Cooper, Patricia J., Ming Lei, Long-Xian Cheng, and Peter Kohl. Selected Contribution: Axial stretch increases spontaneous pacemaker activity in rabbit isolated sinoatrial node cells. J Appl Physiol 89: 2099–2104, 2000.—Isolated, spontaneously beating rabbit sinoatrial node cells were subjected to longitudinal stretch, using carbon fibers attached to both ends of the cell. Their electrical behavior was studied simultaneously in current-clamp or voltage-clamp mode using the perforated patch configuration. Moderate stretch (7%) caused an increase in spontaneous beating rate (by 5%) and a reduction in maximum diastolic and systolic potentials (by 2.5%), as seen in multicellular preparations. Mathematical modeling of the stretch intervention showed the experimental results to be compatible with stretch activation of cation nonselective ion channels, similar to those found in other cardiac cell populations. Voltage-clamp experiments validated the presence of a stretch-induced current component with a reversal potential near \(-11\) mV. These data confirm, for the first time, that the positive chronotropic response of the heart to stretch is, at least in part, encoded on the level of individual sinoatrial node pacemaker cells; all reported data are in agreement with a major contribution of stretch-activated cation nonselective channels to this response.

heart rate; mechanoelectric feedback; stretch-activated channels; modeling; electrophysiology

It has been known for almost a century that increased filling of the right atrium causes heart rate acceleration (2). Still, the (sub)cellular mechanisms that give rise to this response, commonly referred to as the “Bainbridge effect,” remain uncertain.

Initially, the heart’s positive chronotropic response to stretch was believed to be caused by an autonomic reflex. With the confirmation of qualitatively similar responses in isolated heart and sinoatrial node (SAN) preparations, however, it became obvious that the pacemaker’s response to stretch must be, at least in part, caused by mechanisms that are intrinsic to the heart and the SAN (4, 11).

Typically, the SAN responds to stretch with an increase in beating rate and a reduction in both maximum diastolic and systolic potentials (MDP and MSP, respectively; Refs. 11 and 20). This response could be caused by stretch activation of ion channels with a reversal potential between MDP and MSP.

There are at least two major groups of stretch-activated channels (SACs) in cardiac cells: cation nonselective (reversal potential between 0 and \(-20\) mV) and potassium selective (reversal potential negative to MDP). Cation nonselective SACs could therefore form a plausible candidate for the SAN’s response to stretch. These channels have, however, not yet been directly identified in SAN cells (15).

Another group of cardiac ion channels with a reversal potential between MDP and MSP, which are frequently assumed to be mechanically operated, have been identified in rabbit SAN cells: cell-volume activated chloride channels (13). Unlike SACs, these channels require an increase in cytosolic volume for their activation (8, 28). Although volume-activated channels are likely to play a role in cell volume regulation, they are understood to be of little bearing in the context of beat-by-beat variations in cell (or tissue) length and tension, as cell volume is not assumed to change during the cardiac cycle of relaxation or contraction. Swelling of rabbit SAN cells, however, has...
been shown to actually decrease their spontaneous beating rate (22).

Finally, there is the possibility that cardiac nonmyocytes contribute to the positive chronotropic response to stretch. This could include electrical interactions with mechanosensitive connective tissue cells, paracrine effects from endothelial cells, or intracardiac neuronal reflexes (1, 18, 19, 25).

This raises the question of whether the heart’s positive chronotropic response to stretch is a consequence of pacemaker cell properties or whether it necessarily requires the interaction of groups of cells. This study investigates the effects of direct longitudinal stretch on the electrophysiology of isolated, spontaneously beating rabbit SAN pacemaker cells.

MATERIALS AND METHODS

Cell isolation. Cells were isolated according to the procedures described previously by Brown (5). Briefly, 600–1,000-g New Zealand White rabbits were killed by cervical dislocation; hearts were excised quickly and placed in a perfusion dish with the endocardial side pointing upward. After atrioventricular dissection, the right atrium was positioned in a perfused chamber; hearts were excised quickly and placed in 1,000-g New Zealand White rabbits were killed by cervical dislocation; hearts were excised quickly and placed in

The SAN area was separated, and four to five thin tissue strips (∼3 × 1 mm) were cut from the node, perpendicular to the crista terminalis. Tissue strips were continuously superfused with Tyrode solution until reoccurrence of spontaneous beating activity could be confirmed (∼1–3 min).

