A Novel Sulfonylurea Receptor Family Member Expressed in the Embryonic Drosophila Dorsal Vessel and Tracheal System*

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Igor Nasonkin‡§, Ayfer Alikasifoglu‡§, Catherine Ambrose‡, Paula Cahill‡, Michael Cheng‡, Agit Sarınikai‡, Marie Egan‡, and Pamela M. Thomas‡**

From the ‡Department of Pediatrics and Communicable Diseases, University of Michigan, Ann Arbor, Michigan 48109-0646, and the §Department of Pediatrics, Yale University, New Haven, Connecticut 06520

Sulfonylurea receptors (SURx) are required subunits of the ATP-sensitive potassium channel. SURx alone is electrophysiologically inert. However, when SURx is combined with an inward rectifier Kir6.2 subunit, ATP-sensitive potassium channel activity is generated. We report the identification, characterization, and localization of Dsur, a novel Drosophila gene that is highly related to the vertebrate SUR family. The Dsur coding sequence contains structural features characteristic of the ABC transporter family and, in addition, harbors 1.7 kilobases of a distinctive sequence that does not share homology with any known gene. When Dsur alone is expressed in Xenopus oocytes glibenclamide-sensitive potassium channel activity occurs. During Drosophila embryogenesis, the Dsur gene is specifically expressed in the developing tracheal system and dorsal vessel. Studies of the Drosophila genome support that only a single Dsur gene is present. Our data reveal conservation of glibenclamide-sensitive potassium channels in Drosophila and suggest that Dsur may play an important role during Drosophila embryogenesis. The lack of gene duplication in the Drosophila system provides a unique opportunity for functional studies of SUR using a genetic approach.

ATP-sensitive potassium (K_{ATP}) channels serve as a vital link between cellular metabolism and membrane electrical activity in excitable cells, including those of the pancreatic islets, cardiac, smooth, and skeletal muscle, neurons, and epithelia (1). These channels are involved in a variety of important processes, such as control of insulin secretion from pancreatic islet beta cells, the response of cardiac and cerebral cells to ischemia, regulation of vascular smooth muscle tone, and modulation of transmitter release at brain synapses. The pharmacologic characteristics of K_{ATP} channels include blockade by the sulfonylurea class of agents, such as glibenclamide (1).

At the molecular level, a complex of two subunits, the sulfonylurea receptor (SURx) (2) and the inward rectifier Kir6.2 (3), form the K_{ATP} channel. Both subunits are required, as individually neither intact subunit is able to produce a functional K_{ATP} channel and assemble as an octamer in a 4:4 stoichiometry (4). The SURx is a member of the ATP-binding cassette family (2). Characteristic of this family is the presence of two conserved nucleotide binding folds (NBF), each with Walker A and B subsequences forming the nucleic acid binding pocket (5, 6), the ABC signature sequence, and the NBF-2 TIAHRL motif (7). Alignment of SURx sequences with other ATP-binding cassette family members reveals the greatest similarity with members of the multidrug resistance-associated protein (MRP) group (8). In addition to the MRP and SUR genes, this group includes the yeast cadmium factor YCF1, the liver canalicular multispecific organic anion transporter, and the rabbit epithelial basolateral chloride conductance regulator genes (8).

Three vertebrate isoforms of SURx, to which the presence of tissue-specific K_{ATP} channels with different pharmacological sensitivities may be attributed, have been identified. In combination with a Kir6.2 subunit SUR1 forms the pancreatic (3), SUR2A the cardiac (9), and the splice variant SUR2B (10) the smooth muscle types of K_{ATP} channels.

As with many other classes of ion channels, mutation of K_{ATP} channels has been found to be associated with human disease. Loss of function of the pancreatic islet K_{ATP} channel, because of mutation of either the SUR1 or Kir6.2 subunit (11–14), has been demonstrated to lead to persistent hyperinsulinemic hypoglycemia of infancy, an autosomal recessive disorder characterized by unregulated insulin secretion and severe hypoglycemia (15). Disease phenotypes have not yet been assigned to the other K_{ATP} channels. However, based on their importance in the physiology of cardiac and smooth muscle and neurons, one can speculate that abnormalities of them may contribute to disease states.

