CHAPTER 18

Stretch-Activated Conductances in Smooth Muscles

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I. OVERVIEW

The excitability of smooth muscle cells is regulated, in part, by stretch-activated ion channels in the plasma membrane. The response to stretch of a particular muscle or organ (i.e., enhancement or stabilization of excitability) is tuned to specific functional needs by the types of ion channels expressed. Mechanosensitive ionic conductances that yield either inward or outward currents have been observed in and characterized in studies of smooth muscles. In vascular muscles the dominant response to stretch is muscle contraction (the myogenic response). Several mechanisms for the myogenic response have been proposed, and one of these hypotheses involves stretch-dependent

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activation of nonselective cation channels. The inward current resulting from activation of these channels causes plasma membrane depolarization, activation of voltage–gated Ca^{2+} channels, Ca^{2+} entry, and excitation–contraction coupling. Thus, increasing vascular pressure and distension of blood vessels cause responsive vasoconstriction. Other conductances have also been proposed as participants in the myogenic response, and progress characterizing the inward current channels responsive to stretch is summarized. Outward currents responding to muscle stretch are also present in smooth muscles. For example, expression of stretch-sensitive two-pore domain K^+ (K_{2P}) channels has been reported in visceral smooth muscles. These organs resist contraction on filling and provide a reservoir function. Stretch-dependent outward current channels are hypothesized to help stabilize membrane potential until it becomes desirable to empty the stored contents. Mechanosensitive conductances participate in the integrated responses of smooth muscle tissues and this chapter summarizes the state of knowledge about this interesting class of channels found in smooth muscles.

II. INTRODUCTION

Smooth muscle organs are often referred to as "volume organs" because they experience rather dramatic changes in volume during normal physiological processes. The importance of the length or stretch of smooth muscles has long been known to contribute to the regulation of the electrical and contractile states of these muscles. The first experiments linking changes in muscle length to electrophysiological responses were reported by Bülbring (1955), who demonstrated that stretch of the taenia coli of rabbits caused cell membrane depolarization. This response was not due to activation of nerves but was an intrinsic response of the smooth muscle cells (myogenic). Many smooth muscle organs, including blood vessels, urinary bladder, uterus, and gastrointestinal (GI) organs, display intrinsic (myogenic), nonneural responses to stretch, and these responses are tuned to the physiology and functional needs of specific organs. Experiments on isolated smooth muscle cells have shown that a number of ion channels can be activated on cell elongation or cell deformation. In some cases, stretch is transduced to achieve a contractile response through activation of mechanosensitive channels, induction of inward currents, depolarization, and entry of Ca^{2+} through either the mechanosensitive conductance or through voltage-gated Ca^{2+} channels that are ubiquitous in smooth muscles and typically responsive to small levels of depolarization. Mechanosensitive conductances responsible for inward currents include: stretch-activated nonselective cation channels (Kirber *et al.*, 1988; Davis *et al.*, 1992a; Wellner and Isenberg, 1993b), swelling-activated

Cl^- channels (Dick *et al.*, 1998; Yamazaki *et al.*, 1998), and Ca^{2+} channels (Langton, 1993; Farrugia *et al.*, 1999).

In some smooth muscles, contraction in response to stretch would be deleterious to the physiological function of the organ or tissue region. Thus, in some cases stretch activates a net outward current or a balance to stretch-activated inward currents to accomplish membrane potential stabilization and/or relaxation. K^+ channels are the dominant ionic conductances that respond to stretch and stabilize membrane potentials of smooth muscles. Numerous types of K^+ channels have been characterized in studies of smooth muscle cells, including large conductance Ca^{2+}-activated K^+ (BK) channels, small and intermediate conductance-activated K^+ (SK and IK) channels, voltage-dependent K^+ (Kv) channels, ATP-sensitive K^+ (K_{ATP}) channels, inward rectifier K^+ (Kir) channels, and K_{2P} channels (Nelson and Quayle, 1995; Standen and Quayle, 1998; Brayden, 2002; Calderone, 2002; Cole *et al.*, 2005; Jackson, 2005; Sanders and Koh, 2006). Some of these conductances respond to stretch, either directly or in response to secondary cellular signaling.

One basic truth about smooth muscles is that the cells express an abundance of synergistic and contradictory pathways, so nearly every stimulus leads to a highly integrated response. In the case of stretch, channels promoting either inward or outward currents can be activated. There is always a balancing act, and the net conductance determines the effect of stretch on membrane potential. As above, membrane potential is a critical factor, since Ca^{2+} entry and intracellular Ca^{2+} ($[Ca^{2+}]_i$) are dramatically affected by rather small depolarization or hyperpolarization responses in many smooth muscles. This chapter reviews the major conductances that have been linked to cellular stretch or membrane deformation in smooth muscles from vascular and visceral tissues. First, there is a description of the inward conductances that have been imbued with mechanosensitivity. Activation of these channels tends to increase membrane excitability and increased contractile activity. We will also discuss outward conductances that may stabilize membrane potentials in organs or regions of organs that serve as reservoirs.

There have been several modes of mechanical stimulation employed to activate mechanosensitive ionic conductances in smooth muscle cells. These include: longitudinal elongation of cells, stretching of cell membranes by applying negative pressure to pipettes during on-cell patch recording, inflation of cells during whole-cell recording by application of positive pressure to patch pipettes, osmotic swelling by bathing cells in hypoosmotic solutions, and rapid perfusion of extracellular solutions. At present, it is not clear that the stress applied to the cytoskeleton of smooth muscle cells by the various forms of mechanical perturbation is equivalent, and relatively few studies have compared responses to multiple stimulus modalities. In general, cell elongation, while difficult to perform on smooth muscle cells, must be considered the gold

standard for activation of "stretch-activated" ion channels because this form of perturbation is likely to best simulate the type of cellular deformation that occur during filling or increasing the pressure within smooth muscle organs.

III. MECHANOSENSITIVE CONDUCTANCES THAT GENERATE INWARD CURRENTS

An increase in luminal pressure in resistance arteries and arterioles causes an initial passive distension and then constriction that is due to active stress development by vascular smooth muscle cells. This phenomenon, termed the myogenic response by Bayliss (1902), has been investigated for many years by vascular biologists and the mechanism is still not fully understood. Myogenic contractions are associated with a sustained depolarization of smooth muscle cells (Harder, 1984), and the sustained depolarization is insensitive to L-type Ca^{2+} channel-blocking drugs (Knot and Nelson, 1995). Many investigators believe that the membrane potential responses to vascular distension is due to activation of mechanosensitive ion channels in smooth muscle cells (Kirber *et al.*, 1988; Wu and Davis, 2001). It should be noted that the stretch-activated channels in smooth muscles (including those of bladder and stomach) are similar to channels originally described in studies of skeletal muscle and *Xenopus* ooctyes in terms of their single channel conductance, ionic permeability, and blocking effects of multivalent cations [e.g., compare properties of channels described in this chapter with channels described in references Yang and Sachs (1993) and Morris (1990)].

A. Vascular Smooth Muscle

1. Nonselective Cation Conductances

Negative pressure applied to the interior of patch pipettes during on-cell recording of membrane currents activated nonselective cation channels in pig coronary artery smooth muscle cells (Davis *et al.*, 1992a). Membrane stretch increased the open probability of the stretch-activated channels, but did not affect the unitary conductance. These authors compared the effects of stretching the membrane patch with cell elongation. Stretching cells was accomplished by attaching additional patch pipettes and using the strong mechanical properties of giga-seals to increase the length of the cells. These elegant experiments showed that stretching cells caused depolarization and, in some cases, generation of action potentials. Under conditions of voltage clamp stretch caused activation of sustained inward currents. The reversal potential for this current was about −15 mV. Voltage-gated Ca^{2+} channels

were not affected when cells were stretched by about 15–20% of the resting cell length in these studies.

