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Effect of gadolinium on stretch-induced changes in contraction and intracellularly recorded action- and afterpotentials of rat isolated atrium

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1 Atrial arrhythmias, like atrial fibrillation and extrasystoles, are common in clinical situations when atrial pressure is increased. Although cardiac mechanoelectrical feedback has been under intensive study for many years, the mechanisms of stretch-induced arrhythmias are not known in detail. This is partly due to methodological difficulties in recording intracellular voltage during stretch stimulation. In this study we investigated the effects of gadolinium (Gd^{3+}), a blocker of stretch-activated (SA) channels, on stretch-induced changes in rat atrial action potentials and contraction force.

2 By intracellular voltage recordings from rat isolated atria we studied the effects of Gd^{3+} (80 μM) on stretch-induced changes in action potentials. The stretch was induced by increasing pressure inside the atrium (1 mmHg to 7 mmHg). An elastic electrode holder that moved along the atrial tissue was used in the recordings. Thus the mechanical artifacts were eliminated and the cell-electrode contact was made more stable. To examine the influence of Gd^{3+} on atrial contraction we stretched the atria at different diastolic pressure levels (1 to 7 mmHg) with Gd^{3+} application of (80 μM) or diltiazem (5.0 μM). Contraction force was monitored by recording the pressure changes generated by the atrial contractions.

3 Our results show that: (1) atrial stretch induces delayed afterdepolarizations (DADs), increase in action potential amplitude and increase in relative conduction speed; (ii) Gd^{3+} blocks stretch-induced DADs and action potential changes; (iii) Gd^{3+} inhibits pressure-stimulated increase in the atrial contraction force, while similar inhibition is not observed with diltiazem, a blocker of L-type calcium channels.

4 This study suggests that Gd^{3+} inhibits stretch-induced changes in cell electrophysiology and contraction in the rat atrial cells and that the effects of gadolinium are due to rather specific block of stretch-activated ion channels with only a small effect on voltage-activated calcium channels.

Keywords: Gadolinium; stretch; myocyte; electrophysiology; contraction; action potential

Introduction

Increase in the diastolic myocardial tension causes an increase in systolic performance of cardiac muscle. This is the well known Frank-Starling mechanism (for review, see Lakatta, 1986) which is utilized to augment the stroke volume to meet the need for increased cardiac output, e.g. during exercise. In addition to physiological situations, cardiac tissue can be subjected to stretch under a variety of pathophysiological conditions, including heart failure, valve damage and hypertension. In isolated myocardial tissue, stretch has been reported to induce several electrophysiological changes (Penefsky & Hoffmann, 1963; Lab, 1980) and such changes may predispose to arrhythmias (Franz *et al.*, 1989; Hansen *et al.*, 1990) or modulate them (Lammers *et al.*, 1991; Ravelli *et al.*, 1994). In addition, continuous load appears to induce the development of cardiac hypertrophy (Komuro *et al.*, 1991). Neither the stretch-induced effects on cardiac function nor their mechanisms are known in detail at the cellular level.

New insights into stretch-induced changes in heart function came when stretch-activated (SA) channels were discovered (Guharay & Sachs, 1984; for review, see Morris, 1990). These channels are mechanosensitive in the sense that their open probability depends on mechanical stress at the membrane. Several types of SA channels have been documented in adult isolated cardiocytes (Bustamante *et al.*, 1991; Kim, 1992; Van Wagoner, 1993) and in cultured neonatal cardiocytes (Sadoshima *et al.*, 1992; Ruknudin *et al.*, 1993; Kim, 1993). The major SA channels are of a type permeable to both monovalent and divalent cations (Sachs, 1989). In isolated myocytes mechanical loading increases intracellular Ca^{2+} concentration $[Ca^{2+}]_i$ (Le Guennec *et al.*, 1991) and this increase can be inhibited by gadolinium (Gd^{3+}) (Sigurdson *et al.*, 1992), a blocker of SA channels (Yang & Sachs, 1989) and by streptomycin (Gannier *et al.*, 1994). Moreover, it has been demonstrated that stretch-induced secretion of atrial natriuretic peptide (ANP) can be blocked by Gd^{3+} (Laine *et al.*, 1994a).

The problems with investigating the effects of stretch on cardiac function are partly due to the lack of single cell data under physiological conditions. This is a result of difficulties in intracellular recordings from moving tissue (see e.g. Lab, 1978). Most data from stretch-induced electrophysiological changes have been obtained from monophasic action potentials (MAPs) recorded by contact electrodes. However, the interpretation of these extracellular sum potentials is difficult. To overcome this problem we developed a method for intracellular recording of the action potential during stretch of rat isolated atrium. The stretch was induced by increasing pressure inside the atrium within physiological limits. The purpose of the study was to examine the effect of Gd^{3+} on mechanically induced changes in atrial action potentials and contraction force, thus evaluating the role of SA channels in these processes.

