Effects of propafenone on the median frequency of ventricular fibrillation in Langendorff perfused guinea-pig hearts

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Abstract

Objective: The aim was to investigate the antifibrillatory effects of two concentrations of propafenone by means of signal analysis of epicardial ECG recordings in isolated, Langendorff-perfused guinea-pig hearts. Methods: Isolated Langendorff-perfused guinea-pig hearts were used as a model for sustained ventricular fibrillation (VF) during reperfusion after global ischaemia. ECG signals were recorded for the first 20 min of reperfusion. The recording was divided into episodes of 1 s and the median frequency (FM) of the dynamic power spectrum was computed for each episode. Cardiac electrical activity was monitored for an additional 10 min. Additionally steady state conditions (i.e. constant FM values for the remaining observation period) were analysed and the effects of 0.1 μM and 1.0 μM propafenone added at reperfusion on the FM were examined. Results: After initial irregularities, FM remained on a high steady state level in the control group. The addition of propafenone altered the steady state value of FM in a dose-dependent and significant manner but had no effects on the time until steady state was reached. During reperfusion without propafenone, 1 out of 6 hearts spontaneously converted to a stable sinus rhythm. Reperfusion with 0.1 μM propafenone caused spontaneous conversion to stable sinus rhythm in 1 out of 6 hearts and intermittent periods of sinus rhythms in 2 additional hearts. During the first 30 min of reperfusion with 1.0 μM propafenone, 5 out of 6 hearts spontaneously converted to stable sinus rhythm. The sixth heart showed repeated switching between VF and periods of non-sustained sinus rhythm. Conclusion: Propafenone caused a dose-dependent decrease of FM at steady state conditions. The rate of spontaneous termination of VF appeared to be dose dependent and the stability of the sinus rhythm was correlated inversely with the FM immediately before spontaneous defibrillation. Therefore, in this model the FM value prior to spontaneous termination of VF may be useful in the estimation of defibrillation success.

Keywords: Ventricular fibrillation; Reperfusion; Propafenone; Arrhythmias; Spectral analysis; Guinea-pig, heart

1. Introduction

The rate of successful cardiopulmonary resuscitation (CPR) of ventricular fibrillation (VF) depends on the duration of VF preceding CPR. Drug therapy has limited value in the treatment of acute VF because of the inherent time delay to the onset of action. Yakaitis and co-workers investigated the rate of successful CPR in a dog model of VF [1]. The success rate was 100% when CPR was applied 1 min after the onset of VF. The rate dropped to 90% after 3 min and to 30% after 5 min of VF. The duration of sustained VF is reflected by the waveform of the ECG signal and can be estimated by the amplitude of the VF waveform [2]. The electrical activity of the heart as seen in the ECG signal normally changes from a coarse waveform with high-voltage amplitudes to a fine waveform with lower amplitudes. Because of intersubject variability and because of the dependence on the position of the ECG lead, the amplitude of the ECG signal has not proved a useful tool in choosing the most effective treatment [3]. Dynamic spectral characteristics of the ECG signal, such as the median frequency of the power spectrum (FM), appear to be more sensitive because FM describes the frequency distribution of the ECG power spectrum by a single numeric value [4].

The time course of several spectral characteristics has been investigated extensively in the context of self-
terminating ventricular tachyarrhythmias [5,6]. Self-termination of ventricular tachyarrhythmias has been reported in the case of polymorphic ventricular tachycardia, torsade de points and VF [6–8]. Clayton and co-workers were able to demonstrate that at the onset of VF the dominant frequency of both sustained and self-terminating VF increased significantly and then remained constant throughout the VF period [5]. In contrast to the onset of VF, the dominant frequency was decreased immediately before self-termination of VF.

