Induction of Aquaporin-1 mRNA following Cardiopulmonary Bypass and Reperfusion

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ABSTRACT

Background: Cardiopulmonary bypass (CPB) and hypothermic circulatory arrest (HCA) are important components of congenital cardiac surgery. Ischemia/reperfusion injury and inflammatory cascade activation result in endothelial damage and vascular leak, which are clinically manifested as pulmonary edema and low cardiac output postoperatively. Newborns are particularly susceptible. Subtraction cloning is a useful method of isolating induced genes and can be applied to CPB/HCA.

Materials and Methods: We used a newborn lamb model replicating infant CPB with HCA to obtain tissues during various periods of reperfusion. We utilized subtraction cloning to identify mRNA induced in lung following CPB/HCA and reperfusion. Ribonuclease protection was used to quantify mRNA levels.

Results: We isolated a cDNA encoding ovine aquaporin-1 in a subtracted cDNA screen comparing control lung with lung exposed to CPB/HCA and reperfusion. Aquaporin-1 mRNA levels increased 3-fold in lung ($p = .006$) exposed to CPB/HCA and 6 hr of reperfusion. No induction was observed immediately following bypass or after 3 hr of reperfusion. We found no significant induction of aquaporin-1 mRNA following bypass, arrest, and reperfusion in other tissues surveyed, including ventricle, atrium, skeletal muscle, kidney, brain, and liver.

Conclusions: Our finding that aquaporin-1 mRNA is reproducibly induced in lung following CPB/HCA with 6 hr of reperfusion suggests an important role for the water channel in the setting of pulmonary edema. Induction of Aquaporin-1 is late compared with other inflammatory mediators (ICAM-1, E-selectin, IL-8). Further studies are needed to determine if aquaporin-1 contributes to the disease process or if it is part of the recovery phase.

INTRODUCTION

Cardiopulmonary bypass (CPB) and hypothermic circulatory arrest (HCA), which are support techniques for pediatric cardiac surgery, invariably result in vascular injury and inflammatory activation, due in part to ischemia and reperfusion. Clinically, this translates to postoperative morbidity, including pulmonary edema, a transient low cardiac output state, and occasionally, profound capillary leakage. Identification of the inflammatory mediators induced by CPB/HCA is necessary to understand the mechanism of the associated vascular injury.

We have previously shown that genes for proinflammatory adhesion molecules ICAM-1 and E-selectin, as well as the potent neutrophil chemoattractant interleukin-8 (IL-8), are induced immediately following CPB in atrium and skeletal muscle in humans (1,2). Using a lamb model of bypass with circulatory arrest, we can obtain several tissues under varying conditions of bypass and varying times of reperfusion. We used subtraction cloning techniques to learn

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which genes might contribute to pulmonary vascular injury with bypass, circulatory arrest, and reperfusion. In a preliminary screening of genes induced in lung, we have identified the ovine homolog of a known water channel protein, aquaporin 1 (AQP1).

Other studies have employed subtraction techniques to demonstrate that major intrinsic protein (a member of the family of aquaporins) is a delayed early response gene following growth factor stimulation of mouse fibroblast cells (3) and that AQP1 expression is lost during repeated passage of aortic vascular smooth muscle cells in culture (4).

AQP1 (initially named CHIP 28) was first identified in erythrocytes and renal proximal tubules (5,6). It belongs to a family of at least five highly conserved membrane water channels, each with characteristic tissue distribution and physiology. AQP1 is a homotetramer of 30 kD subunits which act as functionally independent transmembrane water channels that are sensitive to inhibition by mercury salts (7–10). In rat tissue surveys, AQP1 is found in several *epithelia*: in ocular ciliary bodies and cornea, choroid plexus, hepatic bile ducts (11), and descending thin limbs of the loop of Henle in the kidney (12). AQP1 has also been localized by immunohistochemical techniques to *endothelium* of cardiac and skeletal muscle capillaries, and to peribronchiolar and perialveolar capillaries in the lung (11). There are no previous reports of the in vivo impact of vascular injury on the expression of AQP1 or other aquaporins. We have found significant elevation of AQP1 mRNA at 6 hr of reperfusion after cardiopulmonary bypass with circulatory arrest. We propose that this water channel gene may participate in pulmonary vascular leakage or response to injury following cardiopulmonary bypass.

MATERIALS AND METHODS

Cardiopulmonary Bypass with Hypothermic Circulatory Arrest

All animals were treated in compliance with the "Principles of Laboratory Animal Care" formulated by the National Society for Medical Research and the "Guide for the Care and Use of Laboratory Animals" (13). The experimental protocol was approved by the Animal Care and Use Committee at Children's Hospital, Boston, MA.

