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Membrane Stretch Increases the Activity of Ca^{2+}-Activated K^+ Channels in Rabbit Coronary Vascular Smooth Muscles

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Abstract

It has been proposed that Ca^{2+}-activated K^+ channels play an essential role in maintaining vascular tone during stretch of blood vessel. However, the underlying mechanism of stretch-induced change of Ca^{2+}-activated K^+ channel activities are still unknown. The present experiment was designed to investigate the effect of membrane stretch on these channels whose activity was measured from rabbit coronary smooth muscle cells using a patch clamp technique. Ca^{2+}-activated K^+ channel were identified by their Ca^{2+} and voltage dependencies and its large conductances as in other preparations. Perfusion of cells with a hypotonic solution, which mimics stretching the cell membrane by making a cell swelling, produced an increase in channel activity in cell-attached patch mode. The similar increase was observed when negative pressure was applied into the patch pipette for stretching the cell membrane within a patch area. In inside-out patch, stretch still increased channel activity even under the conditions which exclude the possible involvement of secondary messengers, or of transmembrane Ca^{2+} influx via stretch-activated cation channels. Pretreatment of arachidonic acid or albumin showed no effect on stretch-induced channel activation, excluding the possibility of fatty acids mediated channel activation during membrane stretch. These results indicate that the stretch may directly increase the activity of Ca^{2+}-activated K^+ channels in our experimental condition.

Key Words: Stretch, Ca^{2+}-activated K^+ channel, coronary smooth muscle, hypotonic solution, arachidonic acid, albumin

INTRODUCTION

Myogenic tone of blood vessel helps to maintain the appropriate amount of blood flow to peripheral organs regardless of their perfusion pressure.[1,2] It is neither related to its supplying nerve nor endogenous hormones. But it disappears when omitting external Ca^{2+} or adding Ca^{2+} channel blockers in the perfusate.[3,4] This means Ca^{2+} influx into the vascular smooth muscle cells should play an important role in preserving vascular myogenic tone.[5,6] $[Ca^{2+}]_i$ is dependent upon the membrane potential of the smooth muscle cell.[6,7] Membrane depolarization enhances Ca^{2+} influx which increases the vascular tone. And membrane hyperpolarization decreases Ca^{2+} influx which induces relaxation of smooth muscle cells.[8,9] This may indicate that the factors affecting the membrane potential can contribute to the regulation of vascular myogenic tone.[6] Experimentally, stretching the vascular smooth muscle induces depolarization by increasing the transmembrane cationic fluxes into the smooth muscle cells, and the degree of depolarization is dependent on the magnitude of the stretch.[4,10-13]

However, this stretch-induced cationic fluxes may cause uncontrolled depolarization and contracture of smooth muscle cells if the depolarization is not opposed by certain protective mechanism.

Ca^{2+}-activated K^+ channel (maxi K^+ channel) in the vascular smooth muscle cells has large unitary conductance (>200 pS) and its density is high (> 15,000/cell). It affects not only the membrane potential of the smooth muscle cells, but also $[Ca^{2+}]_i$ per se.[14-17] This channel is considered as an ideal candidate for a negative feedback against graded depolarization and vascular contracture during membrane stretch, because it is activated by membrane depolarization and cytoplasmic Ca^{2+}. If stretching of the membrane activates the maxi K^+ channel, it can

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prevent excessive $[Ca^{2+}]$ influx into the smooth muscle cells by hyperpolarization of membrane potential and keep the vascular tone in the optimal range in the presence of continuous membrane stretch.[13,18,19] However, the mechanism of activation of maxi K^+ channel by stretch has been unclear yet,[20] whether it is by the direct effect of stretch to the channel or via the secondary messenger system activated by stretch.[21-23]

In this paper we has observed an activation of maxi K^+ channel by membrane stretch which was observed under given conditions of steady state of $[Ca^{2+}]_i$ and membrane potential, and in the presence of arachidonic acids or albumin. Thus, membrane stretch per se may directly activate maxi K^+ channels in rabbit coronary smooth muscle cells.