Spontaneously active SAN tissue strips were placed, for 5 min, in nominally Ca2+-free Tyrode solution before the now quiescent tissue strips were incubated for 35–40 min at 37°C in 400 U/ml collagenase (Yakult) and 3.4 U/ml elastase (Sigma-Aldrich) dissolved in nominally Ca2+-free Tyrode solution. Tissue strips were then washed in Kraft-brühe (KB) storage medium and kept at 4°C for at least 1 h.

Single cells were released from the strips directly into the KB-filled perfusion chamber, using either microforceps or pipette suction for agitation. Reexposure to normal Tyrode solution was preceded by superfusion with low-Ca2+ Tyrode solution; the changeover between solutions was graded and occurred over a period of 5 min.

During experiments, the bath (volume <0.5 ml) was superfused with Tyrode solution at a rate of 2.5–3 ml/min while the superfusate temperature was servo-controlled at 36 ± 0.5°C.

Solutions. Normal Tyrode solution contained (in mM) 140 NaCl, 5.4 KCl, 1.8 CaCl2, 1 MgCl2, 11 glucose, and 8 HEPES and was titrated with NaOH to pH 7.4. Ca2+-free solution contained (in mM) 140 NaCl, 5.4 KCl, 1 MgCl2, 11 glucose, and 8 HEPES titrated with NaOH to pH 7.4. KB solution contained (in mM) 30 KOH, 25 KCl, 80 l-glutamic acid, 10 taunine, 14 oxalic acid, 18 KH2PO4, 0.5 MgSO4, 0.5 EGTA, 10 glucose, and 10 HEPES titrated to pH 7.2 with KOH. The pipette solution contained (in mM) 80 KOH, 50 KCl, 90 aspartic acid, 1 MgCl2, 5 MgATP, 2.5 phosphocreatine di-tris, 2.5 creatine disodium phosphate, 0.1 EGTA, and 10 HEPES titrated to pH 7.4 with KOH. Amphotericin B (200 µg/ml) was added to the pipette solution immediately before the experiments.

Current- and voltage-clamp recordings. The perforated patch-clamp mode was used for electrical recordings from single SAN cells. Patch pipettes were made from 1-mm square, glass capillaries with a 0.5 × 0.5 mm opening (Friedrich & Dimmock) using a Narishige (PB-7) electrode puller. Electrode tip diameters were ∼1–2 µm and resistances ranged from 4 to 8 MΩ. Patch pipettes were positioned with the drift-free PatchMan electronic manipulator (Eppendorf), and electrical signals were recorded with an Axopatch-200B patch-clamp amplifier (Axon Instruments). Data were digitized at 10 kHz with a Digidata 1200B (Axon Instruments) and displayed and analyzed with the help of pCLAMP 8 software (Axon Instruments).

Mechanical stimulation. A pair of carbon fibers, attached to opposite ends of an isolated cell, was used to apply longitudinal stretch while electrophysiological recordings were obtained via a patch pipette positioned half way along the cell (see Fig. 1). This allowed application of bidirectional stretch while reducing transversal shift of the membrane patch under the pipette.

In modification of the original carbon fiber technique by LeGuennec et al. (21), fibers were held by glass capillaries with bent (at 40°) tips. This allowed us to align the carbon fiber tip nearly parallel to the bottom of the perfusion chamber, improving the approach and contact with cells and reducing spatial interference with the centrally located patch pipette (7). Longitudinal stretch was applied using a combination of hydraulic manipulators and a computer-controlled stepper motor (IonOptix, Milton).

Statistics. All results are presented as means ± SE. Statistical significance was determined by ANOVA for repeated measures and Dunnett’s test for multiple comparisons, using Prism software (GraphPad). A probability of P < 0.05 was considered to indicate rejection of the null hypothesis, therefore denoting a significant difference between means.

RESULTS

Current-clamp data. Direct longitudinal stretch of moderate amplitude (5–10% of resting cell length) was applied to eight spontaneously beating rabbit SAN cells, using the carbon fiber technique (Fig. 1). Moderate mechanical stimulation was chosen to avoid cell damage, as witnessed by the reversal of stretch-induced responses on return to control length (see Table 1).