Associating stretch-activated unitary currents with changes in membrane potential when smooth muscle cells are stretched is somewhat difficult because: (1) there are no specific pharmacological blockers for stretch-activated cation channels and (2) the molecular identity of the channels responsible for inward currents is in question so gene inactivation or knock-out experiments are not yet possible. At present comparisons of properties of single channel and whole-cell currents may be the best way to show that specific unitary conductances are responsible for whole-cell and tissue responses. The whole-cell current activated by longitudinal stretch of coronary artery myocytes was a function of the degree of stretch (Fig. 1) and did not experience significant rundown during the course of experiments (Wu and Davis, 2001). Repeated stretches yielded currents of approximately the same magnitude, and phasic stretches at approximately the rate of the heartbeat resulted in sustained inward currents. The current–voltage relationship for the conductance activated by stretch showed that the conductance was weakly, outwardly rectifying positive to −10 mV and the reversal potential was −18 mV. The conductance responsible for the stretch-activated current did not appear to carry much Ca^{2+} under physiological ionic gradients and the current was not reduced when Ca^{2+} was removed from the bath solution. At negative potentials, Na^{+} appeared to be the predominant charge carrier, but at positive potentials, the current was dominated by K^{+} since it did not decrease as potentials approached the Na^{+} equilibrium potential. The properties of the stretch-activated nonselective cation current in coronary myocytes are summarized in Table I.

Wu and Davis (2001) also found an outward current that was enhanced by stretch in vascular smooth muscles. Large-conductance BK channels were responsible for the outward current, and this conductance activated in response to activation of the nonselective cation conductance (Section IV) because activation of the outward current conductance was blocked by reducing extracellular Ca^{2+} during cell elongation and by iberiotoxin (IbTX). Block of BK channels resulted in enhanced depolarization in response to stretch. Thus, stretch-dependent depolarization is an integrated response that is determined by the inward current caused by activation of stretch-sensitive nonselective cation channels, Ca^{2+} entry due mainly to depolarization and activation of voltage-gated Ca^{2+} channels, and activation of BK channels. Activation of K^{+} channels during myogenic responses opposes the dominant depolarization and contractile responses. Thus, blocking K^{+} channels tends to increase the gain of machanosensitivity in vascular muscles.

Stretching cells by greater than 15% of their slack length caused $[Ca^{2+}]_i$ to increase in vascular muscle myocytes (Davis *et al.*, 1992b). The threshold

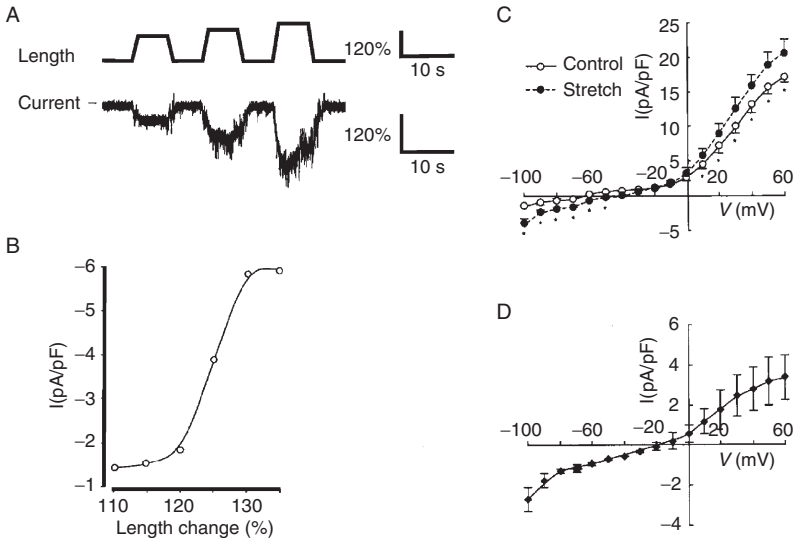

FIGURE 1 Stretch-activated nonselective cation currents in coronary smooth muscle cells. The amplitude of the stretch-activated current in coronary myocytes increased as a function of the magnitude of longitudinal stretch. Panel A shows responses under voltage clamp as cell length was increased to 120%, 125%, and 130% of the resting length. Panel B shows a plot of the amplitude of the stretch-activated current as a function of cell length. The relationship between current and length was fit by a Boltzmann equation and the half-maximal response occurred at 123.3% of the resting length. This experiment was performed with physiological salt concentrations in the bathing solution and the holding potential was −60 mV. Panel C shows the current–voltage relationship for the stretch-activated currents in five cells. Average membrane current was evoked by ramping cells from −100 to +60 mV. Current density was recorded before (open circles) and during a cell elongation to 115% of the resting cell length (filled circles). Panel D shows the difference currents obtained by subtracting the currents elicited before and during stretch. The stretch-activated current reversed at −18 mV. Figure is redrawn and used with permission from Wu and Davis (2001).

for significant changes in $[Ca^{2+}]_i$ was elongation of cells by about 10%. Extracellular Ca^{2+} was the source for stretch-induced rise in $[Ca^{2+}]_i$, but nifedipine blocked only a portion of Ca^{2+} entry stimulated by stretch. Gadolinium, which can block stretch-activated channels in oocytes (Yang and Sachs, 1989), blocked the stretch-induced increase in Ca^{2+} in pig coronary artery myocytes. These data can be interpreted in the following way: stretching cells activate nonselective cation channels, and some Ca^{2+} may enter cells by this pathway. The inward current caused by stretch leads to depolarization and this activates voltage-gated Ca^{2+} channels. These channels facilitate greater entry of Ca^{2+}. This mechanism suggests that stretch-activated

TABLE I

Properties of Nonselective Cation Conductance Activated by Stretch in Vascular Myocytes (Summary of Findings from Wu and Davis, 2001)

1. Effects of stretch were reversible; current could be activated repeatedly
2. Stretch-activated current was graded and increased by increasing stretch up to 135% of resting cell length
3. Stretch-activated current was activated with same time course as depolarization in response to stretch of myocytes
4. Reversal potential in physiological ionic gradients was between −15 and −20 mV
5. Stretch-activated current persisted in Ca^{2+}-free bathing solution
6. Current was unaffected by K^+ channel-blocking drugs
7. Stretch-activated current was blocked by gadolinium[a] and *Grammostola spatulata* venom[a]

[a]It should be noted that these blockers are not specific for non-specific cation channels (NSCC) and can also block L-type Ca^{2+} channels.

cation channels do not have to be the dominant source of Ca^{2+} entry in smooth muscle cells to achieve the required rise in $[Ca^{2+}]_i$ for excitation-contraction coupling. Most vascular muscles have resting potentials within the window current range for L-type Ca^{2+} channels (Vogalis *et al.*, 1991; Fleischmann *et al.*, 1994), so small changes in membrane potential can have a significant impact on Ca^{2+} entry.

Nonselective cation channels can also be activated by cell inflation in mesenteric resistance arteries (Setoguchi *et al.*, 1997). These authors found that positive pressure applied to the patch pipette under whole-cell recording conditions caused noticeable cell inflation and increased a nonselective cation conductance in arterial myocytes. The degree of conductance increase was related to the cross-sectional area of the cells (i.e., amount of cellular inflation). The conductance activated by cell inflation depended on the Na^+ gradient, and extacellular Ca^{2+} reduced the amount of current carried by the stretch-activated channels. Gd^{3+} blocked the stretch-activated conductance with an IC_{50} of 14 μM. Gd^+ also blocked stretch-dependent depolarization of myocytes.

Stretch-activated currents were also recorded from myocytes from resistance arteries of spontaneously hypertensive rats (Ohya *et al.*, 1998). These studies showed, as with myocytes from normotensive animals, that cell inflation evoked Gd^{3+}-sensitive nonselective cation currents. The conductance responsible for these currents showed greater sensitivity to stretch and was increased in amplitude in myocytes from hypertensive rats. Activation of 32-pS cation channels in cell-attached patches was also more sensitive to negative pipette pressure in cells from hypertensive animals. The increase in

sensitivity of stretch-activated nonselective cation channels in hypertensive muscles could lead to enhanced myogenic responses and increased arterial resistance.