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Methods

Animals, preparation and superfusion

Male Sprague-Dawley rats ($n=65$) weighing 290–400 g were used. They were held at 20–22°C and had free access to tap water and standard food. The rats were decapitated and their hearts were rapidly removed and placed in oxygenated warm (25°C) buffer solution (composition, mM: NaCl 137, KCl 5.6, $CaCl_2$ 2.2, HEPES 5.0, $MgCl_2$ 1.2, glucose 2.5, pH 7.4), which was also used for superfusion of the atrial preparation.

A schematic diagram of the experimental arrangement is illustrated in Figure 1. The left atrial appendix was prepared as described previously (Laine *et al.*, 1994b). An X-branch polyethylene adapter was inserted into the lumen of the left auricle and the atrium was placed in a constant temperature (37°C) organ bath. Another tube with a smaller diameter was inserted inside the adapter in order to carry the perfusate inflow into the lumen of the auricle. Outflow from the lumen came from one crossbranch of the X-cannula. The outflow tube was connected to the chamber of a pressure generator (WGA-200, Millar instrument Inc., U.S.A.), by which different waveforms of pressure could be generated into the lumen of the atrium. The other crossbranch of the X-cannula was connected to a pressure transducer (TCB 100, Millar Instruments Inc., U.S.A.), so that pressure in the lumen of the auricle could be monitored with an oscilloscope. Inflow and outflow (3 ml min^{-1}) both to the auricle lumen and to the organ bath with constant temperature were controlled by a peristaltic pump (7553-85, Cole-Parmer Instrument, Co., U.S.A.). All recordings were done within 3 h of decapitation.

Electrophysiological recordings

Membrane potentials were recorded with conventional glass microelectrodes filled with 2 M K-acetate and 5 mM KCl, pH 7.0. The input resistances of the electrodes were 40–70 MΩ. A chloridized silver wire was used as a reference electrode that was placed in contact with the superfusion medium in the organ bath. The electrode holder connected to a micromanipulator was a spring of chloridized silver wire (diameter 0.3 mm) in order to increase the recording time and to eliminate mechanical artifacts caused by movement of the tissue when stimulated by pressure fluctuations. The left atrium was quiescent unless stimulated electrically through bipolar Ag/AgCl electrodes placed in contact with the tissue. This stimulation causes a spreading action potential, as opposed to field stimulation that causes a nearly simultaneous action potential in all atrial cells. The atrium was paced at 2 Hz with rectangular pulses of 1 ms duration and 1.5 times the threshold voltage by a stimulator (S44, Grass Instruments Co., U.S.A.). All electrical signals were amplified with an intracellular amplifier (Dagan 8100-1, Dagan Co., U.S.A.) and stored by a DAT-recorder (Biologic DTR-1800, Biologic LTD., France) for later analysis. Sampling frequency was 3 kHz in all recordings. At the start of the experiment, when the electrode was in contact with the bath medium, the amplifier off-set was set to zero, and the electrode capacitance was compensated (built-in amplifier facility). When the electrode was advanced into the tissue, it was often found to be necessary to increase the capacity compensation to null the larger stray-capacitance.

Estimation of contraction force

To investigate the force of the atrial contracted, we recorded pressure changes generated by the contraction with a pressure transducer (TCB-100, Millar instruments Inc., U.S.A.). It is clear that the pressure developed by the atrial contraction is not a direct measure of contraction force, but other interventions, like the installation of a force transducer in contact with tissue will change the working geometry of the atrium and will lead to a less physiological experiment. Even though the developed pressure cannot be used as a direct measure of maximal contraction force of auxotonic contraction, the relation of force to pressure can be estimated. If the isolated atrium is considered as a thin-walled membrane, the relationship between wall tension, radius of curvature, and the generated pressure (Laplace relationship) can be stated (Lakatta & Maughan, 1990):

$$P = T(1/R_1 + 1/R_2) \quad (1)$$

where T is the local wall tension (force), P is the pressure and R is the local radius of curvature. Because the volume changes are very small with the rather low pressures used in the experiments (less than 10%), we can approximate that $R_1 = R_2$, then

