Ryanodine Receptor Expression in the Kidney and a Non-excitable Kidney Epithelial Cell*

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An oligonucleotide probe to a conserved 3′ region within the three identified ryanodine receptor-calcium release channel isoforms hybridized to a single clone from a rabbit kidney cDNA library. The kidney clone encoded the carboxyl-terminal 338 amino acids within the putative transmembrane domain of the type 2 ryanodine receptor sequence. Reverse transcriptase-polymerase chain reaction with isoform-specific oligonucleotide primers demonstrated the presence of the type 2 ryanodine receptor transcript in rabbit kidney, as well as in a non-excitable cell line, LLC-RK1, derived from rabbit kidney epithelial cells. Amplification by rapid amplification of 5′ cDNA ends indicated the kidney type 2 ryanodine receptor transcript extended >7000 base pairs from the stop codon and is therefore not homologous to the short RyR-1 transcript of ~2500 base pairs previously observed in rabbit brain. [3H]Ryanodine binding and immunoblot analysis with a type 2 ryanodine receptor-specific antibody demonstrated that the native type 2 ryanodine receptor protein is expressed in the kidney. These observations suggest that the type 2 ryanodine receptor isoform may play a functional role in regulating intracellular calcium homeostasis in non-excitable cells.

Two distinct classes of calcium channel are known to be involved in the release of calcium ions from intracellular stores (1). One class is the inositol 1,4,5-trisphosphate-gated calcium channel (IP3 receptor, receptor), which releases Ca2+ from stores upon binding to the second messenger, IP3 (2–4). IP3 receptors are widely distributed and expressed in both excitable and non-excitable tissues, reflecting the ubiquitous nature of the IP3 signal transduction pathway (1). The second class of intracellular calcium channel is sensitive to ryanodine, a neutral plant alkaloid, and is known to be expressed at moderate to high levels in electrically excitable tissues (5, 6). There are currently three known ryanodine receptor isoforms (RyR-1, -2, and -3), each of which comprise subunits of Mr, ~560,000 (5, 6). All three isoforms are present in the brain, whereas skeletal and cardiac muscle express RyR-1 and -3 and RyR-2 and -3, respectively (7–13). The possible role of RyRs in intracellular Ca2+ signaling in non-excitable cells, as well as its precise functional relationship with the ubiquitous IP3 receptors, remains undefined. Physiological and pharmacological observations of ryanodine-sensitive Ca2+ stores have also been made in numerous other cell types, based on the actions of agents that affect muscle RyRs, such as caffeine and ryanodine (14–19). This evidence in disparate, non-excitable cells suggests the possibility of a more widespread involvement of RyRs in fundamental intracellular Ca2+ signaling processes than in only excitable tissues. However, the presence of ryanodine receptor proteins in non-excitable cells has thus far not been demonstrated. In this study, we have used molecular, biochemical, and pharmacological approaches to provide evidence for the specific expression of the RyR-2 isoform in kidney tissue and in the non-excitable kidney epithelial cell line, LLC-RK1. These studies indicate that regulation of intracellular calcium homeostasis via release from internal stores in non-excitable cells may involve complex interactions between specific isoforms of RyR and IP3 receptors.

EXPERIMENTAL PROCEDURES

Materials—The rabbit kidney cortex cDNA library was obtained from Stratagene, the TriClone System from Invitrogen, and the 5′-RACE system from Life Technologies, Inc. The rabbit kidney epithelial cell line, LLC-RK1 (ECACC no. 98050907), was from the European Collection of Animal Cell Cultures (Porton Down, United Kingdom). Tq DNA polymerase was purchased from Perkin-Elmer, sulfo-SMCC from Pierce; activated CH-Sepharose from Pharmacia Biotech Inc.; and adjuvants, proteinase inhibitors, and SDS-PAGE reagents from Sigma. [3H]Ryanodine (specific activity 54.7 Ci/mmol) was obtained from Du Pont NEN, and ryanodine from Agrisystems International (Windy Gap, PA). Peroxidase-conjugated secondary antibodies were purchased from Sigma (guinea pig) and Calbiochem (rabbit), and the ECL detection system from Amersham International. All other reagents were of analytical grade.