Neonatal lambs (age 2–7 days) were anesthetized with intramuscular ketamine (40 mg/

kg, followed by 0.5 mg/kg/hr continuous infusion) and pancuronium bromide (0.3 mg/kg), and mechanically ventilated with 100% oxygen. Femoral artery access was obtained for pressure measurements and arterial blood sampling. An electromagnetic flow probe (Nihon, Kodon, Tokyo, Japan) was placed around the main pulmonary artery for cardiac output measurements. A left atrial sheath (5 Fr) and pulmonary artery catheter (5 Fr) were placed for pressure measurements. Baseline cardiac output, pressure measurements, and arterial blood gases were obtained. After systemic heparinization, a pressure transducer (5 Fr) was placed in the left ventricular apex. A right femoral artery cannula (8 Fr) and a right atrial cannula (24 Fr) were used for cardiopulmonary bypass.

The bypass circuit consisted of a roller pump and membrane oxygenator (VPCML, Cobe). The pump prime (37°C) was 800 cc of Normosol and 600 cc of homologous, heparinized whole blood from adult sheep, to achieve a hematocrit of 20%. The bypass pump flow was 150 cc/kg/min. Phentolamine (0.2 mg/kg) was given prior to cooling and rewarming. After 30 min of bypass and cooling to 15°C, a 2-hr period of hypothermic circulatory arrest was followed by reinitiating bypass and rewarming to 35°C over 30 min (Fig. 1). Sodium bicarbonate (10 mEq) and defibrillation were administered as needed. Cardiac output, pressures, and arterial blood gases were recorded hourly. The lambs were a median weight of 4.9 kg (range 2.6–6 kg).

Tissue Collection

Animals who had undergone CPB/HCA were rewarmed and sacrificed after 0, 3, or 6 hr of reperfusion. Control animals underwent anesthesia and sternotomy only, at room temperature, and were sacrificed after 20 or 260 min. There were 1–3 animals per condition. Organs were harvested immediately and dissected tissue samples (approximately 5 × 5 × 10 mm) were placed directly in polypropylene tubes on dry ice. Long-term storage was at −80°C.

RNA Extraction

Tissue samples were made brittle in liquid nitrogen, broken into small pieces with a hammer, and transferred frozen to RNAzol-B (Cinna/Biotecx, Friendswood, TX) and immediately homogenized (Ultra-turrax T25, Janke & Kunkel, IKA, Germany). RNA was isolated by acid guani-
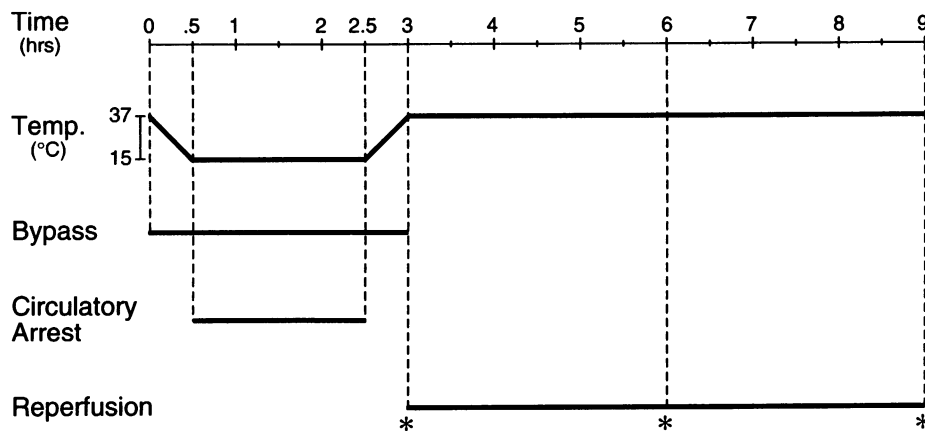
FIG. 1. Experimental time scheme of cardiopulmonary bypass in lambs

Lambs underwent cooling to 15°C for 30 min on bypass prior to 2 hr of circulatory arrest. Lambs were rewarmed to 37°C for 30 min on bypass before reperfusion. Animals were sacrificed after bypass and rewarming (*) at 0, 3, or 6 hr of reperfusion.

dinium-thiocyanate-phenol-chloroform extraction (14). Poly A^+ RNA was isolated by hybridization with Oligo-dT-linked cellulose (Oligo-dT mRNA Kit, Qiagen, Inc., Chatsworth, CA). RNA was quantitated by spectrophotometry and stored at −80°C.

