EXPERIMENTAL STUDIES

Defibrillation Shocks Produce Different Effects on Purkinje Fibers and Ventricular Muscle: Implications for Successful Defibrillation, Refibrillation and Postshock Arrhythmia

HUAGUI G. LI, MB, PhD, DOUGLAS L. JONES, PhD, FACC, RAYMOND YEE, MD, FACC, GEORGE J. KLEIN, MD, FACC
London, Ontario, Canada

Objectives. To understand the mechanisms of postdefibrillation arrhythmias and failed defibrillation, we studied the cellular effects of high voltage shocks on different cardiomyocytes in the dog.

Background. The causes of postdefibrillation arrhythmias and unsuccessful defibrillation are not clear.

Methods. High voltage shocks with voltage differentials of 9.3 to 97.6 V/cm were delivered to isolated canine papillary muscles with attached Purkinje fibers. Transmembrane potentials were recorded simultaneously from the Purkinje fiber and the ventricular muscle using standard microelectrode techniques.

Results. After delivery of high voltage shocks, significant depolarization and rapid firing were observed in Purkinje fibers. The maximal rate of the rapid firing in the Purkinje fibers correlated with shock intensity (r = 0.69, p < 0.05). In contrast, in ventricular muscle, only slight depolarization and a transient refractory state were observed after the shock. The incidence of the refractory state was correlated with both the shock intensity and the rate of the rapid firing in the Purkinje fiber (r = 0.89 and 0.74, p < 0.01 and 0.05, respectively). Propranolol at a concentration sufficient for complete beta-blockade (1 mg/liter) did not change the tissue response to shocks but suppressed or abolished the shock-induced rapid firing of Purkinje fibers at a higher concentration (3 mg/liter). Blockade of the slow calcium channel with verapamil (400 µg/dl) did not alter the responsiveness of the preparation to shocks.

Conclusion. These results indicate that high voltage shocks induce different responses in Purkinje fibers and ventricular muscle. The shock-induced rapid firing in the Purkinje fiber may contribute to postshock arrhythmias and possibly refibrillation in some cases. The shock-induced transient refractory state in the ventricular muscle may prevent the ventricle from responding to the rapid firing and thus may decrease the incidence of postshock arrhythmias.

(J Am Coll Cardiol 1993;22:607-14)
Methods

Hearts were removed from 14 mongrel dogs under general anesthesia with 1.5% isoflurane or 1% halothane. Papillary muscles with attached free running Purkinje fibers were dissected from the right ventricle. A total of 27 useful preparations were derived and tested. The protocol was approved by the University Council on Animal Care of the University of Western Ontario and was carried out in accordance with the Guidelines to the Care and Use of Experimental Animals of the Canadian Council on Animal Care.

The preparation was mounted in the center of a custom-designed tissue bath (Fig. 1) and superfused with 95% oxygen, 5% carbon dioxide and balanced Tyrode solution at a flow rate of 12 ml/min and pH of 7.38 to 7.44. The composition of the Tyrode solution was as follows (mmol/liter): sodium chloride 129, sodium bicarbonate 20, sodium phosphate, monobasic 0.9, magnesium sulfate 0.5, potassium chloride 4.0, calcium chloride 2.5, glucose 5.5. The temperature of the perfusion solution was monitored with a thermistor located in the bath and kept at 37 ± 0.5°C using a heating pump.

The tissue bath was a square chamber with a pair of 1-cm² stainless steel electrode plates mounted on opposing walls and had a volume of 4 ml (Fig. 1). The distance between the electrode plates was 2 cm. The preparation was mounted so that the shock current direction was always approximately parallel to the long axis of the myocardial fibers at the recording sites because previous studies from this laboratory (10) and others (11,12) have shown different shock-induced effects due to shock current direction in relation to myocyte orientation. The long axes of the papillary muscle and the Purkinje fiber were assumed to be parallel to the long axis of the myocytes at the recording site (13). High voltage shocks were delivered to the tissue through the electrode plates using a battery-operated Medtronic 2376 defibrillator. The shock was a truncated monophasic pulse with a duration of 5 ms (with the shock trailing voltage having decreased by ≈65% of the leading edge voltage, referred to as a tilt of 65%) delivered from a 60-μF capacitor. Shocks with leading edge voltages of 50, 100, 150, 200, 250, 300 and 400 V (stored voltage on the defibrillator) were delivered to the bath. Shocks with a stored voltage <50 V were not used. Shocks at a stored voltage of 400 V were delivered to only three preparations; the remaining preparations received shocks at stored voltages from 50 to 300 V. The delivered voltage averaged 85.7 ± 2.0% of the stored voltage. The impedance of the shock circuit averaged 104.1 ± 4.6 Ω.

