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Relationships between Transmembrane Action Potential Changes and Simultaneous Changes in Electrocardiograms of Rats after a One-Month Aortic Pressure Overload

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Summary

An attempt was made to determine the relationship between the characteristics of electrical activity of the hypertrophied myocardium of rats at the cellular level and at the level of the whole heart after a one-month left ventricular pressure overload. Such an animal model has already been demonstrated to be highly resistant to epinephrine-induced arrhythmias. Since severe ventricular arrhythmias often occur in patients with cardiac hypertrophy, ventricular vulnerability might depend on some electrophysiological characteristics of the heart related to the stage of hypertrophy. Using the "floating" microelectrode technique, the computed characteristics of cardiac transmembrane action potentials (AP) of the left and right epicardium cells were compared *in situ* to computed characteristics of the electrocardiograms in anaesthetized control rats (group C) and in rats with left ventricular hypertrophy (group H) induced by a one-month suprarenal constriction of the abdominal aorta. The aortic pressure overload caused a significant ($p<0.001$) and marked increase in AP duration of left ventricular cells: APD 30 and APD 80 were 29 ± 3 ms and 89 ± 6 ms, respectively, in group H and 14 ± 1 ms and 53 ± 2 ms in group C. The same modifications were observed in right ventricular cells when right hypertrophy was present. Simultaneous electrocardiograms exhibited a significant ($p<0.01$) prolongation of P-R, Q-S and T duration and T wave flattening in group H (63 ± 2 ms, 32 ± 3 ms, 109 ± 5 ms and 0.25 ± 0.03 mV as compared with 53 ± 1 ms, 20 ± 1 ms, 88 ± 2 ms and 0.40 ± 0.04 mV in group C). After a one-month aortic overload in rats, both left and right ventricles are hypertrophied and have the same electrophysiological characteristics: in this model, at this stage of hypertrophy, some factors favouring ventricular arrhythmias are missing. The corresponding flattening of the T wave in the ECG might be of clinical relevance.

Key words

Rats – Cardiac hypertrophy – Electrocardiogram – *In situ* cardiac transmembrane potential – Epinephrine-induced ventricular arrhythmia

Introduction

The Framingham study has indicated that patients with electrocardiographically (Kannel 1990) or echocardiographically (Levy *et al.* 1990) determined left ventricular hypertrophy are at high risk of sudden death. The high incidence of serious ventricular arrhythmia in left ventricular hypertrophy (Messerli *et al.* 1984) is now well-recognized (McLenachan and Dargie 1990). Ventricular arrhythmias have been found to correlate with risk of sudden death in patients with hypertrophic cardiomyopathy (McKenna *et al.* 1981). Several mechanisms of sudden death have been proposed, including the association of physical exercise- or emotion-induced increase in plasma catecholamines and a change in left ventricular function leading to ventricular fibrillation (McKenna *et al.* 1980). But firstly, it has been demonstrated that arrhythmogenic doses of epinephrine cause less severe ventricular arrhythmias in rats after a one-month aortic

stenosis than in sham-operated rats (Lessard *et al.* 1992). Secondly, little is known about the cellular mechanisms which generate these arrhythmias. Electrocardiographic recordings are commonly used to study the evolution patterns of cardiac arrhythmias in patients with cardiac hypertrophy. But it is necessary to understand the cellular basis for the genesis of arrhythmia and such research requires detailed studies which are not possible in a clinical setting. Up to now, most studies have used cardiac preparations isolated from different models of hypertrophic mammalian hearts. Nevertheless, the mechanism of sudden death and ventricular arrhythmia generation in hypertrophic hearts without coronary disease remains poorly understood, partly because of the absence of preparations allowing a simultaneous view of the electrical abnormalities at both cellular and whole-heart levels. In this report, we present the results of our study of the *in vivo* electrophysiological characteristics of a model of cardiac hypertrophy without ischaemia or cardiac failure in rats after aortic banding lasting aproximately 5 weeks. We also recorded the same electrophysiological parameters during epinephrine infusion of 25 $mg.kg^{-1}.min^{-1}$.

Methods

Experimental model

The laboratory where this work was done complies with the requirements of the French Ministry of Agriculture (Approval N° A35002) and the authors have been authorized to experiment on living animals (Licence N 005919).

Left ventricular hypertrophy without cardiac failure was induced in male Wistar rats weighing 160–180 g (7 weeks old) by standard coarctation of the suprarenal abdominal aorta with Weck clip forceps (Mercadier *et al.* 1981) after general intraperitoneal anaesthesia with a 50 $mg.kg^{-1}$ dose of sodium pentobarbital and xyphopubic laparotomy. In the first group (H) of 12 rats, the internal diameter of the silver clip was calibrated at 0.35 mm. In the second control group (C) of 13 age-matched sham-operated rats, the aorta was dissected under anaesthesia without placing a clip.