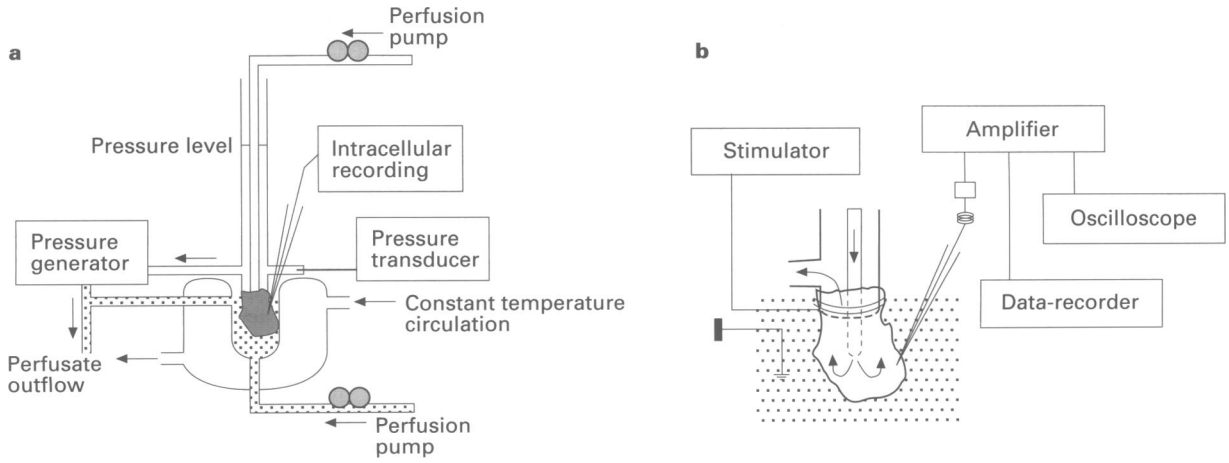

Figure 1 The experimental setup for intracellular recordings in rat atria. (a) The perfusion system. Separate superfusion was supplied to the outside and inside of the atrium. Stretch of the atrium was regulated by controlling the intra-atrial pressure by a pressure generator. Pressure was monitored with a pressure transducer. (b) The recording system. A spring electrode holder was used for intracellular measurements from the contracting atrial tissue. The reference electrode (a chloridized silver wire) was in contact with the medium inside the organ bath. The recorded signals were monitored by an oscilloscope and stored by a digital data-recorder. The atrium was stimulated by a bipolar electrode.

$$T = PR/2 \quad (2)$$

Hence, the pressure generated by atrial contraction is proportional to the tension and can be used as an estimate of contraction force (with an error of less than 5%). The developed pressures were measured from pressure pulses as the difference between diastolic pressure and peak systolic pressure (Figure 2).

Experimental design

The effects of pressure-induced stretch on the action potentials of atrial myocytes were investigated at two different diastolic pressures: 1 mmHg, and 7 mmHg, with and without 80 μM Gd^{3+}. In each experiment the atria were stretched for 3 min at each diastolic pressure before recordings. Three action potential parameters were measured directly: amplitude (mV), duration at 50% and 90% repolarization (ms). A fourth parameter, relative conduction speed (1/s), was determined by use of the stimulation artifact as an indicator of impulse initiation, so that the delay between the stimulation artifact and AP generation was the apparent conduction time. The distance of the microelectrode and the stimulus electrodes was adjusted in 1 mmHg pressure to be equal in all recordings. This means that when the tissue was stretched, the electrical distance (equal amount of cells) between the stimulation electrodes and the microelectrode was constant in each experiment, even though the absolute distance was not (which was increased by stretching). This way the relative conduction speed could be calculated and used for analysis, with the caveat that the treatments might slightly change the excitability at the point of contact of the stimulatory electrodes.

To investigate the force of atrial concentration, we recorded pressure changes generated by the contraction with a pressure transducer (TCB-100, Millar Instruments Inc., U.S.A.) at different diastolic pressures and with different channel blockers (gadolinium and diltiazem). In each experiment the atria were stretched 3 min at each diastolic pressure (between 1–8 mmHg) with and without the blocker, after which the amplitude of the generated pressure pulse as measured. Measured pressure pulses were normalized in each atria to the maximum amplitude (P_{max}) of the pressure pulse generated at each stretching level without the blocker. In alternate experiments the generated pressure were first measured with the blocker and the controls with vehicle were performed after washout. In this way the possible effect of time on the contraction force between different experimental groups was minimized. The contraction returned to the original value in each experiment after washout of either gadolinium or diltiazem.

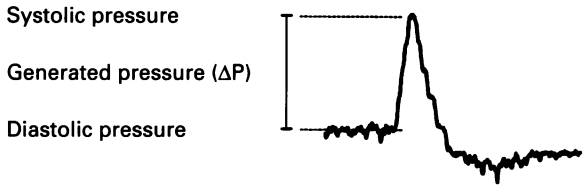

Figure 2 Pressure pulse generated by atrial contraction. Generated pressure (ΔP) was used to estimate the contraction force of the atria at different diastolic pressures.

