Intracellular Calcium Concentration in the Inositol Trisphosphate Receptor Type 1 Knockout Mouse

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Abstract. Recently, mice with a disrupted inositol trisphosphate (IP3) receptor type 1 allele were produced by gene targeting. To examine the role of IP3 receptor type 1 in the regulation of intracellular calcium concentration ([Ca2+]i) of glomerular cells, [Ca2+]i was measured with fura 2-acetoxymethyl-ester in the superfused glomeruli from homozygous and wild-type mice. [Ca2+]i was determined in calcium-free medium before and after the addition of 10−7 M endothelin-1 (ET-1) and 10−6 M angiotensin II (AngII). The expression of mRNA of IP3 receptor isoforms and hormone receptors in the glomeruli from these animals also was measured by quantitative reverse transcription-PCR with specific primers for IP3 receptor isoforms (types 1, 2, and 3), AngII receptor type 1, and ET receptors (types A and B). In homozygous mutants, the shorter mRNA of IP3 receptor type 1, which lacks the first exon, is transcribed. Basal [Ca2+]i and the responses to ET-1 and AngII in homozygous mutants (ET-1, 55 ± 7 nM to 73 ± 7 nM; AngII, 66 ± 6 to 91 ± 8 nM) were significantly lower than those in the wild-type mice (ET-1, 93 ± 13 nM to 162 ± 13 nM; AngII, 87 ± 7 to 147 ± 9 nM; P < 0.05 for both hormones) without significant changes in mRNA expression of hormone receptors. The results with quantitative reverse transcription-PCR also revealed that mRNA expression of the IP3 receptor gene family was not significantly different between the two groups. The present study clearly shows that IP3 receptor type 1 plays a major role in the regulation of [Ca2+]i in the glomeruli and that lack of an isoform of IP3 receptor in the glomeruli does not induce expression of the other isoforms of the IP3 receptor.

Inositol trisphosphate (IP3) plays a key role in regulating cell function in response to various hormone stimuli (1). Stimulation of receptors on the plasma membrane results in hydrolysis of phosphatidyl inositol 4,5-bisphosphate by phospholipase C and the production of IP3 and diacylglycerol. The IP3 produced binds to its specific receptor on the endoplasmic reticulum and activates the calcium channel in the IP3 receptor. This results in the release of calcium stored in the endoplasmic reticulum into the cytoplasm by the action of the calcium pump. The increased intracellular calcium concentration then activates various effectors, such as calcium-activated chloride channels. In the kidney, many different hormones exert their action via this IP3 pathway. Angiotensin II (AngII), endothelin, and various vasoconstrictor substances increase the intracellular calcium concentration in the renal arterioles and mesangial cells (2,3).

Three types of the IP3 receptor have been cloned thus far. The first type was cloned from mouse cerebellum Purkinje cells by Furuichi et al. (4) and is now called the “IP3 type 1 receptor.” IP3 receptor type 1 is expressed in various tissues (5), and its expression is richest in the cerebellum. Moderate amounts of the mRNA of the type 1 receptor are found in the thymus, heart, lung, liver, spleen, kidney, and uterus. Two variants of the IP3 type 1 receptor with alternative splicing have also been reported and are expressed in the cerebellum and various tissues in a tissue-specific manner (6). The second type of IP3 receptor, the IP3 type 2 receptor, was cloned by Südhof et al. from the rat brain cDNA library (7). Type 2 mRNA has been detected in the submandibular gland, kidney, epididymal ducts, and the ovary in the mouse (8). The full-length cDNA of the IP3 type 3 receptor was cloned by Blondel et al. from rat pancreatic islet RNA (9). An in situ hybridization study showed that the mRNA of this type is present in gastric cells, salivary and pancreatic acinar cells, and the epithelium of the small intestine (8). The type 3 receptor is the most abundant type of the three types especially in the pancreas, and is considered to play an important role in the secretion of insulin.

We have previously reported the localization of these three types of IP3 receptors in the kidney (10). The specific monoclonal antibodies (11) were used for immunohistologic detection, and each type of IP3 receptor showed distinctive localization in the rat kidney. Immunostaining of type 1 receptor was observed in the arteries, afferent and efferent arterioles, and mesangial cells. Immunostaining of the type 2 receptor was limited to the tubular cells. Positive cells for anti-type 2 antibody were localized in the cortical to the outer medullary
collecting ducts, and the double staining with the antibody against aquaporin-2 showed that the type 2-positive cells were negative for aquaporin-2, indicating that intercalated cells in the collecting ducts express type 2 receptor. Immunostaining with specific monoclonal antibody against type 3 showed a polarized distribution. Staining in the collecting ducts was most intense in the cytoplasm of the basolateral membrane side and was not seen on the apical side, although plasma membranes were negative for the type 3 immunostaining.