Physiologically, activation of nonselective cation conductances in vascular myocytes seems to be a major factor in mediating myogenic responses; however, other mechanisms may contribute. Endothelia cells, once thought to be important, appear to contribute relatively little to the responses because myogenic responses can still be observed in arteries with the endothelium removed (Nelson *et al.*, 1997). In studies of renal arterioles, which exhibit strong myogenic responses, increased perfusion pressure caused graded afferent arteriolar constriction. This response was largely blocked by 10-μM Gd^{3+} (Takenaka *et al.*, 1998). The myogenic response was also attenuated by reducing extracellular Na^{+}, which appears to be the major charge carrier for stretch-activated channels in vascular myocytes (see above). Entry of Na^{+} is likely linked to depolarization and activation of voltage-gated Ca^{2+} channels, and myogenic constrictions in renal resistance vessels were also reduced in Ca^{2+}-free bathing solutions or by diltiazem to block L-type Ca^{2+} channels.

The molecular identity of stretch-activated nonselective cation channels is unclear and this has limited progress to determine how stretch influences the open probability of channels. Recent studies have tried to link myogenic responses in smooth muscles to specific molecular entities. For example, myogenic tone and depolarization responses to elevated perfusion pressure in cerebral arteries were reduced by antisense oligonucleotides to TRPC6 (Welsh *et al.*, 2002). Park *et al.* (2003) followed up on these observations by comparing the properties of mechanosensitive channels in arterial smooth muscle cells with the properties of canonical transient receptor potential channels (TRPCs). In these studies, negative pressure in the patch pipette during cell-attached recording activated nonselective cation channels of about 30 pS. The open probability of the channels increased as a function of negative pressure. The channels were blocked by Gd^{3+} and an inhibitor of phospholipase C (U73122), but facilitated by diacylglycerol (DAG) and cyclopiazonic acid (CPA). In the presence of DAG or CPA, channels could not be activated without membrane stretch, showing that these drugs facilitate channel activity but stretch is an obligatory stimulus for activation. Park *et al.* (2003) concluded that the 30-pS stretch-activated channels in vascular smooth muscles have properties similar to TRPCs. Unfortunately, the criteria to link whole-cell and single-channel currents to TRPCs is complicated by the extremely nonspecific nature of the pharmacology of TRPCs. Further attempts to test the role of TRPCs in the myogenic response, using specific gene inactivation in smooth muscle cells and other molecular manipulations, are needed to identify the molecular correlates of stretch-activated nonselective cation channels.

Another report suggested that a vanilloid receptor homologue (TRPV2) might contribute to swelling-activated nonselective cation currents in vascular myocytes (Muraki *et al.*, 2003). These authors found that osmotic swelling activated a nonselective cation current and increased $[Ca^{2+}]_i$ in aortic myocytes. The responses were blocked by ruthenium red, a blocker of TRPV2 channels. Aortic myocytes expressed immunoreactivity for TRPV2 antibodies, and TRPV2 immunoreactivity was also observed in mesenteric and basilar arterial myocytes. Treating mouse aorta with TRPV2 antisense oligonucleotides suppressed osmotic swelling-induced nonselective cation currents and the increase in $[Ca^{2+}]_i$ and reduced expression of TRPV2 protein. These authors concluded that TRPV2 is an important mechanosensor in vascular smooth muscle cells that couples membrane stretch to activation of a nonselective cation conductance. Unfortunately, experiments comparing the efficacy of activation of TRPV2 by osmotic stretching of cells were not compared with negative pipette pressure or cell elongation, stimuli that have been linked by previous experiments to the activation of channels responsible for the myogenic responses of vascular muscles. Thus, it is unclear, at present, whether TRPV2 is a central molecular component of the myogenic response.

2. Other Mechanosensitive Inward Currents in Vascular Myocytes

There have been a few reports describing mechanosensitive properties of voltage-gated Ca^{2+} channels in smooth muscles. Basilar artery cells, under whole-cell recording conditions, were exposed to positive and negative pressure applied through the pipette. In most cells, positive pressure caused visible cell inflation and increased L-type Ca^{2+} currents (Langton, 1993; McCarron *et al.*, 1997). There were similar increases in L-type Ca^{2+} currents in perforated-patch recordings when cells were swelled under hypoosmotic conditions (Langton, 1993). It is unlikely that the increase in current was due to incorporation of new channels in response to membrane stretch because there was not an increase in total capacitance while current amplitude increased by up to 50% in some experiments. While it is likely that L-type Ca^{2+} currents are very important in mediating the increase in tone in the myogenic effect in vascular muscles, stretch-dependent depolarization appears to be more dependent on other conductances (e.g., nonselective cation channels) since stretch-induced depolarization persists in the presence of Ca^{2+} channel blockade (Knot and Nelson, 1995).

Stretch-induced depolarization could also be accomplished by efflux of Cl^- ions if longitudinal stretch of cells is coupled to activation of anion selective conductances. The reversal potentials of the stretch-activated conductances characterized in several studies, however, have not been consistent with activation of a Cl^- conductance and are independent of changes in extracellular Cl^- concentration (Davis *et al.*, 1992a; Setoguchi *et al.*, 1997; Wu and Davis, 2001). However, along with nonselective cation conductances, swelling-activated

Cl^- channels are found in vascular smooth muscles that could, under certain circumstances, contribute to the mechanosensitivity of these cells. Hypoosmotic solutions caused cell swelling and activation of an outwardly rectifying Cl^- conductance with a permeability sequence of $SCN^- > I^- > Br^- > Cl^-$ in pulmonary and renal arterial myocytes (Yamazaki *et al.*, 1998). Tamoxifen and 4,4′-diisothiocyanostilbene-2,2′-disulfonic acid (DIDS) blocked the swelling-activated Cl^- current. Expression of the volume-regulated Cl^- conductance was also evaluated and PCR showed that both pulmonary and renal myocytes express ClC-3. Levels of ClC-3 gene expression were slightly higher in pulmonary artery cells than in renal cells; however, there were no quantitative differences in current density in the two cell types. Further studies (e.g., gene inactivation or knockout studies) are needed to be certain that ClC-3 is responsible for the selling-activated Cl^- conductance in vascular myocytes.

Nelson and colleagues (1997) showed that indanyloxyacetic acid (IAA-94) and DIDS hyperpolarized and dilated pressurized cerebral arteries; however, niflumic acid had no effect. The drugs tested had no effect on vascular diameter or membrane potential when the perfusion pressure was low or when myogenic tone was absent. These observations suggested that a Cl^- conductance, but probably not a Ca^{2+}-activated conductance, might participate in myogenic responses in some arteries. It is, however, quite difficult to link Cl^- conductances to physiological responses because the drugs used to block Cl^- channels are notoriously nonselective. If these drugs inhibit myogenic responses, it is possible that part of the inhibitory effect could be due to effects on L-type Ca^{2+} channels (Doughty *et al.*, 1998; Dick *et al.*, 1999) or nonselective cation channels (Park *et al.*, 2003).

B. Bladder Myocytes

There are also stretch-activated channels in myocytes of the urinary bladder. Application of negative pressure to the inside of patch electrodes activated channels with a slope conductance of 39 pS and a reversal potential of 2 mV (Wellner and Isenberg, 1993a). With physiological ionic gradients, the slope conductance of the stretch-activated channels was similar to the conductance of stretch-activated channels in toad gastric myocytes (Kirber *et al.*, 1988). The conductance was nonselective and carried a variety of cations with a selectivity sequence of $K^+ > Na^+ > Cs^+ > Ba^{2+} > Ca^{2+}$. The presence of divalent ions reduced the unitary conductance of the channels with monovalent cations as charge carriers by about half. The single channel conductance and the reversal potential were not affected by substitution of Cl^- with aspartate. The channels, like in vascular muscles, were blocked by Gd^{3+} at μM concentrations. Gd^{3+} reduced long open times of the channels. The authors considered the possibility that the stretch-activated nonselective cation conductance in bladder

myocytes might contribute to the relatively depolarized membrane potentials of intact bladder muscles (i.e., −35 mV in the guinea pig) and the occurrence of spontaneous action potentials. The open probability of stretch-activated nonselective channels in bladder myocytes increased with hyperpolarization, which is usually considered a property of a pacemaker-like conductance; however, the stretch-activated channels in bladder myocytes required mechanical stress for activation even during hyperpolarization.

