Gestational Changes in the Uterine Expression of an Inwardly Rectifying K^+ Channel, ROMK (44156)

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Abstract. **We have examined the repertoire and relative expression levels of voltage-gated K^+ channels in timed-pregnant rat uteri. These studies have revealed the gestation-specific and abundant expression of mRNA encoding an inwardly rectifying K^+ channel, ROMK (originally identified in renal outer medulla), within the gravid uterus. Steady-state levels of ROMK transcripts undergo dynamic gestational changes: they are undetectable in virgin uteri, reach a maximum level by Day 12 of gestation, decline thereafter until, by term, they are again undetectable. Kidney cells also express ROMK transcripts at high levels but do not undergo apparent changes during gestation. Molecular analyses (by "rapid amplification of cDNA ends, or "5'-RACE") of the ROMK mRNAs revealed the presence of two alternative-splicing variants which are likely to arise from distinct transcription-start sites within the same gene. Polymerase chain reaction–based assessments of gravid uteri from other species revealed the expression of ROMK transcripts in the myometrium as well. Uterine expression of ROMK therefore represents a generalized phenomenon, characterized by both gestation- and tissue-specific regulation, and the transcription-regulatory mechanisms of this channel protein are potentially complex. From the biophysical properties of this channel *in vitro* and the observed gestational profile, we hypothesize that this channel modulates both the resting membrane potential and cellular excitability of myometrial cells, and in turn contributes to the observed contractile quiescence of the gravid uterus.**

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During pregnancy, the uterine myometrium undergoes significant changes in order to accommodate both gestation and parturition. Myometrial cells become mechanically quiescent with onset of gestation and maintain this state for the extended duration of pregnancy. Approaching term, spontaneous and synchronized uterine contractions rapidly increase, both in frequency and duration, as the system profoundly changes into a highly active state to facilitate parturition. The underlying physiological mechanisms that promote gestational quiescence, and enable the transformation into labor contractility, largely remain unknown. Since voltage-gated ion channels are critical effectors of smooth muscle activities, it is likely that changes in these systems contribute to the pregnancy-associated functional changes in uterine myometrium. Potassium channels are of particular interest in this context because they comprise the most diverse group of ion channels both in terms of structural and biophysical attributes, and are important effectors of smooth muscle functions (1). Recent studies of uterine K^+ channels as potential targets for tocolysis provide additional support that these channels have a prominent role in the control of myometrial membrane potential and excitability, and thereby potentially impact uterine quiescence as well as contractility (2, 3).

Electrophysiological analyses have provided evidence that the myometrial membrane potential, when at rest, is largely dependent upon K^+ permeability (4). Furthermore, pharmacological agents presumably specific for select K^+ channels have significant tocolytic effects on uterine myo-

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metrial contractility, indicating that these channels are important regulatory components during gestation (5). A variety of K^+ current activities have been identified in the pregnant myometrium, some of which functionally contribute to the maintenance of the myometrial resting potential as well as shaping of the action potentials. The structural basis for these current activities have not been previously characterized. In the present studies, we examined the repertoire of voltage-gated K^+ channels in the gravid uterus and identified cDNA fragments belonging to both the inward rectifier and the K_v families of K^+ channel genes. One such channel, ROMK, manifests a tissue- and gestation-specific profile that temporally correlates with the onset of uterine quiescence. Transcriptional regulation of this channel gene is likely to be complex, because, as shown in this report, it manifests in two mRNA forms that are derived from distinct transcription-start sites; one such form is known to be expressed in the kidney, but the other is novel.

Materials and Methods

Tissue Preparation. Timed-pregnant and age-matched virgin Sprague-Dawley rats were sacrificed, and organs were prepared by Zivic-Miller Laboratories (Selienople, PA). All tissues were immediately snap-frozen under liquid nitrogen and shipped on dry ice. Tissues were maintained at −70°C until processed. Tissues were weighed while frozen, further cooled with liquid nitrogen, and then the entire organ was pulverized. Ten volumes of RNA lysis buffer (4.0 *M* guanidine isothiocyanate; 25 m*M* sodium citrate, pH 7.0; 0.5% sarcosyl, and 0.1 *M* 2-mercaptoethanol) were added to frozen pulverized tissues, which were then homogenized with a sterile disposable tissue grinder (Sage Products, Crystal Lake, IL). Homogenized samples were transferred to Falcon No. 2059 test tubes and then frozen at −70°C until used for RNA preparation.

