The N-methyl-D-aspartate (NMDA) receptor is a glutamate-gated cation channel prevalent in the postsynaptic membranes of central nervous system neurons. The neurotransmitter receptor complex is thought to represent a tetramer where variable NR2 or NR3 polypeptides form heteromeric assemblies with an obligatory NR1 subunit. Recently, we showed that cardiac myocytes from perinatal rats transiently express the NMDA receptor subunit NR2B, the function of which in heart is unknown. To characterize the cardiac NR2B protein, we determined its subcellular distribution and specific molecular interaction partners. By immunostaining of rat heart tissue slices and acutely dissociated cardiac myocytes, the NR2B antigen was localized at the sarcomeric Z-bands. Using immunoprecipitation of detergent-solubilized NR2B protein and subsequent analysis employing matrix-assisted laser desorption/ionization time-of-flight mass spectrometry, ryosidine receptor 2 was identified as a molecular interaction partner of the cardiac NR2B polypeptide. Differences in antibody recognition indicate that the cardiac NR2B polypeptide carries a structurally altered C terminus as compared with the NR2B variant prevalent in central nervous system. Based on its localization and protein interaction, the function of cardiac NR2B protein may relate to mechanosensitivity or play a role in the regulation of the contractile apparatus of neonatal heart.

The NMDA receptor is a subtype of the excitatory glutamate receptor family prevalent in mammalian central nervous system. After activation by glutamate binding, the functional NMDA receptor complex forms an ion channel which permits the influx of Na$^+$ and Ca$^{2+}$ into the cell. The NMDA receptor complex is thought to represent a tetrameric assembly of the subunits NR1, NR2A-D, and NR3A-B (1–3). Members of the NR2A-D subunits as well as some NR1 splice variants use their cytoplasmic C-terminal domains to interact with a variety of intracellular proteins including the cytoskeletal protein α-actinin (4) and, via their PDZ domains, the postsynaptic density proteins PSD-95 and SAP-102 (5–8). In the central nervous system, formation of functional NMDA receptors requires the presence of at least one NR1 subunit in complex with at least one of the NR2 subunits (9, 10). In contrast, NR3 subunits act as regulators that decrease channel currents in NR1/NR2 heteromeric receptors but are not able to form ion channel complexes (2, 3, 11). In the mammalian central nervous system, developmental and regional variations in NMDA receptor subunit composition result in altered channel properties, including differences in drug sensitivity and channel conductance (12).

As evident from developmental distribution studies, the NR1 subunit appears to be uniformly expressed throughout the brain starting early in development. In contrast, the different NR2 and NR3 subunits are strictly regulated, both developmentally and spatially (11, 13). NMDA receptors have been implicated in learning and memory by pattern formation and long term potentiation (14) but are also associated with excitotoxic cell death of neurons during neurodegeneration and stroke (15–17).

While the developmental and regional distribution of NMDA receptor subunits in the central nervous system has been extensively investigated, little is known about the expression of NMDA receptor subunits in non-neuronal tissues. Recently, evidence has accumulated that glutamate receptors may also possess important functions other than synaptic transmission of neuronal impulses in extraneuronal tissues such as lung (18) or pancreas (19). In a previous study, we found NR2B protein to be transiently expressed in rat heart during perinatal development. Here, the NR2B polypeptide was located to sarcomeric Z-bands in both the neonatal heart ventricle and acutely dissociated cardiac myocytes. In contrast to the aorticopulmonary septum and conotruncal cushions (20), cells forming the NR2B expressing ventricle are not of neural crest origin. While the NR2B subunit is known to functionally depend on co-expression with the NR1 subunit, we previously failed to detect either NR1 antigen or transcripts as analyzed by Western blot and RT-PCR, respectively (21). Thus, an identification of potential interaction partners of the NR2B polypeptide was attempted in neonatal rat heart. By immunoprecipitation and matrix-assisted laser desorption/ionization time of flight mass spectrometry-

