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Whole-Cell Mechanosensitive Currents in Rat Ventricular Myocytes Activated by Direct Stimulation

G.C.L. Bett, F. Sachs
Department of Physiology and Biophysics, 124 Sherman Hall, SUNY, Buffalo, NY 14214, USA

Abstract. Mechanosensitive channels may have a significant role in the development of cardiac arrhythmia following infarction, but the data on mechanical responses at the cellular level are limited. Mechanosensitivity is a ubiquitous property of cells, and although the structure of bacteriological mechanosensitive ion channels is becoming known by cloning, the structure and force transduction pathway in eukaryotes remains elusive.

Isolated adult rat ventricular myocytes were voltage clamped and stimulated with a mechanical probe. The probe was set in sinusoidal motion (either in, or normal to, the plane of the cell membrane), and then slowly lowered onto the cell. The sinusoidal frequency was held constant at 1 Hz but the stimulation amplitude was increased and the probe gradually lowered until a mechanically sensitive whole cell current was seen, which usually followed several minutes of stimulation.

The whole cell mechanosensitive current in rat cells had two components: (i) a brief large inward current spike current; (ii) a more sustained smaller inward current. The presence of the initial sharp inward current suggests that some structure within the cell either relaxes or is broken, exposing the mechanosensitive element(s) to stress. Metabolic changes induced by continued stress prior to the mechanosensitive response may weaken the elements that break producing the spike, or simple stress-induced fracture of the cytoskeleton itself may occur.

Key words: Ion channel — Patch clamp — Mechanical — Cytoskeleton

Introduction

Although mechanosensitivity of the heart has been well documented (for reviews *see* Brady, 1991; Sachs, 1991; White, 1996; Sachs & Morris, 1998), little has been uncovered about the behavior of cardiac mechanosensitive channels, particularly in acutely isolated cells. This is due in part to the difficulty of applying a reproducible and nondamaging mechanical deformation to an isolated cell held under voltage clamp.

Mechanosensitive responses can be evoked in the whole heart by inflating a balloon in the ventricle (Franz et al., 1989; Hansen et al., 1991; Zabel et al., 1996) or changing the hemodynamic loading of the heart (Sadoshima et al., 1992*a*). Pulling strips of cardiac muscle by the ends also evokes a mechanosensitive response (Lab et al., 1994). Such mechanical interventions lead to diastolic depolarization (Penefsky & Hoffman, 1963; Zabel et al., 1996), an increase in the rate of spontaneous beating (Bainbridge, 1915; Blinks, 1956), and can even induce arrhythmias (Hansen, Craig & Hondeghem, 1990). Although these multicellular preparations provide insight into the importance of mechanoelectric feedback in the whole tissue, little can be learned about the specific behavior of mechanosensitive channels. The responses are complex, including gene activation (Sadoshima & Izumo, 1997), elevation of intracellular calcium (Sigurdson, Ruknudin & Sachs, 1992), elevation of intracellular cAMP (Watson, Haneda & Morgan, 1989), and release of agents such as ANP (Laine et al., 1994). The response is further complicated because the preparations cannot be held under voltage clamp. Although gadolinium, an inhibitor of most types of mechanosensitive channels (Ruknudin, Sachs & Bustamante, 1993; Suleymanian et al., 1995), has been used to investigate the role of mechanosensitive channels in tissues (Hansen et al., 1991; Stacy, Jr. et al., 1992) and papillary muscle (Lab et al., 1994) it is nonspecific in its action, and has been shown to block L-type calcium channels in ventricular myocytes (Lacampagne et al., 1994). The gadolinium-sensitive current is, therefore, not necessarily due to mechanosensitive current activity alone.

Patch-clamp experiments have shown the presence of pressure-activated single channels in excised or cell-attached patches of cardiac cell membrane from embry-

Correspondence to: G.C.L. Bett

onic chick (Ruknudin et al., 1993), guinea pig (Bustamante, Ruknudin & Sachs, 1991; Sasaki, Mitsuiye & Noma, 1992), rabbit (Hagiwara et al., 1992), rat (Craelius, 1993; Kim, 1993; Van Wagoner, 1993) and mollusc (Sigurdson, Bedard & Morris, 1987). (There is no published single channel data from freshly isolated adult rat ventricular myocytes.) Although the single channel data and the whole heart data are both consistent with the presence of mechanosensitive channels in the heart, there is a lack of data at the whole cell level corroborating the link.

Applying a mechanical deformation to an isolated cell presents many problems. Isolated heart cells can easily be voltage-clamped, but are difficult to stretch in a quantifiable, reproducible and nondamaging manner. Data from nonvoltage clamped cells have shown that there is an increase in the intracellular calcium concentration and a change in action potential shape following stretch (White et al., 1993; Gannier et al., 1996). In contrast to direct mechanical stress, hypoosmotically swelling isolated cardiac cells opens chloride-selective channels which are probably mediated by different pathways (*see* Vandenberg et al., 1996).