Total RNA Preparation. Frozen tissue lysates were thawed and centrifuged at 10,000*g* for 20 min. Two milliliters of supernatant was layered on a 2.5-ml pad of 5.7 *M* CsCl containing 0.1 *M* EDTA. The samples were centrifuged at 35,000 rpm for 15 hr at 18°C with a Beckman SW 50.1 rotor. RNA pellets were resuspended in water previously treated with diethyl pyrocarbonate (DEPC). An aliquot from each RNA sample was diluted into TE buffer (10 m*M* Tris, 0.1 m*M* EDTA, pH 7.4), and the concentration of RNA was estimated spectrophotometrically. Specific amounts of RNA from each sample were aliquotted into microfuge tubes. Sufficient DEPC water was added to each sample to provide the same RNA concentrations throughout all tubes. RNA samples were stored at −70°C as ethanol precipitates until used for either Northern or RNA dot-blot assays.

Northern and Dot Blot Analyses. For Northern analysis, RNA samples were dissolved into 10 μl of denaturing solution comprising 18.5% formaldehyde (v/v), 20% formamide (v/v), 1× MOPS buffer (20 m*M* 3-[*N*-morpholino]propanesulfonic acid, 10 m*M* sodium acetate, 1 m*M* EDTA, pH 7.0) plus 1 μl of ethidium bromide (400 μg/ml). The samples were heated for 10 min at 65°C and then chilled on ice. Samples were electrophoresed in formaldehyde-agarose gels (1.2% agarose [w/v], 6.6% formaldehyde [v/v] in 1× MOPS buffer). Electrophoresis was carried out in 1× MOPS buffer overnight (0.15 V/cm). RNA was transferred by capillary action onto Nytran filters overnight employing 10× SSC (1.5 *M* sodium chloride, 150 m*M* sodium citrate, pH 7.0) as the transfer buffer. The Nytran filters were then rinsed in 5× SSC, air-dried, then baked at 80°C for 1.5 hr. Utilization of ethidium bromide under the above denaturing conditions provided direct evidence that (i) approximately equal amounts of RNA from different tissues were applied to gels for electrophoresis; (ii) RNA integrity was maintained during sample preparation and electrophoresis; and (iii) the transfer of RNA from gel to filter was complete.

RNA dot blots were performed with a VacuoDot-VS manifold (American Bionetics, Cleveland, OH). Nytran filters were pre-wet with 6× SSC prior to applying samples. RNA samples were resuspended in 100 μl of denaturing solution and then heated at 65°C for 15 min. Denatured RNAs were chilled on ice, diluted with three volumes of 6× SSC, and then loaded into manifold wells. The samples were allowed to drain by gravity for 30 min prior to applying vacuum. Each well was vacuum-washed twice with 400 μl of 6× SSC. Filters were air-dried and then heated at 80°C for 1.5 hr. Filters from both Northern blots and dot blots were incubated for 2 hr at 42°C in prehybridization buffer (50% deionized formamide, 5% Denhardt's solution [2% bovine serum albumin, 2% Ficoll, and 2% polyvinylpyrrolidone], 10% sonicated salmon sperm DNA [2 mg/ml], 5% SDS [w/v], 5× SSC, and 10% dextran sulfate [w/v]).

The [^{32}P]-labeled probe for ROMK1 mRNA was generated by random-primed labeling of the ROMK1 cDNA insert (kindly provided by Dr. Barbara Wible) by using a commercial kit (Amersham Life Sciences, Arlington Heights, IL). After prehybridization was complete, the radiolabeled probe was alkaline-denatured and added directly to the prehybridization buffer. Filters were incubated overnight at 42°C. They were then washed twice in 6× SSC plus 0.5% SDS and once in 1× SSC plus 0.1% SDS for 15 min at room temperature. The final wash was carried out in 1× SSC plus 0.1% SDS for 30 min at 56°C. The washed filters were exposed to Kodak X-Omat RP film at −70°C employing intensifying screens. Autoradiographs were scanned with a Microtek MSF-300GS Image Scanner (Microtek, Torrance, CA) that was linked to a Macintosh IIsi computer. The image generated by the Microtek grayscale scanner was captured by Image Studio (Letraset, Paramus, NJ) and then analyzed for intensity of grays relative to background by Scan Analysis (Biosoft, Milltown, NJ). Quantitation of image intensities on film was carried out at less than maximal densities.