Statistical analysis

Results are expressed as mean±s.e.mean. Action potential data were tested by a one way ANOVA followed by Bonferoni correction. Data from contraction studies were tested by a paired t test, with Bonferoni correction and by regression analysis. The results of statistical analysis were accepted at the 5% level (i.e., when $P<0.05$).

Materials

HEPES (N-2-hydroxyethylpiperazine-N'-2-ethansulphonic acid) was obtained from Sigma Chemical Co, St. Louis, MO, U.S.A.; KCl, glucose, $CaCl_2$, $MgCl_2$ from E. Merck, Darmstadt, Germany; NaCl and K-acetate, from FF-Chemicals AB, Sweden; $GdCl_3$ from Aldrich Chemical Company, Milwaukee, U.S.A. and diltiazem was kindly provided by Orion-Farmos Pharmacological Company, Turku, Finland.

Results

The results in this paper were from 65 atrial preparations. The number of successful, stable impalements is large in the case of resting potential parameters ($n=209$), less in paced (contracting) atria ($n=47$). Only cells with relatively normal resting potentials (from −75 mV to −85 mV) were accepted. Action potential data were obtained from 30 different atria (47 cells) and 17 out of 30 atria were pressure stimulated (7 mmHg pressure) at the time of recordings (22 cells). Contraction was studied in 8 atria.

Stretch and Gd^{3+}-induced changes in action potentials

To study the effect of stretch, we recorded stimulated action potentials under low (1 mmHg, $n=14$) and high pressure (7 mmHg, $n=10$). The role of the SA channels in the stretch-induced effect was studied by applying Gd^{3+} to block the channels. The measured parameters of action potentials in the atria with Gd^{3+} buffer were compared to controls without Gd^{3+} in stretched and non-stretched atria. The effects of stretch on the action potential parameters and their block by Gd^{3+} are shown in Table 1. Rise in the diastolic pressure significantly increased the action potential amplitude ($P<0.001$), and relative conduction speed ($P<0.001$). In contrast, no statistically significant differences could be observed in the action potential duration (50% or 90% repolarization) between the stretched and nonstretched atria. Gd^{3+} did not have any effect on action potential parameters at low diastolic pressure ($n=9$), contrary to what would be expected on the basis of a proposed block of L-type Ca^{2+} channels (Lacampagne *et al.*, 1994), but Gd^{3+} at 80 μM inhibited the stretch-induced changes in action potential parameters, sug-

Table 1 Effects of gadolinium on pressure-induced changes in action potentials

	No gadolinium		*Gadolinium*	
	Control	*Stretch*	*Control*	*Stretch*
Amplitude (mV)	85±1	99±3*	85±4	82±1
Relative conduction speed (1/s × 10^3)	7.1±0.3	14.5±1.9*	7.5±0.5	10.4±0.2
Action potential duration 50% (ms)	11.2±0.9	11.6±0.8	14.0±1.0	14.1±0.6
Action potential duration 90% (ms)	33±3	43±4	39±3	39±2

*$P<0.05$

gesting that SA channels may be responsible for these changes.

During 7 mmHg pressure stimulation, delayed afterdepolarizations were observed following each action potential (Figure 3). Occassionally afterdepolarizations resulted in extra action potentials and contractions (Figure 4). Application of 80 μM Gd^{3+} blocked the stretch-induced afterpolarizations entirely (Figure 3) in all experiments ($n=12$). No extra action potentials or extra contractions were observed when gadolinium was used.

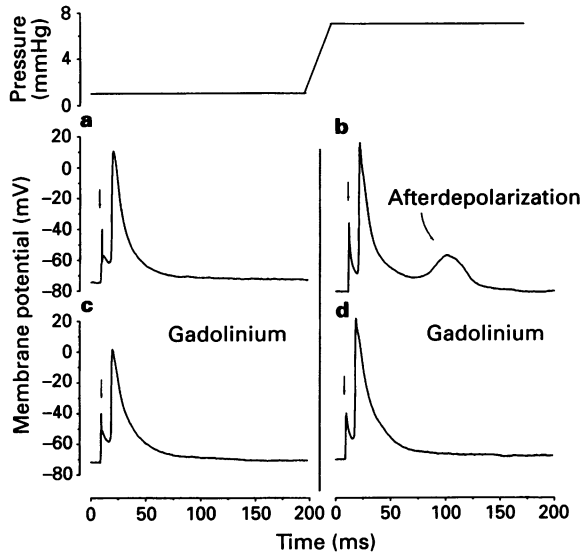

Figure 3 Representative intracellular recordings from rat atrial myocytes. (a) Action potential (AP) with intra-atrial pressure of 1 mmHg. (b) AP and delayed afterdepolarization (DAD) from an atrial myocyte subjected to stretch caused by 7 mmHg intra-atrial pressure. (c) AP from atrium with 80 μM Gd^{3+} superfusion and intra-atrial pressure of 1 mmHg. (d) AP from atria with 80 μM Gd^{3+} superfusation and intra-atrial pressure of 7 mmHg. Note the block of the DAD by Gd^{3+} in (d). The arrows indicated stimulus artifacts.