Sheep Lung Post-CPB cDNA Library

A cDNA library in λZAPII was constructed from poly A^+ RNA isolated from neonatal lamb lung tissue harvested after CPB/HCA and 3 hr of reperfusion (Clontech Laboratories, Palo Alto, CA). Mixed oligo-dT and random primers were used for first-strand cDNA synthesis. The library contained 1.8×10^6 recombinants, with an average insert size of 1.5 kilobases.

Subtraction Hybridization

First-strand $cDNA_{CPB}$ was generated by reverse transcription of 1 μg of oligo-dT-selected RNA isolated from lamb lung after CPB/HCA and 6 hr of reperfusion, by incubating with oligo-dT primer and reverse transcriptase (Subtractor Kit, Invitrogen Corp., San Diego, CA). First-strand $cDNA_{CPB}$ was isolated and used as the "tester" population. $mRNA_{CONTROL}$ from lamb lung harvested after 20 min of anesthesia only was used as a "driver" population. $mRNA_{CONTROL}$ was biotinylated by incubating with photobiotinacetate under a 300 watt light bulb. A large excess of 10 μg of "driver" $mRNA_{CONTROL}$ was hybridized with the "tester" $cDNA_{CPB}$ for 48 hr. After treatment with

streptavidin, the biotin:$mRNA_{CONTROL}$:$cDNA_{CPB}$ duplexes were removed by phenol-chloroform extraction, leaving the subtracted, first-strand $cDNA_{CPB-CONTROL}$; this represented mRNAs induced in lung during CPB/HCA and reperfusion.

Isolation of Subtracted cDNA

A ^{32}P-labeled subtraction probe was generated from the subtracted, first-strand $cDNA_{CPB-CONTROL}$ using random primers and Klenow DNA polymerase (Boehringer-Mannheim, Indianapolis, IN) (15) in a reaction containing 50 μCi [α^{32}P]dCTP (Amersham, Arlington Heights, IL). The sheep lung post-CPB cDNA library described above was plated sparsely (1000 phage per 150-mm plate) to allow isolation of single-phage plaques. Nitrocellulose filter lifts were performed in triplicate. Two lifts were screened with the ^{32}P-labeled subtraction probe. One lift was screened with ovine actin cDNA to identify and discard this common cDNA. Remaining nonactin clones on duplicate filters were selected with a toothpick and amplified in host XL1-blue. Phagemids were excised with helper phage as recommended by the manufacturer (Strategene, La Jolla, CA). Inserts were sequenced by the Children's Hospital sequencing facility using a PRISM instrument (ABI, Foster City, CA).

Synthesis of AQP1 Riboprobes

cDNA templates of ovine AQP1 and nonmuscle actin were chosen of different lengths to allow

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Ovine AQP1   16  MASEFKKKLFWRAVVAEFLAMILFIFISIGSALGFHYPIKSNQTTGAVQD   65
                 MASEFKKKLFWRAVVAEFLAMILFIFISIGSALGFHYPIKSNQTTGAVQD
Bovine AQP1   1  MASEFKKKLFWRAVVAEFLAMILFIFISIGSALGFHYPIKSNQTTGAVQD   50


Ovine AQP1   66  NVKVSLAFGLSIATLAQSVGHISGAHLNPAVTLGLLLSCQISILRAIM    113
                 NVKVSLAFGLSIATLAQSVGHISGAHLNPAVTLGLLLSCQIS+LRAIM
Bovine AQP1  51  NVKVSLAFGLSIATLAQSVGHISGAHLNPAVTLGLLLSCQISVLRAIM    98


Ovine AQP1   16  MASEFKKKLFWRAVVAEFLAMILFIFISIGSALGFHYPIKSNQT         59
                 MASEFKKKLFWRAVVAEFLA   LF+FISIGSALGF YP+ +NQT
Human AQP1    1  MASEFKKKLFWRAVVAEFLATTLFVFISIGSALGFKYPVGNNQT         44
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FIG. 2. Amino acid sequence homology of the 5' end of the subtraction clone from ovine lung, with bovine and human AQP1

This sequence homology was obtained from a BLAST search of the GenBank database. Bold amino acid symbol, identical sequence; +, conservative amino acid substitution.

multiplex RNAse protection analysis in a single sample. A 125-bp fragment of AQP1 cDNA from within the coding region was amplified by polymerase chain reaction with artificial restriction sites placed in the primers to enable cloning into the BamH1 and EcoRI sites of the plasmid vector, pBluescript II KS (Stratagene). Transcription from 0.5 μg linearized AQP1 template produced a full-length, 168-nucleotide (nt) probe with a 125-nt protected sequence. Nonmuscle actin cDNA, which was transcribed from 0.5 μg plasmid vector pBluescript II KS and linearized with AccI, had a full-length, 136-nt probe with a 63-nt protected sequence. Transcription reactions contained 100 μCi [α^{32}P]CTP (Amersham). T7 and T3 RNA polymerase (Promega, Madison, WI) were used for antisense and sense transcription, respectively.