MATERIALS AND METHODS

Preparation of single cell

After anesthetizing a rabbit with pentobarbital sodium (60 mg/Kg), the heart was extracted and the coronary arteries were roughly dissected with their surrounding tissues. In the preparation chamber, meticulous dissection of the coronary arteries from neighboring tissues were done with ophthalmologic scissor and forceps. The coronary artery was exposed longitudinally and bathed in Ca^{2+}/Mg^{2+} free Tyrode solution for 5 minutes. Additional 60 minutes incubation was done in temperature controlled (37°C) water bath after changing with solution containing 0.1% collagenase. After this incubation, the coronary strips was transferred to Ca^{2+}/Mg^{2+} free Tyrode solution, and agitated with fine glass rod. Single coronary smooth muscle cells were stocked in Tyrode solution at 4°C until the experiment.

Measurement of channel currents

Small amount of cell suspension was dropped into the experimental chamber, and waited for 5 to 10 minutes to settle down the cells to the bottom. The experiment was taken under the room temperature, and the bath solution was perfused with gravity at a rate of 1 ml/min. Patch clamping was done according to the method developed by Hamil et al. in either cell-attached or excised inside-out patch mode.[24]

The activity of Maxi K^+ channels was measured from cell-attached and inside-out patch modes using standard patch clamping techniques. Changes of membrane potentials were performed with commercial amplifier (Axopatch 1-C, Axon Instrument Inc., Foster city, CA, USA), and resulting membrane currents were digitized at a rate of 5 KHz with AD converter (Digidata 1200, Axon Instrument Inc., Foster city, CA, USA). The changes of channel activity were analyzed with pClamp 6.0 (Axon Instrument Inc., CA, USA) and expressed as average number of open channels (NP_o) instead of open probability (P_o), because every patch had multiple channels.

Experimental solutions

Tyrode solution for cell preparation contains (mM); NaCl, 140; KCl, 5.4; $CaCl_2$, 1.8; $MgCl_2$, 1.0; HEPES, 10; Glucose 5.5; pH=7.4 adjusted with Tris. Electrode solution contains in mM; KCl, 140; $MgCl_2$, 1; Glucose, 10; HEPES, 10; pH=7.4 adjusted with Tris. Perfusion solution for inside-out patch contains (mM); KCl, 140; HEPES, 10; Glucose, 10; EGTA, 5 and appropriate amount of $CaCl_2$ was added by Fabiato and Fabiato's formula.[25] Isotonic Tyrode solution contains (mM); NaCl, 70; KCl, 5.4; $CaCl_2$, 1.8; $MgCl_2$, 1.0; HEPES, 10; Glucose 5.5; 140 mM Sucrose; pH=7.4 adjusted with Tris. Hypotonic Tyrode solution was made by omitting sucrose from isotonic Tyrode solution. Identically Ca^{2+} free solution was made by removing $CaCl_2$ and adding 5mM EGTA from original composition. Arachidonic acid was dissolved in dimethylsulfoxide (DMSO), and diluted into the perfusate. All chemicals used in our experiment were purchased from Sigma Co.

RESULTS

Effect of membrane stretch on maxi K^+ channel activity in cell-attached patch

We measured the maxi K^+ channel activity of the coronary arterial smooth muscle cells in cell attached patch mode under perfusing isotonic and hypotonic Tyrode solution. As shown in Fig. 1-A, perfusing the smooth muscle cells with hypotonic Tyrode solution caused the marked increase of maxi K^+ channel

activity compared to the control, an isotonic Tyrode solution. And it returned to control level when the perfusing solution was changed from hypotonic to isotonic Tyrode solution (Fig. 1A).

We also tested the effect of local stretch on the activity of maxi K^+ channels by applying negative pressure within the patch pipette in cell attached patch. When we applied a negative pressure of 40 cm H_2O within the pipette, maxi K^+ channel activity was markedly increased compared to the control. The percent increase in NP_o was $214.8 \pm 59.0\%$ of control (n=5), and after releasing negative pressure the activity of maxi K^+ channels decreased to control level (Fig. 1B).

