Proteomic Analysis Reveals Alterations in the Renal Kallikrein Pathway during Hypoxia-Induced Hypertension*

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Obstructive sleep apnea syndrome (OSAS), a disorder characterized by episodic hypoxia (EH) during sleep, is associated with systemic hypertension. We used proteomic analysis to examine differences in rat kidney protein expression during EH, and their potential relationship to EH-induced hypertension. Young male Sprague-Dawley rats were exposed to either EH or sustained hypoxia (SH) for 14 (EH14/SH14) and 30 (EH30/SH30) days. Mean arterial blood pressure was significantly increased only in EH30 (p < 0.0002). Kidney proteins were resolved by two-dimensional-PAGE and were identified by MALDI-MS. Renal expression of kallistatin, a potent vasodilator, was down-regulated in all animals. Expression of α1-antitrypsin, an inhibitor of kallikrein activation, was up-regulated in EH but down-regulated in SH. Western blotting showed significant elevation of B2-bradykinin receptor expression in all normotensive animals but remained unchanged in hypertensive animals. Proteins relevant to vascular hypertrophy, such as smooth muscle myosin and protein-disulfide isomerase, were up-regulated in EH30 but were down-regulated in SH30. These data indicate that EH induces changes in renal protein expression consistent with impairment of vasodilation mediated by the kallikrein-kallistatin pathway and vascular hypertrophy. In contrast, SH-induced changes suggest the kallikrein- and bradykinin-mediated compensatory mechanisms for prevention of hypertension and vascular remodeling. To test the hypothesis suggested by the proteomic data, we measured the effect of EH on blood pressure in transgenic hKLK1 rats that overexpress human kallikrein. Transgenic hKLK1 animals were protected from EH-induced hypertension. We conclude that EH-induced hypertension may result, at least in part, from altered regulation of the renal kallikrein system.

Obstructive sleep apnea syndrome (OSAS),1 a disorder characterized by episodic hypoxia (EH), is a major public health problem (1–3). OSAS affects 4–5% of the general adult population in United States and 1–2% of children (4–6). One of the major consequences of untreated OSAS is systemic hypertension (7, 8), with a prevalence ranging from 15 to 56% (8–10). Indeed, OSAS has been demonstrated to be an independent risk factor for systemic hypertension, and the relative risk is elevated even in young children (8, 11). Moreover, OSAS has also been associated with both proteinuria and end-stage renal disease (12).

The pathophysiology of systemic hypertension in OSAS is complex. Increased sympathetic nervous system activity induced by the intermittent hypoxia that characteristically accompanies OSAS appears to mediate hypertension during OSAS via a mechanism that requires changes in the vasoreactivity of resistance vascular beds such as the kidney (13–19). Indeed, sustained hypoxia (SH) does not lead to hypertension as do pharmacologically-mediated decreases of angiotensin II levels and blockade of angiotensin receptors (20, 21). These data suggest that changes in renal vasoconstrictors play a major role in the development of hypertension during OSAS. Blood pressure is also modulated by vasodilatory components of the kinin cascade that are produced in the kidney, but little is known about their role in OSAS-induced hypertension. Therefore, we hypothesized that alterations in the kinin cascade would contribute to OSAS-induced hypertension.

We wished to study coordinated changes in renal protein expression to examine concomitant alterations in vasodilators and vasoconstrictor proteins that modulate blood pressure control in this organ. Western blotting, enzyme-linked immunosorbent assay (ELISA), and other techniques have been previously used to study renal protein expression (22, 23). However, these techniques are limited by the efficiency of analysis of multiple proteins and by the availability of specific antibodies that identify a protein of interest. Proteomic analysis is an innovative approach to determine coordinated changes in protein expression in tissues and cells (24, 25). One common approach to proteomic analysis is two-dimensional PAGE. The proteins are separated by differential isoelectric point (pI) in the first di-

