## Manganese Action Potentials in Mammalian Cardiac Muscle

$Mn^{++}$ is an impermeant ion that competitively blocks the Ca conductance in crustacean muscles[1-3] and has been widely used to inhibit the Ca conductance in various tissues including cardiac muscle[2,4-8]. However, the specificity of this action of $Mn^{++}$ remains to be established. This report is concerned with the occurrence of $Mn^{++}$ dependent action potentials in mammalian cardiac muscle, a phenomenon that might be expected because of the $Mn^{++}$ dependent slow inward current found in a voltage clamp study of this muscle[5].

The membrane potentials of guinea-pig's papillary muscles, cut from the right ventricle, have been recorded with glass microelectrodes while stimulating pulses have been applied through a sucrose-gap[5]. In principle experiments were performed under Na-free, Ca-free and Mg-free conditions[9]. In the presence of 2–95 m*M* $Mn^{++}$ it was possible to elicit action potentials. They were elicited in all-or-none manner when the membranes were strongly depolarized (Figure 1A) and propagated with little decrement. They showed a definite overshoot; in isotonic 95 m*M* Mn Tyrode's the overshoot amounted to about 50 mV (range 34–55 mV, 10 experiments). The overshoot was dependent on the external $Mn^{++}$ concentration, as shown in Figure 1. There was a decrease in overshoot as $[Mn^{++}]_o$ was reduced in the sequence 95, 50, 20 and 5 m*M* (Figure 1A–D) and an increase as the concentration was raised from 2 to 5 and to 20 m*M* (Figure 1E–G). Figure 2 is a plot of overshoot as a function of logarithm of $[Mn^{++}]_o$. An approximate 30 mV increase in overshoot was obtained with a 10-fold increase in the $Mn^{++}$ concentration as predicted by the Nernst equation for $Mn^{++}$. It seems reasonable to conclude that the cardiac sarcolemma is selectively permeable to $Mn^{++}$ in such Na-free and Ca-free media. It might be argued that this high membrane permeability to $Mn^{++}$ was caused by the deprivation of extracellular $Ca^{++}$, because a zero $Ca^{++}$ solution can cause a general increase of membrane permeability[10]. A series of 7 experiments was performed under more normal conditions. In the presence of 0.6 m*M* $Ca^{++}$, the overshoot still increased with the increase of $[Mn^{++}]_o$ as is shown in Figure 3. The Mn action potential was resistant to terodotoxin ($3 \times 10^{-5}$ *M*) but was suppressed by the addition of 1 m*M* $La^{+++}$. Thus $Mn^{++}$ appears to permeate through the channel for the slow inward current.

In cardiac muscle $Ca^{++}$, $Sr^{++}$ and $Ba^{++}$ can permeate through the channel for the slow inward current as

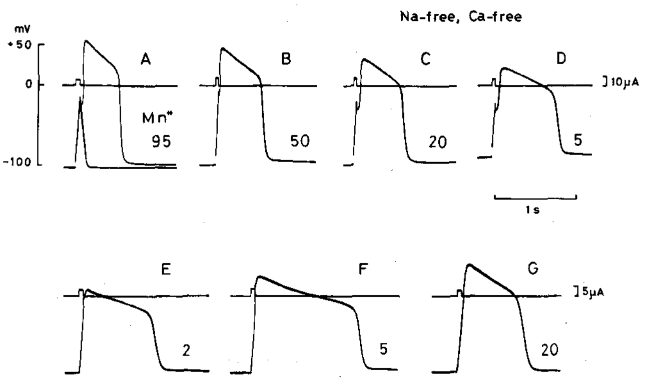


Fig. 1. Action potentials from guinea-pig's papillary muscles in the solution containing various amounts of $Mn^{++}$. The bathing solution lacked $Na^+$, $Ca^{++}$ and $Mg^{++}$. Records A to D and E to G were taken from 2 different muscles and were recorded in alphabetical order. 5 to 10 min were allowed for the exchange of solution. The muscle was stimulated by rectangular depolarizing pulses of 50–100 msec applied through a sucrose-gap. The stimulation was effected less than 5 times in each test solution. The temperature of the perfusing solution was 30 °C.