In six 5 × 10-mm preparations, an estimation of the voltage gradients produced by the shocks was obtained from the tissue during shock delivery. Two Teflon-coated (except at the tip) silver wire electrodes were placed directly on the surface of the tissue along the long axis of the myocardial fiber and the direction of the shock current flow. The vertical distance between these two electrodes was ≈5 mm. The electrode sites were marked and the exact vertical distance was directly measured on the completion of a series of shocks. The voltage difference between these two electrodes was displayed and measured on a storage oscilloscope (Tektronix 5111A) during shock delivery. Voltage differential was calculated as the voltage difference divided by the vertical distance between the electrodes and expressed as V/cm. A calibration curve was thus generated, and the intensity of subsequent shocks was estimated according to this calibration curve. Shocks with a stored voltage of 50 to 400 V produced voltage differentials from 9.3 ± 1.5 to 97.6 ± 1.7 V/cm (mean ± SEM). Shocks of varying intensities were delivered in random order. An interval ≥10 min was allowed between shocks until complete recovery from the previous shock.

Tissue was allowed ≥1 h to equilibrate in the bath. Transmembrane potentials were recorded simultaneously from the Purkinje fiber and ventricular muscle using two glass microelectrodes with tip resistance of 15 to 20 MΩ. To minimize the effect of poor perfusion on the papillary muscle, only the superficial one to two layers of cells were recorded. The distance between these two recording microelectrodes was kept at approximately 5 mm. To eliminate amplifier saturation, a silver-silver chloride reference electrode was positioned in the bath so that the recording reference electrode axis was always parallel to the defibrillation electrode plates.

Transmembrane potentials were processed using a WPI FD 223 Electrometer (World Precision Instruments, Inc.), displayed on Tektronix 5111A storage oscilloscope and recorded on a Gould 2400S ink paper recorder.

The artifacts caused by shocks were recorded before tissue impalement by placing the microelectrodes in the bath, just above the impalement sites, to ensure that the amplifier saturation had been minimized (Fig. 2).

The effects of shocks of different intensities were systematically studied in 12 preparations in a drug-free state. In preparations that did not have baseline spontaneous activity (n = 4), shocks were delivered without external stimulation.
For those with slow baseline spontaneous activity (n = 8), shocks were delivered during phase 4 of the membrane potential.

Tissue responses to shocks synchronized to the upstroke of the action potential were determined in six preparations that had slow baseline spontaneous activity (15 to 29 beats/min). These preparations were also tested during continuous external stimulation at 60 beats/min and twice diastolic threshold voltage. The stimuli were 2-ms rectangular pulses delivered by a WPI 1381 pulse generator (World Precision Instruments).

To evaluate the possible effect of shock-induced norepinephrine release from the nerve terminals on membrane potential, the responses of the tissue to shocks were compared before and during propranolol (1 mg/liter, n = 6) superfusion (14). The tissue response to shocks was determined again at a higher concentration of propranolol (3 mg/liter) in four of six preparations. The effect of verapamil (400 µg/dl, n = 3) was also tested to determine the role that the slow calcium channel might play in shock-induced membrane potential changes. Thirty minutes was allowed for tissue equilibration with the drugs (15).