* The abbreviations used are: OSAS, obstructive sleep apnea syndrome; EH, episodic hypoxia; SH, sustained hypoxia; ELISA, enzyme-linked immunosorbent assay; pI, isoelectric point; Mw, molecular weight; MAP, mean arterial pressure; A1AT, α1-antitrypsin; PDI, protein-disulfide isomerase; DNase I, deoxyribonuclease I; B2R, B2-bradykinin receptor; PDGF, platelet-derived growth factor.
Renal Kallikrein and Hypoxia-induced Hypertension

Proteomic analysis was performed to examine changes in renal protein expression during EH and SH. We observed consistent changes in renal protein expression in response to EH-induced hypertension that differed from SH-induced changes. Two groups of proteins were altered; members of the kallikrein pathway and components of smooth muscle. A decrease in expression of kallistatin, a potent vasodilator, was observed in all groups but only animals exposed to long-term EH developed hypertension. B,R expression and kallikrein levels were significantly increased in normotensive SH-exposed animals, but remained unchanged in hypertensive animals. Overexpression of renal kallikrein in transgenic hKl1 animals prevented EH-induced hypertension. We conclude that changes in the renal kallistatin and kallikrein–kinin pathway are associated with the hypertensive response to EH and maintenance of normal blood pressure in SH. These complex mechanisms may play a significant role in the development of hypertension during OSAS.

MATERIALS AND METHODS

Experimental Animals—Twenty-four Sprague-Dawley male rats weighing 175–200 g were used in this study (12 for 14-day experiments and 12 for 30 days). Also, transgenic hKl1 that express human kallikrein were used. The transgenic hKl1 transgenic line was constructed and maintained as described previously (26). The experimental protocols were approved by the Institutional Animal Care and Use Committee of the University of Louisville. Hypoxic Exposure—Animals were placed in 4 identical commercially designed chambers (30 × 20 × 20; Oxycycler model A44XO, Reming Bioinstruments, Redfield, NY), that were operated under a 12-h light-dark cycle (6:00 AM–6:00 PM). Gas was circulated around each of the chambers, attached tubing and other units at 60 liters/min (i.e., one complete change per 10 s). The O2 concentration was continuously monitored and maintained at ≤0.01% by adjusting overall chamber ventilation. The gas was also circulated through a molecular sieve (Type 3A, Fisher, US) so as to remove ammonia. Humidity was measured and maintained at 40–50% by changing basal ventilatory inputs. Ambient temperature was kept at 22–24 °C. The EH profile consisted of alternating room air and 10% oxygen every 90 s during daylight hours, while oxygen concentration in premixed Tris acetate equilibration buffer with 0.06% SDS, 1.76% ampholytes, 120 mmol/liter dithiothreitol, and 3.2% Triton X-100. The concentration levels of proteins were measured by spectrophotometry using Bio-Rad protein microassay based on Bradford’s method (29).

First Dimension of Two-dimensional PAGE—Tube gel running system (Genomic Solutions Inc., Ann Arbor, MI) was used for first-dimension gel running with 100 mmol/liter sodium hydroxide, the cathode buffer, and 10 mmol/liter phosphoric acid, the anode buffer. Pre-cast Tris, 7.92 mol/liter urea, 0.06% SDS, 1.76% ampholytes, 120 mmol/liter dithiothreitol, and 3.2% Triton X-100. The protein samples of 100 μg were loaded into the tube gels and were focused for 17 h and 30 min to reach 18,000 Vh.

Second Dimension of Two-dimensional PAGE—The gels were extracted from the tubes after completion of focusing and were incubated in premixed Tris acetate equilibration buffer with 0.01% bromphenol blue and 50 mmol/liter dithiothreitol, incubated at 100 °C for 5 min, and transferred to iced. One-tenth volume of a buffer containing with 500 mmol/liter Tris, 50 mmol/liter MgCl2, 1 mg/ml DNase I, and 0.25 mg/ml RNase A was added and incubated for an additional 10 min. The 12,000 rpm supernatants were obtained, 10% trichloroacetic acid was added to precipitate proteins, and the 12,000 rpm pellets were obtained. After several washes with acetone, the pellets were resuspended in a sample buffer containing 40 mmol/liter Tris, 7.92 mol/liter urea, 0.06% SDS, 1.76% ampholytes, 120 mmol/liter dithiothreitol, and 3.2% Triton X-100. The concentration levels of proteins were measured by spectrophotometry using Bio-Rad protein microassay based on Bradford’s method (29).
2E instrument equipped with a 337 nm N₂ laser at 20–35% power in the positive ion reflectron mode. Spectral data were obtained by averaging 10 spectra, each of which was the composite of 10 laser firings. The mass axis was calibrated using known peaks from tryptic autolysis.

