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## Monactin does not influence potassium permeability in the squid axonal membrane

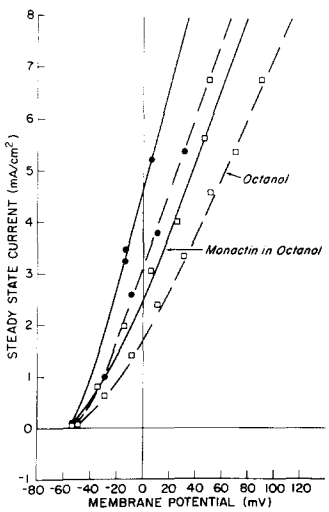
Beginning with the work of MOORE AND PRESSMAN[1] in 1964, it has been convincingly demonstrated that the macrocyclic antibiotics enhance $K^+$ transport across certain natural membranes[2,3] as well as synthetic lipid membranes[4,5].

A set of experiments were performed to observe the effect of monactin on a totally excitable membrane, the squid (*Loligo pealei*) giant axon[6], using conventional voltage-clamp techniques[7] in conjunction with internal perfusion[8].

The squid axon was prepared in artificial sea water containing little or no potassium. The steady state voltage-clamp currents were recorded before, during and after exposing the axon to an aqueous solution of monactin and *n*-octanol. Octanol was always present since the monactin first had to be dissolved in alcohol (0.16 mmole monactin per mole of octanol) prior to mixing with the aqueous salt solutions. Since monactin appears to be more effective at room temperature than at lower temperatures, all experiments were performed at 22–25° and several minutes were usually allotted for the monactin to have its maximum effect.

All external solutions which contained monactin and octanol were present in the concentration of 10 $\mu$M and 6.3 mM, respectively. All internal solutions which contained monactin and octanol were present in the concentrations of 1 $\mu$M and 0.63 mM, respectively.

The effect of the external application of artificial sea water containing 10 $\mu$M monactin *plus* 6.3 mM *n*-octanol on the squid axon is illustrated in Fig. 1. Clearly,



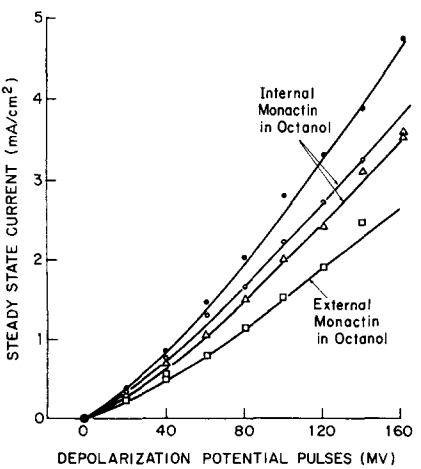




Fig. 1. Effect of monactin externally. ●, control values in artificial sea water; □, experimental values. — — —, octanol experiment in one axon; ———, monactin in octanol experiment on a different axon.

Fig. 2. Effect of monactin internally. ●, control values in artificial sea water; ○ and △, internal monactin in octanol values after 5 min and 15 min, respectively; □, values due to further addition of external monactin in octanol.

the monactin *plus* octanol depressed the steady state $K^+$ outward currents rather than exaggerate them. Note that the octanol alone produced the very same 40 ± 10 % reduction in the $K^+$ currents. This general depressing effect of alcohols on the steady state voltage-clamp currents in squid axons has been previously described by ARMSTRONG AND BINSTOCK[9] in 1964. Thus monactin itself appeared to have little if any effect on the steady state voltage-clamp currents. What happens when monactin and octanol are internally perfused is depicted in Fig. 2. Essentially the same result was obtained, that is, a depression rather than enhancement of the $K^+$ currents. There was less of a depression of the steady state current than with the external exposure simply because a more dilute solution of octanol (*i.e.* 0.63 mM octanol) was used internally. The longer exposure to octanol apparently increased the magnitude of current depression.

It might be argued, with some justification, that having a membrane resistance in the 10–100 Ω range makes the squid axon an unlikely candidate to demonstrate a monactin induced increase in $K^+$ permeability. A truly large increase in $K^+$ transport, however, might possibly be expected to lower the membrane resistance by an order of magnitude. There were also other, perhaps more sensitive, criteria used to discriminate a possible monactin effect. The resting membrane potential (*i.e.* the potential difference between the external and internal solutions at zero current) was one such criterion. If there were a significant increase in the $K^+$ permeability relative

