Stretch Activated Ion Channels in Ventricular Myocytes

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Patch-clamp recordings from ventricular myocytes of neonatal rats identified ionic channels that open in response to membrane stretch caused by negative pressures (1 to 6 cm Hg) in the electrode. The stretch response, consisting of markedly increased channel opening frequency, was maintained, with some variability, during long (>40 seconds) stretch applications. The channels have a conductance averaging 120 pS in isotonic KC\textsubscript{l}, have a mean reversal potential 31 mV depolarized from resting membrane potential, and do not require external Ca\textsuperscript{++} for activation. The channels appear to be relatively non-selective for cations. Since they are gated by physiological levels of tension, stretch-activated channels may represent a cellular control system wherein beat-to-beat tension and/or osmotic balance modulate a portion of membrane conductance.

KEY WORDS: channels; ventricle; myocyte; voltage clamp; stretch receptor.

ABBREVIATIONS: SACs, stretch-activated channels; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

INTRODUCTION

The existence and functional role of ventricular mechanoreceptors have been topics of debate for decades (19, 29). This paper provides evidence for ventricular mechanoreceptors consisting of stretch activated ion channels (SACs).

SACs have been described in erythrocytes (15), frog skeletal muscle (4), chick myoballs (13), oocytes (26), snail heart cells (5, 27), lens epithelia (8), rat aortic endothelium (18), bacteria (20), epithelium of choroid plexus (6) and renal tubule (25). The channels are all activated by suction pressures within the micropipette, in the range 10 to 60 mm Hg. All the SACs on animal cells are selective for cations, with most being relatively non-selective between K\textsuperscript{+} and Na\textsuperscript{+}, others being primarily selective for either K\textsuperscript{+} (27), or Ca\textsuperscript{++} (6). According to present theory (24), SACs are embedded in a cytoskeletal network surround-
ing the cell that pulls the channels to the open state when the cell is stretched. The unique behavior of SACs has led to proposals that they may underly mechanotransduction in sensory organs (12), may provide signaling for vasodilatation (18), and may be involved in osmotic and volume regulation of cells (27).

METHODS AND MATERIALS

Preparation of Myocytes

Hearts were removed from neonatal (0 to 3 days old) rats after decapitation. The isolation procedure was modified from Ahumada et al. (1) and is described briefly. The ventricles, comprising the lower 2/3 portion, were separated, minced and placed in collagenase (Worthington) solutions (1–2 mg/ml) for <40 minutes. After centrifugation, dispersed cells were placed directly in Tyrode’s solution for immediate use or in a high K+ solution (see below) at 4°C for storage until use, which ranged from 0 to 4 days following isolation. Viable, unattached myocytes were round to oval in shape (15), approximately 20 microns in diameter.

Solutions

Standard Tyrode’s contained (in mM) 137 NaCl, 5.4 KCl, 0.02 CaCl₂, 2 MgCl₂, 10 glucose, 10 HEPES, and was adjusted to pH 7.4. Bath temperatures were 21–23°C. High K+ storage solution (16) consisted of (mM) KCl, 80; KH₂PO₄, 30; Na₂ATP, 5; MgSO₄, 5; pyruvic acid, 5; creatine, 5; Na₂EGTA, 0.1, adjusted to pH 7.2 with KOH. The following electrode-filling solutions (all pH 7.2) were employed: El-1, consisting of (mM) 140 KCl, 2 MgCl₂, 1 CaCl₂, 11 K-EGTA, 10 HEPES, and 2 ATP; El-2, 110 BaCl₂, 10 HEPES; El-3, 110 CaCl₂, 10 HEPES; El-4, 140 KCl, 10 HEPES.

Recordings

Standard gigahm seal, cell-attached patch-clamp techniques described by Hamill et al. (14) were used. Patch electrodes were made from Drummond Microcaps and had resistances of 2 to 5 Megohms. Currents and holding potentials were recorded from the List EP/7 on to FM tape and filtered with an 8 pole Bessel filter with cutoff frequency of 600 Hz.