Polymerase Chain Reaction Amplification of Uterine cDNA Fragments. One microgram of total RNA was heat-denatured and then reverse-transcribed into first-strand cDNA by using random hexameric and oligo-d(T) primers, unlabeled deoxynucleotides, and MMLV reverse-transcriptase (Pharmacia, Piscataway, NJ); incubation was conducted at 37°C for 1 hr. The synthesized cDNA products were then used directly as templates for polymerase chain reaction (PCR) amplification. Each PCR reaction incorporated 0.1 μM of each primer, 40 μM of each deoxynucleotide, $MgCl_2$ up to 10 μM (optimal concentration is empirically determined), and 1 unit of *Taq* polymerase (Perkin-Elmer, Norwalk, CT) per 20 μl of reaction volume. Thermocycling condition was typically 1 min at 94°C, 2 min at 60°C, 3 min at 72°C for up to 40 cycles, followed by an additional 10 min at 72°C. Reaction product(s) were enzymatically ligated into a TA-cloning vector (Invitrogen, Carlsbad, CA) and transformed into frozen-competent DH5α bacteria (Life Technologies, Gaithersburg, MD). Resulting clones were broth-amplified and corresponding plasmids were purified through the Wizard MiniPrep (Promega, Madison, WI) columns, and subjected to nucleotide sequencing by using the method of dideoxy chain termination sequencing, using Sequenase II in the presence of 7-deaza-dGTP (US Biochemicals, Cleveland, OH). Attained nucleotide sequences were compared with those of known K^+ channels in the Genbank database.

A pair of degenerate primers was designed based upon regions of nucleotide sequence-homologies shared by members of each K^+ channel gene family. For inward rectifiers, the upstream primer (5′-gccttyctsttctccatngagac-3′) was based upon the sense strand sequences of the channel "pore region," and the downstream primer (5′-ggctcttgckmaggttgc-ccac-3′)was derived from the antisense strand sequences of a conserved region in the carboxyl domain beyond the second putative transmembrane domain (M2). These primers were expected to amplify duplex fragments of about 210 bp in size, and said fragments were expected to contain nucleotide sequences that encode the highly conserved pore region as well as a hydrophobic region reminiscent of inward rectifiers' M2 domain. Similarly, primers were designed from the conserved sequences in the fourth putative transmembrane domain (S4) and pore region of outward rectifiers (upstream, 5′-caagctstcccgccactccaaggg-3′; downstream, 5′-asaccacwgcccaccagaargc-3′), in order to provide products of approximately 250 bp.

To specifically amplify ROMK cDNA fragments from various gestational uteri, oligonucleotide primers were designed to correspond to nt #390–412 (upstream, 5′-gcctttctgttttctctagagac-3′) and nt #636–656 (downstream, 5′-ggctcttcctaagattggccac-3′) of the published rat ROMK-1 cDNA sequence (6). PCR amplicons were subsequently subjected to Southern blotting and hybridization (under very stringent conditions) by a [^{32}P]-labeled, rat ROMK-1 probe.

5′ Rapid Amplification of cDNA Ends (5′ RACE). A ROMK-specific primer (5′-gagaccagccttgccc-gttgccgg-3′) was derived from the antisense strand sequence located approximately 100 bases downstream of the translation-initiation signal. This cDNA region is invariant in all splicing forms of the ROMK mRNA. The primer was used to initiate double-stranded cDNA synthesis with total RNAs of 15-day pregnant uteri. The resulting cDNAs were end-adapted with a duplex adaptor from the Marathon cDNA Amplification Kit (Clontech, La Jolla, CA), and then used as templates in PCR reactions which paired the ROMK-specific primer with an adaptor-derived primer. Amplified products were captured by TA cloning and individually subjected to nucleotide sequencing analysis.

