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Augmentation and subsequent attenuation of Ca^{2+} current due to lipid peroxidation of the membrane caused by *t*-butyl hydroperoxide in the rabbit sinoatrial node

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Cellular electrophysiological effects of membrane lipid peroxidation by *t*-butyl hydroperoxide (TBH) were studied in the rabbit sinoatrial (SA) node. Superfusion for 1–5 min with 300 μM TBH caused an initial increase and subsequent decrease in the spontaneous firing frequency of the SA node. Voltage clamp experiments revealed that TBH initially enhanced but later blocked the Ca^{2+} current. Thus, membrane lipid peroxidation appears to accelerate and then suppress physiological automaticity by causing biphasic changes in the Ca^{2+} current.

Introduction Recent experimental evidence has linked the production of free radicals and resultant lipid peroxidation of the membrane to myocardial reperfusion injury and cardiac arrhythmias (Hess & Manson, 1984). Electrophysiological effects of free radicals on ventricular cells include reductions in the action potential amplitude and maximal rate of depolarization, loss of excitability and generation of abnormal automaticity. The action potential duration is shortened either with or without an initial prolongation, depending on the species and free radical generating systems (Nakaya *et al.*, 1987; Barrington *et al.*, 1988). However, ionic mechanisms underlying these action potential changes have not been elucidated to date. Hence, we studied the changes in the automaticity and ionic currents in the rabbit sinoatrial (SA) node caused by *t*-butyl hydroperoxide (TBH), an agent known to cause lipid peroxidation of the cardiac membrane (Nakaya *et al.*, 1987).

Methods Rabbits weighing 1.5–2.0 kg were anaesthetized by an intravenous injection of sodium pentobarbitone (30 mg kg^{-1}). The heart was quickly removed and the SA nodal tissue was dissected along the crista terminalis. A small strip of tissue, 3 mm long and 0.2 mm wide, was cut from the central portion of the node with a razor blade and ligated with fine silk sutures to obtain small nodal preparations with a final dimension of 0.2 × 0.2 × 0.1 mm. The preparations were superfused in a tissue bath at a flow rate of 5 ml min^{-1} with a modified Tyrode solution having the following composition in mM: NaCl 136.9, KCl 4.0, $CaCl_2$ 1.8, $MgCl_2$ 1.0, NaH_2PO_4 0.33 and glucose 5.5. The pH of the superfusate was adjusted to 7.4 with Na_2HPO_4 and the temperature was maintained at 37 ± 1°C. Partial oxygen pressure of the solution was maintained between 500 and 550 mmHg by 100% O_2 during the experiment. A 70% aqueous solution of TBH (Sigma) was added to the Tyrode solution at desired concentrations before individual experiments.

The membrane potential was recorded by use of conventional glass microelectrodes filled with 3 M KCl and having tip resistances of 20–50 MΩ. Voltage clamp experiments were conducted using double microelectrode techniques. The small preparation size allowed adequate spatial homogeneity. Values were expressed as mean ± s.d. Statistical analyses were made with Student's paired *t* test, and *P* values less than 0.05 were considered significant.

Results *Effects of TBH on the spontaneous action potential* Effects of lipid peroxidation by TBH (10–500 μM) on automaticity and action potential characteristics of the SA node were studied. TBH at 10 and 30 μM did not cause significant changes in the spontaneous action potential. The membrane action became evident at 100 μM, causing an initial increase with subsequent decrease in the spontaneous firing frequency. At 300 μM, these changes were exagger-

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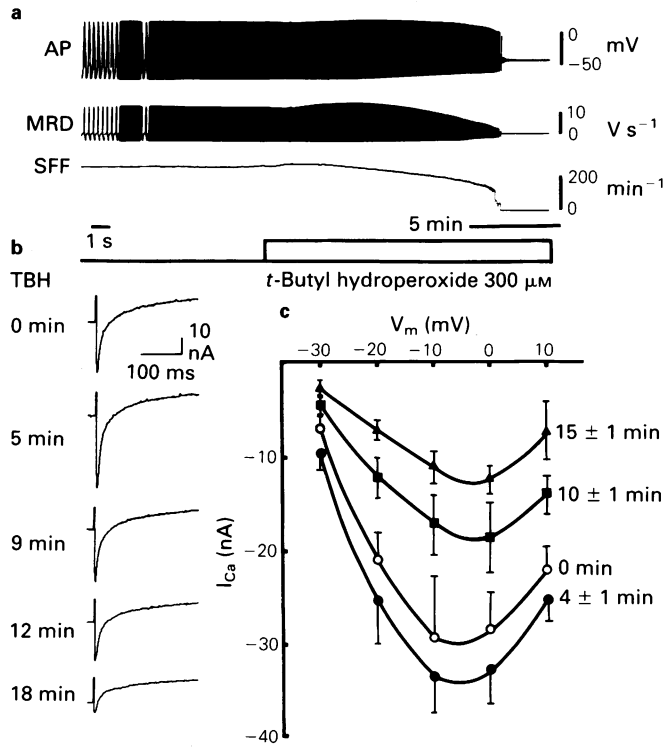

Figure 1 Effects of *t*-butyl hydroperoxide (TBH) on the sinoatrial node: (a) Positive and negative chronotropic actions of 300 μM TBH in a spontaneously beating small preparation. AP = action potential; MRD = maximal rate of depolarization; SFF = spontaneous firing frequency. In (b) is shown the effect of 300 μM TBH on the Ca^{2+} current (I_{Ca}) elicited on step depolarization from −40 to 0 mV. Note an initial increase and subsequent decrease in the current amplitude. (c) Time course of current-voltage relationships for I_{Ca} during 300 μM TBH superfusion ($n = 4$). I_{Ca} became maximal at 4 ± 1 min and then was progressively reduced. 0 min indicates the control curve. V_m = membrane potential.