Isolation of Kidney RyR cDNA Clone—A rabbit kidney cortex oligodT(15)-primed cDNA library in the λ Zap II vector was screened (~1 × 109 plaque-forming units) with the ryanodine receptor consensus oligonucleotide probe, RyRM1 (5′-ATGGGCAACAGGATGACAATGACGAAAG-3′). The RyRM1 oligonucleotide was end-labeled with [32P]ATP using T4 polynucleotide kinase (Boehringer Mannheim), purified on Nensorb 20 cartridges, and approximately 0.5 μg (0.57 MBq) of the probe was used for each screening of the cDNA library, performed according to manufacturer’s instructions (Stratagene). Briefly, overnight hybridization to nitrocellulose filters was at 50 °C in 10% dextran, 1× NaCl, 1% SDS, and 0.1 mg/ml sheared, denatured herring sperm DNA. Filters were then washed at 35 °C in 1× SSC, pH 7.0, and exposed to x-ray film overnight at ~80 °C. After plaque purification by three subsequent rounds of screening, a single positive clone, RK5, was in vitro excised and end-sequenced. Clone RK5 (1908 base pairs) was used to create a series of unidirectional nested deletions in both directions using the Erase-a-Base system (Promega). The nucleotide sequences of nested deletion clones and PCR fragments (see below) were determined using the Taq DyeDeoxyTM terminator cycle sequencing kit.
on a model 373A automated DNA sequencer (Applied Biosystems) with forward and reverse M13 primers. Oligonucleotides were synthesized on an Applied Biosystems model 380B DNA synthesizer, and analysis of nucleotide and protein sequence data was performed using the MacVector and AssemblyLIGN software packages (IBI, Eastman Kodak Co.).

Isolation of Messenger RNA and RT-PCR—Isolation of mRNA, cDNA synthesis, and cloning of PCR products into the pCRll vector were all performed using the TriCloning system (Invitrogen). Poly(A)+ mRNA (2.5–5.0 mg) from ~100 mg of freshly dissected rabbit tissue or ~5 × 10^6 rabbit kidney LLC-RK1 cells. RT-PCR to detect a ~700–900-bp sequence within the region covered by rabbit kidney cDNA clone RK5 was performed using ~1 μg of isolated mRNA and the appropriate pair of isospecific primers. First strand synthesis was primed with oligo(dT) and PCR performed with either RyR-2 (PR1 and PR2) or RyR-3 (PR3, PR4, or RyR-3-specific primers) oligonucleotide primers. The sequences of these primers and their coordinates on the various rabbit RyR isoforms are: PR1, 5'-AGAAT-CAGTGGATTCTGGGACAGTGG-3' (nucleotides 13999–14023); PR2, 5'-TACGCTGTATCCATAGCTGGCAGGCCG-3' (nucleotides 14880–14904); PR3, 5'-CGAGTGGCGACGCTGGGGCAGTTG-3' (nucleotides 14206–14230); PR4, 5'-CAGCGTGGCTCTGGCATGGCGG-3' (nucleotides 15084–15108); PR5, 5'-GCCGTGGTATCGCGCATGTCGTT-3' (nucleotides 13794–13815); PR6, 5'-CACCTTCTGGTACATCTTCCAGC-3' (nucleotides 14535–14558). Amplification by PCR was performed with 30 amplification cycles (one cycle comprised 95°C for 1 min, 55°C for 30 s, and 72°C for 1 min) following a final extension at 72°C for 7 min. Samples were analyzed on a 1% agarose gel using 1-kb DNA ladder molecular weight markers (Life Technologies, Inc.) PCR products were either sequenced directly after purification from excised gel slices (GeneClean, BIO 101), or subcloned into the pCRll vector for propagation in bacteria and subsequent sequencing. RyR-2 sequences amplified with PR1 and PR2 from brain, cardiac muscle, and kidney were identical (see Fig. 2). Control reactions were performed by substituting water for the cDNA samples in the procedures in the absence of reverse transcriptase in the cDNA synthesis step.