Recently, mice with a disrupted IP3 receptor type 1 allele as a result of gene targeting (12) were produced by Mikoshiba’s group, and these mice exhibit ataxic seizures, similar to P400-deficient mice. This finding suggests that a lack of IP3 receptor type 1 does not induce substitutive expression by other members of the IP3 receptor gene family. To examine the effects of lack of the IP3 receptor type 1 in other tissues, we assessed the changes in the intracellular calcium concentration and expression of mRNA of IP3 receptor types 1, 2, and 3 in the glomeruli of these mutant mice.

Materials and Methods

Animals

Male 17- to 20-d-old wild-type and homozygous mutant mice were used in this study. The genotypes of the animals were determined by the tail Southern blotting method. Animals were allowed free access to a regular diet and tap water until the start of the experiments, and they were anesthetized by intraperitoneal injection of pentobarbital before the experiments. All animal experiments described in this study were conducted in accord with the Keio Medical School Guide for the Care and Use of Laboratory Animals.

At about 9 d of age, homozygous mutant mice begin to experience so many severe ataxic seizures that they are unable to consume enough food and liquids to grow normally. As a result, homozygous mutant mice do not survive beyond 20 d after birth. In addition, the birth rate of the homozygous mutant is much lower than that of wild-type mice. Their extremely short longevity and low birth rate of the homozygous mutant is much lower than that of wild-type mice. As a result, single homozygous mutant mice were held in place by using a fine-tipped glass pipette.

Chemicals

Unless stated otherwise, all chemicals were purchased from Sigma Chemical Co. (St. Louis, MO) or Wako Junyaku Co. (Osaka, Tokyo).

Microdissection of the Glomeruli

Microdissection of the glomeruli was performed as reported previously, with minor modifications (13,14). The animals were anesthetized and killed by exsanguination via the abdominal aorta. The abdominal aorta was cannulated and perfused for 5 min with Heps-buffered Dulbecco’s modified Eagle’s medium (DMEM) containing 0.1% bovine serum albumin (BSA) and 0.1% collagenase. The kidneys were removed and cut into small pieces, and the tissue was incubated in the same medium containing BSA and collagenase for 5 min at 37°C. After incubation, tissues were washed in Heps-buffered DMEM containing 0.1% BSA for 30 min. The sample tube was then centrifuged at low speed (300 rpm), and the sedimented glomeruli were transferred to the superfusion chamber. For the subquent superfusion, single glomeruli were held in place by using a water-jacketed line, and the temperature of the superfusion chamber was maintained at 37°C.

Fluorescence was measured with an Argus 50 system (Hamamatsu Photonics, Shizuoka, Japan). Glomeruli loaded with fura-2 AM were excited alternately at 340 and 380 nm by a computer-controlled shutter for 1/30 s, and the fluorescence image at 530 nm was obtained at each excitation wave length (a set of fluorescence images). The ratio of the intensity of fluorescence at 340 nm to that at 380 nm was calculated for each set of images. The mean value of eight sets of images was taken as one measurement value and stored and processed with a personal computer. The eight sets of images were obtained every 5 s. After a 15-min equilibration period, the superfusion solution was changed to Heps-buffered calcium-free solution containing 1 mM ethyleneglycol-bis(β-aminoethyl ether)-N,N,N′,N′-tetra-acetic acid. The [CaCl$_2$] in the original superfusion solution was omitted and replaced with Heps to maintain osmolality. After a 30-s superfusion with calcium-free solution, basal [Ca$^{2+}$], was measured for 1 min, and the solution was quickly switched to calcium-free solution containing 100 nM endothelin-1 (ET-1) or 1 μM AngII. Preliminary experiments showed that these concentrations of ET-1 and AngII induced a maximal [Ca$^{2+}$], response in the glomerulus of the wild-type mouse. The changes in [Ca$^{2+}$], were measured for 3 min and recorded with a personal computer. After acquisition of fluorescence images, the calcium concentration was calibrated with a Calcium Calibration Buffer kit (Calbiochem, Eugene, OR). After the experiments, the images were analyzed. Five areas in the glomerular tuft were chosen, and [Ca$^{2+}$], was calculated using a calibration curve. The mean value of the [Ca$^{2+}$], in the five areas was calculated and taken as the [Ca$^{2+}$], value. [Ca$^{2+}$], was determined in the glomeruli of a homozygous mutant and a wild type littermate on the same day.

