Regulation of Mesangial Cell Ion Channels by Insulin and Angiotensin II

Possible Role in Diabetic Glomerular Hyperfiltration

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Abstract

We used patch clamp methodology to investigate how glomerular mesangial cells (GMC) depolarize, thus stimulating voltage-dependent Ca^{2+} channels and GMC contraction. In rat GMC cultures grown in 100 mU/ml insulin, 12% of cell-attached patches contained a Ca^{2+}-dependent, 4-picosiemens Cl^- channel. Basal NP_o (number of channels times open probability) was < 0.1 at resting membrane potential. Acute application of 1–100 nM angiotensin II (AII) or 0.25 μM thapsigargin (to release $[Ca^{2+}]_i$ stores) increased NP_o. In GMC grown without insulin, Cl^- channels were rare (4%) and unresponsive to AII or thapsigargin in cell-attached patches, and less sensitive to $[Ca^{2+}]_i$ in excised patches. GMC also contained 27-pS nonselective cation channels (NSCC) stimulated by AII, thapsigargin, or $[Ca^{2+}]_i$, but again only when insulin was present. In GMC grown without insulin, 15 min of insulin exposure increased NP_o (insulin ≥ 100 μU/ml) and restored AII and $[Ca^{2+}]_i$ responsiveness (insulin ≥ 1 μU/ml) to both Cl^- and NSCC. GMC AII receptor binding studies showed a B_{max} (binding sites) of 2.44±0.58 fmol/mg protein and a K_d (binding dissociation constant) of 3.02±2.01 nM in the absence of insulin. B_{max} increased by 86% and K_d was unchanged after chronic (days) insulin exposure. In contrast, neither K_d nor B_{max} was significantly affected by acute (15-min) exposure. Therefore, we concluded that: (*a*) rat GMC cultures contain Ca^{2+}-dependent Cl^- and NSCC, both stimulated by AII. (*b*) Cl^- efflux and cation influx, respectively, would promote GMC depolarization, leading to voltage-dependent Ca^{2+} channel activation and GMC contraction. (*c*) Responsiveness of Cl^- and NSCC to AII is dependent on insulin exposure; AII receptor density increases with chronic, but not acute insulin, and channel sensitivity to $[Ca^{2+}]_i$ increases with both acute and chronic insulin. (*d*) Decreased GMC contractility may contribute to the glomerular hyperfiltration seen in insulinopenic or insulin-resistant diabetic patients. (*J. Clin. Invest.* 1993. 92:2141–2151.) Key words: patch clamp • Cl^- channel • nonselective cation channel • Ca^{2+} channel • diabetes mellitus

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Introduction

The glomerular filtration barrier consists of three layers: capillary endothelial cells, basement membrane, and Bowman's capsular epithelial cells. However, a third resident cell type found in the glomerular tuft between and within capillary loops also plays an integral role in filtration (1). These are glomerular mesangial cells (GMC),[1] which phenotypically resemble smooth muscle cells and contain large numbers of myofilaments. In mesangial cells, hormonal and intracellular signaling pathways play an important role in initiating normal physiological and pathologic responses by changing both contractile and growth properties, and thereby altering glomerular filtration.

Mesangial cell contraction depends on membrane depolarization stimulating voltage-dependent Ca^{2+} channels (2–5). In vascular smooth muscle cells, this depolarization process involves Cl^- efflux through Ca^{2+}-dependent Cl^- channels and cation influx through nonselective cation channels (6–8). Several groups have indirect evidence that mesangial cell depolarization induced by vasoactive peptides (e.g., angiotensin II [AII], vasopressin, endothelin-1, platelet activating factor) depends on activation of a Ca^{2+}-dependent Cl^- conductance (9–11). However, specific Cl^- conductances at a single channel level have not been identified in GMC.

GMC contraction in response to vasoactive peptides has been shown to be dependent on the presence of exogenous insulin (12, 13). The physiologic relevance of the latter observation is that decreased GMC contractility has been proposed to contribute to the increased glomerular filtration rate ("hyperfiltration") present in insulinopenic or insulin-resistant diabetic patients (13).

In this study we used patch clamp technology to characterize ion channels capable of mediating membrane depolarization of cultured rat glomerular mesangial cells. The influence of exogenous insulin and the vasoactive peptide, AII, on the regulation of ion channels was also examined. Finally, AII receptor binding studies were performed under various conditions of exogenous insulin exposure.

Methods

Preparation of rat GMC cultures. GMC cultures were established and maintained using previously described methods (14, 15). Briefly, renal cortices from male Sprague-Dawley rats (75–150 g) were dissected. Mesangial cell–enriched glomerular cores were isolated from cortical tissue by differential sieving and incubation for 45–60 min with collagenase (1,200 U/ml) in Ca^{2+}/Mg^{2+}–free Hank's balanced salt solution (Irvine Scientific, Santa Ana, CA). The GMC suspension was washed and plated in RPMI 1640 supplemented with 17% (vol/vol) fetal bo-

1. *Abbreviations used in this paper:* AII, angiotensin II; GMC, glomerular mesangial cells; IP_3, inositol-1,4,5-trisphosphate.

vine serum, 2 mM glutamine, selenium (5 ng/ml), antibiotics (penicillin, 100 U/ml; streptomycin sulfate, 100 μg/ml; amphotericin B, 2 μg/ml), and bovine insulin (100 mU/ml) at 37°C in 5% CO_2/95% air. The RPMI 1640 contained 11 mM D-glucose.

Exogenous insulin exposure to mesangial cell cultures. Mesangial cell outgrowth was usually observed by day 10, and cells reached confluency by day 21, at which time they were trypsinized and subcultured. For chronic insulin exposure experiments, the subcultures were then grown with or without (insulin-deficient GMC cultures) bovine insulin (100 mU/ml) added to the RPMI 1640. For acute insulin exposure experiments, insulin-deficient GMC cultures were exposed to various concentrations of bovine insulin (1 μU/ml, 10 μU/ml, 100 μU/ml, and 100 mU/ml) in the extracellular bath for 15 min immediately before patching. Mesangial cell passages 5–7 were grown on glass coverslips for patch clamp experiments.

Patch clamp recording and analysis. Mesangial cells were visualized with Hoffman modulation optics mounted on a Diaphot-TMD inverted microscope (Nikon Inc., Instr. Group, Melville, NY). Patch pipettes are fabricated from Microhematocrit (blue coded tip) capillary tubes (Fisher Scientific, Pittsburgh, PA) and positioned with a motorized micromanipulator system (Newport Corp., Irvine, CA) as previously described (16). All experiments were conducted at 37°C using a temperature controller and open perfusion micro-incubator (TC-202 and PDMI-2; Medical Sys. Corp., Greenvale, NY). Unitary channel events were obtained using a List patch clamp (EPC-7; Medical Sys. Corp.), digitized by a pulse code modulator (DAS 601; Dagan Corp., Minneapolis, MN), and recorded on a video cassette recorder (SL-HF860D; Sony Corp. of America, Park Ridge, NJ). Data were acquired using a eight-pole Bessel filter (902LPF; Frequency Devices Inc., Haverhill, MA), acquisition hardware and Axotape software (TL-2; Axon Instrs. Inc., Foster City, CA), and a computer (486SX; Mitsuba Southeast, Inc., Norcross, GA) (corner frequency = 1 KHz; sampled at 200 μs/point).

Patch pipettes contained a physiologic saline solution of: (mM) 140 NaCl (final NaCl concentration after titration to pH 7.4 with NaOH), 5 KCl, 1 $CaCl_2$, 1 $MgCl_2$, and 10 Hepes. The extracellular bath solution for cell-attached patches was the same as the patch pipette solution above. The "cytoplasmic" solution for most excised inside-out patches approximated intracellular composition of: (mM) 140 KCl (final KCl concentration after titration to pH 7.4 with KOH), 5 NaCl, 1 $MgCl_2$, 0.001 $CaCl_2$, 2 EGTA, and 10 Hepes (Table I, solution A). For cytoplasmic Ca^{2+} exchange experiments, a computer program using known stability constants calculated the amount of Ca^{2+} needed to vary the final free ionized Ca^{2+} concentration in solution A between 10^{-8} and 10^{-4} M (17).

The convention for applied voltage to the membrane patch ($-V_{pipet}$) represents the voltage deflection from the patch potential (i.e., the resting membrane potential for cell-attached patches; 0 mV for inside-out patches) and is expressed as the potential of the cell interior with respect to the patch pipette interior (i.e., negative values = hyperpolarization; positive values = depolarization). Inward current (pipette to cell) is represented as downward transitions in single channel records. Analysis of data was performed on a computer (486SX; Mitsuba Southeast Inc., Norcross, GA) using locally and commercially developed software.

