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# Effects of hypoxia on renin secretion and renal renin gene expression

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**Effects of hypoxia on renin secretion and renal renin gene expression.** Plasma renin activity (PRA) and renal renin mRNA levels were measured in male rats exposed to hypoxia (8% O<sub>2</sub>) or to carbon monoxide (CO; 0.1%) for six hours. PRA increased fourfold and 3.3-fold, and renin mRNA levels increased to 220% and 200% of control, respectively. In primary cultures of renal juxtaglomerular (JG) cells, hypoxia (lowering medium O<sub>2</sub> from 20% to 3% or 1%) for 6 or 20 hours did not affect renin secretion or gene expression. Renal denervation did not prevent stimulation of the renin system by hypoxia. Because norepinephrine increased 1.7-fold and 3.2-fold and plasma epinephrine increased 3.9-fold and 7.8-fold during hypoxia and CO inhalation, respectively, circulating catecholamines might mediate the stimulatory effects of hypoxia on renin secretion and renin gene expression. Stimulation of  $\beta$ -adrenergic receptors by continuous infusion of 160  $\mu$ g/kg/hr isoproterenol increased PRA 17-fold and 20-fold after three and six hours, respectively, and renin mRNA by 130% after six hours. In rats with a stimulated renin system (low-sodium diet), isoproterenol did not stimulate PRA or renal renin mRNA further. In summary, both arterial and venous hypoxia can stimulate renin secretion and renin gene expression powerfully *in vivo* but not *in vitro*. These effects seem not to be mediated by renal nerves or by a direct effect on JG cells but might be mediated by circulating catecholamines.

Hypoxia may reduce aldosterone secretion by direct inhibition of secretion from adrenal glomerulosa cells [1]. The effects of hypoxia on renin secretion, as reflected by the plasma renin activity (PRA), are less clear, and data on renal renin gene expression are scarce [2–10]. The influence of hypoxia on renin secretion may be modulated by sodium balance [3, 11]. Most studies suggest that acute hypoxia causes natriuresis, which may return to the normal or subnormal range during prolonged exposure [7, 12, 13]. Sodium responses might be affected by different natriuretic mediators, such as a fall in aldosterone secretion and decreased proximal tubular reabsorption, and antinatri-

uretic mediators, such as a fall in renal perfusion pressure, increased angiotensin II and a stimulated sympathetic nervous system [7, 9, 12, 13]. The effects of hypoxia on renin secretion thus might depend partly on the experimental protocol (duration and strength of hypoxia, sodium balance), thereby accounting for divergent findings.

Cell culture and *in vivo* studies indicated that several genes [such as erythropoietin, endothelin-1, platelet-derived growth factor (PDGF)-A, PDGF-B, vascular endothelial growth factor (VEGF), and heat shock transcription factor] are stimulated by hypoxia *in vitro* and/or *in vivo* [14, 15]. We therefore investigated whether hypoxia affects renin secretion and renin gene expression *in vivo* and *in vitro*. Rats were exposed to both acute arterial (8% O<sub>2</sub>) and venous (CO inhalation) hypoxia, and primary cultures of renal juxtaglomerular (JG) cells were exposed to low PO<sub>2</sub> [10].

## METHODS

### Animal experiments

Animal experiments were conducted according to National Institutes of Health and national guidelines. Three groups of male Sprague-Dawley rats (200 to 250 g; food and water *ad libitum*) were studied: untreated controls, hypoxia (six-hour exposure to 8%O<sub>2</sub>/92%N<sub>2</sub>), and CO (six-hour exposure to 0.1% CO in room air). In addition, we tested the effect of  $\beta$ -adrenergic stimulation by infusion of isoproterenol (160  $\mu$ g/kg/h) using osmotic minipumps as previously described [16]. In separate experiments, the effect of dietary salt intake on  $\beta$ -adrenergic stimulation of renin secretion and renal renin gene expression was tested by feeding normal (0.109 mmol/g NaCl), low-salt (0.006 mmol/g NaCl), or high-salt (0.783 mmol/g NaCl) rat chow (Altromin, Lage, Germany) starting eight days before implantation of the minipumps. At the end of the experiments, the animals were decapitated, and blood was collected from the carotid arteries for determination of hematocrit and PRA. Kidneys were removed rapidly, weighed, cut in half, frozen in liquid N<sub>2</sub>, and stored at

**Key words:** plasma renin activity, juxtaglomerular cells,  $\beta$ -adrenergic receptors, isoproterenol, catecholamines.