Longitudinal stretch of guinea pig bladder myocytes by up to 20% caused significant depolarization that was dependent on the change in length (Wellner and Isenberg, 1994). The depolarization was sufficient to increase the frequency of action potentials in many cells. Under voltage clamp, stretch induced an inward current at a holding potential of −50 mV that was blocked by Gd^{3+}. With K^+ currents blocked, the current activated by stretch reversed at 0 mV. The stretch-activated inward current in guinea pig myocytes adapted slowly, decaying in amplitude with time (Wellner and Isenberg, 1995). Internal dialysis of cells with solutions containing dibutyryl cAMP increased the rate of decay, and this was found to be due to activation of BK channels. The authors suggested that entry of Ca^{2+} through the stretch-activated channels and secondary activation of BK channels was responsible for the slow adaptation. Thus, bladder myocytes possess a feedback mechanism to limit the excitatory effects of stretch. Openings of stretch-activated channels were also increased by a protein kinase A (PKA)-dependent mechanism, possibly phosphorylation of the channels. In some cases, treatment of the intracellular surface with dibutyryl cAMP caused openings of nonselective cation channels without application of suction.

Filling of the bladder causes elongation of smooth muscle cells. The activation of stretch-activated inward current channels would have the effect, as in vascular muscles, of stimulating excitation–contraction coupling. As above, Wellner and Isenberg (1994) showed that the frequency of action potentials increased in response to stretch. This response, if unimpeded, would then to defeat the storage function of the bladder since filling would lead rapidly to a contractile response. New evidence suggests that the inward current activated by stretch may be complimented by activation of stretch-sensitive K^+ channels (Section IV).

C. GI Myocytes

1. Nonselective Cation Conductances

Stretch-activated channels in smooth muscles were first identified in toad stomach myocytes (Kirber *et al.*, 1988). Using either cell-attached or excised patch configurations of the patch-clamp technique, channels were activated

when negative pressure was applied to the inside of patch pipettes. The current–voltage relationship for these channels showed inward rectification that was not affected by K^+ substitution for Na^+. The apparent conductance of the stretch-activated channels was about 60 pS with either Na^+ or K^+ as charge carriers. Replacement of Cl^- with aspartate at the intracellular surface of patches had no effect on reversal potentials. Addition of physiological levels of Ca^{2+} to the pipette solutions (i.e., to the extracellular surface of patches) greatly decreased slope conductance and unitary current amplitude. The stretch-activated channels also carried current when Ca^{2+} ions were the only cations in the patch pipette, but the conductance of the channels under this condition was only about 20 pS.

While the cation channels observed in smooth muscle resembled stretch-activated channels previously described in other cell types (cf. Guharay and Sachs, 1984; Morris, 1990, Kirber *et al.* (1988) must be credited with providing the first data supporting the hypothesis that stretch-activated cation channels are responsible for depolarization and contractile responses to stretch in smooth muscles. Thus, their observations contributed significantly to the current state of understanding important physiological responses such as the myogenic response in vascular smooth muscles (Bayliss, 1902) and stretch-sensitive depolarization in visceral smooth muscle (Bülbring, 1955). Kirber *et al.* (1988) calculated that under physiological conditions unitary currents through stretch-activated cation channels at the resting potentials of cells would be about 2.5 pA. Most patches recorded from contained at least two stretch-activated channels. Thus, even a small increase in the activation of these channels would contribute significantly to the total conductance of smooth muscle cells, which normally have input resistances in excess of 1 GΩ. Kirber *et al.* (1988) suggested that inward current through stretch-activated channels might drive membrane potential to more depolarized levels and activate voltage-gated Ca^{2+} currents. Ca^{2+} entry through Ca^{2+} channels, and possibly Ca^{2+} entry through the stretch-activated cation channels, might provide sufficient Ca^{2+} entry to initiate contraction.

Further study of toad gastric myocytes also revealed expression of a hyperpolarization-activated (HA) channel (Hisada *et al.*, 1991). Stretching membrane patches with negative pipette pressure caused a shift in the hyperpolarization sensitivity of the HA channels. These channels were permeable to both Na^+ and K^+, but were not permeable to Cl^-. The activity of HA channels increased transiently after patch excision, but the ability of hyperpolarization to activate these channels disappeared within 3–5 min in excised patches. The HA channels had similar sensitivity to stretch and similar conductance (e.g., 60 pS) to the stretch-activated channels these authors had described previously (Kirber *et al.*, 1988). Thus, it was not easy to distinguish between channels that were and were not activated by hyperpolarization.

The density of stretch-activated channels that were not activated by hyperpolarization far exceeded the HA and stretch-activated type of channels, and stretch activation of the former subclass of channels persisted after patch excision. These observations suggest that there are at least two populations of stretch-activated nonselective cation channels in toad gastric myocytes: one type of these channels may also be activated or sensitized to stretch by hyperpolarization. HA and stretch-sensitive cation channels were also activated by aluminofluoride (Hisada *et al.*, 1993), which has actions such as activation of guanosine triphosphate (GTP)-binding proteins and inhibition of phosphatases (Chabre, 1990).

The question of whether smooth muscle stretch-activated cation channels conduct appreciable amounts of Ca^{2+} and how global Ca^{2+} increases sufficiently to accomplish excitation–contraction coupling when smooth muscle cells are stretched were addressed in elegant studies using digital imaging to monitor $[Ca^{2+}]_i$ levels with fura-2 (Kirber *et al.*, 2000). Unitary currents elicited by stretch of a patch of membrane were monitored simultaneously with $[Ca^{2+}]_i$. Application of negative pressure to patch pipettes activated Ca^{2+}-permeable nonselective cation channels and increased global $[Ca^{2+}]_i$ (Fig. 2). The Ca^{2+} transients recorded from cells also displayed a large focal increase in $[Ca^{2+}]_i$ near the tips of pipettes. When Ca^{2+} was buffered to low levels in the pipette solution, only the global increase in $[Ca^{2+}]_i$ was observed on application of negative pressure to the pipettes. In these experiments, stretch-activated inward currents and depolarization-induced Ca^{2+} entry were preserved, but the focal rise due to local Ca^{2+} entry was inhibited. Removal of Ca^{2+} from the bathing solution with Ca^{2+} present in the pipette caused just the opposite phenomenon: the focal rise in $[Ca^{2+}]_i$ was preserved, but the global transient was absent. Unloading of Ca^{2+} stores prior to stretch of the membrane patch greatly reduced the intensity of the focal rise in $[Ca^{2+}]_i$. These studies show that at least two mechanisms contribute to the rise in $[Ca^{2+}]_i$ and contraction in response to stretch. Stretch-activated channels increased $[Ca^{2+}]_i$ by depolarization and activation of voltage-gated Ca^{2+} channels and by amplification of the focal increase in stretch-induced $[Ca^{2+}]_i$ by release of Ca^{2+} from internal Ca^{2+} stores. The latter appeared due to Ca^{2+}-induced Ca^{2+} release from ryanodine receptors that was induced by Ca^{2+} entering cells via the stretch-activated nonselective cation channels.

