Effect of streptomycin on wall-stress-induced arrhythmias in the working rat heart


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Received 15 March 1996; accepted 20 December 1996

Abstract

Objective: To assess whether streptomycin, an inhibitor of mechano-sensitive cation channels, has an effect on arrhythmias induced by an increase of ventricular wall stress in the rat heart. Methods: The isolated working rat heart preparation was used. Arrhythmias were induced by increasing the afterload (i.e., aortic pressure) against which the left ventricle (LV) pumped for 20 s. This led to an increase of LV pressure, stretch of the LV and an increase in LV wall stress. The number of ventricular premature beats induced by each afterload step was compared in the absence and presence of streptomycin, a compound known to block mechano-sensitive cation channels in the heart. Results: Perfusion with 200 μM streptomycin caused a significant reduction in wall-stress-induced arrhythmias. The effect of streptomycin on arrhythmias reached steady-state within 10 min of application. In the presence of streptomycin, arrhythmias elicited by a 40 mmHg afterload increase were reduced to 38% of control. Arrhythmias induced by an 80 mmHg afterload increase were reduced to 61% of control. Complex arrhythmias (ventricular tachycardia) induced by an afterload increase were also reduced in the presence of 200 μM streptomycin. There was no change in inotropic state with streptomycin, as assessed either by cardiac output or by maximum developed LV pressure. Streptomycin 50 μM (a typical therapeutic plasma concentration in patients) had no effect on wall-stress-induced arrhythmias. Conclusions: The results were inconsistent with streptomycin acting by modulating inositol phosphate production, or altering the level of intracellular calcium or inotropic state. The anti-arrhythmic effect of streptomycin appears more consistent with inhibition of mechano-sensitive cation channels, suggesting that these ion channels might be involved in causing wall-stress-induced arrhythmias.

Keywords: Streptomycin; Stretch-activated channels; Arrhythmias; Rat, heart

1. Introduction

An increase of wall stress in the ventricle is known to induce arrhythmias (ventricular ectopic or premature beats) in the human heart [1–4] and also in the perfused heart of experimental animals [5–12]. Arrhythmias induced by a change in ventricular wall stress might be of importance in clinical situations: for instance, hypertensive patients are known to have a more labile arterial blood pressure [1,13]. In these patients particularly, but also in normal subjects, a rapid fluctuation in arterial pressure will change the afterload against which the heart pumps, and will lead to alteration of intraventricular pressure and thus ventricular wall stress. Relatively rapid increases in ventricular wall stress (occurring over a few beats) may be partly responsible for the increase in arrhythmias (and sudden death) from which hypertensive patients are known to suffer [14].

The mechanism by which an increase of wall stress leads to arrhythmias is not known. One possibility is that increased wall stress might cause stretch of ventricular muscle cells, and that this might activate a stretch-sensitive cellular mechanism [6,15]. Ventricular muscle cells are known to contain at least two types of stretch-activated ion channels [16,17]—one type is permeable to cations [18,19] whilst the other appears to be an anion channel which carries chloride (Cl-) ions [20,21]. In addition, it has also...

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Time for primary review 20 days.

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PII S0008-6363(97)00024-2
been reported that the L-type Ca channel is sensitive to membrane stretch [22]. This gives rise to a distinct possibility that membrane stretch might lead either to a depolarisation of the resting membrane potential, or to an increased influx of Ca or Na ions into the cell, and that these might result in ventricular arrhythmia.

The purpose of this study was to examine whether the aminoglycoside antibiotic, streptomycin, an inhibitor of mechano-sensitive cation channels (e.g., Refs. [15,23]), has an effect on wall-stress-induced arrhythmias. We chose the working rat heart preparation for performing these experiments, since in this model ventricular wall stress can be altered in a graded fashion by increasing the aortic afterload against which the heart pumps. This preparation has been shown previously to be a reliable and sensitive model of wall-stress-induced arrhythmia [8,10,24]. The results suggest that at least a proportion of the arrhythmias caused by an increase of wall stress might involve mechano-sensitive cation channels.

2. Methods

Experiments were performed in accordance with the Home Office Guidance on the Operation of the Animals (Scientific Procedures) Act 1986, published by HMSO, London.

The isolated working heart model [25] including the modifications of Taegtmeyer et al. [26] was used to study arrhythmias induced by a step increase of aortic afterload. Experiments were carried out on the hearts of Wistar rats (250–300 g) which were bred in the Animal house at the School of Medical Sciences, University of Bristol.