Only cells with stable electrophysiological parameters, including MDP, MSP, and beating rate, were selected. However, a considerable variety of parameter values between SAN cells was still observed, which is not atypical for this type of preparation (14, 22). Thus data for individual cells were normalized, relative to control conditions, for the summarized presentation in Table 1.

Under these conditions, we found a stretch-induced increase in spontaneous beating rate in all eight cells (Fig. 1). As an illustration of the variability of parameters between cells, the control cycle length in the eight cells studied ranged from 194.2 to 403.8 ms. Stretch induced a decrease in average cycle length from 290.2 ± 22.3 to 274.7 ± 20.5 ms (n = 8; P < 0.05); on return to control length, average cycle length returned to 289.0 ± 22.4 ms (n = 8; P > 0.05 vs. control).

Normalized data regarding this response, together with other stretch-induced changes of key electrophysiological parameters, are presented in Table 1. The stretch-induced responses of single, isolated SAN cells are representative of those seen in multicellular preparations, that is, a stretch-induced reduction in cycle
length, as well as a reduction in both MDP and MSP (Table 1). These findings are in good agreement with a proposed involvement of SACs in this response.

Mathematical modeling. Using the Oxsoft Heart v4.8 model (Cell MicroControls, Virginia Beach, VA), we simulated moderate stretch of a spontaneously active SAN pacemaker cell (24). We used the standard single SAN cell model contained in the Oxsoft software suite and a previously developed algorithm for modeling of stretch effects on cardiac cells (17), which is an integral part of the package. For the simulations, the following settings were specified: $K_{\text{mode}} = 9$ to simulate the delayed rectifier potassium current as consisting of both rapidly and slowly activating components, $i_{K_r}$ and $i_{K_s}$, $ST_{\text{mode}} = 2$ to introduce stretch-activated conductances, GST = 0.00035 to define the maximum stretch-activated conductance as 350 pS, EST = −20 to set the reversal potential of this conductance to −20 mV, and HST = 1.8 to define half activation of the stretch-activated conductance to occur at a sarcomere length of 1.8 μm (for a detailed account on the mathematical apparatus, see Ref. 17). These settings are based on the attempt to assess stretch effects in a previously established and publicly available mathematical model by modifying as few settings as possible to avoid “overcustomization,” using the most pessimistic of parameters of a plausible range (e.g., a reversal potential of −20 mV).

The simulation was first allowed to run for 20 beats to approach a steady state. A control run was then initiated. Moderate stretch was simulated in a rerun, starting from exactly the same conditions as control, by increasing model sarcomere length at 150 ms into the run from 1.7 to 1.8 μm. This causes a maximum stretch-activated current of $\sim 10$ pA (Fig. 2, bottom), which leads to an increase in beating rate and a reduction in MDP and MSP (Fig. 2, top).

Because the simulated stretch-activated current reverses between MSP and MDP (in this case at −20 mV), the current is outward during systole (i.e., when SAN cell membrane potential is positive to the current’s reversal potential) and inward during diastole (Fig. 2, bottom). In the model, this causes a reduction in both maximum upstroke velocity (Fig. 2, middle trace, positive maxima) and late repolarization velocity (Fig. 2, middle trace, level of plateau after negative velocity maximum). The latter changes were also observed in the current-clamp study of stretch effects on spontaneous SAN cell activity (Table 1). There was no prominent change in spontaneous diastolic depolarization rate, neither in the model nor in the experiments (Table 1), indicating that the effects of stretch-induced activation of a diastolic inward current have been counteracted by other changes, like a reduction in activation of the hyperpolarization-activated inward current and inactivation of the delayed rectifier potas-