Conservation of several channel types, but not the K_{ATP} channel, has been demonstrated in invertebrate species. The study of Drosophila has been an effective approach for the identification and characterization of the structure, function, and gene regulation of other potassium channels (16–18). Here we report the identification and characterization of a novel member of the SURx family, Dsur, and thereby demonstrate conservation of glibenclamide-sensitive potassium channel activity in a lower organism. Dsur is expressed specifically in the developing Drosophila dorsal vessel and tracheal system during embryogenesis. The implication of Drosophila as a model system for structural and functional studies of the K_{ATP} channel is discussed.
EXPERIMENTAL PROCEDURES

Isolation of Dsur cDNA

Three expressed sequence tags (ESTs) that contained novel Drosophila ABC transporter NBF-2 region motifs were identified. Gene-specific primers were used to amplify 400–500-base pair fragments located at the 5' end of each EST clone to create probes for library screening. Approximately 10^6 phage plaques from a Drosophila melanogaster embryonic cDNA library were screened (5'-STRETCH cDNA library, CLONTECH) using standard methods. The full-length SUR homolog was isolated using a combination of several rounds of overlapping library and PCR screening. The 5' end of the cDNA was isolated using the rapid amplification of cDNA ends strategy (Life Technologies, Inc.) according to the manufacturer's recommendations. The final sequence was confirmed as a single message by the sequencing of full-length cDNA products amplified by reverse transcriptase-PCR from Drosophila embryonic mRNA. Sequence analyses, comparison, and alignments were performed using the BCM Search Launcher interface (19) and Lasergene software.

Low Stringency Southern Blot Analysis

Low stringency conditions included a hybridization and wash temperature of 55 °C, wash buffer of 2× SSC, 0.1% SDS, and a final wash of 0.5× SSC, 0.1% SDS. The probe was located between nucleic acids 2131 and 763, relative to the ATG translation initiation codon. Nucleotide binding fold regions are boxed, with the Walker A (GXXGXXG) sequences, ABC transporter signature sequences, and Walker B sequences indicated in bold with a dotted underline and the NBF-2 TIAHRL motif marked with a double underline. The regions in gray are unique to the Dsur sequence. Predicted transmembrane domains are underlined. Single-letter amino acid codes are used.

Electrophysiological Studies

Whole Cell Currents—Stage V–VI Xenopus laevis oocytes were isolated and injected with RNA as described previously (20). The two-electrode voltage clamp technique was used, and recordings were obtained from currents elicited by 20-ms test pulses from -200 to 40 mV in 20-mV increments (V_hold = -65 mV). Microelectrode pipettes (Kimax-51, Kimble Products) typically had resistances of 0.5–2.0 MΩ when filled with 3 M KCl solution. Oocytes were bathed with a control solution containing (in mmol/liter): 105 NaCl, 1 MgCl_2, 1 CaCl_2, and 5 HEPES, pH 7.4. The initial experimental protocol was used to determine whether the current generated was a barium (Ba^{2+})-sensitive potassium current. It consisted of a 10-min equilibration period in control solution, impalement of the cell, a 5-min control period, a 3-min exposure to 2 mM Ba^{2+}, wash in control solution, and a recovery period. In subsequent experiments, the protocol consisted of a 10-min equilibration in control solution, impalement of the cell, a 5-min control period, a 15-min exposure to 500 μM glibenclamide in control bath solution followed by a 3-min exposure to 2 mM Ba^{2+}-containing bath solution, and a 5-min wash in control solution.

Single-channel Currents—Oocytes were placed in a hypertonic solu-
body. Homozygous mutant embryos were identified by the absence of the 3′-noncoding region. Probe detection was performed through use of MDR1-Human, M59076; P-glycoprotein49-Drosophila, YCF1-Yeast, U66261, P-glycoprotein65-Drosophila, M59077; multidrug resistance (MDR1-Human), M14578.

Chromosomal Localization of Dsur

Filter copies of the arrayed P1 library were obtained (Genome Systems, St. Louis, MO) and hybridized with a Dsur cDNA-specific probe, which had been proven to be single copy in the genome by genomic Southern blot. To confirm the result obtained by hybridization against the array, the identified P1 clones were obtained (Genome Systems) and prepped, and the presence of each gene was confirmed by PCR amplification using primers previously demonstrated to be Dsur-specific. Direct sequence analysis of the PCR amplicons confirmed the presence of the Dsur sequence.

In Situ Hybridization and Immunohistochemistry of Whole-mount Embryos

Preparation, in situ hybridization, and immunohistochemistry of whole-mount embryos were performed as described (21). Digoxigenin-labeled RNA probes were synthesized in vitro according to the manufacturer’s recommendations (Roche Molecular Biochemicals) using 1.2 kilobases of Dsur template that extended from 5455 base pairs through the 3′-noncoding region. Probe detection was performed through use of an alkaline phosphatase enzyme conjugated to anti-digoxigenin antibody. Homozygous mutant embryos were identified by the absence of β-galactosidase enzyme staining, which was carried on a balancer chromosome lacZ insert.