Rapid Amplification of 5' cDNA Ends (5'-RACE)—Poly(A)+ mRNA (~1 μg) isolated from brain and kidney tissue as above was used as a template for the 5'-RACE procedure (Life Technologies, Inc.) (20, 21). First strand cDNA synthesis was primed using the RyR-2-specific antisense primer PR2 (see above) according to the manufacturer's protocol. Amplification of the cDNAs was then performed using the universal anchor primer supplied in the kit and the cardiac-specific primer PR7 (CCAGACATAGGTTCTGGTCTG, nucleotides 14804–14829), which anneals adjacent to PR2. The products of each 5'-RACE reaction were purified using GeneClean, and small amounts (~100 ng) analyzed by PCR under the same thermal cycling conditions as for the RT-PCR (see above) reactions. The products were then run on the cDNA clone RK5, which for the 831-bp "homologous" (PR1 and PR7, nucleotides 13999–14829), the 1356-bp "variable" (PR8 and PR9, nucleotides 12504–13839), and the 1096-bp "phosphorylation" (PR10 and PR11, nucleotides 7885–8980) regions of the rabbit RyR-2 sequence. The sequences and locations of PR8–11 are: PR8, GTCAGACATTAAAACGAAGTCTCA (nucleotides 12504–12520); PR9, GATAATAACAGACCATAAATTCGG (nucleotides 13835–13859); PR10, GGGGAATTGTTCTGGCAGATC (nucleotides 7885–7900); PR11, CAGAGCAGAGAGAGCT-3' (nucleotides 8956–8980). Samples from the 5'-RACE and PCR reactions were analyzed on a 1% agarose gel using 1-kb DNA ladder molecular weight markers. DNA bands were excised from the gel and sequenced directly, as described above. A control reaction was performed with kidney mRNA in the absence of reverse transcriptase to eliminate the possibility of amplification due to genomic DNA contamination.

Isolation of Membranes—Crude microsomes were prepared from rabbit brain, cardiac muscle, kidney, liver, and skeletal muscle tissues by differential centrifugation (22, 23). All procedures were carried out at 4°C. Crude mitochondrial and microsomal fractions were homogenized in Ultra-Turrax T25 homogenizer (Janke and Kunkel, 3 × 30 s, high setting) in 10 volumes of homogenization buffer (0.25 M sucrose, 10 mM Hepes, pH 7.2, 0.5 mM EDTA, 1 mM dithiothreitol, 0.2 mM phenylmethylsulfonyl fluoride, 1 mM benzamidine, 10 μM leupeptin, 1 μM pepstatin A, and 100 mM aprotinin). Homogenates were centrifuged for 20 min at 100,000 × g. The supernatant was passed through several layers of cheesecloth and the filtrate centrifuged for 60 min at 100,000 × g. The supernatant was removed, and the pellets were resuspended in four volumes of homogenization buffer using 10 strokes of a Dounce homogenizer. The microsome membranes were stored in 0.5-mL aliquots at ~80°C.

RyR-2 Antisera Production and Purification—A 24-amino acid peptide corresponding to residues 4676–4699 of the rabbit RyR-2, present in the rabbit kidney cDNA RK5, was synthesized on an Applied Biosystems model 430A peptide synthesizer and coupled via an additional COOH-terminal cysteine to keyhole limpet hemocyanin using the bifunctional reagent sulfo-SMCC (Pierce). Rabbits (New Zealand White) were injected subcutaneously with 250 μg of the coupled peptide in Freund’s complete adjuvant, followed by three booster injections at 28-day intervals in Freund’s incomplete adjuvant. Antiserum was boosted by exposure to homogenized rabbit skeletal muscle tissue coated using either peptide or microsomes, or by immunoblot analysis with microsomes. The antiserum pAb129 specifically recognized RyR-2 and was used at an optimum dilution of 1:500 for all the immunoblot studies described. Another polyclonal antiserum, RyR1, was raised in guinea pigs by immunizing with 50 μg of gel-purified rabbit skeletal muscle RyR-2 and was used at an optimum dilution of 1:10,000 for the immunoblots.