Statistical analysis. Data are presented as the mean value ± SEM. The intensity for a given shock is expressed as the stored voltage on the defibrillator and the voltage differential derived from the calibration curve. An unpaired t test was used for comparison of mean values between groups with and without baseline automaticity and groups with synchronized and unsynchronized shocks. The maximal depolarization of Purkinje fibers and ventricular myocytes at different shock intensities was tested with one-way analysis of variance. The relation between shock intensity and recovery time of the rest (maximal diastolic) membrane potential, maximal rate and number of rapid firing action potentials at the onset and end of shock-induced rapid firing in Purkinje fibers was analyzed using linear regression analysis. Statistical analysis was performed only for shocks at stored voltages of 50 to 300 V, because shocks with stored voltage at 400 V were delivered to only three tissues. Unstable recordings, encountered in approximately 5% of the shock episodes, were excluded from the analysis.

Results

Of the 12 preparations systematically studied in the drug-free state, 8 (77%) had stable automaticity at 16 ± 3.4 beats/min (range 6 to 29) before shock delivery. No spontaneous activity was observed in the remaining four preparations.

Prolonged depolarization of the membrane potential. In both Purkinje fibers and ventricular muscle, shocks at a stored voltage of 50 V (9.3 V/cm) induced only a single action potential that rapidly repolarized to the preshock membrane potential level. Shocks at stored voltages of ≥100 V (21.7 V/cm) consistently caused prolonged depolarization of the membrane potentials (Fig. 3). The duration of this depolarization lasted from 2 ± 1.2 s at a shock intensity of 100 V (21.7 V/cm) to 20.8 ± 6.8 s at a shock intensity of 300 V (75 V/cm) in ventricular myocytes and from 5.9 ± 1.6 s at a shock intensity of 100 V (21.7 V/cm) to 48.5 ± 10.2 s at a shock intensity of 300 V (75.0 V/cm) in Purkinje fibers. The duration of the depolarization of the membrane potential correlated positively with the intensity of the shock for both Purkinje fibers and ventricular myocytes (r = 0.98 and 0.99, p < 0.01 and 0.01 respectively).

The magnitude of the shock-induced depolarization of the membrane potential (expressed as maximal depolarization), defined as the membrane potential at the end of the shock-induced action potential, correlated positively with shock intensity for both Purkinje fibers and ventricular myocytes (r = 0.99 and 0.91, p < 0.01 and 0.05, respectively) and was much greater in magnitude in Purkinje fibers than in ventricular myocytes for a given intensity of the shock (Fig. 4).
Rapid firing in Purkinje fibers. Of the 24 shocks delivered at a stored voltage of 50 V (9.3 V/cm) to 12 preparations in the drug-free state, only 1 shock (4.2%) caused a repetitive response in a Purkinje fiber. Except for 3 (3.7%) [1 at a stored voltage of 100 V and 2 at a stored voltage of 150 V] of the 82 shocks at stored voltages of 100 to 400 V (21.7 to 97.6 V/cm), all shocks induced rapid firing action potentials in Purkinje fibers (Fig. 3). These rapid firing action potentials were lowest in amplitude and most rapid immediately after the shock. With progressively increasing amplitude, these fast activities gradually slowed down and usually terminated spontaneously as the membrane potentials approached preshock levels. The maximal firing rate calculated using the shortest interval between the peaks of the rapid firing action potentials ranged from 60 to 300 beats/min. The maximal rate and number of shock-induced rapid firing potentials were both dependent on shock intensity (r = 0.69 and 0.82, p < 0.05 and 0.01, respectively) (Table 1).

Rapid firing action potentials were consistently induced by shocks in preparations with or without baseline automaticity (Fig. 3 and 5). There was no significant difference in the maximal rate of the rapid firing action potentials between the preparations with and without baseline automaticity (at a stored voltage of 300 V [75 V/cm]: 148 ± 17 vs. 145 ± 18 beats/min, p = NS).

In preparations with baseline automaticity before the shock, rapid firing action potentials were induced by shocks either unsynchronized (Fig. 5) or synchronized (Fig. 6) with the upstroke of the action potential. The maximal rate of the rapid firing action potentials in the Purkinje fiber was not significantly different between the synchronized and unsynchronized shocks (at a stored voltage of 300 V [75 V/cm]: 143 ± 5 vs. 157 ± 5 beats/min, p = NS).