The therapeutic effect of antiarrhythmic drugs in VF has received much controversial discussion. It is well accepted that class I antiarrhythmic agents, according to the Vaughan-Williams classification, exhibit a certain antifibrillatory efficacy [9]. Carlisle and co-workers were able to show that the class Ib antiarrhythmic agent lidocaine significantly reduces the dominant frequency in a canine model of sustained VF [10]. In addition, the antifibrillatory efficacy of the class Ic antiarrhythmic agent propafenone was demonstrated by Stefanelli and co-workers using isolated perfused rat hearts [11]. These results support the hypothesis of chemical ventricular defibrillation originally formulated by Sanna and Arcidiacono and later refined by Curtis [12,13]. The present study was designed to investigate the time-course of the spectral characteristics in an in-vitro model for ventricular fibrillation during reperfusion. In addition, the dose-dependent effects of the class Ic antiarrhythmic agent propafenone on the time course of FM were investigated. Thus, this study may lead to a better understanding of the mechanisms of self-terminating ventricular fibrillation and of the antifibrillatory efficacy of propafenone.

2. Methods

2.1. Study protocol

Guinea-pigs of both sexes, approximately 2–4 months of age, weighing 250–350 g, and fed ad libitum, were used. The investigation conforms with the Guide for the care and use of laboratory animals published by the US National Institutes of Health (NIH publication no. 85-23, revised 1985). The animals were injected intraperitoneally with 250 IU of heparin sulphate 30 min before being sacrificed by dislocation of the neck. The chest was quickly opened, the heart removed and attached to a modified Langendorff-type non-recirculating perfusion system (SST-ECG, A. Paar KG, Graz, Austria). Tyrode’s solution, saturated with a mixture of oxygen (95%) and carbon dioxide (5%) and warmed to 36°C was used as a perfusate (in mM: NaCl 132.1, KCl 2.7, CaCl2 2.5, MgCl2 1.15, NaHCO3 24.0, NaH2PO4 0.42, d-glucose 5.6). Using a tube pump the perfusion rate was kept constant at 6 ml/min. The equilibration period was 30 min.

ECG recordings were obtained with two bipolar silver-wire electrodes, placed on the epicardial surface of the spontaneously beating heart. Both pairs of electrodes were positioned in the atrioventricular valve plane. The first pair of electrodes was placed anterior and posterior to the interventricular septum. The second pair was placed in a right angle to the first pair of electrodes. The unfiltered ECG signals were amplified by a factor of 100, digitised at a sampling rate of 1 kHz using a 12-bit A/D converter (Labmaster TL 2-40, Scientific Solutions, Solon, Ohio, USA) and stored on an IBM-compatible PC-AT for further data processing. Ventricular pressure development was monitored using a pressure sensor in the perfusion line (A. Paar KG, Graz, Austria). Details of this high-resolution ECG recording technique have been published previously [14].

After the equilibration period control values of the atrioventricular conduction time, HIS bundle conduction time, intraventricular conduction time, repolarisation period and the heart rate were recorded. Then the hearts were exposed to global ischaemia by reducing the perfusion rate to 0.6 ml/min. Simultaneously the hearts were paced in order to override the spontaneous reduction of heart rate during ischaemia. Pacing was performed with a bipolar silver-wire electrode positioned on the left ventricle at twice the diastolic threshold using a constant current unit (BGS, A. Paar KG, Graz, Austria). The cycle length of 300 ms corresponds to a frequency of 200 beats per minute which is slightly lower than the frequency of the spontaneously beating heart. After a period of 9 to 11 min of global ischaemia, premature ventricular complexes occurred. These premature ventricular complexes were followed instantly by VF. After the onset of VF, electrical stimulation of the heart was stopped. VF was defined as an irregular electrical activity followed by a complete loss of ventricular pressure development. In order to ensure sustained VF the hearts were kept under ischaemic conditions for an additional 13 min after the onset of VF. Reperfusion was initiated by an increase of the perfusion rate to the pre-ischaemic rate of 6 ml/min. The ECG signal was continuously recorded for 20 min after reperfusion onset and transferred on-line to an IBM-compatible PC. Cardiac electrical activity was monitored for an additional 10 min increasing the total observation period to 30 min.

