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Cellular Electrophysiological Changes in Rats with Heart Failure and Ventricular Arrhythmias – *in Vitro-in Vivo* Correlations

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Aim of the study: During heart failure (HF), mechanisms of ventricular arrhythmias are poorly understood. The clinical relevance of elementary mechanisms suggested by in vitro experiments has to be questioned, since in vivo correlations are often lacking. We compared action potential (AP) characteristics, occurrence of automaticity, and triggered activity with in vivo arrhythmic, morphologic, and haemodynamic data in post myocardial infarction (PMI) rats.

Methods and results: Telemetric ECG, morphologic, and haemodynamic data were obtained from 16 live PMI and 13 sham rats, at one and five months after surgery. Action potentials (AP) were studied on the papillary muscle in different solutions, and at different pacing rates. Spontaneous ventricular premature beats (VPBs) were frequent in PMI and were bradycardia-dependent. Abnormal automaticity was the most common in vitro observed mechanism. Time after infarction was related to EAD/DAD in vitro distribution but not with in vivo data. HF induced major alterations of filling pressures and of AP characteristics. AP duration was strongly correlated with the size of infarction but not with the arrhythmic density, in contrast with AP amplitude, resting potential, and V_{max}.

Conclusion: In PMI rats, abnormal automaticity was the major mechanism of in vitro recorded arrhythmias, consistently with the bradycardia-dependence of in vivo observed VPBs. Delay after infarction influenced only EAD/DAD proportion. In this model, AP prolongation could be a simple marker of HF not involved in arrhythmogenic mechanisms. Heterogeneity of the pathobiology of arrhythmias in HF warrants further in vitro-in vivo correlations. **J Clin Basic Cardiol 2005; 8: 23–8.**

Key words: experimental heart failure, ventricular arrhythmias, action potential duration, automaticity, DAD, EAD.

Although the incidence of most cardiovascular diseases has decreased during the past decades, the incidence and prevalence of congestive chronic heart failure has increased in the Western world [1]. Mortality remains very high and up to fifty percent of the deaths are considered unexpected [2]. The mechanisms of sudden death in patients with heart failure seem highly heterogeneous, even when the most frequent cause is ventricular tachyarrhythmia. These mechanisms however have to be more accurately investigated [3]. Among them, the specific weight of the alteration of different electrophysiological parameters by cardiac and peripheral responses is hardly known.

Clinical observations alone are unable to address these important issues. On the other hand, elementary mechanisms identified during *in vitro* experimental studies need the demonstration of their *in vivo* relevance. For example, prolongation of the action potential (AP) is a characteristic of cells and isolated myocardium from ventricles of animals with heart failure, independent of its etiology [4–6]. Recordings however made at unphysiologically slow rates and without physiological mechanical load conditions have questioned the clinical relevance of this observation [7]. Increased dispersion of AP duration has been described and has raised reentry as a major potential cause of ventricular arrhythmias. Moreover, other mechanisms have also to be considered. Abnormal automaticity and triggered activity – favoured by EADs or by DADs – have been shown in experimental conditions. However, the relationships between *in vivo* recorded ventricular arrhythmias and *in vitro* observations obtained on isolated cells or tissular preparations remain unclear. Finally, most of the studies have been performed in animals with cardiac hypertrophy but not necessarily with real heart failure. Nevertheless, mechanisms involved in ventricular arrhythmias might be completely different according to the stage of the disease [8–9].

The post myocardial infarction (PMI) rat is one of the most popular and most documented models of heart failure [10]. In a pioneer study, Qin et al. evaluated some electrophysiological parameters and arrhythmogenic mechanisms in PMI rats [11]. However, these observations have only been performed during the very early phase of ventricular remodelling, a process known to be time-dependent. Reentry, triggered activity, automaticity were suggested by the authors, but again relationships with *in vivo* spontaneous arrhythmias have not been analysed.

As we have demonstrated that spontaneous and chronic ventricular arrhythmias could be recorded by Holter monitoring in this model [12–13], the following points were addressed in our study:

– AP characteristics and their evolution with time were examined in the rat ventricular papillary muscle.
– Occurrence of after potentials, triggered activities, and automaticity in different biochemical conditions were assessed, one and five months after coronary artery ligation.
– Finally, we compared these results with arrhythmias recorded by telemetry in the same awake animals and with morphologic and haemodynamic parameters of failing hearts.

Population, Material, and Methods

A population of 35 rats surviving more than 48 hours after surgical procedure were divided into 4 groups. From the 22 rats undergoing coronary artery ligation, 7 (group PMI1) were studied at 1 month after surgery for telemetric measurements, haemodynamic analysis, infarction size scoring, and cellular electrophysiological experiments. Nine were studied at 5 months (group PMI5). Six died between the first

and the fifth month. From the 13 rats undergoing only a sham operation (same procedure except for the ligation), 6 were analysed at 1 month (group CTRL1) and 7 at 5 months (group CTRL5).