The rapid firing started at various membrane potentials for different shock intensities but usually terminated when the membrane potential approached preshock levels (Table 2). Membrane potentials at the onset and end of rapid firing action potentials correlated positively to shock intensity (r = 0.94 and 0.93, p < 0.01 and 0.01, respectively). In 4 (33%) of the 12 preparations, low amplitude oscillations of the mem-
Figure 6. Shock-induced rapid firing in the Purkinje fiber (upper channel) and transient refractory state in ventricular muscle (lower channel). The preparation had an automaticity at 29 beats/min, and a 1:1 relation between the action potentials can be seen before the shock. After the shock (75.0 V/cm, arrow) that was delivered during the upstroke of the spontaneous action potential, significant depolarization and rapid firing were observed in the Purkinje fiber (A). An increase in diastolic depolarization slope can be seen during the rapid firing. The automaticity returned to the preshock rate approximately 50 s after the shock (B). In contrast, only a slight depolarization and a transient refractory state were observed in ventricular muscle. A and B are continuous recordings. Part of the peak action potential in the Purkinje fiber was missing because of the inadequate frequency response of the paper recorder.

brane potential were also observed at the termination of the rapid firing action potentials (Fig. 7).

Transient refractory state of the ventricular muscle. Before shock delivery, 1:1 conduction of impulses from the Purkinje fiber to ventricular muscle was confirmed in all preparations with automaticity. A pause in ventricular muscle (Fig. 3) occurred after shocks at stored voltages of 100 V (21.7 V/cm) to 400 V (97.6 V/cm), although rapid activities were consistently observed in Purkinje fibers. Thereafter, action potentials, usually at a slower rate than those of the Purkinje fibers, were recorded from ventricular muscle. In the six preparations tested with external stimulation at twice diastolic threshold, consistent 1:1 response of ventricular muscle to the stimulation was observed before the shock. However, no action potential could be induced by the external stimulation transiently after the shock (Fig. 8). This pause in ventricular muscle after the shock was termed the "transient refractory state." The incidence of the transient refractory state in ventricular muscle ranged from 50% with shocks at a stored voltage of 100 V (21.7 V/cm) to 89% with shocks at a stored voltage of 300 V (75 V/cm). The incidence of the transient refractory state correlated positively with shock intensity (r = 0.89, p < 0.01) and the maximal rate of rapid firing in the Purkinje fiber (r = 0.74, p < 0.05). The highest action potential firing rate of a Purkinje fiber after the shock was 300 beats/min, whereas the highest action potential firing rate recorded from a ventricular muscle was only 150 beats/min.

Effects of propranolol and verapamil. Beta-adrenergic receptor blockade with propranolol at a concentration of 1.0 mg/liter did not significantly change the maximal rate of rapid firing in Purkinje fibers after defibrillation shocks (at a stored voltage of 300 V [75 V/cm]: from 141 ± 33 to 141 ± 37 beats/min, p = NS). However, in the four preparations tested with a propranolol concentration of 3.0 mg/liter, the shock-induced rapid firing action potentials in Purkinje fibers had a decreased maximal rate in two preparations and were completely abolished in the other two preparations. No

Table 2. Membrane Potentials (mV) at the Onset and End of Shock-Induced Rapid Firing in Purkinje Fibers*

<table>
<thead>
<tr>
<th>Stored Voltage (V)</th>
<th>100</th>
<th>150</th>
<th>200</th>
<th>250</th>
<th>300</th>
</tr>
</thead>
<tbody>
<tr>
<td>Voltage differential (V/cm)</td>
<td>21.7</td>
<td>34.0</td>
<td>47.6</td>
<td>60.5</td>
<td>75.9</td>
</tr>
<tr>
<td>Onset potential (n = 12)</td>
<td>-57.6 ± 6.6</td>
<td>-51.3 ± 10.3</td>
<td>-32.8 ± 7.2</td>
<td>-25.3 ± 3.4</td>
<td>-27.0 ± 8.6</td>
</tr>
<tr>
<td>End potential* (n = 4)</td>
<td>-72.4 ± 4.6</td>
<td>-70.1 ± 3.2</td>
<td>-66.9 ± 3.5</td>
<td>-66.4 ± 7.2</td>
<td>-56.7 ± 8.5</td>
</tr>
</tbody>
</table>