For seal formation and membrane stretching, suction was applied to the pipette from a side port in the pipette holder. A syringe was used to produce suction and an air manometer in series with the syringe measured the vacuum. In some experiments, a continuous voltage record of applied suction was provided by a strain gauge attached to the manometer. This system provided a resolution of approximately ±2 mm Hg.
RESULTS

Stretch Response

Currents from SACs were recorded from 25 cell-attached patches on myocytes. Figure 1 shows typical SAC currents from a cell-attached patch exposed to El-1 solution (Ca\(^{++}\) free, approximately symmetrical K\(^{+}\)) with pipette potential (V\(_{p}\)) held at 15 mV hyperpolarized from resting membrane potential. Prior to stretch (left of up arrow), there are few openings, with only 2 events being clearly resolved by the chart recording at the top (A), but openings occur with increasing frequency following the onset of stretch indicated by the artifactual baseline shift at the arrow. Channel openings gradually become more frequent during the first few seconds of suction that is maintained at approximately 2 cm Hg. The opening rate remains elevated but considerably variable during the approximately 20 second period of stretch, and returns to a nearly quiescent state immediately after release of suction (off). The expanded segments (B, C) sampled directly from the oscilloscope during the stretch show the currents to be unitary inward currents of approximately 4 pA appearing as bursts, with brief openings followed by several rapid closings. The openings that occurred before and after the stretch appeared identical to those occurring during the stretch. Due to the apparent irregular variation in opening probability during constant stretch, quantitative kinetic or probability analysis of the channels was not done (see Discussion).

Opening rates were clearly related to the magnitude of applied stretch as illustrated in Figure 2 for another SAC exposed to symmetrical K\(^{+}\) (El-1). A continuous record of electrode pressure is at the top, and direct chart recording of current are shown in the middle trace, with V\(_{p}\) = 25 mV. The bottom three traces are expanded recordings directly from an oscilloscope, sampled at the times

Fig. 1. Stretch activation of channel in cell-attached patch of ventricular myocyte of neonatal rat. The bath contained solution (El-1) and was held at 15 mV hyperpolarized from resting potential. Part A shows direct chart recordings during application of negative pressure (approximately 2 cm Hg) between arrows. Baseline artifact is due to mechanical action of suction. Inward currents are shown downward. The response of the channel consists in an immediate increase in burst rate upon suction, maintenance of the elevated rate during the pulses, followed by immediate slowing of opening rate upon relaxation. Note that the apparent variation in current magnitude is caused by distortion from slow pen responses, since digitized samples from an oscilloscope, B, C (filtered at 600 Hz), show them to be unitary 4 pA currents with many brief closings. Time scale is 2 sec for A, 50 msec for B, C.
Fig. 2. Dependence of channel activation on level of suction. Top trace is a record of pressure in micropipette attached by gigaseal to myocyte (different from Fig. 1). Holding potential is 25 mV hyperpolarized from resting. Dashed line represents 0 pressure, negative pressure is downward. Middle trace is a chart record of current from cell-attached path corresponding in time expanded samples taken at the times indicated approximately by the arrows. Scales are shown.

indicated approximately by the arrows. Note that the chart recordings significantly distort the brief unitary opening events, and that there is baseline shifting due to fluid movements in the electrode during suction. For convenience, Figure 2 can be divided into three approximately equal segments of time, each corresponding to a suprathreshold pulse of maintained suction. Pressure in the pipette is seen to vary as the syringe was manipulated. It is seen that the SAC rarely opens when suction is below about 30 mm Hg. When the first pulse is applied, suction initially exceeds 50 mm Hg and is accompanied by frequent openings. During the pulse, suction temporarily falls below 45 mm Hg, but is again increased to 50 mm Hg in the final second. Corresponding to these changes in pressure is a temporary decline in opening rate followed by an increase just prior to release of suction. The next suction pulse (center of record) is lower in magnitude (mostly remaining below 45 mm Hg) than the first, and again opening rate is increased, but to a lesser degree. The third pulse is slightly greater than the first, and produces many openings whose amplitudes are 2 to 3 times larger than events seen at lower pressure. These events apparently represent multiple simultaneous channel openings, indicating the presence of multiple channels in the patch. Most patches exhibited this behaviour during high levels of tension, and occasionally during moderate tension, as during the first suction pulse.

Background, or spontaneous channel opening rate, cannot be stated with certainty since it was not possible to ensure conditions of complete membrane relaxation throughout each experiment. The relatively rare openings that occurred during conditions of minimum tension, could be ascribed to "randomness" as well as to small membrane stresses arising from the electrode or from the surrounding cell. Furthermore, background opening rates usually varied during each trial, often being relatively high immediately after sealing, and then declining after several seconds to a few minutes. This suggests a channel response to the mechanical action of sealing. Background and stretch-induced openings were identical in size and appearance, and neither were affected by voltage changes. It should be noted that the resting membrane tensions of the neonatal myocytes, that are spherical when healthy and unattached to the surface (15), may not be equivalent to that experienced by the cells in their natural state, which is cylindrical.