Surgical Procedure

Induction of HF was performed in male Wistar rats weighing 300–350 g. The animals were anaesthetised with a mixture of ketamine 150 mg/kg and xylasine 0.05 mg/kg. They were directly intubated and ventilated with room air at a rate of 60 ml/min. Body temperature was maintained at 37 °C using a thermostatically controlled blanket. The chest was opened by anterolateral thoracotomy and the pericardium was incised. The atria was removed and a 7-0 suture was passed around the origin of the major branch(es). Complete occlusion was evidenced by a regional color change of the myocardium and immediate dyskinesia. The lungs were re-expanded and before the skin was closed, air in the chest was removed. The rats were extubated and placed in an individual cage with *ad libitum* food, water, and a 12-hours day/ night cycle. Sham-operated rats underwent an identical surgical procedure except for coronary artery ligation.

Telemetric Measurements

Ventricular arrhythmias were recorded at 1 month (n = 6) and 5 months (n = 9) in rats undergoing coronary artery ligation. One day before the electrophysiological study, animals were anaesthetised again and a telemetric transmitter TL11M2-C50-PXT (DSI, St. Paul, Minnesota) was placed subcutaneously in an abdominal position for the recording of electrocardiogram (lead II) by telemetry. Measurements were undertaken in the animal room by means of a RLA bioreceiver (DSI, St. Paul, Minnesota) placed under the cage. The electrocardiogram was recorded for 30-second periods at regular intervals of 15 minutes for 24 hours (total recording 48 minutes). Arrhythmias were counted by the same operator. Results were extrapolated as a mean of ventricular premature beats (VPBs) per hour. Rats with more than 100 VPB/hours were considered "arrhythmic rats".

Heart Failure Evaluation

Left ventricular end-diastolic pressure (LVEDP) was measured in all rats immediately before the sacrifice. Animals were anaesthetised as described above. A polyethylene catheter connected to a STATHAM P23XL transducer and filled with isotonic saline solution was introduced into the right carotid then via the aorta into the left ventricle. LVEDP was considered increased if the measured value was more than 15 mmHg. After this haemodynamic measurement, the heart was quickly removed and placed in an oxygenated Tyrode's solution. A score of necrosis was determinated by macroscopic observation. The surface of the antero-lateral wall of the left ventricle was grossly divided into 6 parts: 3 paraseptal (apical, median, basal) parts and 3 lateral (apical, median, basal) parts. Depending on the number of infarcted segments, a score of 1 to 6 was applied. The papillary muscle was then excised from the left ventricle and immerged in the Tyrode's solution.

Electrophysiological Studies

Three different Tyrode's solutions were used: normal Tyrode's (Tyrode's 1), Tyrode's with low potassium (2 mM), low magnesium (0.25 mM), and caesium (10^{-6} > M) (Tyrode's 2) to favour EADs induction and Tyrode's with isoproterenol (10^{-5} > M) and ouabain (10^{-6} > M) (Tyrode's 3) to favour DADs induction. The composition of normal Tyrode's was: NaCl 135.7 mM, KCl 4, $NaHCO_3$– 24; $MgCl_2$ 0.5; $CaCl_2$ 2.7; NaH_2PO_4– 1.8; glucose 5.5.

Experimental Protocol

Papillary muscles were placed in a 5 ml bath and were constantly irrigated with the oxygenated Tyrode's solution at a rate of 2 ml/min. The bath temperature was maintained at 36.5 ± 0.5 °C. Stimulation pulses were delivered through insulated wires to the papillary muscle. The pulse duration was 2 ms with an amplitude 2-fold of the diastolic threshold. Transmembrane action potential was measured using glass microelectrodes filled with 3M KCl and having a tip resistance of 10–30 MΩ. The microelectrode was coupled via a holder SM002 to a microelectrode amplifier VF180 (Biologic, Claix, France). Transmembrane potentials were monitored and recorded using a personal computer with a Datapac acquisition system (Bio-logic, Claix, France). Simultaneously, action potentials were printed on paper (Gould 8000S recorder). Papillary muscles were equilibrated by stimulations at 1 Hz for 2 hours. Up to 20 impalements were made from different locations in the papillary muscle to obtain cellular action potentials. Analysis of electrophysiological parameters included action potential duration at 50 % and 90 % of repolarisation (APD50, APD90), action potential amplitude (APA), resting potential (RP), and maximum rate of rise of phase 0 (V_{max}). In addition, to obtain a relation between APD and cycle length, the following pacing protocol was tested. We stimulated the muscle at cycle lengths of 10,000, 5000, 1000, 330, and 200 ms for 3 minutes. Every 3 min. train was followed by 20 s pauses. Trains of 5, 10, 15, and 20 stimuli at 3 Hz followed by 2 min. pauses were also tested. These two pacing protocols were consecutively performed for each preparation in Tyrode's 1, 2, and 3 solutions.

Analysis of Data

EAD was defined by a change in the time course of repolarisation of an action potential characterised by an oscillation potential that begins prior to the completion of repolarisation. When an abnormal unique or multiple action potential occurred following an EAD it was defined as an EAD triggered activity. DAD was defined by an oscillation in membrane potential that occurred after repolarisation was completed. Action potentials induced by a train of stimuli were defined as DAD triggered activity. Automaticity was defined as a spontaneous generation of regular sequence of actions potentials, independently of any paced sequence. We used Student's t-tests for independent samples or Kruskall/Wallis non-parametric tests to compare PMI/SHAM groups or smaller groups, respectively, and simple regression tests. The value p = 0.05 was chosen as the significance threshold. Results were expressed as mean ± standard deviation.