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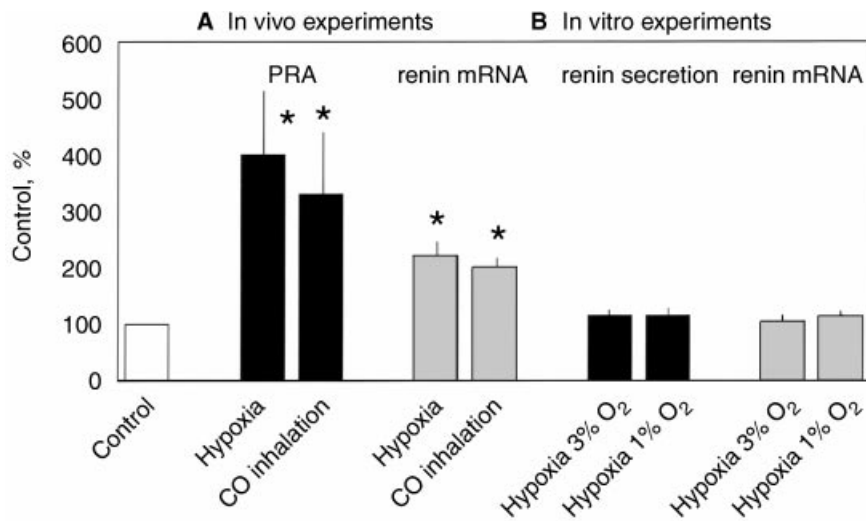


Fig. 1. Renin secretion and renal renin gene expression six hours after induction of acute inspiratory (8% O<sub>2</sub>) and tissue (0.1% CO inhalation) hypoxia in rats *in vivo* and six hours after induction of hypoxia in primary cultures of juxtaglomerular (JG) cells *in vitro*. Data are given as a percentage of control.

–80°C until isolation of total RNA. In other experiments, the left kidney was denervated as described previously [17].

#### Primary culture of renal juxtaglomerular cells

Mouse JG cells were isolated from male C57B16 mice using 0.25% trypsin and 0.1% collagenase as described previously [10], were separated further on Percoll density gradients, and were cultured in RPMI1640 medium (Biochrom, Berlin, Germany). Cultures were made hypoxic by reducing medium PO<sub>2</sub> from 150 (20%) to 20 (3%) or 7 (1%) mm Hg for 6 or 20 hours.

#### Determination of plasma renin activity

Plasma renin activity was determined by incubating plasma samples at 37°C for one hour and measuring generated ANGI using a commercial radioimmunoassay kit (Sorin, Düsseldorf, Germany).

#### Isolation of RNA

Total RNA was isolated according to the protocol of Chomczynski and Sacchi. Kidneys were homogenized in 10-ml solution D [guanidine thiocyanate (4 mM) containing 0.5% N-lauryl-sarcosinate, 10 mM ethylenediaminetetraacetic acid, 25 mM sodium citrate, 700 mM β-mercaptoethanol] with a polytron homogenizer. Sequentially, 1-ml sodium acetate (2 mM, pH 4), 10-ml phenol (water saturated), and 2-ml chloroform were added to the homogenate, with thorough mixing after the addition of each reagent. After cooling on ice for 15 minutes, samples were centrifuged at 4°C (15 minutes, 10,000 g) and the supernatant precipitated with an equal volume of isopropanol at –20°C for one hour. After centrifugation, RNA pellets were resuspended in 0.5-ml solution D, precipitated with an equal volume of isopropanol at –20°C, dissolved in diethylpyrocarbonate-treated water, and stored at –80°C.

#### Determination of preprorenin messenger ribonucleic acid (mRNA)

Renin mRNA was determined by RNase protection in rat kidney using 20 μg RNA and by quantitative reverse transcriptase-polymerase chain reaction in primary cultures of JG cells using 3 μl of a cytoplasmic fraction and 5 pg internal standard as described in detail previously [10, 17].

#### Measurement of plasma catecholamines

The catecholamines epinephrine and norepinephrine were determined in plasma using high-pressure liquid chromatography (HPLC) with Nova-PakC<sub>18</sub> columns and electrochemical detection as described previously [10].

#### Statistics

Levels of significance were estimated by analysis of variance followed by Newman-Keuls test or Student's paired *t*-test. *P* < 0.05 was considered significant.