ated (Figure 1a). In four such experiments, 4 ± 1 min superfusion with TBH initially increased the spontaneous firing frequency from 242 ± 25 to 261 ± 26 beats min^{-1} and the maximal rate of depolarization from 10.1 ± 2.2 to 15.9 ± 4.6 V s^{-1} ($P < 0.05$), but reduced them to 181 ± 23 beats min^{-1} and 8.1 ± 1.3 V s^{-1} ($P < 0.05$) after 8 ± 2 min superfusion. The action potential amplitude also showed a biphasic response with an initial, slight increase from 93.0 ± 0.8 to 94.5 ± 2.1 mV ($P > 0.1$) and a subsequent decrease to 85.9 ± 1.3 mV ($P < 0.05$). These inhibitory actions progressed with time. Finally, the spontaneous activity ceased after 15 ± 3 min with a resting potential of −46 ± 3 mV. At 500 μM, TBH caused similar changes although the time course was somewhat accelerated. Conversely, the action potential duration remained unchanged throughout the experiment. When the preparation was pretreated with butylated hydroxytoluene, a lipid peroxidation inhibitor, the effects of TBH on the spontaneous action potential were slowed, requiring more than 30 min to inhibit the automaticity completely. This observation would support the notion that TBH actions are mediated by membrane lipid peroxidation.

Effects of TBH on the Ca^{2+} current The action potential study suggested that the Ca^{2+} current (I_{Ca}) was initially augmented and subsequently attenuated by TBH-induced lipid peroxidation. Voltage clamp experiments were conducted to verify this assumption. Figure 1b shows representative I_{Ca} traces obtained on step depolarization to 0 mV from a holding potential of −40 mV. TBH transiently increased I_{Ca} at 5 min, and then progressively suppressed this current. The current-voltage relationships in Figure 1c summarize four such experiments. During control, the peak I_{Ca} recorded at −10 mV measured 29.3 ± 6.3 nA. After the addition of TBH, I_{Ca} was increased to 33.6 ± 6.0 nA at 4 ± 1 min

($P < 0.05$), and then decreased to 11.0 ± 1.1 nA at 15 ± 1 min ($P < 0.01$), when peak I_{Ca} was recorded at 0 mV. The delayed rectifying K^+ current was also decreased without an initial increase, whereas the hyperpolarization-activated inward current was increased throughout the experiment (not shown).

Discussion In the present study, membrane lipid peroxidation by TBH caused biphasic changes in SA node automaticity with initial acceleration and subsequent suppression. Voltage clamp experiments revealed that I_{Ca} played a major role in such biphasic changes because other pacemaker currents, the delayed rectifying K^+ current and hyperpolarization-activated inward current, were progressively decreased and increased, respectively. These results verify the contribution of I_{Ca} to pacemaker depolarization in the SA node as reported with the positive chronotropic action of adrenaline (Noma *et al.*, 1980).

The biphasic effects of TBH on I_{Ca} are consistent with recent reports that membrane lipid peroxidation initially increased and subsequently decreased (1) the developed tension of the guinea-pig papillary muscle (Nakaya *et al.*, 1987) and (2) the action potential duration in the canine ventricular muscle (Barrington *et al.*, 1988). The latter authors further noted induction of abnormal automaticity due to early and delayed afterdepolarizations when the action potential duration (and probably I_{Ca}) was increased. We also observed similar triggered activities initiated by early afterdepolarization when physiological SA node automaticity was accelerated by augmented I_{Ca}. Thus, both enhanced physiological automaticity and induction of abnormal automaticity in cardiac cells appear to be mediated by an excess transmembrane Ca^{2+} influx and/or an increased intracellular Ca^{2+} concentration.

The increase in the resting tension caused by TBH suggests an intracellular Ca^{2+} overloading (Nakaya *et al.*, 1987), probably resulting from a depression of Ca^{2+} uptake by the sarcoplasmic reticulum and mitochondria (Hess *et al.*, 1981) and an inhibition of Na,K-ATPase activity (Kramer *et al.*, 1984). Thus, a relatively small rise in intracellular Ca^{2+} concentration may enhance Ca^{2+} entry by promoting Ca^{2+} channel phosphorylation through Ca^{2+}-activated protein kinases (positive feedback regulation of I_{Ca}) (Marban & Tsien, 1982). During the course of lipid peroxidation, intracellular Ca^{2+} concentration may further increase through additional mechanisms such as the opening of Ca^{2+} channels (Lebedev *et al.*, 1982) and destruction of Ca^{2+} channel structure as evidenced by increased membrane fluidity and permeability (Meerson *et al.*, 1982). This, in turn, would attenuate I_{Ca} by (1) a Ca^{2+}-dependent I_{Ca} inactivation mechanism (negative feedback regulation of I_{Ca}) (Eckert *et al.*, 1981), (2) a reduced driving force for I_{Ca}, and (3) a decreased phosphorylation of Ca^{2+} channel due to the lack of adenosine 5'-triphosphate. Hence, lipid peroxidation by TBH appears to regulate I_{Ca} by increased intracellular Ca^{2+} concentrations due to biochemical impairment of Na^+-K^+ and Ca^{2+} pumps, and damaged lipid microenvironment of the Ca^{2+} channel and biomembranes.

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