The experimental design comprised a control group (n = 6) and two treatment groups (n = 6 each). Propafenone was added to the perfusate during reperfusion in concentrations of 0.1 μM in the first group and 1.0 μM in the second group using a syringe pump.

2.2. Signal analysis

Digital signal processing of the data was performed on a Digital DEC 3000-400 workstation. A commercial software package (Matlab Version 4.2c, MathWorks Inc. Natick, MA, USA) was used for performing numeric calcula-
tions. The continuous recordings were divided into 1 s epochs (1000 samples). In a further step, the offset was removed by subtracting the mean of each epoch from each data point. In order to improve the resolution of the computed power spectrum for short data segments the epochs were zero padded on each side with an equal number of zeros to a total length of 4000 points, windowed with a Hanning function and transformed into the frequency domain via a discrete Fourier transformation [15]. Due to this procedure the spacing between adjacent frequency components of each spectrum was 0.25 Hz. The power spectrum of each epoch was calculated by dividing the squared amplitude of each frequency component by the data segment length. FM was then calculated in the power spectrum frequency range between 0.5 and 100 Hz as the sum of products of all frequencies and of all power spectrum components divided by the sum of all power spectrum components. Therefore, FM is the frequency coordinate of the centre of spectral mass of the power spectrum [4]. By applying this procedure to each data epoch the time course of FM was obtained. Steady state condition of FM was determined by calculating a moving average over 10 s.

In addition, FM preceding episodes of sinus rhythms were detected. Minimum requirements for a classification as sinus rhythm were defined as three consecutive sinus beats regardless of whether conversion was transient or sustained. Irregularities in sinus rhythm cycle length were allowed. Conversion to sinus rhythm was classified as sustained when the sinus rhythm was stable until the end of the observation period.

2.3. Statistical analysis

All statistical calculations were performed on an IBM-compatible PC-AT computer using a standard statistical software package (SPSS for Windows 6.0.1, SPSS Inc., NY, USA). Data are expressed as means ± standard deviation. Differences of FM at steady state conditions and of FM preceding conversion to sinus rhythm were assessed by non-parametric analysis of variance according to Kruskal-Wallis. Whenever a significant difference was found, pairwise non-parametric group comparisons according to Wilcoxon-Mann-Whitney were performed. Results of pairwise comparisons were corrected for multiple comparisons according to the Bonferroni method. A 2-sided test design was used and a P value less than 0.05 was considered significant.

3. Results

3.1. Arrhythmias during reperfusion

The time course of mean FM during reperfusion VF for the control group is shown in Fig. 1A. FM first decreased from an initial value of 20.34 ± 6.80 Hz at the onset of reperfusion to an ebb of 15.22 ± 1.40 Hz after 21.0 ± 9.0 s. After this, FM increased, reaching a maximum value of 24.42 ± 2.38 Hz after 152.0 ± 48.6 s. Afterwards FM decreased and finally reached a steady state value of 21.25 ± 1.93 Hz after 516.0 ± 204.0 s. In 4 out of 6 experiments brief periods of sinus rhythm could be observed. Brief periods of sinus rhythm first appeared after 878.0 ± 225.7 s after reperfusion onset. The mean FM value preceding these spontaneous conversions in the 4 experiments was 22.68 ± 5.51 Hz. In one of these 4 experiments conversion to sinus rhythm was sustained after 737 s. FM preceding the sustained conversion to sinus rhythm was 18.3 Hz.
of reperfusion there was an increase of the mean FM value up to a maximum of 28.05 ± 5.38 Hz after 58.0 ± 17.0 s. This increase was not statistically significant and was followed by an exponential decrease of FM. A steady state value of 17.33 ± 2.50 Hz was reached 525.0 ± 288.0 s after onset of reperfusion. In 5 out of 6 experiments brief periods of sinus rhythm could be observed. Brief periods of sinus rhythm first appeared 656.6 ± 206.17 s after the start of reperfusion with an FM value of 18.02 ± 5.78 Hz preceding these periods. The FM values were not significantly different from controls. In one of these 5 experiments conversion to sinus rhythm was sustained after 1184 s. After FM reached steady state conditions, the rhythm patterns of another 2 out of these 5 hearts repeatedly showed intermittent periods of sinus rhythm throughout the remaining observation period.