Ribonuclease (RNAse) Protection Assay

The RNAse protection assay was modified from Kilbridge et al. as follows (2). Total RNA (30 μg) extracted and purified from lamb tissue was hybridized with 300,000 cpm AQP1 riboprobe and 50,000 cpm nonmuscle actin riboprobe. tRNA (30 μg) and sense riboprobes were run as negative controls. After overnight hybridization (37°C), single-stranded RNA was digested (37°C, 90 min) with 80 μg/ml RNase A (Boehringer) and 4 U/ml RNase T1 (Boehringer). Samples

were analyzed on a 5% acrylamide-urea sequencing gel.

Data Analysis

Protected bands were quantified by Phosphor-Imager analysis and ImageQuant software (Molecular Dynamics, Sunnyvale, CA). After subtracting background levels from tRNA negative control lane, AQP1 signal was normalized to its internal nonmuscle actin control, [AQP1-tRNA]/[actin-tRNA]. Different tissue samples from the same experimental condition were combined ($n = 2$–5) and conditions compared using a Student's t-test assuming unequal variances. All experiments were confirmed in duplicate.

RESULTS

Subtraction Cloning of Genes Induced during CPB/HCA and Reperfusion in Lung

mRNA was isolated from lung tissue of a neonatal lamb subjected to CPB/HCA followed by reperfusion, and from a control lamb with anesthesia and sternotomy only. Transcribed cDNA from lung after CPB/HCA and reperfusion was hybridized with excess biotinylated mRNA from control lambs and the mRNA–cDNA complexes were removed, leaving subtracted cDNA that

TABLE 1. Hemodynamic parameters of neonatal lambs at baseline and following CPB/HCA

	Baseline		After CPB/HCA		p Value
	Mean	Standard Error	Mean	Standard Error	
Blood pressure (mm Hg)	67.5	3.5	65.3	2.3	.60
PA pressure (mm Hg)	15.6	1.2	18.8	1.0	.05
LA pressure (mm Hg)	2.8	.43	3.1	.22	.53
CVP (mm Hg)	3.1	.31	4.0	.33	.07
Cardiac output (l/min)	.98	.16	1.2	.14	.32
Heart rate (bpm)	192	11	226	4	.01
Temperature (°C)	36.0	.46	36.1	.18	.79
Hematocrit (%)	30.9	1.3	26.2	.38	.01
PO_2	399	39	320	28	.12
PCO_2	34.1	1.5	36.1	1.5	.37
pH	7.38	.02	7.43	.02	.08

Comparison of hemodynamics and arterial blood gases obtained at baseline compared with hourly samples taken from lambs at conclusion of cardiopulmonary bypass and during reperfusion. Invasive hemodynamics were not available for 20-min sternotomy-only control lambs. CPB, cardiopulmonary bypass; HCA, hypothermic circulatory arrest; PA, pulmonary artery; LA, left atrium; CVP, central venous pressure; l/min, liters per minute; bpm, beats per minute; PO_2, partial pressure of oxygen; PCO_2, partial pressure of carbon dioxide. PO_2, PCO_2, and pH were obtained from arterial blood gas analysis. The remaining values were directly measured.

represented genes induced in lung with CPB/HCA and reperfusion. This subtracted cDNA was labeled and used to probe a post-CPB/HCA cDNA library from lung. In this pilot scale screen, 20 cDNAs were isolated. One scale screen encoded a cDNA fragment highly homologous to human AQP1. This fragment (~700 bp) was ^{32}P-labeled and used to rescreen the post-CPB lung cDNA library. Purification and sequencing of labeled clones confirmed isolation of the full-length cDNA encoding ovine AQP1. A BLAST search (16) of the GenBank database with the 5' end of ovine AQP1 cDNA revealed 98% amino acid homology with bovine AQP1 (CHIP 29) and 84% homology with human AQP1 (CHIP 28) (Fig. 2).