The local Ca^{2+} transients caused by stretch-activated channels were later characterized by imaging with high temporal and spatial resolution (Zou *et al.*, 2002). While it was known from previous studies that stretch-activated nonselective cation channels conduct Ca^{2+} when Ca^{2+} is the only charge carrier, these authors provided direct evidence that appreciable Ca^{2+} enters cells via stretch-activated channels in physiological ionic gradients. With fluo-3-loaded cells, localized Ca^{2+} transients were observed at the tips of patch pipettes used to

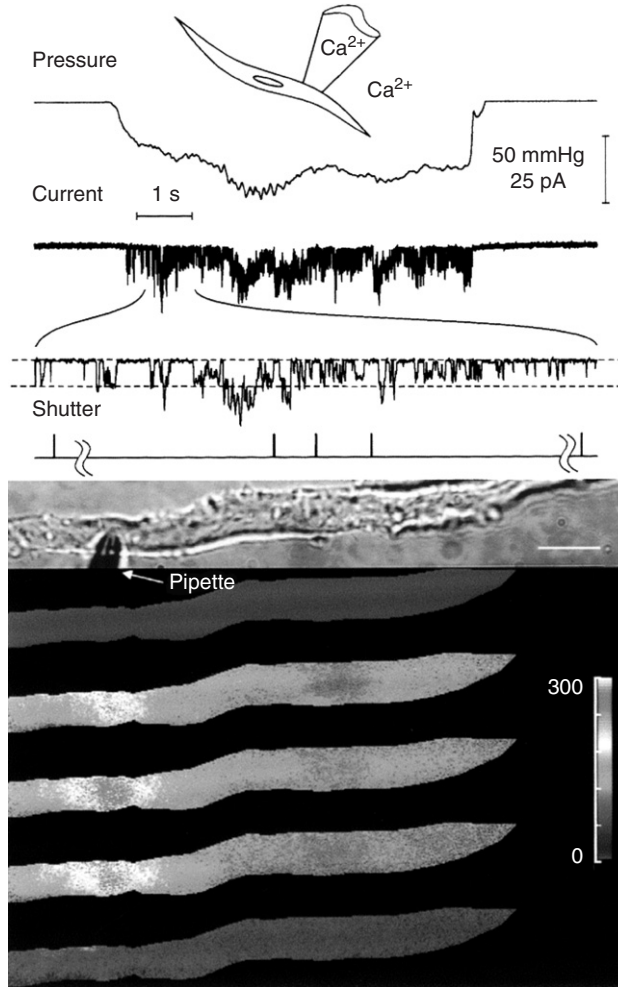

FIGURE 2 Stretch-activated channels in a patch of membrane lead to focal and global increases in $[Ca^{2+}]_i$. $[Ca^{2+}]_i$ was imaged in fura-2 AM-loaded cells. Drawing shows recording configuration where Ca^{2+} was present in both the bath and pipette. First trace shows negative pressure applied to the inside of the pipette and the stretch-activated inward currents activated by negative pressure. Third trace is a blowup of the region of the record denoted. Fourth trace shows the times at which the images below were obtained (vertical lines). Images were taken just before and after negative pressure was applied, and three sequential images were collected during the application of negative pressure (time denoted by wavy marks in fourth trace). During membrane stretch, there was an intense focal increase in $[Ca^{2+}]_i$ near the tip of the pipette and a smaller global increase in $[Ca^{2+}]_i$. From Kirber *et al.* (2000).

measure currents and stretch membrane patches before the global increase in $[Ca^{2+}]_i$ were initiated. The localized Ca^{2+} transients were observed even when Ca^{2+} stores were inactivated. The authors calculated that about 18% of the current through stretch-activated nonselective cation channels in toad myocytes was carried by Ca^{2+} at membrane potentials more negative than resting potential.

In the gut, muscarinic stimulation activates a Ca^{2+}-facilitated nonselective cation conductance in smooth muscle cells (Benham *et al.*, 1985). One study evaluated the sensitivity of this conductance to cell swelling and found that inward current evoked by carbachol increased in peak amplitude by about 50% in hypotonic solutions (Waniishi *et al.*, 1997). Hypertonicity had opposite effects. These effects were not due to changes in muscarinic receptor binding since current activated by GTPγS were also potentiated by hypotonicity. The current activated by hypotonicity was blocked by procaine and Zn (400 μM). The authors also noted that hypotonicity increased the amplitude and duration of depolarization responses to carbachol in cells under current clamp. Studies using hypotonicity to swell cells are always complicated by the possibility that cellular components might be diluted when water enters cells. In the study by Waniishi *et al.* (1997), there was a small shift in equilibrium potential for currents activated by muscarinic stimulation, suggesting changes in ionic concentrations. It is also possible that cell swelling applies fundamentally different forces on the cytoskeleton than cell elongation and it is not clear whether cell swelling and stretch are equivalent stimuli. The mechanism for swelling activation of agonist-sensitive nonselective cation channels was not determined and the possibility existed that stretch-dependent Ca^{2+} release could be responsible for potentiation of currents during hypotonic conditions; this conductance is strongly facilitated by $[Ca^{2+}]_i$ (Pacaud and Bolton, 1991). The authors addressed this problem by taking steps to limit influx of Ca^{2+} and changes in $[Ca^{2+}]_i$ with strong Ca^{2+} buffering, but it is difficult to entirely eliminate effects due to changes in Ca^{2+} in the restricted spaces between sarcoplasmic reticulum (SR) and the plasma membranes of smooth muscle cells. If the effects of hypotonicity in these experiments can be compared to stretch, the data suggest that responses to muscarinic stimulation could be enhanced in muscles that are elongated by filling of GI organs. This would be useful augmentation of muscarinic responses because more force might be needed to do the work of emptying contents. At present, however, neither the mechanism for activation of muscarinic-activated nonselective cation channels nor the significance of these channels to the behavior of GI muscles is understood.

2. Other Mechanosensitive Conductances Expressed in Visceral Smooth Muscle Myocytes

Mechanosensitive Ca^{2+} currents have also been reported in human jejunal circular smooth muscle cells. L-type Ca^{2+} channel currents can be enhanced in these cells by rapid bath perfusion, which was used as a stimulus of mechanosensitive responses (Farrugia *et al.*, 1999; Holm *et al.*, 2000). In jejunal myocytes, entry of Ca^{2+} was linked to activation of Ca^{2+}-activated K^+ channels since increased perfusion enhanced outward current (in voltage clamp) and membrane hyperpolarization (in current clamp). Nifedipine (to block L-type channels) or iberiotoxin (blocker of large-conductance BK channels) blocked the development of outward current and depolarization. The authors suggested that the link between mechanosensitive Ca^{2+} channels and BK channels might provide feedback to limit contractile responses to stretch in jejunal smooth muscles.

The α-subunit of $Ca_V1.2$ channels (molecular basis for L-type Ca^{2+} currents in smooth muscles) may have intrinsic mechanosensitivity. $Ca_V1.2$ channels were cloned from human intestine and expressed in HEK-293 or Chinese hamster ovary cells either alone or with β_2-subunits (Lyford *et al.*, 2002). Currents from the expressed channels were enhanced by rapid bath perfusion. When a proline-rich domain of the C-terminus of $Ca_V1.2$ that may facilitate interactions with integrins was removed, mechanosensitive responses were not disrupted. The authors suggested that the mechanosensitivity may reside in the pore-forming region of the α1C-subunit, but experiments to test this hypothesis were not provided.

