

# Arrhythmogenic effects by local left ventricular stretch: effects of flecainide and streptomycin

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**Abstract** Mechanical stretch has been shown to provoke arrhythmia. We wanted to analyze ventricular arrhythmia induced by local left ventricular stretch in order to find out, where arrhythmias originate and whether they can be prevented pharmacologically. Isolated rabbit hearts (Langendorff technique) were submitted to increased left ventricular stretch at the left wall by insertion of an additional intraventricular balloon and adjusting the end-diastolic pressure (EDP) to 25 mmHg for 10 min followed by 20 min recovery at normal EDP of 5–8 mmHg. Activation and repolarization processes were investigated by ventricular 256 electrode epicardial mapping. The hearts were treated during the whole procedure either with vehicle, 0.5  $\mu$ M flecainide (sodium channel blocker) or 100  $\mu$ M streptomycin (here used as stretch-activated ion-channel blocker). In addition, we performed a series of experiments, in which we enhanced EDP to 30 mmHg (global stretch instead of local stretch) by inflating the left ventricular pressure balloon (strain,  $0.148 \pm 0.034$ ). Each series was performed with  $n=6$ . Stretch resulted in local strain of 25 % at the left wall together with a local slowing of the activation process at the left wall, in a change in the activation pattern, and in ventricular arrhythmia. Coronary flow was not affected. Ventricular arrhythmias originated from the border between the stretched area and the non-stretched region. Flecainide and streptomycin reduced the prolongation of the activation process at the stretched left wall and mitigated the difference in total activation time between left and front wall but only partially prevented arrhythmia. In the additional global stretch experiments relative coronary flow and the

other parameters remained unchanged, in particular TAT. Thus, in contrast to the local stretch series, there was no difference in the change in TAT between left and front wall. Only rare single ventricular extrasystoles ( $<1/\text{min}$ ; originating from LV (front and left wall) i.e. from within the stretched region) were seen during stretch (but not at the beginning) and during recovery. Local left ventricular stretch can elicit ventricular arrhythmias. Local slowing of electrical activation seems involved so that the difference in total activation time of the stretched free left wall and the non-stretched increased.

**Keywords** Cardiac stretch · Ventricular arrhythmia · Flecainide · Streptomycin · Epicardial mapping

## Introduction

Ventricular arrhythmia is still an important problem in clinical medicine. Besides other etiologies one cause of ventricular arrhythmia has been suspected to be mechanical ventricular stretch (Mills et al. 2005). This can be due to, e.g., acute volume overload, aneurysms, medical interventions, commotio cordis, or may also occur during cardiac surgery. However, the underlying mechanisms remain widely unclear (Mills et al. 2005), involving a complex interplay of mechano-electrical feedback, ion channels, and structural factors (Quinn and Kohl 2013).

In order to address the problem of acute ventricular stretch and arrhythmia, we wanted to create an acute local stretch of the left ventricular free wall sustained over a period of 10 min. We hypothesized that (a) this may delay activation of the stretched side, (b) slowing of ventricular conduction by 0.5  $\mu$ M flecainide (Dhein et al. 1993) may suppress arrhythmia, and (c) the local overload could activate stretch-activated ion channels [permeable to  $\text{Ca}^{2+}$  and  $\text{Na}^+$  (Kamkin et al. 2000)] thereby eliciting local electrophysiological effects

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which might act arrhythmogenic. In order to block these channels, we infused streptomycin intracoronarily, since this drug is known to block these channels in concentrations of 40–100  $\mu\text{M}$  (Winegar et al. 1996; Belus and White 2003; Eckardt et al. 2000).

## Methods

### Heart preparation and epicardial mapping

All experiments were performed in accordance with the ethical rules of the Council for International Organization of Medical Science and the German/European laws for animal welfare. The study was approved by the local ethical committee for animal welfare and the investigation conforms to the Directive 2010/63/EU of the European Parliament as well as to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996).

The method of heart preparation and epicardial potential mapping has been described in more detail previously (Dhein et al. 1993) and will be explained only briefly here.

Male white New Zealand rabbits (conventional, normally fed ad libitum, 1,500–1,800 g, Charles River, Erkrath, Germany) were treated with 1,000 IU/kg heparin i.v. 5 min before the hearts was removed in deep anesthesia (0.3 mg/kg medetomidin, 30 mg/kg ketamine). The heart was excised, prepared and perfused according to the Langendorff technique at constant pressure (70 cm  $\text{H}_2\text{O}$ ) with Tyrode's solution of the following composition:  $\text{Na}^+$  161.02;  $\text{K}^+$  5.36;  $\text{Ca}^{2+}$  1.8,  $\text{Mg}^{2+}$  1.05;  $\text{Cl}^-$  147.86;  $\text{HCO}_3^-$  23.8;  $\text{PO}_4^{3-}$  0.42 and glucose 11.1 mM; equilibrated with 95 %  $\text{O}_2$  and 5 %  $\text{CO}_2$  (pH=7.4). The surface temperature of the heart was 37 °C. A water-filled balloon (max. volume 1.0 ml) was inserted into the left ventricle for recording the left ventricular pressure. Thereafter, a second empty small balloon (stretch balloon; max. volume 0.2 ml; see Fig. 1) was inserted in the left ventricle and positioned at the left lateral side wall (location: area, X21/Y3//X21/Y8–X24/Y8//X24/Y8 (electrode number, as displayed in Fig. 4)). End-diastolic left ventricular pressure was adjusted to 6–8 mmHg by injection of 0.7 ml water in the first balloon. The second “stretch balloon” was completely empty.

Next, the hearts were connected to a 256 channel mapping system HAL4 (temporal resolution: 4 kHz/channel; amplitude resolution: 0.04 mV, inter-channel coupling less than –60 dB; bandwidth of the system: 0.5 Hz–20 kHz, data were not filtered) as described previously (Jozwiak et al. 2012). Two hundred fifty-six AgCl electrodes were cast in four polyester plates (in  $8 \times 8$  orthogonal matrices with 1 mm inter-electrode distance), and were attached to the hearts surface in an elastic

manner, so that they could follow the hearts movements easily without dislocation. The hearts were spontaneously beating during the entire experimental protocol.

### Experimental and stretch protocol

After an equilibration period of 45 min the hearts were perfused either with vehicle (Tyrode solution) or with flecainide (0.5  $\mu\text{M}$ ), or with streptomycin (100  $\mu\text{M}$ ) (both drugs were diluted in Tyrode solution).

