BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS **229,** 50–57 (1996)
ARTICLE NO. 1756

Identification and Developmental Expression of Two Activin Receptors in Baboon Lung[1]

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Received October 15, 1996

Activins are members of the transforming growth factor-β (TGF-β) superfamily that exert their effects through interacting with specific cell surface TGF-β superfamily receptors (TSRs). To determine whether activins are involved in lung development, we used a reverse transcription polymerase chain reaction (RT-PCR)-based approach to identify members of the activin receptors from baboon fetal lung mRNAs. Two partial cDNA sequences encoding serine/threonine kinase domains of baboon TSR type I (bTSR1) and type II (bTSR2) were identified by sequencing analysis. bTSR1 displays 96% identity to human activin type I receptor TSR1, whereas bTSR2 shows 80% identity to human activin type II receptor ActRIIB over the kinase domain region. Northern analysis revealed the expression of a 2.1 kb bTSR1 transcript and a 5.0 kb bTSR2 transcript in baboon lung tissues. Both bTSR1 and bTSR2 were expressed throughout embryonic lung development and in adult lung. The expressions of bTSR1 and bTSR2 were developmentally regulated and each had a distinct expression pattern. Furthermore, the expressions of bTSR1 and bTSR2 in fetal baboon lung were altered by oxygen exposure. This study for the first time identifies the presence of the activin receptors in the baboon lung and provides evidence that both bTSR1 and bTSR2 are regulated during lung development, suggesting that activins might play an important role during lung development.

Activins are dimeric glycoproteins originally recognized as gonadal protein hormones by their ability to cause the release of follicle-stimulating hormone from anterior pituitary cells (1,2). They are involved in the regulation of many biological processes, including cell growth, neural cell survival, pituitary hormone secretion, erythropoiesis, and early embryonic development (3,4). Activins are members of a superfamily of polypeptide growth factors that includes the transforming growth factor-βs (TGF-βs), Müllerian inhibiting substance, the decapentaplegic/Vg-related factors, and bone morphogenetic proteins (5,6). Molecules of this multifunctional regulatory polypeptide family may act as carriers of growth and differentiation signals in development events.

Activins and other members of the TGF-β superfamily exert their effects through interacting with specific cell surface receptors, known as type I and type II receptors. Molecular cloning of several type I and type II receptors for activin and TGF-β has shown that both types belong to a novel family of serine/threonine kinases (7-11). Extensive studies of TGF-β superfamily receptors (TSRs) indicate that a complex of type I and type II receptors, but not the individual components, mediates activin and TGF-β signal transduction (12-14). For both activin and

[1] The nucleotide sequences reported in this paper have been submitted to the GenBank with accession numbers U60420 and U60421.

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Abbreviations used: TGF-β, transforming growth factor-β; TSR, TGF-β superfamily receptor; bTSR1, baboon TGF-β superfamily receptor type I; bTSR2, baboon TGF-β superfamily receptor type II; RT-PCR, reverse transcription polymerase chain reaction.

0006-291X/96 $18.00

TGF-β, ligand bound to type II receptor is recognized by type I receptor, which is then phosphorylated by type II receptor, which then allows propagation of the signal to downstream components.

In order to unravel the roles of activin and TGF-β induced signal transduction pathway in lung development processes, it is important to know the identification and molecular nature of the gene family of TSRs in the lung. Comparison of the cloned TSRs has revealed that they share some structural similarities. Each TSR has an extracellular domain with 10 to 13 cysteine residues, a single transmembrane segment, and an intracellular region with a serine-threonine kinase domain. There are some conserved clusters of residues located in the intracellular domains of the TSR family, suggesting that it might be possible to use sequence information present in this region to identify members of the TSR family in the lung. In this report, we used a PCR-based approach to identify members of this gene family in baboon fetal lung with primers to intracellular kinase regions highly conserved across the TSR family. We describe identification of two activin receptors, the baboon TGF-β superfamily receptor type I (bTSR1) and type II (bTSR2), from baboon fetal lung mRNAs. We further demonstrate that the expression of both bTSR1 and bTSR2 is developmentally regulated during baboon lung development, suggesting the involvement of activins in the process of lung development.