Data showing whole cell mechanosensitive cardiac currents comes from pressing on embryonic chick ventricular cells with a mechanical probe (Hu & Sachs, 1996; Bett & Sachs, 1999), stretching guinea-pig ventricular myocytes attached to a cover slip at one end and a suction pipette at the other (Sasaki et al., 1992), and pulling axially on an isolated rat cardiac myocyte under voltage clamp with a pair of concentric pipettes (Zeng, Bett & Sachs, 1999).

In this paper, we present data from acutely isolated voltage-clamped adult rat ventricular myocytes undergoing direct deformation of the membrane with a glass pipette acting as a mechanical probe. The mechanosensitive current was difficult to activate, and always began with a large inward spike of current, which suggests there was structural failure of a protecting element. Once elicited, the characteristics of the mechanosensitive current were highly reproducible. The tension bearing elements in acutely isolated rat ventricular myocytes may not be located on easily accessible parts of the sarcolemma. These elements, and perhaps the channels themselves, may be located either in the T-tubules, or some other internal membrane, which is not easily stimulated. This hypothesis is consistent with the difficulties in obtaining single channel recordings from adult rat ventricular myocytes with either cell-attached or excised patches (*unpublished observations*).

Materials and Methods

Cell Preparation

Hearts were excised from Sprague-Dawley rats (2-to-3-months-old) killed by lethal overdose of anaesthetic (2000 unit/kg heparin followed by 60 mg/kg Nembutal). Ventricular myocytes were enzymatically isolated by retrograde perfusion of the heart (Mitra & Morad, 1985). The heart was perfused in Tyrode's solution (37°C) for 10 min, in calcium-free Tyrode's for 4.5 minutes followed by 30 min in the enzyme solution and finally 5 min in low calcium Tyrode's solution. All solutions were equilibrated with 100% oxygen, and the enzyme solutions were recirculated.

After perfusion, the ventricle was cut off and minced. Cells were dispersed from the tissues by agitation, then filtered into Tyrode's solution through a 200 μM mesh net and stored at 4°C until use. All experiments were performed within 2 to 20 hr after cell isolation.

Mechanical Deformation Protocol

The mechanical probe used to deform the cell was made from 1 mm I.D. capillary glass, one end of which was strongly fire polished to produce a smooth bulb about 20 μM in diameter. No tethers were seen between the probe and cell, and the probe could be lifted cleanly away from the cell once the stimulation sequence was finished. Even though reusing the probe resulted in no apparent alteration of the response a fresh probe was used for each cell to reduce the possibility of contaminants from the previous cell sticking to the glass. The position of the probe was controlled by a tri-axial piezoelectric micromanipulator (Burleigh Instruments), which was driven by computer using Labview®software and a National Instruments interface and/or a sine wave signal generator(Hewlett Packard 8116a Pulse/Function generator) as appropriate. The piezoelectric manipulator had a manual override feature which enabled the height of the probe above the cell to be altered while the probe continued to oscillate in a sinusoidal fashion with unchanged amplitude and frequency. It was not possible to record the change in probe position when the manual override feature was used to raise or lower the probe in the z-direction. Because of this limitation, all figures which include a trace showing the "Manual Override of Z Position" indicate only an approximation of the distance moved by the probe during the experiment.

The isolated ventricular cells were placed on a chamber with a coverslip bottom located on the stage of an inverted Olympus microscope and monitored visually throughout the experiment. The cells were stimulated directly by bringing the mechanical probe into contact with the cell (Fig. 1). The probe was set in sinusoidal motion at 1 Hz (either in the plane of the membrane (X-Y direction), or normal to it (Z direction)) just above the cell, and lowered gently until the pipette was visually observed to make contact with the myocyte. The amplitude of the sinusoidal motion was then slowly increased and the Z position of the probe decreased (i.e., the deformation of the cell was increased) until a mechanosensitive response was seen. Only isolated rat ventricular myocytes (approximately 100 μM by 10 μM) with clear sarcomere stripes and sharp edges were used in these experiments.

Data Recording and Analysis

The whole cell current was monitored via a nystatin-perforated patch (Horn, 1991) using standard electrophysiological patch-clamp recording techniques (Hamill et al., 1981). The patch pipettes had a tip diameter of 1–2 μM and a resistance of 0.5–2 MΩ when the pipette was filled with high potassium solution. The initial seal resistance was greater than 1 GΩ. Once a good seal was obtained, the nystatin was left to permeabilize the patch for 5 min and the resting potential decreased to at least −70 mV. Once a seal was formed, the cell was voltage clamped at −70 mV until the experiments began. All cells were quiescent when released from voltage clamp, and were held at the indicated voltage for the duration of the experiments.