Results

Results of *in vivo* Measurements

Only rats surviving 48 hours after coronary artery ligation have been considered. No death occurred in PMI1, CTRL1, and CTRL5 groups. However, chronic mortality was very high (6/15, 40 %) in infarcted rats surviving after the first month.

Heart failure was validated by a significant increase of left filling pressures in PMI compared with CTRL rats. Left ventricular end-diastolic pressure (LVEDP) was measured only in 6/7 rats in the PMI1 group and in 7/9 rats of PMI5 group, because 3 rats with high cardiac enlargement died immediately after anaesthesia. LVEDP was 22.3 ± 9.0 mmHg in PMI (range 10–40) and 5.2 ± 3.1 mmHg (range 2–10) in CTRL groups, respectively (p < 0.05). The infarction size score ranged between 3/6 and 5/6 in PMI rats (m ± SD). The scar could involve all left ventricular segments but were signifi-

cantly larger in the apex region with large transmural surface areas and calcified fragments. No necrosis was observed in the septum. ECG could be recorded in only 6/7 rats of the PMI1 (one rat died after anaesthesia) and in 8/9 rats of the PMI5 (bad quality of the ECG signal in 1 rat). Telemetric measurements evidenced isolated VPBs without doublets or ventricular tachycardias (Figure 1). Mean density per hour was 970 ± 1671 VPBs (range 6–4705). This VPBs score might have been slightly influenced by the delay after infarction even if the difference was not statistically significant: 277 ± 352 VPBs in PMI1 and 1491 ± 2091 VPBs in PMI5 groups, respectively (p = 0.18). A strong and negative relationship was evidenced between the heart rate and the VPBs score.

Spontaneous arrhythmias appeared to be highly bradycardia-dependent. In each rat, VPBs density was assessed every 15 minutes for 30 seconds. Mean heart rate was measured during this recording. When data from all 14 rats were pooled, HR and VPBs were negatively and significantly correlated (p < 0.01). In all rats beside one (almost no arrhythmic rat), the slope of the linear regression between VPBs and heart rate was always negative. The mean value of this slope for all rats was significantly different from zero (p < 0.05).

No difference was seen in PMI1 and PMI5 groups for the necrosis score (4.4 ± 0.7 vs. 4.3 ± 0.7) or for the LVEDP (21.7 ± 9.3 mmHg vs. 22.9 ± 9.5 mmHg). When it was obtained, LVEDP was also similar in the 2 SHAM groups (3.8 ± 1.6 mmHg in CTRL1 and 4.2 ± 2.1 mmHg in CTRL5, respectively). Finally, no correlation could be established between VPBs score, necrosis score or LVEDP (Tab. 1).
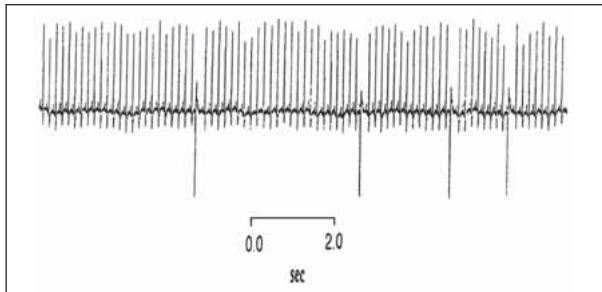

Figure 1. Example of lead II ECG recorded by telemetry from a rat of PMI5 group. Note the presence of frequent spontaneous VPBs.

Action Potential Characteristics in Failing and Control Rats

Action potential parameters are shown in Table 1. Measurements were mostly similar in the twenty measures. For example, the mean variation index (standard deviation/mean) of APD90 was 0.15 ± 0.02, 0.11 ± 0.02, 0.17 ± 0.04, and 0.08 ± 0.01 for CTRL1, PMI1, CTRL5, and PMI5, respectively. Therefore, for each rat, each value was the average of 20 impalements made at the whole surface of papillary muscles.

Heart failure induced major alterations in the cellular electrophysiological parameters. The most prominent changes concerned the duration of APs. Mean APD90 and APD50 were 49 ± 14 ms vs. 128 ± 31 ms and 10 ± 2 ms vs. 37 ± 19 ms for CTRL (1 + 5) and PMI (1 + 5), respectively (p < 0.001). V_{max} was highly modified as well, the amplitude of AP was decreased and the resting potential mildly increased (p = 0.001, 0.11, and 0.06). Moreover, the strong correlations observed between these parameters provided heavy validation of these results. Regression analysis was authorised by the small overlap of values from the SHAM and PMI groups. For example, negative (with APD90, APD50, and RP) or positive (with APA) correlations were shown between V_{max} and the 4 other parameters tested here (p-values: 0.007, 0.02, 0.02, and 0.01, respectively). The resting potential significantly increased, as well, with the decrease of AP amplitude (p < 0.001).