Reverse Transcription-PCR

Glomeruli were microdissected from the kidney, as described above, except that the dissection medium contained 10 mM vanadyl ribonucleoside complex. The microdissected glomeruli were transferred to a tube containing 500 μl of solution D (4 M guanidine-thiocyanate, 25 mM sodium citrate, 0.5% N-lauryl sarcosine sodium, 0.1 M mercaptoethanol). Total RNA was then extracted from 0 to 50 glomeruli from a wild-type and a homozygous mutant mouse by the acid guanidinium thiocyanate-phenol-chloroform method (15). The reverse transcription (RT)-PCR was performed by using a Gene Amp RNA PCR Core kit (Perkin Elmer, Foster City, CA). First-strand cDNA was synthesized from total RNA extracted from 5 to 15 glomeruli by using random hexamers. After incubation at 42°C from one kidney, and transferred to a microtube containing Heps-buffered DMEM with 0.1% BSA, and maintained at 37°C for 1 to 2 h.

Superfusion and Measurement of Glomerular [Ca$^{2+}$]

Glomeruli were superfused as reported previously (14). The glomeruli were loaded with 15 μM fura-2-acetoxyethyl-ester (AM) in Heps-buffered DMEM containing 0.1% BSA for 30 min. The sample tube was then centrifuged at low speed (300 rpm), and the sedimented glomeruli were transferred to the superfusion chamber. For the subsequent superfusion, single glomeruli were held in place by using a fine-tipped glass pipette.

The superfusion solution, composed of 115 mM NaCl, 5 mM KCl, 2.3 mM Na$_2$HPO$_4$, 2.3 mM CaCl$_2$, 1 mM MgSO$_4$, 15 mM Na-glucanate, 5 mM Na-Hepes, 5 mM H-Hepes, and 8.3 mM d-glucose, pH 7.4, was changed continuously with a peristaltic pump (Gilson Medical Electronics, Villiers-le-Bel, France) at a flow rate of 5 ml/min. The solution was preheated through a water-jacketed line, and the temperature of the superfusion chamber was maintained at 37°C.

Fluorescence was measured with an Argus 50 system (Hamamatsu Photonics, Shizuoka, Japan). Glomeruli loaded with fura-2 AM were excited alternately at 340 and 380 nm by a computer-controlled shutter for 1/30 s, and the fluorescence image at 530 nm was obtained at each excitation wave length (a set of fluorescence images). The ratio of the intensity of fluorescence at 340 nm to that at 380 nm was calculated for each set of images. The mean value of eight sets of images was taken as one measurement value and stored and processed with a personal computer. The eight sets of images were obtained every 5 s. After a 15-min equilibration period, the superfusion solution was changed to Heps-buffered calcium-free solution containing 1 mM ethyleneglycol-bis(β-aminoethyl ether)-N,N,N′,N′-tetra-acetic acid. The [Ca$^{2+}$], in the original superfusion solution was omitted and replaced with Heps to maintain osmolality. After a 30-s superfusion with calcium-free solution, basal [Ca$^{2+}$], was measured for 1 min, and the solution was quickly switched to calcium-free solution containing 100 nM endothelin-1 (ET-1) or 1 μM AngII. Preliminary experiments showed that these concentrations of ET-1 and AngII induced a maximal [Ca$^{2+}$], response in the glomerulus of the wild-type mouse. The changes in [Ca$^{2+}$], were measured for 3 min and recorded with a personal computer. After acquisition of fluorescence images, the calcium concentration was calibrated with a Calcium Calibration Buffer kit (Calbiochem, Eugene, OR). After the experiments, the images were analyzed. Five areas in the glomerular tuft were chosen, and [Ca$^{2+}$], was calculated using a calibration curve. The mean value of the [Ca$^{2+}$], in the five areas was calculated and taken as the [Ca$^{2+}$], value. [Ca$^{2+}$], was determined in the glomeruli of a homozygous mutant and a wild type littermate on the same day.
for 45 min, the samples were heated for 5 min at 99°C to terminate the reactions, and they were then stored at 4°C until further use.