The total number of functional channels (N) in the patch are estimated by observing the number of peaks detected on current amplitude histograms. As a measure of channel activity, NP_o (number of channels times the open probability) is calculated (18).

$$NP_o = \sum_{n=0}^{N} \frac{n \cdot t_n}{T}, \tag{1}$$

where T is the total record time, n is the number of channels open, and t_n is the record time during which n channels are open. Therefore, NP_o can be calculated without making assumptions about the total number of channels in a patch or the open probability of a single channel. The probability that any one channel is open (P_o) is calculated from the expression (16):

$$P_o = (\sum_{n=1}^{N} P_n)/N, \tag{2}$$

where P_n is, the probability that n channels are open, calculated as the amount of time in the open state divided by the total record time for each unitary current level. Summation of P_n's for each level are then divided by N. The assumptions for this calculation are that the channels function independently and identically, and that n channels are open when the current is between $(n - ½)i$ and $(n + ½)i$, where i is the unit current.

Relative ion permeability ratios for GMC channels were calculated using a modification of the Goldman-Hodgkin-Katz equation (given below).

$$E_{rev} = \frac{RT}{F} ln \frac{P_K[K]_o + P_{Na}[Na]_o + P_{Cl}[Cl]_i}{P_K[K]_i + P_{Na}[Na]_i + P_{Cl}[Cl]_o}, \text{ where} \tag{3}$$

$[K]_o$, $[Na]_o$, and $[Cl]_o$ are the concentration of these ions on the outside surface of the apical membrane (pipette solution); $[K]_i$, $[Na]_i$, and $[Cl]_i$ are the concentrations on the inner surface (cytoplasmic bath solution); and P_K, P_{Na}, and P_{Cl} are the relative ion permeabilities.

Statistics. Experiments in the cell-attached or excised inside-out patch configuration were conducted in a paired fashion; data from each patch membrane served as the control for an experimental manipulation. Data are reported as mean NP_o or P_o values±1 SD.

The average change in NP_o or P_o for a group of patches, compared before and after an experimental manipulation, was also analyzed using the paired t test (19):

$$t = \frac{\bar{x} - \mu}{s/\sqrt{n}}, \tag{4}$$

where $\bar{x}$ is the average change in NP_o or P_o, μ (hypothesis that $\bar{x}$ will be different from zero) = 0, s is the SD for $\bar{x}$, and n is the number of patches. Significance was $P < 0.05$. This approach reduces the variability in the observations due to differences in ion channel activity between individual patches and yields a more sensitive test than comparing the mean NP_o or P_o responses (19).

AII receptor binding assay. AII receptor binding assays were performed as previously described (20–23). Rat GMC were grown to ~ 80–90% confluency in 24-well plates (Falcon 3047; Fisher Scientific) under the same exogenous insulin exposure conditions described above. GMC were washed twice with 0.4 ml of ice-cold binding buffer containing 50 mM Tris-HCl, 100 mM NaCl, 5 mM $MgCl_2$, and 0.2% BSA, pH 7.4. The cells were then incubated in the above buffer with ^{125}I-AII at varying concentrations for 90 min at 4°C to prevent receptor internalization and achieve saturation binding conditions. Incubations were performed with or without unlabeled AII added in 1,000-fold excess of labeled AII. To terminate AII binding and remove the unbound AII, the GMC were washed rapidly four times with 0.4 ml of ice-cold binding buffer. Solubilization of GMC was accomplished using 0.25 ml of cell lysis buffer containing 0.1% SDS and 0.1 N NaOH. Specific AII binding equaled total binding (^{125}I-AII, 0.1–10 nM; sp act, 2,000 Ci/mmol) minus nonspecific binding (unlabeled AII). Bradford protein analysis (Bio-Rad Laboratories, Richmond, CA) was performed on 50-μl aliquots of the solubilized GMC. AII receptor binding dissociation constants (K_d) and AII receptor binding sites (B_{max}) were calculated as previously described (20–23).

Chemicals. Insulin, AII, and thapsigargin (Sigma Chemical Co., St. Louis, MO) were of the highest commercial grade available. ^{125}I-AII was purchased from Amersham Corp. (Arlington Heights, IL).

Results

Mesangial cells contain low-conductance, Ca^{2+}-activated Cl^- channels. Several groups have presented indirect evidence that depolarization of GMC in response to vasoactive peptides is dependent on activation of a Ca^{2+}-dependent Cl^- conductance (9–11). However, identification of a Cl^- conductance with the

appropriate characteristics has not been accomplished at a single channel level in GMC.

In 10 of 81 (12%) successful cell-attached patches (pipette, 140 mM NaCl) on cultured rat GMC grown in the presence of insulin (100 mU/ml), inward current with a unitary conductance of 2–5 picosiemens (pS) (mean g = 3.6±1.1 pS) was identified (Figs. 1 and 2). At resting membrane potential ($-V_{pipet}$ = 0 mV), NP_o (number of channels · open probability) was always low (mean NP_o = 0.05±0.04) in the cell-attached configuration (n = 10). No significant voltage dependence was detected for NP_o between $-V_{pipet}$ of −80 and +80 mV. The current–voltage (I-V) relationship revealed slight outward rectification and the reversal potential (E_{rev}) was near 0 mV.

To investigate the 4-pS channel's ion selectivity, excised inside-out patches were studied (Fig. 2 *B*). Results suggested a channel that was either selective for Cl^- or nonselective for cations, E_{rev} was again ~ 0 mV with pipettes containing 140 mM NaCl and "cytoplasmic" bath containing 140 mM KCl (Table I, solution A) (n = 6). A small increase in inward current conductance (4.2±0.2 pS) and amplitude was observed after patch excision into cytoplasmic bath solution A with 10^{-6} M Ca^{2+}. Progressively replacing cytoplasmic bath K^+ with Na^+ (solutions A-C) did not shift the I-V curve, suggesting this channel was equally permeable to K^+ and Na^+. However, raising the cytoplasmic bath Cl^- concentration from 12 mM Cl^- (solution D) to 242 mM Cl^- (solution E) shifted the I-V curve positively (E_{rev} depolarized from −31 to +15 mV). If this channel were perfectly selective for Cl^-, the expected E_{rev} under these ionic conditions would have shifted from −34 to +13 mV. Thus, the selectivity of this channel is higher for Cl^- than for Na^+ or K^+. Assuming there is no significant permeability to gluconate, from Eq. 3 it can be calculated that the permeability to Cl^- relative to Na^+ was > 50 for this 4-pS channel.

At resting membrane potential, acute application of 100 nM AII to the extracellular bath outside the cell-attached patch pipette abruptly increased mean NP_o (0.28±0.13) for the 4-pS Cl^- channel (n = 5). Fig. 3 is a single-channel record showing Cl^- channel activation by AII. Comparing data obtained from each patch before adding AII, the average change in NP_o after AII was significant by paired t test (see Methods) (Fig. 4). Cl^-

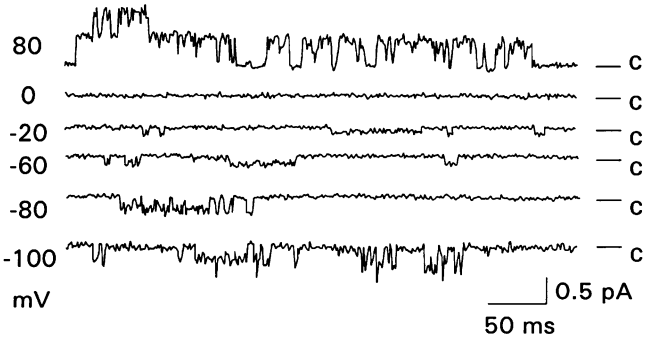

Figure 1. Single low-conductance Cl^- channel events. Cell-attached patch depicts inward current (outward Cl^- movement) as downward deflections. Horizontal bars mark the zero current level (*C*, closed state). Voltage (mV) represents the applied patch pipette voltage ($-V_{pipet}$) displacement away from resting membrane potential (see Methods). Each trace was recorded at a corner frequency (Fc) of 1 KHz, sampled at 5 KHz (200 μs/point), and depicted without software filtering.