Physiologic Parameters of Neonatal Lambs

There was no significant difference in mean blood pressure, pulmonary artery pressure, left atrial pressure, central venous pressure, cardiac output, temperature, PO_2, PCO_2, or pH obtained at baseline compared with hourly measurements obtained from lambs during reperfusion following CPB/HCA at 15°C or 20°C (Table 1). There was a statistically significant increase in heart rate, and a decrease in hematocrit following CPB/HCA, compared with baseline levels. However, all values were within physiological range.

Tissue Expression of AQP1 mRNA

We examined the tissue distribution of AQP1 mRNA in neonatal lambs. Quantitative comparison of AQP1 mRNA levels in tissues pre- and post-CPB/HCA (at 15°C), were performed. RNA was isolated under three conditions: (1) control: 20 min sternotomy only; (2) following CPB/HCA with 30 min rewarming and no additional reperfusion; and (3) after CPB/HCA followed by 6 hr of reperfusion. Using the RNAse protection assay, we examined AQP1 mRNA levels in lung, ventricle, atrium, brain, liver, kidney, and skeletal muscle (Fig. 3A). AQP1 signals were normalized to an internal nonmuscle actin control. Results are quantified in Fig. 3B. AQP1 mRNA was undetectable by the sensitive RNAse protection assay in brain and liver. There was minimal expression in atrium and skeletal muscle, and easily detectable levels in ventricle, kidney, and lung. The lung demonstrated a significant change during the time course of CPB/HCA, which is further described in the following section.
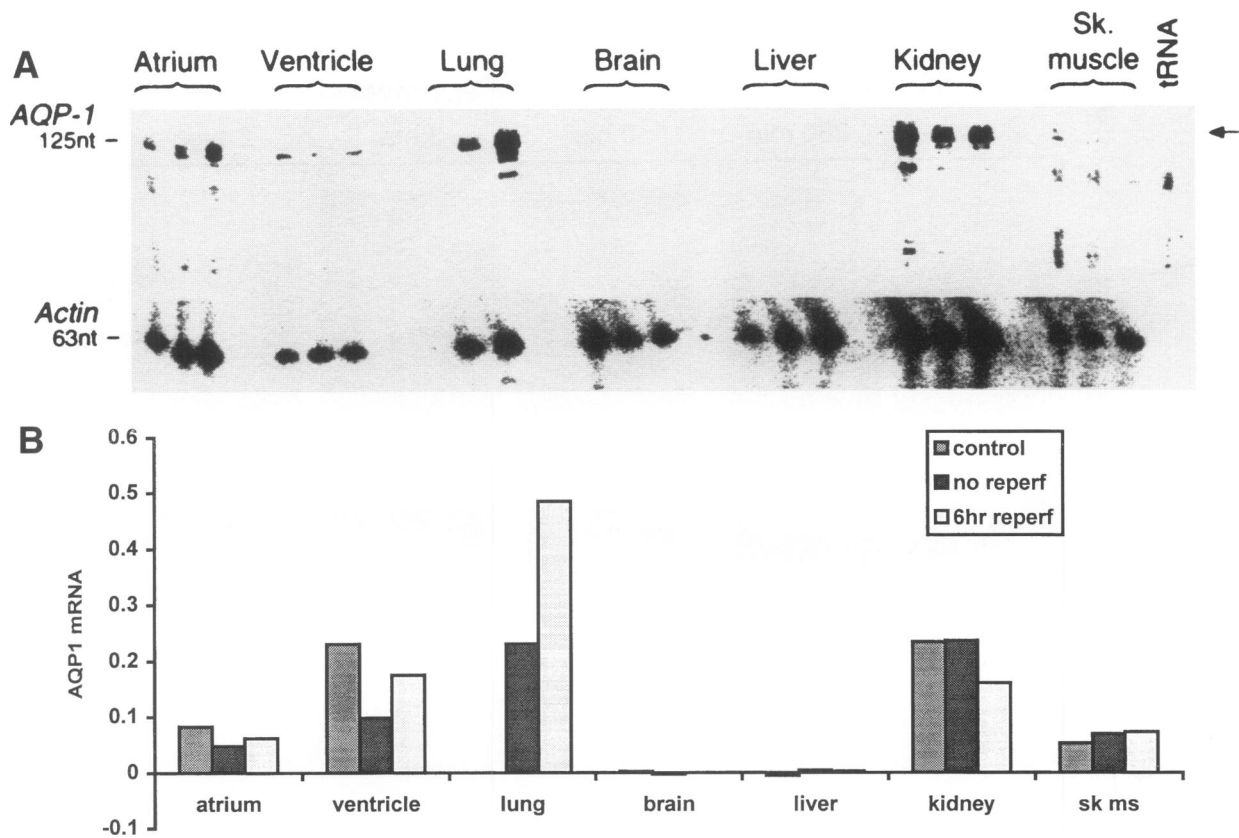
FIG. 3.   AQP1 mRNA levels in lamb tissues before and after CPB with HCA at 15°C