PCR was performed with AmpliTaq DNA polymerase for each set of specific primers, β-actin (16), type 1 (4), type 2 (17), and type 3 IP3 receptors, AngII receptor type 1 (AT1) (18), and endothelin receptors type A (ET_A, GenBank accession no. AF039892) and type B (ET_B) (19). The primers and the probe for type 3 IP3 receptor was originally designed from rat cDNA sequence (20), and we confirmed that mouse cDNA of these sequences was identical to rat cDNA. The tubes were placed in a DNA Thermal Cycler 480 (Perkin Elmer), and after initial

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<th>Receptor</th>
<th>Primer</th>
<th>Base number</th>
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<td>Type 1 IP3 receptor</td>
<td>Forward 5'-AGTTTGCCCAACGATTTCCGTG-3' (7781–7801 bp)</td>
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<td>Reverse 5'-GTTGACATTCATGTGGAGG-3' (8540–8560 bp)</td>
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<td>Probe 5'-GCACCCAAAGAGAGCTGCTGGTG-3' (7850–7874 bp)</td>
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<td>Type 2 IP3 receptor</td>
<td>Forward 5'-GCCGACATATGACCTTAACT-3' (824–844 bp)</td>
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<td>Reverse 5'-ATGTTTCTAGGGGTGTGTT-3' (1574–1594 bp)</td>
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<td>Probe 5'-ATCCCGCTCTGGAATGCACTCC-3' (887–911 bp)</td>
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<td></td>
<td>Reverse 5'-TCACCGCAGCTGATCAGGTTCTG-3' (8127–8147 bp)</td>
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<td></td>
<td>Probe 5'-GTCTCGTGCCAGATCCTAGAG-3' (7437–7461 bp)</td>
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<td>Reverse 5'-ATTATCCTAAAGATGCTCA-3' (777–796 bp)</td>
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<td>Probe 5'-GTCACCTGCATCATCTCGG-3' (563–583 bp)</td>
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<td>ET_A receptor</td>
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<td>Probe 5'-GACCAAGATGTCTCTGGGTGAGT-3' (152–172 bp)</td>
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<tr>
<td>ET_B receptor</td>
<td>Forward 5'-TTACAAGACAGCCAAAGATT-3' (1017–1027 bp)</td>
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<td></td>
<td>Reverse 5'-TGCTTCCCCAGAGCGACTGTA-3' (1518–1537 bp)</td>
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<td>Probe 5'-GACCAAGCAATCCACACAGG-3' (1254–1274 bp)</td>
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<td>β-actin</td>
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<td>Probe 5'-GAGATGGGCACTGCGGCCACTCTCTT-3' (756–780 bp)</td>
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For 45 min, the samples were heated for 5 min at 99°C to terminate the reactions, and they were then stored at 4°C until further use.

Each primer and probe were designed from mouse cDNA for IP3 receptors, hormone receptors, and β-actin. Base number of primers and probe for type 3 IP3 receptor reflects rat cDNA. We confirmed that these sequences of rat cDNA were identical to mouse cDNA. IP3, inositol trisphosphate; AT1, angiotensin II type I receptor; ET_A, endothelin receptor type A; ET_B, endothelin receptor type B.
incubation at 93°C for 3 min, 20 to 40 cycles of amplification were carried out. The cycler was programmed as follows: 93°C for 1 min (denaturation), 62°C for 2 min (annealing), and 72°C for 2 min (extension). An additional final incubation at 72°C for 10 min (final extension) was then performed.

In these experiments, it was possible to detect mRNA of IP3 type 1 receptor by the following procedure. As described in the previous article (12), in the homozygous mutants, targeting vector was placed just 3' to the translation initiation codon, and the mutated product was expected to lack IP3 binding activity. Indeed, IP3-induced Ca2+ release from cerebellar microsomes was almost completely abolished in homozygous mutant mice. On the other hand, Northern blotting showed that poly(A)+ RNA from the cerebella of homozygous mutant mice contained a hybridizable transcript, which is similar in size to the wild-type IP3 receptor type 1 mRNA. This transcript, however, lacks the first exon, and Western blotting with crude membrane fraction of cerebellum revealed no expression of IP3 receptor type 1 protein. In our experiments, primers for IP3 receptor type 1 were designed downstream of exon 1, and so we could detect mRNA of this type receptor by RT-PCR.