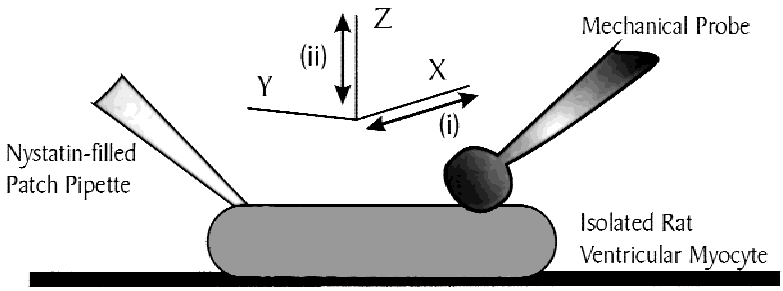

Fig. 1. Experimental setup. Acutely isolated rat ventricular myocytes were voltage clamped using a nystatin permeable patch. A heavily fire polished glass capillary tube was used as a mechanical probe. The probe was guided by a piezoelectric micromanipulator driven by a signal generator with manual override in the Z direction. In the experiments presented here, the probe was set in sinusoidal motion either in (i) the X-Y plane, (i.e., the plane of the membrane) or (ii) the Z direction (normal to the membrane), while it was lowered slowly onto the cell using the manual positioning feature.

The voltage and current signals were amplified and filtered by an Axopatch 200-B patch-clamp amplifier (Axon Instruments, Foster City, CA), and passed through an Instrutech VR-10 Digital Data Recorder to enable storage on VHS videotapes (SONY Videocassette Recorder SLV-675HF). Some records were also stored directly on computer (DELL XPS 200n) through a National Instruments data acquisition board (AT-MIO-16E) and Labview (National Instruments) graphically programmed Virtual Instruments.

Some signal generation (commands for voltage and probe position) was performed using Labview Virtual Instruments (National Instruments), but the main continuous sine-wave motion was initiated by a Hewlett Packard 8116A Pulse/Function Generator.

Solutions

Tyrode's solution (mM): 137 NaCl, 5.4 KCl, 0.5 $MgCl_2$, 1.8 $CaCl_2$, 10 HEPES, 5.0 glucose, pH 7.4 with NaOH. *Nominally calcium-free solution:* as for Tyrode's, but without the added calcium chloride. The bath solution was nominally calcium-free to reduce the possibility of interference from spontaneous contractions. *Electrode tip solution* (mM): 150 KCl, 5.0 NaCl, 10 HEPES, 0.5 EGTA. (The electrode was tip was dipped in Nystatin-free solution before being back filled with the antibiotic-containing solution to ensure formation of a good seal.) *Electrode solution:* A supersaturated concentration of Nystatin (200 μg per ml) was added to the electrode solution. All experiments were conducted at room temperature (20–25°C).

Results

Direct Stimulation of the Ventricular Cell Activates a Mechanosensitive Current

A brief deformation of an isolated rat ventricular myocyte with a mechanical probe is not sufficient to elicit a mechanosensitive response. In all the data presented here, the probe was in continuous contact (though with a sinusoidal oscillation) with the cell for at least five minutes before any mechanosensitive response was seen. The degree of cellular deformation was not the sole factor in eliciting a response, for a large initial movement of the probe in the Z direction would not elicit a mechanosensitive response whereas a smaller movement of the probe following a period of continued stimulation would activate the current. Despite the resistance to initiating a mechanosensitive current, once activated, the response was consistent.

Figure 2 shows typical whole cell responses from an isolated rat ventricular myocyte voltage clamped at −70 mV. In both traces shown (Fig. 2*A* and *B*) the cell was deformed for several minutes before a response was seen, i.e., time $t = 0$ on the graph corresponds to about 5 minutes since initial contact. In Fig. 2*A*, the probe was set to oscillate in the Z direction while it was slowly lowered onto the cell. As soon as the mechanosensitive current appeared, the probe was raised (via the manual override) in the Z direction to reduce the magnitude of the mechanical stimulus. The probe remained in contact with the cell throughout.

There were three distinct phases to the mechanosensitive current: (i) a large brief inward current spike, (ii) a slowly rising current which reached a steady plateau after about 5 sec, and (iii) a rapid decay of the current back to the resting current level once the stimulus was reduced. If the Z position of the probe remained unchanged once the mechanosensitive current was initiated, the inward current increased, reaching a plateau. If the plateau current was allowed to persist, (i.e., the mechanical stimulation was not reduced) it would usually result in spontaneous cell contractions that eventually lead either to loss of the membrane seal or to rounding up of the cell. In Fig. 2*A* the mechanosensitive current decayed rapidly once the mechanical strain was reduced, but the cell continued to respond to the sinusoidal stimulus for a short while afterward. This modulated current decayed slowly, even though the mechanical stimulus was not changed during this time.