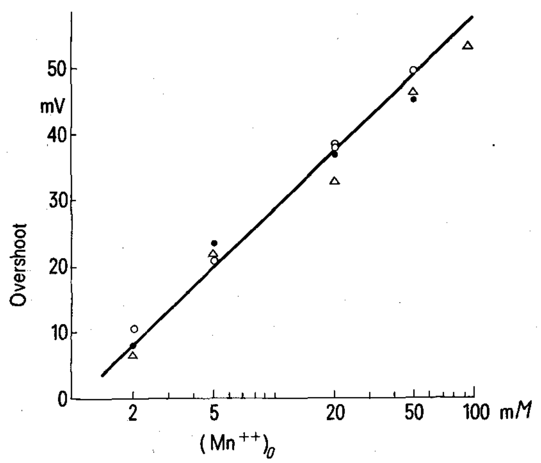


Fig. 2. The relationship between the peak potential of Mn action potential and the concentration of Mn ions in the external solution, constructed from data obtained from 3 different muscles. The experiment plotted as triangles was performed in the order of 95 (Figure 1A), 50 (B), 20 (C), 5 (D) and 2 m*M*-$Mn^{++}$; filled circles in the order of 2 (Figure 1E), 5 (F), 20 (G) and 50 m*M*; open circles in the order of 20, 50, 20 5 to 2 m*M* $Mn^{++}$. The straight line has a slope of 30 mV/decade.

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[9] Composition of the normal Tyrode's solution was: NaCl 142, KCl 2.7, $CaCl_2$ 1.8, $MgCl_2$ 1, *Tris*-HCl 10, glucose 5 m*M*; pH 7.3. Composition of $Mn^{++}$ Tyrode's was: $MnCl_2$ 95, KCl 2.7, *Tris*-HCl 10, glucose 5 m*M*; pH 7.3. $MnCl_2 \cdot 4H_2O$ from BDH Chemicals was used. The concentration of $Mn^{++}$ was varied by substituting $MnCl_2$ by an equivalent amount of tetraethylammonium chloride (cf. R. S. ARONSON and P. F. CRANEFIELD, J. gen. Physiol. *61*, 786 (1973).
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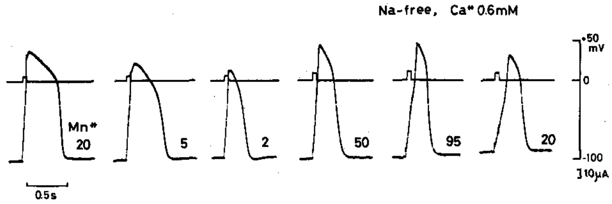


Fig. 3. $Mn^{++}$-dependent action potentials from guinea-pig's papillary muscles in Na-free, $Ca^{++}$-containing solution. The bathing solution lacked $Na^+$ and $Mg^{++}$ but contained 0.6 m*M* $Ca^{++}$ and various amounts of $Mn^{++}$ as illustrated. The experiment was performed in the order of left to right.

charge carriers[7,11,12]. $Mn^{++}$ has usually been regarded to be impermeant and it has been used as an inhibitor of Ca conductance. Antagonistic action of $Mn^{++}$ to the Ca, Sr and Ba action potentials has been demonstrated in guinea-pig's atria, when it was used in low concentrations (0.15–1 m*M*)[6]. In the present study, action potentials with overshoot were elicited under Na-free and Ca-free conditions in the prsence of 2–95 m*M* $Mn^{++}$. The relatively close agreement between the experimental points and the theoretical slope for a Mn electrode (30 mV/decade at 30 °C) *in Figure 2* is consistent with the view that $Mn^{++}$ conductance is large during the peak of the overshoot. Because of the occurrence of Mn action potential and of Mn slow inward current[5] in guinea-pig's ventricular muscle and similar action potentials elicited in frog heart (Dr. D. Ellis, personal communication), membrane permeability to $Mn^{++}$ is hardly negligible in cardiac muscle.