The effects of 1.0 μM propafenone on the time course of the mean FM during reperfusion is shown in Fig. 1C. Initially, the mean FM slightly increased, reaching a maximum value of 28.32 ± 3.14 Hz at 119 ± 52.0 s. After this FM decreased to a steady state value of 12.90 ± 1.84 Hz. Steady state conditions were reached after 578.0 ± 198.0 s of reperfusion. All hearts showed brief episodes of sinus rhythm that first occurred after 705.33 ± 193.38 s. Sinus rhythms occurred significantly earlier with 1.0 μM propafenone than in the control hearts (P < 0.05) and 0.1 μM propafenone (P < 0.05). FM preceding the first occurrences of sinus rhythm was 13.02 ± 1.92 Hz and was significantly different from control value (P < 0.05). In 5 out of 6 hearts conversion to sinus rhythm was sustained after 722.00 ± 193.40 s with FM preceding conversion of 12.10 ± 2.30 Hz. The remaining heart repeatedly switched between VF and periods of non-sustained sinus rhythm.

3.2. FM at steady state

Time until steady state of FM was 516.7 ± 204.1 s for the control group, 525.0 ± 288.3 s for 0.1 μM propafenone and 578.3 ± 198.6 s for 1.0 μM propafenone. The tendency to prolonged intervals with increased dosages of propafenone was not statistically significant. Fig. 2 gives the FM values at steady state condition. FM is significantly reduced after addition of either 0.1 μM propafenone (P < 0.05) or 1.0 μM propafenone (P < 0.01). In addition, FM at steady state was significantly more reduced by 1.0 μM than by 0.1 μM propafenone (P < 0.05).

FM preceding non-sustained episodes of sinus rhythms was 18.13 ± 6.21 Hz (n = 74) and 13.19 ± 2.97 Hz (n = 7) preceding sustained conversion to sinus rhythm (P < 0.05).

4. Discussion

4.1. Model characteristics

VF is a fatal arrhythmia associated with a complete loss of cardiac pump function. American Heart Association guidelines for the treatment of VF dictate immediate termination by a direct current electrical shock [16]. Although defibrillation is the most effective treatment of VF there are some major disadvantages. Delivery of multiple countershocks is associated with a steady increase of energy requirements for successful defibrillation which is due to the damage of cardiac tissue [17,18]. Therefore, defibrillation should be attempted when minimum energy is required for successful defibrillation. A parameter, derived from the body surface ECG signal, indicating whether high or low energy is necessary for successful defibrillation would be helpful in this case because of the reduction of ineffective shocks and time to successful defibrillation.

The amplitude of the ECG signal during sustained VF decreases over time and, therefore, reflects the down time (DT), i.e., the time between onset of VF and initiation of resuscitation [2]. Because of major intersubject and interlead variations a reliable estimate of DT based on the amplitude of the body surface ECG signal is difficult to achieve. Dzwonczyk and co-workers describe a model for the estimation of DT using dynamic changes in the frequency distribution of VF [4,19]. The authors tracked FM of the ECG power spectrum and found a characteristic time course where mean FM value decreased immediately after onset of VF and increased later. After reaching a peak value FM decreased for the remaining observation period.