Figure 2*B* shows the results from a cell where the probe was oscillating in the plane of the membrane (X-Y direction) while being lowered on to the cell in the Z direction. The probe remained in contact with the cell for more than 10 sec after the mechanosensitive current appeared, and the inward current reached a plateau. The cell was briefly switched from voltage clamp to current clamp ($I = 0$) during the mechanosensitive response. In both traces there is a clear increase in the noise level associated with the sustained inward current which suggests that the opened channels have a significant conductance relative to the mean. Once the mechanical

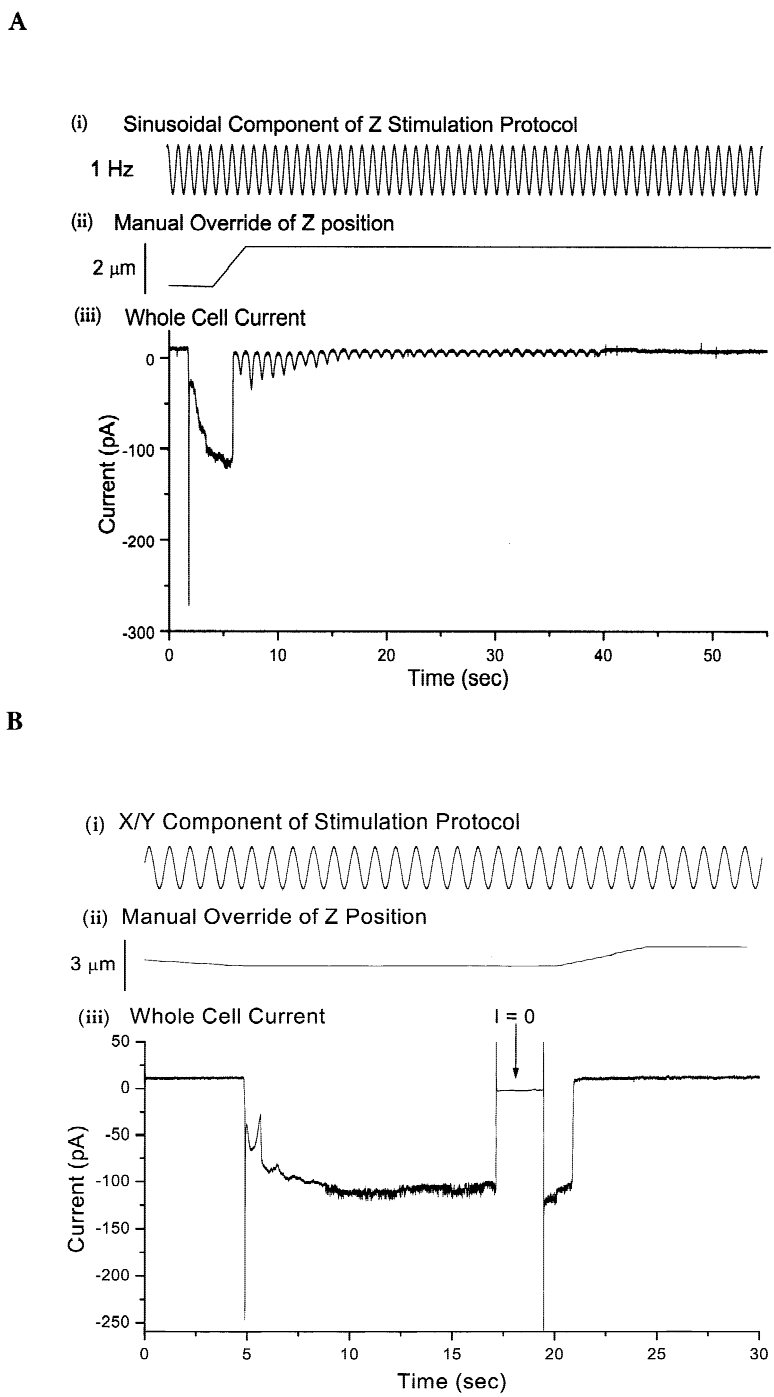

Fig. 2. Typical responses of an adult ventricular myocyte to direct stimulation. (*A*) (i) Sinusoidal stimulus protocol for the mechanical probe in the Z direction, perpendicular to the cell membrane. (ii) Manual override of the Z position (the total movement in the Z direction is the sum of traces (i) and (ii)). Manual alterations to the probe position could not be recorded, so this trace reflects an approximate reflection of the movement of the mean probe position in the Z direction. Note that the probe was lifted off the cell as soon a mechanosensitive response was observed to prevent a persistent inward current. The frequency (1 Hz) and amplitude of the sinusoidal stimulus remained unchanged throughout. (iii) The whole cell mechanosensitive current. There is an initial brief inward spike of current, then a smaller inward current, followed by a period in which the whole cell current followed the stimulus, and then slowly decayed back to the pre-response levels. (*B*) (i) Sinusoidal stimulation protocol in the plane of the membrane (X-Y direction). (ii) Manual control of the Z direction position. (iii) Whole cell current. Notice that there is an increase in current noise when the mechanosensitive current is activated. The cell was briefly clamped to $I = 0$ during this recording. The recovery of the cell from mechanosensitive current was rapid, and returned to the pre-stimulation level. Both cells were held at −70 mV.

probe was raised in the Z direction, the current quickly fell back to the normal background level for the cell, indicating there was no long-term alteration in the current induced by the mechanical stimulus.