2.1. Preparation of hearts for perfusion

Anaesthesia was induced using 95% O2/5% CO2 with 4% halothane, and maintained using between 2 and 2.5% halothane [10]. The O2/CO2 mixture ensured that the animal hyperventilated, thereby achieving optimal tissue oxygenation. Heparin (500 IU) was injected into a femoral vein 2 min before heart removal. The heart was excised and placed immediately in 4°C Tyrode’s solution, the aorta was mounted on a cannula and retrograde perfusion initiated. The time taken from heart excision to establishment of perfusion was usually less than 60 s. The heart was perfused initially in a Langendorff fashion. The pulmonary veins were identified and cannulated establishing a pathway for solution to pass into the left atrium. Once a tight seal with no leaks had been attained for perfusion into the left atrium, anterograde (‘working heart’) perfusion was commenced at a constant left atrial preload (15 cmH2O). The afterload systolic pressure was set by adjusting the height of the aortic column of fluid to achieve a standard systolic pressure of 80 mmHg. This level of afterload pressure was chosen to give a coronary flow rate of close to 18 ml·min−1—which is similar to the coronary flow rate found in many previous studies (e.g., Ref. [27]), and ensured hearts were unlikely to become ischaemic.

2.2. Experimental solutions

The perfusion solution (Tyrode’s) was made fresh each day, and contained (mM): NaCl 114; NaHCO3 25; NaH2PO4 1.0; CaCl2 2.6; MgCl2 1.0; and glucose 11.1. KCl was added to give a perfusate K concentration (Kc) of 2.4 or 6.0 mM. It was gassed continuously with 95% O2 and 5% CO2 (pH 7.4) and maintained at 37°C. Streptomycin was dissolved in Tyrode’s solution to give a concentration of 50 or 200 μM.

2.3. Baseline cardiac function tests

All hearts were perfused initially with 6 mM Kc Tyrode’s solution and allowed to stabilise for a 15 min period prior to the start of the experiment. Baseline cardiac function was then measured. Heart rate in control animals (mean ± s.e.m.) was 225 ± 10.1 beats·min−1, and perfusion with 200 μM streptomycin had no significant effect (P > 0.05, non-parametric statistics, see later; n = 6 animals). Aortic flow was measured using a mechanical flow meter placed in the aortic outflow line. Hearts were accepted only if they could produce a cardiac output (= aortic flow + coronary flow) of greater than 30 ml·min−1, and also if there was a less than 5% decline in cardiac output during each 90 min experiment. Coronary flow was collected and measured from the perfusion chamber outflow cannula. With the standard 80 mmHg aortic afterload, it was 18.2 ± 0.73 ml·min−1 (n = 6 hearts). With an imposed increase of afterload by 80 mmHg, coronary flow increased by a further 16.4 ± 0.74 ml·min−1. This suggests that hearts were unlikely to become ischaemic during an increase in afterload. Perfusion with 200 μM streptomycin had no significant effect on coronary flow (18.8 ± 0.58 ml·min−1 with streptomycin, n = 6 hearts; P > 0.05 compared to control hearts). We used two indices to monitor inotropic state. First, we measured cardiac output when the heart was stable in either control or streptomycin solution. Second, we measured the maximum systolic pressure (Pmax) which could be generated against the cross-clamped aorta whilst electrically pacing the heart at 300 beats·min−1 (e.g., Refs. [8,10,11,28]). (This was the only time that hearts were electrically paced. During the normal step increases in aortic afterload, hearts were not electrically paced. With pacing, it was necessary to set a rate faster than intrinsic heart rate, and we were concerned that this may have partially attenuated any arrhythmias normally observed during an afterload step.) Cross-clamping the aorta distal to the pressure transducer prevented outflow from the left ventricle (LV), and since the ventricle continued to fill during diastole, this will have caused LV end-diastolic pressure (EDP) to increase to a standard level.
in each heart. The level of EDP is expected to determine the maximum systolic pressure which can be produced by the LV (i.e., Frank-Starling relation) and thus P\text{max} might be expected to reflect the inotropic state of the heart. One caveat to this is that if streptomycin altered passive LV properties, then a given rise in EDP might produce a different end-diastolic volume, and might influence P\text{max}. However, there is no evidence for an effect of streptomycin on passive LV properties. By assessing the data obtained from cardiac output and P\text{max} measurements in combination, we assumed that we were able to detect whether streptomycin altered inotropic state. Baseline cardiac function parameters were recorded at the start of each experiment and monitored at 30 min intervals, when the heart reached a steady state in each perfusate.