APD90 and APD50, but no other electrophysiological parameters, were strongly related to the size of infarction (necrosis score, p = 0.003 and 0.001, respectively). The VPBs density was highly related to AP amplitude (p = 0.04) and a trend was noted between this arrhythmic score, the resting potential (p = 0.07), and V_{max} (p = 0.09). For example, in group PMI5, rats with more than 100 VPBs/hour showed 88 ± 15 mV vs. 104 ± 5.6 mV for the AP amplitude, –72 ± 7 mV vs. –81 ± 6 mV for the resting potential, and 164 ± 59 V/s vs. 196 ± 42 V/s for the V_{max}, compared with less arrhythmic rats, respectively. No relationship was found between these parameters and the severity of filling pressures (LVEDP, p = 0.45, 0.70, 0.53, 0.41, and 0.82, respectively). Moreover, differences observed between 1 and 5 months values of APD90, APD50, APA, RP, and V_{max} were not significant (120 ± 40 ms vs. 134 ± 22 ms, 37 ± 25 ms vs. 37 ± 14 ms, 100 ± 4 mV vs. 96 ± 13 mV, –75 ± 2 mV vs. –77 ± 6 mV, 145 ± 15 V/s vs. 185 ± 27 V/s, respectively, for PMI1 and PMI5).

Table 1. Characteristics of action potentials as a function of LVEDP, necrosis score and arrhythmias

		LVEDP < 20 mmHg	LVEDP > 20 mmHg	necrosis score > 4	necrosis score > 4	VPSs < 100 VPB/hours	VPSs > 100 VPB/hours
APA	PMI1	98 ± 4.3	102 ± 5.9	98 ± 4.5	103.8 ± 5.4	98 ± 4.3	102 ± 5.9
	PMI5	104 ± 4.7	101 ± 6.4	98 ± 8.1	94.8 ± 17.8	104 ± 5.6	88 ± 15.2
RP		–74 ± 5.1	–75 ± 4.8	–74 ± 4.9	–76 ± 5.0	–74 ± 5.1	–75 ± 4.8
		–79 ± 6.9	–78.1 ± 6.8	–78 ± 6.8	–75 ± 8.9	–81 ± 6.2	–72 ± 7.1
V_{max}		158 ± 29.4	171 ± 27.7	165 ± 28.9	166 ± 29.4	158 ± 29.4	171 ± 27.7
		199 ± 39.5	179 ± 48.7	182 ± 53.8	189 ± 48.9	196 ± 42.0	164 ± 59.1
APD30		20 ± 15.5	19 ± 8.7	11 ± 3.9	34.6 ± 8.1	20 ± 15.5	19 ± 8.7
		18 ± 3.3	21 ± 8.7	29 ± 7.4	48 ± 14.8	21 ± 8.6	16 ± 6.1
APD50		38 ± 33.6	31 ± 8.2	19.9 ± 8.0	60.6 ± 20.5	38 ± 33.6	31 ± 8.2
		35 ± 8.2	44.3 ± 16.5	29 ± 7.4	74 ± 14.8	43 ± 16.8	31 ± 10.4
APD90		110 ± 52.1	119 ± 18.9	95.1 ± 30.9	154 ± 20.7	110 ± 52.1	119 ± 18.9
		–79 ± 6.9	145 ± 16.7	119 ± 17.6	152 ± 14.1	134 ± 27.4	134 ± 20.2

APA = action potential amplitude; RP = resting potential; APD = action potential duration at 30, 50 and 90 % of repolarisation. LVEDP: left ventricular end-diastolic pressure. VPB = ventricular premature beat. Data given as mean ± standard deviation.

APD90 as a Function of Basic Cycle Length in Failing and Control Rats

Figure 2 summarises results of AP duration rate dependence in groups CTRL1, CTRL5, PMI1, and PMI5. For each stimulation rate, APD90 was significantly increased in the failing myocardium group compared with the control group. Moreover, an increase of APD90 at 0.1 Hz was observed in PMI5 when compared with PMI1, but the difference was not statistically significant. Nevertheless, as we observed irreversible automaticity or EAD at a slow stimulation rate, three rats from the PMI5 were excluded from the results. At 0.5 Hz and faster stimulation rates, APD90 prolongation were similar in PMI1 and PMI5 groups. Rate-induced reduction of APD was considerably more pronounced in failing than in normal hearts. The estimated slope of the curve APD90/cycle length was 28.5 and 38.5 in PMI1 and PMI5, respectively, vs. 8.2 and 10.7 in CTRL1 and CTRL5.

After-Depolarisations, Triggered Activity, and Automaticity

DAD were never observed in CTRL rats. DAD occurred in PMI1 in Tyrode's 2 solution containing low potassium, low magnesium, and caesium and following a train of stimulation. Figure 3 illustrates a DAD following a train of 20 stimuli at 3 Hz. The first action potential reached a threshold producing a triggered activity. The final oscillation (15 mV) recorded is a DAD that failed to reach a threshold. Four of the seven PMI1 gave rise to triggered activity following 5, 10, 15,

and 20 pulses at 3 Hz. This triggered activity persisted less than two minutes and was not followed by an apparent DAD. Only one PMI5 presented DADs after a train of 10 and 15 pulses in normal Tyrode's solution.