Swelling-activated Cl^- channels are also found in visceral smooth muscle cells. For example, the ClC-3 gene is expressed in canine colonic smooth muscle cells and a current similar to that generated by expressed ClC-3 channels (outwardly rectifying and activated by reduced hypoosmotic extracellular solutions and inhibited by PKC) is present in colonic myocytes (Dick *et al.*, 1998). This conductance was blocked by tamoxifen and DIDS, but niflumic acid, nicardipine, and removal of Ca^{2+} did not affect currents. The swelling-activated Cl^- conductance was inhibited by extracellular ATP and negatively regulated by PKC. The phorbol ester, phorbol 12,13 dibutyrate, decreased the swelling-activated Cl^- and chelerythrine activated current, even in the presence of isotonic extracellular solutions. As discussed in the Section III.A.2, it is difficult to evaluate the physiological significance of the Cl^- conductances because blockers have significant nonspecific effects. For example, tamoxifen, a promising inhibitor of the swelling-activated Cl^- current in colonic myocytes, blocked L-type Ca^{2+} currents and DIDS had similar effects (Dick *et al.*, 1999). These Cl^- channel-blocking drugs also inhibited delayed rectifier K^+ currents. Thus, the effects of compounds on intact tissues would tend to be obscure.

IV. MECHANOSENSITIVE CONDUCTANCES THAT GENERATE OUTWARD CURRENTS

A. *Vascular Muscles*

As discussed in Section III, a general increase in vascular luminal pressure induces depolarization (Nelson *et al.*, 1990), and mechanosensitive channels play key functions in depolarization and contractile responses (Meininger and Davis, 1992; Setoguchi *et al.,* 1997). In contrast to this dominant response, several studies have discussed the functional role of BK channels (Benham and Bolton, 1986; Brayden and Nelson, 1992; Nelson and Quayle, 1995; Nelson *et al.*, 1995; Brenner *et al.*, 2000; Pluger *et al.*, 2000) and delayed rectifier K^+ channels (Kv, Cheong *et al.*, 2001a,b; Albarwani *et al.*, 2003; Ahmed *et al.*, 2004; Cole *et al.*, 2005) in myogenic tone and vasoconstriction. BK channels are voltage-and Ca^{2+}-dependent, but some investigators have suggested that these channels are also regulated by stretch. For example, application of negative pressure to membrane patches increased openings of BK channels in mesenteric arterial smooth muscle cells without affecting the unitary conductance or the voltage sensitivity (Kirber *et al.*, 1992; Dopico *et al.*, 1994). BK channels were also activated by intracellular application of fatty acids, but this occurred only in the presence of basal channel openings. When $[Ca^{2+}]_i$ is low or when membrane potential is at negative levels (i.e., when the open probability of BK channels is low), fatty acids would not enhance the open probability of BK channels (Kirber *et al.*, 1992). Thus, stretch activation of BK channels from low open probability was independent of fatty acid generation. Pretreatment with fatty acids did not prevent activation of BK channels by stretch in coronary arterial smooth muscle cells (Lee *et al.*, 2000), and pretreatment with albumin, which binds to free fatty acids, did not affect activation of BK channels by stretch. These data suggest that the activation of BK by stretch is independent of fatty acid production in rabbit coronary artery. In other experiments on coronary myocytes, repetitive negative pressure pulses were applied to patches. In these experiments, BK channel activity progressively increased, but there was no increase in stretch-activated cation channels with repetitive negative pressure pulses. The authors suggested that this might be due to different sensitivities of these channels to dynamic changes in membrane tension (Wu *et al.*, 2003), but it is not clear how $[Ca^{2+}]_i$ was held constant in these experiments. To date, there have been no reports demonstrating that Kv channels express mechanosensitivity in vascular smooth muscles.

Even though the activation of BK channels has been demonstrated in many vascular smooth muscle cells (SMCs), the mechanism of activation of these channels has not been clarified. Two possible mechanisms have been

discussed: (1) direct activation by stretch and/or (2) inhibitory control of BK channel openings by interactions with the cytoskeleton. Evidence for cytoskeleton regulation of BK channels is based on the use of cytochalasin-D (actin depolymerizer) and phalloidin (actin stabilizer). Treatment with cytochalasin-D increased the open probability of BK channels, and this effect was reversed by treatment with phalloidin. These findings suggest that interactions with actin filaments may inhibit the open probability of BK channels and stretch might reduce this form of negative regulation (Piao *et al.*, 2003). These are preliminary observations, however, and the actual mechanism of actin regulation of channel open probability needs further study.

There has been some discussion regarding the physiological significance of stretch-activation of BK channels. Activation of BK channels in response to stretch may oppose or provide feedback for the contractile response to cell elongation mediated by nonselective cation channel activation (Kirber *et al.*, 1992). Thus, activation of BK channels may limit stretch-induced vasoconstriction. There is controversy, however, about the role of BK channels in the myogenic responses in vessels of different sizes. Blockers of BK channels (iberiotoxin or tetraethylammonium) did not affect the resting diameter of arterioles (Jackson and Blair, 1998) as others have shown in small arteries (Paterno *et al.*, 1996). Therefore, the contributory role of BK channels in stretch-dependent responses may vary as a function of vascular region and also with species.

B. Bladder Smooth Muscle

The bladder can store considerable volumes of urine before voiding becomes necessary. During filling, the bladder wall stretches to accommodate the increase in volume. The compliance of the detrusor muscle to stretch is of critical importance for the storage function of the bladder. For adequate filling to occur, the detrusor smooth muscle must be able to stretch and accommodate the increase in bladder volume without a significant rise in pressure. It is known that smooth muscle from the bladder detrusor exhibits phasic contractions in response to spontaneous action potentials or transmural nerve stimuli (Levin *et al.*, 1986; Brading, 1992). Since spontaneous contractions occur locally and do not readily spread throughout the tissue (Hashitani *et al.*, 2000), it has been suggested that the poor electrical coupling of detrusor smooth muscle facilitates muscle bundles to adjust their length to achieve the minimum surface area/volume ratio during bladder filling without contraction or rise in intravesicular pressure (Levin *et al.*, 1986; Kinder and Mundy, 1987; Brading, 1994).

As discussed above, stretch of bladder myocytes activates nonselective cation channels in guinea pig myocytes (Wellner and Isenberg, 1993a,b). This mechanism, which is excitatory in nature, does not correlate with the observation that stretch does not activate contraction in bladder but leads to stabilization of membrane potential or even relaxation. Thus, influx and/or release of Ca^{2+} must couple to other mechanisms that blunt the excitatory responses to stretch. Ji *et al.* (2002) failed to find stretch-activated nonselective channels in mouse and rabbit bladder myocytes. Instead, stretching these muscles caused gating of ryanodine receptors, release of Ca^{2+} from the SR, and generation of Ca^{2+} sparks or propagated Ca^{2+} waves. This mechanism coupled to activation of a Ca^{2+}-activated Cl^- conductance and occurred in the absence of Ca^{2+} influx. Activation of Cl^- channels would have much the same effect as activation of nonselective cation channels (i.e., depolarization and enhanced open probability of voltage-gated Ca^{2+} channels; Ji *et al.*, 2002). This mechanism also fails to explain the stabilization of membrane potential in response to filling in the bladder.

Phosphorylation of BK channels might increase coupling between Ca^{2+} sparks and BK channels in bladder myocytes (Wellner and Isenberg, 1993a). This might switch the coupling of sparks from activation of net inward to net outward current, but this phenomenon has not been demonstrated. BK channels clearly participate in the regulation of membrane potential and repolarization of action potentials in bladder smooth muscles (Heppner *et al.*, 1997; Hashitani and Brading, 2003), but this does not mean BK channels are involved in stabilization of membrane potential during stretch-dependent responses unless it can be shown that a specific stretch-dependent mechanism regulates BK channel open probability in bladder myocytes. Other mechanisms, such as other classes of stretch-activated K^+ channels (see below) are likely to mediate the membrane stabilization response to stretch in bladder muscles.

C. *Uterine Smooth Muscle*

The uterus is a unique smooth muscle organ that undergoes extreme changes in volume during pregnancy. Muscle quiescence during pregnancy is another example of stabilization of excitability during conditions of stretch. How muscle relaxation is maintained during pregnancy has been investigated widely, and some studies have suggested that the expression of BK channels is regulated by female steroid hormones (Mironneau and Savineau, 1980; Kihira *et al.*, 1990; Toro *et al.*, 1990; Anwer *et al.*, 1992; Khan *et al.*, 1993). The functional role of BK channels and the response to

stretch in controlling basal contractility is controversial (Khan *et al.*, 1993, 1997; Aaronson *et al.*, 2006).