Martin and co-workers found that the time course of mean FM was highly reproducible although species specific [20]. The findings of the present study are consistent with these results. Immediately after onset of reperfusion we observed the same oscillations of the mean FM value that have been described by other authors [21]. A slight decrease and increase of FM has been reported by Dzwon-
czyk and co-workers reported a more complex oscillation pattern immediately after onset of VF using human data [4,19,20]. The initial time course of mean FM during VF in isolated Langendorff-perfused guinea-pig hearts best resembles the initial time course of FM recorded in a porcine model because our model showed the same initial decrease and increase of FM in the early phase of reperfusion [20].

In contrast to the porcine model of Martin and co-workers, FM remained constant in our model for the remaining observation period. A decrease of mean FM could not be observed in our model. We attribute this difference to the constant rate of coronary perfusion because in Langendorff-perfused hearts coronary flow is independent of the cardiac pump function [4,21]. There is evidence for a direct relationship between the FM value and the coronary blood flow [21]. Furthermore, in a canine model the dominant frequency of the ECG power spectrum was maintained when coronary perfusion was improved by cardiopulmonary bypass or by administration of calcium channel antagonist [22,23].

4.2. Effects of propafenone

In the control experiments FM reached steady state conditions after approximately 9 min. This parameter was not influenced by the presence of either 0.1 μM or 1.0 μM propafenone. In contrast to this, the time course of FM displayed a dose-dependent decrease after the initial oscillating period when 0.1 μM or 1.0 μM propafenone was added to the perfusion solution. These effects are due to the antiarrhythmic agent. Predominantly propafenone is a blocker of the fast sodium channel in the myocardium [11]. Because of the slow binding kinetics, propafenone has been classified as class Ic according to Vaughan-Williams [9]. Blocking of the fast sodium channel by propafenone reduces the upstroke velocity of the action potential of the cardiac myocyte and, therefore, reduces conduction velocity [24].

This effect is most likely to be responsible for the decrease of FM during reperfusion and the defibrillatory effects of propafenone in this model. Clayton and co-workers analysed the dominant frequency of the ECG power spectrum of transient and sustained VF [5,25]. During the onset phase of both sustained and transient VF the amplitude of the dominant frequency of the power spectrum increased and the dominant frequency shifted towards higher values, indicating an acceleration of myocardial activation. However, immediately before spontaneous termination of VF the amplitude of the dominant frequency decreased and the dominant frequency shifted towards lower values again. The decrease of the dominant frequency of the ECG power spectrum indicates a slowing down of myocardial activation preceding termination of VF. These results are in good accordance with the data of the present study. Administration of propafenone resulted in a decrease of mean FM, indicating reduced myocardial activation. This effect was more pronounced in the presence of 1.0 μM propafenone, thus indicating a dose-dependent effect. In addition, conversion to sustained sinus rhythm was more frequent after administration of 1.0 μM propafenone.

Additionally, the pharmacological profile of racemic propafenone exhibits moderate antagonistic effects on the L-type calcium channel and weak β-adrenoceptor blocking effects [11,24]. However, calcium channel antagonists have been found to stabilise both power spectrum characteristics and pre-existing VF and, therefore, a significant contribution of the calcium channel antagonistic effect is not likely in this in-vitro model [22]. Additionally, Langendorff-perfused hearts are totally denervated and endogenous catecholamine stores should have been emptied by the time of reperfusion onset. These facts rule out a possible contribution of β-adrenoceptor blocking effects in termination of VF in this experimental setting.

The main limitation of the present study is the fact that the exact mechanism of the induced arrhythmia is not known. Additionally, VF induced in a Langendorff-perfused heart preparation differs from VF occurring in the clinical setting. Nevertheless, our results are in good agreement with the power spectrum characteristics (FM) of human ECG recordings of VF [20,25].

4.3. Conclusion

In our model for sustained VF, propafenone had a clear antifibrillatory effect. The findings of this study indicate that a slowing of the myocardial conduction velocity is the main cause of the termination of VF by propafenone in this model. The steady state value of FM was inversely related to the conversion rate to sustained sinus rhythm and may be useful in defining optimum conditions for defibrillation.

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References

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