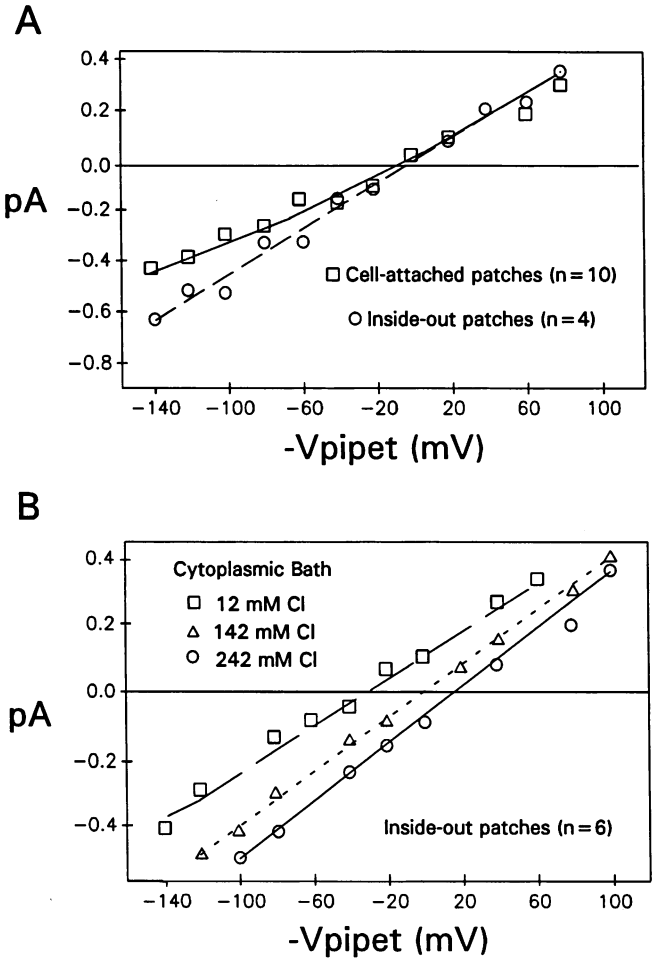

Figure 2. Current–voltage (I-V) relationship for low conductance Cl^- channel. (*A*) Cell-attached patches (*squares*) and inside-out patches excised into a 140-mM KCl, 10^{-7} M $CaCl_2$ (solution A; *circles*) cytoplasmic bath reveal slight outward rectification. Unit conductance was calculated from the I-V curve slope near resting membrane potential ($-V_{pipet}$ = 0 mV). (*B*) Cytoplasmic ion replacement experiments (mean current amplitude for six inside-out patches): I-V curves with cytoplasmic bath 12 mM Cl^- (solution D; *squares*), 142 mM Cl^- (solution A; *triangles*), and 242 mM Cl^- (solution E; *circles*) show reversal potentials (E_{rev}), indicating a P_{Cl}/P_{Na} ratio > 50:1. Cytoplasmic Ca^{2+} was 10^{-6} M for all ion replacement experiments.

Table I. Solution Composition for Patch Clamp Experiments

Solution	A	B	C	D	E	F
NaCl	5	50	95	10	240	14
KCl	140	95	50	0	0	0
$MgCl_2$	1	1	1	1	1	1
$CaCl_2$	10^{-8}–10^{-4}	10^{-6}	10^{-6}	10^{-6}	10^{-6}	10^{-6}
EGTA	2	2	2	2	2	0
Na gluconate	0	0	0	124	0	0
Mannitol	0	0	0	0	0	240
Hepes	10	10	10	10	10	10
pH	7.4	7.4	7.4	7.4	7.4	7.4

Data shown are millimolar concentrations, except for $CaCl_2$ which is reported as the final free Ca^{2+} molar concentration. NaCl and KCl are final concentrations after titration of pH with NaOH or KOH.

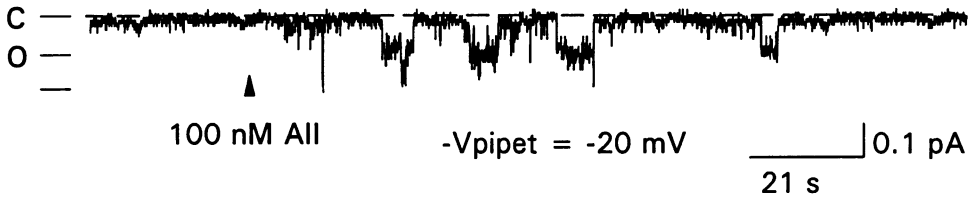

Figure 3. Low conductance Cl^- channel activation by AII. 100 nM AII was slowly added to the extracellular bath outside the cell-attached patch pipette over 15 s to prevent disruption of the membrane seal. Recording of 4-pS Cl^- channel at $-V_{pipet} = -20$ mV depicts activation of inward current events within seconds of AII exposure. Fc = 1 KHz; sampling = 5 KHz; and software filtering = 400 Hz.

channel activation was also observed at AII doses of 1 nM (n = 4) and 10 nM (n = 4).

Hydrolysis of mesangial cell membrane phospholipids, initiated by vasoactive peptides including AII, is associated with release of inositol-1,4,5-trisphosphate (IP_3)-sensitive intracellular Ca^{2+} pools (1, 24). This effect can be mimicked by thapsigargin, which releases Ca^{2+} from intracellular pools without hydrolysis of inositol polyphosphates (16, 25, 26). Acute exposure to 0.25 μM thapsigargin in the extracellular bath also increased Cl^- channel activity (n = 4) (Fig. 4). The average change in NP_o was again significant. In the excised inside-out patch configuration, directly raising the free "cytoplasmic" Ca^{2+} concentration from 10^{-8} to 10^{-4} M increased P_o by ~ 10-fold and confirmed this was a Ca^{2+}-dependent Cl^- channel (n = 6) (Fig. 5, *triangles*).

Mesangial cells contain Ca^{2+}-activated nonselective cation channels. A second channel type with a unitary conductance of 25–29 pS (mean g = 27.4±1.9 pS) and a linear I-V relationship was identified in 16 of 81 (20%) successful cell-attached patches (pipette, 140 mM NaCl) on rat GMC grown in the presence of insulin (100 mU/ml) (Figs. 6 and 7). In the cell-attached configuration, this channel was rarely open at resting membrane potential (mean NP_o = 0.03±0.05). NP_o was insensitive to either membrane depolarization or hyperpolarization ($-V_{pipet}$ between −120 and +120 mV). The E_{rev} in the cell-attached configuration was close to 0 mV.

When excised inside-out patches (pipette, 140 mM NaCl) were exposed to a cytoplasmic bath containing 140 mM KCl (solution A), the E_{rev} was also very near 0 mV (n = 5) (Fig. 7 *B*). Unitary conductance did not change with patch excision nor did E_{rev} change appreciably with progressive replacement of K^+ for Na^+ in the cytoplasmic bath (solutions A–C). In anion exchange experiments, the cytoplasmic bath was switched from 147 mM Cl^- (solution A) to 124 mM gluconate (solution D), but there was little change in E_{rev} (+1.2±2.0). However, exchanging both intracellular cations and anions for the nondiffusible osmole, mannitol (solution F), shifted the E_{rev} (+43.1±4.2 mV) toward E_{Na} (+58 mV). The last two experiments indicate that P_{Cl}/P_{Na} for this channel is only ~ 0.1:1. When monovalent cations were replaced with divalent cations in the pipette solution (pipette, 110 mM $CaCl_2$, 10 mM glucose, 10 mM Hepes, pH 7.4), inward current channel events (ie., Ca^{2+} influx) could not be distinguished (n = 4). In contrast to the 4-pS Cl^- channel, cytoplasmic ion substitution

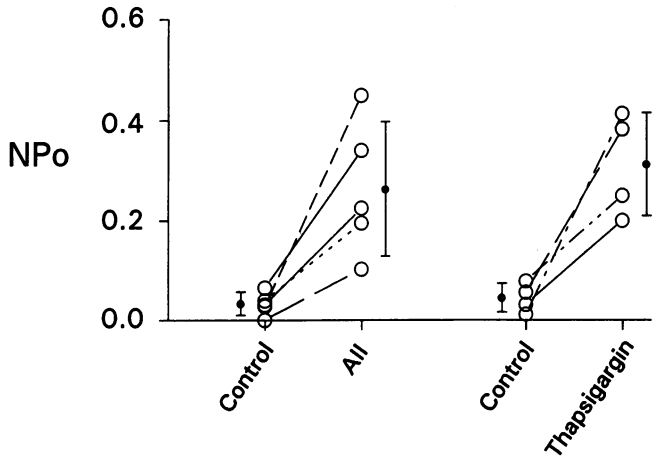

Figure 4. Activation of Cl^- channels by AII is mimicked by release of intracellular Ca^{2+} stores. (*Left*) Cl^- channel activity, NP_o (number of channels × the open probability), is depicted before and after 100 nM AII exposure for cell-attached patches at $-V_{pipet} = -20$ mV. Mean NP_o increased from 0.033±0.023 to 0.28±0.13 (n = 5). (*Right*) Cl^- channel activity is depicted before and after 0.25 μM thapsigargin exposure for cell-attached patches at $-V_{pipet} = -20$ mV. Mean NP_o increased from 0.045±0.029 to 0.31±0.10 (n = 4). Control NP_o was calculated for the 3-min recording period just before AII or thapsigargin exposure. AII or thapsigargin were added to the extracellular bath outside the cell-attached patch pipettes over 15 s to prevent disruption of the membrane seal. NP_o's were then calculated for 1–2-min recordings immediately after exposure. Symbols connected by lines represent relative change in channel activity for the same cell-attached patch.