*Summary.* The membrane potential in guinea-pig's papillary muscles from right ventricle was recorded by glass microelectrodes and stimulation was effected by current pulses applied through a sucrose-gap. Action potentials with overshoot were recorded in the solution lacking $Na^+$ and $Ca^{++}$ but containing 2–95 m*M* $Mn^{++}$. The overshoot was inecease with the increased of $[Mn^{++}]_o$ by about 30 mV/decade. Similar $Mn^{++}$-dependent action potentials were also obtained in Na-free solution containing 0.6 m*M* $Ca^{++}$. The results indicate that Mn inward current is sufficient to generate action potentials in cardiac muscle.

R. Ochi[13]

*Department of Physiology, Jichi Medical School, Minamikawachi-machi, Tochigi-ken (Japan 329-04),*
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[13] This work was supported by grants No. 921814 and No. 957299 from Japanese Ministry of Education.

## Chemical Excitability of Axons: Excitatory and Inhibitory Effects of Putative Neurotransmitters and Modulators on Frog Sciatic Nerves

According to a widely accepted view, neurons and other excitable cells contain two fundamentally different types of electrogenic membranes: chemically-excitable (and usually electrically-inexcitable) synaptic membranes, and electrically-excitable axonal membranes[1]. Most theories of drug action regard synapses as the target for neurotropic drugs, thus assuming explicitly or implicitly that the electrically-excitable membrane of axons is chemically-inexcitable. Although local ionic currents[2] rather than chemical mediators such as acetylcholine[3] appear to be responsible for the propagation of action potentials, drug interaction studies on isolated sciatic nerves[4] have provided experimental evidence for the presence in axonal membranes of specific receptors for acetylcholine, L-epinephrine, histamine and serotonin. We have recently shown (in non-anesthetized, gallamine-paralyzed rabbits)[5,6] that the microiontophoretic administration of acetylcholine, tryptamin, norepinephrine and other putative neurotransmitters to corpus callosum fibres can elicit or inhibit the occurrence of action potentials. Since few if any synapses have been observed in the corpus callosum, these results suggest that axonal membranes may be chemically excitable. We have tested this hypothesis in isolated sciatic nerves (*Rana pipiens* and *Rana catesbeiana*) bathed in a modified Ringer's solution (NaCl 112 m*M*; KCl 3.5 m*M*; $CaCl_2$ 1.8 m*M*; $PO_4H_2K$/NaOH buffer, pH 7, 0.07 m*M*; dextrose 11 m*M*).

A 5 barrel micropipette system was placed into a desheathed portion of the nerve. A 4.8 *M* NaCl barrel was used for extracellular recording of multiple unit activity; 3 other barrels were used for the microiontophoretic administration (ejecting and holding currents: 5–20 nA) of drugs (0.5 *M*, ph 5) (acetylcholine, norepinephrine, dopamine, 2-phenylethylamine, tryptamine, histamine). A 3 *M* NaCl barrel was used as output for an automatic current balancing system[7] and also to test for current artifacts. We have often recorded spontaneous firing of nerve, suggesting that the preparation was not in a truly physiological state. At most sites, the firing rate was not modified by the administration (5 sec to 3 min) of synaptic activating drugs (0.5 *M*). However, we found units whose rate of firing was modified by these agents. These sites appeared to be highly localized because minor displacements of the electrode rendered the drug ineffective. Acetylcholine (Figure 1) often induced firing. Norepinephrine, 2-phenylethylamine and tryptamine either induced firing or inhibited spontaneous firing, depending on the site. The most common response to histamine administration was a brief period of firing followed by inhibition. In many instances, the effects of acetylcholine (Figure 1), histamine, or norepinephrine outlasted for sec or even min the ejecting current. Different units showed different patterns of response to the drug tested. The effects of most drugs at a given site were usually reproducible, but tachyphylaxis was observed with 2-phenylethylamine, histamine and acetylcholine. Comparing dopamine, norepinephrine, 2-phenylethylamine and tryptamine on the same units, several instances were found in which one amine was excitatory, another inhibitory, and another without effect. Figure 2 shows one example of units in which dopamine (but not norepinephrine) decreased the rate of firing. Whenever norepinephrine and 2-phenylethylamine exerted opposite effects, inhibition usually dominated.

These on-going experiments raise interesting possibilities, but it would be premature to conclude that the drugs tested are able to elicit action potentials in axonal membranes. The results obtained might be due to the artificial experimental conditions of our in vitro set-up

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