CTRL and PMI1 preparations failed to show either EAD at any cycle length in either Tyrode's solution. APD90 was particularly increased at 0.1 Hz during which EADs developed in five PMI5 preparations in normal Tyrode's solution. When the stimulation rate was increased from 0 to 5 Hz, EADs could still be induced in only one PMI5 at 0.5 and 1 Hz. In conditions which favoured EADs (low potassium, low magnesium, and caesium), EADs occurred in five PMI5 at 0.1 and three PMI5 at 0.5 Hz. EADs were observed in three PMI5 during the 2 minutes of quiescence between two trains of stimulation. The first upstroke was spontaneous and EADs induced triggered activity in all cases (Fig. 4).

Abnormal automaticity was the most commonly recorded arrhythmia. Figure 5 summarises the occurrence of spontaneous activity induced at different cycle lengths and in different Tyrode's solutions. In normal Tyrode's and at low frequency (0.1 Hz), automaticity was very frequent in PMI1 (5/7) and PMI5 (7/9). At this frequency, however, some CTRL rats also displayed automaticity (1/6 and 2/7 at 1 and 5 months, respectively, but the difference was very significant). When the stimulation rate increased, automaticity decreased in all groups. At 5 Hz, automaticity was never observed. Spontaneous activity could be stopped by rapid stimulation.
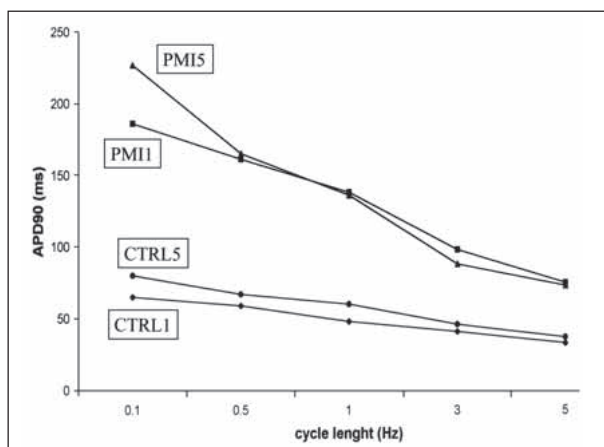

Figure 2. AP duration (APD 90) as a function of basic cycle length in the 4 groups (CTRL1, CTRL5, PMI1, PMI5).

Figure 4. Illustration of an EAD that occurred during the 2 minutes of quiescence between 2 trains of stimulation. Abnormal sustained rhythm develops after the second action potential with EAD.
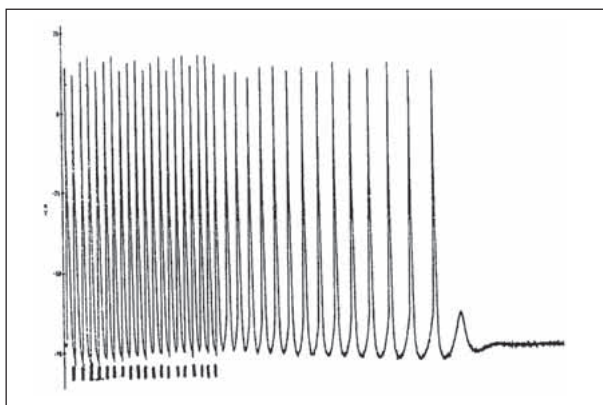

Figure 3. Illustration of a DAD following a train of 20 stimuli at 3 Hz. The first action potential reached threshold producing a triggered arrhythmia. The final oscillation (15 mV) is a DAD that failed to reach threshold.
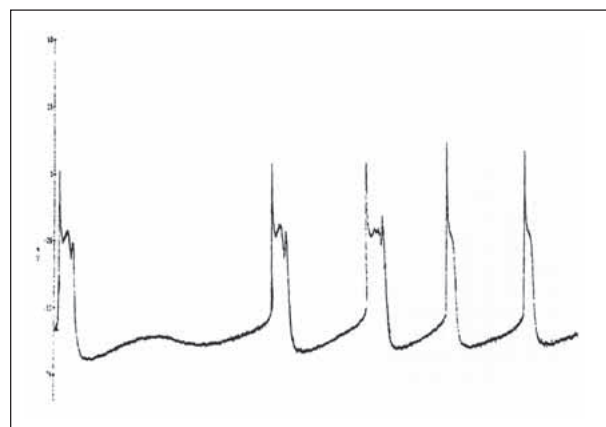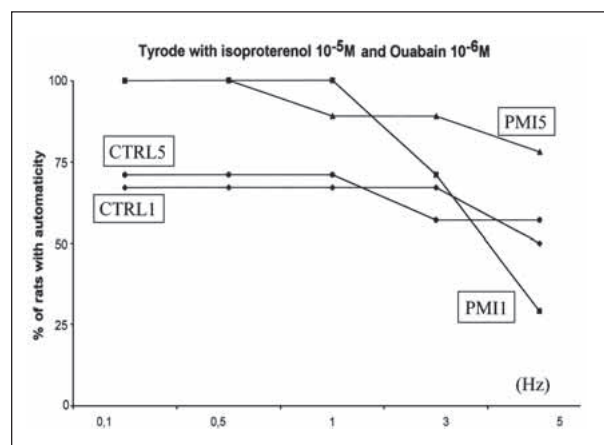

Figure 5. Proportion of automaticity in rats of the 4 groups at different pacing rates (0.1–5 Hz) in modified Tyrode's solution.