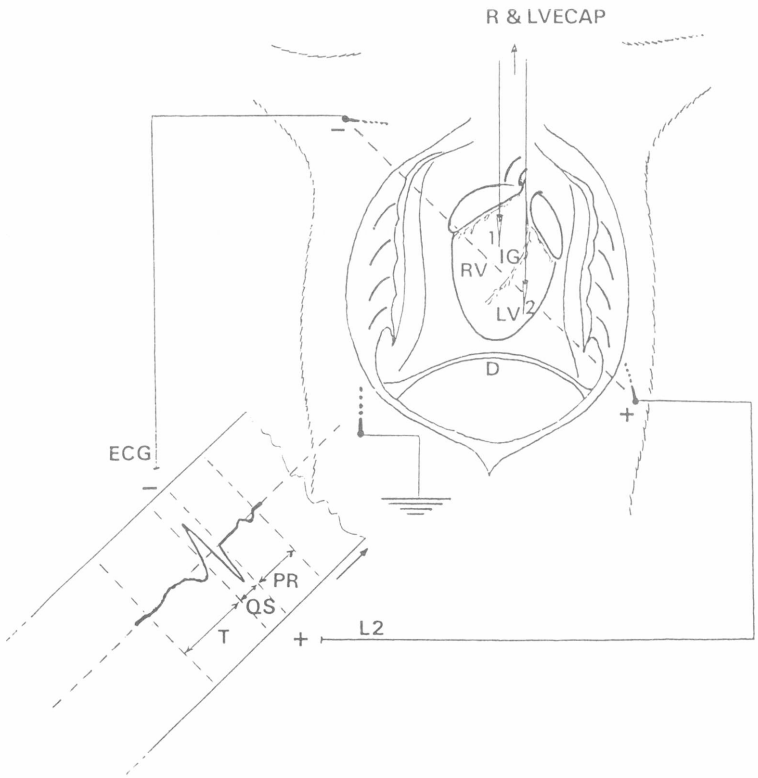

Fig. 1. *Scheme of the technical arrangements for simultaneous recording of transmembrane potentials in rats. The location of the two subcutaneous electrodes shows that the ECG trace represents the global differential activity of the left and right hearts (see "Methods"). D: diaphragm; ECG L2: electrocardiogram lead 2; IG: interventricular groove (anterior descending branch of the left coronary artery); LV: left ventricle; RV: right ventricle; R & LVECAP: right and left ventricular epicardial cell transmembrane action potential; 1: right ventricular location of the floating microelectrode; 2: left ventricular location of the floating microelectrode.*

Recording of cardiac electrical activity

The experiment was performed 4 or 5 weeks later, after stable marked hypertrophy had been obtained. A tracheotomy was performed on the animals under anaesthesia, so that a tracheal cannula could be inserted. The global electrocardiogram (ECG) was recorded from subcutaneous electrodes in standard lead 2. After sternotomy, the animals were ventilated with air using a respirator (Braun), the rate and volume of which were adjusted to maintain the pre-operation spontaneous physiological conditions (about 80 $cycles.min^{-1}$ and 4 cm^3). The lungs were

moved aside and the pericardium was opened. Cardiac transmembrane action potentials (AP) were recorded by glass capillary electrodes (Clark Electromedical Instruments GC 150 F) pulled by a Narishige PE-2 electrode puller and filled with 3 $mol.l^{-1}$ KCl (resistance 5–25 MΩ). After suspending only the light tip of the electrode to a microelectrode holder with a flexible and thin silver wire (diameter 25 μm), the "floating" microelectrode was impaled into a left or a right ventricular cell of the epicardial surface with a micromanipulator (Prior POO-44). To preserve the uniformity of sampling, the left and right ventricular recording sites were selected according to their proximity to major coronary artery segments (see Fig. 1). The room was heated to maintain the epicardial surface above 35 °C which was controlled with a hand-held probe (Ellab TE-3). The microelectrode holder was linked to the upper trace of an oscilloscope (Tektronix 5301 N) through an impedance adaptator (WPI S-7000 modular microprobe electrometer system). The ECG was recorded on the lower trace after fifty-fold preamplification. The dissection of a jugular vein allowed analogous recordings during under infusion of epinephrine at the rate of 25 $mg.kg^{-1}.min^{-1}$, a dose which had previously been demonstrated to cause ventricular arrhythmia in control rats (Lessard *et al.* 1992).

Computer analysis

The two analog AP and ECG signals were analyzed by computer (Logabax Persona 1600). After on-line electronic measurement of the maximum depolarization speed and of the peak potential, original software was applied, allowing digitization of the recorded signals at different rates of sampling. One kHz sampling (8-bit resolution) over a 4-second period was generally sufficient to measure the following AP mean characteristics which were added to the maximum rate of rise of the upstroke (dV/dt max) and to the maximum value of the peak voltage or "overshoot" (OS): diastolic resting membrane potential (RMP), action potential amplitude (APA), action potential duration to 30 % (APD 30) and 80 % (APD 80) of complete repolarization to RMP, cardiac period duration (R-R) and frequency (HR).

Because of the possible presence of unwanted signals such as superimposed electromyogram, the user himself moved a cursor along the screen trace to determine the durations of the different ECG intervals and wave shapes. The computer calculated the durations of the P-Q interval (PQ), QRS deflexion (QS) and T wave (T) over a single chosen cardiac cycle within the 4-second recording. Since the ECG of a normal rat does not present any intervening isoelectric potential or baseline segment between S and T waves, the point where the upward trace crossed the zero reference potential line could be defined (Sambhi and White 1960) in lead 2 as the end of the S wave and the beginning of the T wave. Measurements of the QT interval were corrected for the heart rate using the following equation (Kissin *et al.* 1948) :

$$QTc = QT\ (ms)/\sqrt{R\text{-}R\ (ms)}$$

The mean T maximum magnitude above the zero potential reference line (TM) was also measured over 6 or 8 cardiac cycles to evaluate the maximum difference between left and right ventricular repolarization levels, since the axis of the recording lead was perpendicular to the ventricular septum (see Fig. 1 and Discussion).

