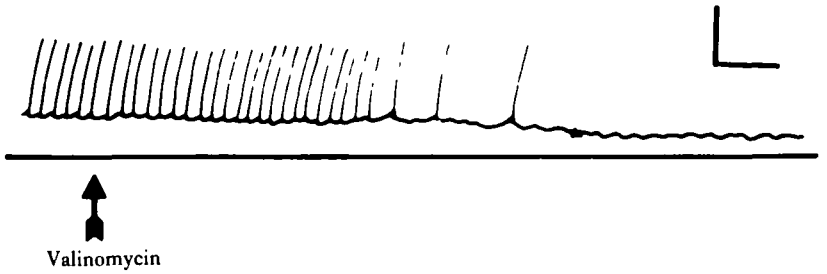


Fig. 2. Intracellular recording from a rabbit ventricular cell. Arrow indicates the addition of $10^{-6}$ M valinomycin. Valinomycin reduced the frequency of spontaneous activity and finally abolished the action potentials. A small hyperpolarization was also produced. Scale bar: 15 mV, 2 s.

to limit changes in the resting potential. Since valinomycin increases the permeability of the membrane to potassium, it effectively clamps the membrane potential to the potassium diffusion potential. From the potassium concentration in the medium (4·7 mM), and assuming an activity coefficient of 0·745 (Lee & Fozzard, 1975), an intracellular potassium activity of 75 mM can be calculated. This value compares well with that reported by Lee & Fozzard (1975) for rabbit papillary muscle. It can, therefore, be estimated that the intracellular sodium concentration must have increased by 10–50 mM during treatment with ouabain and monensin. This change may be sufficient to produce a significant alteration in intracellular calcium which, we presume, is the basis for the observed inotropic effect of monensin (Pressman, 1976).

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