## RESULTS AND DISCUSSION

After six hours acute hypoxia or CO exposure, PRA increased fourfold and 3.3-fold, respectively, versus controls (control PRA 3 ng ANGI/hr/ml), and renin mRNA levels to 220% and 200%, respectively (Fig. 1). Renin secretion or gene expression in primary JG cell cultures was not affected by hypoxia (Fig. 1). Renal denervation did not prevent stimulation of the renin system by arterial hypoxia (2.3-fold increase of renin mRNA levels in denervated kidneys and 1.8-fold increase of renin mRNA levels in contralateral kidneys). Norepinephrine increased 1.7-fold and 3.2-fold from a baseline concentration of 735 pg/ml and plasma epinephrine 3.9-fold and 7.8-fold from a baseline

concentration of 352 pg/ml during hypoxia and CO inhalation, respectively [10]. Stimulation of  $\beta$ -adrenergic receptors by infusion of isoproterenol increased PRA 17-fold and 20-fold after three and six hours, respectively, and increased renal renin mRNA by 130% after six hours but no change at three hours (Holmer et al, unpublished observation). Isoproterenol had no additional stimulatory effects on PRA or renal renin mRNA in rats on a low-sodium diet in contrast to animals on normal- or high-salt diets.

In summary, both inspiratory and tissue hypoxias stimulate renin secretion *in vivo* in rats on a normal-sodium diet in accordance with several, but not all, reports in the literature [2–9]. The effects of hypoxia on PRA are consistent with the marked stimulation of renin gene expression already after six hours [10]. Because several genes (such as, erythropoietin, endothelin-1, PDGF-A, PDGF-B, VEGF, heat shock transcription factor) are stimulated by hypoxia *in vitro*, the direct effects of hypoxia on the renin system were studied in JG cells [14, 15]. Hypoxia had no effect on baseline renin secretion in isolated JG cells *in vitro*, suggesting that hypoxia does not affect JG cells directly. This is consistent with findings in isolated perfused kidneys, also suggesting that hypoxia has no direct effect on renin secretion [18], and with the lack of effect of hypoxia on forskolin-stimulated renin responses (although forskolin-induced stimulation of renin gene expression is partly attenuated after prolonged exposure to the severest hypoxia, possibly due to energy depletion) [10]. These results suggest that stimulation of renin secretion during hypoxia is indirect. One possible mediator is the sympathetic nervous system. Although renal nerve activity is increased during hypoxia, there was no clear effect of renal denervation on stimulation of renin gene expression by hypoxia, thus arguing against a relevant role of sympathetic nerves in conscious rats *in vivo* [19, 20]. Circulating catecholamines, however, are markedly stimulated during hypoxia and might contribute to stimulation of the renin system. Stimulation of  $\beta$ -adrenergic receptors by isoproterenol infusion markedly stimulates both renin secretion and gene expression in rats *in vivo* [21]. The effect of isoproterenol is modulated by sodium intake, such that catecholamines in low-sodium animals have no additional stimulatory effect on hypoxia-induced renin secretion. Consistent with these data during hypoxia, circulating catecholamines, but not sympathetic nerves, mediate hemorrhage-induced stimulation of renin secretion and gene expression [17]. However, the presently available data do not exclude participation of the macula densa mechanism, the renal baroreceptor mechanism, or locally released autacoids in hypoxic stimulation of the renin system [10]. Several mediators or mechanisms might therefore contribute to the final response of the renin system during hypoxia, such as stimulatory effects via catecholamines and inhibitory effects of

endothelin-1, which is induced by hypoxia and inhibits renin secretion [15, 22].

In conclusion, both inspiratory and tissue hypoxia stimulate renin secretion and gene expression powerfully *in vivo* but not *in vitro*. These effects are not mediated by renal nerves or by direct effects on JG cells but might be mediated by circulating catecholamines.