The results of such experiments were not accepted if either the AP contours or RMP levels were unstable during single cell impalement or if the values of any of these parameters changed from one impalement to another in the same limited area of the epicardium: these changes indicated inadequate penetration of the microelectrode into the cell. Conversely, reproducible afterpotentials with stable AP shapes and stable RMP values from one recording to another were easily distinguished from movement artifacts of the microelectrode tip within the cell membrane.

Hypertrophy estimate

The heart was removed and rinsed with heparinized saline immediately after the various electrical recordings. The ventricles were weighed before dissecting the left ventricle (including the septum) and the right ventricle which were then weighed separately in order to calculate different hypertrophy indexes (HI, $mg.g^{-1}$): LVHI equal to the left ventricular/body weight ratio, RVHI equal to the right ventricular/body weight ratio and

$$HI\ \% = \frac{\text{observed - theoretical (LV + RV) weight (mg)}}{\text{theoretical (LV + RV) weight (mg)}} \cdot 100$$

with theoretical (LV + RV) weight (mg) = [2.081 *x* body weight (g)] + 155 as reported by Swynghedauw *et al.* (1968) for male Wistar rats. In our experiments we checked the validity of this equation for our control rats.

Statistical analysis

The results are expressed as means ± S.E.M. Paired Student's t-test or Wilcoxon's *z* test were used whenever possible for simple two-way comparison of the data. However, multiple comparisons required analysis of variance and Scheffé's *F* test (ANOVA). Statistical significance was set at $p<0.05$.

Table 1 : anatomical data. Aortic stenosis or sham operation was performed when the rats were 2 months old. Aortic stenosis (H) and control sham-operated (C) animals were recorded and sacrificed after 4 or 5 weeks. Values are means ± SEM.

groups	body weight g	(LV + RV) weight mg	HI%	LV weight mg	LVHI $mg \cdot g^{-1}$	RV weight mg	RVHI $mg \cdot g^{-1}$
C (n = 13)	366±14	916±30	0.03±1.71	725±25	1.98±0.04	191± 6	0.53±0.02
H (n = 12)	286±14***	1242±68***	66.81±8.50***	1020±57***	3.63±0.22***	222±16*	0.79±0.06***

n = number of rats ; * : $p \leq 0.05$; ** : $p \leq 0.01$; *** : $p \leq 0.001$ (comparison H vs C)
C : control sham-operated animals ; H : animals with experimental aortic stenosis ; HI : hypertrophy index ; LV : left ventricle ; RV : right ventricle

Table 2 : basic electrophysiological changes in epicardial left and right ventricular cells due to a 1-month aortic overload and electrophysiological changes due to 25 $\mu g \cdot kg^{-1} \cdot min^{-1}$ epinephrine infusion (E). Values are means ± SEM.

groups	LV					RV				
	RMP mV	APA mV	dV/dt max $V \cdot sec^{-1}$	APD 30 msec	APD 80 msec	RMP mV	APA mV	dV/dt max $V \cdot sec^{-1}$	APD 30 msec	APD 80 msec
C (n = 13)	-74±1	89±1	146±10	14±1***	53±2***	-70±2	85±2	167±14	10±1***	48±3***
C + E	-76±3	90±2	134±11	18±1††	79±3†††	-73±2	88±2	155±15	14±1††	74±3†††
H (n = 12)	-74±2	92±2	129±9	29±3†††	89±6†	-73±1	89±2	131±11	21±3†††	82±7†††
H + E	-77±2	94±2	121±10	38±4***	113±6***	-73±2	93±2	139±15	27±4**	110±7***

n = number of rats ; * : $p \leq 0.05$; ** : $p \leq 0.01$; *** : $p \leq 0.001$ (comparison H vs C)
† : $p \leq 0.05$; †† : $p \leq 0.01$; ††† : $p \leq 0.001$ (comparison E vs base)
APA : action potential amplitude ; APD 30 and 80 : action potential duration to 30 and 80% of repolarization ; C : control sham-operated animals ; dV/dt max : maximum depolarization speed (first derivative) ; E : epinephrine ; H : animals with experimental aortic stenosis ; LV : left ventricle ; RMP : diastolic resting membrane potential ; RV : right ventricle