The Mechanosensitive Current is in Phase with the Stimulus

When the mechanical probe was raised in the Z direction, though still remaining in contact with the cell, the inward mechanosensitive current decayed sharply back to the background current level. In six responses from three different cells, the current followed the sinusoidal behavior of the probe, either modulating the plateau current or just after the rapid decrease in mechanosensitive current when the probe was lifted. This modulation gradually decayed to zero.

Figure 3 shows the details of the sinusoidal movement of the probe and the mechanosensitive whole cell current. The sinusoidal stimulation is in phase with the

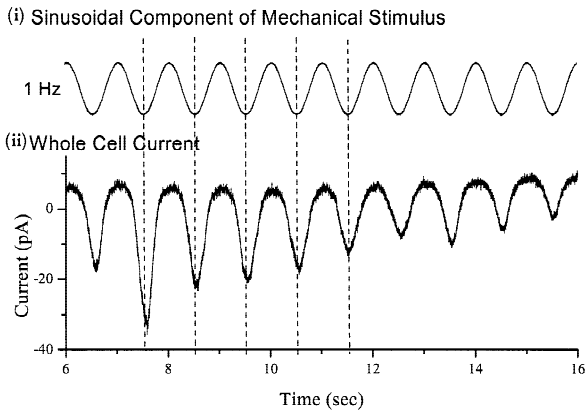

Fig. 3. The oscillations seen in the whole cell mechanosensitive current were in phase with the sinusoidal stimulation. (i) Movement of the probe in the Z direction. (ii) Whole cell current. The dashed lines show that the oscillations of the mechanosensitive current are in phase with the sinusoidal movement of the mechanical probe, and the peak inward current coincides with the maximum depression of the mechanical probe.

mechanical response. This exact relation between input stimulus and output current indicates that the membrane current is not due to some uncontrolled cellular response, such as cyclic release of calcium from intracellular stores, or other intracellular substances or messengers. The rapidity of the response (with minimal phase delay) suggests that mechanosensitive ion channels are involved.

On a few occasions, the cell began spontaneous contractions which were localized in the region where the probe and the cell had been previously in contact. The bath solution was nominally free of calcium, so the calcium involved in the contraction process was probably released from intracellular stores.

The Mechanosensitive Current Always Starts with a Large Spike of Inward Current

Every mechanosensitive current recorded from in these cells was preceded by a large and brief inward current. Apart from an occasional small inward current preceding the spike (*see* Fig. 4*Ba*) there was no visual or electrical indication that the cell was about to produce the current spike and become mechanosensitive. The inward current spike lasted about 10 msec.

A cell that had previously exhibited mechanosensitivity but had subsequently inactivated to the baseline level would have a spike of current at the beginning of the next mechanosensitive period elicited a few minutes later. This was true even if the mechanical probe was not moved in the X-Y direction between succeeding experiments.

The spike was also present in mechanosensitive currents that were separated by only a few seconds. Figure 4*B* shows two separate sensitive phases, activated only 15 seconds apart and both started with a current spike of ~400 pA. Figure 4*A* shows that even when the mechanical stimulus is continuous, and the current decays back to the baseline level for less than 500 msec, the next mechanosensitive phase begins with a large inward spike.

These cells typically produced inward spikes of ~300–400 pA and plateau currents of ~100 pA. Even when a very small mechanosensitive current was evoked in a cell (*see* Fig. 4*C*), it too was preceded by an inward current spike. The absolute magnitude of the spike was reduced; however, suggesting it is related to the magnitude of the subsequent mechanosensitive current.

The Rate of the Early Spike Development Depends on Probe Position

The spike is clearly a fundamental part of the mechanosensitive response, as this is when the cell becomes mechanically sensitive. The characteristics of the current spike did not vary from cell to cell apart from the shape within the first few msec.

When the mechanical probe was made to follow a sinusoidal stimulus in the plane of the membrane (i.e., the X-Y direction), the initial shape of the inward current spike was determined by the direction and position of the probe at the time when the current first appeared.

For a given Z position, when the mechanical probe was oscillating sinusoidally in the X-Y direction, the maximum cell deformation occurred at both the peaks in each cycle. The minimum strain was produced when the probe was near the mean, or centerline of the sinusoid. The initiation of the spike depended on whether the cell deformation was increasing or decreasing.

When the strain was increasing, i.e., the probe was moving away from the centerline toward a peak, the appearance of the current was rapid (*see* Fig. 5*A*). However, if the probe was headed toward the centerline of sinusoidal variation, i.e., the mechanical deformation was decreasing when the current started, the current had a much slower start (*see* Fig. 5*B*). Figure 5 shows examples of the current being initiated at different points in the cycle. The Z position of the probe remained constant throughout the time shown.