2.4. Induction of arrhythmias

Following the initial 15 min stabilisation period, the perfusate was changed from 6 mM K\text{aq} Tyrode’s to one containing 2.4 mM K\text{aq}. A small number of wall-stress-induced arrhythmias can be elicited in the presence of 6 mM K\text{aq}, and these become greatly increased with 2.4 mM K\text{aq} [10,28]. Since the aim of this study was to assess the effect

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**Fig. 1.** (A) Schematic diagram of the experimental protocol (see text). (B) The change of intra left ventricular pressure (middle trace) induced by a step increase of aortic pressure (afterload; lower trace). ECG shown in upper trace. All traces are from the same heart; however, the two pressure traces were not obtained simultaneously. First, we placed a needle in the left ventricle (see text) and measured the change in intra-left-ventricular pressure caused by a step increase of aortic pressure afterload by 80 mmHg. Then we removed the needle from the left ventricle cavity, placed it in the aorta and 20 s later, we made the same step change in afterload and recorded directly the change in aortic pressure.
A  
pre-control

ECG

Aortic Pressure

200 μM Streptomycin

Aortic Pressure

40 mmHg Afterload Increases

80 mmHg Afterload Increases

Number of VPB

Number of VPB

PRE-CONTROL  TEST  POST-CONTROL

PRE-CONTROL  TEST  POST-CONTROL
of streptomycin on arrhythmias, we preferred to establish a situation in which a significant number of arrhythmias could be observed under control conditions.

After 15 min equilibration in 2.4 mM $K_o$, we began the afterload step protocol (see Fig. 1A). Arrhythmias were induced by short (20 s) increases in afterload. By turning a 3-way tap in the aortic outflow line, the aortic outflow could be switched from the basal level of afterload (80 mmHg systolic) to a pre-set higher afterload producing either a 40 or 80 mmHg rise in aortic systolic pressure. Data obtained using a 23-gauge needle placed within the LV showed that a step increase in aortic afterload produced an increase in intra-LV peak systolic pressure which took between 3 and 10 s to reach a maximal level (Fig. 1B). There was only a minimal increase in LV EDP during an afterload increase of 80 mmHg. From the Law of Laplace (wall tension is proportional to the product of intra-ventricular pressure and ventricular radius), a graded increase in intra-LV systolic pressure will produce a graded increase in LV wall stress. Hearts were allowed a stabilisation period of 2 min between each afterload rise. The order of afterload increase applied was randomised and 3 runs were performed for each level of afterload. The series of afterload steps lasted for the final 15 min of the 30 min initial pre-control period in 2.4 mM $K_o$.

The perfusate was then changed to either an identical 2.4 mM $K_o$ solution (for control hearts), or to 2.4 mM $K_o$ with added 50 or 200 $\mu$M streptomycin (for test hearts). After 15 min equilibration in this solution, another series of 40 and 80 mmHg afterload steps was applied. These lasted for the remaining 15 min of the 'test' period. Finally, the perfusate was changed to a 2.4 mM $K_o$ streptomycin-free solution, and the heart left to equilibrate for 15 min ('post-control' period, Fig. 1). The series of afterload steps was repeated during the final 15 min of this period.

2.5. Data acquisition and analysis

The electrocardiogram (ECG) was recorded from electrodes attached to the metal aortic and left atrial perfusion cannulae and a third metal cannula draining the perfusion chamber. The height of the cannula within the perfusion chamber allowed the chamber to be partially flooded to a level submerging the apex of the heart, thus establishing an electrical contact with the apex.

The ECG and aortic pressure were recorded continuously on a chart recorder (Gould 8000 series). Pressure was monitored distal to the aorta using a Gould diaphragm pressure transducer (model P23 ID). Arrhythmias were analysed in accordance with the Lambeth convention [29]. Ventricular premature beats (VPBs) were defined as discrete and identifiable QRS complexes having a typically bizarre morphology. The number of VPBs during a 20 s increase in afterload was counted. Ventricular tachycardia (VT) was defined as 3 or more consecutive VPB's (in this study a series of 3 or more VPBs had to be of identical morphology and coupling interval to be accepted as VT). For analysing VT specifically, we counted the number of complexes in each salvo of VT which was elicited by each afterload step.