After another 10 min, we injected 0.2 ml water into the second “stretch balloon” so that the end-diastolic left ventricular pressure was elevated to 22–23 mmHg (start of stretch period). Additionally, we measured the resulting local strain in the stretch balloon area and in the rest of the left ventricle by setting small marks. Strain  $\varepsilon$  was calculated as the change in length related to the initial length as

$$\varepsilon = X' - X / X$$

with  $X$  as the initial length, which is stretched to a final length  $X'$ . Thus, the strain measure  $\varepsilon$  is defined as the ratio of elongation with respect to the original length and is dimensionless (Salameh and Dhein 2013). The resulting local strain which was applied thereby to an area of 14  $\text{mm}^2$  of the left ventricular wall (in the area of the stretch balloon) was about 0.25 (see Fig. 1). After a 10-min stretch we deflated the “stretch balloon” completely, so that end-diastolic left ventricular pressure recovered to 6–8 mmHg. Subsequently, we registered all parameters for a recovery period of 20 min.

Maximum systolic developed left ventricular pressure (LVP), end-diastolic LVP (EDP), heart rate measured as basic cycle length (BCL) and coronary flow (CF) were assessed continuously as described (Dhein et al. 1993).

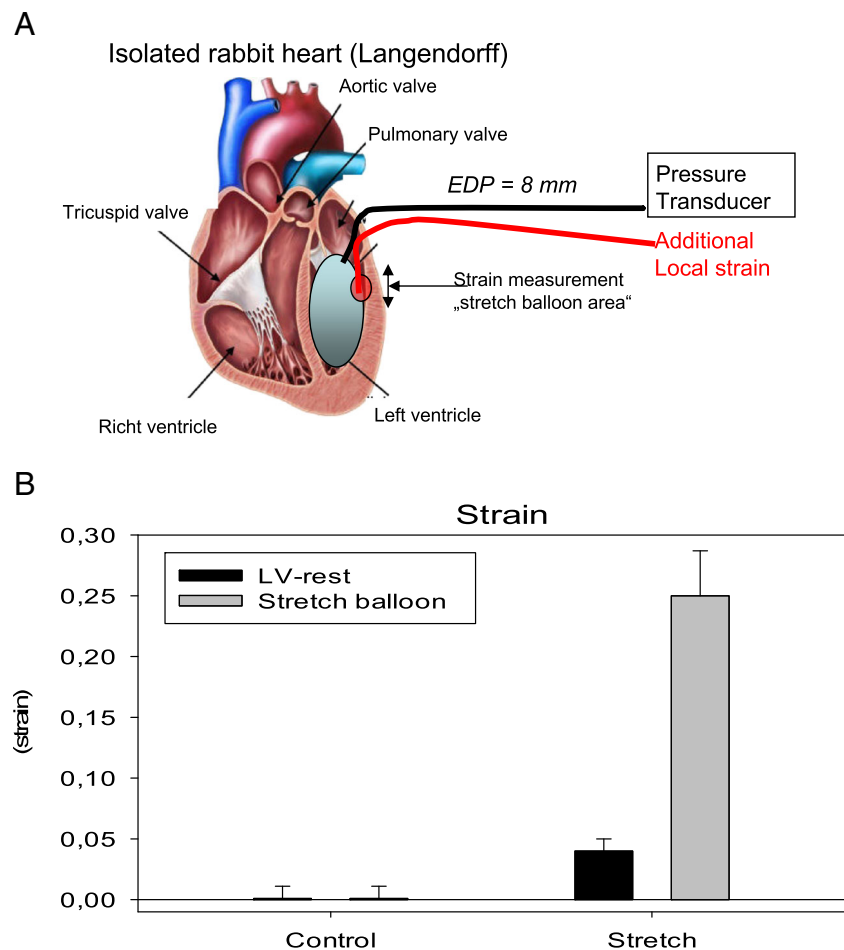
In addition, we performed a series of experiments, in which we enhanced EDP to 30 mmHg (global stretch instead of local stretch) by inflating the left ventricular pressure balloon. This resulted in a strain of  $0.148 \pm 0.034$ . Moreover, we performed a time control series over a period of 120 min without treatment.

### Analysis of parameters

Epicardial potential mapping was carried out in each experimental phase during rhythmic periods of constant cycle length of at least 3 min, in order to make it possible to compare the activation patterns (of single heart beats) or their alterations.

For evaluation of the mapping data, the activation time points at each electrode were determined as  $t(dU/dt_{\min})$  during the activation complex, i.e., time of fastest intrinsic deflection (Dhein et al. 1993; Durrer and Van der Tweel 1954; see also inset to Fig. 3a). Next, the repolarization time points were determined as  $t(dU/dt_{\max})$  during the T-wave as described (Dhein et al. 1993; Millar et al. 1985). After automatic

**Fig. 1** Panel **a** position of the “local” stretch balloon in the left ventricle. Below (panel **b**) find the results for the strain measurements as means $\pm$ SEM (calculation and definition of stretch see “Methods”). The “stretch balloon” columns indicate the strain which was measured at the left ventricular wall within the area beneath which the stretch balloon was positioned. The “rest of LV” columns indicate the strain which was measured in the left ventricle (mainly at the front wall) outside the “stretch balloon” area



determination activation and repolarization times were verified (or corrected if necessary manually by the investigator). From these data, for each electrode an activation recovery interval (ARI, corresponding the epicardial potential duration) was calculated. Basic cycle length (BCL) was assessed for determination of frequency.

The distribution of ARI was analyzed for each area of the heart (i.e., front, left, right or back wall) calculating the standard deviation of ARI at 64 electrodes and expressed as ARI-dispersion. From the activation time points an activation sequence was determined. We determined those electrodes which were activated before any of the neighboring ones and defined them as “breakthrough-points” which can be considered as the origins of epicardial activation (Arisi et al. 1983). This analysis and the construction of activation patterns allowed detection of the origin of a ventricular arrhythmia.

In a similar way, the spread of epicardial excitation was analyzed. In order to allow a quantitative and comparative description of the activation process for each electrode an activation vector was calculated from the activation times and the locations of the surrounding electrodes which were

activated after the central electrode (i.e., a maximum number of 8), as described by Müller et al. (1991). These vectors give direction and apparent velocity of local activation. The percentage of similar vectors (VEC) between heart beats in the various experimental phases compared with those under control conditions was determined (vectors deviating not more than 5° from their original direction were considered to be similar) (Dhein et al. 1993; Müller et al. 1991).

In addition, the total activation time (TAT, [ms]) was assessed as the delay between activation of the first and activation of the last electrode of all 256 electrodes. More in detail, we also determined local TAT at each of the four sides as the delay between activation of the first and the last electrode of the 64 electrodes within one plate (right, front, left, or back).

Moreover, the ST-segments of the 256 ECG’s were analyzed. We summed up all deviations from the isoelectrical level at 50 % of mean ARI and calculated the total ST-deviation of 256 leads in arbitrary units [a.u.]. An increase in that parameter points to an increase in efflux of positively charged ions during the action potential and is indicative for ischemic regions (Coronel et al. 1988, 1992).

## Statistics

All values are given as MEANS±SEM of  $n=6$  experiments in each series. Significance was analyzed using analysis of variance for comparison of multiple groups. If ANOVA indicated significant differences Wilcoxon rank test for paired observations or Mann  $U$  test for unpaired observations were performed. The occurrence of arrhythmia and the possible suppression by the treatment was investigated using Chi square test. For all statistics, the level of significance was  $p<0.05$ .