Results

Identification of Uterine K^+ Channels. The majority of known voltage-gated K^+ channel proteins can be categorized, based upon their respectively deduced protein sequences and likely membrane topologies, as members of either "inwardly" or "outwardly" rectifying channel gene families (7). The latter gene family includes all known K_v channels. Because of the presence of significant nucleotide sequence homologies between members of each gene family (8), reverse-transcription polymerase chain reaction (RT-PCR) is capable of specifically amplifying cDNA fragments for the individual members of these gene families. We used this approach to examine the structural repertoire of both families of voltage-gated K^+ channels in the gravid uterus. While outwardly rectifying current activities have been previously described in the uterine myometrium during late-pregnancy stages, inwardly rectifying currents have yet to be detected in this tissue.

A pair of oligonucleotide primers was designed based upon the available sequence homologies between members of each channel gene family. Each primer pair was used in amplification reactions incorporating, as starting templates, single-stranded cDNAs reverse-transcribed from total uterine RNAs of 18-day pregnant and labored rat uteri. PCR reactions corresponding to outward and inward rectifiers provided duplex products with the expected sizes. Amplified products were subcloned into plasmid vectors and individually sequenced. The majority of the attained sequences (59 out of 77) presented the requisite structural features of three known K^+ channels, indicating that this approach toward the identification of uterine K^+ channels was relatively efficacious.

Both 18-day pregnant and labored uteri yielded outward rectifier fragments that were identical with rat $K_v1.5$, a member of the *Shaker* family of voltage-gated K^+ channels (*Shaker* being the prototypical voltage-gated K^+ channel, which was the first to be structurally identified) that renders a delayed rectifier current activity. This channel is widely expressed in a variety of tissue systems and is present in rat uteri at both of the gestational time points examined herein. Uteri at 18 days of pregnancy repetitively yielded two known inward rectifiers: one that bears ex-

tremely high homology (>94% at the nucleotide level) with mouse IRK-1 (9), and the other being identical to rat ROMK-1 (6). The sequence that is highly homologous with IRK-1 bears point mutations at the cDNA level but rendered no change in the deduced protein sequence. Since the corresponding region of the rat IRK-1 cDNA sequence is identical to that of mouse (Chang *et al.,* unpublished observations), the variant that we have identified from gestational uteri is likely to represent a polymorphic form of IRK-1. The uterine sequence that corresponds to ROMK-1 contains point deviations from the known rat cDNA sequence in infrequent instances; such sequence deviations were probably introduced by *Taq* polymerase during the PCR process.

The relative abundance of uterine mRNAs corresponding to these three identified channel sequences was initially assessed by blotting analyses of total RNAs prepared from 18-day pregnant uteri. This study revealed that, while the uterine ROMK sequence readily identified as 3-kb transcript (consistent with the renal ROMK transcript) (6), the $K_v1.5$ and the IRK-1 sequences did not reveal discernable hybridization signal even following extended autoradiography. These findings immediately suggested that the relative abundances of mRNAs encoding uterine K^+ channels were highly disparate, and those encoding ROMK were far more abundant (by almost 100-fold) than those for the other channel proteins. Subsequent analyses using poly(A)$^+$-tailed mRNAs revealed the presence of $K_v1.5$ and IRK-1 transcripts in gestational uteri (data not shown).

Gestational Expression of ROMK. The detected presence of ROMK mRNA transcripts in 18-day pregnant but not labor uteri suggested that ROMK expression was altered during these gestational stages. This possibility was examined in further detail. Northern blot analysis was conducted by using total RNAs extracted from entire uteri at various gestational time points including adult virgin, 12-, 15-, 18-, 21-day pregnant as well as labored uteri. The results indicated that the virgin tissue was devoid of detectable signal, while 12-day pregnant uteri presented the highest level of ROMK transcripts (with the expected size of about 3 kb) (Fig. 1A). Time points subsequent to the 12th day of pregnancy presented steadily decreasing but detectable levels of said transcripts, which became undetectable by labor. This temporal profile was consistently observed with three separate sets of timed-pregnant uteri, and was characterized by an over 20-fold change in steady-state ROMK mRNAs (Fig. 1B). Uteri from nine virgin animals were separately analyzed, and all provided consistent lack of detectable ROMK expression, suggesting that ROMK expression in virgin animals was most probably not influenced by the estrus cycle.