Analysis of Peptide Sequences—Peptide mass fingerprinting was used for protein identification from tryptic fragment sizes by using the MASCOT search engine (www.matrixscience.com) based on the entire NCBI and SwissProt protein data bases using the assumption that peptides are monoisotopic, oxidized at methionine residues, and carbamidomethylated at cysteine residues. Up to 1 missed trypsin cleavage was allowed although most matches did not contain any missed cleavages. Mass tolerance of 150 ppm was the window of error to be allowed for matching the peptide mass values. Probability-based MOWSE scores were estimated by comparison of search results against estimated random match population and were reported as $-10 \times \log_{10}(P)$, where $P$ is the absolute probability. Scores greater than 71 were considered significant ($p < 0.05$). All protein identifications were in the expected size range based on its position in the gel.

Western Blotting—The kidneys were homogenized in phosphate saline buffer (pH 7.0) and centrifuged at 1,000 rpm for 5 min, and the supernatants were saved. Deoxycholate was added to 0.5% into the

### TABLE 1

|                          | MAP (mm Hg) | Baroreceptor Gain (bpm/mmHg) |
|--------------------------|-------------|------------------------------|
| Control                  | 89.7 ± 3.7  | 4.15 ± 0.17                  |
| EH14                     | 92.3 ± 3.9  | 2.68 ± 0.13                  |
| EH30                     | 104.7 ± 4.2 | 2.22 ± 0.10                  |
| SH14                     | 88.7 ± 3.4  | 4.13 ± 0.18                  |
| SH30                     | 90.4 ± 3.9  | 4.18 ± 0.21                  |

* $P < 0.0002$.

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**FIG. 1.** Mass spectra and peptide mass fingerprinting. Typical mass spectra of kallikrein-binding protein (kallistatin) obtained by MALDI-TOF mass spectrometry (A). Peptide mass fingerprinting was performed using the Mascot search engine to query the NCBI protein data base. Results of the search are shown in B. MOWSE scores >71 were considered statistically significant for matching. Observed masses were matched to the theoretical masses of kallistatin. Measured peptide masses covered 40% of the kallistatin sequence.
samples, and the mixtures were incubated at 4 °C for 30 min. The mixtures were centrifuged at 12,000 rpm for 30 min, the supernatants were saved, and the protein concentrations were measured by Bradford’s method. SDS sample buffer (Tris-HCl, glycerol, SDS, dithiothreitol, and bromphenol blue) was added 1:1 to the protein solution, the mixture was heated at 100 °C for 5 min, and 50 μg of protein was loaded

**Fig. 2. The proteome map of differentially expressed proteins.** The proteome map was created by representing two-dimensional gel of renal proteins from RA30 (A), EH30 (B), SH30 (C), RA14 (D), and EH14 (E) rats. The proteins were resolved by differential isoelectric point (pI) for the first dimensional running (x-axis) and differential molecular weight (Mw) for the second dimension (y-axis). The differentially expressed proteins were labeled and subjected to in-gel tryptic digestion and peptide mass fingerprinting. The quantitative analysis of each protein spot is shown in Tables II and III. Homer-1b was expressed only in 30 days SH but three forms of A1AT precursor were absent in 30 days SH. Several proteins expressed as multiple forms of the same protein that most likely to be from post-translational modifications that cause changes in their pI and Mw. K, kallistatin; F, ferritin.
on 10% SDS-PAGE. Proteins on the gel were transferred to a nitrocellulose membrane by electroblotting. The membrane was treated with primary antibody against mouse B2-bradykinin receptor (BD Transduction Laboratories, Franklin Lakes, NJ) 1:1000 in 5% milk/TTBS at 4°C overnight and immunoreactive protein was detected by radiography using goat anti-mouse IgG-conjugated with horseradish peroxidase.