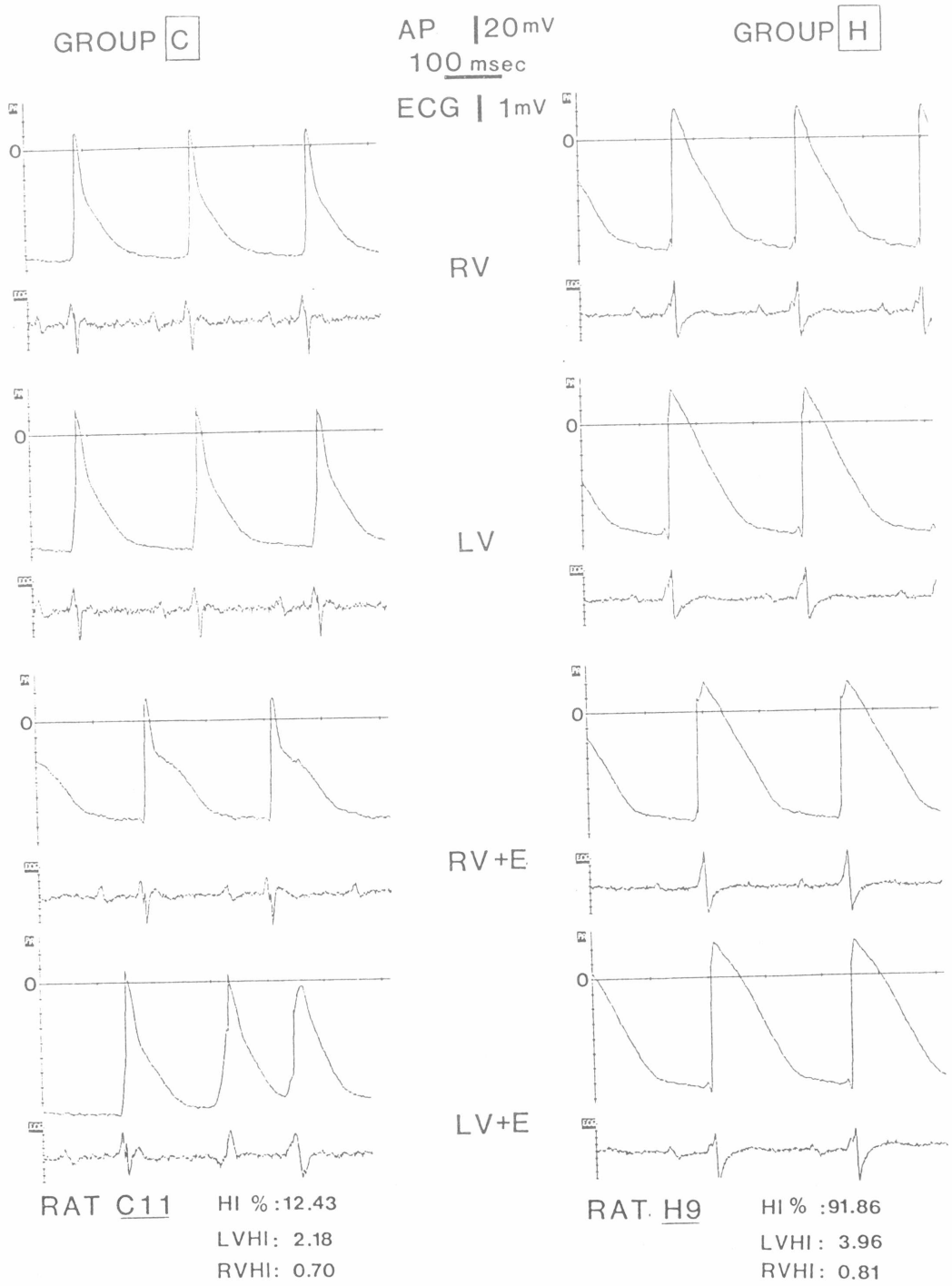

Fig. 2. *Sample records obtained from a control sham-operated rat (group C, left) and a rat with one-month aortic stenosis (group H, right). In each panel the upper trace shows transmembrane action potentials (AP) from epicardial ventricular cells and the lower trace lead 2 of the electrocardiograms (ECG). Note that hypertrophy of both right (RV) and left (LV) ventricles is associated with the marked prolongation of right and left AP durations and associated with the prolongation and the flattening of T waves. Epinephrine (E) infusions (4 lower panels) preserve the flattening of T waves in the right tracings (group H) while the T wave magnitude is slightly increased in the left tracings (group C) and two propagated ventricular premature beats can be observed. E: epinephrine; HI: hypertrophy index.*

Results

Anatomical characteristics of the two groups (Table 1 and Fig. 2)

Aortic stenosis resulted in pronounced cardiac hypertrophy of 67 % with greater ventricular weight in group H, although the body weight was lower than that of group C. Left ventricular hypertrophy was very marked, since LVHI increased by 83 %, but there was also a significant RVHI increase of 49 %. It is important to note that after a one-month aortic overload both ventricles were hypertrophied.

Basic cellular electrophysiological data – effects of aortic stenosis (Table 2 and Fig. 2)

A statistical comparison shows that, in group C, several differences in the values of various parameters between right and left ventricles were significant: RMP, APA, APD 30 ($p<0.01$) and APD 80 ($p<0.05$) were higher in left ventricular epicardial cells; dV/dt max seemed to be lower in the left ventricle, but this difference was of borderline significance ($p=0.081$).

In both left and right ventricles, one-month aortic stenosis caused an almost twofold increase in APD 30 and APD 80. Though this surgical treatment seemed to reduce dV/dt max in both types of cells, this reduction was not significant here and dV/dt max did not correlate with HI in the corresponding ventricle.

However, this cardiac overload tended to cancel left/right electrophysiological differences: APD 30 was the only parameter that remained significantly higher in the left ventricle ($p=0.012$).

Cellular electrophysiological data under epinephrine infusion (Table 2 and Fig. 2)

The main effect of epinephrine concerned a significant prolongation of APD 30 and APD 80 in both left and right ventricular epicardial cells. As for the reduction due to aortic stenosis, dV/dt max was not significantly modified by epinephrine. A statistical comparison between left and right ventricles shows that the epinephrine infusion preserved the significantly higher value of APD 30 in left ventricular epicardial cells of the control group ($p=0.006$).

Bifactorial variance analysis and Scheffé's *F* test show that the effects of the aortic stenosis and of epinephrine were both significant and simply additive. In group H, in both left and right hypertrophied ventricular epicardial cells, APD 30 and APD 80 were higher during epinephrine infusion than under basic conditions and higher than in ventricular cells of the controls during epinephrine infusion. Except for APD 30 which remained higher in the hypertrophied left ventricle cells ($p=0.013$), infusion of epinephrine enhanced the reduction of the left/right differences which was initiated by aortic stenosis. This was especially true for APD 80 which was considerably prolonged in each ventricle by both the surgical and pharmacological treatments.