**Analysis of PCR Products**

Analysis of PCR products was performed as reported previously (21), except that Express Hybridization Solution (Clontech Laboratories, Palo Alto, CA) was used in this study. The PCR products were separated by electrophoresis on a 2% agarose gel. After ethidium bromide staining, DNA bands were visualized with an ultraviolet transilluminator. For Southern blot analysis, gels were denatured, neutralized, and blotted onto Hybond N+ nylon membranes (Amer sham International, Buckinghamshire, United Kingdom). After fixation, the membranes were hybridized at 37°C for 1 h in Express Hyb ridization Solution containing [γ-32P]dATP-labeled synthetic oligonucleotide probes designed within the forward and reverse primers for each cDNA (Table 1). After hybridization, the membranes were washed under high-stringency conditions and an imaging plate was then exposed to them. The imaging plate was analyzed with the BAS 2000 image analyzing system (Fuji Film Institution, Tokyo, Japan). Band intensity was measured for each PCR product, and the ratios of IP3 and hormone receptor band intensity to the β-actin band intensity were calculated.

**Histologic Studies**

After anesthetizing the animals, both kidneys were removed and fixed in 4% paraformaldehyde. Tissue sections were stained by periodic acid-Schiff reagent and methenamine silver-periodic acid-Schiff methods and examined by light microscopy.

**Statistical Analyses**

Values are expressed as means ± SEM. t test was used to compare data sets. One-way analysis of covariance was used to analyze the effect of ET-1 and AngII. P < 0.05 was considered statistically significant.

**Results**

As shown in Figure 1, there were no obvious differences between the histologic appearance of the kidneys from the homozygous mutant mouse and the wild-type animals. The development of the mesangial cells and arterioles in the glomeruli seemed normal.

A representative response of [Ca2+]i to endothelin is shown in Figure 2. In the homozygous mutant mouse, basal [Ca2+]i was lower than in the normal glomeruli, and the response to ET-1 was also smaller than normal. The results are summarized in Figure 3. Basal [Ca2+]i (55 ± 7 nM) was significantly lower in homozygous mutants than in wild-type mice (93 ± 13 nM, P < 0.05), and the response to endothelin was also significantly lower in homozygous mice (73 ± 7 nM) than in the wild type (162 ± 13 nM, P < 0.05). To examine whether this attenuated response of [Ca2+]i is specific to endothelin, we next determined the response to AngII. As shown in Figure 4, basal [Ca2+]i, and the response to AngII was also significantly lower in homozygous mutants (66 ± 6 to 91 ± 8) than in wild-type mice (87 ± 7 to 147 ± 9 nM, P < 0.05 for basal and the response to AngII), as well as the response to endothelin.

In quantitative RT-PCR studies, we first determined the relationship between band intensity and cycle number and number of glomeruli in wild-type mice. The band intensity in Southern blotting was linear between 20 and 30 cycles for actin and between 25 and 35 cycles for IP3 receptor type 1, AT1, and ETA (data not shown). On the basis of these results, 23 cycles were chosen for actin, and 32 cycles for IP3 receptor isoforms and hormone receptors. The band intensity in the Southern blotting was linear between 5 to 15 glomeruli for actin and IP3 receptor type 1 under these conditions (data not shown). Accordingly, total RNA was extracted from 50 glomeruli and dissolved in 10 μl of distilled water. Two microliters of this RNA solution was used for each reverse transcription and PCR with specific primers for type 1, type 2, type 3, and actin. In different series of experiments, total RNA was extracted from 50 glomeruli and used for RT-PCR with specific primers for AT1, ETα, ETβ, and actin.

The Southern blotting of RT-PCR products showed that
types 1 and 3 were expressed in the glomeruli (Figure 5), but no expression of IP3 receptor type 2 could be detected by this method. As described in Materials and Methods, in the homozygous mutant, it was possible to detect mRNA expression of IP3 receptor type 1, since the short form of mRNA of IP3 receptor type 1, which lacks the first exon, is expressed in these animals (12). In Figure 6, the representative Southern blottings of RT-PCR for AT1, ETA, and ETB are shown. In homozygous mutants, expression of AT1 tended to be higher than that in wild type, although the difference did not reach statistical significance. The ratios of IP3 receptor type 1, type 3, AT1, ETA, and ETB to actin are shown in Table 2. Expression of type 1 was clearly the most abundant of the three types of IP3 receptors in the wild-type and the homozygous mutant mice, and the difference in the two types of receptors between the wild-type and homozygous mutant mice was not significant.