Decay of the Mechanosensitive Current is Constant

If the mechanical probe was raised slightly in the Z direction once the mechanosensitive current appeared the current fell back to the background level indicating that there was no long-term effect of stimulation on the cell. Following withdrawal of the stimulus the recovery was rapid, and altered little from cell to cell. The current decreased exponentially with a time constant of $\tau = 0.0286 \pm 0.0004$ sec.

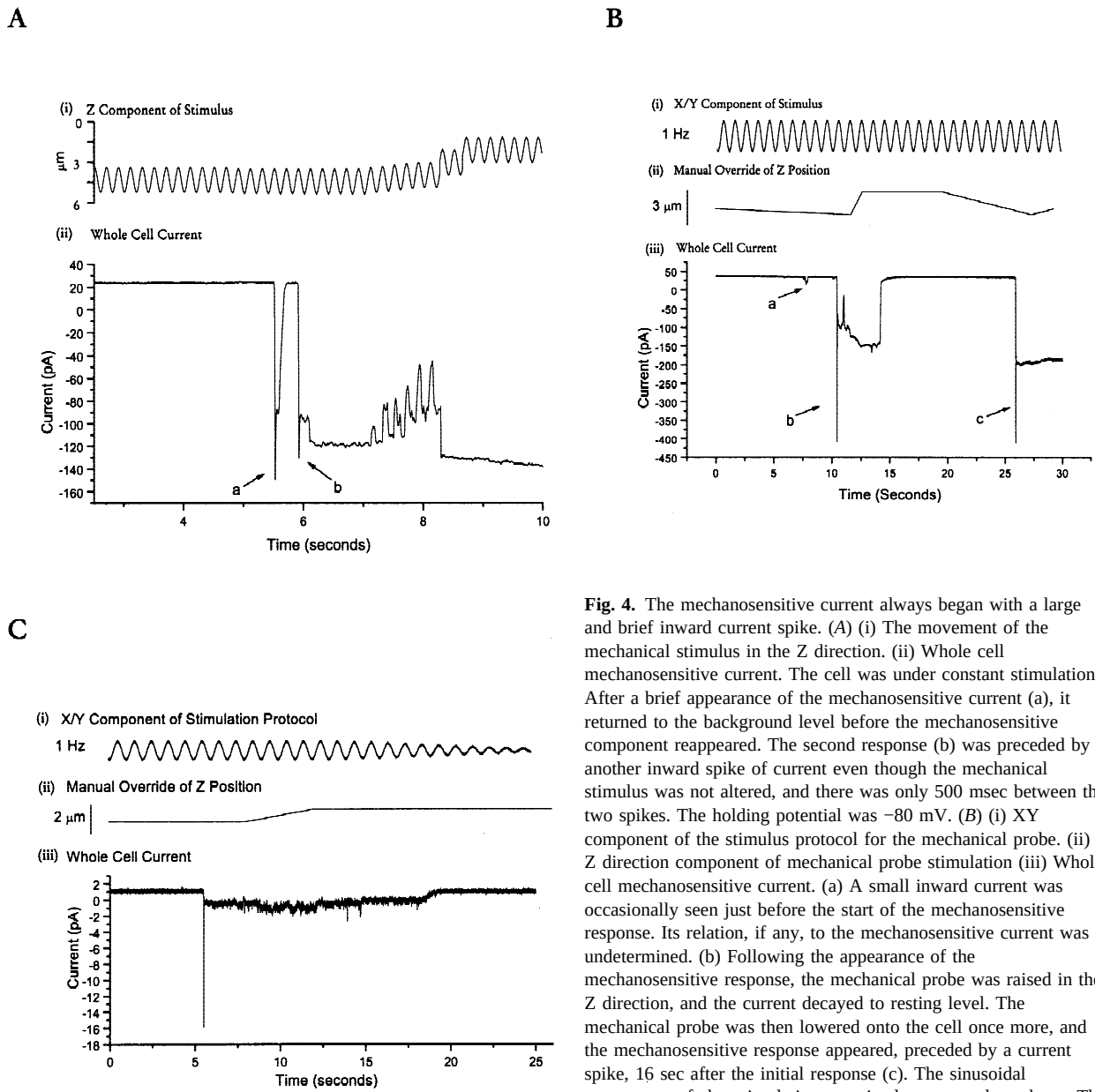

Fig. 4. The mechanosensitive current always began with a large and brief inward current spike. (*A*) (i) The movement of the mechanical stimulus in the Z direction. (ii) Whole cell mechanosensitive current. The cell was under constant stimulation. After a brief appearance of the mechanosensitive current (a), it returned to the background level before the mechanosensitive component reappeared. The second response (b) was preceded by another inward spike of current even though the mechanical stimulus was not altered, and there was only 500 msec between the two spikes. The holding potential was −80 mV. (*B*) (i) XY component of the stimulus protocol for the mechanical probe. (ii) Z direction component of mechanical probe stimulation (iii) Whole cell mechanosensitive current. (a) A small inward current was occasionally seen just before the start of the mechanosensitive response. Its relation, if any, to the mechanosensitive current was undetermined. (b) Following the appearance of the mechanosensitive response, the mechanical probe was raised in the Z direction, and the current decayed to resting level. The mechanical probe was then lowered onto the cell once more, and the mechanosensitive response appeared, preceded by a current spike, 16 sec after the initial response (c). The sinusoidal component of the stimulation remained constant throughout. The holding potential was −70 mV. C Even the smallest mechanosensitive current was accompanied by a relatively large inward spike and an increase in the noise level. The cell was held at −100 mV.