*Membrane potentials at the end of the rapid firing could be accurately determined only in the four preparations that did not have baseline automaticity because the transition between rapid firing and the recovery of baseline automatic activity was gradual in some cases and thus the end of the shock-induced firing could not be reliably determined. End potential and onset potential = membrane potentials at the end and onset, respectively, of rapid firing action potentials.
Depolarization and rapid firing in Purkinje fibers. Aronson and Cranefield (17) have described prolonged depolarization of the membrane potential caused by strong stimulation in Purkinje fibers. In their study, although low amplitude potentials were observed, the intrinsic activity of the Purkinje fiber after the strong stimulation was not clear because the preparation was continuously stimulated. The present study demonstrated that over a wide range of shock intensities, high voltage shocks frequently induced rapid firing action potentials in Purkinje fibers. The maximal rate and number of the shock-induced rapid firing action potentials in the Purkinje fiber increased as the shock intensity was increased. It is likely that in the intact heart, this shock-induced rapid firing in the Purkinje fiber may be a source of postshock arrhythmias. Because rapid firing showed a dependence on shock intensity, it may also be the cause of the higher incidence of postshock arrhythmias with energy much higher than the defibrillation threshold (4). Whether this shock-induced rapid firing in the Purkinje fiber also contributes to the unsuccessful defibrillation of some shocks with energy much higher than the defibrillation threshold needs to be investigated. Conversely, this shock-induced rapid firing in the Purkinje fiber does not seem to be the mechanism of unsuccessful defibrillation during subthreshold defibrillation shock delivery because the incidence and rate of this shock-induced rapid firing increased as the shock intensity was increased. The reinitiation of ventricular fibrillation after subthreshold shocks is found to originate from areas with the lowest voltage gradient.

Refractoriness in ventricular muscle. A transient refractory state occurred in ventricular muscle during the rapid firing activity in the Purkinje fiber after the high voltage shock. This transient refractory state prevented ventricular muscle from following the fast firing rate of the shock-induced rapid activity in the Purkinje fiber. It may be expected that in the in vivo situation, this transient refractory state of ventricular muscle may protect the heart from the rapid tachysystolic impulses. Thus, despite the shock-induced rapid firing action potentials in the Purkinje fiber, successful defibrillation may still be achieved as a result of the refractory state of the ventricular myocyte. In contrast, it is conceivable that this rapid firing in the Purkinje fiber could be conducted through the Purkinje system into a suitable region where conduction block into the myocardium was not achieved, such as the region of low voltage gradient.

The mechanism of shock-induced rapid firing action potentials remains to be determined. All rapid firings occurred during membrane depolarization and usually ceased as the membrane potential approached the preshock diastolic potential. It is possible that the rapid firing may be related to the magnitude of the membrane depolarization. Shock-induced depolarization may bring the membrane potential of the automatic tissue closer to its firing threshold and thus favor the increase in automaticity. This is supported by the finding that an increase in diastolic depolarization was often observed during rapid firing. However, the different mem-
brane potential levels at the onset and end of rapid firing after various shock intensities suggest that the magnitude of membrane depolarization may not be critical to the development of rapid firing. In addition, membrane potential oscillations, similar to delayed afterdepolarization potentials, were observed in some preparations after termination of shock-induced rapid firing. However, a mechanism similar to delayed afterdepolarization seems unlikely to be involved in the shock-induced rapid firing because verapamil, which has been shown to inhibit delayed afterdepolarization (17), did not alter the shock-induced rapid firing in this study. Finally, defibrillation shocks cause a delay in repolarization, which may facilitate the development of early afterdepolarization, which in turn may result in triggered activity. In fact, intracellular injection of depolarization current has been shown to induce triggered activity as a result of early afterdepolarizations in canine Purkinje fibers (18).

