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Both membrane stretch and fatty acids directly activate large conductance Ca^{2+}-activated K^+ channels in vascular smooth muscle cells

Michael T. Kirber[a], Richard W. Ordway[a], Lucie H. Clapp[b], John V. Walsh Jr.[a] and Joshua J. Singer[a]

[a]*Department of Physiology, University of Massachusetts Medical School, Worcester, MA, USA* and [b]*Department of Pharmacology, United Medical and Dental Schools, St Thomas's Hospital, London, UK*

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Large conductance Ca^{2+}-activated K^+ channels in rabbit pulmonary artery smooth muscle cells are activated by membrane stretch and by arachidonic acid and other fatty acids. Activation by stretch appears to occur by a direct effect of stretch on the channel itself or a closely associated component. In excised inside-out patches stretch activation was seen under conditions which precluded possible mechanisms involving cytosolic factors, release of Ca^{2+} from intracellular stores, or stretch induced transmembrane flux of Ca^{2+} or other ions potentially capable of activating the channel. Fatty acids also directly activate this channel. Like stretch activation, fatty acid activation occurs in excised inside-out patches in the absence of cytosolic constituents. Moreover, the channel is activated by fatty acids which, unlike arachidonic acid, are not substrates for the cyclo-oxygenase or lypoxygenase pathways, indicating that oxygenated metabolites do not mediate the response. Thus, four distinct types of stimuli (cytosolic Ca^{2+}, membrane potential, membrane stretch, and fatty acids) can directly affect the activity of this channel.

Ca^{2+}-activated K^+ channel; Stretch-activated channel; Fatty acid; Vascular smooth muscle; Rabbit pulmonary artery

1. INTRODUCTION

Stretch-activated ion channels (channels activated by increases in membrane tension) were first reported by Guharay and Sachs [1] and have since been found in a wide variety of preparations [2]. We reported channels activated by membrane stretch in smooth muscle cells isolated from toad stomach [3]. In this preparation these channels may play a role as mechanoelectric transducers in the initiation of stretch-induced contraction, a characteristic of a wide variety of smooth muscle cell types (for references see [3]).

We have since studied two types of ion channels which are affected by membrane stretch in another preparation of smooth muscle cells, cells isolated from rabbit pulmonary artery. One type is apparently cation-selective and may play a role in the initiation of contraction of blood vessels in response to stretch [4]. The second type, a large conductance K^+ channel, activated by increases in Ca^{2+} concentration at the intracellular surface of the channel, is the subject of this report. As shown below, the effects of membrane stretch on this channel appear to be directly on the channel itself or some closely associated component (and not secondary to an elevation of internal Ca^{2+} that might occur by some sort of stretch induced influx of Ca^{2+} through other stretch-activated channels or release from intracellular stores).

This same Ca^{2+}-activated K^+ channel is activated by fatty acids. Fatty acids are integral components of the biological membranes in which ion channels reside and are liberated as 'free' or unesterified fatty acids by a number of stimuli [5]. One fatty acid, arachidonic acid, is known to act as a signaling molecule in a number of systems [6]. While arachidonic acid clearly acts as a precursor to a variety of biologically active metabolites [6], it is now also clear that this and other fatty acids themselves exert many effects on biological systems, independent of such metabolites [5]. Our previous studies of a different type of K^+ channel in gastric smooth muscle cells [7], as well as studies in a number of other preparations (reviewed in [5]), have demonstrated that these 'free' fatty acids regulate the activity of ion channels and have raised the possibility that these compounds are endogenous channel regulators. Like the K^+ channel in gastric smooth muscle cells, activation of the Ca^{2+}-activated K^+ channel in pulmonary artery by fatty acids appears to result from the interaction of the fatty acids themselves with a protein or lipid site in the membrane.

This channel is of interest for two principal reasons. First, a large body of literature exists documenting the vasoactive effects of both arachidonic acid (and related metabolites) (for example [8]) and mechanical stretch [9]. Our findings suggest that these stimuli may, at least in part, exert their influence by affecting the gating behavior of this large conductance Ca^{2+}-activated K^+

Correspondence address: Department of Physiology, University of Massachusetts Medical School, 55 Lake ave. North, Worcester, MA 01655, USA.