**Tissue Kallikrein Level**—The kidneys were homogenized in phosphate saline buffer (pH 7.0) and centrifuged at 1,000 rpm for 5 min, and the supernatants were saved. Deoxycholate was added to 0.5% to the supernatants were saved, and the mixtures were incubated at 4°C for 30 min. The mixtures were centrifuged at 12,000 rpm for 30 min, the supernatants were saved, and the protein concentrations were measured by Bradford’s method. Kallikrein level was measured by ELISA as previously described (31).

**Statistical Analysis**—For physiological variables, differences between the various treatment groups were compared by two-way analysis of variance and the Newman-Keuls multiple range test for multiple comparisons. Mann-Whitney Test by SPSS software was used for analysis of variance and the Newman-Keuls multiple range test for multiple comparisons. Mann-Whitney Test by SPSS software was used for analysis of variance and the Newman-Keuls multiple range test for multiple comparisons. Mann-Whitney Test by SPSS software was used for analysis of variance and the Newman-Keuls multiple range test for multiple comparisons.

### RESULTS

**Blood Pressure**—Table I shows mean arterial blood pressure and baroreceptor gain for normoxic rats and those exposed to EH and SH. EH14 animals had mild reductions in baroreceptor gain, but blood pressure was within normal limits. However, all of the EH30 animals were hypertensive and had marked attenuation of baroreceptor gain. In contrast, none of the animals in the SH group developed hypertension or attenuation of baroreceptor gain at either SH14 or SH30.

**Examples of Proteomic Analysis**—The expression of 248 protein spots was analyzed on each gel. An average image was established from multiple gels as a reference gel for each group. The intensity of each matched spot was compared. All of the differentially expressed protein spots were excised, underwent in-gel tryptic digestion and analyzed by MALDI-TOF. Fig. 1A demonstrates typical mass spectra, in this instance obtained from the gel spot corresponding to the kallikrein-binding protein (kallistatin). Fig. 1B illustrates the results of the peptide mass fingerprinting method performed using the Mascot protein search engine to query the NCBI database.

