HYDROGEN PEROXIDE ACTIVATES A LARGE OUTWARD K^+ CURRENT IN ISOLATED VENTRICULAR MYOCYTES FROM CAVIA COBAYA (GUINEA PIG) AND MUS RATTUS (RAT)

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Free radicals generated during myocardial ischemia are thought to contribute significantly to ischemic damage. Hydrogen peroxide (H_2O_2) is a byproduct of superoxide free radical formation and may also result in formation of the hydroxyl free radicals. The purpose of this study was to determine whether H_2O_2 has direct electrophysiological effects in heart. Guinea pig and rat ventricular myocytes were isolated enzymatically and were voltage clamped using the patch clamp technique (whole cell clamp configuration). The pipet (intracellular) solution contained in mM: 120 KCl, 10 NaH_2PO_4, 14 EGTA, 1 $CaCl_2$, 10 HEPES, with or without 5 MgATP, pH = 7.1 with KOH (total [K] = 150). The bath (extracellular) solution was modified Tyrode's solution consisting of: 137 NaCl, 5.4 KCl, 1 $MgCl_2$, 0.33 NaH_2PO_4, 1.8 $CaCl_2$, 10 HEPES, 10 glucose, pH = 7.3 to which 1 mM H_2O_2 or 5 μM (CCCP) and 20 mM 2-deoxyglucose were added (pH 7.3). Exposure to 1 mM H_2O_2 caused transient prolongation of the action potential duration followed by marked shortening (without depolarization of the resting membrane potential) leading to inexcitability after an

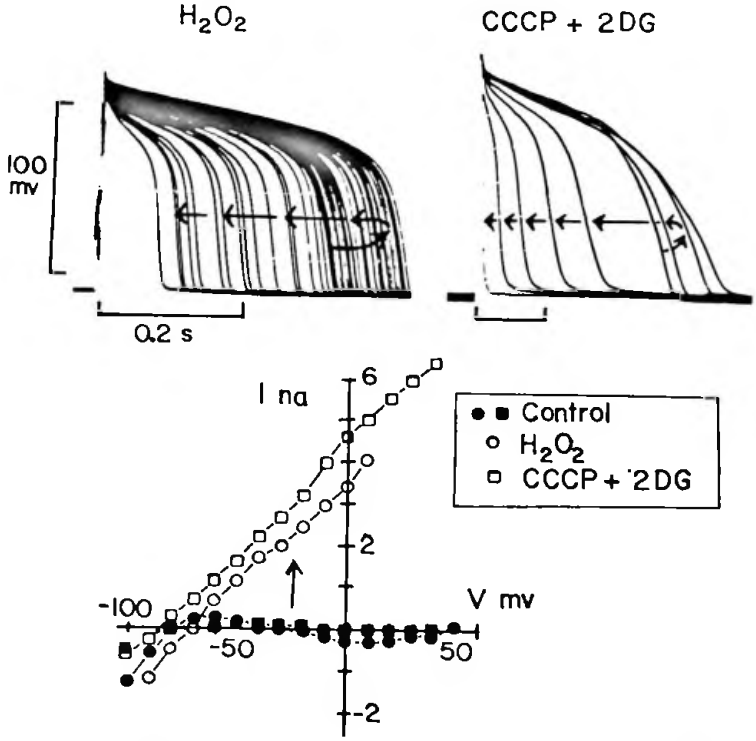

FIG 1: Effect of 1 mM H_2O_2 and 5 μM CCCP + 20 mM 2-deoxyglucose on the action potential (top) and I-V relations at the end of a 50 ms voltage clamp pulse (bottom) in guinea pig ventricular myocytes. The patch pipet contained 5 mM MgATP during exposure to H_2O_2, but no ATP during exposure to the metabolic inhibitors. Currents were not corrected for leakage.

average of 14 ± 6 min (n=9) (Fig 1, top). Voltage clamp results demonstrated that H_2O_2 activated a large outward K^+ current (Fig 1, bottom). The magnitude and voltage dependence of this current was similar to that induced by exposure of myocytes to metabolic inhibitors such CCCP (5 μM) and 2-deoxyglucose (20 mM). Previous studies have demonstrated that metabolic inhibitors activate an ATP sensitive K^+ channel in cardiac myocytes (Noma, Nature, 305:147, 1963). Unlike metabolic inhibitors, however, including 5 mM MgATP in the patch pipet dialysing the interior of the cell did not prevent or prolong the onset of activation of this K^+ current by H_2O_2. It is unlikely that activation of this K^+ current by H_2O_2 resulted from extensive nonspecific membrane destruction since, i) the myocytes had not shortened significantly and appeared structurally intact with clear striations remaining visible, ii) the effect of H_2O_2 was reversible sometimes if H_2O_2 was removed quickly after the onset of action potential shortening, iii) the Ca^{++} current remained intact at the onset of activation of the outward K^+ current, iv) exposure to the detergent duodecyl sulfate (400 μM) to induce nonspecific membrane damage did not produce similar results. These findings indicate that H_2O_2 either directly or via generation of hydroxyl free radicals activates a large abnormal K^+ current in heart. The H_2O_2-activated K^+ current has a similar magnitude and voltage dependence as the ATP sensitive K^+ current raising the possibility that H_2O_2 may interfere with the ability of ATP to suppress the ATP sensitive K^+ channel in heart. Single channel studies will be performed to test this hypothesis. H_2O_2 accumulation during myocardial ischemia may contribute to electrophysiological abnormalities causing arrhythmias by activation of this K^+ current.