Candidates for stretch induced activation of maxi K^+ channel

The activity of maxi K^+ channel was subjected to many factors, such as intracellular Ca^{2+}, membrane potential, and intracellular metabolites. Stretch-induced change of intracellular Ca^{2+} concentration or cellular metabolite may contribute to the change of maxi K^+ channel activity during membrane stretch. To rule out these possibilities, we made a cell-free, inside-out patch to change the intracellular constituents on purpose, and measured the effect of negative pressure within patch pipette on maxi K^+ channel activity.

In inside-out patch perfused with pCa 7 solution (no GTP, no nucleotide and no ATP in perfusing solution), negative pressure within the pipette could still increase the activity of K^+ channel as those in cell attached patch. Also the activity of maxi K^+ channel was increased along with the magnitude of negative pressure within the patch pipette (Fig. 2). However, beyond 50 cm H_2O, huge leakage currents were developed, and the seal resistance was decreased

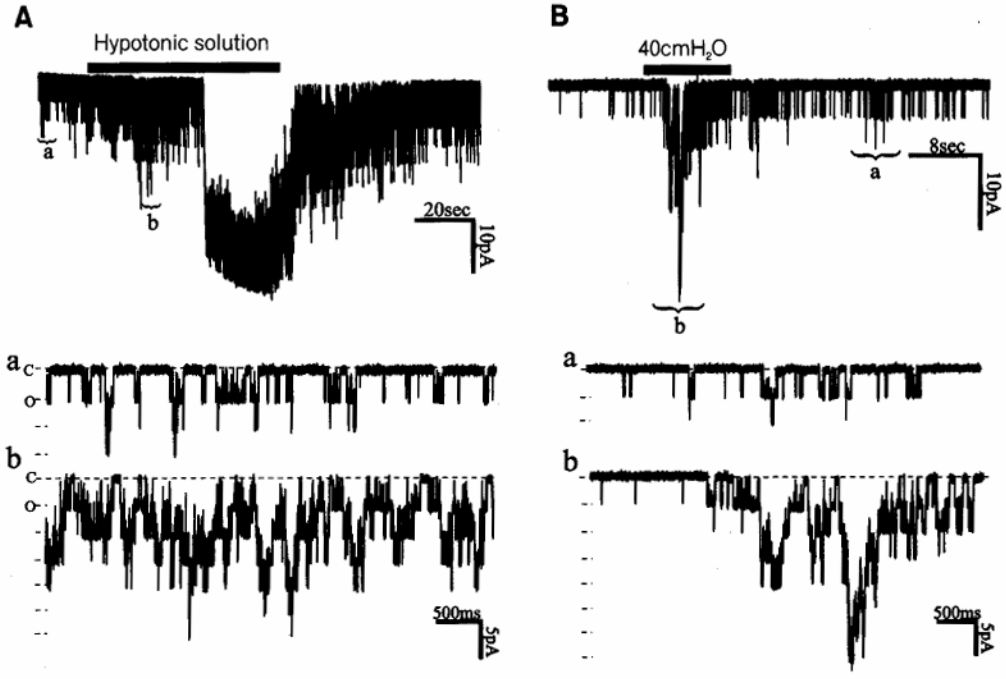

Fig. 1. Activation of the maxi K^+ channel by perfusion of hypotonic solution. A) Effect of hypotonic bath solution on the K^+ channel activity was measured in cell-attached patch configuration, and the pipette potential was held at 0 mV. Hypotonic Tyrode solution was perfused as indicated by bar in the figure. Lower panel shows experimental traces (a: control, b: hypotonic solution) with expanded time scale. Horizontal dashed bar represent open channel amplitude. B) The K^+ channel activity was recorded in cell-attached mode and the path membrane potential was clamped to 0 mV. Application of negative pressure (−40 cm H_2O) causes a reversible increase of the K^+ channel activity. 0 Ca^{2+} Tyrode solution was used as a bath solution and a high K^+ solution (mM) (140 KCl, 1 $CaCl_2$, 1 $MgCl_2$, 10 HEPES, 10 Glucose, pH 7.4) was used as a pipette solution. Lower panel shows experimental traces (a: control, b: negative pressure within pipette) with expanded time scale. Horizontal dashed bar represent open channel amplitude.