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## REFERENCES

- BRICKNER RC, JANKOWSKI B, RAFF H: The conversion of corticosterone to aldosterone is the site of the oxygen sensitivity of the bovine zona glomerulosa. *Endocrinology* 130:88–92, 1992
- MOLTENI A, ZAKHEIM RM, MULLIS KB, MATTIOLI L: The effect of chronic alveolar hypoxia on lung and serum angiotensin I converting enzyme activity. *Proc Soc Exp Biol Med* 147:263–265, 1974
- SUTTON JR, VIOL GW, GRAY GW, MCFADDEN M, KEANE PM: Renin, aldosterone, electrolyte and cortisol responses to hypoxic decompression. *J Appl Physiol* 43:421–424, 1977
- MILLEDGE JS, CATLEY DM, WARD MP, WILLIAMS ES, CLARKE CRA: Renin-aldosterone and angiotensin-converting enzyme during prolonged altitude exposure. *J Appl Physiol* 55:699–703, 1983
- OPARIL S, NARKATES AJ, JACKSON RM, ANN HS: Altered angiotensin-converting enzyme in lung and extrapulmonary tissues of hypoxia adapted rats. *J Appl Physiol* 65:218–227, 1988
- LAWRENCE DL, SKATRUD JB, SHENKER Y: Effects of hypoxia on atrial natriuretic factor and aldosterone regulation in humans. *Am J Physiol* 258:E243–E248, 1990
- JAIN S, WILKE WL, TUCKER A: Age-dependent effects of chronic hypoxia on renin angiotensin and urinary excretions. *J Appl Physiol* 69:141–146, 1990
- OLSEN NV, KANSTRUP IL, RICHALET JP, HANSEN JM, PLAZEN G, GALEN FX: Effects of acute hypoxia on renal, and endocrine function at rest and during graded exercise in hydrated subjects. *J Appl Physiol* 73:2036–2043, 1992
- NEYLON M, MARSHALL J, JONES EJ: The role of the renin-angiotensin system in the renal response to moderate hypoxia in the rat. *J Physiol (Lond)* 491:479–488, 1996
- RITTHALER T, SCHRICKER K, KEES F, KRÄMER B, KURTZ A: Acute hypoxia stimulates renin secretion and renin gene expression *in vivo* but not *in vitro*. *Am J Physiol* 272:R1105–R1111, 1997
- KENTERA D, SUSIC D, CEMERIKIC D: Plasma renin activity and hypertrophy of the right ventricle in hypoxic rats. *Basic Res Cardiol* 74:445–450, 1979
- OLSEN NV: Effect of hypoxaemia on water and sodium homeostatic hormones and renal function. *Acta Anaesthesiol Scand Suppl* 107:165–170, 1995
- SWENSON ER, DUNCAN TB, GOLDBERG SV, RAMIREZ G, AHMAD S, SCHOENE RB: Diuretic effect of acute hypoxia in humans: Relationship to hypoxic ventilatory responsiveness and renal hormones. *J Appl Physiol* 78:377–383, 1995
- BENJAMIN IJ, KROGER B, WILLIAMS RS: Activation of heat shock transcription factor by hypoxia in mammalian cells. *Proc Natl Acad Sci USA* 87:6263–6267, 1990
- KRÄMER BK, BUCHER M, SANDNER P, ITTNER KP, RIEGGER GAJ, RITTHALER T, KURTZ A: Effects of hypoxia on growth factor expression in the rat kidney *in vivo*. *Kidney Int* 51:444–447, 1997
- MODENA B, HOLMER S, ECKARDT KU, SCHRICKER K, RIEGGER G, KAISLING B, KURTZ A: Furosemide stimulates renin gene expression in the kidneys of salt-supplemented rats. *Pflügers Arch* 424:403–409, 1993

17. HOLMER S, RINNE B, ECKARDT KU, LEHIR M, SCHRICKER K, KAISLING B, RIEGGER G, KURTZ A: Role of renal nerves for the expression of renin in adult rat kidney. *Am J Physiol* 266:F738–F745, 1994
18. SPATH JA, DAUGHERTY RM, SCOTT JB, JADDY FJ: Effect of acute local hypoxia on renin activity in renal venous plasma. *Proc Soc Exp Biol Med* 137:484–488, 1971
19. CZYZYK-KRZESKA MF, TRZEBSKI A: Respiratory-related discharge pattern of sympathetic nerve activity in the spontaneously hypertensive rat. *J Physiol (Lond)* 426:355–368, 1990
20. MALPAS SC, SHWETA A, ANDERSON WP, HEAD GA: Functional response to graded increases in renal nerve activity during hypoxia in conscious rabbits. *Am J Physiol* 271:R1489–R1499, 1996
21. HOLMER SR, KRÄMER BK, KAISLING B, PUTNIK K, RIEGGER AJG, KURTZ A: Role of beta-adrenergic stimulation on renal renin expression in normal, high and low salt diet in rats. (abstract) *J Hypertens* 14(Suppl 1):S18
22. KRÄMER BK, SCHRICKER K, SCHOLZ H, CLOZEL M, RIEGGER GAJ, KURTZ A: Role of endothelins for renin regulation. *Kidney Int* 49(Suppl 55):119–121, 1996