Attenuated response of renal mechanoreceptors to volume expansion in chronically hypoxic rats

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Multifiber renal afferent nerve activity responds to volume expansion in sea level rats but not in chronically hypoxic (high altitude) rats. We performed single-unit recordings of renal afferent nerve activity to characterize renal sensory receptors and their responses to volume expansion in these animals. Hypoxia was induced by placing Wistar rats in an altitude chamber (380 Torr, 5,500 m) for 4 wk. Spontaneously firing renal R2 chemoreceptor and arterial, ureteropelvic, and venous mechanoreceptors were identified. The basal activity of each receptor was similar among these rats. In response to specific stimulus, the increasing impulse of R2 chemoreceptor was similar between two groups of rats, but the increasing activity of each mechanoreceptor was less in hypoxic rats. When challenged with saline load, R2 chemoreceptor activity decreased, but all mechanoreceptors activated in all rats. Despite similar increases of arterial, venous pressure during saline load, the increasing activity of each mechanoreceptor was significantly less in hypoxic rats. These results indicated chronic hypoxia attenuates the sensitivity of renal mechanoreceptors in response to the stimulation of saline load.

chronic hypoxia; renal chemoreceptor; renal mechanoreceptor; renal nerve; saline load

Like other visceral organs, the kidneys have a profuse sensory innervation. Electrophysiological studies have identified two major classes of sensory receptors in the mammalian kidney: chemoreceptors (CRs), sensitive to ionic composition of renal pelvic perfusate and/or renal ischemia (26, 27), and mechanoreceptors (MRs), sensitive to arterial perfusion (21), venous (2), and ureteral pressure (4). These renal afferent nerves could initiate renorenal reflexes that affect the kidney through its efferent innervation or through circulatory changes (19, 28). In addition, afferent nerves may have a direct effect on the function of visceral organs by releasing neuropeptides (18). Thus these renal sensory receptors symbolize specific messages from the different areas of the intrarenal vascular and interstitial beds and play an important role in the regulation of the body fluid homeostasis (13).

Human beings or animals subjected to long-term hypoxia develop physiological adjustments to adapt the hypoxic stress (29). Despite severe polythemia and hypervolemia after chronic hypoxia, renal regulation of body fluids is maintained (7, 22). For example, the urinary excretion of water and sodium during and after saline loading (5% of body wt) was not affected in chronically hypoxic rats [high altitude (HA) for 4 wk] compared with that in sea level (SL) control rats. This is despite the fact that the regulatory functions of hormones (7–9, 15, 24, 25) and of renal sympathetic nerves (10, 11, 12, 16) are altered in the chronically hypoxic subjects. Nonetheless, the multifiber recordings of renal efferent nerve activity (RENA) and afferent nerve activity (RANA) in response to volume expansion were different in SL and HA rats (10, 11). During saline loading, RANA in SL rats but not in HA rats increased twofold within a few minutes. The mechanism and the significance of the reduced RANA in hypoxic rats have yet to be determined.

In this study, we performed single-unit recordings of the activity of the peripheral end of the cut nerve filaments to clarify the nature of the increased RANA in response to saline load. Our study showed that, although CRs were not affected, the three types of renal MRs were altered in chronically hypoxic rats. Therefore, in normal control rats, the enhanced MR activation outweighed the decreased CR activation during volume expansion, resulting in an increased multifiber RANA, whereas, in hypoxic rats, lower MR activation merely counterbalanced the decreased CR activation, and the multifiber RANA remained unchanged during volume expansion. Our study provided detailed descriptions of the responsiveness of intrarenal receptors to volume expansion. The method (single-unit recordings) used in this study can be applied in the future to examine the responses of the intrarenal environment to other stimuli.

METHODS

Induction of chronically hypoxic rats. Female Wistar rats weighing 190–220 g were placed for 15 h/day for 4 wk in a high altitude chamber (HA rats) at a constant temperature and a consistent light cycle (light from 0700 to 1800), whereas control animals were maintained at sea level (SL) at the same temperature and light cycle. A level of 380 Torr (5,500 m) was selected, because it represents the maximal altitude to which most rats can successfully adapt. The animals were exposed to hypoxia from 1700 to 0800 and then returned to room air. The body weight of the animals was measured once a week. Food and water were provided ad libitum.