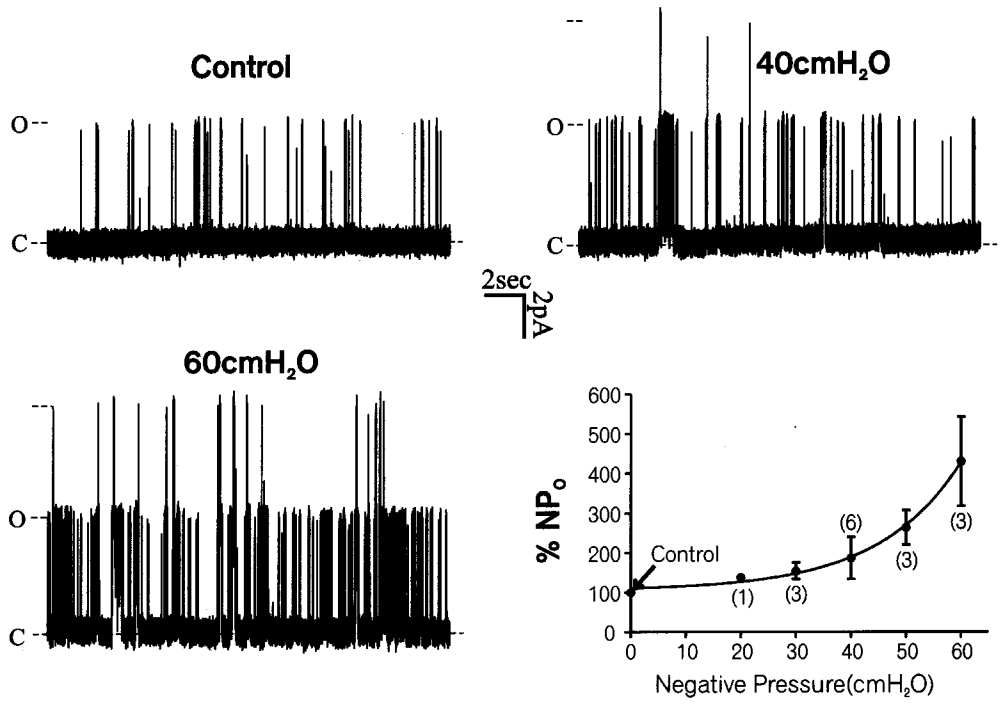

Fig. 2. Pressure dependent increase of maxi K^+ channel activity in rabbit coronary smooth muscle cell. After making an inside-out patch, the membrane potential was clamped at +20 mV. Magnitude of negative pressure within the pipette was increased stepwise from 0 to −70 cm H_2O and the resulting K^+ channel activity change was recorded. A High K^+ solution and a pCa 7 solution were used as a pipette solution and a bath solution (see material and method for details). Summarized result of negative pressure effect on maxi K^+ channel activity. The numbers of patch examined expressed within the figure. The change of activity was expressed as percentile of control maxi K^+ channel activity.

to less than 10 giga ohm as well. Therefore, we used 40 cm H_2O of negative pressure during the experiment for elucidating the mechanism of stretch-induced activation of maxi K^+ channel.

During membrane stretch there might be a movement of Ca^{2+} from pipette solution to bath solution through stretch-activated non-selective cation channels. This Ca^{2+} influx may increase the local $[Ca^{2+}]_i$ underneath the patch membrane and increase maxi K^+ channel activity. To rule out this possibility. we changed the pipette and bath solution to an identically Ca^{2+} free solution (<pCa 9), and examined the effect of negative pressure on maxi K^+ channel activity. As seen in Fig. 3A, application of negative pressure could still increase the maxi K^+ channel activity in the identically Ca^{2+}-free solution where percent change of NP_o was set to 195.0±49.8 of control (n=3).