SDS-Polyacrylamide Gel Electrophoresis and Immunoblot Analysis—SDS-PAGE was performed using 5% (w/v) separating and 3% (w/v) stacking gels (23) cast in the PROTEAN II minigel system (Bio-Rad). Microsomes (~50 μg) were denatured for 5 min at 95°C in loading buffer (0.1% Tris-HCl, pH 6.8, 2% SDS, 2% β-mercaptoethanol, 10% glycerol, 50 μg/ml bromphenol blue) prior to loading. Electrophoresis was performed at a constant current (20 mA/gel) and the separated proteins either stained with Coomassie Brilliant Blue R-250 or electrotransfered onto polyvinylidene difluoride Immobilon membranes (Millipore) at 15°C for 1 h at 400 mA, then 1500 mA for 15 h. Transfer membranes were blocked for 1 h with 4% nonfat dry milk proteins, then incubated with either skeletal muscle RyR-2 antiserum RyR1 or RyR-2-specific antiserum pAb129. Peroxidase-coupled secondary antibodies were used at a dilution of 1:1000 and the blot developed with 3,3'-diaminobenzidine/H2O2. Alternatively, the enhanced chemiluminescence technique was performed according to the manufacturer's protocol with the secondary antibodies at a 1:2000 dilution and with three 15-min phosphate-buffered saline wash steps between incubations. After incubation with substrate, the blot was exposed to x-ray film for 30 s.

Measurement of [3H]Ryanodine Binding—Crude microsomes (~0.5 mg) of protein) were incubated for 2 h at 37°C with 1.6 nM [3H]ryanodine (54.7 Ci/mmol) in 200 μl containing 1 mM NaCl, 5 mM ATP, 20 mM Na-Pipes, pH 7.0, 100 μM EGTA, 150 μM Ca2+, and 0.2 mM phenylmethylsulfonyl fluoride. Samples (~25 μl) of each incubation were taken, placed into scintillation vials and counted to determine the total radioactivity. Further samples (50 μl) were taken and passed through Whatman GF/B filters, followed by three 5-ml washes under vacuum with ice-cold water, and then analyzed by scintillation spectrometer to determine the amount bound. The bound fraction was calculated from the presence of 100 μM unlabeled ryanodine and deduced from the final calculation of specific ryanodine binding.

RESULTS

Rabbit Kidney RyR cDNA Clone, RK5—Examination of the optimally aligned rabbit RyR-1, -2, and -3 isoform nucleotide sequences revealed the highest sequence identity in the putative channel-forming transmembrane domain located at the carboxyl terminus of the protein (data not shown). An oligonucleotide probe from this conserved 3' region of the RyR nucleotide sequence was used to investigate the expression of RyR isoforms in kidney, a tissue where a functional RyR protein has not previously been demonstrated. Screening of 102 recombinants from a kidney cDNA library with the consensus probe resulted in isolation of one cDNA clone, RK5, that contained a 1.9-kb insert. Determination of the RK5 nucleotide sequence revealed identity with the 3' region of the RyR-2 nucleotide sequence at bp 13894–15801 (Fig. 1), previously elucidated from rabbit cardiac muscle (25, 26). Translation of the open reading frame in RK5 showed that it encodes the carboxyterminal 398 amino acids of RyR-2, as well as 893 bp of the 3' untranslated region. The observation of a single RyR-2 cDNA clone with a probe that can hybridize to all three isoforms suggests the specific expression of this RyR isoform in rabbit kidney.

RT-PCR with RyR Isospecific Oligonucleotides—To investigate the apparently specific presence of only the RyR-2 transcript in kidney, RT-PCR was performed using isospecific...
specific primer pairs designed to amplify a 906-bp region of
RyR-2 (PR1 and PR2; Fig. 1), a 903-bp region of RyR-1 (PR3 and PR4), or a 765-bp region of RyR-3 (PR5 and PR6). In
controlexperiments, amplification of the correct size DNA frag-
ment was observed using mRNA isolated from rabbit brain
(RyR-1,-2, and -3), skeletal (RyR-1 and -3), and cardiac (RyR-2
and -3) muscle tissues, in agreement with previous studies (12).
The identity of each of the PCR products from brain, skeletal,
and cardiac muscle as encoding the appropriate RyR isoform
was further verified by sequencing of the amplified DNA (data
not shown).