Since uterine ROMK transcripts were at the highest level in 15-day pregnant uterus, we compared this level of expression with other tissues at the same gestational age. Prominent expression of ROMK transcripts has been reported for kidney (6, 10). Consistent with this tissue pattern, we observed that at 15 days of pregnancy, ROMK expression is most prominent in the kidney, quite notable in the uterus, and not detectable in liver or placenta (Fig. 2). We further examined whether gestational changes in ROMK expression were also evident in the renal system. Northern blot analysis of kidney total RNAs attained from the same gestational time points revealed that ROMK transcript levels remained elevated throughout gestation and, relative to virgin animals, did not change with onset of either pregnancy or labor (Fig. 3). Thus, the transcriptional mechanisms that regulate uterine expression of ROMK are highly dynamic, possessing tissue- and gestation-specific properties.

Molecular Diversity of Uterine ROMK Transcripts. Multiple forms of ROMK mRNA have been identified in rat and human kidneys, presumably due to alternative splicing of transcripts arising from a single ROMK gene in each species (10, 11). These variations largely arise from rearrangements of exons located 5′ of the "core exon," which contains the vast majority of the protein-coding sequence of the gene. The resulting variants differ from each other in the 5′ UTR and the N-terminal coding regions of the mRNAs. We examined whether such structural diversity also existed in pregnant rat uteri. The experimental approach entailed rapid amplification of

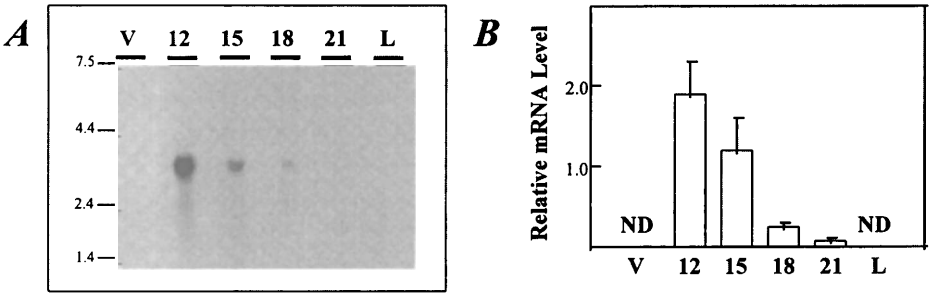

Figure 1. (A) Northern analysis of uterine ROMK expression during pregnancy. Total RNAs (10 μg/lane) were prepared from entire rat uteri of virgin (V), 12-day (12), 15-day (15), 18-day (18), 21-day (21) pregnant as well as labor (L) animals. RNA samples were hybridized with the ROMK cDNA probe. Electrophoretic positions of RNA size standards (in kb) are indicated along the left margin. (B) Relative expression level of ROMK mRNA at each gestational stage (mean ± SD, $n = 3$), as determined by densitometric scanning of dot-blot autoradiograms (against arbitrary scale). ND, not detected.

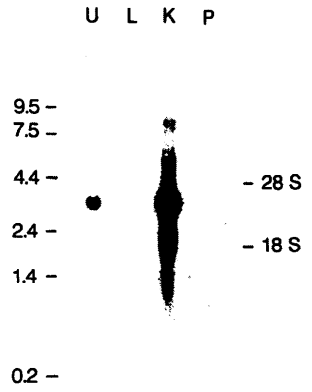

Figure 2. Northern analysis of ROMK expression, at 15 days of pregnancy, in uteri (U), liver (L), kidney (K) and placenta (P). The RNA samples were hybridized with [^{32}P]-labeled, ROMK cDNA probe. Electrophoretic positions of RNA size standards (in kb) are indicated along the left margin.

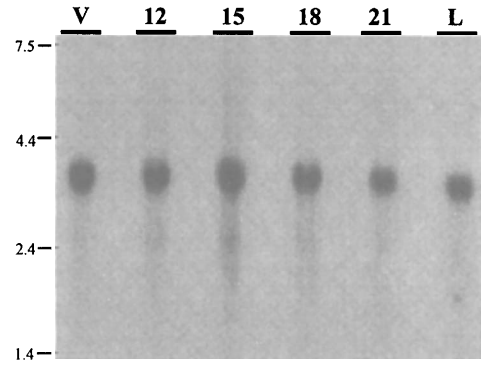

Figure 3. Northern analysis of renal ROMK expression during pregnancy. Total RNAs (10 μg/lane) were prepared from kidneys of virgin (V), 12-day (12), 15-day (15), 18-day (18), 21-day (21) pregnant as well as term-labor (L) rats. The RNA samples were hybridized with [^{32}P]-labeled, ROMK cDNA probe. Electrophoretic positions of RNA size standards (in kb) are indicated along the left margin.

cDNA ends (RACE) of uterine ROMK mRNAs to specifically amplify their 5′ UTR and N-terminal coding regions. Sequence analysis of the amplified products revealed the presence of two distinct isoforms of ROMK mRNAs (summarized in Fig. 4).