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Oligonucleotides used for RT-PCR amplification of the entire coding region of NMDA receptor subunit NR2B

| Sequence | Position | Size   |
|----------|----------|--------|
| NR2B I forward: | 5’-CGTACGGCAAGGAGGCTACGACGTCG-3’ | nt324-1624 | 1300 bp |
| NR2B I reverse: | 5’-GGATTACGGCTCTCTCCCGGACAA-3’ | | |
| NR2B II forward: | 5’-TTCGGAGACAGTGAGGACGAGGGAA-3’ | nt1532-2187 | 655 bp |
| NR2B II reverse: | 5’-GGACCGAGATACCCAAATGGCGCTG-3’ | | |
| NR2B III forward: | 5’-TGTTGCACCTTCCTGCTCTTACAG-3’ | nt1982-2633 | 651 bp |
| NR2B III reverse: | 5’-ATGTCGATGGATAAGCTGCCCG-3’ | | |
| NR2B IV forward: | 5’-CGTACGGCAAGGAGGCTACGACGTCG-3’ | nt2498-3554 | 1056 bp |
| NR2B IV reverse: | 5’-GGATTACGGCTCTCTCCCGGACAA-3’ | | |
| NR2B V forward: | 5’-CGTACGGCAAGGAGGCTACGACGTCG-3’ | nt3456-4834 | 1378 bp |
| NR2B V reverse: | 5’-GGATTACGGCAAGGAGGCTACGACGTCG-3’ | | |

**Complexes of NR2B and Ryarodine Receptor 2 in Myocard**

**Generation and Use of NR2B Antibodies—**Antibodies were raised in rabbits (Chinshilla Bastard) against a synthetic peptide representing the N-terminal region of subunit NR2B (50-EPRISENKTDDEEPGYC-55) as described before (21, 22). Numbers indicate the position of the first and last amino acid in the mature subunit sequence. The underlined cysteine residue was added to the C terminus for coupling to keyhole limpet hemocyanin (Calbiochem, Bad Soden, Germany) or EAH-Sepharose (Amersham Biosciences, Freiberg, Germany) with the heterobifunctional cross-linker sulfosuccinimidyl-4-([maleimidomethy]cyclohexane-1-carboxylate (sulfos-SMCC, Pierce). Peptide-specific antibodies were purified by affinity chromatography on immobilized peptides to sulfos-SMCC activated EAH-Sepharose. A polyclonal anti-NR2B antibody from rabbit recognizing a C-terminal epitope consisting of 19 amino acids was purchased from Santa Cruz, CA.

**Reverse Transcriptase PCR—**Total RNA from heart was reverse transcribed into cDNA using the Superscript II reverse transcriptase (Invitrogen, Eggenstein, Germany) and random hexamer primers. Aliquots of the cDNA obtained were amplified by PCR performed in 15 µl Tris (pH 8.3), 2 mM MgCl2, 50 µM KCl, 125 µM concentration of each deoxynucleoside triphosphate, and 10 pmol of each oligonucleotide obtained in Table I (all purchased from MWG, Munich, Germany). Reaction conditions were as follows: heating to 94 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 2 min. The cycles were performed 30

**Preparation of Membrane Proteins and Immunoprecipitation—**For preparation of membrane proteins, heart and cerebral cortex tissue from Wistar rats (P 5 or P 74) was dissected and homogenized for 3 min in buffer HP1 (0.32 mM sucrose, 20 mM Tris-HCl (pH 7.4), 5 mM Mg-phenanthroline, 2 µg/ml E64, 10 µg/ml pepstatinA, 10 µg/ml leupeptin, 30 µg/ml Pefabloc, 2.5 mM EDTA, 2.5 mM EGTA) (Sigma) using a glass homogenizer. The homogenate was centrifuged (1000 x g; 5 min), and the low speed supernatant was pelleted at 20,000 x g for 20 min. The resulting crude membrane fraction was resuspended in hypotonic buffer HP2 (HP1 without sucrose), homogenized for 30 s, and recentrifuged at 20,000 x g for 20 min. The final pellet was resuspended in HP2 at a final protein concentration of 5–10 mg/ml. Immunoprecipitations were performed as described previously (22). In short, membrane proteins were solubilized from crude membrane fractions by incubation with 1% Triton, 0.5% sodium deoxycholate in 50 mM Tris-HCl (pH 7.0) for 1 h at 30 °C followed by centrifugation at 100,000 x g for 30 min. Solubilized proteins were reacted with affinity-purified antisem against NR2B or NR1 or a monoclonal antibody against ryarodine receptor 2 (Alexis, Cologne, Germany) (antibody concentration: ~20 µg/ml). Antibody-coupled protein complexes were precipitated using protein A-conjugated Sepharose beads. Protein complexes were precipitated (3000 x g; 5 min), washed three times with 50 mM Tris-HCl containing 1.5 mM NaCl, and resuspended in Laemmli buffer containing 2 mM urea. All steps were carried out at 4 °C.

