Effect of 2,4 Dinitrophenol on Electrical Activity in Purkinje and Ventricular Muscle Fibers of Hog Heart

By Morris Kleinfeld, M.D., John Magin, E.E., Bernard Murphy, M.S., and Edward Stein, Ph.D.

In a recent study, it was observed that 2,4 dinitrophenol, in concentrations of 2 to 5 \( \times 10^{-5} \) M, produced a shortened action potential duration and a decrease in the amplitude of the action potential in the atrial fiber of the isolated mouse heart and in the ventricular fibers of the intact and isolated perfused frog heart. The membrane resting potential was unchanged even during the period of cardiac arrest. Similar findings had been reported in the rat atrium and cat ventricular muscle fiber. In the study reported here, the investigation was extended to the specialized conductive (Purkinje) fiber in order to make comparative observations of the effects of 2,4 dinitrophenol on the electrical activity of this fiber as compared to its effect on ventricular muscle fiber. A false tendon-ventricular muscle preparation of the hog heart was used because its greater mass compared to a frog heart preparation facilitated visualization and employment of the technical procedures suitable for this study.

Methods

Hog hearts obtained within approximately 15 minutes after slaughter were washed in Tyrode's solution. From each heart, several segments of ventricular muscle, to which false tendons were attached, were excised and immediately placed in a thermost bottle containing oxygenated Tyrode's solution. Approximately one to two hours later, one of the excised segments was transferred to a perfusion chamber and bathed in a slow-flowing Tyrode's solution through which 96 per cent \( \text{O}_2 \) and 4 per cent \( \text{CO}_2 \) was bubbled continuously. The remaining segments were then stored in another container of Tyrode's solution through which the same mixture of gases was bubbled. The temperature of the perfusion chamber was maintained between 34 and 36 C. The tissue was stimulated by rectangular pulses at 60 beats per minute by means of a Grass stimulator, and this rate was maintained throughout the experiment. The driven rate exceeded the intrinsic rate of the spontaneously beating false tendon. The action potentials of the Purkinje and ventricular muscle fibers were obtained by the ultramicroelectrode technique of intracellular recording and monitored on a dual beam oscilloscope (Tektronix 545A). Dinitrophenol was administered as a constant perfusion in concentrations of 2 to 5 \( \times 10^{-5} \) M, and the changes in electrical activity were recorded at periodic intervals on a Sanborn Twinbeam model 62 electrocardiograph. The drug was perfused for 20 minutes after which the control solution was readministered to ascertain if the changes induced by 2,4 dinitrophenol were reversible. Twenty-two such procedures were conducted in this experiment.

Results

Dinitrophenol produced changes in the transmembrane action potentials in both the Purkinje and ventricular muscle fibers; however, the time of initiation and rate of change of the different phases of the action potential in the two fibers varied. In the Purkinje fiber, the earliest and most pronounced alteration was a loss in overshoot resulting in a decrease in the amplitude of the action potential. Shortly thereafter, the action potential duration shortened, but the percentage change was less than that which occurred in the overshoot. The membrane resting potential decreased slightly. In the ventricular muscle fiber, the earliest and most prominent change was a shortening of the action potential duration. This was followed by a loss in overshoot and a slight decrease in the amplitude of the action potential. The membrane resting potential declined but was not significantly different.
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from that noted in the Purkinje fiber. A progressive increase in the electrical threshold for excitability occurred in both fibers as demonstrated by the occurrence of dropped beats and the fact that the control rate of the heart could only be maintained by increasing current strengths. The effects in the electrical activity in both fibers were reversed completely by washing the tissue with Tyrode’s solution. Figure 1 illustrates a typical experiment showing the simultaneous effects of 2,4 dinitrophenol on the action potential in the Purkinje and ventricular muscle fibers and the reversal of these effects after washing the tissue with Tyrode’s solution. A summary of the data demonstrating the changes in the different phases of the transmembrane action potential in both fibers is shown in figure 2.

Discussion

The occurrence of a shortened action potential duration in both the Purkinje and ventricular muscle fibers after the administration of 2 to 5 × 10^-5 M dinitrophenol can be explained by an enhanced K^+ efflux from the cell. Macfarlane has suggested that 2,4 dinitrophenol increases the permeability of the membrane to K^+ and hence the increased K^+ efflux. Webb and Hollander, on the other hand, conjectured that the presence of negatively charged adenosine triphosphate molecules within the membrane could impede the diffusion of positively charged K^+. Hence, an inhibition of oxidative phosphorylation produced by 2,4 dinitrophenol with its resulting decrease in adenosine triphosphate could account for the enhanced K^+ efflux from the cell and a shortened action potential duration. Of interest is the finding of Neil that 0.3 mM dinitrophenol produced a loss of intracellular K^+ in the rat intestine, presumably by its interference with oxidative phosphorylation. The decrease in the amplitude of the action potential produced by 2,4 dinitrophenol has been ascribed to a decrease in inward Na^+ current brought about by a lowering of the membrane resting potential. A decrease in the amplitude of the action potential may also be explained on the basis of a depression of the sodium-carrying system by 2,4 dinitrophenol, resulting in a decrease in sodium influx. The decrease (<20 per cent) in membrane resting potential with 2,4 dinitrophenol observed in these experiments varied from the appreciable decrease in the rabbit heart (>50 per cent) observed by de Mello. Perhaps the larger dosage (10^-4 M), the longer duration of exposure (45 minutes), and the different species (rabbit) used by de Mello account for the differences observed. The progressive rise in the electrical threshold for excitability in