Out of 108 patches tested for stretch response with approximately
Fig. 3. Current-voltage relationship of SAC. Top traces are chart recordings of current from cell attached patch (different from Figs. 1 and 2) sampled at the holding potentials indicated. \(-V_p\) denotes the negative of the holding potential applied to the pipette such that positive numbers represent depolarizations and negative numbers represent hyperpolarizations, relative to resting level. Moderate stretch is applied during all samples. The graph at the bottom shows the I-V relationship for the SAC. Current measurements were made from oscillographic samples, not from traces above.
symmetrical K⁺ in the electrode (El-1 or El-4), 25 showed responses similar to those of Figures 1 and 2, while most of the remainder had channels that did not respond to stretch. Based on these data, a conservative estimate of SAC density is 0.1 per μm², assuming our electrodes each sampled a 5 μm² membrane area. Density could actually be higher since our data indicate that multiple channels are often present in one patch. SACs were not found with CaCl₂ or BaCl₂ (El-2 and El-3) in the electrode (12 patches).

Current-Voltage Relations

SAC currents behaved ohmically as illustrated in Figure 3. The top half shows chart recordings of currents at the pipette potentials (−Vp) indicated (relative to resting membrane potential) obtained during relatively low tensions to preclude multiple openings. At −Vp's of 80, 70 and 60 mV (lower traces), currents are outward (upward deflections), and change to inward (see traces at −Vp = 10, 0, −25, −45 mV) as the trans-patch potential becomes more negative than the reversal potential. At increasing hyperpolarizations, the baseline becomes less regular due to the appearance of outward currents coming from a non-stretch-sensitive channel in the patch. The I–V relationship shown at the bottom indicates that conductance is linear, with a magnitude of 116 pS and reversal potential of 36 mV depolarized from resting potential. The current-voltage relationships of a total of 11 channels were analyzed from cell-attached patches exposed to approximately symmetrical K⁺, yielded a mean slope conductance of 120 ± 45 pS, and reversal potential 31 mV ± 13 mV depolarized from resting level.

DISCUSSION

The stretch sensitivities of channels reported here for ventricular myocytes bear similarities to those found for SACs in yeast and bacterial membranes as well as on a variety of animal membranes (for review see Ref. 17). The threshold for activation of these channels by suction has ranged between 5 to 25 mm Hg, with the responses saturating at suctions of 50–60 mm Hg. These levels of suction, when applied to a patch of 2 microns diameter, would produce membrane tensions ranging between 1 and 4 dynes/cm (24). Such membrane tensions could exist physiologically from millimolar differences in transmembrane osmolarity, or in the case of the mammalian heart, by normal diastolic pressures.

Our data clearly show that SACs generally open in bursts whose amplitude is unaffected by moderate levels of tension. The primary effect of tension appears to be a marked decrease in the interburst interval, suggesting, as others have (17), that the stretch-sensitive kinetic component is an interburst closed state. We have postponed detailed kinetic analyses of SACs until the irregular variations in opening probability apparent from our records (see Figs. 1A, 2), as well as those of others (8), are better understood.

On the basis of the reversal potential of approximately 31 mV depolarized
from resting potential in approximately isotonic KCl, the SACs appear to be relatively non-selective cationic channels. Since all our SAC recordings were done with \(0 \text{Ca}^{++}\) externally, it is not known whether \(\text{Ca}^{++}\) may have a modulating effect on the channel. Such effects were reported for the lens SAC, which is highly selective for K\(^{+}\) in low external \(\text{Ca}^{++}\), but becomes non-selective between K\(^{+}\) and Na\(^{+}\) when \(\text{Ca}^{++}\) is raised above 2 mM (8).

SACs cannot yet be definitively associated with a macroscopic whole cell current in any cell type, including cardiac cells. A recently described macroscopic current of unknown origin found in guinea pig ventricular myocytes (3), bears some similarities with the SAC current, since it has a reversal potential in between that of K\(^{+}\) and Na\(^{+}\) and is blocked by a known SAC antagonist, lanthanum (28). \(I_{S}\) is a well-known macroscopic current of cardiac cells (7, 10, 22) carried by both K\(^{+}\) and Na\(^{+}\), with conductance dependent on external K\(^{+}\), properties that are also characteristic of SACs. While there is little direct evidence linking stretch sensitivity with any macroscopic current in the ventricle, certain relationships imply such a link. It is known, for example, that stretching induces a pacemaker current in the chick embryonic ventricle (23), reduces the duration of the cardiac action potential (2) and increases ventricular contractility (21).

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