The cycle length of automatic rhythm varied constantly, increasing and decreasing beat by beat and it was not possible to identify a single value. Mean automatic cycle length, however, varied from 4 s to 200 ms with the most frequent values in the range 300–400 ms. This phenomenon appeared similar in PMI1 and PMI5. In modified Tyrode's solution, automaticity increased in all groups and was even recorded at 5 Hz. When isoproterenol and ouabain were added (Tyrode's 3), up to 100 % of failing myocardium displayed automaticity at low frequencies (0.1 and 0.5 Hz).

Because of the relatively small number of preparations, results were then pooled with the 3 Tyrode's solutions to test potential correlations of occurrence of EAD, DAD, and automaticity with the other parameters of the study. For example, each rat would have been considered positive for EADs, if one or more EAD had been induced in either of the 3 solutions. Strong relationships were shown between EADs, DADs occurrence and the delay after infarction. More DADs were observed at one month, 5/7 rats vs. 1/9 at 5 months (p = 0.003). On the contrary, more EADs were seen at 5 months (6/9 vs. 0/7 at 1 month, p = 0.01). EADs and abnormal automaticity seemed more frequent in arrhythmic rats (more than 100 VPBs/h).

Discussion

Main Results of the Study
The major goal of our work was to analyse correlations between cellular electrophysiological *in vitro* changes induced by heart failure and *in vivo* arrhythmic, morphologic and haemodynamic data from live animals (VPBs density, necrosis score, LVEDP), according to the delay after infarction. As yet, such a correlation has only been reported in rabbits with heart failure [7–24]. To our knowledge, these relationships have been investigated in rats for the first time in our study. We used a very popular rat model of heart failure in which we have documented very frequent spontaneous and chronic arrhythmias [12–13]. This model was characterised by predominance of ventricular dilatation over hypertrophy [14–15]. Observations consistent with data obtained in human heart failure have suggested its clinical relevance [16].

The main results of this study can be summarised as follows:

– Abnormal automaticity was found to be the major mechanism of arrhythmias recorded in muscle preparations. Their occurrence was so high that other mechanisms might have been underestimated. Nevertheless, the very clear bradycardia-dependent characteristics of *in vivo* recorded arrhythmias had already been observed in another study from our lab and could be consistent with the predominance of an automatic mechanism in this post-infarction rat model [13, 17].
– The arrhythmic score on Holter recordings was found related only to changes in AP amplitude, resting potential and V_{max}, which are indexes of severe cell alterations but, surprisingly, not to AP duration modifications, which is the major electrophysiologic alteration described in the literature. Nevertheless, AP lengthening was observed and correlated with infarction size. This result could have consequences on our understanding of the genesis of arrhythmias.
– Although infarction geometry or arrhythmias could be connected to AP measurements, the influence of the delay after infarction or of filling pressures – two well-known parameters of ventricular remodeling – appeared less clear. However, DADs were more frequent at 1 month than later. On the contrary, EADs were observed only after 5 months.

Methodological Considerations
In this post-infarction rat model, we aimed to correlate *in vitro* and *in vivo* data. The pathophysiological significance of *in vitro* studies of ventricular arrhythmias, in a number of hypertrophy and heart failure models, has been weakened, as yet, because of the lack of *in vivo* related data [5]. The clinical relevance of AP modifications observed at stimulation rates significantly slower than physiological rates, or in animal models without any *in vivo* arrhythmia, has to be questioned. For example, AP duration lengthening and changes in ionic currents have been described in the Syrian hamster with dilated cardiomyopathy [18]. However, Holter monitoring demonstrated clearly that death in this model was induced by a progressive cardiac rate decrease rather than arrest [19]. Moreover, no ventricular arrhythmias were ever seen on continuous recordings. Cellular electrophysiological modifications could in this case only be markers of heart failure and not a cause for sudden death or even arrhythmias.

In our study all PMI rats showed large infarction scars; all rats presented with VPBs. LVEDP was always more than 10 mmHg in PMI rats and less than 10 mmHg in sham rats. AP characteristics were strongly modified by heart failure. A strong validation of our results was provided by repeated measurements (20 data per preparation), by highly significant correlations between the 5 cellular electrophysiological parameters (APD90, APD50, APA, RP, V_{max}) and by the low variability of measured parameters. For these reasons, the surprising correlation between *in vivo* arrhythmias and AP amplitude (and the lack of relationship with AP lengthening) cannot be due to artefacts.

Potential Mechanisms of Ventricular Arrhythmias
Few studies are available in the literature concerning the electrophysiological remodeling of infarct-induced heart failure in the rat. Santos described ventricular AP and L-type calcium channels in this model, one to two months after infarction. He described a decrease in the current density, independent of the AP lengthening [20].