One isoform bears a nucleotide sequence that is identical to the recently identified renal ROMK-2 (10). Like its renal counterpart, uterine ROMK-2 encodes a protein sequence which is N-terminally truncated by 19 amino acids relative to that of ROMK-1. The 5′ UTR region of ROMK-2 shares perfect homology only with a portion of the 5′ UTR of ROMK-3 (12). The other uterine isoform, which we refer to as ROMK-4, is entirely novel and lacks significant homology in the 5′ UTR region with any of the other isoforms, even though its protein-coding region and the deduced N-terminal protein sequence are identical with those of ROMK-1. Beyond these regions the remaining protein-coding sequences of these isoforms are identical. From alignment of these nucleotide sequences, it is clear that the rat ROMK gene consists of multiple exons and that their differential usages give rise to the various splicing variants. Existence of multiple rat ROMK exons is also in accord with the structural organization of the human ROMK gene (11). Heterogeneities of the 5′ UTR regions of uterine isoforms indicate that two or more transcription-start sites are operational during gestation within the ROMK gene.

ROMK Expression in Other Gravid Uterine Models. Gestational ROMK expression in the gravid rat uterus prompted our examination of possible ROMK expression in other gravid uterine models. For this purpose, we attained whole uteri (devoid of implantation sites) from midgestational baboon (85 days pregnant), as well as late-pregnant guinea pig (65 days pregnant). Total RNAs prepared from these tissues were used for PCR-based detection of ROMK transcripts. By this approach, we observed the presence of ROMK transcripts in both baboon and guinea pig uteri, as revealed by specific hybridization signals of the expected sizes (Fig. 5). These findings further suggested that the nucleotide sequences of guinea pig and baboon amplicons must be highly homologous with that of rat. Late-pregnant guinea pig uteri were also used to dissect apart the longitudinal and circular muscle layers, which were subsequently used for PCR assessment of ROMK transcript presence. Appropriately sized amplicons hybridized specifically with the ROMK probe in both preparations. While the dissection scheme employed herein served only to enrich, and not purify, the respective myometrial layers, the persistent presence of ROMK in these findings provided strong indication that ROMK is indeed expressed in the uterine myometrium.

Discussion

In the present studies, we have detected the gestational expression of three voltage-gated K^+ channel proteins in rat uteri. Among these, one inwardly rectifying K^+ channel, ROMK, exhibits abundant and dynamic expression throughout gestation but is undetectable in both virgin and labored states. This temporal profile contrasts that of renal ROMK expression, which remains elevated throughout gestation, suggesting that uterine regulation of ROMK expression contains gestation-specific components that are absent in the renal system. The dynamic changes in uterine ROMK expression also attests to dramatic changes in transcriptional regulation occurring during mid-pregnancy which might be shared by other uterine effectors and thereby profoundly relevant to a variety of uterine changes throughout pregnancy. The gravid uterus also manifests two distinct splicing variants of the ROMK transcript, which arise from distinct transcription-start sites in the same structural gene. Such diversity presents added complexity in ROMK's gestational uterine expression. Gestational expression of uterine ROMK expression seems to be a generalized phenomenon, as baboon and guinea pig uteri also manifested detectable ROMK transcripts. Since dissected myometrial cells of guinea pig uteri maintained detectable ROMK ex-