RESULTS

Isolation of Dsur—We used an approach based on computer searching of the EST data base to identify a Drosophila gene that is highly related to the vertebrate SUR family. Three novel ESTs were identified. However, only one retained consistent homology with SUR sequences through its 5′ end, and we named this gene Dsur. The full-length Dsur cDNA contains an open reading frame of 2167 amino acids (Fig. 1), with a predicted molecular mass of 241,858 daltons. Northern analysis performed on Drosophila embryonic mRNA revealed a message size of approximately 6.5 kilobases, supporting the hypothesis that the full-length cDNA had been isolated (data not shown). The methionine chosen as the translation start site met the Kozak criteria for having the ATG codon flanked by a favorable context for initiation, stop codons in all three reading frames 5′ to this location, and no strong upstream ATG codons in any frame (22, 23). A consensus polyadenylation site (AATAAA) is located 205 base pairs after the stop codon.

Structural Features of Dsur—Similar to its vertebrate counterparts, Dsur contains structural features characteristic of the ABC transporter family, including the presence of two NBF groups each with a Walker A and B consensus sequence, the presence of two NBF regions each following a transmembrane domain between the NBF domains are present in each of the 15 membrane-spanning regions, in a nine + six pattern, with the two cytoplasmic NBF regions each following a transmembrane domain (25). This is consistent with the structure predicted for the MRP subfamily of ABC transporters (8).

Two regions of hydrophilic sequence that flank the transmembrane region between the NBF domains are present in Dsur but not in either vertebrate SUR or other ABC transporter sequences (Fig. 1). These unique regions of sequence are contained within the Dsur transcript and do not represent...
either cloning artifacts or retained intronic sequences. This was demonstrated by amplification and sequencing of the full-length Dsur cDNA from Drosophila mRNA template and determination of genomic organization upon comparison of cDNA and genomic sequences. A computer search of public sequence data bases with these unique portions of Dsur does not reveal significant homology with any known sequence, including potassium channel pore sequences. These two regions of unique sequence are responsible for the difference in size of 585 codons between the vertebrate and Drosophila SUR molecules.

To assess the complexity of the Drosophila KATP channel system, we sought other SUR isoforms and an inward rectifier subunit. Neither was detected by computer search of the Drosophila EST data base, PCR screening of subunit. Neither was detected by computer search of the Dro-

SUR system, we sought other using a libraries. Southern blot analysis of Drosophila channel pore regions, or low stringency screening of DNA template using degenerate primers against conserved sequence are responsible for the difference in size of 585 codons between the vertebrate and Drosophila SUR molecules.

Electrophysiological Activity of Dsur—Because we were unable to isolate an inward rectifier subunit sequence from Drosophila and Dsur contains additional sequences beyond that present in vertebrate SUR family members, we postulated that Dsur would be sufficient to generate KATP channel activity. Two microelectrode voltage clamp techniques were used to measure outward whole cell currents from Xenopus oocytes injected with Dsur, human SUR1, or human Kir6.2 cRNA (Fig. 4). In oocytes expressing Dsur, average current was 224 ± 5 nA (n = 12) (Fig. 4A), which is significantly different from the average current of 57 ± 5 nA found in control noninjected oocytes (n = 11, p = 0.001) (Fig. 4D). To determine the amount of whole cell current that was sensitive to sulfonylureas, the oocytes were perfused with a solution of glibenclamide, a sul-

fonylurea agent and prototypical inhibitor of the KATP channel (1). 96.4 ± 0.6% of this Ba2+-sensitive potassium current was found to be irreversibly inhibited by glibenclamide (n = 10). The residual glibenclamide-insensitive current was not different from the base-line current found in uninjected cells (n = 10, p = 0.12) and was neither Ba2+- nor amiloride-sensitive. No statistically significant increase in whole cell current was observed in those oocytes expressing either human SUR1 (n = 8) or Kir6.2 (n = 3) (Fig. 4, B and C). Average whole cell current for oocytes expressing human SUR1 was 87.8 ± 4.5 nA, and for those expressing Kir6.2, the whole cell current was 77.3 ± 7 nA.

Measurement of single-channel currents from inside-out patches, made from Xenopus oocytes expressing the full-length Dsur cRNA, confirmed the whole cell current studies (Fig. 5). In excised patches in the absence of ATP, a low conductance inwardly rectifying potassium channel was observed (n = 3). This channel activity was inhibited by glibenclamide.