(A) Autoradiograph of RNAse protection assay. For each organ: left, 20 min sternotomy only; middle, CPB/HCA, no reperfusion; right, 6 hr of reperfusion following CPB/HCA. The expected AQP1-protected band is 125 nucleotides (nt) and the expected actin-protected band is 63 nt. Total RNA was lost from the lung control sample.
(B) Quantitation of AQP1 mRNA levels obtained from PhosphorImager analysis of the sequencing gel in A. Background was determined by tRNA control. AQP1 was normalized to the internal actin control, [AQP1-tRNA]/[actin-tRNA]. Negative numbers represent AQP1 signals weaker than background tRNA. Control, 20 min sternotomy only; no reperf, CPB/HCA without reperfusion; 6 hr reperf, 6 hr of reperfusion following CPB/HCA. Lung control data were not obtained in this experiment.

Induction of AQP1 mRNA during CPB/HCA and Reperfusion

Induction of AQP1 mRNA during cardiopulmonary bypass (3 hr) with hypothermic circulatory arrest (2 hr at 15°C) followed by reperfusion (up to 6 hr at 37°C) was determined by RNAse protection assay of parallel samples from the same tissue type but harvested under different experimental conditions (Fig. 1). Samples included: 20 and 260 min, sternotomy only, controls; and CPB/HCA followed by 0, 3, or 6 hr of reperfusion (37°C). Two to three animals per condition were evaluated and 1 to 4 separate tissue samples per animal were analyzed. All AQP1 mRNA levels were normalized to an internal nonmuscle actin control and background was subtracted from tRNA negative control.

The lung demonstrated a reproducible 3-fold induction in AQP1 mRNA after CPB/HCA followed by 6 hr of reperfusion ($3.38 \pm .28$ compared with 20 min control, $1.0 \pm .15$, p value, .005) (Fig. 4). This induction was not seen immediately following CPB ($1.74 \pm .58$) nor after 3 hr of reperfusion ($1.24 \pm .05$). There was a significant increase in AQP1 mRNA between 3 hr of reperfusion and 6 hr of reperfusion (p value, .016).

Ventricle AQP1 mRNA levels were not induced in neonatal lambs during CPB/HCA or during 6 hr of reperfusion. AQP1 mRNA levels normalized to internal actin control were not significantly different between the 20-min control (0.203; ± 0.037) and CPB/HCA with no reperfusion (0.198; ± 0.023, p value, .90), CPB/
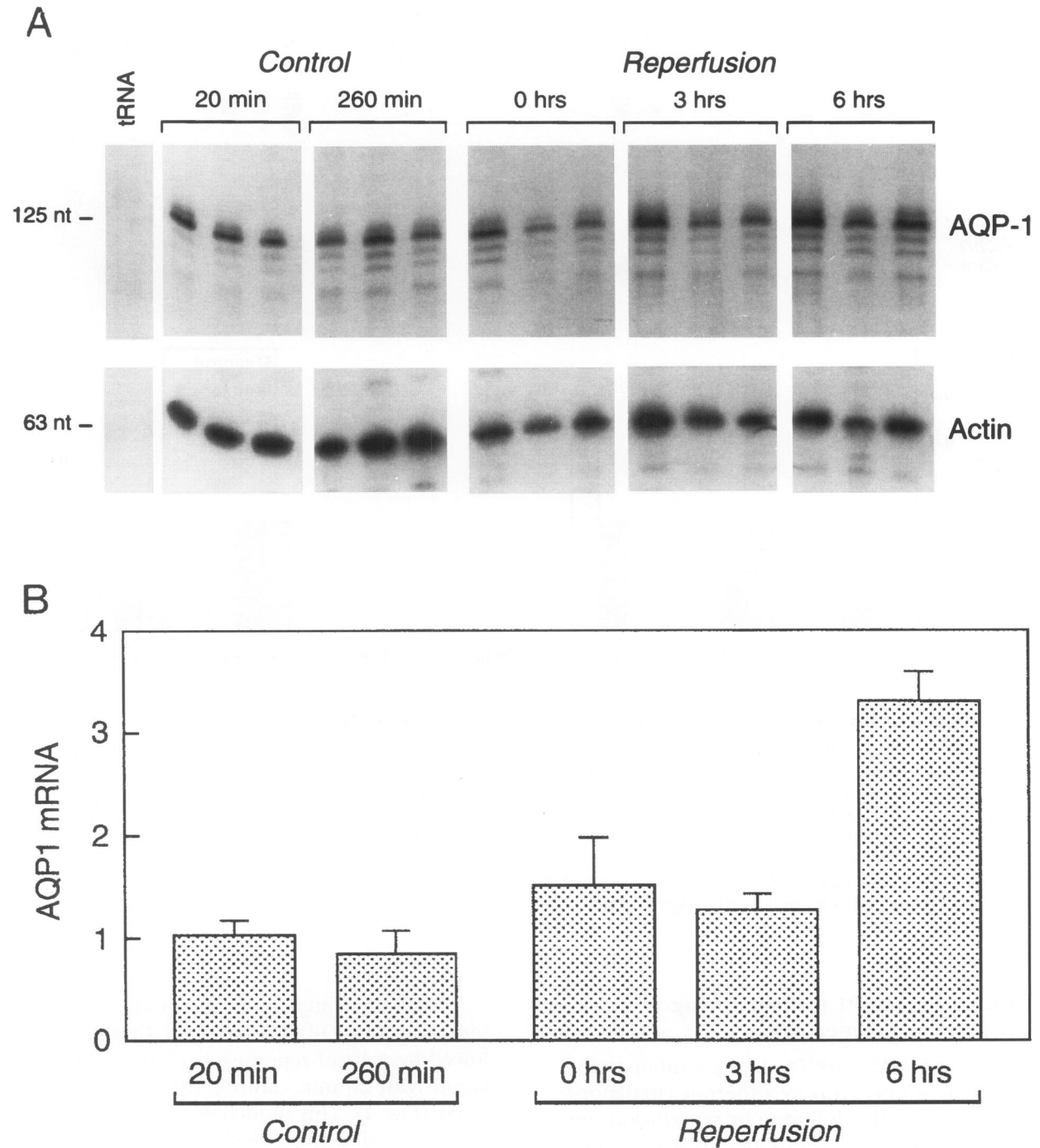
FIG. 4. AQP1 mRNA in lung before and after CPB/HCA followed by reperfusion