General surgery. After 4 wk, the rats were anesthetized with pentobarbital sodium (40 mg/kg ip). The trachea was exposed via a midline cervical incision and then intubated. Catheters were placed in the left femoral artery for continuous blood pressure recordings and in the left femoral vein for drug and saline administration. Heart rate was determined using a tachograph triggered by the arterial pulsations. All hemodynamic responses were recorded by a Gould polygraph (RS-3400). The rat was placed on its right side, and the left kidney was exposed via a flank incision and dissection from the surrounding tissue. The kidney surface was continuously bathed with paraffin oil warmed to 38°C and illuminated with
a fiber-optic light source. With the help of a stereoscopic dissecting microscope (Olympus, SZ-STU2), the left renal nerves at the angle between the aorta and left renal artery were carefully isolated from the surrounding tissues on each side of the left renal artery for recording of the renal nerve activity.

Recording of renal nerve activity. The recording technique has been reported previously (11). Briefly, recordings from the multifiber preparations were made by placing the intact renal nerve fibers in two pairs of thin bipolar stainless steel electrodes. The renal nerves and electrodes were continuously bathed in a pool of warm paraffin oil (38°C) to prevent drying. The electrical signals were amplified 20,000-fold and filtered (high-frequency cutoff, 3,000Hz; low-frequency cutoff, 30 Hz) by a Grass model P511 AC preamplifier and continuously displayed on a Gould oscilloscope (1604). In the meantime, the amplified signals were fed into a window discriminator (World Precision Instruments model 121) and counted on a Gould integrator (13–4615–70). The neural activity was transformed into a spike number. All recordings were performed under normoxic conditions. The renal nerves were transected, and the multifiber RANA was evaluated by several stimuli to determine its activity. This procedure was performed before the single RANA dissection.

Recording of single RANA. There were 24 SL and 26 HA rats. After general surgery, a short section of the renal artery was carefully cleared of connective tissue to allow complete occlusion by clamping with forceps during the single RANA experiment. A three-way adapter constructed of PE-90 tubing was first connected to three saline-filled lengths of PE-50 tubing and then to a short (1–2 cm) length of PE-50 tubing, which had been previously inserted into the left upper ureter and tied at the ureteropelvic junction. This arrangement enabled continuous measurement of intrapelvic pressure during normal urine flow and during backflow of urine, as described previously by Recordati et al. (27). Renal venous pressure was measured from another catheter (PE-10) put into the renal vein from the ovarian vein. Multifiber RANA was recorded from the peripheral end of the cut nerve filaments. The greater splanchnic and all other visible nerves at the angle between the aorta and left renal artery were severed to isolate the left kidney from efferent neural influences. The nerve bundle was repeatedly split with fine forceps until a single-unit impulse was identified. Nerve activity and hemodynamic variables were stored on a Gould oscilloscope (RS3400).

Data. All hemodynamic and single RANA responses were displayed on a Gould polygraph (RS3400).

Volume expansion. In this experiment, only one single unit was studied in each rat. After identification of each fiber, the resting activity of the sensory receptor was determined for 4 to 5 min to serve as a control period. Intravenous infusion of isotonic saline of 5% of body weight over a 10-min loading period was then begun. After saline loading, we determined the single RANA during a 10-min recovery period. To compare the hemodynamic changes, we recorded the arterial blood pressure, ureteral pressure, and renal venous pressure simultaneously throughout the experiment.

Statistical analysis. The activity of each unit was displayed graphically as instantaneous frequency of each impulse and as counts of the number of impulses per unit time. Quantitative data are expressed as means ± SE. Differences in absolute unit activity expressed as impulses per unit time were assessed by the Student's paired t-test when appropriate. Data of units between groups were analyzed by the unpaired t-test.

RESULTS

General observations. After 4 wk of intermittent exposure to 5,500 m hypoxia, all rats were in apparent good health, although the final body weight of the HA rats was significantly less than that of SL rats (204 ± 6 to 233 ± 9 vs. 208 ± 5 to 255 ± 9 g, P < 0.05). The hematocrit level in HA rats was significantly higher than that of SL rats (66 ± 3 vs. 45 ± 1%, P < 0.01). Single RANA. A typical recording of single unit of RANA, arterial pressure, and transformed spikes in a SL rat is illustrated in Fig. 1. The transformed spikes...
were counted by a Gould integrator. Four types of renal sensory receptors, CR2, MRa, MRu, and MRv, were recorded in both SL and HA rats. In this experiment, all the renal sensory receptors we chose were of the spontaneous type because they could represent the basal firing rate of multifiber RANA. The basal discharge of the four types of sensory receptors was similar in SL rats and HA rats (Table 1). In addition to their responses to specific stimuli, MRs are also different from CR2 in the waveform of recordings (especially the amplitude of the action potential) and higher signal-to-noise ratios, since they might be from a different class of fibers.