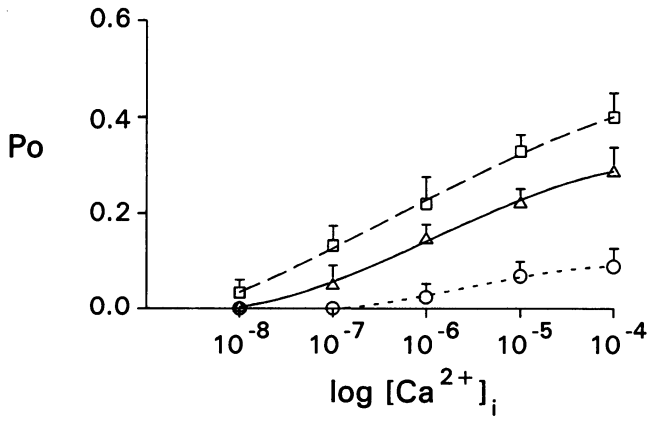

Figure 5. Intracellular Ca^{2+} activates Cl^- channel. Cl^- channel activity (mean P_o's from six inside-out patches) is plotted with increasing free Ca^{2+} concentrations (10^{-8}–10^{-4} M; see Methods) bathing the cytoplasmic surface of the excised patch membrane. Plots are for cultured GMC grown with insulin (*triangles; n* = 6), insulin-deficient GMC cultures (*circles; n* = 3), and insulin-deficient GMC cultures after acute insulin exposure (*squares; n* = 8). Since the Ca^{2+} activation curves were similar for low- and high-dose acute insulin exposure, the data are combined.

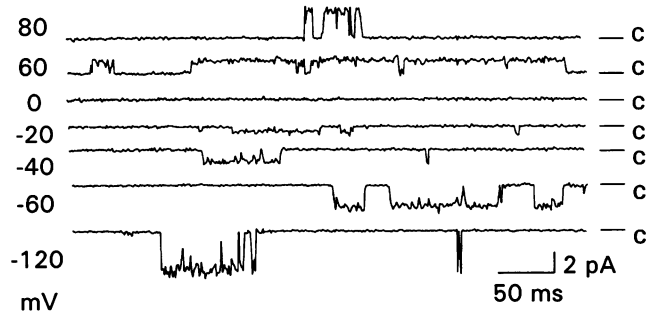

Figure 6. Single nonselective channel events. Cell-attached recording shows inward current events at $-V_{pipet} < 0$ mV. Fc = 1 KHz; sampling = 5 KHz; and no software filtering.

experiments revealed that the 27-pS channel was nonselective for Na^+ over K^+, but relatively impermeable to Cl^-.

Matsunaga et al. (15) have previously described a 25-pS nonselective cation channel (NSCC) in cultured rat GMC that was activated by vasoactive peptides (vasopressin or AII), or by raising cytoplasmic Ca^{2+}. Similarly, when we exposed cultured GMC to AII (100 nM) in the extracellular bath, a significant increase in NSCC activity (mean NP_o = 0.20±0.12) was observed in cell-attached patches (n = 6) (Fig. 8). NSCC activation was also observed at AII doses of 1 nM (n = 3) and 10 nM (n = 5). Releasing intracellular Ca^{2+} pools with 0.25 μM thapsigargin also caused NSCC activation (mean NP_o = 0.20±0.06) in cell-attached patches (n = 4). Confirming the Ca^{2+} dependency of this NSCC, direct exposure of the cytoplasmic surface of inside-out patches to progressively higher Ca^{2+} concentrations (10^{-8}–10^{-4} M) also increased P_o (n = 5) (Fig. 9, *triangles*).

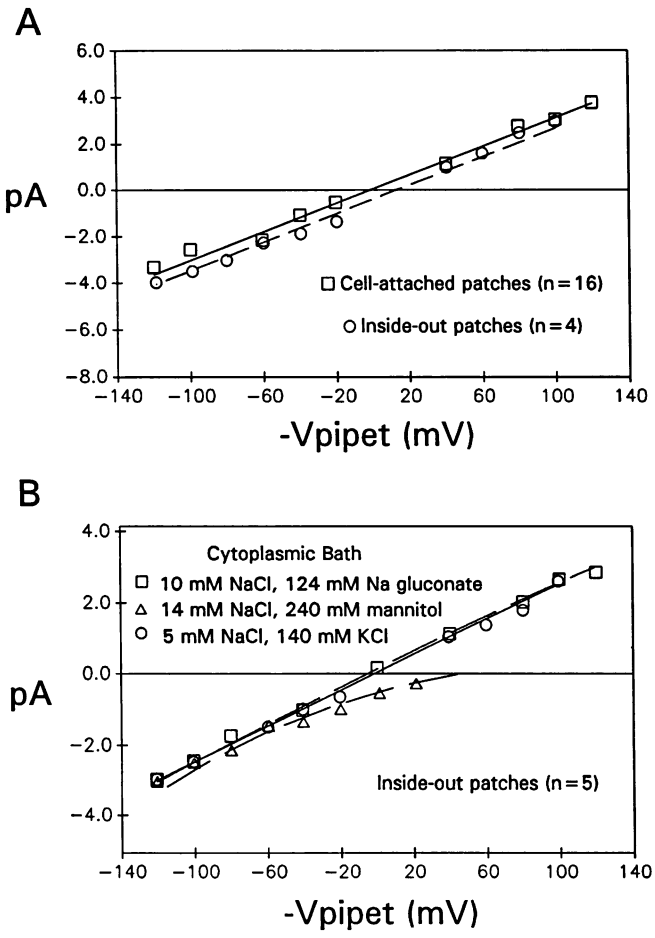

Figure 7. I-V relationship for nonselective cation channel. (*A*) Cell-attached (*squares*) and inside-out configurations with 140 mM bath KCl (solution A; *circles*) reveal a channel that is relatively nonselective for Na^+ over K^+ ions ($E_{rev} \sim 0$ mV). Linear regression analysis reveals a unitary conductance of 27.4±1.9 pS. (*B*) Cytoplasmic ion replacement experiments (mean current amplitude for five inside-out patches): I-V curve with cytoplasmic bath containing 5 mM NaCl/140 mM KCl (solution A; *circles*) is depicted. Replacement of bath KCL with 124 mM Na gluconate (solution D; *squares*) did not significantly alter E_{rev}. Replacement with 240 mM mannitol (solution F; *triangles*) shifted E_{rev} positively. Calculated $P_{Cl}/P_{Na} \sim 0.1{:}1$. Cytoplasmic Ca^{2+} was 10^{-6} M for all ion replacement experiments.

Cl^- and nonselective cation channel activation is dependent on exogenous insulin. In cultured rat GMC grown in the absence of insulin (insulin-deficient GMC cultures), the 4-pS Cl^- channel was observed in only 4% (3/74) of cell-attached patches. Moreover, there was no stimulation of channel activity in 3 patches with or 20 patches without baseline Cl^- channel activity when 100 nM AII was added to the extracellular bath. 0.25 μM thapsigargin also failed to activate the Cl^- channel.