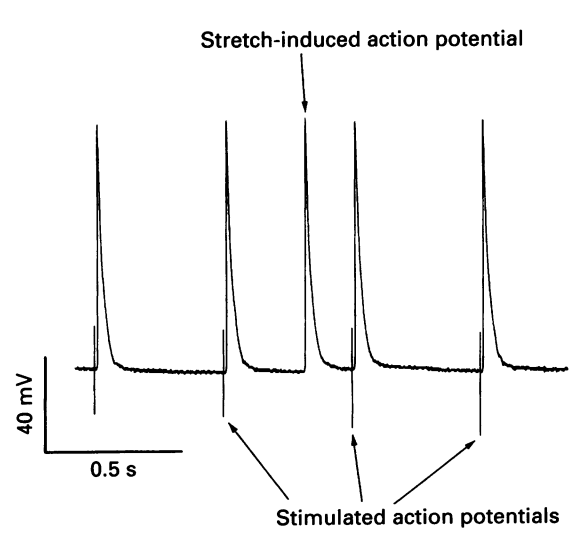

Figure 4 Representative intracellular voltage recording from a rat atrial myocyte where a pressure-induced action potential (i.e. AP that is not caused by electrical pacing) is illustrated. At the time of recording the atrium was subjected to 5 mmHg diastolic pressure. The extras were not normally seen under low (~1 mmHg) pressures. The pressure-induced action potential is probably generated in some other cell and then conducted into the recorded cell. The resting (diastolic) potential of the recorded cells was −80 mV.

Contraction force during stretch

The dependence of the contractile performance of heart myocyte on preceding stretch is a well-known phenomenon, the Frank-Starling effect. This can be observed also with the isolated left atrial preparation (Figure 5), where the relative contraction, as determined by developed pressure, increases as a function of the diastolic pressure (between 1 and 8 mmHg, $n=8$). The amplitude of the generated pressure is only 13% with 2 mmHg pressure compared to that of 8 mmHg diastolic pressure (normalized to 100%).

Generated pressure pulses were recorded at five different diastolic pressure (between 1 and 8 mmHg) with ($n=4$) and without ($n=8$) 80 μM Gd^{3+} superfusion (Figure 6). As a negative control we used 5 μM diltiazem (to block voltage-activated L-type Ca^{2+} channels) in order to compare the effects on contractility of Gd^{3+} and of diltiazem with each other (Figure 6). If the high concentration (80 μM) of gadolinium blocks a major part of the L-type Ca^{2+} channels as has been suggested in cultured myocytes (Lansmann, 1990) a similar reduction of contraction force with both gadolinium and diltiazem would ensue. As can be seen from Figure 6 the effects of gadolinium and diltiazem were different. Diltiazem at 5 μM blocked almost 50% of the contraction compared to control, at all diastolic pressures ($n=4$, $P<0.05$). The block of contraction by 80 μM

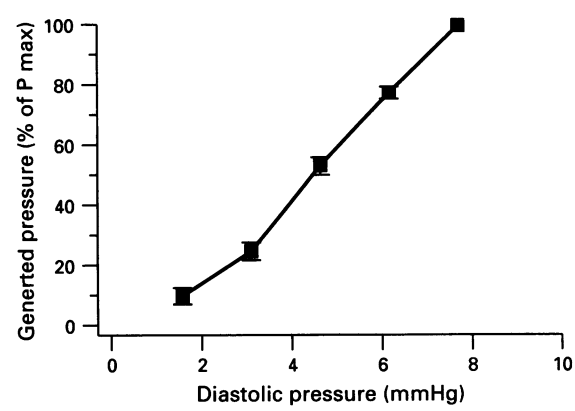

Figure 5 Increase in generated pressure in rat atrial appendix at different diastolic pressures, illustrating the Frank-Starling behaviour. The contraction force was estimated by the amplitude of pressure pulse generated by the atrial contraction ($n=8$), the amplitude with 8 mmHg basal pressure is 100%.