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channel and consequently the membrane potential of the smooth muscle cell. Second, it is unusual to find a channel the gating behavior of which can be directly affected by multiple diverse types of stimuli (internal Ca^{2+}, membrane potential, membrane stretch, and fatty acids). The further investigation of these diverse modes of channel activation and their interrelationships may be of significant biophysical interest. Preliminary reports of our studies have appeared in abstract form [10,11].

2. METHODS

Freshly isolated smooth muscle cells from rabbit pulmonary artery were obtained using the methods of Clapp and Gurney [12]. Single channel currents were recorded from excised inside-out patches using standard patch-clamp techniques and equipment [3]. Unitary current amplitudes were estimated by inspection using custom software [13]. As a measure of channel activity we used the product of the number of channels in the patch and the probability that a particular channel is open (NP_o), which is equivalent to the average number of open channels. NP_o was determined either by dividing the time averaged current by the unitary current amplitude or by using the program IPOCH, which performs the computation based on an idealized channel record derived from the raw data [13].

The compositions of the various pipette solutions are given in the figure legends. Unless otherwise stated, the bathing solution contained in mM: 130 K^+, 5 EGTA, 1 Mg^{2+}, 10 HEPES, 10 glucose, 114.5 Cl^- (pH 7.4). Fatty acids (Nu Chek) were first dissolved in DMSO before being diluted in bathing solution [7]. The final concentration of DMSO was 0.1% or less, and control applications of DMSO had no noticeable effect on channel activity. Agents were applied by pressure ejection from a glass micropipette and the concentration given is that in the pipette [14]. The patch was stretched by applying suction to the back end of the patch pipette. Suction was monitored using a pressure transducer [3]. All studies were carried out at room temperature.

3. RESULTS

Large conductance Ca^{2+}-activated K^+ channels in rabbit pulmonary artery smooth muscle cells appear to be similar to channels of this type found in a variety of preparations [15]: they are highly selective for K^+ (the reversal potential is near zero in symmetric K^+ solutions and is shifted in a negative direction when most of the K^+ at the extracellular surface is replaced with sodium to mimic physiological conditions) and have a conductance of 270 pS with 130 mM K^+ on both sides of the membrane (Fig. 1). Moreover, the channel is activated by increasing the concentration of Ca^{2+} at the intracellular surface of the patch (Fig. 2) and by applying more positive membrane potentials. However, as described below, the activity of the channel from rabbit pulmonary artery is also increased by membrane stretch and by the application of fatty acids to the cytosolic surface of the patch.

3.1. *Membrane stretch*

Activation of the channel by stretch was obtained under a variety of experimental conditions which together point to a direct mechanical effect on the channel itself or on a component closely associated with it. Release of Ca^{2+} from intracellular stores does not mediate the response since activation by stretch was readily and repeatedly obtained in excised inside-out patches. To demonstrate a direct effect of stretch we still had to eliminate the possibility that stretching the membrane caused the channel to be indirectly activated by somehow permitting the transmembrane movement of an ion which could in turn alter the gating behavior of the channel. For example, stretching the membrane could have allowed Ca^{2+} to pass through the membrane through another type of undetected stretch-activated channel or through a stretch-induced leak. This would have elevated the Ca^{2+} concentration on the cytosolic surface of the channel and caused its activation. The possibility of activating the channel indirectly by stretch induced Ca^{2+} flux was ruled out by showing stretch