AP duration (APD) changes might be involved in reentry processes, particularly when an important dispersion of APD between different areas of the ventricle was noted. Enhancement of interregional variations has been suggested in hypertrophied hearts [5]. Qin et al., in the same model of post-infarction rat, reported intramural differences between epicardial and endocardial areas. Both APD25 and APD50 were significantly longer in epicardial post-MI cells [11] (p < 0.01). These data, nevertheless, were obtained in the very early phase of ventricular remodeling (3 weeks). Induced arrhythmias were obtained but spontaneous VPBs were not available. AP characteristics were reported from isolated cells and not from tissue preparations. Regional differences have been checked in our laboratory and reported elsewhere [21]. Myocytes isolated from lateral free wall, septum, apex of the left ventricle, and from the right ventricle showed no difference in the cell dimensions. Basal L-type Ca^{2+} did not exhibit regional dependence neither in control nor after infarction. As in other models of heart disease in rats I_{to} was significantly decreased but the basal heterogeneity of I_{to} density was not enhanced by the disease, with the right ventricular cells showing the largest values [22]. In our study, intra-individual measurements concerned 20 different impalements in the papillary muscle. Inhomogeneity was evaluated by the coefficient of variation and did not exceed 17 %. However, all regions of the left ventricle were not involved. Finally, a reentry process, in this model, could be favoured by mechanisms other than AP dispersion, like inhomogeneous fibrosis or

modification of the gap junctions. AP lengthening was related to the scar size and not with recorded arrhythmias and could rather be a marker of the disease than a cause for arrhythmic disturbance. It could be different, however, for non-recorded or more severe arrhythmias.

In our study, triggered activities induced by EADs or DADs and automaticity were related to cellular alterations (AP amplitude, V_{max}, resting potential). They might represent triggers of sustained ventricular arrhythmias and have a different meaning according to the stage of the disease. The predominance of cellular automaticity is consistent with the bradycardia-dependence characteristics of *in vivo* recorded arrhythmias [17].

In a different heart failure model, Vermeulen, Rademaker, and Opthof, from the Janse's group tried also to correlate *in vivo* telemetric recordings and *in vitro* electrophysiological data. New Zealand rabbits with volume and pressure overload suffered from ventricular arrhythmias. Rabbits dying from sudden death had developed a shorter cycle length than surviving rabbits [23]. A more accurate analysis did not isolate a unique mode of onset: some arrhythmias were preceded by pauses, and some by acceleration [24]. DADs and automaticity, but no EADs occurred in failing hearts but only during superfusion with modified Tyrode's solution (hypokaliaemia). As in our model, the rate of automatic and triggered rhythms was slow, suggesting a role in triggering but not maintaining malignant ventricular arrhythmias [7].

Limitations

Chronic mortality was high in this model but permanent recording of the ECG was unfortunately not possible and a lethal arrhythmia has never been observed. In our population, however, we only documented spontaneous arrhythmias. We did not try to induce tachyarrhythmias with programmed ventricular stimulation. This technique needs aggressive thoracotomy in the rat and could have led to artefactual results in hypoxic preparations. We decided to protect the viability of hearts to obtain physiologically relevant measurements. Although far less dangerous, simple anaesthesia used to perform haemodynamic measurements or to implant a telemetric device unfortunately killed some of the post-infarction animals, as mentioned above. Moreover, rats killed by anaesthesia were probably the most severe and the most interesting animals. Unfortunately, haemodynamic, electrocardiographic and cellular electrophysiological data were lost for the study. Nevertheless, induced arrhythmias have been described in this model. Fast ventricular tachycardias have been recorded in our lab and reported by other groups [25–26]. They could be triggered and stopped by pacing, suggesting a reentry mechanism.

Finally, during the development of heart failure, not only cellular electrophysiological modifications are induced. Fibrosis processes, tissular changes of refractoriness and of conduction velocity can predispose to an increase in arrhythmic death. The sympathetic nervous system, the renin-angiotensin-aldosterone system and other neurohumoral components are strongly activated. These parameters have not been investigated in our study.

Conclusion

Consistency of some *in vivo* and *in vitro* results suggests that the variability of arrhythmic mechanisms described in HF is not only induced by methodological artefacts but reflects heterogeneity in the pathobiology of the disease. In the post-infarction rat model of heart failure, automaticity seems to represent the major mechanism for recorded ventricular arrhythmias. AP lengthening might be only a marker of the disease. Further *in vitro-in vivo* studies are warranted, with the aim to understand sudden death mechanisms as closely as possible.

