Diuretic effect of hypoxia, hypocapnia, and hyperpnea in humans: relation to hormones and $O_2$ chemosensitivity

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Hildebrandt, Wulf, Andy Ottenbacher, Markus Schuster, Erik R. Swenson, and Peter Bärtsch. Diuretic effect of hypoxia, hypocapnia, and hyperpnea in humans: relation to hormones and $O_2$ chemosensitivity. J. Appl. Physiol. 88: 599–610, 2000.—We studied the contributions of hypoxemia, hypocapnia, and hyperpnea to the acute hypoxic diuretic response (HDR) in humans and evaluated the role of peripheral $O_2$ chemosensitivity and renal hormones in HDR. Thirteen healthy male subjects (age 19-38 yr) were examined after sodium equilibration (intake: 120 mmol/day) during 90 min of normoxia (NO), poikilocapnic hypoxia (PH), and isocapnic hypoxia (IH) (days 1–3, random order, double blind), as well as normoxic voluntary hyperpnea (HP; day 4), matching ventilation during IH. $O_2$ saturation during PH and IH was kept equal to a mean level measured between 30 and 90 min of breathing 12% $O_2$ in a pretest. Urine flow during PH and IH (1.81 ± 0.92 and 1.94 ± 1.03 ml/min, respectively) but not during HP (1.64 ± 0.96 ml/min) significantly exceeded that during NO (control, 1.38 ± 0.71 ml/min). Urine flow increases vs. each test day's baseline were significant with PH, IH, and HP. Differences in glomerular filtration rate, fractional sodium clearance, urodilatin, systemic blood pressure, or leg venous compliance were excluded as factors of HDR. However, slight increases in plasma and urinary endothelin-1 and epinephrine with PH and IH could play a role. In conclusion, the early HDR in humans is mainly due to hypoxia and hypocapnia. It occurs without natriuresis and is unrelated to $O_2$ chemosensitivity (hypoxic ventilatory response).

diuresis; urodilatin; endothelin; peripheral arterial chemoreceptor

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The separate contributions of hypoxia, HP, and hypocapnia to HDR were studied by the following four experimental conditions applied on 4 consecutive days over 90 min each: normoxia (NO), poikilocapnic hypoxia (PH), and isocapnic hypoxia (IH).
matching ventilation during IH was studied on day 4. We considered urine flow the primary efficacy variable and its increase of 1 SD (e.g., from ~1.00 to 1.50 ml/min) sufficient to explain hypoxic diuresis, as a volume loss of an additional 30 ml/h or 720 ml/day would be in line with the hypoxic diuresis observed during comparable hypoxia under normobaric (41) or high-altitude field conditions (2, 3). To assess such an increase in urine flow with P < 0.05, the number of subjects (n = 13) in the present study was sufficient (26). The investigations were carried out under conditions of sodium balance and postural volume equilibration. As this study addresses several hypotheses on the mediation of HDR, it is considered to be a pilot study.

METHODS

Subjects

Thirteen healthy male subjects volunteered for the study, which was approved by the ethics committee of the Medical Faculty of the University of Heidelberg. Their age, body weight, body height, and body mass index are given in Table 1. All subjects had been familiarized with the laboratory facilities, especially with breathing through a mouthpiece while wearing a noseclip. Subjects were nonsmokers and had abstained from alcohol, caffeine, and any medication. They had not been above an altitude of 2,000 m within 6 mo before the study nor were they involved in competitive sports during the study. All measurements were carried out in a quiet environment with calming music provided by earphones. The ambient room temperature was maintained between 22 and 25°C.

Study Procedure

Sodium equilibration. Four days before and throughout the 4 consecutive test days, the subjects adhered to a fixed sodium diet (120 mmol/day). During the same time, their 24-h urine production was measured to determine daily sodium excretion and the resulting sodium balance. The subjects’ fluid and food intake, urine output, as well as body weight and daily physical activity, were documented in an individual diary.

Pretests. Within 2 wk before the 4 consecutive test days, all subjects underwent measurement of their HVR under HVRiso and pokilocapnic (HVRpoi) conditions and their hypercapnic ventilatory response (HCVR). These tests were performed with the subjects in a semireclined, comfortable sitting position. In addition, a 90-min period of breathing 12% inspiratory O2 fraction (FiO2) was performed to determine individual mean arterial O2 saturation (SaO2) between 30 and 90 min of PH exposure for use as matched controlled targets during IH and PH.