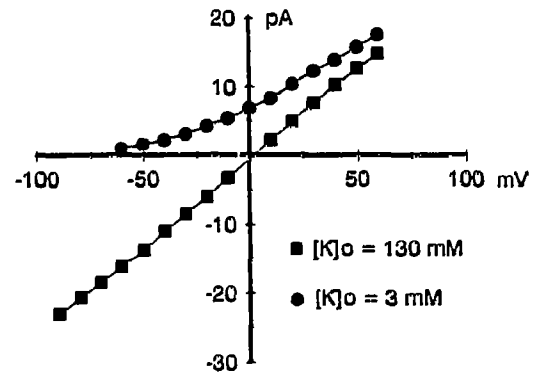

Fig. 1. Unitary current–voltage relationship in symmetric 130 mM K^+ and 3 mM $[K^+]_o$, 130 mM $[K^+]_i$ solutions. The slope conductance near 0 mV is 270 pS in symmetric K^+. For the symmetric K^+ patch the pipette solution contained the identical solution to the bath with the exception of the 10 mM glucose. The low $[K^+]$ pipette solution contained (in mM) 137 Na^+, 3 K^+, 1 Mg^{2+}, 10 HEPES, 2 EGTA, 135 Cl^- (pH 7.8). Channel openings at potentials where the channel was not tonically active were elicited by applying solutions identical to the bathing solution with 4.8 and 5 mM $CaCl_2$ added.

Fig. 2. Application of Ca^{2+} to the intracellular surface of an excised inside-out patch increases channel activity. The bathing solution contained (in mM) 130 KCl, 10 HEPES, 1 $MgCl_2$, 10 glucose. The patch pipette solution contained (in mM) 110 Na^+, 5 EGTA, 1 Mg^{2+}, 3 K^+, 97.5 Cl^-, 10 HEPES. The application pipette contained bathing solution with 10 μM $CaCl_2$ (0 EGTA) added. The application pipette was located at some distance from the patch pipette so that the responses would not be offscale; this accounts for the observed delay in activation. The pH of all solutions was 7.4. The membrane potential was 0 mV.

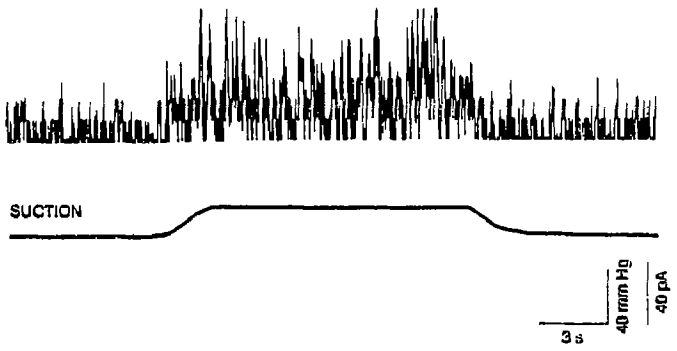

Fig. 3. Application of suction to the patch pipette causes a readily reversible increase in channel activity with identical Ca^{2+}-free (5 mM EGTA) solutions at both surfaces of the excised membrane patch. The standard bathing solution without glucose was used in the bath and in the patch pipette, and the membrane potential was held at +60 mV.

activation of the channel in excised inside-out patches with 0 Ca^{2+} (5 mM EGTA) at both surfaces of the patch (Fig. 3).

For the experiment described above, when the effects of stretch were tested using identical solutions at both surfaces of the patch in the absence of Ca^{2+}, positive potentials were necessary to produce appreciable channel activation and permit observation of the added activation by membrane stretch. These positive potentials might have resulted in the movement (driven by the electrical field) of some ion other than Ca^{2+} that may have caused activation of the channel. We carried out a set of experiments to eliminate this possibility: we tested for the effects of stretch above, below (+ 10 mV and −10 mV) and at 0 mV patch potential using solutions which differed only in their Na^+ and K^+ composition (K^+ higher at the cytosolic surface) and which contained the same Ca^{2+} concentration at both surfaces of the patch (3 mM Ca^{2+}, 5 mM EGTA). We also tested for the effect of stretch at +20 mV and −20 mV using symmetric 130 mM KCl solutions with no EGTA and 10 μM added Ca^{2+}. In all cases membrane stretch repeatedly and reversibly caused increases in channel activity making it highly unlikely that activation by stretch is secondary to transient increases in ion concentrations via small unobserved channels or some sort of undetectable membrane disruption.