The expression of three hormone receptors, AT1, ETA, and ETB, was not significantly different between the wild-type and the homozygous mutant mice.

Discussion

The present study revealed that the lack of the IP3 receptor type 1 resulted in a low basal intracellular calcium concentration and decreased responses to ET-1 and AngII in the glomerulus, with no clear changes in the histology of the glomeruli or the development of the kidney. Furthermore, the lack of type 1 receptor did not induce a compensatory increase in the other types of IP3 receptors in the glomeruli.

IP3 receptor, Ca$^{2+}$-ATPase in the endoplasmic reticulum, and Ca$^{2+}$ channels on the plasma membrane play important roles in regulating intracellular calcium concentration (1). Activation of Ca$^{2+}$ channels and IP3 receptors induces an increase in [Ca$^{2+}$], while Ca$^{2+}$-ATPase causes an influx of calcium into the endoplasmic reticulum and decreases [Ca$^{2+}$]. Phospholipase C is activated by stimulation with various hormones, IP3 is generated in the cell, and IP3 receptor is activated. Activation of the IP3 receptor induces an efflux of calcium from the endoplasmic reticulum and results in an increase in [Ca$^{2+}$]. Activation of Ca$^{2+}$ channels induces an influx of calcium from the extracellular fluid and also induces an increase in [Ca$^{2+}$]. In the present study, to identify the role of IP3 receptors in the changes in [Ca$^{2+}$], we superfused glomeruli with calcium-free solution to eliminate the influx of calcium from the extracellular fluid. In this solution, the glomeruli from the wild-type mice showed a sharp increase in [Ca$^{2+}$], in response to ET-1 and AngII, whereas the glomeruli...
from the IP3 receptor type 1-deficient mice showed a greatly attenuated response to the stimulation. This clearly demonstrated the importance of the presence of IP3 receptor type 1 in the regulation of \([\text{Ca}^{2+}]_i\) in the glomerulus. The response to ET-1 and AngII, however, was not completely abolished. This decreased response was expected in the homozygous mutant mouse, since our previous immunohistologic studies showed that type 1 and type 3 immunostaining was present in the glomerulus (10). In the present study, consistent with the results of the immunohistologic studies, expression of the mRNA of type 1 and 3 was confirmed in wild-type and mutant mouse by quantitative RT-PCR, and no expression of type 2 receptor was detected. Yang et al. also reported the absence of expression of mRNA of IP3 receptor type 2 in rat kidney (22). We speculate that the response to ET-1 can be explained by the expression of the type 3 receptor. On the other hand, the levels of expression of type 3 did not differ in the wild-type and mutant mice. Expression of type 1 was also similar in both animals. In the homozygous mutant mouse, the lack of function of intact receptors may induce upregulation of mRNA expression and other types of IP3 receptors. Our findings in this study did not support the possibility of compensatory regulation of IP3 receptors, although it is still possible that expression of protein of IP3 receptor type 3 might be changed without upregulation of mRNA. In the homozygous mutant mice, basal

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**Figure 4.** Intracellular Ca\(^{2+}\) concentration in a superfused glomerulus under basal conditions and after angiotensin II stimulation. \(n = 9\) for each animal group.

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**Figure 5.** Representative picture of Southern blotting of reverse transcription (RT)-PCR products with specific primers for IP3 receptor types 1 and 3 and actin.

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**Figure 6.** Representative picture of Southern blotting of RT-PCR products with specific primers for angiotensin II type 1 receptor, endothelin receptor types A and B, and actin. AT1, angiotensin II type 1 receptor; ETA, endothelin receptor type A; ETB, endothelin receptor type B.
was also significantly lower than in the wild-type animals. The level of \([Ca^{2+}]_i\) is regulated by IP3 receptor, \(Ca^{2+}\)-ATPase, and \(Ca^{2+}\) stores in the endoplasmic reticulum, and \(Ca^{2+}\) channels, as discussed above. In the homozygous mutant mice, it is possible that the alteration in these factors, in addition to the lack of IP3 type 1 receptor, results in the decreased level of basal \([Ca^{2+}]_i\). The exact reasons for these changes in basal \([Ca^{2+}]_i\), remain to be determined.