## Chemicals

All chemicals used in this study were of analytical grade. All chemicals were purchased from Sigma (Taufkirchen, Germany), except heparin which was from Serva (Heidelberg, FRG).

## Results

Inflation of the second balloon resulted in significant elevation of left ventricular end-diastolic pressure in all three experimental series during the 10 min of stretch application by approximately 20 mmHg (see Fig. 2a). The developed left ventricular systolic pressure, however, was not significantly affected (Fig. 2b), and remained nearly constant in all three groups. However,  $dP/dt(\max)$  as a measure of contractility was increased upon stretch from  $1,294\pm 49$  to  $1,354\pm 54$  mmHg/s, i.e. by about 5 % ( $p<0.05$ ).

Regarding the resulting pressure and strain, it needs to be noted that the use of two balloons leads to elevated pressure in all areas of the ventricle but to an eccentric dilatation. Consequently, we also determined the strain in the balloon area and in the rest of the left ventricle, by setting small marks in these areas and measuring the distance before and during inflation of the balloon. The results are shown in Fig. 1 (lower panel): as can be seen there was a strain of about 0.25 in the stretch balloon area and only a moderate strain of ca 0.04 in the rest of the left ventricle.

Atrioventricular conduction time (PQ), and basic cycle length remained unaffected (Fig. 2c–d) during the experiments. BCL was slightly but significantly longer in the drug treatment series, but did not change over time.

Moreover, coronary flow also was not significantly reduced during stretch, so that the relative CF (CF/LVP) was unaltered during the entire experiment (Fig. 2e).

With regard to the electrophysiological parameters, we first investigated the ST-segments and found that they were not elevated and remained unchanged during stretch and recovery in all series (Fig. 2f).

The epicardial activation recovery intervals ARI (all 256 sites) also did not change significantly during stretch or during recovery. In flecainide- and streptomycin-treated hearts the

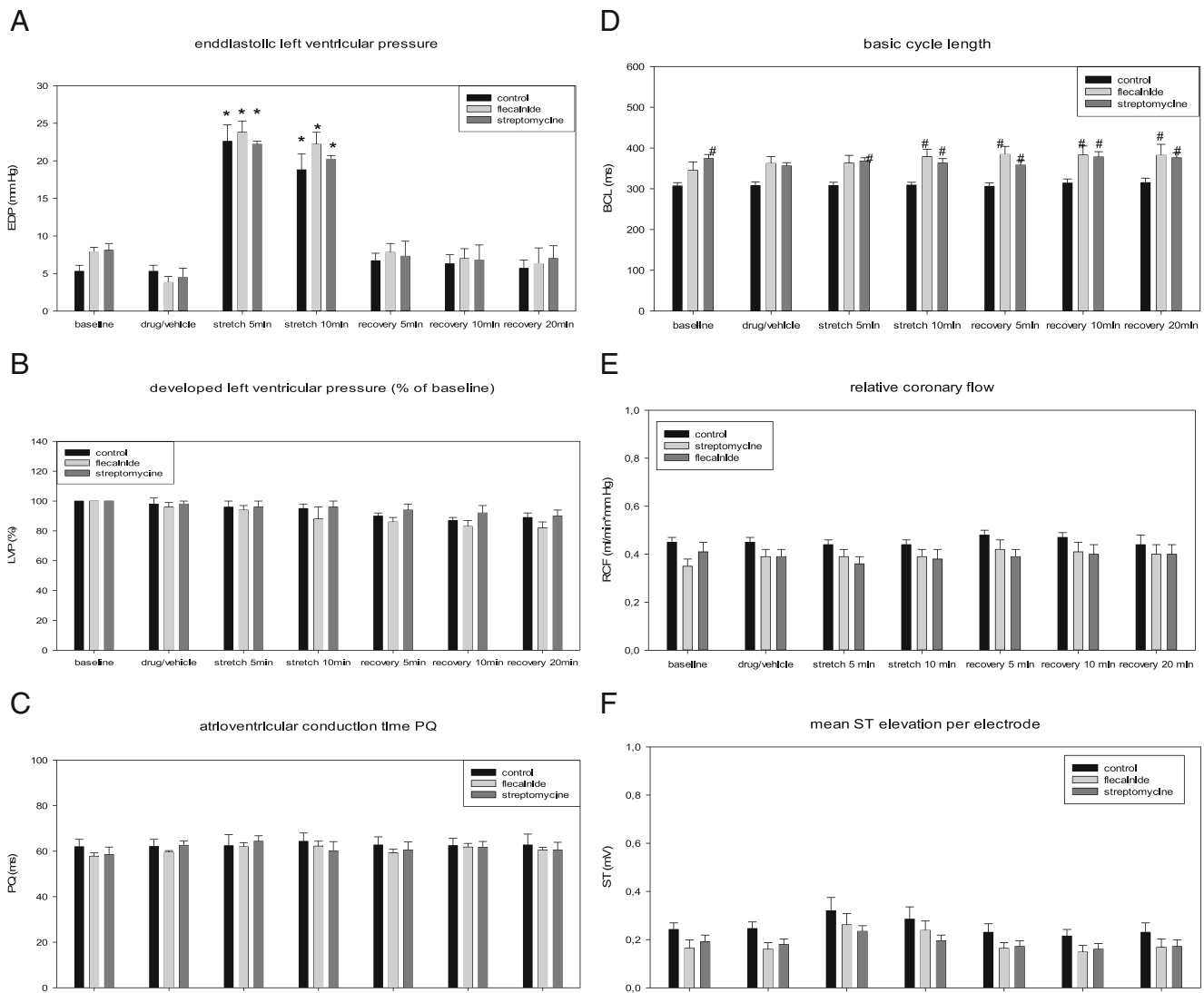
ARI were slightly longer than in control hearts (Fig. 3a). In addition, we evaluated the local ARI at the left wall (i.e., in the stretched region). Both drug treatments led to a small prolongation of ARI. Stretch and recovery did not significantly alter ARI (Fig. 3a). However, regarding the stretched left ventricular wall, in the first 5 min of stretch, there was a small, not significant change in ARI at the stretched left wall in untreated hearts by  $+4.3\pm 3.8$  ms which, however, was not significantly altered by the drug treatments, and did not depend on BCL.

Next, we focused on the homogeneity of the epicardial activation and recovery. First, we investigated the dispersion of ARI. However, there was only a small and not significant increase in dispersion during stretch in all 3 series followed by a slight, also not significant, decrease during recovery (Fig. 3c). Again there were no differences between the three series. Moreover, the changes in dispersion were independent from BCL ( $p=0.403$ ).

In the next step, we analyzed the activation process and determined the local direction of activation for heart beats under stretch and recovery conditions and compared these to the vectors under baseline conditions. We found that the percentage of vectors with similar directions significantly dropped under stretch in untreated hearts and thereafter slightly recovered (Fig. 3b).