MATERIALS AND METHODS

RNA isolation. Frozen baboon lung tissues were kindly provided by Dr. Jacqueline J. Coalson from the Southwest Foundation for Biomedical Research (San Antonio, TX) as part of a NIH-sponsored project that allowed multiple investigators to use tissues from the animals. Fetal baboons (Papio cynocephalus, gestation 180 days) were delivered by hysterotomy at different gestational stages. Some early gestation fetuses were resuscitated and supported with mechanical ventilation and 100% oxygen for up to 14 days. Total RNA was prepared from baboon lung tissues by the guanidine thiocyanate/cesium chloride method as described (15). The pellets were extracted with phenol/chloroform and followed by ethanol precipitation. Poly(A)$^+$ RNAs were selected with the poly ATtract mRNA isolation kit (Promega, Madison, WI). The integrity and quantity of the RNA were evaluated by UV spectrophotometry and by denaturing agarose gel analysis stained with ethidium bromide.

RT-PCR, cloning, and sequencing. cDNA was synthesized from 1-5 μg of total RNA primed with oligo $(dT)_{12\text{-}18}$, and reverse transcribed in a final volume of 20 μl using superscript II reverse transcriptase (GIBCO/BRL, Grand island, NY) in 1 $\times$ synthesis buffer containing 20 mM Tris-HCl, pH 8.4, 50 mM KCl, 2.5 mM $MgCl_2$, 100 μg/ml BSA, 10 mM DTT, 500 μM each dNTP. The mixture was incubated at 42°C for 50 min, then heated at 70°C for 15 min. The original mRNA was destroyed by incubation with RNase H at 42°C for 10 min. Two degenerate oligonucleotide primers for PCR (sense: 5′d[GTGGC(T/A)GTCAAGATCTT(C/T)]3′; antisense:5′d[GTCGTGGTCCCA(G/A)CA(G/T)TC]3′) were synthesized based on human TβRII cDNA sequences (9). This reaction was used in a PCR with Taq DNA polymerase (Boehringer Mannheim) according to the supplier's instructions. The cDNA was amplified with a set of sense and antisense primers in a final volume of 50 μl. PCR cycling conditions were: 94°C for 5 min, 60°C for 2 min, and 72°C for 5 min, followed by 92°C for 1 min, 60°C for 1 min, 72°C for 2 min, and a 10 min final extension at 72°C.

cDNAs amplified by PCR were gel-purified, cloned into plasmid pNoTA (5prime-3prime, Boulder, CO), and were characterized by sequencing analysis. Sequencing was carried out in both directions by the dideoxy chain termination method (16) using Sequenase version 2.0 (U.S. Biochemicals, Cleveland, OH) kit and [^{35}S]-dATP (Amersham, Arlington Heights, IL). Overlapping regions of the DNA were sequenced using specific internal primers.

Northern analysis. Poly (A)$^+$ RNA was fractionated on an agarose gel, transferred to Nytran nylon membrane (ICN, Costa Mesa, CA), and fixed by a Stratalinker UV cross-linker (Stratagene, La Jolla, CA). Filters were hybridized at 42°C in 50% formamide solution containing 5XSSPE (1XSSPE is 0.18 M NaCl, 10 mM Na_2HPO_4, and 1 mM EDTA), 5X Denhart's solution (1X Denhart's is 0.02% (w/v) each of polyvinylpyrolidone, bovine serum albumin, and Ficoll), 0.1% SDS, and 0.1 mg/ml of denatured and sonicated fish sperm DNA with 10^6 cpm of [^{32}P]-labeled bTSR1, bTSR2 or TβRII cDNA probes. Equivalent RNA loading and transfer were confirmed by subsequent reprobing with a rat glyceraldehyde phosphate dehydrogenase (GAPDH) cDNA probe. Filters were washed twice with 2XSSC (1X SSC is 0.15 M NaCl, 15 mM trisodium citrate), 0.1% SDS for 15 min at room temperature and finally washed with 0.1XSSC, 0.1% SDS for 20 min at 60°C. The filters were autoradiographed.