(A) Autoradiograph of RNase protection assay. Each lane represents a separate tissue specimen; each condition represents 2–3 animals. Control animals underwent sternotomy only for 20 or 260 min. Reperfusion animals underwent CPB/HCA (2 hr of circulatory arrest at 15°C) followed by 0, 3, or 6 hr of reperfusion at 37°C. The non-muscle actin-protected band is 63 nucleotides (nt). Three fragments of AQP1 mRNA are seen below the expected 125 nt AQP1-protected band. (B) Quantitation of AQP1 mRNA levels obtained from PhosphorImager analysis of the sequencing gel in A. Background was determined by tRNA control. AQP1 was normalized to the internal actin control, [AQP1-tRNA]/[actin-tRNA]. All samples were then normalized to a mean of 1 for the 20-min control samples. Comparative values of AQP1 normalized to actin were similar, regardless of whether the single 125-nt AQP1 band or all 4 bands were quantitated.

HCA with 3 hr of reperfusion (0.157; ± 0.007, p value, .27), or CPB/HCA with 6 hr of reperfusion (0.285; ± 0.035, p value, .21).

There was considerable variance in kidney AQP1 mRNA levels without a specific trend during CPB/HCA and reperfusion. This variability most likely reflects the random harvesting of tissue from within the kidney with samples containing various amounts of proximal tubules and thin descending limbs where AQP1 is known to be localized. More specific harvesting in the future would be required to obtain comparable samples and to determine if AQP1 mRNA is induced.

Because AQP1 mRNA levels are known to increase in rat lung 12 hr following corticosteroid administration (17), it is possible that the induction we observed in lamb lung was a result of an endogenous stress response to anesthesia, surgery, bypass, and/or hypothermia. We compared AQP1 mRNA levels in lung from a lamb treated with 30 mg/kg methylprednisolone, followed by 260 min of anesthesia, to control animals with 260 min of anesthesia alone. No difference was observed (steroid lamb 70%, and 260 min control lamb 60%, of 20 min control AQP1 mRNA levels).