Discussion

Mechanosensitive channels in acutely isolated rat ventricular myocytes are not easily stimulated. These experiments show that a whole cell inward mechanosensitive current can be locally activated by mechanical stimulation but only following continuous stimulation of more than 5 minutes. A rapid and large cell deformation did not elicit a whole cell mechanosensitive current, instead, it usually resulted in cell death. Only a slow increase in the magnitude of stimulation (to a previously quiescent level) resulted in initiation of a mechanosensitive current. Once elicited, the mechanosensitive current had two distinct components: a brief and large inward current spike, which lasted no more than 10 msec, followed by a smaller sustained inward current. The magnitude of the sustained current was comparable to that obtained from these cells undergoing axial strain ~(100 pA) (Zeng et al., 1999), although whole cell current seen with axial stretch was not accompanied by the initial inward current spike.

The direct stimulation used in this study permitted longer-term stimulation than the axial strain experiments

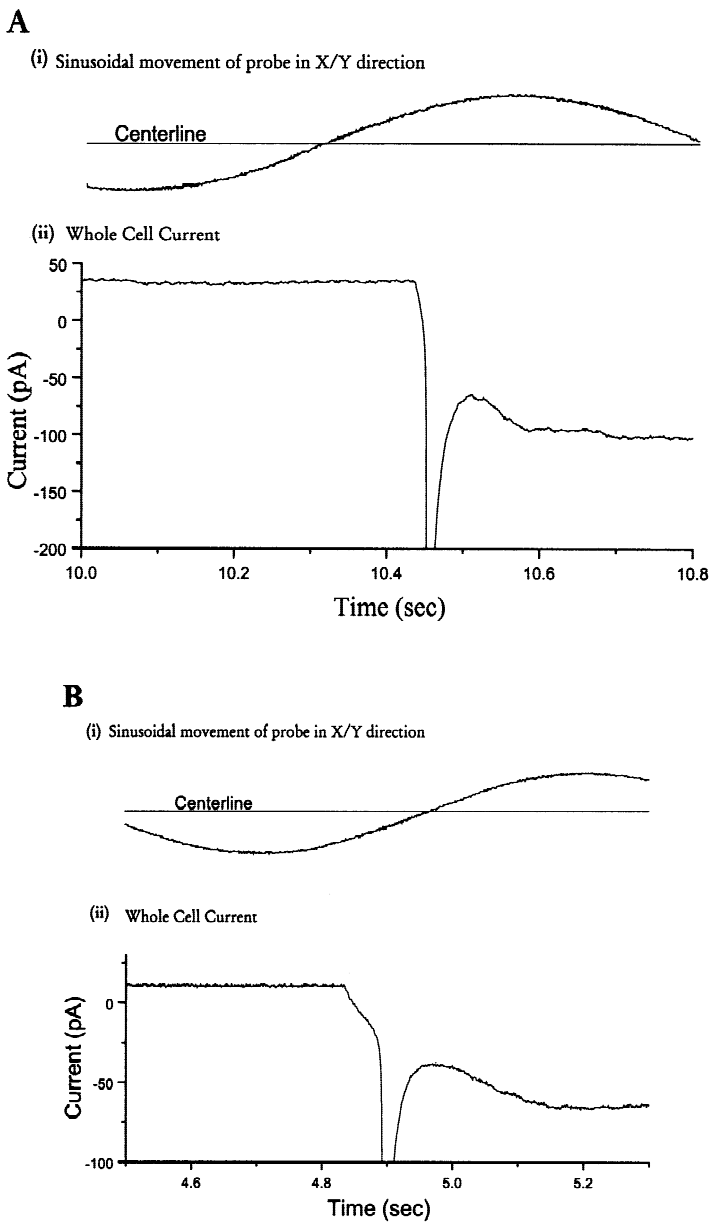

Fig. 5. The shape of the beginning of the mechanosensitive current was determined by the motion of the mechanical probe. (A and B) (i) sinusoidal motion of the mechanosensitive probe in the X-Y plane (in the plane of the membrane), so the maximum deformation is at both peaks within one cycle. (ii) Whole cell current. In *A*, the inward current spike begins when the probe is moving away from the centerline, and the mechanical deformation is increasing. The onset of the current is rapid. In *B*, the current spike appears as the mechanical probe is returning to the mean of the sinusoidal stimulation. The development of the current in this circumstance is much slower than in *A*. The position of the probe in the Z direction was unchanged throughout the interval of the traces shown.

done on these cells by (Zeng et al., 1999). A major difference is that in these experiments the probe stimulation was local whereas the axial strain was global. The differences in sensitivity may reflect the amount of membrane being stimulated or differences in the other processes which are activated by stretching, such as the changes in the affinity of calcium binding to Troponin C (Gulati, Sonnelblick & Babu, 1991; Hoffman & Fuchs, 1987) the amount of available intracellular calcium (Allen & Kentish, 1985), or changes in cAMP (Watson et al., 1989).