References:
1. Anonymous. Changes in mortality from heart failure. United States, 1980–1995. Morbidity Mortality Week Rep 1998; 47: 633–7.
2. Luu M, Stevenson W, Stevenson L, Baron K, Walden J. Diverse mechanisms of unexpected cardiac arrest in advanced heart failure. Circulation 1989; 80: 1675–80.
3. Vermeulen J. Mechanisms of arrhythmias in heart failure. J Cardiovasc Electrophysiol 1998; 9: 206–21.
4. Aronson R. Afterpotentials and triggered activity in hypertrophied myocardium from rats with renal hypertension. Circ Res 1981; 48: 720–7.
5. Tomaselli G, Marban E. Electrophysiological remodeling in hypertrophy and heart failure. Cardiovasc Res 1999; 42: 270–83.
6. Hart G. The Cellular electrophysiology in cardiac hypertrophy and failure. Cardiovasc Res. 1994; 28: 933–46.
7. Vermeulen J, McGuire MA, Opthof T, Coronel R, de Bakker JM, Klopping C, Janse MJ. Triggered activity and automaticity in ventricular trabeculae of failing human and rabbit hearts. Cardiovasc Res 1994; 28: 1547–54.
8. Cohn JN, Archibald DG, Ziesche S, Franciosa JA, Harston WE, Tristani FE, Dunkman WB, Jacobs W, and Flohr KH. Effect of vasodilator therapy on mortality in chronic congestive heart failure. Results of a Veterans Administration Cooperative Study. N Engl J Med 1986; 314: 1547–52.
9. Packer M. Lack of relation between ventricular arrhythmias and sudden death in patients with chronic heart failure. Circulation 1992; 85: I 50–6.
10. Pfeffer MA, Pfeffer JM, Fishbein MC, Fletcher PJ, Spadaro J, Kloner RA, Braunwald E. Myocardial infarct size and ventricular function in rats. Circ Res 1979; 44: 503–12.
11. Qin D, Zhang ZH, Caref E, Boutjdir M, Jain P, El-Sherif N. Cellular and ionic basis of arrhythmias in postinfarction remodelled ventricular arrhythmias. Circ Res 1996; 79: 461–73.
12. Aimond F, Beck L, Gautier P, Cherif OK, Davy J-M, Lorente P, Nisato D, Vassort G. Cellular and in vivo electrophysiological effects of dronedarone in normal and post myocardial infarcted rats. J Pharmacol Exp Ther 2000; 292: 415–24.
13. Beck L, Blanc-Guillemaud V, Cherif O, Jover B, Davy J-M. The effects of spironolactone and fosinopril on the spontaneous and chronic ventricular arrhythmias in a rat model of myocardial infarction. Cardiology 2001, 96: 85–93.
14. Pfeffer MA and Braunwald E. Ventricular remodeling after myocardial infarction. Experimental observations and clinical implications. Circulation 1990; 81: 1161–72.
15. Litwin SE, Katz SE, Morgan JP, Douglas PS. Serial echocardiographic assessment of left ventricular geometry and function after large myocardial infarction in the rat. Circulation 1994; 89: 345–54.
16. Pfeffer MA, Pfeffer JM, Steinberg C, Finn P. Survival after experimental myocardial infarction: beneficial effects of long-term therapy with captopril. Circulation 1985; 72: 406–12.
17. Gilmour RF Jr. Enhanced automaticity. In: Podrid PJ, Kowey PR (eds). Cardiac arrhythmia: mechanisms, diagnosis and management. Williams and Wilkins, Baltimore, 1994; 78–87.
18. Thuringer D, Deroubaix E, Coulombe A, Coraboeuf E, Mercadier JJ. Ionic basis of the action potential prolongation in ventricular myocytes from syrian hamsters with dilated cardiomyopathy. Cardiovasc Res 1996; 31: 747–57.
19. Lessard Y. Relation décès-arythmie dans la cardiomyopathie dilatée du hamster. Arch Mal Cœur 1999; 92: 482 (Abstr.).
20. Santos PE, Barcellos LC, Mill JG, Masuda MO. Ventricular action potential and L-type calcium channel in infarct induced hypertrophy in rats. J Cardiovasc Electrophysiol 1995; 6: 1004–14.
21. Aimond F, Alvarez JL, Rauzier J-M, Lorente P, Vassort G. Ionic basis of ventricular arrhythmias in remodeled rat heart during long-term myocardial infarction. Cardiovasc Res 1999; 42: 402–15.
22. Bénitah JP, Gomez AM, Bailly P. Heterogeneity of the early outward current in ventricular cells isolated from normal and hypertrophied rat hearts. J Physiol (Lond) 1993; 469: 111–38.
23. Opthof T, Coronel R, Rademaker HM, Vermeulen JT, Wilms-Schopman FJ, Janse MJ. Changes in sinus node function in a rabbit model of heart failure with ventricular arrhythmias and sudden death. Circulation 2000; 101: 2975–80.
24. Rademaker H. Arrhythmogenesis during the development of heart failure in rabbits. Academic thesis. University of Amsterdam 1997.
25. Bélichard P, Savard P, Cardinal R, Nadeau R, Gosselin H, Paradis P, Rouleau JL. Markedly different effects on ventricular remodeling result in a decrease in inducibility of ventricular arrhythmias. J Am Coll Cardiol 1994; 23: 505–13.
26. Nguyen T, El Salibi E, Rouleau JL. Postinfarction survival and inducibility of ventricular arrhythmias in the spontaneously hypertensive rat. Effects of ramipril and hydralazine. Circulation 1998; 98: 2074–80.