Recently, there is evidence showing that K_{2P} channels are expressed in myometrium. K_{2P} channels are a family of K^+ channels that may contribute to regulation of membrane potential in both electrically excitable and non-excitable cells.Within the K_{2P} channel family, TREK-1, TREK-2, and TRAAK have unique functional properties and represent mechanosensitive K^+ channels (Fink *et al.*, 1996; Patel *et al.*, 1998; Maingret *et al.*, 1999a,b; Lesage and Lazdunski, 2000; Lesage *et al.*, 2000). The expression of TREK-1 in human myometrium has been shown in molecular, immunoblot, and immunohistochemistry studies (Bai *et al.*, 2005). Transcripts for TREK-1 occur at higher levels than for TREK-2, and a significant increase in TREK-1 expression was seen in pregnant tissues (Tichenor *et al.*, 2005). These data suggest the hypothesis TREK channels could participate in the regulation of muscle excitability during pregnancy, but this hypothesis has not yet been tested.

D. GI Smooth Muscle

There are mechanosensitive inward current channels GI myocytes (as described in Section III.C), but contraction is not the dominant response to stretch in many regions of the GI tract. For example, the colon and fundus display a reservoir function that allows volume expansion (muscle elongation) without increasing intraluminal pressure. Filling of these organs does not cause myogenic contraction. Thus, in addition to neural reflexes that contribute to volume accommodation, a myogenic mechanism is likely to exist that stabilizes membrane potential and limits excitability during cell elongation.

Stretch-activated K^+ channels have been observed in studies of toad gastric myocytes (Petrou *et al.*, 1994; Ordway *et al.*, 1995; Kirber *et al.*, 2000), guinea pig gastric myocytes (Li *et al.*, 2002), and murine and canine colonic myocytes (Koh and Sanders, 2001). Single-channel studies in toad gastric myocytes revealed 20-pS K^+ channel at 0 mV in an asymmetrical K^+ gradient (3/130 mM) that were activated by negative pipette pressure. Open probability was augmented by fatty acids. These authors suggested that fatty acids may be liberated from membrane phospholipids in response to stretch to enhance K^+ channel activity. The properties of the channels in toad gastric myocyte channels are similar to TREK-1 channel, in terms of the activation by fatty acids, stretch, and intracellular acidic pH (Zou *et al.*, 2001). In murine colonic myocytes, negative patch pipette pressure activated K^+ channels that were voltage-independent with a slope conductance of

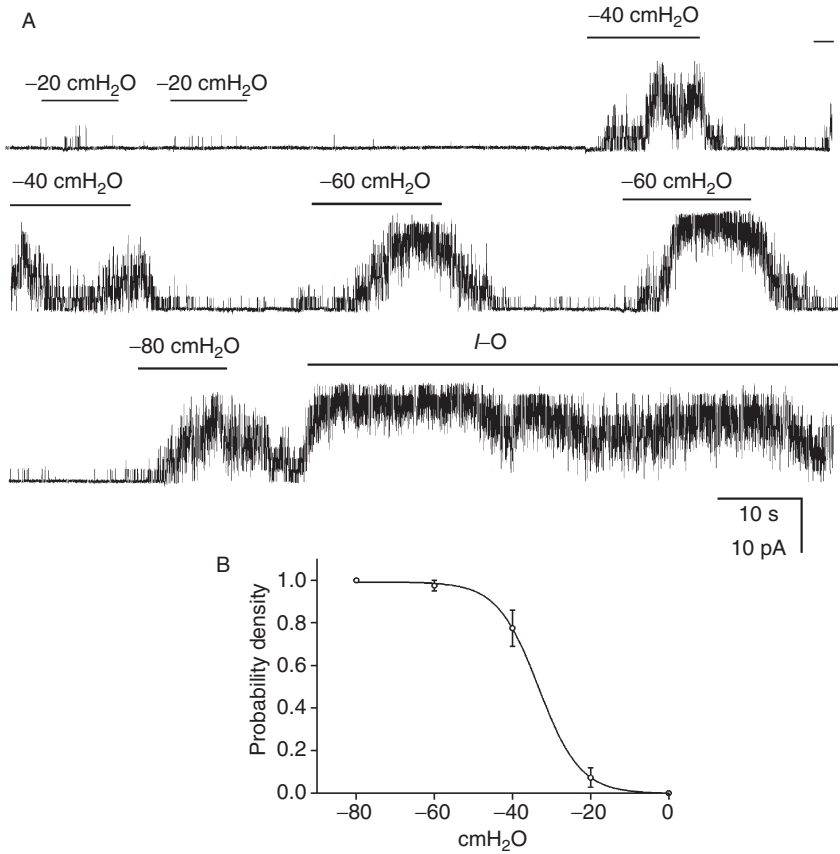

FIGURE 3 Relationship between pressure and open probability of channels activated by negative pressure in murine colonic myocytes. Patches from five cells were exposed to pressure ranging from −20 to −80 cmH_2O. In order to be sure that the effects of negative pressure were reversible and lacked desensitization, different levels of negative pressure were applied to the same patch and each pressure was tested twice. Panel A, a negative pressure of −20 cmH_2O had little effect on channel activity. However, greater negative pressures (−40 cmH_2O) applied to the same patch increased NP_o to 6.2. Further negative pressure (−60 and −80 cmH_2O) increased NP_o to the maximal level. After restoration of atmospheric pressure in each step, the open probability returned to near zero. After application of pressure pulses, the patch was excised. This caused maximal activation of channels in the patch. Panel B, the graph summarizes the relationship between pressure and NP_o in patches from five cells. *I-O* denotes inside-out patches. Figure redrawn from Koh and Sanders (2001).

95 pS in symmetrical K^+ gradients (Fig. 3). The effects of negative pressure on open probability were graded as a function of pressure and reversed when atmospheric pressure was restored. Cell elongation also caused activation of

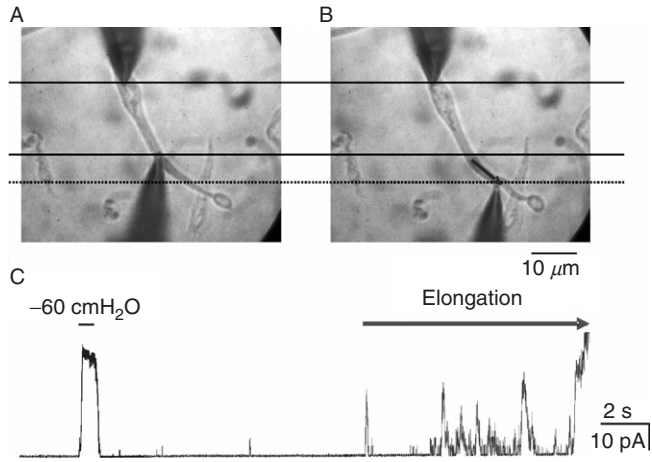

FIGURE 4 Activation of stretch-dependent K^+ (SDK) channels via cell elongation in murine colonic myocytes. On-cell patches of murine colonic myocytes were held at 0 mV in asymmetrical K^+ gradients (5/140 mM). The pipette solution contained charybdotoxin (200 nM) to inhibit large conductance Ca^{2+}-activated K^+ channels. Panel A, two patch pipettes were sealed to the same cell. Single-channel currents were measured via one pipette, and the other pipette was used to stretch the cell. Panels B and C, after confirming that negative patch pressure (−60 cmH_2O) activated SDK channels in this patch, the cells were elongated (in this example by 8 μm). Cell elongation caused activation of channels with the same properties as negative pressure. Figure redrawn from Koh and Sanders (2001).