Besides an activation of non-selective cation channels, membrane stretch can activate membrane bound phospholipase activity and increase local concentration of free fatty acids, especially arachidonic acids.[23] These free fatty acids were reported to increase the activity of many type of K^+ channels, including maxi K^+ channels.[26,27] These reports suggest that stretch-induced activation of maxi K^+ channel might be due to the stretch-induced increase of free fatty acids concentrations. To test this possibility, we exposed the cytosolic surface of inside-out patches to arachidonic acid (4 μM) to activate the maxi K^+ channel and then determining whether the maxi K^+ channel activity increased in response to negative pressure in the presence of arachidonic acid. After making an inside out patch, arachidonic acid (4 μM) was applied to the bath. Within a few seconds K^+ channel activity increased and reached to a steady state. When channel activity reached to a steady state, a negative pressure was again applied to the pipette, which produced a further increase in channel activity of the same type of channels (% change of

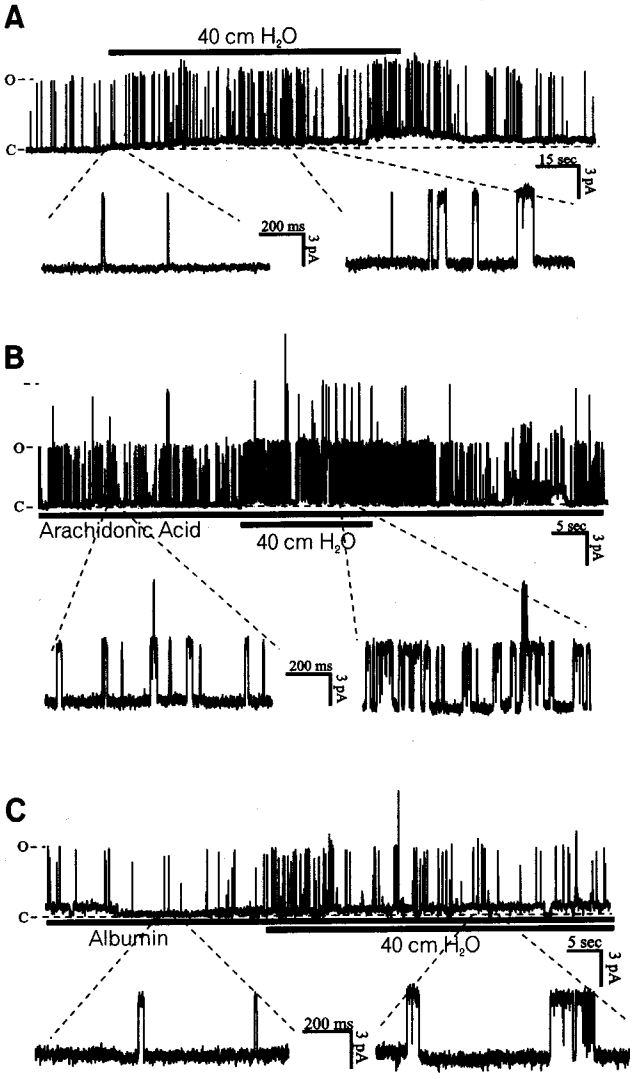

Fig. 3. Effect of various conditions on the negative pressure-induced activation of maxi K^+ channel. A) Maxi K^+ channel activity was recorded using identically Ca free bath and pipette solutions. Application of negative pressure (−40 cm H_2O) to the patch membrane causes a reversible increase of K^+ channel activity. Membrane potential was clamped at +40 mV in inside out configuration. B) After making an I-O patch, patch membrane potential was held at +40 mV. Application of arachidonic acid (4 μM) to the bath solution causes a increase of K^+ channel activity and negative pressure (−40 cm H_2O) within the pipette further increase the K^+ channel activity in spite of the presence of arachidonic acid. A high K^+ solution and a pCa 7 solution were used as a pipette solution and a bath solution. C) Effect of negative pressure (−40 cm H_2O) on K^+ channel activity was recorded in the presence of Albumin (10 μM). Application of negative pressure cause a marked increase of K^+ channel activity. The membrane potential was held at +40 mV. A High K^+ solution and a pCa 7 solution were used as a pipette solution and a bath solution.