**SDS Electrophoresis and Western Blotting—**Protein concentrations were estimated by the method of Bradford (25) using BSA as a standard. Membrane preparations or immunoprecipitates were incubated (56 °C; 20 min) in Laemmli sample buffer containing 2 mM urea. Proteins (50 µg of protein per lane) were separated by SDS-polyacrylamide gelelectrophoresis on 6% polyacrylamide slab gels under reducing conditions. Proteins were visualized with SyproRuby™protein stain or transferred onto nitrocellulose for subsequent in-gel digestion or Western blot analysis, respectively. Following SDS gelelectrophoresis, proteins were transferred on nitrocellulose using a semi-dry blotting apparatus (Biometra, Göttingen, Germany). Protein transfer was controlled by reversible staining with Ponceau S. Blots were blocked with 5% BSA in PBS containing 5% nonfat dry milk powder and 0.1% Triton X-100 for 1 h. Incubation with affinity-purified polyclonal antisem K9 (10 µg/ml) or antibodies against the ryarodine receptor 2, NR1, and NR2B proteins (dilution 1:500) was carried out overnight in 0.5% Blotto at 4 °C, followed by three washing steps with PBS. Antibodies were detected by fluorescence using Cy5-conjugated goat anti-rabbit IgG.
In heart tissue from adult rats, antibody yielded an immunosignal in the same molecular mass range in 180 kDa in cerebral cortex preparations. Only K39 and the N-terminal right panel) of NR2B protein. All antibodies recognized specific bands of 27–76 that is situated within the epitope formed by amino acids 27–76 that is situated within the epitope formed by amino acids 27–76 that is situated within the epitope formed by amino acids 27–76 that is situated within the epitope formed by amino acids 27–76 that is situated within the epitope formed by amino acids 27–76.

To corroborate these results, we performed peptide mass fingerprinting of a K39 immunoprecipitate of the 180-kDa antigen from rat heart. Following SDS electrophoresis and in-gel digest, an informative peptide fragment pattern was obtained and sub-

Complexes of NR2B and Ryanodine Receptor 2 in Myocard

FIG. 1. Antibody binding to NR2B antigen from central nervous system and heart. A, transmembrane topology and antibody epitopes of the NR2B polypeptide. The NR2B protein is characterized by an extracellular N terminus that is followed by three transmembrane domains. The ion pore permeable for Na\(^{+}\) and Ca\(^{2+}\) is formed by a loop of the polypeptide chain. The glutamate binding pocket is composed of two extracellular domains. The C terminus is intracellular. The epitopes of the antibodies used in this study are marked. B, membrane proteins from cerebral cortex from P 12 rats and heart from adult and P 4 rats were subjected to Western blot analysis using antiserum K39 (left panel), an antibody against the very N terminus (amino acids 27–76; middle panel), and the C terminus (amino acids 1436–1457; right panel) of NR2B protein. All antibodies recognized specific bands of 180 kDa in cerebral cortex preparations. Only K39 and the N-terminal antibody yielded an immunosignal in the same molecular mass range in preparation from neonatal heart (P 5). In heart tissue from adult rats, (1:200; Dianova, Hamburg, Germany) for 30 min, followed by extensive washing with PBS. Finally, fluorescence was visualized using the Storm Fluorescence Imager (Amersham Biosciences).