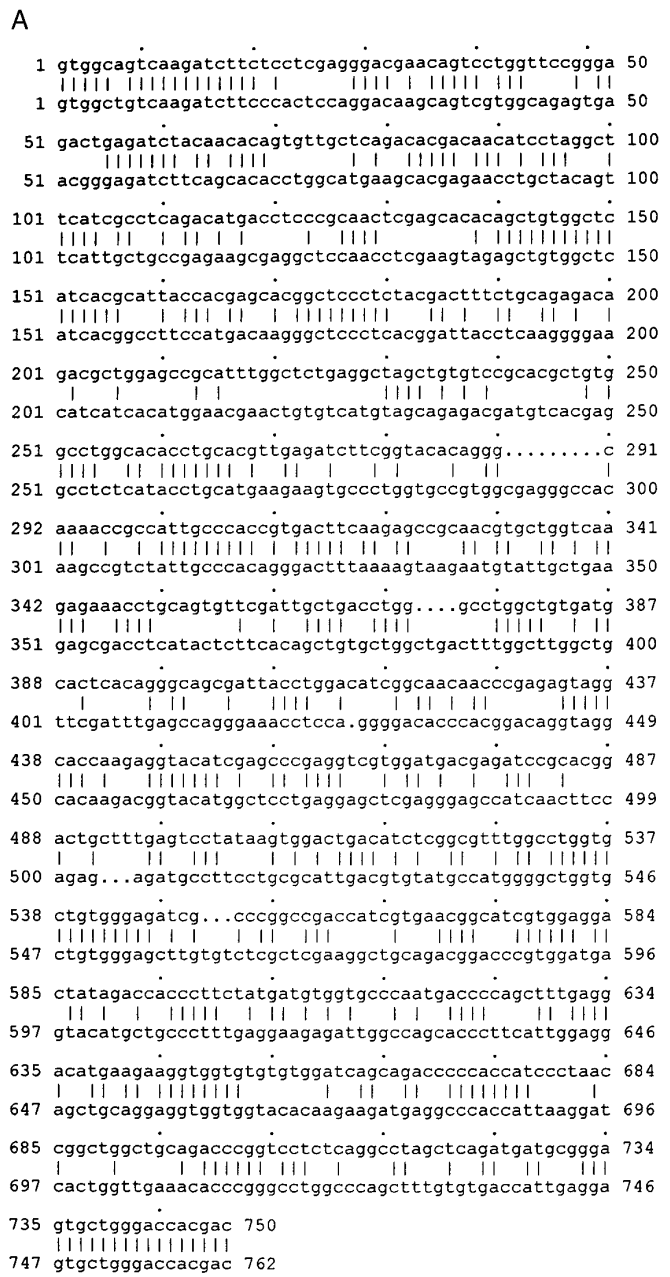

FIG. 1. Sequence alignment of bTSR1 and bTSR2. (A). Alignment of nucleotide sequences of bTSR1 and bTSR2. Identical nucleotides are connected with vertical bars; (B). Comparision of amino acid sequences of the intracellular kinase regions of the TSRs. The sources for the amino acid sequences are human TSR1 (7), human SKR1 (20), human ALK-3 (22), human ALK-4 (22), human ALK-5 (10), human ALK-6 (23), human ActRIIB (21), human ActRII (24), and human TβRII (9). The nucleotide sequences of bTSR1 and bTSR2 have been deposited in the GenBank database under accession numbers U60420 and U60421, respectively.