Table 3 : basic changes in electrocardiogram due to a 1-month aortic overload and changes due to 25 $\mu g \cdot kg^{-1} \cdot min^{-1}$ epinephrine infusion (E). Values are means ± SEM.

groups	HR	PR	QS	T	QT	QTc	TM
	beats/min	msec	msec	msec	msec	msec	mV
C (n = 13)	335±10*	53±1***	20±1***	88±2**	107±2***	253±5***	0,40±0,04**
C + E	304±18†	65±2†††	21±1	107±4†††	128±4†††	284±8††	0,53±0,05†
H (n = 12)	283±18†	63±2†††	32±3	109±5††	141±7††	300±6†	0,25±0,03
H + E	245±12*	76±2**	35±2***	129±6**	164±8***	326±10**	0,26±0,05**

n = number of rats ; * : $p \leq 0.05$; ** : $p \leq 0.01$; *** : $p \leq 0.001$ (comparison H vs C)

† : $p \leq 0.05$; †† : $p \leq 0.01$; ††† : $p \leq 0.001$ (comparison E vs base)

c : corrected for heart rate (see text) ; C : control sham-operated animals ; E : epinephrine ; H : animals with experimental aortic stenosis ; HR : heart rate ; PR : duration of the P-Q interval : QS : duration of the QRS deflexion ; T : duration of the T wave ; TM : magnitude of the T wave

Basic characteristics of the EG – effects of aortic stenosis (Table 3 and Fig. 2)

Heart rate values were slightly but significantly lower in group H. Cardiac overload prolonged atrial and ventricular conduction intervals PR and QS; the same was true for the ventricular repolarization phase, even when corrected for the heart rate. The maximum amplitude TM of the T wave was significantly reduced.

Effects of epinephrine infusion on the ECG (Table 3 and Fig. 2)

The effects of epinephrine infusion were superimposed on those of the aortic stenosis on HR, PR and QTc. QS remained unchanged in groups C and H. TM was increased by epinephrine in group C, but not in group H.

Ventricular arrhythmias under epinephrine infusion (Fig. 2)

The technique used in our experiments did not permit to observe all the arrhythmic periods that may have occurred while infusing epinephrine, but this was not the aim of this work. The same dose of epinephrine had previously been demonstrated to cause less severe and less frequent ventricular arrhythmias in similar rats of group H than in control rats. These rhythm disturbances have been precisely quantified elsewhere (Lessard *et al.* 1992). In the present study we observed epinephrine-induced ventricular rhythm disorders in 5 rats in group C and none in rats in group H. One example of ventricular premature beats (VPBs) in a control rat is shown in Figure 2.

Discussion

Cellular electrophysiology of the hypertrophic myocardium after a one-month left ventricular pressure overload

Studies on the effects of ventricular hypertrophy in cardiac cell electrophysiology have yielded conflicting results (see review by Hart 1994). Using pressure-induced right or left ventricular hypertrophy the majority of investigators have been working on isolated preparations: whole hearts, papillary muscles and cardiac cells of different species including rats, cats, guinea-pigs and ferrets. A significant lengthening of APD has generally been observed. Controversial results concern RMP, APA, upstroke velocity and duration of the early phase of repolarization in which significant or non-significant changes have been described. Indeed, the use of isolated preparations has been useful to demonstrate that these alterations are linked to changes in the individual properties of each cell. Conversely, the study of cardiac cellular characteristics *in situ* avoids additional interfering alterations due to the non-physiological conditions of the preparations such as low bath temperature, low stimulation frequency or inadequate ionic, oxygen or energy supplies. For example, in a study which has been often quoted (Gülch *et al.* 1979), the mean maximum velocity obtained in cells of the left ventricular muscle isolated from control rats was 52 V/s, a value which is three times lower than that generally observed by us *in situ*. Therefore, we can firstly assert that, after a one-month pressure overload, the hypertrophied cardiomyocytes of the rat exhibit no significant change in RMP, APA and dV/dt max. As is often described in the papillary muscle and in endocardial fibres, the most consistent abnormality associated with cardiac hypertrophy is a prolongation of the entire course of repolarization, even in epicardial cells. However, in two different studies on cardiomyocytes with pressure overload-induced hypertrophy a prolongation of only the latter half of repolarization was shown in left ventricular epicardial fibres of rats (Gülch 1980, Keung and Aronson 1981). Working on single myocytes isolated from hypertrophied rat and guinea-pig hearts, the authors of the last group (Aronson and Nordin 1984, Nordin *et al.* 1989) and of another research group (Ryder *et al.* 1993) did not mention such differences between epicardial and endocardial cells. This difference in duration of the early phase of repolarization between epicardial and endocardial hypertrophied cardiomyocytes might be ascribed to the experimental model (Goldblatt hypertension) and the duration (2 months) of hypertrophy. Under our experimental conditions, we observed the same delay for the entire epicardium repolarization as that usually mentioned for the endocardium.