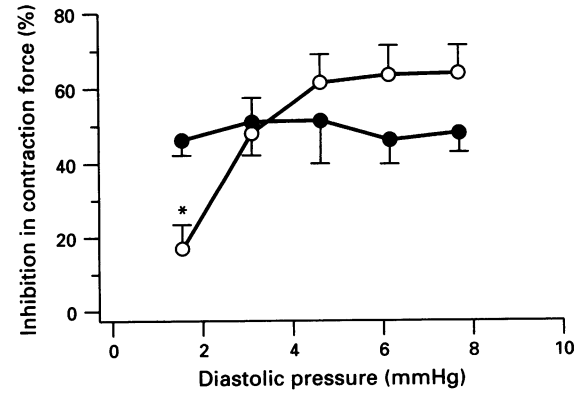

Figure 6 Effects of gadolinium (80 μM, ○) and diltiazem (5 μM, ●) on atrial contraction. The result shows the relative inhibition (compared to the control contraction of the same atria) at different diastolic pressures; *indicates significant statistical difference ($P<0.05$) between contraction pulse amplitudes in gadolinium and diltiazem superfused atria as compared to control.

Gd^{3+} was highly pressure-dependent: at 2 mmHg basal pressure it blocked only $17 \pm 12\%$ of the contraction ($n=4$) while at 7 mmHg pressure Gd^{3+} blocked $64 \pm 14\%$ compared to control ($P<0.05$, $n=8$). The effect of Gd^{3+} was thus significantly different at low pressure from that with higher pressures and from the effects of diltiazem at any pressure.

Discussion

The findings in the present study show that gadolinium has an effect on stretch-induced changes in contraction force and on intracellularly determined electrophysiological properties in rat isolated atria. Our main findings were: (i) stretch can induce proarrhythmic afterdepolarizations in single cells; (ii) stretch increases the amplitude and the relative conduction speed of the action potential (iii) Gd^{3+} at 80 μM inhibits afterdepolarizations and action potential changes; and (iv) Gd^{3+} at 80 μM reduces contraction in proportion to the preceding stretch.

Gadolinium as a blocker of stretch-activated ion channels

The specificity of gadolinium in blocking the SA channels has been debated for some time. Ability of Gd^{3+} to block the L-type calcium current is well known (Lansmann, 1990), and it has been shown that even low concentrations (10 μM) of gadolinium can block the majority of L-type calcium channels (Lacampagne *et al.*, 1994) in isolated heart cells. These findings are interesting, because in the present study even high concentrations of gadolinium (80 μM) did not have such a dramatic effect on action potentials or contraction under low diastolic pressure, moderate stretch conditions. The variability of the effects of Gd^{3+} block in various experiments seems to have a natural explanation. The non-specific block (in the sense of affecting other than stretch-activated channels; delayed rectifier K^+-channels: Hongo *et al.*, 1995; L-type Ca^{2+}-channels: Lacampagne *et al.*, 1994) is dependent on the expression of the channels in the particular cells type (e.g. atrial vs. ventricular) or in the animal model used. To be more specific, the delayed rectifier K^+-channels are expressed more in guinea-pig cardiocytes than in the rat (Varró *et al.*, 1993), and also more in rat atria than in rat ventricles (Boyle & Nerbonne, 1991). Similarly, there seems to be quantitative differences in the recorded Ca^{2+}-current, implying differences in the expression of the Ca^{2+}-channel types and their kinetics (Varró *et al.*, 1993). An additional complication is the occassional use of Gd^{3+} chelating substances, like bicarbonate, in the buffer solutions (Boland *et al.*, 1991).

If the effects of gadolinium are similar in isolated cells and in semi-intact tissues, 80 μM Gd^{3+} superfusion would cause a total inhibition of tissue contraction via blocking L-type calcium channels. Instead the contraction was reduced by only 17%. Hence, it seems likely that the specificity of gadolinium for blocking SA channels is quite different in isolated cells when compared to intact or semi-intact tissue. It is also possible, but unlikely, that some unknown mechanism increases the affinity of L-type calcium channels for gadolinium during stretch, so that a block of L-type Ca^{2+}-current would be more prominent when the tissue is stretched. Even so, the effect would be the same, namely a specific block of the stretch-induced effect.