When analyzing these activation processes we found that not only the direction of the waves changed but also the time required for activation of all electrodes (total activation time, TAT) was increased upon stretch (Fig. 4a, b). As can be seen from the figure, the activation process was slowed at the left ventricular wall, i.e., in the area of the “stretch balloon” and its circumference. Therefore, we next calculated the increase in total activation time at the free left wall (where the second balloon was positioned; strain=0.25) and at the front wall (strain=0.04). This clearly showed significant prolongation of the activation process at the left wall in relation to the activation of the front wall under stretch ( $p<0.05$ ). Thus, the activation process becomes inhomogeneous and is significantly more slowed in the area of the “stretch balloon” (i.e., at the left side wall) during stretch than at the front wall (Fig. 3e).

Next, we wanted to find out whether these changes might be sensitive to pharmacological treatment using either a classical antiarrhythmic drug (flecainide) or a drug which blocks stretch-activated ion channels (streptomycin). When applying the sodium channel blocker flecainide we observed a slight prolongation of the whole hearts TAT ( $p<0.05$ ) ( $p<0.05$ ; Fig. 3f). The stretch-induced local prolongation of TAT at the left wall was significantly but not completely antagonized by flecainide ( $p<0.05$ ) (Fig. 3f), while the TAT at the front wall was prolonged, so that the difference in TAT between left wall and front wall was always insignificant. The effects of stretch on TAT or the derived parameters were not dependent on BCL ( $p=0.703$ ).



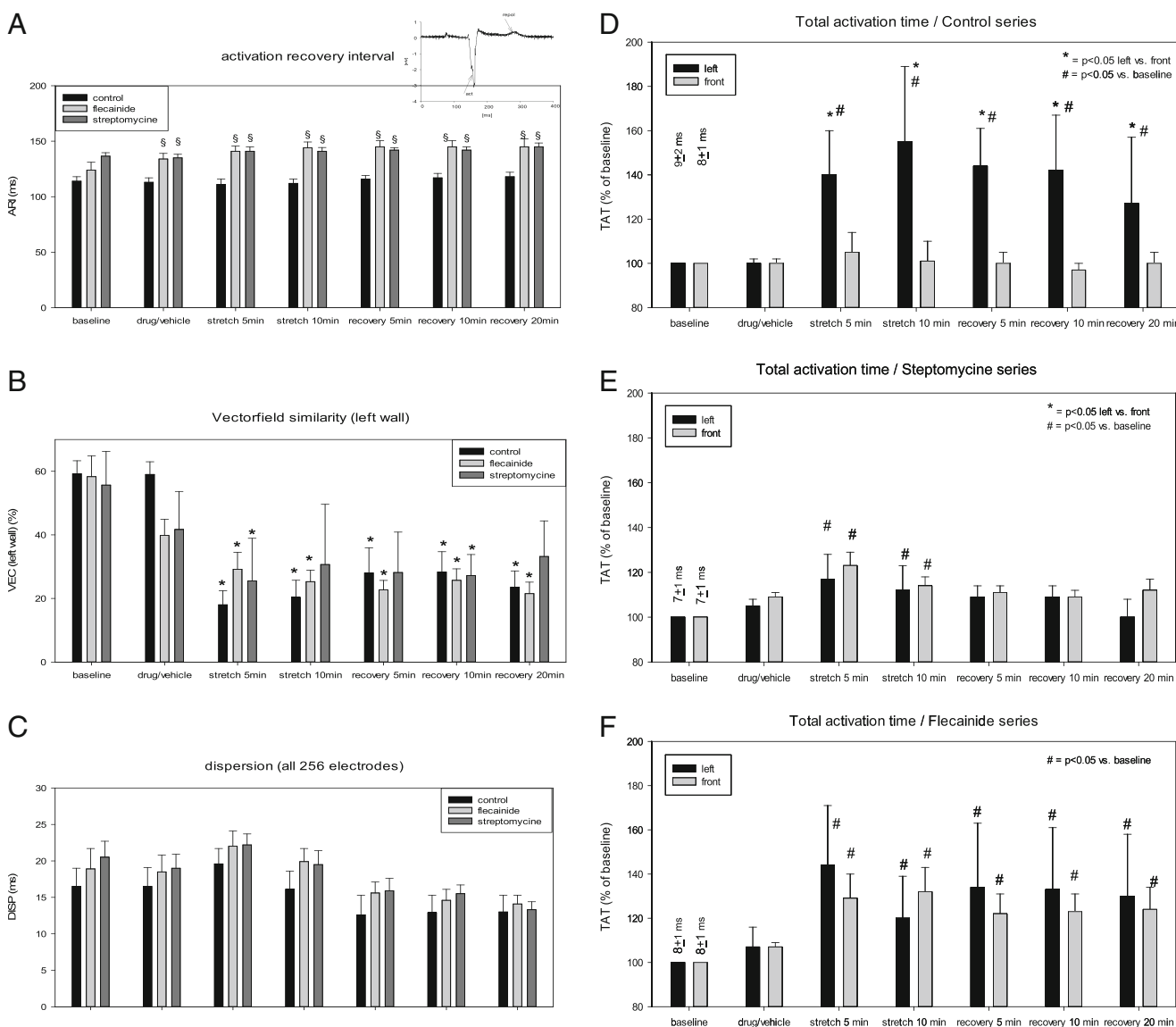
**Fig. 2** Effects of the pre-treatment, local stretch and recovery under treatment with either vehicle, flecainide ( $0.5 \mu\text{M}$ ) or streptomycin ( $100 \mu\text{M}$ ) on end-diastolic pressure (a), left ventricular developed systolic pressure (b) ( $100 \%$ =control:  $81 \pm 6$ ; flecainide:  $95 \pm 5$ ; streptomycin:  $106 \pm 8$  mmHg), atrioventricular conduction time

(c), basic cycle length (d), coronary flow (e), and ST elevation (f) given as means $\pm$ SEM of  $n=6$  experiments in each series. For 10 min, stretch was applied by elevating the end-diastolic pressure ( $*p < 0.05$  vs. baseline;  $\#p < 0.05$  vs. untreated control series)

The application of streptomycin also very slightly prolonged TAT before stretch. Thereafter, the stretch-induced prolongation of TAT was significantly antagonized (see Fig. 3e). There was only a slight stretch-induced prolongation of TAT, which however was significantly lower than in control hearts ( $p < 0.05$ ). Of note, there was no difference in TAT between left and front wall during the entire experiment (Fig. 3e). Both drug treatment significantly reduced the left-front TAT difference ( $p < 0.05$ ).