Expression and Chromosomal Localization of Dsur—To determine the embryonic expression pattern of Dsur, whole-mount in situ hybridization was performed using a specific cDNA probe (Fig. 6). The very early embryonic expression is consistent with remnant maternal messages. Specific embryonic expression was noted beginning at stage 10 in the dorsal vessel, the Drosophila homolog of the heart and circulatory system, and beginning at stage 15 in the tracheal system (including tracheal pits, trees, and placodes), posterior spiracles, and salivary glands. Once initiated, expression remained pres-

ent throughout embryogenesis. Dsur is expressed in the larval and adult stages, as demonstrated by reverse transcription and polymerase chain reaction amplification of RNA isolated from wild-type Drosophila (data not shown).

Using the arrayed P1 library generated by the Berkeley Drosophila Genome Project, we demonstrated that P1 clones DS07249, DS04407, and DS05801 contain the Dsur sequence, placing it in the 31B1–2 region of the second Drosophila chromosome. Chromosomal deficiencies Df(2L)J2 and Df(2L)J1 span this region (26). As assessed by in situ hybridization, animals with homozygous mutants for either genotype or heterozygous mutants for both genotypes lack the Dsur message, confirming the chromosomal localization of Dsur (data not shown).

DISCUSSION

We have isolated a novel member of the SUR family, Dsur, from the Drosophila genome. Sequence analysis places Dsur into the MRP group of ABC transporters. In general, ABC transporters of various types exhibit the greatest homology in the characteristic and conserved NBF regions. Little or no homology may be present in other regions of the molecules, although the membrane topology is anticipated to be similar
The sequence homology between Dsur and vertebrate SUR family members is not limited to the NBF regions but rather extends the length of the molecule to the amino terminus. Together, sequence analysis results and electrophysiologic characterization support the assignment of Dsur into the SUR family of ABC transporters. These data support the hypothesis that Dsur encodes a glibenclamide-sensitive potassium channel with inward rectification characteristics like those of IK(ATP) generated by the complex of SUR and Kir6.2 subunits (3). Dsur appears novel among SUR family members because expression of it alone in Xenopus oocytes results in measurable channel activity. We cannot discount the possibility that an endogenous protein present in Xenopus oocytes may interact with Dsur and confer the ability for production of channel activity. However, heterologous expression of mammalian SUR alone in Xenopus oocytes results in measurable channel activity. We cannot discount the possibility that an endogenous protein present in Xenopus oocytes may interact with Dsur and confer the ability for production of channel activity. However, heterologous expression of mammalian SUR alone in Xenopus oocytes results in measurable channel activity. We cannot discount the possibility that an endogenous protein present in Xenopus oocytes may interact with Dsur and confer the ability for production of channel activity. However, heterologous expression of mammalian SUR alone in Xenopus oocytes results in measurable channel activity. We cannot discount the possibility that an endogenous protein present in Xenopus oocytes may interact with Dsur and confer the ability for production of channel activity. However, heterologous expression of mammalian SUR alone in Xenopus oocytes results in measurable channel activity. We cannot discount the possibility that an endogenous protein present in Xenopus oocytes may interact with Dsur and confer the ability for production of channel activity. However, heterologous expression of mammalian SUR alone in Xenopus oocytes results in measurable channel activity. We cannot discount the possibility that an endogenous protein present in Xenopus oocytes may interact with Dsur and confer the ability for production of channel activity. However, heterologous expression of mammalian SUR alone in Xenopus oocytes results in measurable channel activity. We cannot discount the possibility that an endogenous protein present in Xenopus oocytes may interact with Dsur and confer the ability for production of channel activity. However, heterologous expression of mammalian SUR alone in Xenopus oocytes results in measurable channel activity. We cannot discount the possibility that an endogenous protein present in Xenopus oocytes may interact with Dsur and confer the ability for production of channel activity. However, heterologous expression of mammalian SUR alone in Xenopus oocytes results in measurable channel activity.

The embryonic expression pattern of Dsur implies a potential role for the sulfonylurea receptor family in cell migration, as the tracheal system is undergoing directed cell migration but...
not cell division during the developmental stages of Dsur expression (29). Indeed, our preliminary analysis of deletion mutants in the region of Dsur reveals normal tracheal cell specification but marked abnormalities in the architecture of the tracheal system, which can be attributed to aberrant cell migration. Branched tubular epithelial structures like the Drosophila tracheal system are common in nature (29) and include the cardiovascular system, lung, and pancreas. Therefore, additional understanding of upstream regulators, downstream effectors, and physiological function of Dsur gained from the study of the Drosophila model system may have much broader implications.

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REFERENCES

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