Stretch-induced changes in action potentials

Stretch-dependent changes in ventricular or atrial action potential duration have been reported previously (Lab, 1978; Franz *et al.*, 1989; Ravelli *et al.*, 1993). Most of these studies were carried out using contact electrode techniques that recorded extracellular monophasic action potentials (MAPs). MAPs reflect sums of potentials in many cells, and their extracellular nature makes it difficult to compare them to intracellular recordings. For example, alterations in MAPs' durations are not only due to changes in action potential durations but also possibly caused by changes in conduction velocities. In a few successful intracellular recordings the rapid stretch of the cardiac tissue has been reported to induced fast depolarizations and extra action potentials (Lab, 1980; White *et al.*, 1993). The effect of stretch on intracellularly recorded action potentials parameters has remained controversial, even more so when we consider that the reported depolarizations up to 30 mV (if real and not artefactual), would have rendered the cells unexcitable by inactivation of Na^{2+}-channels.

We found that stretch causes a small increase in $APD_{90\%}$, but this increase was not statistically significant. In contrast, stretch significantly increased action potential amplitude, and relative conduction velocity (Table 1). Increase in conduction velocity during stretch was also observed by Dominguez and Fozzard (1979) in cardiac Purkinje fibres. It is clear that stretch causes changes in electrophysiology of individual myocytes. These changes could be caused directly by currents through SA channels at the time of contraction, or by secondary effects caused by changes in intracellular ion concentrations (calcium, sodium), which are due to changes in steady-state permeability of the plasmalemma by activation of SA channels. The rise in action potential amplitude observed in this study does not support the hypothesis that stretch causes a transient depolarization of membrane potential, as this would have an opposite effect. However, it is possible that membrane potential responses to stretch are strongly dependent on the rate of rise or duration of the stretching stimulus (Franz *et al.*, 1992). In that case, rapid increase in stretching level could induce a transient depolarization whereas slow stimulus would not. An additional point to discuss is the possible adaptational mechanisms involved. The cells may adapt to stretch, e.g. via a mechanism whereby the increased intracellular resting Ca^{2+} (Allen *et al.*, 1988) induces an increase in tension of contractile elements over a period of minutes (Allen & Kurihara, 1982), thus opposing the stretch. The resting Ca^{2+} may rise, in addition to direct stretch-induced conductance, also via an increased activity of Na^{2+}-Ca^{2+}-exchanger. This could be caused by the increased Na^+ current through SA-channels or through fast voltage-activated channels (larger AP amplitude, see Table 1). Furthermore, the mechanosensitive cells have adaptive properties. MacBride and Hamill (1993) show that the open probability of stretch-activated channels in oocytes is dramatically reduced over a period of seconds by a sustained stretching stimulus.

Delayed afterdepolarizations

Delayed afterdepolarizations (DADs) are oscillations of membrane potential that occur after complete repolarization of the cardiac action potential (January & Fozzard, 1988). Therefore, they are initiated during electrical and mechanical diastole. The prototypical experimental method used to induced DADs is to expose heart tissue to cardiac glycosides. There are known to inhibit the Na-K pump (Ebner *et al.*, 1986), raising the intracellular Na^+ concentration. This in turn leads to rise of $[Ca^{2+}]_i$ via Na^+-Ca^{2+} exchange and causes calcium overload, which is believed to be the main cause of delayed afterdepolarizations (Levy, 1989). The afterdepolarizations are generated by a depolarizing, i.e. inward, current, which in case of cardiac glycosides is known as a transient inward curent (TI) (January & Fozzard, 1988). Delayed afterdepolarizations can also be generated by altering the ionic environment. Reducing $[K^+]_o$ causes similar currents and aftercontractions as does poisoning by digitalis.

The block of afterdepolarizations by Gd^{3+} in the present study suggests that the effects of stretch could be mediated by specific changes in ionic currents underlying the action potentials. Two hypotheses have been proposed to explain the afterdepolarizations and the TI. Both are based on the idea of intracellular calcium overload. The first, originally proposed by Kass *et al.* (1978a,b) suggests that a non-selective cation channel regulated by intracellular Ca^{2+} is involved. Under

Ca^{2+} overload, there could occur a repetitive release of Ca^{2+} from SR, which would in turn activate a non-selective cation channel in the cell membrane, causing TI and afterdepolarizations. The other hypothesis is based on the fact that intracellular Ca^{2+} activates Na^{+}-Ca^{2+} exchange, increasing Ca^{2+} efflux and Na^{+} influx, resulting in an inward depolarizing current (Arlock & Katzung, 1985). However, the basis of afterdepolarizations is still unclear.