Finally, regarding the occurrence of ventricular arrhythmia, we observed ventricular extrasystoles in three of six control experiments during the stretch period (frequency of VES:  $>1/\text{min}$ ). Interestingly, we did not observe ventricular arrhythmia in the first 5 min of stretch, but in the subsequent 5 min. The

origin of these arrhythmias was never in the area of the “stretch balloon” itself but in two cases in non-adjacent areas and in one case in an area adjacent to the stretched area. During recovery from stretch, in untreated control hearts, we observed both VES and non-sustained ventricular tachycardia, nearly all originating from the left wall (only one came from the back wall), but in no case from the area of the “stretch balloon”. All arrhythmia came from sites more or less adjacent to the stretched area. In total, five of six hearts of the control series exhibited VES/VT during the experiment. VES rate was mostly  $>1/\text{min}$ . In Table 1, the origin of every arrhythmia is indicated with the X-electrode/Y-electrode position. None of these origins located within the area of the “stretch balloon” (X21/Y3//X21/Y8–X24/Y8//X24/Y8; see Table 1 and



**Fig. 3** Effects of the pre-treatment, local stretch and recovery under treatment with either vehicle, flecainide (0.5  $\mu$ M) or streptomycin (100  $\mu$ M) on activation recovery intervals (ARI) (a), vectorfield similarity (VEC) (b), dispersion (c) (\* $p$ <0.05 vs. baseline;  $\S$  $p$ <0.05 vs. untreated control series). In panels d–f a comparison of the total activation time at front and left wall (TAT) in untreated (d), streptomycin (e) and flecainide-

treated hearts (f) is shown (\* $p$ <0.05 left vs. front;  $\#p$ <0.05 vs. baseline). All data are given as means $\pm$ SEM of  $n=6$  experiments in each series. The inset in panel a depicts a unipolar epicardial electrogram and the time of the fastest deflection ( $t(dU/dt_{min})$ ) (activation time) and ( $t(dU/dt_{min})$ ) (repolarization time) (see “Methods” sections for details)

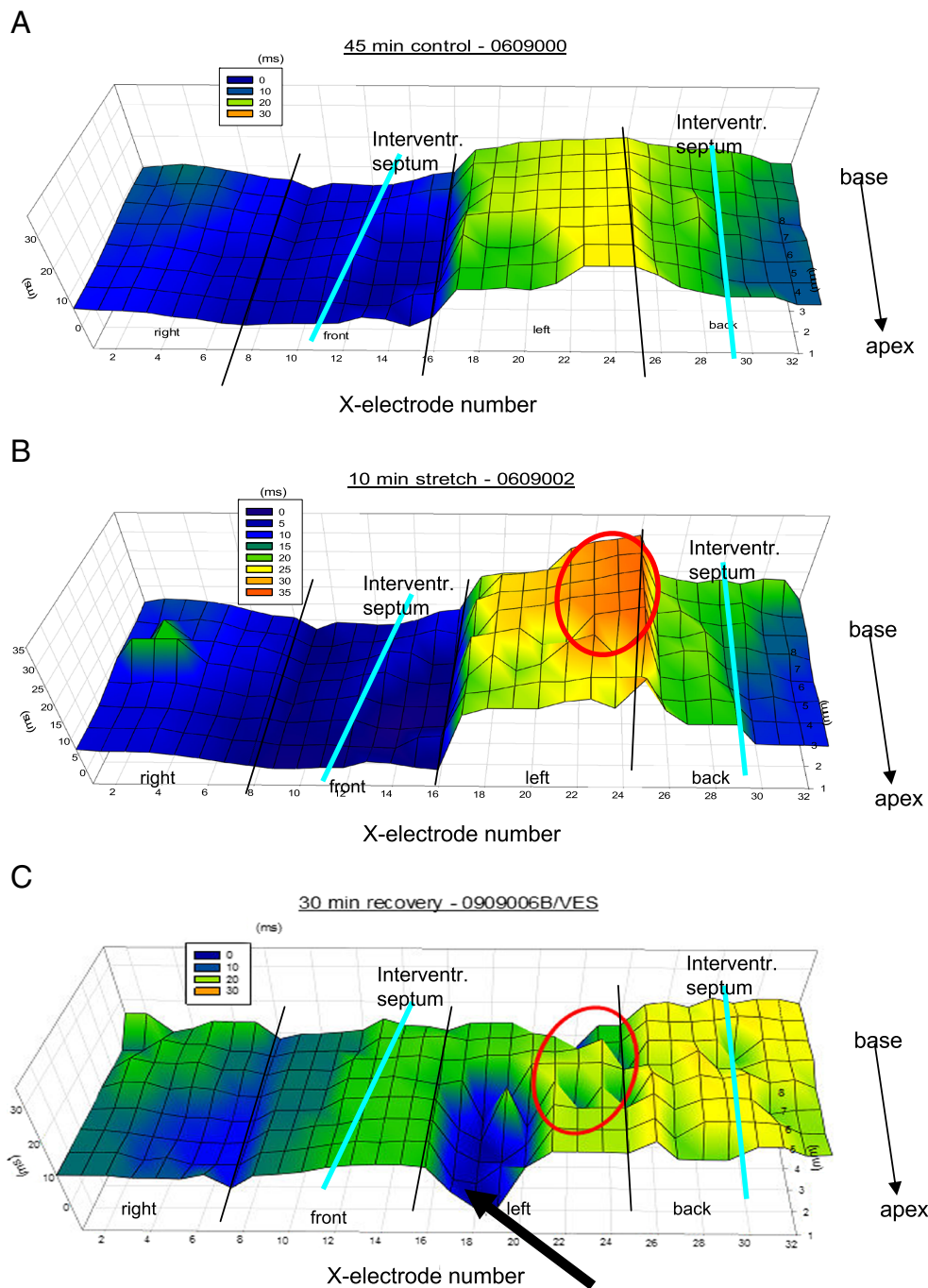
Fig. 4c). In some cases, arrhythmia was initiated at the back wall, but in these cases always from the left ventricular side of the back wall near to the interventricular septum. In no case did arrhythmia originate from the right ventricle.

Moreover, while in the control group VES were seen during both stretch and recovery period, VT was only observed during recovery from stretch.

Treatment with flecainide significantly reduced VES at 10 min stretch. However, a non-sustained VT was observed at this time in the flecainide series. Moreover, flecainide significantly reduced VES in late (20 min) recovery from

stretch, but had no effect on VT at that time. In total, two of six hearts of the flecainide series showed arrhythmia during the experiments. In contrast to the control series, in flecainide-treated hearts no arrhythmia originated from the left wall.

Although under streptomycin the total number of hearts with arrhythmia was only three of six, only at late recovery (20 min) there was a reduction in VES, but not in VT (Table 1). In one case, we saw VES before stretch and after drug application. This was the only arrhythmia which originated from the right ventricle near to the anterior septum. The other three



**Fig. 4** Original activation maps during the 45-min equilibration (panel **a**) and after 10-min of local stretch (panel **b**). The Y-axis gives the local activation time. The orientation on the hearts surface is given with the maps. The location of the second “stretch”-balloon is *encircled*. The location of the interventricular septum on the front and back wall is indicated by a *light-blue line*. Panel **c** original activation map of a

ventricular extrasystole in a heart of the control series after 20 min recovery from local stretch. As can be seen, the earliest activation came from the lower front wall at the border to the left wall (*arrow*). The location of the second “stretch”-balloon is *encircled*. The location of the interventricular septum on the front and back wall is indicated by a *light-blue line*. This is a different experiment than displayed in panel **a**

arrhythmias under streptomycin were initiated at the left ventricle (left, front, or back wall) but always outside the area of the stretch balloon.