A second important finding of our study is that both ventricles were hypertrophied without any signs of heart failure. We did not observe the first indices of heart failure until 11 to 12 months of aortic constriction and then only in a few rats. Moreover, it is well known that our model remains in the compensatory phase for months (Swynghedauw and Delcayre 1982). It has also been demonstrated that RVH and LVH are associated in dogs after pulmonary artery banding (Buccino *et al.* 1969), in Goldblatt rats (Gülch 1980) and in patients with hypertension (Nunez *et al.* 1987), but the electrical and some functional consequences have received little attention. In our experimental conditions, the same electrical changes were associated in both directly and indirectly stressed ventricles. This means that whatever the origin of the hypertrophic process, a left or a right ventricular pressure overload will sooner or later have anatomical and electrical repercussion throughout the entire myocardium. After different durations of the pressure overload, these changes may be more or less marked. In accordance with our measurements, APD 50 had previously been shown to be 88 % longer in the

left ventricles of control rats than in the right ventricles (Gülch 1980), but after a 2 to 3-month chronic loading (Goldblatt) which caused a biventricular increase in APD 50, the left/right difference was much more marked (+157 %). In our model, the left/right difference in APD 80 was no longer significant after a one-month pressure overload. The other left/right differences also became less significant, or no longer significant, after a one-month pressure overload.

Thirdly, we noted in both ventricles the same, although non-significant, tendency to a decrease in dV/dt max. A significant reduction in the rate of rise of the AP was mentioned by some authors (Gelband and Bassett 1973, Tritthart *et al.* 1975) in animals at a later stage of cardiac hypertrophy or even presenting heart failure. This observation suggests that more severe hypertrophy or longer duration of the overload would also have caused reduction in the maximum upstroke velocity in our model.

Table 4 : basic changes in activation time (AT) and in repolarization time (RT) in epicardial left and right ventricular cells due to a 1-month aortic overload and changes due to 25 $\mu g \cdot kg^{-1} \cdot min^{-1}$ epinephrine infusion (E). Values are means ± SEM.

groups	LV		RV	
	AT 80 msec	RT 80 msec	AT 80 msec	RT 80 msec
C (n = 13)	13±1*	66±2***	15±1*	63±4***
C + E	14±1	92±3†††	15±1	89±4†††
H (n = 12)	15±1	104±6††	18±1	100±6††
H + E	15±1	129±6**	18±1	129±7**

n = number of rats ; * : $p \leq 0.05$; ** : $p \leq 0.01$; *** : $p \leq 0.001$ (comparison H vs C)
† : $p \leq 0.05$; †† : $p \leq 0.01$; ††† : $p \leq 0.001$ (comparison E vs base)

AT 80 : activation time to 80% of repolarization ; C : control sham-operated animals ; E : epinephrine ; H : animals with experimental aortic stenosis ; LV : left ventricle ; RT 80 : repolarization time to 80% of repolarization (RT = AT + APD : details in text) ; RV : right ventricle

Changes in the ECG

Let us examine the consequences of these cellular electrical changes on the aggregate ECG.

Although the atrial electrical activity was not the purpose of this study, we could note a prolonged PR interval which is an expression of the reduction in atrio-ventricular conduction velocity.

Despite the increased cell diameter in such a model of cardiac hypertrophy (Rapaport 1982), a factor which has a positive influence on the conduction velocity, we also observed a longer QS which is expression of depressed ventricular conduction. QS correlated with HI% ($r = 0.68$; $p = 0.0002$). The QRS complex was lengthened without any obvious change in its shape. The cause of such an increase in QRS duration can hardly be attributed to cellular uncoupling. However, since a significant and progressive reduction in dV/dt max has already been demonstrated and logically associated with a decrease in conduction velocity in the course of progressive ventricular hypertrophy (Tritthart *et al.* 1975), we consider the non-significant decrease in dV/dt max we observed in both ventricles to be related to a small but progressive reduction in intracellular conduction velocity at the epicardial level.

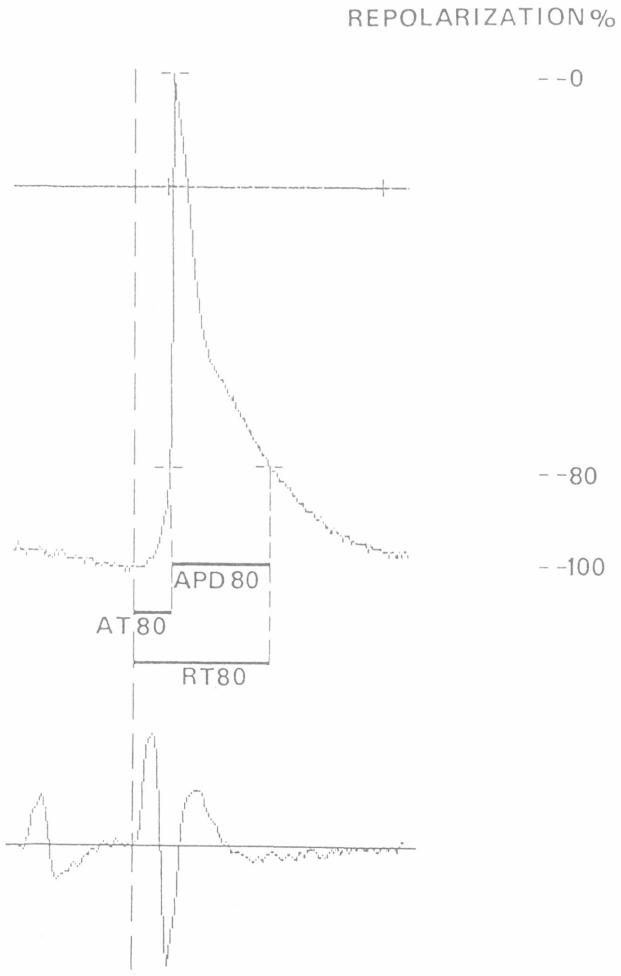

Fig. 3. *Method used to compare the state of repolarization at different recording points. The time from the activation of the earliest activated ventricular site to 80 % of repolarization at the recording point by adding the duration of the action potential from the upstroke to 80 % of repolarization to the delay from the onset is obtained of the QRS complex to the upstroke of the action potential (RT = AT + APD) (see "Discussion", third part). APD 80: action potential duration to 80% of repolarization; AT 80: activation time to 80 % of repolarization; RT 80: repolarization time to 80 % of repolarization.*