DISCUSSION

The recent discovery of a highly conserved family of water channels, aquaporins, has significantly increased understanding of rapid *trans* membrane water fluxes. A majority of research has focused on the role of aquaporins in the kidney, where mutations in AQP2 cause nephrogenic diabetes insipidus (18–20). High-resolution immunoelectron microscopy studies have demonstrated reversible migration of AQP2 from intracellular vesicles to the apical plasma membrane in response to vasopressin (21). AQP1 is expressed at the cell surface and is sensitive to inhibition by mercury salts (7,8,10). In studies of sheep lung, Folkesson et al. demonstrated that a mercury-sensitive water channel plays an important role in water permeability across capillary endothelium and into alveolar spaces (22). Using an isolated lung perfused continuously with an iso-osmotic dilute blood solution, they measured the movement of water (determined by dilution of radiolabeled albumin) from the capillaries to the alveolar spaces which contained hypertonic (900 mOsm/l) fluid instilled bronchoscopically. In the control lung, the osmotically induced wa-

ter permeability had a $t_{1/2}$ of .85 min. Water permeability in the contralateral lung was reversibly attenuated ($t_{1/2}$ 2.7 min) by mercury (0.5 mM $HgCl_2$). In addition, in isolated rat alveolar type II epithelial cells, they measured an osmotic water permeability (P_f 0.015 ± .002 cm/sec, 10°C) which is comparable to the water permeability reported in AQP1-rich erythrocytes (P_f, 0.02 cm/sec) (23) and in isolated renal tubules (P_f, 0.007 − 0.06 cm/sec) (21). As a known mercury-sensitive water channel, AQP1 may play an important role in water permeability between alveolar spaces and perialveolar capillaries. Additional studies demonstrated a significant induction in AQP1 mRNA and protein expression (17,24) in perinatal rat lung, implying a potential role for AQP1 in lung water clearance at birth. This effect was augmented by corticosteroids, suggesting that under some circumstances, AQP1 induction may be part of an intrinsic stress response (17).

Using subtraction cloning techniques, we isolated full-length ovine AQP1 cDNA from neonatal lamb lung. This ovine cDNA was highly homologous to bovine and human AQP1. In the setting of cardiopulmonary bypass in neonatal lambs, we found that AQP1 mRNA is increased 3-fold in lung after cardiopulmonary bypass and circulatory arrest (15°C) followed by 6 hr of reperfusion (37°C). This increase was not seen after 3 hr of reperfusion, nor was it seen to a significant degree in the other tissues. Substantial increases in AQP1 mRNA levels in lung have been demonstrated during the perinatal period in rats as a function of age (17,24). In our experiments, mean lamb age among different conditions was similar (control, mean = 3 days; no reperfusion, mean = 5 days; 3 hr reperfusion, mean = 4.5 days; 6 hr reperfusion mean = 5 days). Therefore, age was not the sole determinant of increases in AQP1 mRNA levels, but it may have contributed to variation among samples. We demonstrated that high-dose corticosteroids do not result in AQP1 mRNA induction in lung over a 4-hr time course in an anesthetized lamb. Further study would be needed to determine if endogenous stress response hormones contribute to AQP1 induction observed at a later time following CPB/HCA.

Nieslen et al., studying tissue distribution of AQP1 in rats, demonstrated an abundance of AQP1 in the kidney and erythrocytes, moderate levels in lung, lesser amounts in the heart and liver, and none in the brain (11). In lambs, we found that AQP1 mRNA was essentially unde-

tectable in brain and liver, minimally expressed in skeletal muscle and atrial muscle, and expressed significantly in ventricle, kidney, and lung.

Identification of AQP1 by differential gene expression after CPB/HCA is important and relevant for understanding bypass-related lung injury for two reasons. First, AQP1 mRNA levels are significantly induced by the subtraction conditions (CPB/HCA and 6 hr of reperfusion versus control). Second, increased AQP1 mRNA levels during a period of susceptibility to bypass-related, acute pulmonary edema is functionally consistent with the observation of mercury-sensitive water movement from capillaries to the alveolar space (22) and the observation of induction of AQP1 mRNA and protein expression in rat lung during the perinatal period (17,24). Further studies are needed to document increases in AQP1 protein expression and localization to perialveolar capillary endothelium associated with pulmonary vascular leak.

Induction of AQP1 mRNA in lung following CPB/HCA with reperfusion is late compared with induction of inflammatory mediators (ICAM-1, E-selectin and interleukin-8) (1,2), which participate in leukocyte recruitment, vascular injury, and associated capillary leak. Other studies imply a role for AQP1 in clearance of lung water (17,22,24). The late induction of AQP1 mRNA in lung may reflect a response to the pulmonary edema that occurs as a result of endothelial damage following CPB/HCA. Further investigations into the role of AQP1 in the lung could have significant clinical impact on understanding and managing other processes involving pulmonary vascular endothelial or alveolar epithelial damage, such as sepsis, neonatal respiratory distress syndrome, or adult respiratory distress syndrome.

ACKNOWLEDGMENTS

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