B

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                1                                                  50
hTSR1           VAVKIFSSRD EQSWFRETEI YNTVLLRHDN ILGFIASDMT SRNSSTQLWL
bTSR1           VAVKIFSSRD EQSWFRETEI YNTVLLRHDN ILGFIASDMT SRNSSTQLWL
hSKR1           VAVKIFSSRD EKSWFRETEL YNTVMLRHEN ILGFIASDMT SRHSSTQLWL
hALK5           VAVKIFSSRE ERSWFREAEI YQTVMLRHEN ILGFIAADNK DNGTWTQLWL
hALK5           VAVKIFSSRE ERSWFREAEI YQTVMLRHEN ILGFIAADNK DNGTWTQLWL
hALK3           .AVKVFFTTE EASWFRETEI YQTVLMRHEN ILGFIAADIK GTGSWTQLYL
hALK6           VAVKVFFTTE EASWFRETEI YQTVLMRHEN ILGFIAADIK GTGSWTQLYL
hActRIIB        VAVKIFPIQD KQSWQNEYEV YSLPGMKHEN ILQFIGAEKR GTSVDVDLWL
bTSR2           VAVKIFPLQD KQSWQSEREI FSTPGMKHEN LLQFIAAEKR GSNLEVELWL
hTβRII          VAVKIFPYEE YASWKTEKDI FSDINLKHEN ILQFLTAEER KTELGKQYWL
hActRII         GALAVSGRPS S*MTL*LSRS SHSRTSSRGR VNGRSSAHLA *STRTCYSSL
Consensus       VAVKIFSSR- EQSWFRETEI YQTVLLRHEN ILGFIAADMK GTGSWTQLWL

                51                                                 100
hTSR1           ITHYHEHGSL YDFLQRQTLE PHLALRLAVS AACGLA.... ......HLHV
bTSR1           ITHYHEHGSL YDFLQRQTLE PHLALRLAVS ARCGLA.... ......HLHV
hSKR1           ITHYHEMGSL YDYLQLTTLD TVSCLRIVLS IASGLA.... ......HLHI
hALK4           VSDYHEHGSL FDYLNRYTVT IEGMIKLALS AASGLA.... ......HLHM
hALK5           VSDYHEHGSL FDYLNRYTVT VEGMIKLALS TASGLA.... ......HLHM
hALK3           ITDYHENGSL YDFLKCATLD TRALLKLAYS AACGLC.... ......HLHT
hALK6           ITDYHENGSL YDYLKSTTLD AKSMLKLAYS SVSGLC.... ......HLHT
hActRIIB        ITAFHEKGSL SDFLKANVVS WNELCHIAET MARGLA.... ......YLHE
bTSR2           ITAFHDKGSL TDYLKGNIIT WNELCHVAET MSRGLS.... ......YLHE
hTβRII          ITAFHAKGNL QEYLTRHVIS WEDLRKLGSS LARGIA.... ......HLHS
hActRII         LPRSEAPTSK *SCGSSRPSM TRAPSRITSR GTSSHGTNCV M*QRRCHEAS
Consensus       ITDYHEHGSL YDYLKR-T-D -EELLKLALS AASGLA---- ------HLHV

                101                                                150
hTSR1           EI...FGTQG KPAIAHRDFK SRNVLVKSNL ...QCCIADL GLAVMHSQGS
bTSR1           EI...FGTQG KTAIAHRDFK SRNVLVKRNL ...QCSIADL GLAVMHSQGS
hSKR1           EI...FGTQG KPAIAHRDLK SKNILVKKNG ...QCCIADL GLAVMHSQST
hALK4           EI...VGTQG KPGIAHRDLK SKNILVKKNG ...MCAIADL GLAVRHDAVT
hALK5           EI...VGTQG KPAIAHRDLK SKNILVKKNG ...TCCIADL GLAVRHDSAT
hALK3           EI...YGTQG KPAIAHRDLK SKNILIKKNG ...SCCIADL GLAVKFNSDT
hALK6           EI...FSTQG KPAIAHRDLK SKNILVKKNG ...TCCIADL GLAVKFISDT
hActRIIB        DIP.GLKDGH KPAISHRDIK SKNVLLKNNL ...TACIADF GLALKFEAGK
bTSR2           EVPWCRGEGH KPSIAHRDFK SKNVLLKSDL ILFTAVLADF GLAVRFEPGK
hTβRII          DHTPC..GRP KMPIVHRDLK SSNILVKNDL ...TCCLCDF GLSLRLDPTL
hActRII         HTCMRMCPGA VARATSRLLP TGTLKVRMYC *RATSQPCWL TLAWLFDLSQ
Consensus       EIP-CFGTQG KPAIAHRDLK SKNILVKKN- ---TCCIADL GLAV-FD-GT