Although stretch can cause afterdepolarizations which are similar to those induced by digitalis, the origins of these two phenomena are not necessarily the same. Hence, we could block intracellularly recorded afterdepolarizations by 80 μM Gd^{3+}, in line with the block of afterdepolarizations by Gd^{3+} in MAP-recordings (Hansen *et al.*, 1991; Stacy *et al.*, 1992). These findings suggest that SA channels are invovled in stretch-induced afterdepolarizations. A variety of different SA channels have been identified in heart muscle cells of various species (Morris, 1990). Although channels of differing ionic selectivity are known to exist, there is little information about stretch-induced currents in cardiac myocytes. Kim (1993) recorded a whole-cell current induced by stretch in atrial myocytes. At negative holding potentials this inward current was pressure-dependent and could be activated by rather small pressures. The channels are underlying this current were permeant to cations including Ca^{2+}. Opening this channel would increase $[Ca^{2+}]_i$, which is known to occur when heart cells are stretched (Sigurdson *et al.*, 1992). Interestingly, this increase in $[Ca^{2+}]_i$ has been shown to be blocked by Gd^{3+} (Sigurdson *et al.*, 1992). Because $[Ca^{2+}]_i$ overload is the only explanatory link between the various interventions and the afterdepolarizations in all reported cases (digitalis poisoning, altering ionic environment), we assume that $[Ca^{2+}]_i$ is the main factor causing DADs. Therefore, SA channels may cause calcium overload and indirectly lead to DADs via the same mechanisms (Kass *et al.*, 1978a, b). These results suggest that blockade of DADs by Gd^{3+} ions is achieved by block of cation-selective SA channels, that inhibits the stretch-induced increase in calcium concentration inside the cell.

Stretch-induced changes in atrial contraction force

Although the Frank-Starling law of heart function has been under intensive study (see e.g. Lakatta, 1986; Lab *et al.*, 1994), there are still many unsolved problems concerning the mechanism that causes this phenomenon. This study suggests membrane permeability changes may modulate the contraction during stretch. It seems likely that SA channels participate in the regulation of intracellular calcium concentration, and thus partly determine the twitch force in cardiac myocytes (Le Guennec *et al.*, 1991).

To check for an inhibition in contractility (via the block of L- and T-type calcium channels) by Gd^{3+}, we recorded contractions from Gd^{3+} superfused atria. Gd^{3+} at 80 μM did change the contractility at low pressure (1 mmHg), but the reduction was small (17%) compared to control. This inhibition of contraction at low pressure can be interpreted as caused by non-specific block of voltage-activated calcium channels (SA channels are not substantially activated). But with higher pressures inhibition of the contractility by gadolinium was more prominent (64% at 7 mmHg) (Figure 6). In contrast, diltiazem (L-type calcium channel blocker) at 5 μM blocks an equal amount of contraction (50%) independent of the stretching level. Thus diltiazem block has no stretch sensitivity, whereas Gd^{3+}-block has. These findings are in agreement with a previous report where 80 μM Gd^{3+} superfusion was found to have only a minor effect on contraction in a left atrial strip preparation with mild stretch (Laine *et al.*, 1994a). It must be noted that in another multicellular preparation, the papillary muscle strip, gadolinium similarly causes a stretch-dependent reduction of the contraction (Lab *et al.*, 1994). The data expressed in Figures 5 and 6 suggest that although Gd^{3+} may have blocked part of the voltage-gated Ca^{2+}-channels, the main function was block of SA channels, because 64% of stretch-induced increase in contraction was inhibited with gadolinium. These results do not completely rule out the possibility that physiological effects of gadolinium in stretched heart tissue are due to both blockade of SA channels and blockade of voltage-gated Ca^{2+}-channels.

Arrhythmic and antiarrhythmic effects of stretch

Stretch may, by causing afterdepolarizations, induce extra action potentials (Figure 4., also Lab, 1980; White *et al.*, 1993). This can be called a proarrhythmic effect of stretch, which may also have its basis in the SA-channels (Nazir *et al.*, 1995). Opposing this, stretch also has at least one antiarrhythmic consequence, namely the increase in conduction speed (Table 1). However, the latter may, be also proarrhythmic, depending on the location of the stretched myocytes in the conduction pathway in the heart. If the increased conduction velocity is accompanied by changes in excitability caused by alterations in resting potential (Nakagawa *et al.*, 1988; Tavi & Weckstrom, 1995), the situation is even more complicated. It seems clear, that by interpreting results obtained with isolated cells, or even with a multicellular *in vitro* models as in the present study, the proarrhythmic or antiarrhythmic potential of stretch cannot be easily judged. As pointed out above, the cells may also adapt to stretch, and, consequently, the dromotropic effects of stretch should be rigorously investigated in a more suitable animal model.

In conclusion, gadolinium blocks stretch-induced changes in cell electrophysiology, namely the changes in action potential parameters and the appearance of delayed afterdepolarizations. Moreover, gadolinium blocks a part of the stretch-induced increase in contraction force in a stretch-dependent manner.

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