In additional control experiments, we inflated the pressure balloon to achieve end-diastolic pressure of 30 mmHg, i.e.,

without a local eccentric dilatation. This resulted in a strain of  $0.148 \pm 0.034$  ( $p < 0.05$ ). The relative coronary flow and basic cycle length remained unchanged. There was no significant alteration of the observed parameters—in particular there was only a slight (not significant) increase in total activation time

(left wall). Thus, in contrast to the local stretch series, there was no difference in the change in TAT between left and front wall, i.e., at all times in the course of this experiment the difference in TAT between left and front wall remained the same. In addition, we did not observe VT or VF in these experiments (Table 2; for comparison a time control is given in Table 3). Ventricular extrasystoles were seen during stretch (but not at the beginning) and during recovery. These VES were rarely with a frequency of VES: <1/min, and were all single VES. During stretch these VES originated from the front and left wall (near the apex), and during recovery also from front and left wall (two from the middle, two from an apex-near region), i.e., from within the stretched region.

## Discussion

The experiments show that 10 min of local left ventricular stretch can make the heart more prone to ventricular arrhythmia (Table 1). While this is in principle not new, we here show (a) that ventricular arrhythmia originates from areas in the circumference of the stretched area, (b) that local stretch leads to local conduction slowing, (c) that the resulting difference in the activation propagation between the stretched and non-stretched area seems to play a pathophysiological role in the

elicitation of stretch-induced ventricular arrhythmia, and (d) that in control hearts ventricular tachycardia only was seen during recovery, while VES were also observed during stretch. This, together with the finding that both drugs could only suppress VES but not VT, indicates that both are pathophysiologically different.

First of all, one could imagine that local ischemia could play a role. However, as can be seen, coronary flow was not affected (Fig. 2e) and similarly the ST-segments also remained unchanged (Fig. 2f). This is clearly against the assumption of an ischemic pathogenesis. In further support, ARI remained nearly unaltered (Fig. 3a, b), while ischemia typically would lead to marked local ARI shortening (Coronel et al. 1988, 1992; Jozwiak and Dhein 2008).

In isolated cells, stretch can elicit a mild action potential prolongation by activation of stretch-activated ion channels (Kamkin et al. 2000; White et al. 1993; Zeng et al. 2000). We also found a small non-significant prolongation in ARI in the first minutes of stretch (by  $+4\pm 2$  ms). However, it is necessary to state that stretch in isolated cells cannot be directly compared to the situation in the whole heart in the present experiments, which are a combination of local tissue stretch and elevated end-diastolic pressure (Salameh and Dhein 2013). The four-fold elevation in end-diastolic pressure (from 5–8 to 20–25 mmHg) does not simply mean a four-fold dilation. The local strain, which was applied to a  $14\text{ mm}^2$  left ventricular

**Table 1** Number of hearts exhibiting ventricular extrasystoles (VES)/tachycardia (VT) in the experimental series using the local stretch protocol (local strain=0.25).

	Arr.	BL	Drug/veh	Stretch 5 min	Stretch 10 min	Recov. 5 min	Recov. 10 min	Recov. 20 min
Control	VES	0/6	0/6	0/6	3/6 <sup>a</sup> (F,L,B) (X28/Y4; X21/Y2; X16/Y8)	2/6 <sup>a</sup> (F, F) (X10/Y8; X16/Y8)	3/6 <sup>a</sup> (B,L,L) (X29/Y1; X18/Y2; X17/Y5)	3/6 <sup>a</sup> (L,L,L) (X17/Y2; X18/Y1; X18/Y6)
	VT	0/6	0/6	0/6	0/6	0/6	1/6 (B) (X29/Y1)	1/6 (L) (X18/Y2)
Flecainide	VES	0/6	0/6	0/6	0/6*	0/6	1/6 <sup>a</sup> (F) (X12/Y2)	0/6*
	VT	0/6	0/6	0/6	1/6 (B) (X25/Y2)	0/6	0/6	1/6 (F) (X12/Y2)
Streptomycin	VES	0/6	1/6 (F) (X10/Y2)	0/6	1/6 (L) (X19/Y4)	0/6	0/6	0/6*
	VT	0/6	0/6	0/6	0/6	0/6	1/6 (F) (X15/Y4)	1/6 (B) (X28/Y2)

Behind the occurrence of arrhythmia, the origin of the VES/VT is given (F front, B back, R right, L left). The “stretch balloon” was located X21/Y3//X21/Y8–X24/Y8//X24/Y8. The origin of the VES or VT is additionally given in the table with X-position and Y-position of the electrode

\* $p < 0.05$  significant arrhythmia reduction by treatment

<sup>a</sup> Rate of VES > 1/min



**Table 2** Effects of a 15 min elevation of global pressure to an EDP of 30 mmHg (strain=0.148±0.034) followed by 15 min recovery

	BL	1 min stretch	10 min stretch	15 min stretch	15 min recovery
EDP (mmHg)	6±1	30±3*	30±4*	30±3*	8±2
LVP (mmHg)	93±6	85±7	86±5	88±6	84±6
RCF (ml/mmHg)	0.42±0.01	0.44±0.02	0.43±0.03	0.43±0.01	0.39±0.02
BCL (ms)	369±29	380±25	381±27	386±23	379±23
PQ (ms)	58±1	61±2	60±2	60±2	60±2
ST (mV)	0.18±0.03	0.19±0.04	0.18±0.02	0.20±0.04	0.17±0.04
VEC (%)	55.8±3.9	46.4±3.9	45.6±4.4	31.0±2.9*	37.4±4.4*
ARI (ms)	126±6	128±5	127±5	126±6	134±6
DISP (ms)	18±1	20±1	19±1	18±1	17±1
TAT left (ms)	8.3±1.4	9.6±1.3	8.8±1.2	8.5±1.0	12.2±4.7
TAT front (ms)	11.0±1.4	10.9±1.2	10.7±1.0	11.7±1.2	11.6±0.9
VES	0/6	0/6	2/6 <sup>a</sup>	1/6 <sup>a</sup>	3/6 <sup>a</sup>
VT	0/6	0/6	0/6	0/6	0/6

EDP end-diastolic pressure; LVP left ventricular pressure; RCF relative coronary flow; BCL basic cycle length; PQ atrioventricular conduction time; ST ST-deviation; VEC vectorfield similarity; ARI activation recovery interval; DISP dispersion; TAT total activation time; VES ventricular extrasystoles (number of hearts showing VES); VT ventricular tachycardia (number of hearts showing VT)

\* $p < 0.05$  versus baseline (BL)

<sup>a</sup> Rate of VES < 1/min

area, was about 0.25. Although this is in a similar range as the stretch which has been applied to isolated cells, e.g., 0.22 (Kamkin et al. 2000), in tissue consisting of connective tissue, cardiomyocytes, and vasculature the micro-distribution of stretch is highly complex and also depends on the microarchitecture of the tissue.