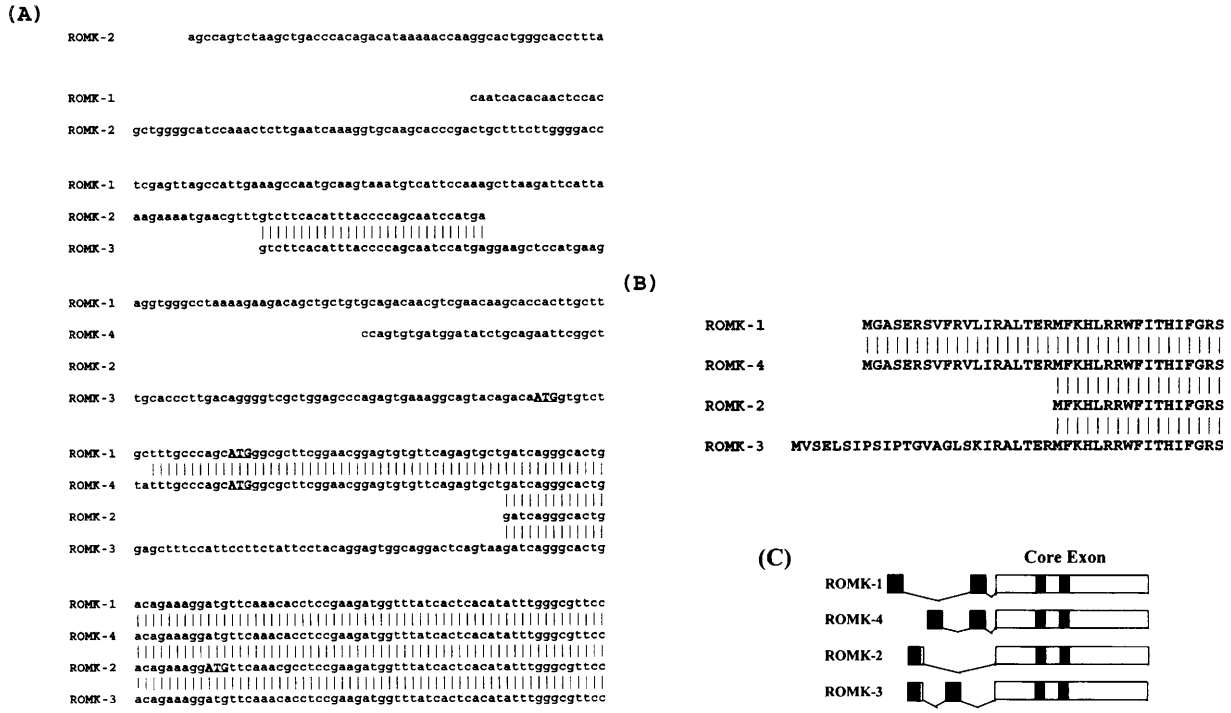

Figure 4. Summary of ROMK isoforms. Forms 2 and 4 were discerned by 5′ RACE of pregnant rat uteri mRNAs, and forms 1, 2, and 3 were identified from rat kidney. (A) Alignment of 5′ UTR and N-terminal coding regions of ROMK isoforms. Putative translational-initiation codons are capitalized and underlined. Vertical lines, nucleotide sequence conservations between the isoforms. Note that ROMK-3 bears a 5′ UTR sequence which is partly shared by ROMK-2. (B) Alignment of deduced N-terminal sequences of ROMK proteins. Vertical lines, conserved residues. While forms 1 and 4 are identical, form 3 is N-terminally extended by seven more residues and form 2 is truncated by 19 residues. (C) Schematic of ROMK exons to account for all identified isoforms. Core Exon, the entire coding region of ROMK-2 and most of the coding regions of the other isoforms. Sequence variations in the 5′ UTR regions of ROMK-1, -2, and -4 suggest variable splicing of upstream exons (bearing the respective N-termini of the protein-coding region). ROMK-1 and -4 share a common upstream exon, while each contains an exon not present in the other isoform. ROMK-2 and ROMK-3 also share a common exon (distinct from that shared by ROMK-1 and ROMK-4), but ROMK-3 additionally contains an exon not present in any other isoform.

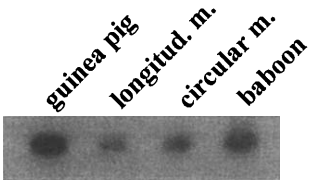

Figure 5. Presence of ROMK transcripts in the gravid uteri of guinea pig and baboon. Total RNAs were prepared from these tissue samples and used in reverse-transcription reactions to generate first-strand cDNAs, which then served as templates for PCR-amplifying a specific segment of the ROMK cDNA.

pression, this channel is very likely to be localized to the uterine myometrium.