### Table II

| Protein Spot | NCBI identifier | 30 days Control | 30 days EH | 30 days SH |
|--------------|-----------------|----------------|------------|------------|
| Kallistatin 1 | gi|92335 | 18764 ± 4563 | 9670 ± 3125** | 11376 ± 3035 |
| Kallistatin 2 | gi|92335 | 11371 ± 2609 | 6114 ± 2059** | 6711 ± 1312** |
| Kallistatin 3 | gi|92335 | 8397 ± 1805 | 3360 ± 1305** | 4004 ± 766** |
| Kallistatin 4 | gi|92335 | 15742 ± 4185 | 8635 ± 4443** | 11113 ± 3581** |
| Kallistatin 5 | gi|92335 | 8282 ± 2754 | 4360 ± 1698** | 5539 ± 593** |
| A1AT precursor 1 | gi|203063 | 3297 ± 924 | 9293 ± 2068# | 0 ± 0 |
| A1AT precursor 2 | gi|203063 | 3271 ± 607 | 9328 ± 2596# | 0 ± 0 |
| A1AT precursor 3 | gi|203063 | 1910 ± 512 | 4464 ± 1226# | 0 ± 0 |
| A1AT precursor 4 | gi|203063 | 969 ± 36 | 2592 ± 946# | 963 ± 204 |
| A1AT precursor 5 | gi|203063 | 2514 ± 446 | 8159 ± 2284# | 4257 ± 1665 |
| PDI 1 | gi|6981324 | 2191 ± 1095 | 6308 ± 1984# | 1095 ± 178 |
| PDI 2 | gi|6981324 | 2782 ± 1077 | 6425 ± 2089# | 859 ± 211** |
| Smooth muscle myosin | gi|127148 | 17238 ± 3844 | 73845 ± 22111# | 2243 ± 588** |
| Beta-actin 1 | gi|113270 | 3192 ± 111 | 7014 ± 2183# | 8164 ± 2350# |
| Beta-actin 2 | gi|113270 | 1948 ± 470 | 5016 ± 1516# | 3208 ± 481# |
| Vimentin | gi|5030431 | 9380 ± 1763 | 16489 ± 4549# | 2756 ± 881** |
| Ferritin (light chain) | gi|2119695 | 83476 ± 40352 | 144071 ± 54247# | 633736 ± 159904# |
| Ferritin 2 (heavy chain, fragment) | gi|111625 | 1179 ± 771 | 2048 ± 1041 | 7947 ± 1830# |
| Ferritin 3 (heavy chain, fragment) | gi|111625 | 18384 ± 11864 | 14854 ± 11131 | 69750 ± 16898# |
| Ferritin 4 (heavy chain, fragment) | gi|111625 | 2822 ± 2057 | 3611 ± 3109 | 7988 ± 720# |
| Dnase I 1 | gi|494869 | 3070 ± 1294 | 2439 ± 875 | 13439 ± 2656# |
| Dnase I 2 | gi|494869 | 7397 ± 4361 | 5782 ± 2357 | 54515 ± 4333# |
| Dnase I 3 | gi|494869 | 9933 ± 5564 | 8261 ± 2369 | 886990 ± 6206# |
| Calbindin 1 | gi|2119348 | 33278 ± 16822 | 16894 ± 5181 | 2765 ± 1379# |
| Calbindin 2 | gi|2119348 | 193670 ± 56828 | 23574 ± 107266 | 2197 ± 215# |
| Tropomyosin | gi|205924 | 23592 ± 36620 | 36699 ± 10125 | 6990 ± 2241# |
| Apolipoprotein A-I | gi|2145143 | 14662 ± 6699 | 14707 ± 4767 | 1597 ± 519** |
| AT_Pase, delta chain | gi|1326036 | 121163 ± 11370 | 107597 ± 30854 | 6852 ± 2808# |
| Homer-1b | gi|3834625 | 0 ± 0 | 0 ± 0 | 2934 ± 929# |

* Less than control, p < 0.05.
* Greater than control, p < 0.05.
* Absent after 30 days SH.
* Expressed only in 30 days SH.
ined the effect of EH and SH on B2R expression. Extracted kidney tissue was analyzed by immunoblotting, and a single 42-kDa band was observed consistent with the B2R. Densitometry was performed to compare signal intensity of each sample. As shown in Fig. 3, A and B, B2R expression was significantly increased in kidneys exposed to either EH or SH for 14 days. However, B2R expression remained elevated only in kidneys from SH30 animals. B2R expression fell to basal levels in animals exposed to EH30 that developed hypertension (Fig. 3C).

Effect of EH and SH on Renal Kallikrein Level—Because kallikrein can directly activate the B2R and thereby directly promote vasodilation, we wished to determine if hypertensive EH30 animals had changes in kallikrein expression. Renal kallikrein levels were measured by ELISA in SH- and EH-exposed animals and are summarized shown in Fig. 4. Only SH30 animals had increased kallikrein levels (2.17 ± 0.19 versus 1.64 ± 0.13 ng/mg of total protein, p < 0.05). Kallikrein levels in EH14 and EH30 animals were unchanged.

Effect of Renal Kallikrein on Blood Pressure During Hypoxia—To examine the relationship between kallikrein levels and EH-induced hypertension, we employed a transgenic rat that expresses the human tissue kallikrein gene, TGR (hKLK1). Translation of hKLK1 mRNA was verified previously by the demonstration of human kallikrein in the urine of transgenic rats (700 ± 127 ng/ml) (26). The TGR (hKLK1) animals were exposed to RA and EH, and the blood pressure was compared with the control rats (see Fig. 6, A and B). Systolic and diastolic BP of the control EH animals were significantly increased after 4 weeks and were higher at longer exposure (at 6–9 weeks). Both systolic and diastolic BP of the control RA and TGR (hKLK1) RA animals remained at the basal level during the entire experiment. Overexpression of renal kallikrein in TGR (hKLK1) animals prevented the animals from hypertension during EH at 4–8 weeks. Although the systolic BP of TGR (hKLK1) EH was significantly increased at 9 weeks, systolic levels were much lower than the control EH animals, and diastolic BP remained at the basal level.