NP_o=398.8±80.3, n=3). After returning to an atmospheric pressure, the channel activity returned to the same level observed just before application of negative pressure (Fig. 3B). Futhermore, the negative pressure within the pipette could still increase the activity of maxi K^+ channel even in the presence of defatted albumin (10 μM), which was reported to bind with circulating free fatty acid and lower its blood level.[28] (Fig. 3C, % change of NP_o=219.8± 13.5, n=7).

DISCUSSION

The K^+ channel activity recorded in our experiment was strongly dependent on membrane depolarization and intracellular Ca^{2+} concentration. These findings are compatible with the maxi K^+ channel.[15,29-32] Maxi K^+ channel is distributed over various vascular smooth muscle cell membrane in high density and these channels affect the level of membrane potential and vascular contractility.[13,17] Activity of maxi K^+ channel is regulated by various modulators such as membrane potential, intracellular Ca^{2+}, lipid, and nucleotide.[33] The stretch of membrane is also considered to be one of the modulators.[20,22,34] However, it is still uncertain whether the stretch of the membrane can directly increase the activity of maxi K^+ channels.

When renal proximal tubular cells were exposed to hypotonic solution, the activity of maxi K^+ channel was increased which was dependent on the concentration of Ca^{2+} in the hypotonic solution,[21,35,36] as it was in our experimental result in Fig 1A. Stretching the membrane by exposing the cell with hypotonic solution may allow Ca^{2+} influx through a stretch-induced leak or stretch-activated non-selective cation channel. This would elevate intracellular concentration of Ca^{2+} and cause the activation of maxi K^+ channel.[22] However, the activity of maxi K^+ channel in rabbit coronary smooth muscle cells, unlike renal proximal tubular cells, increased in identically Ca^{2+}-free solution (Fig. 3A) which eliminates the possibility of Ca^{2+} influx during membrane stretch. It suggests that in rabbit coronary smooth muscle cells, increased Ca^{2+} influx is not a prerequisite for stretch-induced activation of maxi K^+ channels.

In our experiment, there was 10−30 seconds of lag

phase before channel activation after an exposure to hypotonic solution or application of negative pressure to the pipette, and slow recovery of channel activity was observed after removing the stimuli (see Fig. 1 and 3). These slow onset of stretch effect may suggest involvement of intracellular metabolites or signal transduction system to the activation of maxi K^+ channel. In cell-free, excised inside-out patch perfused with pCa 7 solution which has no substrates for signal transduction system, application of negative pressure to the pipette still increased maxi K^+ channel activity reversibly (Fig. 2). This means that the stretch may increase the activity of maxi K^+ channel of coronary smooth muscle cells directly rather than by the effect of intracellular metabolites.[37,38]

Free fatty acid, such as arachidonic acid can increase the activity of various types of K^+ channels.[39-43] In toad gastric smooth muscle cells, a K^+ channel activated by fatty acids was also sensitive to membrane stretch.[22] Ordway et al. suggest that the membrane stretch increases fatty acid level by activating the membrane bound phospholipase in pulmonary smooth muscle cells, and these fatty acids mediate the stretch induced increase of maxi K^+ channel activity.[23] To test this possibility, we pretreated sufficient amount of arachidonic acid in bath solution and investigated the effect of stretch on maxi K^+ channel activity.[26] As shown in Fig. 3B, pretreatment of arachidonic acid could not prevent the stretch-induced increase of maxi K^+ channel. Also the pretreatment of albumin, which bounds with free fatty acid and decreases the concentration of fatty acids[28] had no effect on stretch induced increase of maxi K^+ channel activity (Fig. 3C). Therefore, it is unlikely that fatty acids act as second messengers in stretch-induced increase of maxi K^+ channel activity in rabbit coronary smooth muscle cells.

In summary, both hypotonic stimulation and stretch increased the activity of maxi K^+ channel of coronary vascular smooth muscle cells regardless of the presence of Ca^{2+}. These same phenomena were also observed in cell attached and cell free excised inside-out patch without the involvement of any intracellular metabolites, such as GTP, ATP, divalent cation, arachidonic acid. The stretch can thus directly increase the maxi K^+ channel activity in rabbit coronary smooth muscle cells.