Peptide Mass Fingerprinting—After SDS-gel electrophoresis and SyproRuby\textsuperscript{TM} staining, protein gels were visualized using UV light and bands of interest excised. Gel bands were crushed into 1 mm\(^3\) pieces and destained using 50% acetonitrile, 25 mm ammoniumhydrogencarbonate. Following complete drying in a vacuum centrifuge, gel pieces were resuspended in 50 \(\mu\)l of 25 mm ammonium carbonate containing 0.2 \(\mu\)g/\(\mu\)l sequencing grade trypsin (Promega, München, Germany). After enzymatic digest at 37 °C overnight, the resulting peptide fragments were eluted from the gel by adding 5% acetic acid, 50% acetonitrile and sonication (20 min; room temperature). Supernatants were collected, and elution was repeated four times. Finally, supernatants were dried completely in a vacuum centrifuge, and peptides were resuspended in 10 \(\mu\)l of \(H_2O\) containing 0.1% trifluoroacetic acid. For MALDI-TOF-MS, digests were diluted with \(H_2O\) (1:10 to 1:50). The sample (0.5 \(\mu\)l) was mixed with 0.5 \(\mu\)l of 3-hydroxy-\(\alpha\)-cyanocinnamic acid matrix (saturated solution in 30% acetonitrile, 0.1% trifluoroacetic acid), dotted onto a steel target, and air-dried. MALDI-TOF analysis was performed on a Biflex III (Bruker Daltonics, Bremen, Germany) in the reflector mode. Desorption of the samples was carried out using a nitrogen laser (337 nm), and the probes were accelerated with 19 kV after a delay of 3500 ns. For one sample spectrum, 25–50 individual spectra of the respective probes were performed and averaged. A peptide standard mix served as an external calibration. The masses obtained by MALDI-TOF-MS were used for a peptide mass fingerprint search. Experimentally obtained masses were aligned to theoretical peptide masses for trypsin digestion of all proteins available in the NCBI data base. For this purpose, we employed the search program MS-Fit, available at prospector.ual.edu (26). Data base search settings were as follows: protein mass (apparent molecular mass of the protein in SDS-PAGE according to the molecular mass of the standard proteins), 7.50 kDa of the respective cut out protein band; species, rodentia; mass tolerance, 0.05%; maximum number of missed cleavages, 1; standard modifications, none; minimum number of peptides required for match, 4; data base, NCBI Protein.

RESULTS

Immunological Variations of NR2B Antigen in Rat Heart and Central Nervous System—During perinatal development, the NMDA receptor subunit NR2B is detectable in rat heart (21) using an affinity-purified rabbit antiserum (K39) directed against an extracellular epitope formed by amino acids 439–454 that is adjacent to transmembrane region 1 (TM1) (Fig. 1A). In contrast, studies using antibodies directed against the very C-terminal part of NR2B failed to detect the corresponding antigen in rat heart at any age analyzed (27–29). To elucidate these apparently contradictory reports, we employed three antibodies defining distinct epitopes of NR2B. In Western blot analysis, affinity-purified antiserum K39 stained a 180-kDa protein present in both heart and cerebral cortex tissue. In the immunostains from central nervous tissue, a microheterogeneity of the NR2B antigen was observed. An identical result was reproducibly obtained in with an antibody directed against an epitope formed by amino acids 27–76 that is situated within the N-terminal extracellular domain of NR2B. In contrast, an antibody against the C-terminal part of NR2B detected this antigen in cerebral cortex but not in heart (Fig. 1B). Numbers indicate the first and last nucleotide of the mature transcript. Sizes of a molecular mass marker are given in bp. All fragments revealed the expected sizes as predicted from gene bank data.

none of the antibodies recognized a corresponding antigen. Depending on the antibody used, the experiment was performed 3–12 times, C, analysis of the entire coding sequence of NR2B transcript by RT-PCR. RNA from neonatal rat heart (P 4) was prepared and subjected to reverse transcription. The coding sequence of the NR2B transcript was amplified in five overlapping fragments. Numbers indicate the first and last nucleotide of the mature transcript. Sizes of a molecular mass marker are given in bp. All fragments revealed the expected sizes as predicted from gene bank data.
TABLE II
Peptide mass fingerprints of proteins precipitated from neonatal heart
by anti NR2B antiserum K39

| Sample band | Masses (m/z) | Candidate proteins |
|-------------|-------------|--------------------|
| BSA         | 927.570     | BSA                |
|             | 1001.640    |                    |
|             | 1249.770    |                    |
|             | 1305.880    |                    |
|             | 1402.470    |                    |
|             | 1464.890    |                    |
|             | 1479.970    |                    |
|             | 1505.020    |                    |
|             | 1946.080    |                    |
| No. 1       | 1010.2318   | NMDA receptor subunit 2B, |
|             | 1038.9552   | rat RNA polymerase 1-4   |
|             | 1094.1754   | (194-kDa subunit), nitric- |
|             | 1104.2162   | oxidase synthase, rat    |
|             | 1155.3628   | voltage-gated sodium     |
|             | 1180.0897   | channel, type VIII (α-   |
|             | 1210.9887   | polypeptide)             |
|             | 1261.2813   |                    |
|             | 1284.7112   |                    |
|             | 1309.9639   |                    |
|             | 1337.9856   |                    |
|             | 1346.9864   |                    |
|             | 1486.2567   |                    |
|             | 1507.4509   |                    |
|             | 1566.8834   |                    |
|             | 1804.9401   |                    |
|             | 2391.7336   |                    |
|             | 2700.3953   |                    |
|             | 3336.0561   |                    |
| No. 6       | 937.4432    | Spectrin, rat ryanodine |
|             | 971.5962    | receptor cardiac, rodent |
|             | 993.1034    | sacsin, rodent myosin   |
|             | 1002.0651   | actin cross-linking family |
|             | 1034.4806   | protein 7, mouse IP3    |
|             | 1045.5512   | receptor, mouse dynein.  |
|             | 1055.4878   |                    |
|             | 1146.5867   |                    |
|             | 1187.3013   |                    |
|             | 1291.6429   |                    |
|             | 1307.8300   |                    |
|             | 1494.3620   |                    |
|             | 1526.6619   |                    |
|             | 1650.8332   |                    |
|             | 2062.9863   |                    |
|             | 2212.8385   |                    |
|             | 2327.9869   |                    |
|             | 2391.7336   |                    |
|             | 1804.9401   |                    |