                151                                                200
hTSR1           DYLDIGNNPR VGTK...... .......... .......... ..RYMAPEVL
bTSR1           DYLDIGNNPR VGTK...... .......... .......... ..RYIEPEVV
hSKR1           NQLDVGNNPR VGTK...... .......... .......... ..RYMAPEVL
hALK4           DTIDIAPNQR VGTK...... .......... .......... ..RYMAPEVL
hALK5           DTIDIAPNHR VGTK...... .......... .......... ..RYMAPEVL
hALK3           NEVDVPLNTR VGTK...... .......... .......... ..RYMAPEVL
hALK6           NEVDIPPNTR VGTK...... .......... .......... ..RYMPPEVL
hActRIIB        SAGD..THGQ VGTR...... .......... .......... ..RYMAPEVL
bTSR2           PPGD..THGQ VGTR...... .......... .......... ..RYMAPEEL
hTβRII          SVDDLANSGQ VGTA...... .......... .......... ..RYMAPEVL
hActRII         GNLQGTPTDR *ARDGTWLLR CSREPSTSRE MPSCALTCMP WGWCCGSLCL
Consensus       D--DI--N-R VGTK------ ---------- ---------- --RYMAPEVL

                251                                                300
hTSR1           DEQIRTDCFE S..YKWTDIW AFGLVLWE.. ...IARRT.I VNGIVEDYRP
bTSR1           DDEIRTDCFE S..YKWTDIS AFGLVLWE.. ...IARPT.I VNGIVEDYRP
hSKR1           DETIQVDCFD S..YKRVDIW AFGLVLWE.. ...VARRM.V SNGIVEDYKP
hALK4           DETINMKHFD S..FKCADIY ALGLVYWE.. ...IARRC.N SGGVHEEYQL
hALK5           DDSINMKHFE S..FKRADIY AMGLVFWE.. ...IARRC.S IGGIHEDYQL
hALK3           DESLNKNHFQ P..YIMADIY SFGLIIWE.. ...MARRC.I TGGIVEEYQL
hALK6           DESLNRNHFQ S..YIMADMY SFGLILWE.. ...IARRC.V SGGIVEEYQL
hActRIIB        EGAINFQR.D A..FLRIDMY AMGLVLWE.. ...LASRCTA ADGPVDEYML
bTSR2           EGAINFQR.D A..FLRIDVY AMGLVLWE.. ...LVSRSKA ADGPVDEYML
hTβRII          ESRMNLENAE S..FKQTDVY SMALVLWE.. ...MTSRCNA V.GEVKDYEP
hActRII         AARLQTDPWM STCCPLRKRL ASTLRWRSCR RWWCTRR*GP PLKITG*NTR
Consensus       DESIN-DHF- S---KRADIY AFGLVLWE-- ---IARRC-I VGGIVE-YQL

                351                                                400
hTSR1           PFYDVVPNDP SFED.MKKVV CVDQQTPTIP NRLAADPVLS GLAQMMRECW
bTSR1           PFYDVVPNDP SFED.MKKVV CVDQQTPTIP NRLAADPVLS GLAQMMRECW
hSKR1           PFYDVVPNDP SFED.MRKVV CVDQQRPNIP NRWFSDPTLT SLAKLMKECW
hALK4           PYYDLVPSDP SIEE.MRKVV CDQKLRPNIP NWWQSYEALR VMGKMMRECW
hALK5           PYYDLVPSDP SVEE.MRKVV CEQKLRPNIP NRWQSCEALR VMAKIMRECW
hALK3           PYYNMVPSDP SYED.MREVV CVKRLRPIVS NRWNSDECLR AVLKLMSECW
hALK6           PYHDLVPSDP SYED.MREIV CMKKLRPSFP NRWSSDECLR QMGKLMTECW
hActRIIB        PFEEEIGQHP SLED.MQEVV VHKKKRPVLR DYWQKHAGMA MLCETIEECW
bTSR2           PFEEEIGQHP SLEE.LQEVV VHKKMRPTIK DHWLKHPGLA QLCVTIEECW
hTβRII          PFGSKVREHP CVES.MKDNV LRDRGRPEIP SFWLNHQGIQ MVCETLTECW
hActRII         AWPSFV*PSR SAGTMMQRLA CPRAVWRSGC P*FGGRST.. ALPRTVSFPW
Consensus       PFYDLVPSDP SFED-MR-VV CV-KLRP-IP NRW-SD-GLR -LAK-MRECW
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FIG. 1—*Continued*