Using mRNA from kidney tissue and the kidney epithelial
cell line LLC-RK1, a 900-bp product was consistently ampli-
fied only with the RyR-2 primers (Fig. 2A), and not with the
RyR-1 and -3 primers (data not shown). Control reactions in
which water was substituted for tissue (Fig. 2, Water), or where
the reverse transcriptase was omitted from the cDNA synthesis
step (Fig. 2, *), did not result in any amplification, confirming
the absence of genomic DNA contamination. Determination of
the sequence of the kidney 900-bp product amplified with
RyR-2 primers confirmed identity with the RyR-2 sequence (25,
26). In contrast, the 900-bp product derived from the LLC-
RK1 cells revealed a novel sequence that was very closely
related to that of the RyR-2. Fig. 2 shows an alignment of the
RyR-2 nucleotide sequence amplified from kidney with that of
the RyR-2 cDNA clone RK5 from rabbit kidney (Fig. 1) and
the cardiac muscle RyR-2 (28). Notably, both the RK5 cDNA clone (Fig. 1) and the 906-bp RyR-2 fragment amplified by PCR (Fig. 2A) were within the 3'-end 2500 bp of the RyR-2 isoform. We therefore performed 5'-RACE on kidney RNA to determine whether a truncated form of the RyR-2 transcript might also exist in these rabbit tissues (see “Experimental Procedures”), as it does for the RyR-1 in rabbit brain. Three pairs of primers were designed to amplify discrete domains of the RyR-2 sequence with increasing distance from the 3'-end (termed homologous (B), variable (C), and phosphorylation (D); see Fig. 3 schematic), following cDNA synthesis from brain and kidney mRNA. Fig. 3 shows that amplification occurred with all three sets of primers (Fig. 3, lanes B–D), suggesting that the RyR-2 transcript in these tissues extends at least 7000 bp from the stop codon. The authenticity of each of the amplified bands as RyR-2 fragments was confirmed by DNA
sequencing (data not shown). The control reactions performed
with kidney mRNA in the absence of reverse transcriptase
resulted in no amplification (Fig. 3, Kidney-RT), indicating
that genomic DNA contamination was absent. These results from
5'-RACE are therefore not consistent with the specific expres-
sion of a short RyR-2 transcript in brain and kidney.

Immunoblot Analysis—A synthetic peptide, corresponding to
a RyR-2-specific sequence located within clone RK5 (4677–4703, Fig. 1), was used to raise an antibody for immunoblot analysis with skeletal muscle, brain, cardiac muscle, and kidney microsomes. Fig. 4 illustrates the Coomassie-stained gel (panel A) and identical immunoblots incubated with antibodies specific for RyR-1 (panel B) and RyR-2 (panel C). A single high \( M_r \) band immunoreactive with the RyR-2 antibody was readily observed in brain and cardiac muscle (panel C, lanes 2 and 3), consistent with the predicted \( M_r \), 565,000 for the native RyR-2 protein (25, 26). A high \( M_r \) band, with similar mobility to the brain and cardiac muscle RyR-2, was also observed in kidney microsomes when immunoblots were developed using enhanced chemiluminescence (panel C, lane 4), indicating the presence of the full-size, native RyR-2 protein. The detection of the kidney RyR-2 protein only by enhanced chemiluminescence, and not with standard immunostain development procedures (data not shown), indicates the low level of RyR expression in this tissue, consistent with the results from cDNA library screening (Fig. 1). Immunoreactivity of the RyR-1 antibody specifically with the high \( M_r \) RyR-1 protein present abundantly only in skeletal muscle, together with a minor degradation product, is also illustrated (Fig. 4, panel B, lane 1).