DISCUSSION

We used proteomic analysis to study renal protein expression in a clinically relevant model of hypertension induced by long-term EH exposures. The expression of 248 protein spots was visualized, and we constructed rat renal proteome maps for the differentially expressed proteins during EH and SH exposures.

The changes in blood pressure and baroreceptor gain were anticipated based on previous studies using EH and are compatible with the blood pressure elevations reported (33, 34). However, we now show that the initial effects of EH are to reduce baroreceptor gain, and that these changes are then followed by the emergence of arterial hypertension. In contrast, SH was not associated with any identifiable changes in either blood pressure or baroreceptor function.

Analysis of the proteomic maps revealed that changes in the kallistatin pathway correlated with onset of hypertension. Indeed, kallistatin was consistently down-regulated by EH exposures, and this effect was greater at 30 days. Kallistatin is a novel serine protease inhibitor (35) whose main functions include potent vasodilation and inhibition of kallikrein-kinin activity (35, 36). Decreases in kallistatin would be expected to reduce the vasodilating capacity of the kidney and cause or aggravate hypertension (37, 38).

AIAT is another serine protease inhibitor and acts like other...
trypsin inhibitors to inhibit the activity of kallikrein-kinin system (39–41). During EH, kallistatin and A1AT had decreased and increased expression, respectively. Based on previous work, we would expect kallistatin to be the more potent of the two inhibitors of kallikrein (42).

\[B_2R\] expression was significantly increased in kidneys exposed to either EH or SH for 14 days and remained modestly elevated in kidneys from animals exposed to SH30, but fell to

**Fig. 5. Proposed schema for pathophysiology of EH-induced hypertension and a compensatory mechanism in SH.** Panel A, proposed scheme of EH-induced hypertension after a 30-day hypoxia exposure. Panel B, proposed scheme of compensation in SH characterized by increased \(B_2R\) expression and elevated kallikrein levels.

**Fig. 4. Tissue kallikrein level.** Renal kallikrein level was significantly increased only in SH30 animals. However, we observed a trend of increased kallikrein in the SH14 group. *, \(p < 0.05\) compared with the control.

**Fig. 6. Effect of renal kallikrein on the blood pressure during hypoxia.** Transgenic rat line harboring the human tissue kallikrein gene, TGR (hKLK1) was generated. Systolic and diastolic BP of the control EH animals were significantly increased after 4 weeks and were higher at longer exposure (at 6–9 weeks). Both systolic and diastolic BP of the control RA and TGR (hKLK1) RA animals remained at the basal level during the entire experiment. For the TGR (hKLK1) EH animals, diastolic BP remained at the basal level, whereas systolic BP started to be significantly increased at 9 weeks but significantly less than the control EH. *, \(p < 0.05\) compared with the control RA; **, \(p < 0.05\) compared with the control RA and TGR (hKLK1) EH.
basal levels in animals exposed to EH30. In animals exposed to EH14 that were not hypertensive, all 5 forms of AIAT were increased whereas only 2 of 5 forms of kallistatin were decreased. Also, B2R expression was significantly increased in EH14 animals. Therefore, EH14 animals retained vasodilatory pathways, and this may explain the fact that they remained normotensive. However, in hypertensive EH30 animals, the vasodilatory kallistatin, kallikrein, and bradykinin receptor pathway(s) were down regulated or at basal levels. Interestingly, in normotensive SH30 animals, kallikrein levels and B2R expression were both increased. Therefore, these data suggest a new hypothesis that EH-induced hypertension results, at least in part, from a decrease in the vasodilatory effect of kallistatin and a failure to increase B2R expression to compensate. The proposed scheme for this mechanism is shown in Fig. 5A. Also, these data suggest the hypothesis that increases in kallikrein and B2R expression serve as a compensatory mechanism to prevent hypertension during exposure to sustained hypoxia (Outlined in Fig. 5B).