2.6. Statistical analysis

For each heart, the average value for the 3 runs performed for each afterload level (40 and 80 mmHg) was taken. Results were expressed as mean ± standard error of mean (s.e.m.). Statistical comparisons were made using non-parametric statistics, using the Bonferroni correction procedure for the data in Figs. 2–4, to take into account any family-wise error rate. We used non-parametric statistics because it was not possible to predict accurately that the data were normally distributed. For analysing the time course over which streptomycin acted to attenuate arrhythmias (data not shown, but mentioned in relation to Fig. 2) we used non-parametric statistics with the Friedman test to determine the time at which arrhythmias became significantly different with streptomycin. Analysis was performed using Microsoft Excel, Systat and SPSS statistical software packages.

3. Results

3.1. Effect on ventricular premature beats of 200 $\mu$M streptomycin

The first objective was to determine whether streptomycin had an effect on wall-stress-induced arrhythmia in the working rat heart. After the pre-control period, one concentration (50 or 200 $\mu$M) of streptomycin was added to the perfusate, and after 15 min the afterload steps were
A. Pre-control

B. 50μM Streptomycin

C. 40mmHg Afterload Increases

D. 80mmHg Afterload Increases
repeated. Other experiments (data not shown) showed that streptomycin had a significant effect on VPB’s within 7.5 min ($P < 0.05$), and its inhibitory effect on arrhythmias reached steady state within 12.5–15 min. After the test period we removed streptomycin, allowed re-equilibration in normal Tyrode’s solution and repeated the afterload steps to obtain post-control data.

Fig. 2A,B illustrates original recordings. The ECG and aortic pressure were recorded routinely, and panel A shows the arrhythmias elicited by an 80 mmHg afterload step during the pre-control period. The heart was initially in sinus rhythm. After a step increase in afterload, it was a consistent finding that VPB’s were not observed immediately after the step increase, but took between 3 and 10 s to become evident (consistent with intra-LV pressure taking 3–10 s to reach its maximal level during an afterload step). Once arrhythmias appeared, either they occurred singly with periods of sinus rhythm in between, or else they occurred in runs as VT (see latter half of afterload step, Fig. 2A). Panel B of Fig. 2 shows an identical afterload step applied to the same heart 15 min later, during perfusion with 200 μM streptomycin. Fewer VPB’s were elicited by the afterload step—in the presence of streptomycin, the heart remained in sinus rhythm for almost the entire period of the afterload step.

Fig. 2C,D shows mean data for arrhythmias obtained from control and 200 μM streptomycin-treated hearts. We analysed only the arrhythmias which occurred during the step increase of afterload. (Arrhythmias also sometimes occurred at the end of the afterload step, when raised afterload was reduced to 80 mmHg, but these arrhythmias were less reproducible. In addition, we have shown previously that these arrhythmias are always single ectopics and never complex arrhythmias [10,11,28]. In contrast, both single and complex arrhythmias occurred reproducibly during the increase of afterload.) On the basis that complex arrhythmias are potentially more dangerous and thus perhaps more relevant clinically, we confined our analysis of arrhythmias to the period during the afterload increase. Fig. 2C shows arrhythmias elicited by 40 mmHg afterload steps; Fig. 2D shows arrhythmias elicited by 80 mmHg steps. These plots also illustrate one complicating factor—the number of VPB’s elicited by a given afterload step declined during the experiment. Thus, it was important that we carried out parallel experiments in control hearts not exposed to streptomycin. For control experiments an identical protocol was performed, except that streptomycin was not added during the ‘test’ period and hearts remained perfused with 2.4 mM K+, Tyrode’s. In Fig. 2C, comparing control hearts ($n = 6$) with those exposed to 200 μM streptomycin ($n = 10$ hearts), it is clear that streptomycin-treated hearts had a similar number of arrhythmias during the pre- and post-control periods, but fewer arrhythmias during streptomycin exposure. The streptomycin-treated hearts developed only 38% of the arrhythmias elicited in control hearts during this period. Using a non-parametric statistical analysis, this difference was significant ($P < 0.05$).