It has been shown that the effects of left ventricular volume load (1.0 ml) on refractoriness were small at normal (slow) pacing rates with only 8 % shortening at a pacing rate of

350 ms and became more evident at high pacing rates (Reiter et al. 1997). Since in our experiments BCL was about 340 ms and did not change during the experiment, it is reasonable that the changes in ARI were very small. Moreover, the interesting study of Reiter and colleagues (1997) cannot be easily compared with our study since they used a specialized preparation where the left ventricular wall was thinned by a cryosurgical procedure to a wall thickness of only 1 mm, so that the passive elastic properties of this

**Table 3** Time control series

	BL (30 min)	45 min	60 min	90 min	120 min
EDP (mmHg)	6±1	6±2	7±2	6±2	6±3
LVP (mmHg)	96±6	98±5	100±6	99±3	93±4
RCF (ml/mmHg)	0.37±0.02	0.35±0.02	0.36±0.02	0.37±0.02	0.37±0.03
BCL (ms)	427±35	428±41	423±34	405±31	407±32
PQ (ms)	59±2	60±3	59±2	58±3	61±2
ST (mV)	0.14±0.04	0.17±0.05	0.13±0.03	0.11±0.02	0.11±0.02
VEC (%)	55.8±2.6	36.8±4.8	36.8±4.7	34.8±5.1*	29.5±3.5*
ARI (ms)	174±33	177±32	181±31	176±24	179±21
DISP (ms)	12±1	14±2	11±1	11±2	12±2
TAT left (ms)	10.4±1.6	13.9±2.4	11.4±0.8	11.8±1.4	12.1±0.4
TAT front (ms)	9.7±1.1	9.5±0.9	9.3±0.9	9.9±0.7	9.7±1.0
VES	0/6	0/6	0/6	0/6	0/6
VT	0/6	0/6	0/6	0/6	0/6

EDP end-diastolic pressure; LVP left ventricular pressure; RCF relative coronary flow; BCL basic cycle length; PQ atrioventricular conduction time; ST ST-deviation; VEC vectorfield similarity; ARI activation recovery interval; DISP dispersion; TAT total activation time; VES ventricular extrasystoles (number of hearts showing VES); VT ventricular tachycardia (number of hearts showing VT)

\* $p < 0.05$  versus baseline (BL)

preparation are different. Stretch can depress L-type  $\text{Ca}^{2+}$  current, activate stretch-activated ion channels and inwardly rectifying  $\text{K}^+$  currents (Kamkin et al. 2000), although the effect on  $I_{\text{Ca,L}}$  is still a matter of debate (Calaghan and White 1999). These currents can exert opposite effects on the action potential plateau so that the net effect on potential duration may vary. Thus, it was shown that stretch can shorten the plateau and prolong the late repolarization phase of an action potential. Besides reports on prolonged action potentials, others found that action potential duration can be reduced by stretch (White et al. 1993). This effect may be explained by a  $\text{K}^+$ -specific stretch-activated current (SAC)  $I(\text{Ko})$  which results in action potential shortening (Healy and McCulloch 2005). Moreover, it should be stated that in the present experiments of our study stretch was sustained over 10 min. Stretch-activated ion channels are known not only to depend on force but also on the rate of change in force, and may inactivate during prolonged stretch. In addition, it was also shown that stretch can activate sarcoplasmic  $\text{Ca}^{2+}$ -release (Iribe et al. 2009), and can activate the sodium-proton-exchanger NHE leading to elevated intracellular  $[\text{Na}^+]$  and  $[\text{Ca}^{2+}]$  (Kocksämper et al. 2008). All these effects (depending on model, type of stretch, duration of stretch, etc.) can contribute to alterations in intracellular ion homeostasis which may change cardiac electrophysiology and rhythm. For a final statement on this point however, it would be necessary to monitor local  $[\text{Ca}^{2+}]$ , together with epicardial mapping which at present was technically not possible.

Other authors investigated the effect of short volume-overload pulses (50 ms up to 60 s) on the ventricle (Zabel et al. 1996). They described that early systolic pulses caused a short repolarization, while diastolic pulses had the opposite effect. Sustained pulses of 60 s were similar to an integral over many systolic and diastolic pulses, and led to a reduction in action potential amplitude. This might be related to a slight membrane depolarization, which has been described to be associated with stretch (Franz 1996) and which will result in reduced sodium channel availability due to an enhanced number of channels trapped in the inactivated state. This would typically lead to slowed conduction and enhanced total activation time (Buchanan et al. 1985; Dhein et al. 1993). For interpretation of our present results, it is necessary to note that in our experiments sustained stretch over minutes (10 min) was applied in order to simulate the clinical situation.

Local stretch affected the activation process: total activation time was locally enhanced, i.e., the activation wave was slowed at the (stretched) free left ventricular wall (Fig. 3f) rather than at the front wall or the other sides. This may be explained by the depolarization accompanying stretch (Franz 1996) or by enhanced membrane capacitance. It has been shown that the calculated membrane capacitance which is a function of the membrane-surface-to-cellular-volume-ratio (Kootsey 1991) is enhanced by

stretching and that this is related to conduction slowing (Mills et al. 2008). However, this study cannot be directly compared with our study, since these authors used a preparation which was mechanoelectrically uncoupled by 2,3-butanedione monoxime, so that mechanoelectrical and electromechanical feedback were excluded. Nevertheless, the idea of a local stretch-induced change in membrane-surface-to-cellular-volume-ratio and thereby in membrane capacitance together with a depolarization would fit well to our data.

Next, activation of stretch-activated ion channels causes depolarization (Franz 1996) and thereby would reduce sodium channel availability together with conduction velocity. However, the process is more complicated since it is also known that stretch can modulate fast sodium channels (Beyder et al. 2012a). Acute stretch is known to cause increased peak  $\text{Nav}1.5$  current, stabilization of inactivation and accelerated kinetics of the channel (Morris and Juranka 2007; Beyder et al. 2010; Strege et al. 2003). Thus, besides dampening effects on  $I(\text{Na})$  (by depolarization), activating effects also can occur. Interestingly, this mechanosensitivity of  $\text{Na}_v 1.5$  can be blocked by lidocaine (Beyder et al. 2012b) and ranolazine (Beyder et al. 2012a).

The local slowing observed in our study causes inhomogeneities in the activation pattern, which quantitatively is evident from a decrease in vectorfield similarity.