Insulin-deficient GMC cultures were then exposed to various levels of insulin (1 μU/ml, 10 μU/ml, 100 μU/ml, and 100 mU/ml) for 15 min before patching. Acute exposure to insulin concentrations of 1 or 10 μU/ml (low dose) did not change Cl^- channel activity in cell-attached patches when compared with insulin-deficient GMC cultures (n = 7) (Fig. 10, *right*). However, after acute insulin exposure, 100 nM AII stimulated Cl^- channel activity in six of seven patches. When compared with insulin-deficient cultures, acute exposure to 100 μU/ml or 100 mU/ml insulin (high dose) alone increased baseline inward current NP_o and amplitude for the 4-pS Cl^- channel (n = 10) (Fig. 10, *left*). In six experiments, high-resistance patch seals were stable enough to measure the stimulatory effects of high-dose insulin on the 4-pS Cl^- channel in the same patch for > 15 min (Fig. 11). Within 5 min of applying

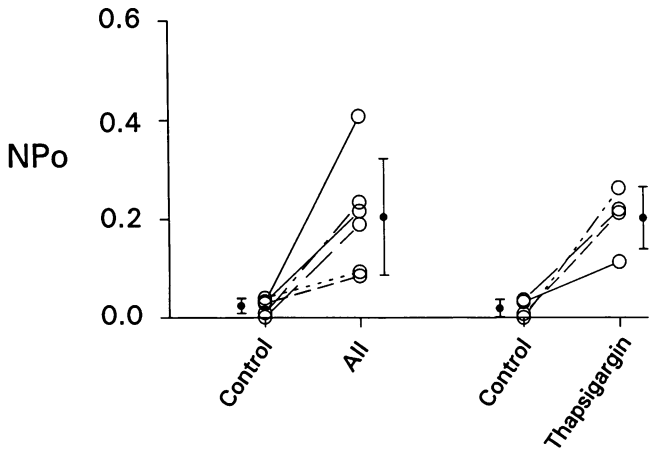

Figure 8. Activation of NSCC by AII is mimicked by release of intracellular Ca^{2+} stores. (*Left*) NSCC activity: NP_o is depicted before and after 100 nM AII exposure for cell-attached patches at $-V_{pipet}$ = −20 mV. Mean NP_o increased from 0.024±0.015 to 0.20±0.12 (n = 6). (*Right*) NSCC activity: NP_o is depicted before and after 0.25 μM thapsigargin exposure for cell-attached patches at $-V_{pipet}$ = −20 mV. Mean NP_o increased from 0.019±0.018 to 0.20±0.06 (n = 4). NP_o's were calculated as in Fig. 4.

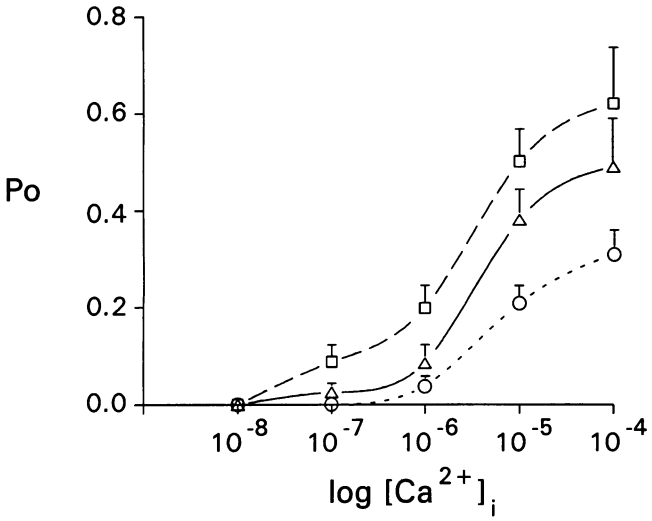

Figure 9. Intracellular Ca^{2+} activates NSCC. NSCC activity (mean P_o's from six inside-out patches) is plotted with increasing free Ca^{2+} concentrations (10^{-8}–10^{-4} M; see Methods) bathing the cytoplasmic surface of the excised patch membrane. Plots represent cultured GMC grown in insulin (*triangles; n* = 5) and insulin-deficient GMC cultures (*circles; n* = 4), and insulin-deficient GMC cultures exposed to high- or low-dose acute insulin (*squares; n* = 8).

100 μU/ml insulin to the extracellular bath, an increase in Cl^- channel NP_o was observed. An additional stimulation of Cl^- channel activity was observed within seconds of adding 100

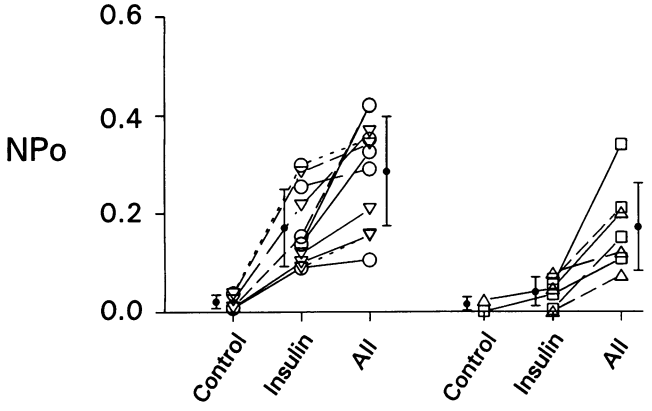

Figure 10. Effect of acute insulin exposure on Cl^- channel. Cultured GMC were grown in the absence of insulin and then acutely exposed to exogenous insulin at various concentrations. (*Left*) High-dose insulin exposure (*inverted triangles,* 100 μU/ml insulin; *circles,* 100 mU/ml insulin). Cl^- channel activity: NP_o is depicted before and 15 min after high-dose insulin exposure for cell-attached patches at $-V_{pipet} = -20$ mV. Mean NP_o increased from 0.021±0.014 to 0.17±0.08. After 15 min of high-dose insulin exposure, 100 nM AII was added to the extracellular bath. An additional increase in mean NP_o to 0.29±0.11 was observed (*Right*) Low-dose insulin exposure (*triangles,* 10 μU/ml insulin; *squares,* 1 μU/ml insulin). Scant Cl^- channel activity is observed before or 15 min after low-dose insulin exposure for cell-attached patches at $-V_{pipet} = -20$ mV. NP_o was unaffected by 15-min exposure to low-dose insulin, but subsequent addition of 100 nM AII to the extracellular bath increased mean NP_o to 0.17±0.09. Control NP_o was calculated for the 3-min recording period just before acute insulin exposure. NP_o's were then calculated for 1–2 min of recording 15 min after insulin exposure and immediately after AII exposure. Symbols connected by lines represent relative change in channel activity for the same cell-attached patch.

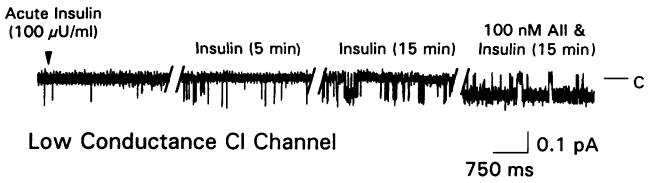

Figure 11. Effect of high-dose insulin exposure on Cl^- channel. Single channel recordings from the same cell-attached patch at $-V_{pipet} = -20$ mV. Progressive increase in Cl^- channel activity is observed after 5- and 15-min exposure to 100 μU/ml insulin. A further increase in channel activity is seen with the subsequent addition of 100 nM AII. Fc = 1 KHz; sampling = 5 KHz; and no software filtering.

nM AII. Restoration of Cl^- channel sensitivity to AII in cell-attached patches was observed with all acute insulin concentrations (1 μU/ml, 10 μU/ml, 100 μU/ml, and 100 mU/ml).

The Ca^{2+} activation curve for the 4-pS Cl^- channel in excised patches shifted to the left when comparing cultured GMC grown with insulin (Fig. 5, *triangles; n* = 6) with insulin-deficient GMC cultures (Fig. 5, *circles; n* = 3). A further leftward shift was observed for excised patches from insulin-deficient GMC cultures exposed to insulin acutely (Fig. 5, *squares; n* = 8). Since the Ca^{2+} activation curves were similar for low- and high-dose acute insulin exposure, the data are combined in Fig. 5.

The 27-pS NSCC was observed in 11 of 74 (15%) cell-attached patches obtained in insulin-deficient GMC cultures. Usually closed at $-V_{pipet} = 0$ mV, NP_o for this NSCC was not altered by adding 100 nM AII and 0.25 μM thapsigargin in seven of seven cell-attached patches. NSCC NP_o increased in cell-attached patches obtained on insulin-deficient GMC cultures exposed to high-dose insulin (100 μU/ml or 100 mU/ml) for 15 min before study (*n* = 9) (Fig. 12, *left*). GMC exposure to low-dose insulin (1 μU/ml or 10 μU/ml) for 15 min did not increase NP_o, but did restore the stimulatory response of NSCC to subsequent application of 100 nM AII in seven patches (Fig. 12, *right*). Fig. 13 is a single channel record depicting restoration of AII responsiveness by acute exposure to 10 μU/ml insulin.