A similar picture was observed for 80 mmHg afterload steps (Fig. 2D). Exposure to 200 μM streptomycin reduced arrhythmias to 61% of those observed in control hearts, and this difference was significant ($P < 0.05$). Therefore, it appears that 200 μM streptomycin inhibited VPB’s elicited by an increase in wall stress in the working rat heart.

3.2. The effect of 50 μM streptomycin

Streptomycin 200 μM is within the concentration range used experimentally to block mechano-sensitive cation channels (e.g., Refs. [15,23,30–33]). However, therapeutic plasma streptomycin concentration in patients is usually near 50 μM, therefore we also tested the effect of this concentration on wall-stress-induced arrhythmias.

Fig. 3A,B illustrates the effect of perfusing 50 μM streptomycin. Panel A shows the pre-control period; as before, VPB’s and VT occurred during the 80 mmHg afterload step. Panel B shows an identical afterload step applied to the same heart in the presence of 50 μM streptomycin. VPB’s and VT could still be observed.

Mean data for the effect of 50 μM streptomycin on arrhythmias is shown in Fig. 3C (40 mmHg steps) and Fig. 3D (80 mmHg steps). Once again, it was important to compare the effect of 50 μM streptomycin with data obtained from control hearts not exposed to streptomycin. There was no significant effect of 50 μM streptomycin on the number of VPB’s elicited, for afterload steps of either 40 or 80 mmHg ($P > 0.05$), for arrhythmias elicited in control and streptomycin hearts, for both afterload step amplitudes. Thus, in contrast to 200 μM, 50 μM streptomycin had no detectable anti-arrhythmic effect.

3.3. Does streptomycin affect contractility?

In addition to block of mechanosensitive cation channels, there are other possible mechanisms for the anti-
The arrhythmic effect of 200 μM streptomycin (e.g., effects on L-type Ca channels [34], effects on IP₃ release [35]) which require consideration. Since these alternative mechanisms might lead to a reduction in an inotropic state, we determined the effect of 200 μM streptomycin on inotropic state. We assessed this firstly by measuring cardiac output. Cardiac output in the absence of streptomycin was 58.0 ± 4.8 ml · min⁻¹, and in the presence of streptomycin was 58.7 ± 4.8 ml · min⁻¹; there was no significant difference. Second, we measured P_max. Our first choice was to assess the effect of streptomycin on P_max when hearts were perfused with 2.4 mM Kₒ solution. However, we found that repetitive cross-clamping of the aorta in the presence of 2.4 mM Kₒ led frequently to irreversible and terminal arrhythmias, so that P_max became unstable and unreliable. Arrhythmias leading to instability of P_max were less common when cross-clamping was performed in the presence of 6 mM Kₒ. For these experiments, working heart preparations were stabilised in 6 mM Kₒ for 10 min. For the following 10 min in 6 mM Kₒ, the aorta was cross-clamped every 2.5 min and P_max reached steady-state during this pre-control period (Fig. 4A). For the 6 test hearts, 200 μM streptomycin was added to the perfusate for 20 min whilst P_max measurements continued to be taken. Another 6 control hearts remained in 6 mM Kₒ, without streptomycin added. The mean results (Fig. 4A) demonstrate there was no significant effect of 200 μM streptomycin on P_max (P > 0.05 for every time point). Together with the cardiac output data (obtained in 2.4 mM Kₒ), the fact that streptomycin did not alter P_max suggests that 200 μM streptomycin did not have a significant negative inotropic effect under these conditions in the working rat heart.

3.4. Effect of streptomycin on complex arrhythmias

In the clinical situation, it is complex ventricular arrhythmias (e.g., VT, and also ventricular fibrillation) that are associated with the greatest risk of fatal arrhythmia. We therefore assessed the effect of streptomycin on VT. We counted the number of complexes in each salvo of VT that occurred during each 80 mmHg afterload step, in the absence and presence of 200 μM streptomycin (Fig. 4B). Hearts developed less VT complexes during streptomycin application than did control hearts. The difference in number of VT complexes was significant (P < 0.05). These data show that streptomycin inhibits more complex arrhythmias, in addition to single ventricular ectopics.