3.2. *Fatty acids*

Activation of the channel by fatty acids also appears to be direct and not due to the formation of biologically active metabolites or to activation of another channel type which might in turn allow the flux of ions that could affect the gating behavior of the channel. As in our study of gastric smooth muscle cells [7], we began in pulmonary artery smooth muscle cells by examining the effects of arachidonic acid (20 μM) in excised, inside-out patches (Fig. 4a). Upon application of arachidonic acid, Ca^{2+}-activated K^+ channel activity increased from an initial basal level. Arachidonic acid appears to have no effect on other channel types under the recording conditions used here.

In order to test whether channel activation resulted from an action of the fatty acid itself or from the generation of an arachidonic acid metabolite, fatty acids that are not substrates for the enzymes that convert arachidonic acid to active metabolites [5] were also tested. For example, the saturated fatty acid, myristic acid (14:0) mimicked the effect of arachidonic acid (Fig. 4b) as did the *trans*-unsaturated fatty acid, linoelaidic acid (18:2 *trans*-9,12; not shown).

The effectiveness of fatty acids that are not converted to active metabolites indicates that such metabolites do not mediate the fatty acid activation. Since these effects were seen in excised membrane patches in the absence of nucleotides and calcium (5 mM EGTA on both sides of the membrane), they also did not require signal transduction mechanisms involving phosphorylation,

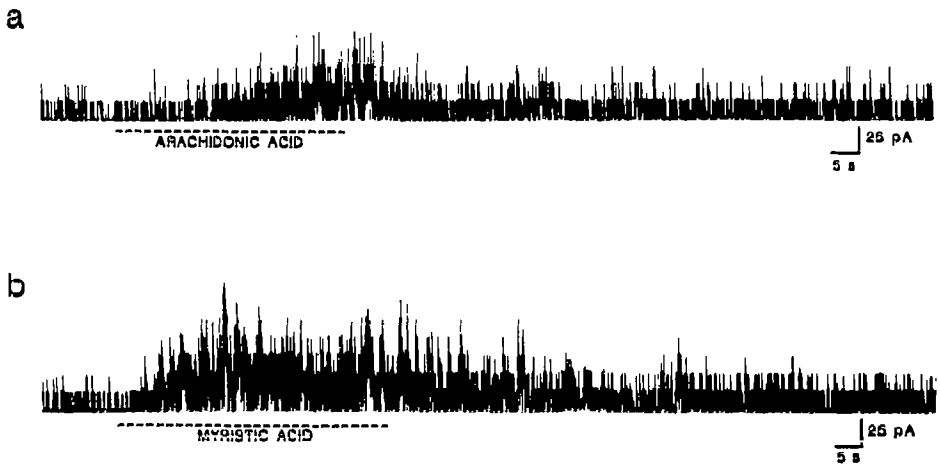

Fig. 4. Fatty acids, arachidonic acid 20 μM (a) and myristic acid 20 μM (b), applied to the intracellular surface of the patch activate large conductance K^+ channels. In both cases the pipette solution contained in mM 20 K^+, 110 Na^+, 114.5 Cl^-, 1 Mg^{2+}, 5 EGTA, and 10 HEPES, pH 7.4. The membrane potential across the patch was +50 mV in (a) and +60 mV in (b).

GTP-dependent proteins, cyclic nucleotides, calcium, or metabolites of the NADPH-dependent cytochrome P450 enzyme. Rather, fatty acids, like stretch, appear to directly activate these K^+ channels by acting on the channel itself or a closely associated component.

3.3. *Membrane potential, stretch and fatty acids*

To begin to understand the relationships between these diverse modes of channel activation, we examined the effects of membrane potential on channel activity in the presence and absence of other activating stimuli. Assuming that the channels in a patch behave identically and act independently of each other, and provided that the probability that a channel is open (P_o) is low, the potential sensitivity of channel gating can be determined in patches containing multiple channels without knowing the actual number of channels in the patch [16]. Moreover, if the voltage activation is well described by a Boltzman relationship then a plot of the natural log of the product of the number of channels in the patch (N) and the probability that a single channel is open (P_o) versus potential should be linear when P_o for a single channel is small. The reciprocal of the slope (a measure of the potential sensitivity) is the voltage required to produce an e-fold change in P_o at low P_o.