If these local inhomogeneities involve  $I(\text{Na})$ , one might assume that blockade of  $I(\text{Na})$  by flecainide might modulate the stretch effects. To test this hypothesis, we applied the sodium channel blocker flecainide in a therapeutic concentration. As expected, it prolonged TAT, which can be explained by the reduced sodium channel availability leading to reduced propagation velocity. However, in the local stretch region there was an attenuation of the stretch-induced TAT prolongation, so that the local differences were mitigated by flecainide together with a reduction in VES-arrhythmia. Thus, one might speculate that there are two effects of flecainide: the well known sodium channel blockade (slowing of propagation) and modulation of mechanosensitivity of  $I(\text{Na})$  (as recently reported for lidocaine and ranolazine (Beyder et al. 2012a, b)) which could attenuate the stretch-induced TAT prolongation, so that TAT prolongation in stretched and non-stretched region might be equalized.

Thus, one may assume that one of the arrhythmogenic mechanisms in these stretch-induced ventricular arrhythmias is the local difference in the activation time. On the other hand, the alteration of the activation pattern (i.e., reduced vectorfield similarity, Fig. 3b) was not significantly affected by flecainide, which means that the alteration of propagation is a more complex process. Of note, VT as the more dangerous arrhythmia was not prevented by flecainide. This may indicate that this type of stretch-induced arrhythmia is due to another arrhythmogenic mechanism than VES.

To clarify whether stretch-activated ion channels may be involved, we applied streptomycin, which is known to block these channels in the concentration used (Belus and White 2003; Eckardt et al. 2000). Since the relative prolongation of total activation time could be antagonized by streptomycin (see Fig. 3d–f), one may conclude that this slowing of the activation process is carried by stretch-activated ion channels. This view is further supported by the findings of Kamkin et al. (2000) that these channels can cause depolarization mainly by a slow  $\text{Na}^+$  influx, which then would reduce sodium channel availability, and thereby reduce propagation velocity. On the other hand, it needs to be stated that streptomycin is not specific for stretch-activated ion channels and may also affect  $I_{\text{Ca,L}}$ ,  $I_{\text{Kr}}$ , and  $I_{\text{Ks}}$  (Belus and White 2002). However, as with flecainide, streptomycin also did not prevent from stretch-induced alteration of the vectorfields (Fig. 3b), which again supports the view that activation pattern is a more complex phenomenon. Since streptomycin could not fully suppress stretch-induced arrhythmia, other factors seem also involved.

Both flecainide and streptomycin only reduced VES, but not VT incidence. This could mean reduced excitability (by blocking  $I(\text{Na})$ ) or reduced triggered activity (by blocking stretch-activated ion channels) possibly due to altered calcium handling. In that case the observed effects in TAT would be a bystander effect facilitating VT and modulation of intracellular calcium handling may be expected to act antiarrhythmically.

For comparison, we also performed a series with elevated global pressure ( $\text{EDP}=30$  mmHg). Again, there were no indications for ischemia (RCF was not reduced; ST was not elevated; ARI was not shortened). To our surprise, the hearts tolerated this elevated pressure very well and only some VES were seen (with lower frequency (i.e.  $<1/\text{min}$ ) than in the local stretch series), and no case of VT occurred. In contrast to the local stretch, in these cases VES originated from the stretched region, i.e., from the left ventricle. In that regard, local stretch with eccentric dilatation and global stretch seem to be different. It seems that stretch in general can provoke ventricular arrhythmia, in particular VES. Moreover, if there is an inhomogeneity in stretch, then the VES seem to originate predominantly from LV regions outside the most stretched zone.

Another interesting aspect was that in both models arrhythmia was found during recovery, and in particular in late recovery, i.e., after  $>10$  min. This was against our expectations, since we assumed the highest incidence during stretch and directly upon release. This finding, however, indicates that stretch seems to cause a longer lasting effect on cardiac electrophysiology and activation process.

In conclusion, we could show that local stretch can elicit ventricular arrhythmias and that these arrhythmias do not originate from the stretched area but from the border or other areas of the left ventricle, and occur during late stretch (10 min) and in particular during recovery and late recovery

( $>10$  min). Local slowing of activation seems to play a role in particular for the generation of VES so that total activation time of the stretched free left wall and the non-stretched areas drifted apart. VT, however, seems to involve other factors.

#### Limitations

Of course, streptomycin is an antibiotic and thus not a specific stretch-activated ion channel blocker. However, it has been widely used in experimental research to block these channels, which seems possible in concentrations of 40–100  $\mu\text{M}$  (Belus and White 2003; Eckardt et al. 2000). Millimolar concentrations ( $>2$  mM) of streptomycin are required to inhibit other cardiac ion channels (Belus and White 2002). Thus, regarding cardiac effects, streptomycin can be considered as stretch-activated channel blocker in submillimolar concentrations (Miura et al. 2010; Belus and White 2002, 2003). Alternatively, lanthanides such as  $\text{La}^{3+}$  or  $\text{Gd}^{3+}$  have been used, but these exert also strong effects on L-type calcium current (Lacampagne et al. 1994). We tried to apply  $\text{Gd}^{3+}$  in our hearts in another series, but the problem was that  $\text{Gd}^{3+}$  extremely slowed the heart rate and dramatically decreased LVP, while streptomycin did not affect these parameters. Another alternative would be the use of the tarantula toxin GsMTx-4, but the amounts which are necessary for a 30-min perfusion of a rabbit heart with coronary flows around 35 ml/min, are extremely expensive. Epicardial mapping does not give information about endocardial or deep intramural activation, and thus the exact origin of activation might differ from its apparent localization in epicardial mapping. While pacing of the hearts may help to reduce variability in the parameters we decided to investigate spontaneously beating hearts in order to mimic the physiological situation. At last, as in all animal models, the results primarily refer to the species investigated, i.e., the rabbit, and may not be uncritically transferred to other species.

It needs to be mentioned that the two balloons will probably interact: inflation of the second balloon will increase the pressure in the whole left ventricular cave, while there will be only limited eccentric dilatation. Technically, it was not possible to define exactly the amount of eccentric dilatation, since the mapping electrode plate was on top of the area. Although it seems reasonable to assume that the stretch in the area of the second balloon might be somewhat higher, we refer here to left ventricular stretch and to the location of this stretch-balloon. The strain measurement was two-dimensional and, thus, is only a rough approximation, since strain distribution in the ventricular wall is three-dimensional. Moreover, 3D strain is inhomogeneous due to the structure of the myocardial tissue with complex twisting of the fibers and inhomogeneous collagen strands. An exact analysis of the micro-distribution of 3D strain within the ventricular wall is technically not possible (Horowitz 1991) together with simultaneous epicardial

mapping. However, our measurements give a rough estimation of strain and in particular show that the local stretch led to higher strain in the stretch balloon area as compared with the LV rest (see Fig. 1b).

The incidence of VT was low, so that for a final statement on VT the model needs to be adapted to produce a higher VT incidence. Thus, here, we focus on VES.

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