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**FIGURE 1**

The effect of 5 × 10^-5 M dinitrophenol on the Purkinje and ventricular muscle fibers of the isolated perfused hog heart. Simultaneous records are of the transmembrane action potential of Purkinje fiber (above) and ventricular muscle fiber (below). The significant changes shown are progressive shortening of the action potential duration, loss in overshoot with decrease in amplitude of action potential, and decline in membrane resting potential of both types of fibers. The degree of change in the various phases of the transmembrane action potential between the two fibers varied. Discussion appears in the text. A. Control; B. 12 minutes after administration of 5 × 10^-5 M dinitrophenol as constant perfusion; C. 20 minutes after; D. 40 minutes after return to Tyrode’s solution. Ordinates = millivolts; abscissae = time. Time lines, 0.04 second. Gain, 1.0 cm. = 50 mv. for transmembrane action potential. All measurements were made from top of action potential trace. Horizontal lines to right of each action potential indicate zero level, (0) for membrane potential.

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The effects of 2,4 dinitrophenol (5 × 10^{-5} M) on the different phases of action potential in Purkinje and ventricular muscle fibers of isolated perfused hog heart. Figure represents average results of 16 different experiments. At zero time, 5 × 10^{-5} M dinitrophenol was administered as a perfusion. Temperature of bath was maintained between 34 and 36 C. , membrane resting potential; , overshoot; , action potential duration; , transmembrane action potential.

The presence of an adequate membrane resting potential can be explained on the basis of an inactivation by 2,4 dinitrophenol of the system which is responsible for carrying Na^+ ions through the surface membrane, resulting in a lowered intracellular Na^+.

The differences, both in time of initiation and rates of change, observed in the various phases of the action potential between the Purkinje and ventricular muscle fibers following exposure to 2,4 dinitrophenol are attributable in part to their differences in metabolic energy requirements. If Hoffman and Suckling are correct in assuming that the maintenance of the plateau phase of the action potential is dependent on metabolic energy, then one may conjecture that the earlier and more pronounced shortening of the plateau in the ventricular muscle fiber, as compared to the Purkinje fiber, is due to the greater need of the former for metabolic energy. Since oxidative phosphorylation is inhibited by 2,4 dinitrophenol, less adenosine triphosphate is available to the ventricular muscle for its activity. It is of interest that Eccles et al. found a significantly greater degree of glycogenolysis in the ventricular as compared to the Purkinje tissue of ox hearts exposed to room temperature for six hours. They indicated that the appreciably slower rate of glycogenolysis in the Purkinje tissue afforded the conducting tissue some degree of protection against anoxia. This suggests that the Purkinje tissue might be highly dependent on glycolytic metabolism and hence is less oxygen dependent than ventricular muscle. It may be speculated that the greater shortening of the action potential duration in the ventricular as compared to the Purkinje fiber is due to a more marked inhibitory effect on K^+ influx by 2,4 dinitrophenol in the former, with the K^+ influx coupled to metabolic energy. To account for the earlier and more pronounced loss in overshoot in the Purkinje as compared to ventricular action potential after 2,4 dinitrophenol administration, it is suggested that 2,4 dinitrophenol has a greater modifying effect on the Purkinje fiber membrane or on its Na^+ carrying system. This results in an earlier and more pronounced decrease in Na^+ influx in this fiber.

Summary and Conclusions

DNP (2,4 dinitrophenol), in concentrations of 2 to 5 × 10^{-5} M produced changes in transmembrane action potential in both the Purkinje and ventricular muscle fibers of the isolated perfused hog heart; however, the time of initiation and rate of change of the different phases of the action potential in the two fibers varied. In the Purkinje fiber, the earliest and most pronounced alteration was a loss in overshoot resulting in a decrease in amplitude of the action potential. Shortly thereafter, the action potential duration shortened, and membrane resting potential decreased. In the ventricular muscle fiber, the earliest and most prominent change was a shortening of action potential duration. This was followed by loss in overshoot and decrease in amplitude of action potential. The membrane resting potential declined but was not significantly different from that noted in Purkinje fiber. The changes in both fibers
were reversed completely by washing with Tyrode's solution. The earlier and more pronounced shortening of action potential duration in ventricular muscle fiber as compared to Purkinje is attributed to a greater need of the former for metabolic energy. It may also be conjectured that the greater shortening of action potential duration in ventricular fiber is due to a more marked inhibitory effect of K⁺ influx by 2,4 dinitrophenol in this fiber with the K⁺ influx coupled to metabolic energy. To account for the more enhanced loss in overshoot in Purkinje fiber, it is suggested that 2,4 dinitrophenol has a greater modifying effect on Purkinje fiber membrane or on its Na⁺ carrying system. This results in an earlier and more pronounced decrease in Na⁺ influx in this fiber as compared with ventricular muscle fiber.

References
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