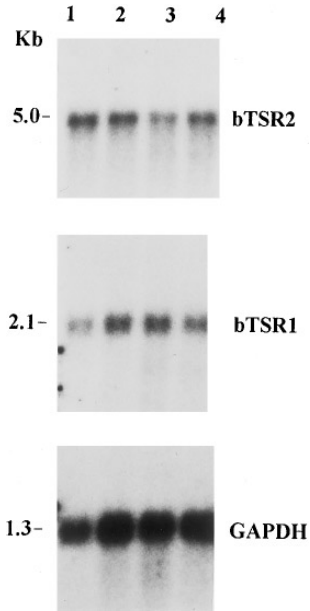

FIG. 2. Expression of activin receptors in embryonic baboon lung tissues. Poly(A)+ RNA (1.5 μg) isolated from baboon lung was subjected to electrophoresis through a formaldehyde denaturing 1.0% agarose gel and after Northern blotting, hybridized with radiolabeled bTSR1 or bTSR2 cDNA probe and reprobed with a glyceraldehyde phosphate dehydrogenase (GAPDH) cDNA as a control for loading. The size of the transcripts in kilobase is shown on the left. Lane 1, embryonic day 124; lane 2, embryonic day 142; lane 3, embryonic day 161; and lane 4, embryonic day 180.

RESULTS AND DISCUSSION

TGF-βs are known to act as mutifunctional cytokines involved in controlling many cellular activities including cell growth, differentiation, and extracellular matrix deposition in lung (6,17-19). Both type I and type II receptors for TGF-β are present during lung development and are involved in TGF-β-dependent growth regulation (18,19). In an effort to determine if other members of the TGF-β superfamily are also involved in lung development, we used a PCR-based approach to identify TSRs in baboon lung. Members of the TSR gene family were amplified by using two degenerate primers flanking the intracellular serine/threonine kinase domains. The identity of the PCR amplified cDNAs was characterized by sequencing analysis. Two cDNA clones (bTSR1 and bTSR2) displayed significant sequence similarity to the known activin receptors (Figure 1). bTSR1 and bTSR2 are 55% identical in nucleotide sequence, but only 38% identical in amino acid sequence. When compared to TSRs known so far, bTSR1 was found to be highly homologous to human activin type I receptor TSR1 (7) and SKR1 (20). The deduced amino acid sequences of bTSR1 kinase domain displays 96% identity to human TSR1 and 81% identity to human SKR1. The high identity between bTSR1 and human TSR1 indicates that bTSR1 could be the baboon counterpart for human TSR1. bTSR2 displays high homology to human activin type IIB receptor ActRIIB (21) and human TGF-β type II receptor TβRII (9). bTSR2 shows 80% identity to human ActRIIB and 69% identity to human TβRII over the kinase region and it might be a previously unrecognized member of the activin receptor family. Activins are known to play important roles in ovarian and testicular development. The