REFERENCES

1. Johnson PC. Autoregulation of blood flow. Circ Res 1986; 59:483-95.
2. Meininger GA, Zawieja DC, Falcone JC, Hill MA, Davey JP. Calcium measurement in isolated arterioles during myogenic and agonist stimulation. Am J Physiol 1991; 261:H950-9.
3. Nakayama K. Calcium-dependent contractile activation of cerebral artery produced by quick stretch. Am J Physiol 1982;242:H760-8.
4. Laher I, Bevan JA. Stretch of vascular smooth muscle activates tone and $^{45}Ca^{++}$ influx. J Hypertens 1989;7 Suppl:17-20.
5. Knot HJ, Nelson MT. Regulation of membrane potential and diameter by voltage dependent K channels in rabbit myogenic cerebral arteries. Am J Physiol 1995;269: H348-55.
6. Nelson MT, Patlak JB, Worley JF, Standen NB. Calcium channels, potassium channels, and voltage dependence of arterial smooth muscle tone. Am J Physiol 1990;259: C3-18.
7. Nelson MT, Quayle JM. Physiological roles and properties of potassium channels in arterial smooth muscle. Am J Physiol 1995;268:C799-822.
8. van Breeman C, Cauvin C, Yamamoto H, Zschauer A. Vascular smooth muscle cells calcium channels. J Cardiovasc Pharmacol 1987;10:S10-5.
9. Rubart M, Patlak TB, Nelson MT. Ca currents in cerebral artery smooth muscle cells of rat at physiological Ca concentration. J Gen Physiol 1996;107:459-72.
10. Bürbling E, Kuriyama H. The effect of adrenalin on the smooth muscle of guinea-pig taenia coli in relation to the degree of stretch. J Physiol 1963;169:198-212.
11. Coburn RF. Stretch-induced membrane depolarization in ferret trachealis smooth muscle cells. J Appl Physiol 1987; 62:2320-5.
12. Davis MJ. Myogenic response gradient in an arteriolar network. Am J Physiol 1993;264:H168-79.
13. Brayden JE, Nelson MT. Regulation of arterial tone by activation of calcium-dependent potassium channels. Science 1992;256:532-5.
14. Bolton TB, Lang RJ, Takewaki T, Benham CD. Patch and whole cell voltage clamp of single mammalian visceral and vascular smooth muscle cells. Experientia 1985;41:887-94.
15. Yamaguchi H, Honeyman TW, Fay FS. Beta adrenergic actions on membrane electrical properties of dissociated smooth muscle cells. Am J Physiol 1988;254:C423-31.
16. Trieschmann U, Isenverg G. Ca^{++} activated K^+ channels contribute to the resting potential of vascular myocyte. Ca^{++} sensitivity is increased by intracellular Mg^{++} ions. Pflugers Arch 1989;414 Suppl 1:183-4.
17. Carl A, McHale NG, Publicover NG, Sanders KM. Participation of Ca^{2+}-activated K^+ channels in electrical activity of canine gastric smooth muscle. J Physiol 1990; 429:205-21.
18. Ubl J, Murer H, Kolb HA. Hypotonic shock evokes open-