The effects of membrane potential on channel gating were examined at low P_o in the presence and absence of free Ca^{2+} at the cytosolic surface of the channel (Fig. 5a). As for other channels of this type [15], both Ca^{2+} and more positive potentials increase P_o with the voltage sensitivity of the channel appearing unaffected by the presence of free Ca^{2+} (near 11.5 mV required for an e-fold change in P_o for the channel in rabbit pulmonary artery).

Like Ca^{2+}, both membrane stretch (Fig. 5b) and fatty acids (Fig. 5c) increase the activity of the channel apparently without altering its voltage sensitivity. Thus, Ca^{2+}, stretch, and fatty acids all appear to shift the curve relating P_o to membrane potential along the voltage axis to more negative potentials. The most straightforward interpretation of these data is that none of these modes of activation affect the voltage sensing portion of the channel molecule.

3.4. *Basal activity and activating stimuli*

The effects of stretch or fatty acids on these Ca^{2+}-activated K^+ channels appear to be different from other stretch or fatty acid activated channels which we have studied in smooth muscle [3,4,7]. The other channels can be activated by the application of either suction to the patch pipette or fatty acids in the absence of basal activity. Application of either to the large conductance Ca^{2+}-activated K^+ channel has little effect in the absence of basal activity (for example at more negative membrane potentials and/or the absence of Ca^{2+} at the cytosolic surface). However, under conditions where a moderate amount of basal activity is present, membrane stretch or fatty acids cause a profound increase in activity.

4. DISCUSSION

The activity of large conductance K^+-selective channels found in pulmonary artery smooth muscle cells is increased by elevations of intracellular Ca^{2+}, more positive membrane potentials, membrane stretch and fatty acids (Figs. 2–4). At low P_o, $[Ca^{2+}]_i$, fatty acids, and stretch all increase channel activity by a constant multiplicative factor at various potentials suggesting that none of these affect the voltage sensor portion of the channel molecule (Fig. 5). Both membrane stretch and fatty acids seem to directly affect either the channel itself or a membrane component closely associated with it.

Although increases in the activity of Ca^{2+}-activated K^+ channels have been reported to be correlated with