A correction of QT for the heart rate showed a significant prolongation of ventricular repolarization in the hypertrophied myocardium. In addition, QTc strongly correlated with HI% ($r=0.81$, $p=0.0001$). In the hypertrophied group, the lengthening of the T wave was generally associated with its flattening (despite this flattening, the rates of sampling which our technique allows were sufficient to determine the precise shape and duration of the T waves). The existence of the T wave implies a regional difference in the state of repolarization of the cardiac fibres on both sides of the plane perpendicular to the axis of the recording lead and including the zero reference potential point of the heart. The genesis of the T wave has generally been associated with both apex-basis and epi-endo differences, but can also be linked to other regional differences (Burgess 1979). Since the recording axis was orthogonal to the partition plane of the two ventricles (Fig. 1) and since the course of the repolarization in epicardial fibres does not differ appreciably from that always observed in endocardial fibres (see previous section), the flattening of the T wave, objectified by the depressed TM, reflects a tendency to a more simultaneous repolarization of the left and right ventricles. Any decrease in the difference in AP durations has been known for years to reduce the T wave (Cohen *et al.* 1976). Figure 2 clearly illustrates that a reduction in T wave magnitude is associated with a prolonged and more "triangular" AP in both left and right hypertrophied ventricles.

Comparison of the state of repolarization in left and right ventricles

Better than measuring APD on the intracellular recordings and better than a study of the T wave alone, the simultaneous recording of ECG and transmembrane potentials allows us to study the variations of the state of repolarization resulting from hypertrophy and epinephrine perfusion in the two recording sites. Accordingly, we performed further measurements. The state of repolarization is determined by the local repolarization time (RT) which is linked to APD at the same level of repolarization: RT = AT + APD (Franz *et al.* 1987). The activation time AT and the other features are illustrated in Figure 3. Left and right equalization of aggregate RT at 80 % of repolarization (RT 80) reflects the tendency to simultaneous repolarization of both ventricles. Although our intracellular recordings only concerned one region in each ventricle and although these two regions had different basic activation times AT 80, we compared the left and right evolutions of AT 80 and RT 80 in relation to hypertrophy and epinephrine perfusion. Table 4 shows that AT 80 was significantly increased after the aortic overload in the right as well as in the left ventricle epicardium. This confirms a lower conduction speed in the hypertrophied ventricles, despite the non-significant decrease in dV/dt max (Table 2). Epinephrine infusion at our arrhythmogenic concentrations neither improved nor worsened ventricular conduction. As cardiac overloading also increased APD 80, RT 80 was considerably lengthened in both hypertrophied ventricles and epinephrine generally increased this effect. Interesting findings were obtained by a left/right comparison (Wilcoxon paired

test) of these parameters (Fig. 4). By reducing intraventricular conduction, the aortic overload also decreased (p=0.027) the significant initial difference (p=0.002) between the activation times (AT 80) of the two recording sites. As a consequence of these APD and AT changes, the small and non-significant (p=0.279) left/right difference in the state of repolarization (RT 80) between the two sites, which was increased (p=0.173) by epinephrine in the control rats, was reduced (p=0.530) in the hypertrophied rats and minimized (p=0.814) by epinephrine. Figure 4 allowed us to compare the effects of aortic overload and epinephrine infusion on the left/right differences in APD 80, RT 80, AT 80 and dV/dt max on the one hand and on T wave magnitude on the other: it confirms the tendency to a simultaneous repolarization of left and right ventricles.