The decreased response to endothelin and AngII was apparently induced by the lack of IP3 type 1 receptor, although it was possible that the altered expression of hormone receptors induced these attenuated responses in the homozygous mutant mice. To examine this possibility, we determined the expression of mRNA of AngII and endothelin receptors. Both ET\(\alpha\) and ET\(\beta\) receptors are known to be present in the glomerulus (23), and both isoforms induce the increase in \([Ca^{2+}]_i\) (24). In the present experiments, the expression of mRNA of ET\(\alpha\) and ET\(\beta\) was not significantly different between the two groups, suggesting that the decreased expression of endothelin receptor did not result in the attenuated response of \([Ca^{2+}]_i\) in the glomeruli from the homozygous mutant mice. Moreover, we determined the expression of mRNA of AngII receptors. In the glomerulus, it has been reported that two isoforms of AT1, AT1a and AT1b, are present in the rat glomerulus (25), and both isoforms activate phospholipase C and increase \([Ca^{2+}]_i\) (26). These two isoforms possess very similar cDNA sequences. In the present study, our major objective was the measurement of the response of \([Ca^{2+}]_i\) in the glomeruli; therefore, we did not determine the two isoforms separately. The expression of AT1 receptor tended to be higher in the homozygous mutant mice, probably due to dehydration, although this difference did not reach statistical significance. From these results, however, it is unlikely that the attenuated response of \([Ca^{2+}]_i\) to hormones was induced by the decreased expression of hormone receptors.

It has been reported that microinjection of the nonselective IP3 receptor antagonist heparin had no effect on the endothelin-induced increase in \([Ca^{2+}]_i\) in Chinese hamster ovary (CHO) cells (27), nor did blockade of IP3 receptor type 1 by specific antibody abolish the effects of endothelin. These findings indicate that neither IP3 receptor type 1 nor the other types of IP3 receptor mediate the increase in \([Ca^{2+}]_i\) by endothelin in CHO cells. The present findings in glomeruli, however, showed that lack of type 1 receptor resulted in an attenuated response of \([Ca^{2+}]_i\) to endothelin, indicating that a major part of the \([Ca^{2+}]_i\) response is mediated by the type 1 receptor in wild-type mice. The exact reasons for this discrepancy between the two studies are not known, although the difference in cell types may be related to the different response to endothelin. On the other hand, it is possible that the remaining \([Ca^{2+}]_i\) response in the glomeruli of the homozygous mutant mouse can be explained by the signal transduction pathway proposed on the basis of the study in CHO cells.

In the present study, we determined \([Ca^{2+}]_i\) in a superfused single glomerulus. As described in Materials and Methods, it is impossible to obtain enough animals for primary culture of specific types of cells in the kidney, and we therefore had to choose the present preparation for our study. A whole glomerulus consists of at least three components: visceral epithelial cells, mesangial cells, and endothelial cells. We probably observed the sum of the changes in these three cell types in our determinations of \([Ca^{2+}]_i\). Furthermore, the body weight of the homozygous mutant mice did not exceed 2 g, and their extremely small bodies made determination of BP and kidney function impossible. The results of this study show that IP3 receptor type 1 plays an important role in the regulation of \([Ca^{2+}]_i\), although functional significance should be determined in the future.

Acknowledgments

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References

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<table>
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<th>Receptor</th>
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<th>Homozygous Mutant</th>
</tr>
</thead>
<tbody>
<tr>
<td>IP3 receptor type 1/actin</td>
<td>0.63 ± 0.13</td>
<td>0.84 ± 0.16</td>
</tr>
<tr>
<td>IP3 receptor type 3/actin</td>
<td>0.051 ± 0.009</td>
<td>0.055 ± 0.019</td>
</tr>
<tr>
<td>AT1 receptor/actin</td>
<td>0.84 ± 0.07</td>
<td>2.13 ± 0.76</td>
</tr>
<tr>
<td>ET(\alpha) receptor/actin</td>
<td>0.45 ± 0.22</td>
<td>0.22 ± 0.58</td>
</tr>
<tr>
<td>ET(\beta) receptor/actin</td>
<td>0.73 ± 0.10</td>
<td>1.83 ± 0.77</td>
</tr>
</tbody>
</table>

*Values are means ± SEM. n = 5 for each animal group.