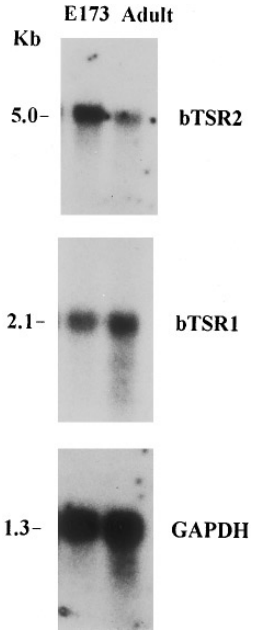

FIG. 3. Expression of activin receptors in fetal and adult baboon lung tissues. Each lane contained 2 μg of mRNA prepared from day 173 gestational age fetal baboon lung tissue or adult baboon lung tissue. mRNA was subjected to electrophoresis through a formaldehyde denaturing agarose gel and after Northern blotting, hybridized with radiolabeled bTSR1 or bTSR2 cDNA probe and reprobed with a GAPDH cDNA as a control for loading. The size of the transcripts in kilobase is shown on the left.

finding of activin receptors in the lung suggests that activins might also play an extragonadal role in lung development.

The expression of bTSR1 and bTSR2 during embryonic lung development was examined by Northern analysis (Figure 2). A 2.1 kb bTSR1 mRNA and a 5.0 kb bTSR2 transcript were detected in baboon lung tissue. Expression of bTSR1 was found in baboon fetal lung tissue early in development at 124 days of gestation, increased as development proceeds, and then decreased at term (180 days). Expression of bTSR2 mRNA was high at 124 days of gestation, decreased as development proceeds, and then increased at the term. The pattern of bTSR1 expression during embryonic lung development was distinct from that of bTSR2. The expression of activin receptors by adult and fetal lung were evaluated. Fetal lung expressed a significantly higher level of mRNA for bTSR2 than adult lung, whereas, adult lung displayed a much higher level of bTSR1 (Figure 3). The results demonstrate that the expressions of both bTSR1 and bTSR2 are developmentally regulated in baboon lung.

The effects of oxygen exposure on the expressions of these activin receptor genes in fetal baboon lung were also examined. The expressions of both bTSR1 and bTSR2 mRNAs were greatly increased by100% oxygen exposure for 48 hours, however, they were down-regulated by 100% oxygen exposure for 14 days (Figure 4). We speculate that bTSR1 and bTSR2 may play a role in oxygen-induced lung pathologic changes.

In summary, we identified two activin receptors bTSR1 and bTSR2 from baboon lung by RT-PCR cloning, and demonstrated that the expressions of both bTSR1 and bTSR2 are regulated during embryonic baboon lung development. Our finding provides evidence for

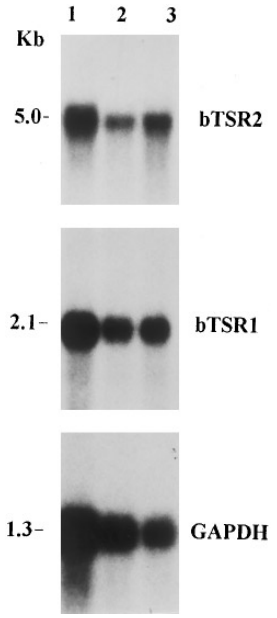

FIG. 4. Changes of bTSR1 and bTSR2 gene expression in baboon lungs during hyperoxic exposure. Poly(A)+ RNA (1.5 μg) was isolated from baboon lung tissues exposed to 100% oxygen, subjected to electrophoresis through a formaldehyde denaturing agarose gel and after Northern blotting, and hybridized with radio-labeled bTSRI or bTSR2 cDNA. The size of the transcripts in kilobase is shown on the left. Lane 1, day 141 gestational age fetal baboon lung exposed to 100% oxygen for 48 hours; lane 2, day 140 gestational age fetal baboon lung exposed to 100% oxygen for 14 days; and lane 3, day 138 gestational age fetal baboon lung as a control.

a novel function of activins and indicates that, besides TGF-β, other members of this superfamily might also be involved in lung development. The distinct expression profile of each TSR suggests possible different roles for bTSR1 and bTSR2 in development of lung.

ACKNOWLEDGMENTS

This work was supported by grants from the Department of Veterans Affairs, the American Lung Association, the National Institutes of Health (HL32188) and the National Institutes of Health sponsored bronchopulmonary dysplasia resource center at the Southwest Foundation for Biomedical Research (HL52636, San Antonio, TX).

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