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Calcium Channel Current in Cultured Rat Mesangial Cells

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SUMMARY

The presence of voltage-dependent calcium channels has been suggested in mesangial cells by using calcium-sensitive fluorescent probes. However, direct electrophysiological evidence for voltage-dependent calcium channels has not yet been presented. In this study voltage-dependent calcium channels were studied in cultured rat mesangial cells. Whole-cell patch-clamp experiments were done with 50 mM Ba^{2+} as a charge carrier. Step depolarizing pulses from a holding potential of −50 mV produced an inward barium current at potentials more positive than −10 mV, and a peak current (10–45 pA) was obtained at a membrane potential of approximately +30 mV. The inward current was augmented by 100 nM Bay K 8644, attenuated by 1 μM nifedipine, and abolished by 50 μM Cd^{2+}. These results indicate that the inward current is a barium current flowing through L-type calcium channels. This may be the first study that demonstrates the presence of L-type calcium channels in mesangial cells.

Mesangial cells have recently been thought to play an important role in modulating renal hemodynamics and glomerular filtration rate (1). The cells contract in response to many kinds of vasoconstrictors such as angiotensin II and vasopressin, and the contraction is thought to be responsible for the modulation of glomerular filtration rate. Calcium seems to be essential for the contraction of mesangial cells. Several groups, using calcium-sensitive fluorescent probes, suggested the presence of voltage-dependent calcium channels in mesangial cells (2, 3). For example, cytosolic free calcium was increased by membrane depolarization with high potassium and this response was inhibited by calcium channel blockers, i.e., nifedipine or verapamil. On the other hand, a calcium channel agonist, Bay K 8644, increased cytosolic free calcium in the resting state. However, direct electrophysiological evidence for voltage-dependent calcium channels has not been presented. In this study we investigated under whole-cell clamp conditions whether voltage-dependent calcium channels exist in cultured rat mesangial cells and we demonstrated for the first time inward current flowing through L-type calcium channels.

Materials and Methods

Glomerular mesangial cell cultures. Glomerular mesangial cells were isolated from rat kidneys by the method described elsewhere (4). Briefly, kidneys were dissected from 5–6-week-old male Sprague-Dawley rats weighing 150–200 g and were decapsulated. Cortical tissue was minced and passed successively through a series of stainless steel sieves of different pore sizes (106, 180, and 62 μm). The fraction accumulating on the sieve with 62-μm pore size was collected in HBSS buffered with 20 mM HEPES, pH 7.4 (buffered HBSS), and was allowed to sediment by gravity. The sedimentation step was repeated three times with buffered HBSS. The glomeruli were observed to be stripped of their capsules and virtually free of tubule tissue microscopically. Glomeruli were treated with 0.1% collagenase for 15–20 min at 37° and then plated onto 100-mm plastic tissue culture dishes in RPMI 1640 medium containing 20% fetal bovine serum, 100 units/ml penicillin, and 100 μg/ml streptomycin. Plates were incubated in a humidified atmosphere at 37° in 5% CO_2 in air. Mesangial cells usually reached confluence in 10–14 days, and the cells were harvested by trypsinization and subcultured.

Whole-cell clamp experiments. Membrane current recordings were made under voltage-clamp conditions using a whole-cell clamp technique (5). On the day of the experiment, mesangial cells of passage 1 or 2 were harvested by trypsinization and plated on glass coverslips (5-mm square). After the cells had adhered on the surface of the coverslips, one coverslip was transferred onto the floor of the experimental chamber (0.5 ml in volume) and perfused with modified Tyrode solution at a rate of 1–2 ml/min. The temperature of the perfusate in the experimental chamber was maintained at 32–33°.

Patch electrodes were fabricated from glass capillaries of 1.5-mm outer diameter, using a double-step microelectrode puller (PP-83; Narishige, Tokyo, Japan). After the tip was polished with heat, the electrode was filled with internal solution of following composition (in mM): CsCl, 140; $MgCl_2$, 2.5; HEPES, 5; EGTA, 5; and ATP, 2. The pH was adjusted to 7.1 with CsOH. The outer diameter of the electrode tip

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ABBREVIATIONS: HBSS, Hanks' balanced salt solution; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N,N,N′,N′*-tetraacetic acid; HEPES, 4-(2-hydroxyethyl)-1-piperazine ethanesulfonic acid.