In the inside-out configuration, Ca^{2+} activation curves for the 27-pS NSCC also shifted to the left when comparing cultured GMC grown in insulin (Fig. 9, *triangles; n* = 5) with insulin-deficient GMC cultures (Fig. 9, *circles; n* = 4). Acute exposure of insulin-deficient GMC cultures to high- or low-dose acute insulin again resulted in a further leftward shift in the Ca^{2+} activation curve (Fig. 9, *squares; n* = 8). Restoration of NSCC sensitivity to activation by AII in cell-attached patches or cytoplasmic Ca^{2+} in excised patches was seen with all acute insulin concentrations (1 μU/ml, 10 μU/ml, 100 μU/ml, and 100 mU/ml).

Chronic, but not acute insulin exposure increases AII receptor density. Since exogenous insulin exposure could conceivably affect AII ligand–receptor interactions, AII receptor binding studies were performed. Fig. 14 shows that AII binds to rat glomerular mesangial cells in a saturable manner when measured at 4°C to prevent AII receptor internalization. For cultured rat GMC grown in the absence of insulin, the number of binding sites (B_{max}) was 2.44±0.58 fmol/mg protein and the apparent binding dissociation constant (K_d) was 3.02±2.01 nM. Acute exposure of insulin-deficient GMC cultures to low-dose (10 μU/ml) or high-dose (100 mU/ml) insulin for 15

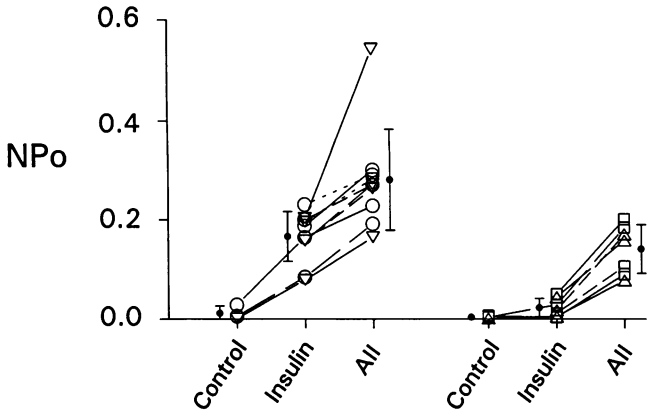

Figure 12. Effect of acute insulin exposure on NSCC. Cultured GMC were grown in the absence of insulin and then acutely exposed to exogenous insulin. (*Left*) High-dose insulin exposure (*inverted triangles*, 100 μU/ml insulin; *circles*, 100 mU/ml insulin). NSCC activity: NP_o is depicted before and 15 min after high-dose insulin exposure for cell-attached patches at $-V_{pipet} = -20$ mV. Mean NP_o increased from 0.011±0.014 to 0.18±0.05. After 15 min of high-dose insulin exposure, 100 nM AII addition increased mean NP_o to 0.28±0.10. (*Right*) Low-dose insulin exposure (*triangles*, 10 μU/ml insulin; *squares*, 1 μU/ml insulin). Before and 15 min after low-dose insulin exposure, NSCC activity was negligible for cell-attached patches at $-V_{pipet} = -20$ mV. After 15 min of low-dose insulin exposure, subsequent addition of 100 nM AII increased mean NP_o to 0.14±0.05. NP_o was measured as in Fig. 10.

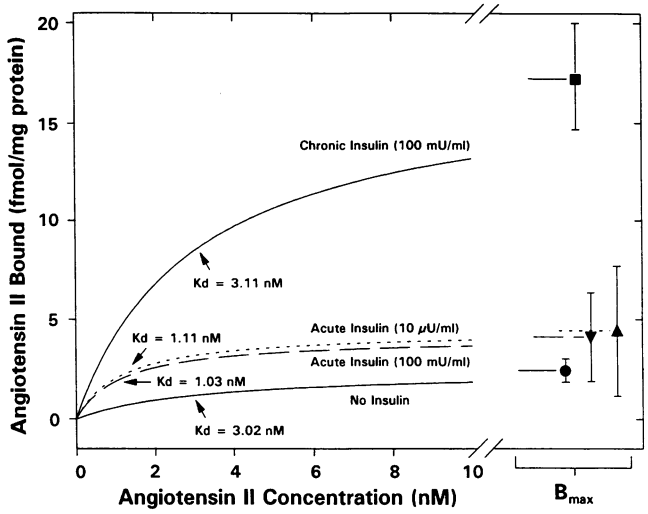

Figure 14. AII receptor binding assays. Specific binding of ^{125}I AII to rat GMC at equilibrium (90 min) as a function of ^{125}I-AII concentration under different exogenous insulin exposure conditions. Plots are for cultured GMC grown with 100 mU/ml insulin (*square*), insulin-deficient GMC cultures (*circle*), and insulin-deficient GMC cultures after acute low-dose (10 μU/ml; *triangle*) or high-dose (100 mU/ml; *inverted triangle*) insulin exposure. The binding affinity constants (K_d) are marked by arrows (*left*), and the number of binding sites (B_{max}) are shown on the right.

min before AII binding resulted in B_{max} values of 4.43±3.28 and 4.12±2.24 fmol/mg protein and K_d values of 1.37±2.45 and 1.18±2.77 nM, respectively. The latter B_{max} and K_d values were not significantly different from values from insulin-deficient GMC cultures. When GMC were chronically exposed to 100 mU/ml insulin in the growth medium, there was a marked increase in AII binding, due to a significant increase in B_{max} (17.4±2.6 fmol/mg protein), while K_d (3.11±1.81 nM) was not significantly different from insulin-deficient GMC cultures.

Discussion

The measured membrane potential of glomerular mesangial cells or phenotypically similar vascular smooth muscle cells ranges from −40 to −55 mV (1, 6), the same physiologic range in which Ca^{2+} channels are strongly voltage dependent in contractile vascular smooth muscle cells. Evidence indicates that GMC contraction also depends on the activation of voltage-dependent Ca^{2+} channels (2–5). In cultured rat GMC, the intracellular Ca^{2+} transients induced by membrane depolarization or vasoactive peptides are sensitive to classic Ca^{2+} channel blockers (dihydropyridines, benzothiazepines, phenylalkylamines) (2, 4, 5). The membrane depolarization response to vasoactive peptides is thought to involve activation of Ca^{2+}-dependent Cl^- channels and nonselective cation channels in vascular smooth muscle cells (6–8). In renal afferent arteriolar smooth muscle cells, membrane depolarization, elevation in intracellular Ca^{2+}, and contraction in response to the vasoactive peptide endothelin-1 is attenuated by Cl^- channel blockers (8). Kurokawa and Okuda (10), Kremer et al. (9), and Palant and Ross (11) present indirect evidence that GMC depolarization after exposure to vasoactive peptides is dependent on a Ca^{2+}-dependent Cl^- conductance. However, the mechanism by which vasoactive peptides might depolarize GMC and activate voltage-dependent Ca^{2+} channels has not been investigated at a single channel level. Using patch clamp methodology, we have identified two Ca^{2+}-dependent ion channels in rat mesangial cell cultures that are capable of membrane depolarization and are activated by the vasoactive peptide, AII, but only in the presence of insulin (Table II).

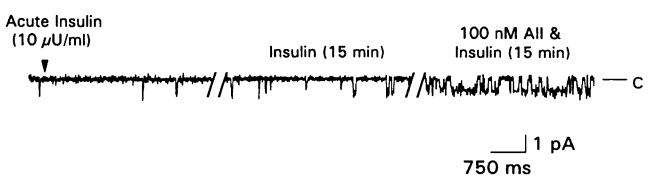

Figure 13. Effect of low-dose insulin exposure on NSCC. Single-channel recordings from the same cell-attached patch at $-V_{pipet} = -20$ mV. No change in NSCC activity is observed after 15-min exposure to 10 μU/ml insulin, but channel activity is stimulated by the subsequent addition of 100 nM AII. Fc = 1 KHz, sampling = 5 KHz; and no software filtering.