ing of Ca^{++} activated K^+ channels in opossum kidney cells. Pflugers Arch 1988;412:551-3.
19. Bregestovski PD, Bolotina VM, Serebryakov VN. Fatty acid modifies Ca^{2+}-dependent potassium channel activity in smooth muscle cells from the human aorta. Proc R Soc Lond B 1989;237:259-66.
20. Taniguchi J, Guggino WB. Membrane stretch: a physiological stimulator of Ca^{2+}-activated K^+ channels in thick ascending limb. Am J Physiol 1989;257:F347-52.
21. Kawahara K, Ogawa A, Suzuki M. Hypoosmotic activation of Ca^{++}- activated K^+ channels in cultured rabbit kidney proximal tubule cells. Am J Physiol 1991;260: F27-33.
22. Kirber MT, Ordway RW, Clapp LH, Walsh Jr JV, Singer JJ. Both membrane stretch and fatty acids directly activated large conductance Ca^{2+}- activated K^+ channels in vascular smooth muscle cells. FEBS Lett 1992;297: 24-8.
23. Ordway RW, Petrou S, Kirber MT, Walsh Jr JV, Singer JJ. Two distinct mechanisms of ion channel activation by membrane stretch. Evidence that endogenors fatty acids mediate stretch activation of K^+ channels. Biophys J 1992;61:A390.
24. Hamil OP, Marty A, Neher E, Sackman B, Sigworth FJ. Improved patch-clamp techniques for high resolution current recording from cells and cell-free membrane patches. Pflugers Arch 1981;391:85-100.
25. Fabiato A, Fabiato F. Calculation programs for computing the composition of the solutions containing multiple metals and ligands used for experiments in skinned muscle cells. Journal de Physiologie 1979;75:463-505.
26. Ahn DS, Kim YB, Lee YH, Kang BS, Kang DH. Fatty acids directly increase the activity of Ca-activated K channels in rabbit coronary smooth muscle cells. Yonsei Med J 1994;35:10-24.
27. Ordway RW, Petrou S, Kirber MT, Walsh Jr JV, Singer JJ. Stretch activation of a toad smooth muscle K^+ channels may be mediated by fatty acids. J Physiol 1995; 484:331-7.
28. Ordway RW, Singer JJ, Walsh Jr JV. Direct regulation of ion channels by fatty acids. TINS 1991;14:96-100.
29. Benham CD, Bolton TB, Lang RJ, Takewaki T. Calcium-activated potassium channels in single smooth muscle cells of rabbit jejunum and guinea-pig mesenteric artery. J Physiol 1986;371:45-67.
30. Blatz AL, Magleby KL. Calcium-activated potassium channel. TINS 1987;10:463-7.
31. Singer JJ, Walsh Jr JV. Characterization of calcium-activated potassium channels in single smooth muscle cells using the patch-clamp technique. Pflugers Arch 1987;408: 98-111.
32. Hu SL, Yamamoto Y, Kao CY. The Ca^{2+}-activated K^+ channel and its functional roles in smooth muscle cells of guinea pig taenia coli. J Gen Physiol 1989;94:833-47.
33. Toro L, Stefani E. Calcium-activated K^+ channels: metabolic regulation. J Bioenerg Biomembr 1991;23:561-76.
34. Johnson RM, Tang K. Induction of a Ca^{2+}-activated K^+ channel in human erythrocytes by mechanical stress. Biochem Biophys Acta 1992;1107:314-8.
35. Filipovic D, Sackin H. A calcium-permeable stretch-activated cation channel in renal proximal tubule. Am J Physiol 1991;260:F119-29.
36. Suzuki M, Kawahara K, Ogawa A, Morita T, Kawaguchi Y, Kurihara S, et al. $[Ca^{2+}]_i$ rises via G protein during regulatory volume decrease in rabbit proximal tubule cells. Am J Physiol 1990;258:F690-6.
37. Kim D, Clapham DE. Potassium channels in cardiac cells activated by arachidonic acid and phospholipids. Science 1989;244:1174-6.
38. Ordway RW, Walsh Jr JV, Singer JJ. Arachidonic acid and other fatty acids directly activate potassium channels in smooth muscle cells. Science 1989;244:1176-9.
39. Piomelli D, Voltrra A, Dale N, Siegelbaum SA, Kandel ER, Schwartz JH, et al. Lipoxygenase metabolites of arachidonic acid as second messengers for presynaptic inhibition of Aplasia sensory cells. Nature 1987;328:38-43.
40. Kim D, Duff RA. Regulation of K^+ channels in cardiac myocytes by free fatty acids. Circ Res 1990;67:1040-6.
41. Wallert MA, Ackerman MJ, Kim D, Clapham DE. Two novel cardiac atrial K^+ channels, $I_{K\text{-}AA}$ and $I_{K\text{-}PC}$. J Gen Physiol 1991;98:921-39.
42. Kim D. A mechanosensitive K^+ channel in heart cells; activation by arachidonic acid. J Gen Physiol 1992;100: 1021-40.
43. Vandrope DH, Morris CE. SAK channel/S channel from Aplasia neurons. Comparison of stretch, FMRF amide and 5-HT effects. Biophys J 1992;61:A251.