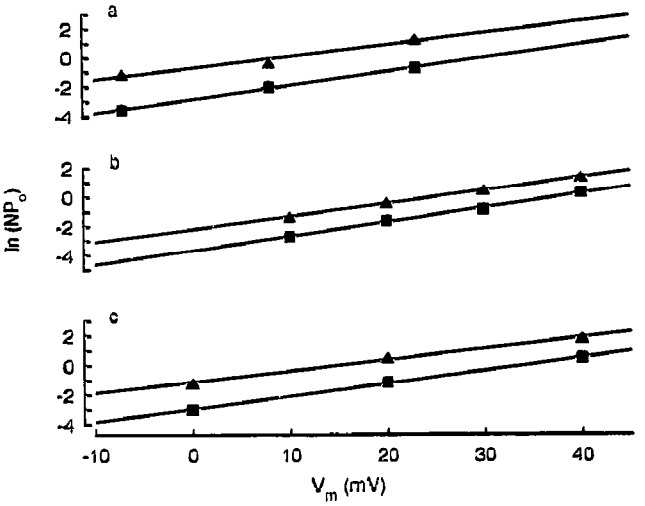

Fig. 5. Three distinct stimuli [Ca^{2+}] (a), stretch (b), and myristic acid (c) increase channel activity without affecting the voltage sensitivity (■ control, ▲ with stimulus). (a) *Elevation of* $[Ca^{2+}]_i$. The voltage sensitivity is respectively 12.3 mV and 10.5 mV per e-fold change in NP_o in the presence and absence of Ca^{2+}. NP_o was calculated from data segments 15 s in duration at each potential. The patch contained at least 18 Ca^{2+}-activated K^+ channels. The bathing solution was the standard solution without glucose. The pipette solution contained (in mM) 110 Na^+, 20 K^+, 114.5 Cl^-, 1 Mg^{2+}, 5 EGTA, 10 HEPES, (pH 7.4). Ca^{2+} containing solution was bathing solution with 4 mM $CaCl_2$ added. (b) *Application of stretch.* The voltage sensitivity is respectively 11.2 mV and 10.2 mV per e-fold change in NP_o in the presence and absence of 30 mmHg suction. Segments used to calculate NP_o ranged in duration from 9 to 30 s but were typically 28 s in duration. The patch contained a minimum of 12 Ca^{2+}-activated K^+ channels. The bathing solution contained (in mM) 130 K^+, 1.1 Mg^{2+}, 10 HEPES, 2 EGTA, 0.755 Ca^{2+}, 126.7 Cl^- (pH 7.2). The patch pipette contained (in mM) 110 Na^+, 3 K^+, 1 Mg^{2+}, 10 HEPES, 5 EGTA, and 97.5 Cl^- (pH 7.4). (c) *Myristic acid (20 μM).* The voltage sensitivity is respectively 13.3 mV and 11.4 mV per e-fold change in NP_o in the presence and absence of 20 μM myristic acid. NP_o was calculated from data segments 10 s in duration at each potential. The patch contained at least 14 Ca^{2+}-activated K^+ channels. The pipette contained (in mM) 110 Na^+, 20 K^+, 114.5 Cl^-, 1 Mg^{2+}, 5 EGTA, 10 HEPES (pH 7.4). Myristic acid containing solution was bathing solution with 20 μM myristic acid added.

membrane stretch in other preparations [17–23], activation in these preparations appeared to be indirect, i.e. secondary to an influx of Ca^{2+} through other stretch-activated channels or to possible release of Ca^{2+} from intracellular stores. Our studies suggest that this does not appear to be the case for the channel in pulmonary artery.

Direct fatty acid activation of Ca^{2+}-activated K^+ channels has been reported for channels of this type in two other types of vascular smooth muscle cells of mammalian origin [24–26] suggesting that this phenomenon might be quite widespread among vascular smooth muscles. However, in the case of at least one of these, cultured human aorta, the time course of onset and recovery from the effects of fatty acids seems to be prolonged compared to the results from the smooth muscle cells studied here [24,25]. In toad stomach smooth muscle cells, fatty acids activate another different type of K^+ channel (smaller conductance and relatively insensitive to membrane potential) [7].

That this large-conductance K^+ channel could be activated by four different types of stimuli is a new finding. It remains to be determined whether these stimuli indeed act independently of one another on different parts of the channel molecule. It is possible that some interaction between these stimuli might occur. For example, membrane stretch could affect channel activity by activating a membrane bound phospholipase, producing free fatty acids, which could, in turn, activate the channel.

4.1. *Role in vascular smooth muscle function*

Activation of these large conductance K^+ channels under physiological conditions will produce outward, hyperpolarizing, currents in the smooth muscle cells, which would tend to inhibit contraction involving voltage sensitive mechanisms. When the membrane potential is at normal resting levels and the intracellular Ca^{2+} concentration low, as in a relaxed cell, we would not expect to see significant stretch or fatty acid activation of these channels. We would, however, expect to see activation in cells which exhibited some degree of contraction. A somewhat paradoxical role for stretch which depends on the contractile state of the cell is suggested. Stretch can induce contraction in relaxed cells (perhaps via cationic stretch-activated channels); however, when a cell is already somewhat contracted further excitation due to stretch may be countered by the stretch-activation of these large conductance K^+ channels. Thus, these channels may have a protective role in limiting additional myogenic activation when a high degree of basal tone is present, thereby assuring that arteries remain patent.

The vasoactive effects of arachidonic acid appear to be complex, and can depend on a variety of factors including the particular preparation, the presence or absence of an intact endothelium, and level of basal tone [8,27]. In several arterial preparations, in the presence and absence of endothelium, with some adrenergically induced basal tone, arachidonic acid application induced relaxation [27]. Such relaxation may, at least in part, be due to a direct effect of the fatty acid on large conductance K^+ channels. (This of course does not preclude further vasoactive effects of arachidonic acid metabolites.) In addition, fatty acid activation of this class of channels may have relevance in conditions such as ischemia where circulating levels of fatty acids are elevated [28,29].