In both human and rat kidneys, the ROMK gene has been shown to give rise to multiple transcripts through alternative splicing, and most of the encoded proteins have essentially the same channel-biophysical properties *in vitro* (10–12). The nature of alternative splicing, however, readily suggests that multiple promoter elements are functional within the same ROMK gene and that transcriptional regulation of the various splicing variants is likely to be complex. The present studies revealed the uterine presence of two ROMK transcripts, one of which is novel and has yet to be identified in renal cells. The very nature of the "upstream" exons that comprise the 5′ UTRs of ROMK transcripts is of particular interest because they imply simultaneous usage of distinct promoter/regulatory elements in the ROMK gene, and such elements are individually associated with the upstream exons. In other words, the ROMK gene is likely to possess and gestationally utilize multiple, functional promoter elements which might be differentially regulated, and which may cross-regulate each other. It should be noted that RNA splicing of ROMK transcripts was recently shown to occur in the 3′ UTR region as well (13). It is therefore possible that additional splicing variants beyond those presently described may exist in the gravid uterus.

The dynamic nature of ROMK expression in the gravid uterus, compared with the lack of gestational change in renal ROMK expression, provides a clear indication that the gene-regulatory mechanisms are highly tissue specific in addition to being highly gestation specific. Insights into the regulatory mechanisms of uterine ROMK expression will further advance our understanding of this channel's physiological attributes during pregnancy. More interestingly,

discernment of these transcriptional-regulatory mechanisms may in turn reveal the nature and diversity of molecular changes instigated by uterine cells during gestation. It is noteworthy that this temporal pattern of ROMK expression has been observed for other myometrial molecular effectors including several G-protein subunits (14, 15), a voltage-gated sodium channel α subunit ($Na_v2.1$ [16]) as well as pituitary peptide 23 (17). It is tempting to speculate that this shared pattern of gestational profile reflects possible mechanistic commonalities at the level of gene regulation.

Earlier studies provided evidence to suggest that myometrial quiescence during gestation is accompanied by gradual but significant changes in the electrical properties of muscle cells (reviewed in Refs. 18 and 19). Most notably, the myometrial membrane potential hyperpolarizes during early pregnancy and, by midgestation, reaches the most electronegative level (about −80 mV) relative to the nonpregnant level (close to −50 mV). During late gestation and approaching onset of parturition, the resting membrane potential begins to depolarize back towards the nonpregnant level (20). Concomitantly, the amplitude and frequency of spontaneous myometrial action potentials also undergo changes during gestation, presumably due to suppressed action potential generation resulting from significantly hyperpolarized resting potential (21). Studies in other smooth muscles have indicated that voltage-gated K^+ channels have prominent roles in the stabilization of resting membrane potentials at hyperpolarized levels, as well as in the shaping of spike frequencies and amplitudes (1). In the context of the gravid uterus, the temporal expression profile of ROMK highly parallels the known hyperpolarization of the myometrial membrane potential through mid-pregnancy, suggesting that this channel's role during gestation is likely to participate in, and might be principally responsible for, the stabilization of myometrial membrane potential during gestational progression. In this context, ROMK would contribute to the myometrium's contractile quiescence by antagonizing external stimuli that would otherwise depolarize the cellular resting membrane potential and consequently activate the intracellular contractile machineries.

ROMK's channel properties *in vitro* also support its functional involvement in myometrial quiescence. ROMK channels exhibit modest rectification relative to other inward rectifiers, is regulated by intracellular ATP, and maintains channel-opening probabilities at extremely high levels with little voltage-dependent variation (6, 22). Its modest rectifying property is attributed to its enhanced ability, relative to other inward rectifiers (e.g., IRK-1), to conduct outward current at membrane potentials more depolarized than the K^+ reversal potential (E_K), while preserving its ability to conduct inward current at membrane potentials more hyperpolarized than E_K (13). This functional duality enables ROMK channels to more actively maintain the resting membrane potential near the E_K level than other inward rectifiers; the unusually high channel-opening probabilities of this channel directly facilitate its role in membrane potential control. Such mechanistic postulations of ROMK channel functions in the gravid myometrium, however, require further substantiations from studies aimed to define more precisely the uterine distribution of ROMK expression.

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