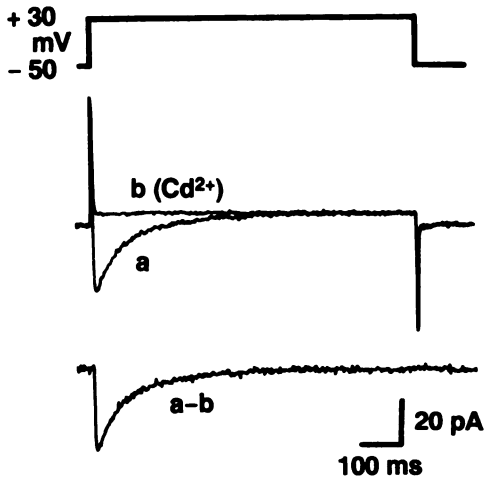

Fig. 1. Inward current in a rat mesangial cell. The membrane was held at −50 mV under voltage-clamp conditions, with the external solution containing 50 mM Ba^{2+}, and step depolarizing pulses to +30 mV for 800 msec were applied (*upper*). Current traces before (*a*) and after (*b*) application of 50 μM Cd^{2+} are superimposed (*middle*). The difference between the currents in the absence (*a*) and presence (*b*) of 50 μM Cd^{2+} is also shown (*lower*).

was 1–2 μm and the electrode resistance ranged between 3 and 5 MΩ. After the whole-cell clamp configuration was achieved, the external solution was switched from modified Tyrode solution to a solution of the following composition (in mM): $BaCl_2$, 50; NaCl, 70; CsCl, 5; tetraethylammonium chloride, 25; HEPES, 5; and glucose, 25. The pH was adjusted to 7.4 with NaOH. A patch-clamp amplifier (CEZ-2200; Nihon Kohden, Tokyo, Japan) was used to record the whole-cell membrane currents. The current signals were digitized using a PCM recording system (RP-880; NF Circuit Design Block, Nagoya, Japan) and were stored on a videocassette (NV 730, National, Osaka, Japan) for later analysis.

Drugs used were nifedipine (Sigma, St. Louis, MO) and Bay K 8644 (Calbiochem, San Diego, CA).

Experimental values are given as mean ± standard error.

Results

Inward current recorded in the mesangial cells. When the mesangial cells were studied under voltage-clamp conditions with 50 mM Ba^{2+} as a charge carrier, step changes in membrane potential from a holding potential of −50 mV to +30 mV produced a capacitive transient followed by an inward current, which was rapidly activated and decayed slowly within a 800-msec step pulse (Fig. 1, *middle*, *a*). The inward current was abolished by externally applied Cd^{2+} (50 μM) (Fig. 1, *middle*, *b*), revealing a time-independent outward current (background current). The current trace recorded in the external solution containing Cd^{2+} was subtracted from that recorded in normal solution to show the Cd^{2+}-sensitive fraction of the inward current (Fig. 1, *lower*). The absolute magnitude of this inward current was in the range from 10 to 45 pA.

A typical family of inward currents elicited by depolarizing steps from a holding potential of −50 mV is shown in Fig. 2A. Differences between the current traces before and after application of 50 μM Cd^{2+} were measured at the test potential indicated to the left of each trace. Fig. 2B shows a current-voltage relationship, in which the current amplitude was measured at the peak of the inward current obtained in Fig. 2A and is plotted as a function of the test potential. Current flowed inwardly at potentials ranging from −10 to +60 mV and reached its maximum at approximately +30 mV. The inward current was identified as a barium current flowing through the voltage-dependent calcium channel, because it was elicited by depolarization from a holding potential of −50 mV and was abolished by 50 μM Cd^{2+}, an inorganic calcium channel blocker.

Effect of nifedipine. To test further the pharmacological properties of the inward barium current, the effect of the dihydropyridine calcium channel blocker nifedipine was examined. Nifedipine (1 μM) decreased the inward currents remarkably, without affecting the outward background current (Fig. 3). The inhibitory effect of nifedipine on the barium current was produced at all membrane potentials tested (0 to +40 mV) (Fig. 3B). The maximum reduction of peak inward current was 78 ± 11% at +20 mV ($n = 3$).

Effect of Bay K 8644. The inward barium current elicited by a depolarizing step to +10 mV was enhanced by the dihydropyridine calcium channel agonist Bay K 8644 (Fig. 4). Fig. 4B shows the current-voltage relationships before and after external application of 100 nM Bay K 8644. The activation curve shifted to less positive potential in the presence of Bay K 8644. The peak inward current at +20 mV was increased by Bay K 8644 to 162 ± 11% of the control value ($n = 6$).