4. Discussion

The results of this study show that in the working rat heart, application of 200 μM streptomycin led to a significant reduction in the number of wall-stress-induced arrhythmias. Streptomycin 200 μM exerted its steady-state anti-arrhythmic effect within 10–15 min of application. We found in this preparation that 50 μM streptomycin (a concentration within the therapeutic range in man) had no inhibitory effect on arrhythmias. The data from this study are in agreement with preliminary results from a different study using 80 μM streptomycin on Langendorff-perfused guinea-pig hearts [31], and also with a previous study which used gadolinium (Gd; a stretch-activated channel blocker) on perfused canine hearts [6].

4.1. Anti-arrhythmic effect of 200 μM streptomycin

Streptomycin 200 μM reduced wall-stress-induced arrhythmias, and it also reduced the incidence of more
complex (and clinically more serious) VT. Streptomycin is known to block mechano-sensitive cation channels (e.g., Refs. [15,19,23]). We found that a step increase of afterload caused a progressive increase of peak LV systolic pressure over the following 3 to 10 s, and also a small rise of LV EDP. These changes will lead to an increase of LV wall stress, and will result in an increased degree of mechanical stretch of individual ventricular muscle cells. This might be anticipated to open stretch-activated channels in the cell membrane, which will increase the permeability of the membrane both to cations and to Cl ions. This could cause a depolarisation of membrane potential (e.g., [36]), and if this reaches threshold and results in opening of TTX-sensitive Na channels, then it might result in an ectopic action potential. An increase in membrane permeability to cations will also increase influx of Ca ions into the cell (e.g., [36]). Elevation of intracellular Ca in the rat heart is known to cause spontaneous Ca release from the sarcoplasmic reticulum, leading to transient oscillations of intracellular Ca and generation of arrhythmias (e.g., [7,37–42]).

Streptomycin 200 μM did not abolish arrhythmias completely, but rather inhibited them by between 40 and 60%. One possibility is that this dose of streptomycin might not be maximally effective. The evidence from the present study is consistent with the mechano-sensitive cation channel at least being involved in wall-stress-induced arrhythmia. However, there may be other mechanisms which account for some arrhythmias still present during streptomycin. For instance, with an increase of afterload there was an increase of coronary perfusion and this may lead to a change in cardiac metabolism which may result in arrhythmias. Streptomycin does not inhibit stretch-activated Cl channels, and activation of these by increased wall stress might cause depolarisation. Another possibility is that the L-type Ca channel itself might be sensitive to stretch, since a recent study has reported an increase of L-type Ca channel current in myocytes exposed to mechanical and osmotic stresses [22].

4.2. Possible alternative mechanisms for the anti-arrhythmic effect of streptomycin

There are other potential possibilities for the anti-arrhythmic effect of streptomycin, in addition to block of the mechano-sensitive cation channel. Streptomycin has been reported previously to reduce L-type Ca channel current [34]. However, at least in smooth muscle myocytes, inhibition of the L-type Ca channel by streptomycin occurs with a K_i of 2.1 mM [43], and there is little effect of 200 μM streptomycin. A reduction of Ca entry via the Ca channel will reduce the Ca loading state of heart cells and since changes of intracellular Ca (Ca_i) are linked to development of arrhythmia [44,45], a fall of Ca_i with streptomycin might be expected to cause a reduction of wall-stress-induced arrhythmia. Aminoglycoside antibiotics have also been reported to inhibit production of IP_3 [46] and, at least in the ischaemic/reperfused heart, there is evidence that IP_3 may be involved in causing arrhythmias [35]. In skeletal muscle IP_3 has been reported to be involved in excitation–contraction coupling and to modulate Ca release from the sarcoplasmic reticulum [33] and a similar mechanism may also exist in cardiac muscle.

Since both these alternative mechanisms might be expected to cause a reduction in the inotropic state of the heart, we assessed involvement of them by investigating the effect of streptomycin on inotropic state. We found no effect of 200 μM streptomycin on inotropic state, as assessed by measuring both cardiac output and P_max. This is consistent with another recent study, which found little effect of 100 μM streptomycin on inotropic state of the rabbit heart [30]. These results indicate that streptomycin under these conditions might not cause a reduction of Ca entry via the L-type Ca channel, or a reduction in IP_3 release. With these mechanisms less likely, this would appear to point in the direction that the anti-arrhythmic effect of 200 μM streptomycin might be due to inhibition of the mechano-sensitive cation channel.