We further examined the hypothesis generated by the proteomic analysis that increased renal kallikrein expression serves as a compensatory mechanism to prevent hypoxia-induced hypertension. We employed TGR (hKLK1) rats that express human tissue kallikrein in the kidney (26). Overexpression of kallikrein prevented hypoxia-induced hypertension during 4–8 weeks of exposure. Although the systolic BP started to increased at 9 weeks, the pressure of TGR (hKLK1) EH animals was much less than in the control EH animals (Fig. 6A).

The long-term (30 days SH) increase in the B2R expression was modest. However, the B2R may exert effects on the kidney other than through vasodilation. A recent report demonstrated a potent anti hypertrophic effect of B2R on renal vasculature (43). We observed in EH changes in protein expression that have been associated with vascular smooth muscle proliferation and the attendant vascular remodeling. EH30 animals had increased expression of smooth muscle myosin, PDI, vimentin and α-actin. Smooth muscle myosin is a component of vascular smooth muscle cells (44). PDI plays an important role in homocysteinic changes and tissue remodeling (45) and is up-regulated in models of renal vascular hypertrophy and hyperplasia induced by angiotensin II or platelet-derived growth factor (PDGF) (46). Vimentin and α-actin are filamentous and cytoskeletal proteins, respectively, and are found in a wide variety of cells. However, their expression has been shown to change in a coordinated manner during vascular hypertrophy and hyperplasia (46). Alteration of these proteins in EH was time-dependent, as no changes occurred at EH14 but increases became apparent at EH30. In contrast, expression of smooth muscle myosin, PDI, tropomyosin, and vimentin decreased in the kidneys of animals exposed to SH30, i.e., in those animals exposed to hypoxia that did not develop hypertension. These data are consistent with the hypothesis that kallikrein and B2R act to inhibit vascular remodeling.

The proteomic analysis was also consistent with a number of other changes in protein expression previously observed in hypertension. For example, in our experiments apolipoprotein-AI levels were increased in the kidney in hypertensive animals and were decreased when compared with controls in normotensive animals. In hypertension-induced renal vascular wall thickening, vascular wall thickness correlates more strongly with lipid deposition than with high blood pressure (47) and apolipoprotein-AI is observed in the intimal and medial layers of atherosclerotic vessels (48).

Interestingly, the protein homer-1b was expressed only in kidneys from animals exposed to 30 days SH. Homer-1b is a 30 kDa PDZ-domain-containing protein that interacts with metabotropic glutamate receptors and inositol trisphosphate receptors in postsynaptic neurons (49, 50). Homer-1b predominantly localizes to the rat hippocampus. Binding of homer-1b with mGlur1α and mGlur5α receptors may lead to stabilization and cell-surface targeting of the receptors in their signaling pathways (51). Homer-1b is a novel protein that has been described in the protein data base since 1999 (ca.expasy.org). No available data exists that reports expression of this protein in the kidney. We are currently examining the role of homer-1b in the kidney exposed to long-term SH.

In summary, we have shown that both EH and SH alter renal protein expression. However, the differential changes induced by these exposures are consistent with the hypothesis that kallistatin and AIAT play important roles in the pathogenesis of EH-induced hypertension via altered regulation of renal kallikrein system and a decrease in vasodilatory effect. This effect may be one of the multiple factors contributing to the hypertension of OSAS. Additionally, two conditions in which kallikrein levels were elevated were associated with normalization of blood pressure. TGR (hKLK1) rats that overexpressed human tissue kallikrein and rats exposed to sustained hypoxia that had elevated kallikrein levels had normalization of their blood pressure. These data are consistent with the hypothesis that increase of renal kallikrein expression serves as a compensatory mechanism to prevent hypoxia-induced hypertension. Alterations in B2-bradykinin receptor expression indicate the activation of a compensatory mechanism that prevents hypertension and vascular remodeling in SH-exposed rats. Additional changes in other proteins suggest several hypotheses regarding the renal response to intermittent hypoxia that may manifest as a result of vascular hypertrophy and remodeling.

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