Fig. 2. Immunodetection of NR2B in rat heart tissue slices. A, heart tissue (P 5) stained with TRITC-conjugated phallodin. The striated staining pattern indicated that this area contained cardiac myocytes. B, heart tissue (P 5) stained with anti-NR2B antiserum. Significant NR2B immunosignals were detectable in juvenile heart slices. C, heart tissue (P 5) stained with anti-NR2B antiserum preincubated with antigenic peptide prior to staining. The NR2B signal could be blocked completely by antigen competition. D, enlargement of the indicated area of picture B, NR2B immunosignal showed a striated pattern (arrows). E, adult heart tissue stained with TRITC-conjugated phallodin. F, adult heart tissue stained with anti-NR2B antiserum. In adult tissue no NR2B immunosignal was detectable. Magnification bar: A–C, E, F, 100 μm; D, 50 μm.
analyzed by confocal microscopy, all myocytes showed significant immunosignals for NR2B. In addition to a weak intracellular reticular staining pattern, an intense accumulation of antigen was observed at distinct sarcomeric bands (Fig. 3, A, D, and G). By immunological co-localization with α-actinin, a protein forming the Z-bands, these structures could be confirmed to represent the Z-bands (Fig. 3C). We also found a less defined co-distribution of NR2B antigen with filamentous actin (Fig. 3F). In contrast, the reticular staining pattern of tubulin and the striated pattern of NR2B antigen was clearly distinct and did not overlap (Fig. 3I).

**Different Molecular Interaction Partners of NR2B in Cerebral Cortex and Heart**—To identify proteins interacting with the cardiac NR2B polypeptide, detergent extracts from membranes from heart and cerebral cortex were subjected to immunoprecipitation using affinity-purified anti-NR2B antisera. The protein complexes precipitated were separated on SDS gels, stained with SyproRuby™, and visualized under UV light. In particular in the higher molecular mass range, we found significant differences in the composition of protein complexes precipitated from either from heart or from central nervous system preparations. In samples from cerebral cortex, signals were prominent in molecular mass ranges of 180 kDa (Fig. 4A: band 1) and 120 kDa (Fig. 4A, band 5), most likely representing the NR2B and NR1 proteins, respectively. In addition, we observed strong signals at molecular masses of 170 and 150 kDa (Fig. 4A, bands 2 and 3). Similar to cerebral cortex, immunoprecipitates from juvenile heart contained proteins of 180 kDa (Fig. 4A, band 1), 170 and 150 kDa (Fig. 4A, bands 2 and 3). However, the 120-kDa protein apparent in the central nervous system preparation could not be detected in juvenile heart. Co-precipitates from heart contained proteins of 130 kDa (Fig. 4A, band 4) and >205 kDa that were absent from cerebral cortex (Fig. 4A, band 6).

**Identification of Ryanodine Receptor 2 as an Interaction Partner of Cardiac NR2B Polypeptide**—To identify the putative interaction partners of the cardiac NR2B polypeptide, the 130-kDa and >205-kDa bands from heart precipitate were excised from the SDS gel and subjected to a trypsin digest. The 180-kDa bands from cerebral cortex and heart, which most likely represented the NR2B polypeptide, served as internal controls. The peptide mass fingerprints resulting from the protein bands analyzed (Fig. 4B) were aligned to theoretical peptide mass fingerprints of proteins listed in the NCBI data base (26). By comparison of the fragment sizes produced by tryptic digest, the 180-kDa protein from heart could be confirmed as NMDA receptor subunit NR2B (Table II), while the 130-kDa protein appeared as a proteolytic product of NR2B (data not shown).