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**Table 1. Stretch-induced changes of key electrophysiological parameters of spontaneously beating rabbit SAN pacemaker cells**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control (absolute values)</th>
<th>Stretch, % of control</th>
<th>Recovery, % of control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cycle length</td>
<td>290.2 ± 22.3 ms</td>
<td>95.0 ± 0.7*</td>
<td>99.8 ± 0.9</td>
</tr>
<tr>
<td>Maximum systolic potential</td>
<td>45.2 ± 3.6 mV</td>
<td>97.8 ± 0.4*</td>
<td>100.6 ± 0.4</td>
</tr>
<tr>
<td>Maximum diastolic potential</td>
<td>−54.3 ± 3.2 mV</td>
<td>97.6 ± 0.4*</td>
<td>99.6 ± 0.3</td>
</tr>
<tr>
<td>Spontaneous depolarization rate</td>
<td>0.115 ± 0.015 V/s</td>
<td>111.2 ± 3.3</td>
<td>106.7 ± 3.4</td>
</tr>
<tr>
<td>Maximum upstroke velocity</td>
<td>22.98 ± 7.56 V/s</td>
<td>89.0 ± 1.8*</td>
<td>94.6 ± 2.7</td>
</tr>
<tr>
<td>Early repolarization velocity</td>
<td>−0.50 ± 0.06 V/s</td>
<td>105.6 ± 1.9*</td>
<td>102.3 ± 1.4</td>
</tr>
<tr>
<td>Late repolarization velocity</td>
<td>−1.59 ± 0.17 V/s</td>
<td>96.3 ± 0.9*</td>
<td>99.7 ± 1.0</td>
</tr>
<tr>
<td>Spontaneous depolarization time</td>
<td>137.0 ± 4.6 ms</td>
<td>92.7 ± 1.4*</td>
<td>102.7 ± 1.8</td>
</tr>
<tr>
<td>Threshold to peak time</td>
<td>14.8 ± 0.9 ms</td>
<td>109.3 ± 2.9*</td>
<td>100.6 ± 2.3</td>
</tr>
<tr>
<td>Repolarization time</td>
<td>141.0 ± 3.4 ms</td>
<td>97.1 ± 0.7*</td>
<td>99.2 ± 0.6</td>
</tr>
</tbody>
</table>

Values are means ± SE; $n = 8$ cells. Early repolarization velocity, rate of initial fast repolarization; late repolarization velocity, rate of secondary slow phase of repolarization; spontaneous depolarization time, time from maximum diastolic potential (MDP) to threshold; threshold to peak time, time from threshold to maximum systolic potential (MSP); repolarization time, time from MSP to MDP. SAN, sinoatrial node. *$P < 0.05$ (ANOVA).
Control activity (bright curves) and simulated stretch effects (dark curves) involving autocrine blocking agents, and so forth (3). The application of tension-bearing elements, release of changes in cell attachment, membrane "softening," rearrangement of tension-bearing elements, release of autocrine blocking agents, and so forth (3). The application of well-controlled levels of "external" stretch does not, by itself, guarantee reproducible levels of "internal" stretch activation of (sub)cellular mechanisms (27).

In our experiments, isolated SAN cells were voltage clamped to either +40 mV (n = 5) or −60 mV (n = 6) to mimic MSP and MDP conditions before moderate stretch was applied. In all cases, stretch elicited an outward current at +40 mV and an inward current at −60 mV. This is in keeping with the proposed activation of an ion channel population with a reversal potential between MSP and MDP.

To obtain a more quantitative measure of stretch effects on whole cell currents, a second study design was introduced, based on repetitively performing voltage-clamp step protocols at different levels of longitudinal stretch.

Five SAN cells were subjected to a sequence of depolarizing voltage steps (from a holding potential of −60 to +40 mV, in steps of 10 mV), in the presence and absence of stretch (Fig. 3, A and B). The difference current (Fig. 3C) shows the presence of an inward current at the holding level of −60 mV, which was reduced and turned outward by progressive depolarization (for clarity, Fig. 3, A-C, shows 20-mV steps only). The amplitude of the stretch-induced current ranges from −30 to +20 pA, which is the same order of magnitude as predicted by the modeling.

Figure 3D summarizes the current-voltage relation obtained on the basis of steady-state pulse currents from five SAN cells, assuming a linear current-voltage relationship of the stretch-activated current, as reported in mammalian ventricular myocytes (9, 10, 27) (curve fitting by linear regression, 95% confidence interval indicated by dotted lines). The stretch-induced current has a conductivity of 6 nS/pF (average slope of current-voltage curve, r² = 0.93). These data are in keeping with the hypothesis of a stretch-induced activation of a cation nonselective SAC with a reversal potential at −11.0 mV (the 95% confidence interval for the reversal potential ranges from −4.4 to −17.8 mV).