The time involved between the initial contact of the mechanical probe and the cell and the resulting mechanosensitive current meant that there was sufficient time for the cell to undergo secondary changes due to the stimulation. The absolute magnitude of the strain was not the critical factor in initiating a mechanosensitive current, for a rapid deformation to a given point would not produce in a current whereas a gradual deformation to that same degree would produce a mechanosensitive current after a period of prolonged stimulation. Stretching cardiac myocytes leads to a number of secondary cellular responses including rapid activation of immediate-early genes, hypertrophy (Sadoshima et al., 1992*a*), increased levels of cAMP (Watson et al., 1989) the appearance of phosphatidylinositol, protein kinase C, Raf-1 kinase, extracellular signal-regulated protein kinases (ERKs) and angiotensin II (*see* Yamazaki, Komuro &

Yazaki, 1998), the activation of VEGF gene expression (Li et al., 1997) and an increase in pH (Cingolani et al., 1998). Yamazaki et al. (1996) working with neonatal rat cardiocytes showed that stretch-induced activation of Raf-1 peaked after 2 min of mechanical stimulation, MAPKK at 8 min, and MAPKs after 8 min, which is within the time period of the experiments presented here.

If the development of mechanical sensitivity was precipitated by the buildup of a substance within the cell, there would presumably be no correlation between the initiation of the mechanosensitive current and the probe position. However, Fig. 5 shows there is a strong relationship between the shape of the foot of the inward current spike and the position of the mechanical probe. If the stimulation is increasing when the current starts, the spike is initiated rapidly whereas a current that starts as the mechanical deformation is decreasing rises slowly. There is clearly a direct link between the movement of the probe and the initiation of the mechanosensitive current. Although there is a connection between the short-term mechanical input and the mechanosensitive response, the metabolism of the cell in response to the prolonged mechanical stimulation may alter the structural integrity of the cytoskeletal components involved in force transmission.

The presence of the initial sharp inward current suggests that some structure within the cell either relaxes or is broken, exposing the mechanosensitive element(s) to stress. The inward spike was seen at the start of every appearance of the mechanosensitive current, whether there were only a few seconds between the currents, or several minutes, and was present even if the position of the probe was unchanged. Figure 4*a* shows that under continuous stimulation the mechanosensitive current can appear, disappear and reappear with 500 msec. Metabolic changes induced by continued stress prior to the mechanosensitive response may weaken the elements that break producing the spike, or simple stress-induced fracture of the cytoskeleton itself may occur.

The speed with which the current activates, coupled with ability of the current to switch on, off, and on again within 500 msec indicates that whatever changes within the cell make it mechanosensitive is capable of rapid transformation. A similar inward current spike can be seen in voltage-clamped embryonic chick cardiac cells when suction is applied to the cell membrane (Bett & Sachs, 1999).

There are no published data on single channel mechanosensitive currents from acutely adult isolated ventricular myocytes, only neonatal (Craelius, 1993; Hu & Sachs, 1996), cultured (Ruknudin, Bustamante & Sachs, 1991; Sadoshima et al., 1992*b*; Ruknudin et al., 1993) or atrial (Hoyer, Kohler & Distler, 1997; Kim, 1993) myocytes. One of the major differences between the cell types in which single channel currents have been seen and adult rat ventricular myocytes is the that the former have few T-tubules.

The absence of single channel data from freshly isolated cells suggests that the mechanosensitive channels are either located in the T-tubules (or some other internal membrane) or the mechanotransduction mechanism is disrupted on formation of a patch. Zeng et al. (1999) calculated that the absence of single channel data is not likely to be a result of low channel density given the magnitude of their whole cell current and the typical values for single mechanosensitive channel conductances.

Comparing mechanosensitive data from single channels in membrane patches and whole cell currents may lead to contradictions, because the mechanical properties of the membrane are disrupted by the formation of the patch (Sokabe, Sachs & Jing, 1991; Hamill & McBride, Jr., 1992; Akinlaja & Sachs, 1998), but the lack of single channel data may be related to the difficulties seen in evoking whole cell mechanosensitive currents.

Understanding the factors affecting the appearance of the inward current spike, i.e., the factor(s) that make the cell become responsive to mechanical stimuli will not only ease the study of mechanosensitive ion channels, but may have clinical significance. If the mechanically protective components are compromised in conditions such as hypoxia and repeated stretching of weakened regions of the heart during systole, the heart will become susceptible to excitation during phase three resulting in fibrillation and sudden death.

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