Discussion

The present voltage-clamp study clearly shows the presence of a calcium channel in cultured rat mesangial cells. The calcium channel shares the voltage dependency, kinetics, and pharmacological properties of L-type calcium channels that have been demonstrated in other tissues such as cardiac muscle

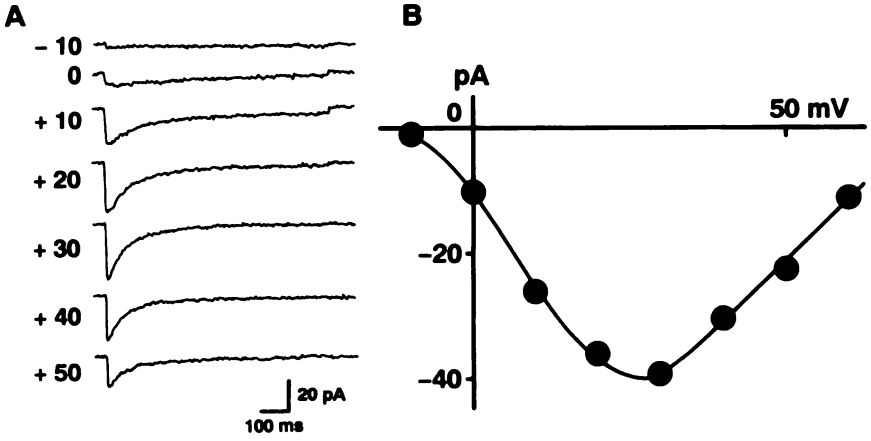

Fig. 2. Family of inward barium currents and the current-voltage relationship. A, Current traces in response to depolarizing pulses for 800 msec to various voltages from the holding potential of −50 mV. Command potentials are indicated to the *left of each trace* (in mV). Current traces are those after subtraction of the traces recorded in the presence of 50 μM Cd^{2+}. B, Current-voltage relationship for the Cd^{2+}-sensitive peak inward current, in which the current amplitude was measured at the peak of the inward current obtained in A and is plotted as a function of the test potential.

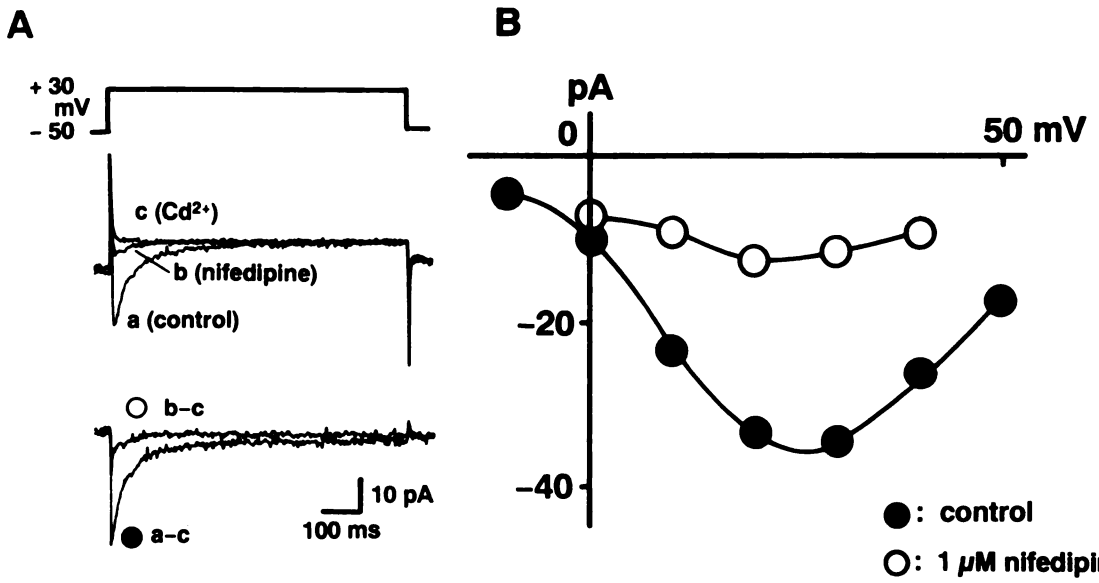

Fig. 3. Effect of nifedipine on the inward current. A, Depolarizing test pulses for 800 msec to +30 mV from the holding potential of −50 mV (*upper*) were applied. Current traces before (*a*) and after application of 1 μM nifedipine (*b*) or 50 μM Cd^{2+} (*c*) are superimposed (*middle*). The difference between the current traces in the absence and presence of 50 μM Cd^{2+} is shown (*a* − *c*) (● in *lower*). The difference between the current trace in the presence of 1 μM nifedipine and that in the presence of 50 μM Cd^{2+} is also shown (*b* − *c*) (○ in *lower*). B, Current-voltage relationships before (●) and after (○) application of 1 μM nifedipine.