K^+ channels with the same properties as those activated by negative pressure (Koh and Sanders, 2001) (Fig. 4). The stretch-activated channels were maximally activated by patch excision, suggesting that either an intracellular messenger or interactions between the channels and the cytoskeleton regulate open probability.

Sodium nitroprusside (SNP), an NO donor, activates stretch-activated K^+ channels in smooth muscle myocytes, and 8-Br-cGMP mimics these effects (Koh and Sanders, 2001). Thus, the effects of NO may be mediated through activation of cGMP-dependent protein kinase (PKG). These findings revealed a novel property of stretch-activated K^+ channels in GI myocytes: the activity of these channels may be dually regulated by both stretch and the primary enteric inhibitory neurotransmitter, NO.

The hypothesis that K_{2P} channels might be responsible for the stretch-activated K^+ conductance in visceral myocytes was investigated. Six functional subfamilies of K_{2P} channels have been described (e.g., TWIK, TREK, TASK, TASK-2, THIK, and TRESK), and these are classified based on functional domains (Franks and Honore, 2004). TREK family channels are activated by stretch and include TREK-1, TREK-2, and TRAAK (KCNK2,

KCNK4, and KCNK10, respectively). RT-PCR revealed that TREK channels, but not TRAAK channels, are expressed in murine colonic smooth muscle (Koh *et al.*, 2001). TREK channels appear to be good molecular candidates for stretch-activated K^+ channels (Sanders and Koh, 2006), based on the following similarities between homologously expressed TREK-1 channels and native stretch-activated K^+ channels: both channels exhibit similar unitary conductances, pharmacology, stretch sensitivity, and regulation by NO, cGMP, and PKG (Koh *et al.*, 2001).

TREK-1 channels are inhibited by PKA and PKC. Maingret *et al.* (2002) used site-directed mutagenesis of a PKA consensus sequence in TREK-1 and showed that mutation at S333 abolished regulation by PKA. Exposure of cells expressing murine TREK-1 channels to SNP or 8-Br-cGMP, however, resulted in a sustained increase in open probability of mTREK-1 channels. Mutation of the PKG consensus site (S351A) in mTREK-1 eliminated the increase in open probability evoked by SNP or 8-Br-cGMP (Koh *et al.*, 2001). S351 is potentially phosphorylated by PKG or PKA, but the data demonstrated that the initial decrease in channel activity after exposure to 8-Br-cAMP is due to PKA phosphorylation at S333, and channel activation is due to PKG or PKA phosphorylation at S351. The initial decrease in channel activity on exposure to 8-Br-cAMP, due to phosphorylation at S333, remained intact in S351A mutant channels. 8-Br-cGMP did not cause an initial or sustained decrease in open probability of either wild-type or S351A channels, suggesting that PKG cannot phosphorylate S333. Thus, phosphorylation near the C-terminus of TREK-1, via different second-messenger signaling pathways, differentially regulates and finely tunes channel open probability.

Stretch of cell membranes is an activator of TREK family channels (Fig. 5), but others stimuli are also effective, including shear stress, cell swelling, and negative pressure in patch pipettes (Maingret *et al.*, 1999a,b; Patel *et al.*, 2001). Disruption of the cytoskeleton by either biochemical or mechanical means affects responses of TREK channels to stretch, and it appears that these channels are normally inhibited by the cytoskeleton (Lesage and Lazdunski, 2000). Regulation of the channels through interactions with the cytoskeleton may be the predominant mechanism that increases channel open probability when membranes are excised from cells (Sanders and Koh, 2006).

The specific domain of K_{2P} channels that confers mechanosensitivity has not been determined. Indeed, studies suggest that two separate domains may be involved: one domain may facilitate cytoskeletal interactions and another domain may actively sense membrane stretch (Kim *et al.*, 2001; Lauritzen *et al.*, 2005). Lauritzen *et al.* (2005) have studied the associations between channels and the cytoskeleton in TREK-1 channel protein by performing mutagenesis on residues within the C-terminus. Even in the presence of these mutations, sensitivity to stretch was retained. Kim *et al.* (2001) made chimeric

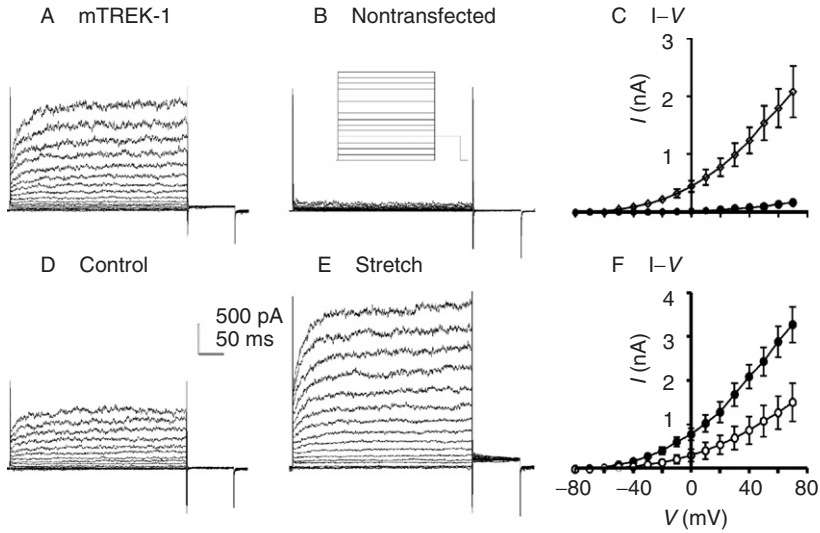

FIGURE 5 Characterization of the mTREK-1 current expressed in COS cells. Currents were recorded in whole-cell voltage clamp. The membrane potential was stepped from −80 to +70 mV in 10-mV increments for 400 ms. COS cells were stretched by micromanipulator. Panel A shows representative currents mTREK-1 transfected COS cells. Panel B shows nontransfected COS cells. Panel C shows average current–voltage (*I-V*) relationships in transfected (Δ) and nontransfected (●) COS cells. Panel D shows representative currents mTREK-1 transfected COS cells. Panel E is after cell elongation using manipulator. Panel F shows the average current-voltage relationship in control (○) and after stretch (●). Figure redrawn from Koh *et al.* (2001).

channels to define functionally important regions. These authors found that replacing the C-terminal tail of mechanosensitive channels with nonmechanosensitive channels caused loss of arachidonic acid regulation, but the channels retained sensitivity to stretch. Thus, more mutational studies are needed to understand the basis for mechanosensitivity in this class of channels.

The role of stretch-activated K^+ channels (and specifically the role of TREK family channels) in physiological responses has been complicated by the lack of highly specific blockers of these channels. The channels in toad myocytes were inhibited by very high concentrations of TEA (25 mM), but stretch-dependent K^+ channels in colonic myocytes were not blocked by TEA (up to 10 mM) or 4-aminopyridine (5 mM). Stretch-activated K^+ channels in colonic myocytes were also insensitive to changes in $[Ca^{2+}]_i$ (Koh and Sanders, 2001). We found that sulfur-containing amino acids (L-cysteine, L-methionine, or DL-homocysteine) block stretch-activated

K^+ channels in GI muscles and myocytes and inhibited hyperpolarization responses to nitrergic nerve stimulation (Park *et al.*, 2005). These compounds also blocked expressed TREK-1 currents in COS cells. L-methionine was the most selective blocker of this series of compounds, and it had no effect on the other types of K^+ channels expressed in colonic myocytes.

In conclusion, investigation of mechanosensitive K^+ channels is an exciting new area of research for the regulation of function in visceral smooth muscles. The possibility that these channels are one of the main effectors for nitrergic enteric inhibitory neural regulation is potentially of fundamental importance to understanding the regulation of whole organ function and how volume accommodation and reservoir functions occur in whole visceral organs. A better understanding of these channels, interactions with cytoskeletal elements, and changes in expression during development and pathophysiological circumstances may provide new methods of therapy for motility disorders.

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