Chemoreceptor type 2. At rest, the CR2 was identified by its spontaneous firing and was activated by RAO. This procedure was used to distinguish another type of chemoreceptor, CR1, which is silent at rest (26, 27). The result also showed that CR2 was unresponsive to the elevation of ureteral pressure with saline (Table 1). Meanwhile, CR2 did not respond to the elevation of arterial blood pressure or to RVO. The excitation level of CR2 in response to RAO or back flow of nondiuretic urine was similar in HA and SL rats (Table 1).

Renal mechanoreceptors. As seen in Fig. 2, the MRa was activated by intravenous administration of noradrenaline (2 µg) in one SL and one HA rat. The excited level of MRa responding to increasing arterial blood pressure was significantly less in the HA rats than in the SL rats (P < 0.05, Table 1), although the elevated level of arterial blood pressure was similar in HA rats and SL rats (178 ± 6 vs. 183 ± 6 mmHg, respectively). On the other hand, decreased renal perfusion pressure by RAO depressed the firing rate of MRa similarly between HA and SL rats.

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The increase in ureteral pressure to 35 ± 1 mmHg by elevating ureteral catheter with both urine and saline activated the MRu of HA and SL rats (Fig. 3). The activated level of MRu in response to elevated ureteral pressure was significantly less in HA rats than in SL rats (P < 0.05, Table 1).

RVO activated MRv in both SL and HA rats (Fig. 4). The activated level of MRv in response to RVO was also significantly less in HA rats than in SL controls (P < 0.05, Table 1). RAO, increasing ureteral pressure, or

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<th>Receptor Group</th>
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<th>Activities (spikes/10 s) During</th>
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<td>RAO</td>
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<tr>
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<td>9.2 ± 1.9</td>
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<td>SL</td>
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<td>6.8 ± 1.1</td>
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Values are means ± SE. SL, rats at sea level; HA, chronic hypoxic (high altitude) rats; RAO, renal artery occlusion; RPP, increasing renal perfusion pressure by norepinephrine (2 µg iv); UP, increasing ureteral pressure to 35 ± 1 mmHg with saline and nondiuretic urine; RVO, renal vein occlusion; CR2, type 2 chemoreceptor; MRa, renal arterial mechanoreceptor; MRu, renal ureteropelvic mechanoreceptor; MRv, renal venous mechanoreceptor. *P < 0.05 vs. basal activity. †P < 0.05 vs. SL.
arterial blood pressure had no effect on the activity of MRv.

Volume expansion on renal sensory receptors. A representative recording of one MRa responding to volume expansion in one SL rat is demonstrated in Fig. 5B. The activity of MRa increased ~3 min after starting the volume expansion and gradually recovered to the control level ~10 min after the expansion period. Group data for response of the CR2, MRa, MRu, and MRv to saline loading is shown in Fig. 6. During the saline loading and recovery periods, the activity of CR2 was significantly decreased, and the response was similar in both HA rats and SL controls. Conversely, activities of MRa, MRu, and MRv to saline loading were enhanced in both HA and SL rats. However, the peak activity of MRa during saline loading was less in HA rats than in SL rats (20.0 ± 2.5 vs. 41.3 ± 5.7 spikes/10 s, P < 0.05, respectively). The peak firing of MRu in response to saline loading was less in HA rats than in SL rats (24.3 ± 6.0 vs. 43.0 ± 5.1 spikes/10 s, P < 0.05, respectively). The peak value of MRv in response to saline loading was also less in HA rats than in SL rats (17.1 ± 3.3 vs. 33.6 ± 3.7 spikes/10 s, P < 0.05, respectively).

The responses of arterial blood pressure, ureteropelvic pressure, and renal venous pressure to saline loading are shown in Fig. 7. The data showed that ureteropelvic pressure and renal venous pressure were markedly
DISCUSSION

The significant findings in this study were summarized as follows. 1) The basal activities of renal sensory receptors were similar in both chronically hypoxic (HA) rats and control (SL) rat, and each renal sensory receptor could be activated and identified by its specific stimulus. 2) The excited responses of the CR2 were similar in both groups of rats. Intravenous saline load depressed CR2 activity in both HA and SL rats to the same degree. 3) The saline loading activated renal arterial, ureteropelvic, and venous MRs in both groups of rats, but the degree of response was significantly less in HA rats than in SL rats.