4.3. Assessment of streptomycin on LV inotropic state

We assessed LV inotropic state using two methods—cardiac output and P_max. Measurement of P_max has been used in previous investigations employing the working rat heart preparation (e.g., Refs. [8,10,11,28]). Nevertheless, it is still useful to examine this test more closely. P_max was measured by cross-clamping the aortic outflow tube distal to the pressure transducer, whilst electrically pacing at 300 beats min⁻¹. This largely prevents outflow from the heart, and any small outflow which does occur will be ejected into the slightly compliant silicon tubes. Diastolic filling of the LV will still occur, so both the end-diastolic volume and pressure must increase. The EDP of the LV will increase up to the maximum level that can be attained, and that is the preload applied to the left atrium (15 cmH₂O). According to the Frank-Starling relation, a rise in EDP will lead to increased developed tension by the myocytes in the ventricular wall, and the level of systolic tension and systolic intra-LV pressure will be a measure of inotropic state. We have shown in previous experiments that P_max does appear to reflect the inotropic state of the heart. Negatively inotropic agents (low external Ca, Ca channel blockers, low-dose ryanodine) lead to a fall in P_max: in contrast, positively inotropic agents (high external Ca, low external Na, isoprenaline, cardiac glycosides, EMD 57033) increase P_max (e.g., [47,48]). One caveat must be added here—if streptomycin affected passive LV properties, then a given rise in EDP might cause a different amount of diastolic stretch of the ventricle. This would then influence P_max and make it less reliable as an index of inotropic state. However, it has not been reported so far that streptomycin might influence passive LV properties.
4.4. Relation to previous studies

Some previous studies have investigated the role of stretch-activated channels in causing ventricular arrhythmias. Hansen et al. [6] showed in the canine heart that Gd (1–10 μM) inhibited arrhythmias induced by a rapid and brief diastolic stretch. However, Gd is also a potent blocker of the L-type Ca channel in guinea-pig myocytes over an identical concentration range [49]. In relation to this, Hansen et al. provided evidence that the anti-arrhythmic effect of Gd under their conditions was not due to inhibition of the Ca channel. One advantage of streptomycin over Gd might be that it does not (at 200 μM) block the Ca channel, and thus may be a more selective agent for studying the role of mechano-sensitive cation channels in the rat heart.

Little is known about the dose–response curve for streptomycin on mechano-sensitive cation channels in the heart. Gannier et al. [15] found that 40 μM streptomycin inhibited the rise in Ca2+ induced by mechanical stretch of guinea-pig myocytes. In comparison, Nazir et al. [31] in the Langendorff-perfused guinea-pig heart found that 80 μM streptomycin had only a partial inhibitory effect on arrhythmias induced by a brief diastolic stretch of the ventricle. In the working rat heart we found that 50 μM streptomycin had no detectable effect on stretch-induced arrhythmias, whereas 200 μM streptomycin had a partial inhibitory effect. Some of the difference between these studies may reflect a species difference, and it is possible that the mechano-sensitive channel in the rat may be less sensitive to streptomycin. It is possible that isolated myocytes might possibly have an altered sensitivity for streptomycin, as evidenced by the apparent higher sensitivity of guinea-pig myocytes to streptomycin, compared to the perfused intact guinea-pig heart [15,31].

Some previous information about streptomycin has come from studies on the cochlea of the ear, where streptomycin inhibits stretch-activated channels in the hair cells, and also has a toxic effect on the auditory system. Ohmori [23] found that streptomycin inhibited the stretch-activated channel in doses between 100 and 500 μM, whilst Kroese and van den Berken [32] found effects between 20 and 200 μM. The doses used in this study are within the same concentration range, and this is at least consistent with the possibility that in this study, streptomycin acted primarily by inhibiting mechano-sensitive cation channels.

Acknowledgements

We are grateful to the Wellcome Trust, British Heart Foundation, Physiological Society, Royal Society and United Bristol Healthcare Trust for financial support. Geoff Dalton was supported by a Training Fellowship from the British Heart Foundation, Andrew Salmon and Joseph Mays were Medical Students and were awarded Summer Vacation Scholarships by the Physiological Society. We thank Stephen Evans for advice, and Jon Issberner and Lesley Arberry for excellent technical help. We are grateful to Dave Clements, Merv Higgins and John Vinnicombe for skilful engineering help, to Malcolm Fowler for glassblowing expertise, and to Jeff Croker, Jenny Pasterfield and Mike Rickard for expert electronic and computer assistance.

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