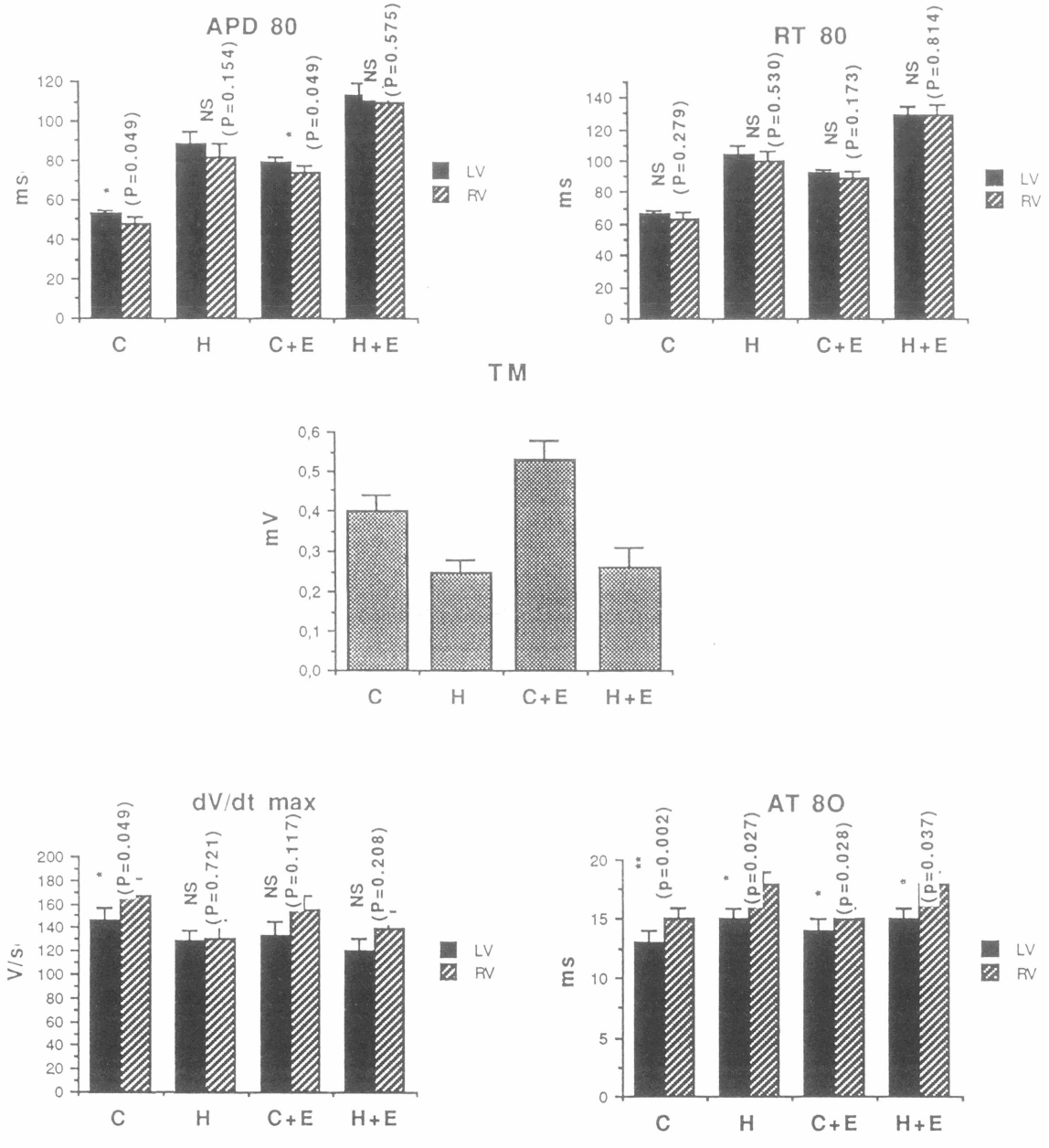

Fig. 4. *Left/right comparison of some cardiac electrophysiological characteristics in sham-operated rats (C) and in rats after a one-month aortic overload (H) under basic conditions or after epinephrine infusion. APD 80: action potential duration to 80 % of repolarization; AT 80: activation time to 80 % of repolarization; dV/dt max: maximum depolarization speed (first derivative); E: epinephrine; LV: left ventricle; RV: right ventricle; RT 80: repolarization time to 80% of repolarization; TM: magnitude of the T wave.*

Action of epinephrine and its relationship to ventricular arrhythmias

The depression of conduction velocity and temporal dispersion of the recovery of excitability (whether the refractory period is reduced or increased) are recognized as important factors favouring the development of ventricular arrhythmias (Han and Moe 1964, Lessard and Paulet 1986). Adrenergic influences are known to facilitate the induction of ventricular arrhythmias mainly by increasing the non-uniformity of cardiac excitability during repolarization (Han *et al.* 1964). Whether the refractory periods are increased or reduced, more temporal dispersion in action potential downstrokes will result in a more marked (positive or negative) T wave, and less temporal dispersion in action potential downstrokes will result in a flatter T wave (Burgess 1979). Giant T waves are often associated with ventricular arrhythmias (Han and Goel 1972). In our study, epinephrine enhanced T wave magnitude in rats of group C and caused ventricular arrhythmia. We already demonstrated that rats of group H after a one-month aortic stenosis exhibit less severe epinephrine-induced ventricular arrhythmias than sham-operated rats. Such a mechanism is not likely to be dependent on a general downregulation of cardiac beta-adrenoceptors (Lessard *et al.* 1992). It might be linked to a more uniform distribution of these receptors due to biventricular hypertrophy. The lack of epinephrine-induced arrhythmia in rats of group H after a one-month pressure overload is associated with lowered T waves and simultaneous repolarization of the left and right ventricles. At this stage of hypertrophy, in spite of a depressed ventricular conduction velocity, the similar repolarization states in both ventricles do not favour re-entries at the left and right junctional areas of the myocardium.

Mainly from observations in patients and from studies on *in vitro* preparations of different experimental animal models, it is usually stated that the hypertrophied myocardium is always associated with an increased propensity for ventricular arrhythmias. Considering our previous investigations and the present results, this statement should be somewhat modified: ventricular arrhythmia susceptibility depends on the electrophysiological characteristics of cardiomyocytes and hence on the characteristics of the model of hypertrophy including the duration of the cardiac overload. As the left, right ventricular weight ratio continuously evolves during pressure-dependent hypertrophy (Nordlander 1980), consequent changes in left/right electrophysiological characteristics induce changes in ventricular vulnerability to arrhythmias. According to the duration of the cardiac overload, ventricular arrhythmia vulnerability may be increased, unchanged or decreased. After a one-month aortic overload, ventricular vulnerability is decreased. The study of the T wave form in a lead exploring the left-right differences in ventricular repolarization might be useful for predicting the likelihood of development of ventricular arrhythmias in the course of cardiac hypertrophy.

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List of abbreviations

APA: action potential amplitude
APD: action potential duration
AT: activation time
c: corrected for heart rate
dV/dt max: maximum depolarization speed (first derivative)
E: epinephrine
HI: hypertrophy index
HR: heart rate
LV: left ventricle
RMP: diastolic resting membrane potential
RV: right ventricle
RT: repolarization time (RT = AT + APD)
TM: magnitude of the T wave

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