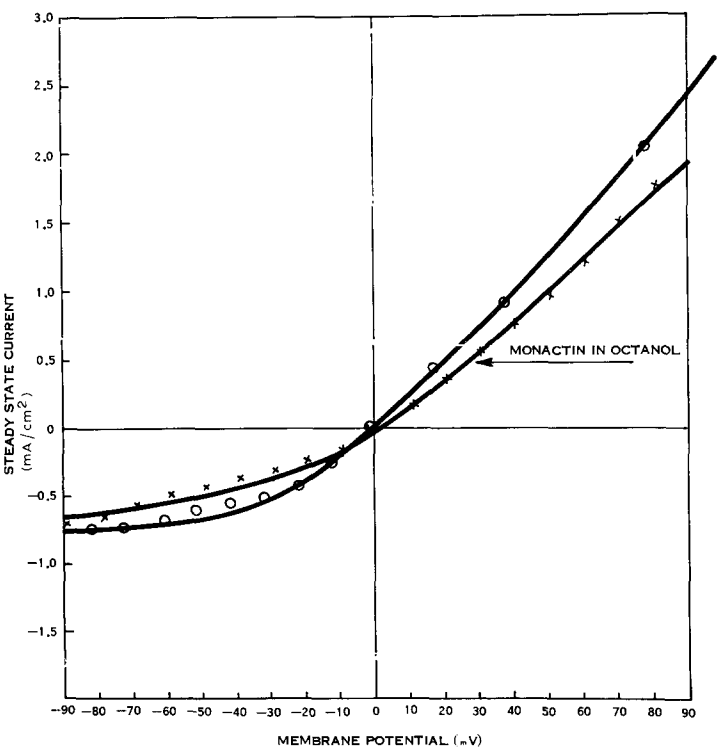



Fig. 3. Steady state current *versus* membrane potential in high external $K^+$ solutions. ○, control values in 440 mM $K^+$, 10 mM $Ca^{2+}$, and 50 mM $Mg^{2+}$; ×, values obtained with the addition monactin in octanol.

to that of $Na^+$, one should have observed a hyperpolarization of the resting membrane potential. No such hyperpolarization occurred in any of these experiments.

Another gauge of possible monactin effect was to depolarize the axon by entirely replacing the $Na^+$ in the external sea water solution with $K^+$ and then to monitor the current–voltage curves under voltage-clamp conditions[10]. With nearly equimolar concentrations (approx. 0.5 M) of $K^+$ both inside and out, the membrane resistance is relatively high, so that $K^+$ transport *via* monactin should noticeably augment the $K^+$ currents. Fig. 3 illustrates the absence of any increased $K^+$ transport.

Furthermore, if these same steady state current–voltage curves are translated into membrane chord conductance with $K^+$ as the primary (if not sole) charge carrier, then the resting membrane conductance of the axon prior to and following the application of monactin may be compared. Fig. 4 shows that monactin did not improve the $K^+$ conductance. On the contrary, there was a 40% reduction in resting membrane conductance (*i.e.* membrane conductance in the limit of zero current) consistent with an uncomplicated octanol effect. According to ARMSTRONG AND BINSTOCK[9], alcohols decreased the $K^+$ conductance near the resting membrane potential. If monactin had substantially improved $K^+$ permeability this would presumably have shown up in the limiting values of the chord conductance of the axonal membrane

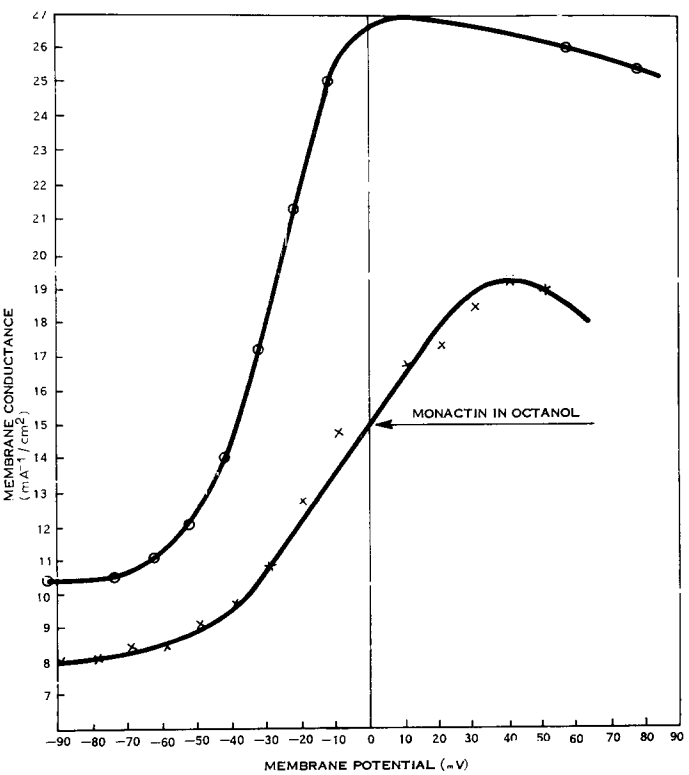



Fig. 4. Steady state conductance *versus* membrane potential. Calculated chord conductances from Fig. 3. Symbols are same as in Fig. 3.

where the currents were small and the membrane separated nearly identical concentrations of potassium.

A monactin effect might very possibly manifest itself by an absolute increase in leakage current, as it is usually assumed that the leakage represents a $K^+$ current. Furthermore, the leakage resistance in these axons was in the 200–300 $\Omega$ range so that the currents would tend to be quite small unless the monactin provided an additional leakage path (*i.e.* a new transport mechanism). Compared with the control, monactin in octanol did not produce any increase in the leakage current.

It may be concluded that monactin did not exhibit any striking effect on $K^+$ transport in the squid axon, using the several criteria that are ordinarily employed to measure $K^+$ permeability in an excitable membrane. Perhaps, these negative results are due to the presence of cholesterol in the squid axon membrane[11], which has been shown to greatly attenuate the $K^+$ conductance in synthetic membranes[12].

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