**[H]Ryanodine Binding**—The binding of [\( ^{3}H \)]ryanodine to microsomes from rabbit skeletal muscle, brain, cardiac muscle, kidney, and liver was performed in the presence of 150 \( \mu M \) Ca\(^{2+} \) and 1 M NaCl (Table I). Specific ryanodine binding to skeletal muscle, brain, and cardiac muscle microsomes ranging from 159 to 1198 fmol of ryanodine/mg of protein was observed,
sensing the existence of a functional RyR protein in electrically non-excitabile as well as excitable cells (14–17, 29–31). The level of RyR isoform transcripts has been observed to be most abundant in excitabile tissues such as skeletal muscle, smooth muscle, heart, and brain, with much lower levels detected in other tissues (9, 12, 13, 25, 26, 32). Hence, there have been no reports describing the presence of a RyR protein in a non-excitabile tissue, which would account for the ryanodine- and caffeine-sensitive Ca^2+ pools observed. There may be a novel RyR homologue present in these tissues, or there may be a low level expression of an existing RyR isoform. We have addressed this question in the current study by using a consensus oligonucleotide probe to screen for RyR-related cDNAs in a library derived from rabbit kidney cortical tissue. Our finding, that a single clone corresponding to the RyR-2 isoform was detected (Fig. 1), suggests that this is the sole RyR isoform present in this tissue. Further analysis by RT-PCR with kidney mRNA using isoform-specific primers confirmed this observation of specific expression of RyR-2, and not of the RyR-1 and -3 isoforms (Fig. 2). It may be possible that the RyR-2 detected in the kidney library had originated from some contaminating smooth muscle present in blood vessel walls (32). We therefore employed an established rabbit kidney epithelial cell line, LCC-RK1, to determine for the presence of an RyR isoform in this non-excitabile cell. The specific identification of an RyR-2-related transcript encoding a protein sequence with 99.7% identity to that of the RyR-2 indicates that expression of the RyR-2 protein is conserved in this non-excitabile kidney cell line. Furthermore, these results suggest that the RyR-2 may therefore play a fundamental role in intracellular calcium homeostasis in this cell type. The observation of a short, ~2.5-kb transcript of RyR-1 in brain (28) led us to speculate on the possible existence of a comparable short RyR-2 transcript. However, we were unable to detect a short transcript in Northern blots of brain and kidney (data not shown). In addition, the 5'-RACE experiments performed using brain and kidney mRNA suggest that the RyR-2 transcript in these tissues extends >7 kb from the carboxyl-terminal stop codon (Fig. 3) and are therefore not consistent with the existence of a short RyR-2 transcript in these tissues. Finally, the presence of a native, full-length, and pharmacologically active RyR was demonstrated in our observation of specific binding to a high M_r protein by the RyR-2 antibody (Fig. 4) and also by the specific binding of kidney microsomes to [3H]ryanodine (Table I). Overall, our results provide clear evidence for the expression of RyR-2 in kidney and a kidney epithelial cell and suggest this isoform may mediate the ryanodine- and caffeine-sensitive intracellular Ca^2+ store that has been shown to be regulated by vitamin D metabolites in cultured kidney proximal tubules (33).

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Table I

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<tr>
<th>Tissue</th>
<th>Binding</th>
<th>n</th>
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<tbody>
<tr>
<td>Whole brain</td>
<td>158 ± 54</td>
<td>6</td>
</tr>
<tr>
<td>Cardiac muscle</td>
<td>408 ± 108</td>
<td>4</td>
</tr>
<tr>
<td>Kidney</td>
<td>12.6 ± 4.6</td>
<td>6</td>
</tr>
<tr>
<td>Liver</td>
<td>4.2 ± 3.2</td>
<td>6</td>
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FIG. 3. 5'-RACE with brain and kidney mRNA. Products from 5'-RACE (A) and subsequent PCR using RyR domain-specific primers (B, 831 bp; C, 1365 bp; D, 1096 bp), using brain and kidney mRNA, were analysed on a 1% agarose gel. A control reaction without reverse transcriptase was performed with kidney mRNA (Kidney-RT). Below is a schematic showing the location of the various regions defined by the PCR primers (for coordinates see “Experimental Procedures”). A single band of the expected size was amplified for all the regions with both brain and kidney.

FIG. 4. SDS-PAGE and immunoblot analysis of rabbit kidney microsomes. Panel A, skeletal muscle (lane 1), brain (lane 2), cardiac muscle (lane 3), and kidney (lane 4) microsomes (50 μg of protein) were electrophoresed through a 5% SDS-polyacrylamide gel and stained with Coomassie Brilliant Blue R250. Panels B and C show identical protein transfers probed with purified skeletal muscle RyR antiserum RyR1 (panel B) and with RyR-2 peptide antiserum pAb129 (panel C).

DISCUSSION

Many electrophysiological and pharmacological studies in disparate cell types have provided consistent evidence for ryanodine- and caffeine-sensitive intracellular calcium stores, consistent with the levels reported in previous studies (7, 22). Although relatively low, a significant level of specific binding (12 fmol/mg) was reproducibly observed with kidney microsomes, consistent with the low level of RyR-2 detected by immunoblot analysis (Fig. 4). The ryanodine binding to liver microsomes under the same conditions was 4.2 fmol/mg, suggesting the kidney binding was specific and significant. No specific binding occurred in the absence of micromolar calcium in the binding assays (data not shown), in accord with previous observations of the properties of ryanodine binding to excitabile tissues. These observations confirm that the native, functional ryanodine receptor is expressed in the rabbit kidney.
REFERENCES

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