Low-conductance, Ca^{2+}-activated Cl^- channels sensitive to AII. The first is an outwardly rectifying Cl^--selective channel with a unitary conductance of 2–5 pS. With a reversal potential near the resting membrane potential of GMC and a low open probability under baseline conditions ($-V_{pipet} = 0$ mV, 37°C, physiologic extracellular saline, cell-attached configuration), this low-conductance Cl^- channel would generate little Cl^- flux. However, NP_o for this Cl^- channel increased > 100% with exposure to the vasoactive peptide, AII. Stimulation of Cl^- channel activity was seen at 1-, 10-, and 100-nM concentrations of AII. Circulating human plasma AII levels are usually ≤ 50 nM and physiological effects are mimicked by infusion rates that maintain plasma levels in this range (27).

Table II. Mesangial Cell Ion Channels

	$P_{Cl}/P_{Na}/P_K$*	Current–voltage relationship‡	Direct activation by acute insulin‡	Responsive to AII‡	Open probability at $[Ca^{2+}]_i = 10^{-7}$ M
4-pS Cl^- channel					
Cultures without insulin		Outwardly rectifying		No	0.0002±0.0008
Cultures with insulin	>50:1:1	Outwardly rectifying		Yes	0.05±0.04
Acute insulin (low dose)		Outwardly rectifying	No	Yes	0.14±0.04
Acute insulin (high dose)		Linear	Yes	Yes	0.18±0.10
27 pS NSCC					
Cultures without insulin		Linear		No	0.0006±0.0014
Cultures with insulin	0.1:1:1	Linear		Yes	0.03±0.02
Acute insulin (low dose)		Linear	No	Yes	0.09±0.03
Acute insulin (high dose)		Linear	Yes	Yes	0.12±0.03

Cutlures with or without insulin = rat mesangial cell cultures grown with or without insulin (100 mU/ml). Acute insulin = rat mesangial cell cultures grown without insulin are exposed to insulin for 15 min before patching. Low dose = 1 μU/ml or 10 μU/ml insulin; high dose = 100 μU/ml or 100 mU/ml insulin. * Excised inside-out patches. ‡ Cell-attached patches.

In cultured rat GMC, both AII and vasopressin stimulate a macroscopic Cl^- permeability associated with the release of intracellular Ca^{2+} stores (9–11). In response to vasoactive peptides, there is hydrolysis of mesangial cell membrane phospholipids and release of IP_3-sensitive intracellular Ca^{2+} pools (1, 24). Thapsigargin is a cell-permeable tumor promotor that rapidly releases Ca^{2+} from intracellular stores without hydrolysis of inositol polyphosphates in both contractile (vascular smooth muscle, platelets) and noncontractile cells (neuronal, parotid acinar, and cortical collecting tubule) (16, 25, 26). Supporting the hypothesis that AII activates this 4-pS Cl^- channel by mobilizing intracellular Ca^{2+}, we found that adding thapsigargin caused an ~ 200% increase in NP_o. Ca^{2+} dependency was confirmed by our observation that directly raising free Ca^{2+} at the cytoplasmic surface of inside-out patches also increased channel activity.

Palant and associates have described two anion-permeable channels in cultured rat GMC, but with much larger conductances of 48 pS (28) and 62 pS (29). Dependency of these latter two anion channels on intracellular Ca^{2+} or vasoactive peptides was not reported. Stimulation of low-conductance (2–5 pS) Cl^- channels by intracellular Ca^{2+} has been described in amphibian distal nephron cells (30, 31), mouse juxtaglomerular cells (32), rat lacrimal gland cells (33), Xenopus oocytes (34), and porcine intermediate lobe cells (35).

Ca^{2+}-activated nonselective cation channels sensitive to AII. The second channel has a unitary conductance of 25–29 pS, a linear I-V relationship, no voltage dependency, and is nonselective for cations, but relatively impermeable to Cl^-. We expect this channel to contribute little to basal ion fluxes since it is quiescent at resting membrane potential and E_{rev} is near $-V_{pipet} = 0$ mV. This NSCC was also activated by AII and thapsigargin in cell-attached patches, and cytoplasmic Ca^{2+} in excised inside-out patches. Stimulation of NSCC channel activity was also observed at physiologic nanomolar concentrations of AII.

Matsunaga et al. (15) recently characterized a 25-pS Ca^{2+}-dependent NSCC that is activated by AII or vasopressin in cultured rat GMC. Palant and associates have also described an osmotically activated NSCC (36) and a 62-pS stretch-activated NSCC (29) in cultured rat GMC. Responses to cytoplasmic Ca^{2+} and vasoactive peptides were not reported for these latter two NSCC. A family of 20–35-pS NSCC that are activated by intracellular Ca^{2+} have also been described in nonmesangial cell types, including vestibular dark cells (37), rat insulinoma (38), cardiac ventricular (39), neuroblastoma (40), pancreatic acinar (41), Schwann (42), lacrimal gland (33), thyroid follicular (43), and neutrophils (44).

Modulation of mesangial cell ion channels by exogenous insulin. Studies by Kreisberg (45), and Dunlop and Larkins (12) have demonstrated in cultured rat GMC that the presence of insulin in the growth medium was required for normal intracellular Ca^{2+} transients and contraction to occur in response to 1 nM to 10 μM AII or other vasoconstrictive peptides (e.g., platelet-activating factor, endothelin-1). We found that activation of both the 4-pS Cl^- channel and the 27-pS NSCC by AII or thapsigargin also depended on chronic exposure to 100 mU/ml insulin. Acute exposure of insulin-deficient GMC cultures to insulin (15 min) directly increased the activity of both channels at dosages ≥ 100 μU/ml and restored channel responsiveness to AII at dosages ≥ 1 μU/ml. Excised patch experiments revealed that insulin deficiency decreased the sensitivity of the Cl^- channel and NSCC to activation by cytoplasmic Ca^{2+}. Comparing cultured rat GMC grown with and without insulin, open probability for both the Cl^- channel and NSCC increased 100-fold for excised patches exposed to 10^{-7} M Ca^{2+} in the cytoplasmic bath (Table II). Mesangial cell intracellular Ca^{2+} concentrations range from 10^{-7} to 10^{-6} M under baseline or vasoactive peptide–stimulated conditions (9, 24). Using fura-2-loaded rat GMC, Dunlop and Larkins (12) have shown that IP_3-sensitive Ca^{2+} release itself is also attenuated in insulin-deficient GMC cultures.

Variable GMC responsiveness to AII under our different insulin exposure conditions could be mediated by alterations in AII ligand–receptor binding. Our results show that ^{125}I AII binds to rat GMC cultures in a saturable manner. AII binding decreased by 86% when 100 mU/ml insulin was removed from our standard growth medium. This decreased binding in the absence of chronic insulin exposure was due to a marked reduction in the number of AII receptor binding sites, rather than a change in receptor binding affinity. In contrast, acute exposure of insulin-deficient GMC to either low- (10 μM/ml) or high-dose (100 mU/ml) insulin for 15 min did not significantly change the number of binding sites, or the binding affin-

ity. In GMC cultured without supplemental insulin, other investigators have shown that AII binds only to a single class of AII receptor subtype 1 (AT_1) receptors with K_d's ranging from 0.37 to 2.8 nM, and B_{max}'s ranging from 6.93 to 43.5 fmol/mg protein in rat (passage unknown) (22), murine (continuous cell line) (20), and human (passages 3–6) (21) GMC. Binding studies for human fetal and adult GMC (passages 4–11) grown with 8 μM (1 U/ml) insulin show a K_d of 1.25–1.6 nM and B_{max} of 65–70 fmol/10^5 cells (23) (insulin concentration conversions: 7.175 μM = 1 U/ml [46] and 1 μg/ml = 24 mU/ml [Sigma Chemical Co.]). Using rat GMC passages 5–7, our K_d and B_{max} values are consistent with these previous studies. Our results indicate that the insulin-dependent responsiveness of Cl^- and NSCC to activation by AII and intracellular Ca^{2+} is time dependent. Acute exposure (min) of GMC to exogenous insulin has no affect on AII receptor binding affinity or receptor density. However, chronic exposure (d) to insulin results in an increase in AII receptor density.

Both acute and chronic insulin exposure shifts the Ca^{2+} activation curves for the Cl^- and NSCC, making them more sensitive to intracellular Ca^{2+}. This effect was observed in patches excised from GMC and independent of AII receptor interactions. Therefore, channel activation and restoration of AII responsiveness after acute or chronic insulin exposure must also be mediated by another signaling mechanism, independent of insulin's affects on AII ligand–receptor binding.