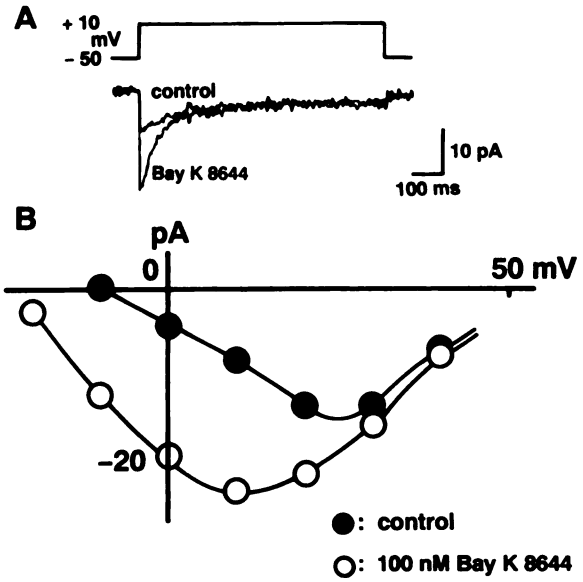

Fig. 4. Effect of Bay K 8644 on the inward current. A, Current traces before (control) and after application of 100 nM Bay K 8644 (Bay K 8644) are superimposed. The current trace in the presence of 50 μM Cd^{2+} has been subtracted from each trace. B, Current-voltage relationships before (●) and after (○) application of 100 nM Bay K 8644.

(6), skeletal muscle (7), and smooth muscle (8), that is, 1) the current is elicited by depolarization from a holding potential of −50 mV and reaches its peak at approximately +30 mV, 2) the current is activated rapidly and decays slowly with Ba^{2+} as a charge carrier, 3) the current is inhibited by the application of calcium channel blockers Cd^{2+} and nifedipine, which are thought to be selective for L-type calcium channels at the concentrations used in the present experiments, and 4) the current is enhanced by the dihydropyridine calcium channel agonist Bay K 8644. Because the mesangial cell is phylogenetically related to vascular smooth muscle, it is reasonable that the properties of the calcium channels in mesangial cells bear some resemblance to those of the channels in smooth muscle cells.

Little is known about the functional role of voltage-dependent calcium channels in mesangial cells. However, elevation of intracellular Ca^{2+} concentration resulting from extracellular Ca^{2+} influx was reported in rat mesangial cells when the cells were exposed to angiotensin II (2, 9) or arginine vasopressin (10). Because the elevation of intracellular Ca^{2+} concentration was inhibited by chelation of extracellular Ca^{2+} or by the voltage-dependent calcium channel blockers verapamil or nifedipine (9), the elevation was attributed to Ca^{2+} influx through the voltage-dependent calcium channels. The mesangial cells also contract in response to angiotensin II or arginine vasopressin in an extracellular Ca^{2+}-dependent manner (10, 11). It is suggested that the L-type calcium channel found in the present study may be involved in such responses.

The following sequence of intracellular events has been recently suggested in the action of several vasoactive peptides including angiotensin II (12), arginine vasopressin (13), and endothelin (14) in mesangial cells: initial generation of inositol 1,4,5-triphosphate and subsequent mobilization of intracellular Ca^{2+} stores, which in turn activates chloride channels and finally results in membrane depolarization. Ca^{2+} influx through the L-type calcium channels during membrane depolarization would account for the late and sustained increase in intracellular Ca^{2+} concentration observed in the responses to these vasoactive peptides.

Voltage-dependent calcium channels have also been suggested to play an important role in modulation of mesangial cell proliferation, because proliferation of mesangial cells is inhibited by several kinds of calcium channel blockers (15). Because proliferation of mesangial cells is observed in a variety of immune- and nonimmune-mediated diseases (16), the voltage-dependent calcium channels may play a pathogenic role in these proliferative renal diseases.

In addition to L-type calcium channels, the presence of T-type calcium channels has been reported for various kinds of smooth muscle cells (8). However, the shape of the inward current and the current-voltage relationship in the rat mesangial cells were nearly identical for two different holding potentials (−50 mV and −90 mV).[1] Therefore, it is likely that the L-type calcium channel is a predominant component of voltage-dependent calcium channels in rat mesangial cells. In addition, the presence of nonselective cation channels and potassium channels has also been demonstrated with single-channel recordings in rat mesangial cells (17).

Acknowledgments

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