In contrast, analysis of the peptide pattern representing the high molecular mass protein of >205 kDa generated several candidates of different prediction probabilities (Table II), including structural proteins (spectrin, dynein, myosin) and two proteins involved in Ca²⁺ homeostasis (ryanodine receptor 2 and IP₃ receptor). In central nervous system neurons, NMDA receptor subunits form heteromeric complexes that serve as cation channels permeable to Ca²⁺ and Na⁺. Given their roles in cardiac muscular Ca²⁺ regulation, we focused on the ryanodine receptor 2 and the IP₃ receptor as candidate proteins. Immunoprecipitations were performed using the affinity-purified anti-NR2B antisera K39 as the precipitating antibody. The resulting pellets were subjected to Western blot analysis using monoclonal antibodies against IP₃ receptor and ryanodine re-

![Fig. 4. Immunoprecipitation of NR2B protein from juvenile heart and cerebral cortex tissue.](https://example.com/fig4.png)
When anti-NR2B immunoprecipitates from rat heart were analyzed, the anti-IP$_3$ receptor antibody failed to generate any significant immunosignal (Fig. 5D). Western blots reacted with anti-ryanodine receptor 2 antibody, however, produced significant signals with anti-NR2B precipitates from neonatal heart. No ryanodine receptor 2 signal was detectable in precipitates from cerebral cortex or adult heart tissue (Fig. 5A). When the reverse experiment was carried out, using the anti-ryanodine receptor 2 antibody as the precipitating agent and the anti-NR2B antisera K39 as a probe, NR2B protein was detectable only in the anti-ryanodine receptor 2 precipitates from neonatal heart. Precipitates from cerebral cortex or adult heart failed to generate NR2B signals (Fig. 5B). When anti-NR1 antibodies were used for immunoprecipitation as an antibody control, NR1 signals were restricted to detergent extracts and precipitates from cerebral cortex but were absent from those of neonatal heart (Fig. 5C). Neither the anti-NR2B nor the anti-ryanodine receptor 2 antibody were found to produce any immunosignal with anti-NR1 immunoprecipitates from neonatal heart (Fig. 5, A and B). To analyze the subcellular distribution of NR2B and ryanodine receptor 2, cardiac myocytes were double-stained with corresponding antibodies after 36 h in culture. Confocal laser scanning microscopy produced a clearly detectable colocalization of the signals for the NR2B (Fig. 5E) and ryanodine receptor 2 antigens (Fig. 5F), both accumulating at the Z-bands of the sarcomers. In addition, some weaker signals were detectable for both antigens lacking subcellular co-localization (Fig. 5G).

**DISCUSSION**

While the classical hypothesis of synaptic transmission restricts NMDA receptors to the postsynaptic membranes of central nervous neurons, recent evidence indicates that some of the receptor subunits also serve physiological functions in non-neuronal tissues (18, 19, 29, 30). We recently detected a strictly developmentally regulated expression of the NMDA receptor subunit NR2B in embryonic and neonatal rat heart (21). However, the cardiac NR2B antigen differed from the NR2B antigen present in brain; polypeptides from both tissues were recognized by antibodies directed against epitopes within the N-terminal domain or adjacent to transmembrane region TM1, whereas the cardiac NR2B variant escaped immunodetection by an antibody directed against an epitope present on the C terminus of the neuronal NR2B polypeptide. Although differences in antigenic properties may be attributed to alternative splicing, transcript variability affecting the C terminus of NR2 subunits has not been observed. While one short alternative exon is known for the N-terminal part of NR2B (31), our data suggest structural variations in the C terminus to underlie these differences in antibody binding. In particular, the NR2B transcripts analyzed revealed no sequence heterogeneity regardless of their origin from heart or central nervous system.
and no significant differences have been observed in the electrophoretic mobility behavior of NR2B antigens from these tissues. Most likely, this phenomenon may be attributed to masking of the C-terminal antibody binding site by posttranslational modifications in heart, e.g. phosphorylation of the C-terminal domain of NR2B (32). This aspect is, however, in need of further investigation.

The NR2B antigen detectable in heart was not found on the cell surface of myocytes but accumulated apparently intracellularly at the Z-bands of the cardiac sarcomers. This accumulation at the Z-bands was fully consistent with the previous observation that, in striatum, NR2B tightly interacts with the systolic contraction phase of the myocyte (35). This function of further investigation.

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