Renal MRs are nerve endings that monitor hydrostatic pressure changes within the kidney. At rest, systemic and renal hemodynamics were similar in both SL and HA rats (6, 22), and this could explain why the basal discharge of MRs was similar in SL and HA rats. In the present study, three types of renal MRs (i.e., MR_a, MR_u, and MR_v) were excited in response to saline loading in both HA and SL rats. Because MR_a is identified with its activation by acute elevation in arterial blood pressure (14, 17, 21), it is suggested that the increased activity of MR_a during saline load is ascribed to the elevated arterial blood pressure. However, in our experiments, the arterial blood pressure was slightly but insignificantly elevated during saline loading. Similar responses were observed by Astrom and Crafoord (2, 3) when renal vasodilation was produced by infusion of isotonic saline into the renal artery. They suggested that the excitation of RANA was attributed to an increase in interstitial (hydrostatic) pressure rather than to the dilation of vasculature or an elevated arterial pressure (4). In this study, we suggest MR_a could be also possibly stimulated by the increase of blood volume (through volume stretch) and/or blood flow (through shear stress). However, an advanced study is needed to explore the mechanism. On the other hand, both renal ureteral pressure and renal venous pressure significantly increased during volume expansion, which can activate MR_u and MR_v, respectively (3, 4). A similar result, demonstrated by Genovese et al. (13), showed that RANA increases when diuresis is activated within a moderate physiological range and that this increase is due to the activation of renal MRs. Pelvic pressure and/or interstitial pressure appear to be the major determinants of the neural response. The MR activation in response to volume expansion was less in HA rats than in SL rats, although the responses of arterial, ureteropelvic, and venous pressures during volume expansion were similar between SL and HA rats. This result showed that attenuated response of MRs to volume expansion in HA rats is not dependent on the pressure gradients. A chronic volume adaptation of the increased blood volume (40% higher) may account for to the change (or decrease) in the MR sensitivity of HA rats (1). The less-increased responses of MRs to various elevated pressures in the HA rats could also be attributed to hypervolemia-induced structural adaptation, because chronic hypoxia increases total blood volume (22) and total capillary area (1, 22). However, this mechanism remains to be explored.

Renal CRs respond to alterations in the chemical composition of the renal interstitial or pelvic environment. Two types of renal CRs have been identified (26, 27), CR1 and CR2. CR1 has no basal activity and responds to renal ischemia. CR2 is spontaneously active, with the resting discharge rate being highest in nondiuretic conditions. CR2 activity is related to the hydrated state of the animal. Recordati et al. (26) reported that the multifiber resting discharge of renal chemosensitive activity declines during extracellular fluid volume expansion in normoxic rats. On the other hand, hydropenia leads to concentrated urine and increases the discharge of CR2 (20, 26). In this study, CR2 was activated by RAO (renal ischemia) and backflow of the nondiuretic urine but not activated by increasing renal perfusion pressure, ureteropelvic pressure with saline, and RVO. The similar basal resting discharge of CR2 between SL and HA rats may be due to their similar chemical components of the plasma and urine in these animals (6, 7, 22). During RAO and backflow of nondiuretic urine, the extent of CR2 activation was almost the same in both groups of rats. However, CR2 activity was similarly depressed in both HA and SL rats. Some
chemical substances, such as endogenous adenosine, bradykinin, and potassium ion, could have been diluted below the threshold concentrations and thus have decreased CR2 discharge during and after volume expansion. Whether the sensitivity of CR is altered in hypoxic rats cannot be determined in test parameters used in this study.

In conclusion, although CR2 activity was depressed by volume expansion, the three types of renal MRs were activated in SL and, to lesser degree, in HA rats. In SL rats, the enhanced MR activation outweighed the decreased CR2 activation during volume expansion, thus increasing multifiber RANA, whereas, in HA rats, lower MR activation counterbalanced the decreased CR2 activation, and thus the multifiber RANA remained unchanged during volume expansion. Based on our results, we conclude that chronic hypoxia attenuates the sensitivity of renal MRs and results in a decreased response to the stimulation of saline loading.

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