What insulin-mediated signaling mechanisms might affect the Ca^{2+}-dependent, 4-pS Cl^- channel and 27-pS NSCC in glomerular mesangial cells? Insulin regulates membrane phospholipid metabolism: hydrolysis of phosphatidylinositol-glycan, stimulation of phosphatidic acid synthesis, and hydrolysis of phosphatidylcholine by phospholipases C and D (47). All of these phospholipid effects generate diacylglycerol and IP_3, the latter stimulating the release of intracellular Ca^{2+} stores and potentiating the activation of these Ca^{2+}-activated channels.

Generation of diacyglycerol by insulin also activates Ca^{2+}- and phospholipid-dependent protein kinase C (47), which inhibits atrial natriuretic peptide (ANP)-induced stimulation of particulate guanylyl cyclase in GMC (48). AII-induced GMC contractions and elevations in intracellular Ca^{2+} are inhibited by guanylyl cyclase stimulation and elevated intracellular cGMP levels (48, 49). If cGMP levels were elevated in GMC grown without insulin, it could explain the observed insensitivity of 4-pS Cl^- channels and 27-pS NSCCs to AII under insulin-deficient growth conditions. However, in the presence of AII, basal cGMP levels are reduced, not elevated, in rat GMC and murine mesangial cell cultures grown without supplemental insulin (20, 48). ANP is the only receptor-mediated agonist of guanylyl cyclase that has been identified in GMC (1). But, even after exposure to both ANP and AII, cGMP levels in GMC are similar to unstimulated basal values (20). GMC also contain a soluble form of guanylyl cyclase that is activated by endothelial cell–derived nitric oxide (49). While nitrates also appear to affect intracellular Ca^{2+}, GMC were not cocultured with endothelial cells in the present study. Therefore, changes in intracellular cGMP probably do not explain the difference in AII responsiveness between insulin-treated and insulin-deficient GMC.

Marunaka and Eaton (30, 31) have characterized a 3-pS Ca^{2+}-dependent Cl^- channel in an amphibian distal nephron cell line (A6) that was stimulated by insulin. Insulin increased P_o, linearlized the outwardly rectifying I-V curve, and caused a 100-fold decrease in the threshold cytoplasmic Ca^{2+} concentration required for channel activation. They found that alkaline phosphatase mimicked the effects of insulin and hypothesized that dephosphorylation was responsible for the observed channel activation. We noted that insulin promotes the hydrolysis of phosphatidylinositol-glycan, which in turn generates products that activate intracellular phosphatases (47). Modulation of intracellular Ca^{2+} sensitivity for Ca^{2+}-dependent ion channels by dephosphorylation/phosphorylation reactions has been proposed by others (9, 30, 31, 38, 50, 51).

Role for mesangial cell ion channels in the pathogenesis of diabetic glomerular hyperfiltration. Our findings suggest that impaired activation of the mesangial cell ion channels described in this study would be expected in patients with insulinopenic and, perhaps, insulin-resistent diabetes mellitus. This would result in impaired depolarization in response to vasoactive peptides, decreased Ca^{2+} influx through voltage-activated Ca^{2+} channels, decreased GMC contractility, increased glomerular capillary ultrafiltration coefficient (K_f), and an increase in glomerular filtration (Fig. 15). Impaired contraction of vascular smooth muscle cells is also seen in the afferent arterioles of streptozotocin-treated diabetic rats and is improved by insulin therapy (52). Renal micropuncture and clearance studies have shown that the elevated glomerular filtration rate, single nephron filtration rate, glomerular hydraulic pressure, and renal plasma flow observed in streptozotocin-treated diabetic rats is reduced by intrarenal infusion of insulin and calcium (13). Diabetic glomerular hyperfiltration was restored if the Ca^{2+} channel blocker, verapamil, was infused after insulin and calcium.

Previous work on cultured rat GMC studied the effects of relatively high insulin concentrations (insulin concentration conversions: 7.175 μM = 1 U/ml [46] and 1 μg/ml = 24

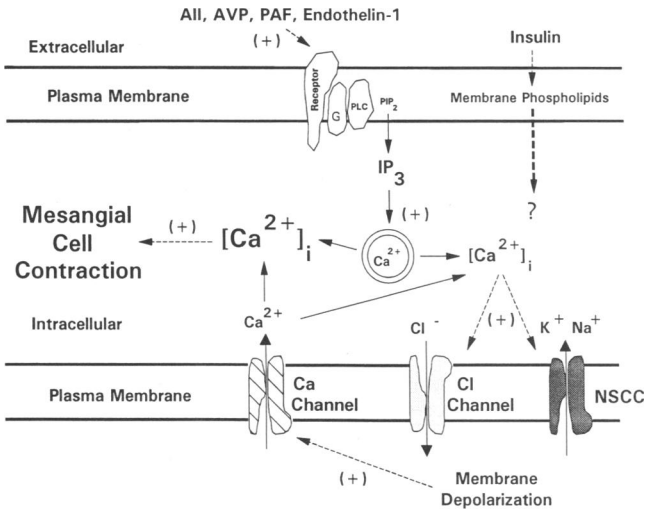

Figure 15. Model for regulation of glomerular mesangial cell ion channels. (*Top*) Vasoactive peptides stimulate receptor-mediated phospholipid hydrolysis generating IP_3. (*Bottom right*) Intracellular Ca^{2+} release stimulates Ca^{2+}-dependent, Cl^- channels and NSCCs. (*Bottom*) Cl^- efflux and cation influx, respectively, cause membrane depolarization and activation of voltage-dependent Ca^{2+} channels. (*Middle*) Ca^{2+} influx results in mesangial cell contraction and decreased glomerular capillary ultrafiltration coefficient (K_f). (*Top*) Exogenous insulin modulates Cl^- channel and NSCC activity, likely through affects on mesangial cell membrane phospholipid metabolism (see Discussion).

mU/ml [Sigma Chemical Co.]): 0.7 nM (98 μU/ml), 4 μg/ml (96 mU/ml), and 7 μM (976 mU/ml) (12, 45). However, the glomerular hyperfiltration observed in streptozotocin-treated diabetic rats is acutely decreased with intrarenal infusion of much lower doses of insulin (0.5 μU/ml) (13, 53). Therefore, we evaluated the responses of insulin-deficient rat GMC cultures acutely exposed to more physiologic insulin concentrations. Cl^- and NSCC were not directly stimulated, but responsiveness to AII and cytoplasmic Ca^{2+} was restored with insulin concentrations of 1 and 10 μU/ml. Higher insulin dosages (100 μU/ml and 100 mU/ml) also restored channel responsiveness to vasoactive peptide and Ca^{2+}, in addition to directly stimulating Cl^- and NSCC activity.

Normal fasting plasma insulin levels in humans are 5–20 μU/ml, and in response to a high carbohydrate meal, levels increase to $\sim$ 70 μU/ml in normal, 175 μU/ml in hypertensive, and 250 μU/ml in obese individuals (46, 54). In normal subjects receiving a constant insulin infusion (0.05 U/kg per h), steady-state plasma insulin levels are 134 μU/ml, with even higher peak levels expected with intermittent injections (55). These data indicate that physiologic concentrations of insulin restore or mediate the response of glomerular mesangial cells to vasoactive peptides and cytoplasmic Ca^{2+}.

Conclusions. We present single channel data showing two ion channels capable of depolarizing glomerular mesangial cells in response to the vasoactive peptide, AII: Ca^{2+}-dependent, 4-pS Cl^- channels promoting Cl^- efflux; and Ca^{2+}-dependent, 27-pS nonselective cation channels promoting cation influx.

The activity of both channels and their responsiveness to AII and intracellular Ca^{2+} are dependent on the presence of exogenous insulin. Both acute and chronic insulin exposures raise the sensitivity of both channels to intracellular Ca^{2+} through a non-AII receptor–mediated mechanism. Chronic, but not acute, insulin exposure also increases AII receptor density by > 85%, while K_d is unaffected. Regulation of the 4-pS Cl^- channel and the 27-pS NSCC by insulin provides a mechanism for the impaired Ca^{2+} uptake by glomerular mesangial cells observed in association with insulin deficiency. Thus, insulinopenia or insulin resistance could play an essential role in the early glomerular hyperfiltration that is observed in diabetes mellitus and that correlates with the development of proteinuria and diabetic nephropathy (13).

Acknowledgments

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