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REFERENCES

[1] Guharay, F. and Sachs, F. (1984) J. Physiol. 352, 685–701.
[2] Morris, C.E. (1990) J. Membr. Biol. 113, 93–107.
[3] Kirber, M.T., Walsh Jr., J.V. and Singer, J.J. (1988) Pflügers Arch. 412, 339–345.
[4] Kirber, M.T., Clapp, L.H., Gurney, A.M., Walsh Jr., J.V. and Singer, J.J. (1989) J. Gen. Physiol. 94, 37a–38a.
[5] Ordway, R.W., Singer, J.J. and Walsh Jr., J.V. (1991) Trends Neurosci. 14, 96–100.
[6] Needleman, P., Turk, J., Jakschik, B.A., Morrison, A.R. and Lefkowith, J.B. (1986) Annu. Rev. Biochem. 55, 60–102.
[7] Ordway, R.W., Walsh Jr., J.V. and Singer, J.J. (1989) Science 244, 1176–1179.
[8] Singer, H.A. and Peach, M.J. (1983) J. Pharmacol. Exp. Ther. 226, 790–795.
[9] J. Hypertens. Suppl. (1989), 7 (Sept. suppl. 4), s1–s173.
[10] Ordway, R.W., Clapp, Gurney, A.M., L.H., Singer, J.J. and Walsh Jr., J.V. (1989) J. Gen. Physiol. 94, 37a.
[11] Ordway, R.W., Kirber, M.T., Clapp, L.H., Walsh Jr., J.V. and Singer, J.J. (1990) Biophys. J. 57, 310a.
[12] Clapp, L.H. and Gurney, A.M. (1991) Pflügers Arch. 418, 462–470.
[13] Vivaudou, M.B., Singer, J.J. and Walsh Jr., J.V. (1986) Pflügers Arch. 407, 355–364.
[14] Lassignal, N.L., Singer, J.J. and Walsh Jr., J.V. (1986) Am. J. Physiol. 250 (Cell Physiol. 19), C792–C798.
[15] Latorre, R., Oberhauser, A., Labarca, P. and Alvarez, O. (1989) Annu. Rev. Physiol. 51, 385–399.
[16] Singer, J.J. and Walsh Jr., J.V. (1987) Pflügers Arch. 408, 98–111.
[17] Christensen, O. (1987) Nature 350, 66–68.
[18] Taniguchi, J. and Guggino, W.B. (1989) Am. J. Physiol. 257 (Renal Fluid Electrolyte Physiol. 26), F347–F352.
[19] Davidson, R.M., Tatakis, D.W. and Auerbach, A.L. (1990) Pflügers Arch. 416, 646–651.
[20] Dubé, L., Parent, L. and Sauvé, L. (1990) Am. J. Physiol. 259 (Renal Fluid Electrolyte Physiol. 28), F348–F356.
[21] Filipovic, D. and Sackin, H. (1991) Am. J. Physiol. 260 (Renal Fluid Electrolyte Physiol. 29), F119–F129.
[22] Kawahara, K., Ogawa, A. and Suzuki, M. (1991) Am. J. Physiol. 260 (Renal Fluid Electrolyte Physiol. 29), F27–F33.
[23] Ubl, J., Murer, H. and Kolb, H.A. (1988) Pflügers Arch. 412, 551–553.
[24] Bregestovski, P.D., Bolotina, V.M. and Serebryakov, V.N. (1989) Proc. R. Soc. London Ser. B 237, 259–266.
[25] Bolotina, V., Omelyanenko, V., Heyes, B., Ryan, U. and Bregestovski, P. (1989) Pflügers Archv. 415, 262–268.
[26] Katz, G., Roy-Contancin, L., Bale, T. and Reuben, J.P. (1990) Biophys. J. 57, 506a.
[27] De Mey, J.G. and Vanhoutte, P.M. (1982) Circ. Res. 51, 439–447.
[28] Bazan Jr., N.G. (1970) Biochim. Biophys. Acta 218, 1–10.
[29] Katz, A.M. and Messineo, F.C. (1981) Circ. Res. 48, 1–16.