**DISCUSSION**

Thus moderate stretch of rabbit SAN pacemaker cells, by 5–10% of their resting length, causes a fully reversible increase in their spontaneous beating rate and a reduction in MDP and MSP. This is the first observation of a cellular substrate for the Bainbridge effect (2), the positive chronotropic response of the heart to stretch.

Both experimental studies and mathematical modeling suggest that this response may be caused by activation of cation nonselective SACs with a reversal potential between MSP and MDP of the SAN cell. There is no evidence of a predominant role of potassium-selective SACs, as this would be predicted to cause an outward current at all potential levels tested, therefore shifting both MSP and MDP in a negative direction. Our voltage-clamp data suggest a reversal potential for the stretch-induced current roughly be-

![Fig. 2. Computer simulation of the effect of stretch activation of a linear cation nonselective current (reversal potential of −20 mV) on pacemaker cell electrophysiology. Top trace (voltage) illustrates changes in action potential generation: simulated stretch causes an increase in beating rate and a reduction in diastolic and systolic potential maxima. This is accompanied by a reduction in maximum depolarization and late repolarization velocities (middle traces, dV/dt). Bottom trace illustrates the total stretch-activated current (iₚ). Control activity (bright curves) and simulated stretch effects (dark curves) are overlaid; onset of stretch is marked by an arrow.](http://jap.physiology.org/Downloaded from http://jap.physiology.org/)

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**Stress curve:**

- **Control activity (bright curves):**
- **Simulated stretch effects (dark curves):**

- **Stretch-activated current:**
  - **voltage:**
  - **di/dt:**
  - **iₚ:**

- **Fig. 2:** Computer simulation of the effect of stretch activation of a linear cation nonselective current (reversal potential of −20 mV) on pacemaker cell electrophysiology.
between 0 and −20 mV, which, again, is typical for cation nonselective SACs observed in mammalian ventricular myocytes (9, 10, 27). However, because the stretch-induced whole cell current was obtained as a difference current, we cannot exclude that this net current is made up of more than one underlying current component. This theory will require further investigation [for example, using specific SAC blockers such as GsMTx-4 (26)].

When the most “pessimistic” (i.e., least “depolarizing”) of these levels is used to simulate SAC effects on SAN cell activity, the mathematical model predicts that a stretch-activated current in the 10−11-A region would be sufficient to cause a positive chronotropic response. This is consistent with the experimentally observed stretch-induced currents. Thus it is likely that SACs are an important subcellular mechanism of the positive chronotropic response to stretch.

Alternatively, a stretch-induced increase in cAMP (6) could cause enhanced activation of the hyperpolarization-activated “pacemaker” current, (12), which should cause a significant increase in the diastolic depolarization rate. The latter could not be confirmed in this study. Also, the time course of cAMP synthesis would suggest that this mechanism is less likely to explain the pacemaker’s instant response to stretch.

Furthermore, it is interesting to see that the stretch-dependent current component, revealed as the current difference between stretched and nonstretched states (Fig. 3C), is almost completely flat. This makes it unlikely that, under our experimental conditions, other voltage- or time-dependent currents were significantly affected by stretch. This, together with the reduction in maximum upstroke velocity during stretch (Table 1), does not support the notion of a stretch-induced increase in open probability of the L-type Ca2+ channel in rabbit SAN pacemaker cells (23) during moderate stretch.

Finally, we would like to stress the importance of working in perforated patch mode and of selecting only SAN pacemaker cells that show spontaneous rhythmic beating and steady action potential parameters, as these investigations depend crucially on cell quality.

Thus we have shown that the Bainbridge effect has its origins, at least in part, at the level of individual SAN cells and that it does not necessarily require interaction with other cells. This does not, however, exclude an additional contribution of other (multi)cellular mechanisms to the positive chronotropic response to stretch (11, 16, 18).

In conclusion, the heart’s positive chronotropic response to stretch is, at least in part, accomplished by mechanisms present at the level of individual SAN cells and that it does not necessarily require interaction with other cells. This does not, however, exclude an additional contribution of other (multi)cellular mechanisms to the positive chronotropic response to stretch (11, 16, 18).

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combination with newly identified selective blockers of these SACs (26).

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