Effects of verapamil and propranolol. The specific ionic channels responsible for shock-induced rapid firing is unknown. The fast sodium channel seems unlikely to be the major contributor to this rapid activity. As can be seen in Table 2, most of the rapid firing action potentials occurred at membrane potential levels at which the fast sodium channel is known to be inactivated (19). There is also no direct evidence to support the role of the transsarcolemmal voltage-dependent calcium channel in the generation of shock-induced rapid firing because verapamil at a high concentration did not significantly modify the rapid firing induced by the defibrillation shock. However, oscillatory potentials resulting from intracellular calcium alteration cannot be excluded. High voltage shocks have been demonstrated to cause microlesions of the myocardial sarcolemma and nonspecifically increase the permeability of cellular membrane (20-22). The electrochemical gradient of calcium facilitates its influx in the presence of increased membrane permeability and may result in intracellular calcium overload. This influx of calcium is likely to be nonspecific and would not be expected to be blocked by a calcium channel blocker such as verapamil. The increased intracellular calcium concentration could trigger an oscillatory release of calcium from sarcoplasmic reticulum and facilitate the development of oscillatory potentials.

Because propranolol at a concentration sufficient for complete beta-adrenergic receptor blockade (14) did not alter the shock-induced rapid firing, beta-receptor activation resulting from the release of norepinephrine from endogenous nerve terminals is unlikely to be responsible for the rapid firing in the Purkinje fiber after defibrillation shocks. The suppression or elimination of shock-induced rapid firing by the high concentration of propranolol in this study may be the result of a membrane-stabilizing effect (14).

It has been shown that defibrillation shocks prolong the refractory period in the isolated cardiomyocyte (8) and ventricle (9,23) of the intact heart. We (24) have also demonstrated that a refractory state occurred after high voltage shocks in a guinea pig papillary muscle preparation. The present study demonstrated that a transient refractory state prevented ventricular muscles from responding to the rapid firing impulses in the Purkinje fibers. A transient conduction block between the Purkinje fiber and ventricular muscle also may be manifested as a pause after the shock. Indeed, a transient conduction disturbance has been observed in high voltage gradient areas after defibrillation shocks during mapping of the intact heart (25). However, the failure of the constant external stimulation to induce action potentials after the shock indicates that the occurrence of the transient refractoriness is an important cellular component of the pause in ventricular muscle, which is not dependent on the Purkinje-myocyte junction conduction.

The mechanism underlying the different responses of Purkinje fibers and ventricular muscle cells to defibrillation shocks is not well understood. It may, however, be related to the different ionic basis of membrane potentials (25).

Limitations of the study. Shock intensity cannot be expressed accurately as delivered energy (joules) in the present study because only a small part of the total delivered energy passed through the tissue, with the majority of the energy shunted through the lower impedance Tyrode solution. This made the precise measurement of shock energy received by the tissue impossible. A rough estimate of voltage gradient was made by measuring the "voltage differential" between two electrodes on the surface of the tissue. Shock intensities estimated by this method were within the range of voltage gradients mapped during defibrillation of the intact heart (1,2), although generally higher than those in the "low voltage gradient areas." Although the measurement of voltage differential provided an estimate of the shock intensity, the voltage differential may not be equivalent to voltage gradient measured in the intact heart. Because of the small size of the tissue preparation, only two points were used for the measurement.

The defibrillation shock was purposely delivered to the nonfibrillating tissue preparation in this study. The response of myocytes in a fibrillating heart may be different. Thus, the relevance of the present study to the intact fibrillating heart deserves further study. Nevertheless, the fact that the response of the tissue preparation to shocks was found to be unrelated to the phase of the membrane potential or the state of automatice suggests that a defibrillation shock may produce similar effects in a fibrillating heart.

Conclusions. The present study demonstrated that high voltage shocks over a wide range of intensities induced rapid firing action potentials in Purkinje fibers. In contrast, a transient refractory state occurred after the shock in ventricular muscle. The shock-induced rapid firing in the Purkinje fiber may contribute to postshock arrhythmias and possibly re fibrillation, whereas the shock-induced transient refractory state in ventricular muscle may protect the ventricle from responding to rapid activation and thus may be the mechanism of successful defibrillation.
References


17. Aronson RS, Cronefield PF. The effect of resting potential on the electrical activity of canine cardiac Purkinje fibres exposed to Na-free solution or to ouabain. Pflugers Arch 1974;347:101-16.