Test conditions for the diuretic response. The following four test conditions, which lasted 90 min each, were investigated on 4 consecutive days in sodium-equilibrated subjects: 1) NO (control), 2) PH, 3) IH, and 4) voluntary normoxic (isocapnic) HP. The test conditions of NO, PH, and IH were studied in random order on days 1, 2, and 3, whereas HP was performed on day 4 with HP matching minute ventilation (Ve) during IH (Table 2, Fig. 1A).

One investigator controlled FiO2 throughout PH and IH such that the individual SaO2 determined by the pretest (see Pretests above) was reached and kept constant (Table 2, Fig. 1B and D). Moreover, for IH and HP, he kept the end-tidal PCO2 (PETCO2) at a level observed at normoxic baseline by adding CO2 to the inspiratory air provided by a reservoir Douglas bag (Fig. 1C). Table 2 presents mean PETCO2 values of the last 80 min of all four experimental conditions.

To match the ventilation of HP to ventilation during IH, subjects breathed normoxic air from a small, visible inspiratory air reservoir (Douglas bag), which was filled at a flow rate equal to the ventilation during IH. The flow rate was adjusted every minute according to the gliding average of 3-min intervals during IH, and subjects were instructed to keep the size of the inspiratory air reservoir constant.

The conditions of NO, PH, and IH were performed in a double-blind fashion, i.e., the inspired gas mixture was prepared and controlled by one investigator placed behind a screen out of sight of the subject. A second investigator observed the subject and performed measurements (blood sampling, venous compliance measurement, etc.) but was unaware of the test conditions. All subjects except for two with extensive previous experience in respiratory response testing failed to guess correctly any test condition.

Table 1. Individual anthropometric and chemosensitivity

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age (yr)</th>
<th>Body Weight (kg)</th>
<th>Body Height (cm)</th>
<th>BMI (kg/m²)</th>
<th>HVRiso (l·min⁻¹·%⁻¹)</th>
<th>HVRpoi (l·min⁻¹·%⁻¹)</th>
<th>HCVR (l·min⁻¹·mmHg⁻¹)</th>
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<tr>
<td>1</td>
<td>20</td>
<td>57</td>
<td>171</td>
<td>19.5</td>
<td>0.59</td>
<td>0.07</td>
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<td>2</td>
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<td>170</td>
<td>27.3</td>
<td>0.81</td>
<td>0.17</td>
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<tr>
<td>3</td>
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<tr>
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<td>5</td>
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<td>22.5</td>
<td>0.10</td>
<td>0.09</td>
<td>1.04</td>
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<td>6</td>
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<td>21.5</td>
<td>0.30</td>
<td>0.15</td>
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<td>23.0</td>
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<td>1.36</td>
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<tr>
<td>12</td>
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<td>179</td>
<td>24.0</td>
<td>0.35</td>
<td>0.12</td>
<td>0.76</td>
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<tr>
<td>13</td>
<td>37</td>
<td>77</td>
<td>163</td>
<td>23.0</td>
<td>0.71</td>
<td>0.24</td>
<td>1.51</td>
</tr>
<tr>
<td>Mean</td>
<td>27.2</td>
<td>70.5</td>
<td>175.9</td>
<td>22.8</td>
<td>0.67</td>
<td>0.20</td>
<td>1.66</td>
</tr>
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<td>±SD</td>
<td>±5.8</td>
<td>±7.8</td>
<td>±6.2</td>
<td>±2.1</td>
<td>±0.66</td>
<td>±0.11</td>
<td>±0.53</td>
</tr>
</tbody>
</table>

*Individual values and means ± SD are given for n = 13 subjects. Shown are age, body weight, body height, body mass index (BMI), and measures of chemosensitivity: isocapnic hypoxic ventilatory response (HVRiso), pokilocapnic hypoxic ventilatory response (HVRpoi), and hypercapnic ventilatory response (HCVR).*
On the morning of each test day, the subject drank 400 ml of water at 7:00 AM. On arrival at the laboratory at 8:00 AM, he was asked to empty his bladder. Thereafter, he resumed a supine position in which complete emptying of the bladder was immediately determined by three-dimensional (3D) ultrasonic volumetry (see below). This supine rest position was maintained for 55 min to reach postural volume equilibration. After 45 min in this position, venous compliance was recorded in duplicate at two calf circumferences. After 55 min, venous blood samples were drawn for normoxic daily baseline measurements of Hb, hematocrit (Hct), plasma creatinine, electrolytes, osmolality, and ET-1. Thereafter, the subject emptied his bladder again for determination of normoxic baseline urine flow during this postural volume equilibration. After 45 min in this position, venous compliance was recorded in duplicate at two calf circumferences. After 55 min, venous blood samples were drawn for normoxic daily baseline measurements of Hb, hematocrit (Hct), plasma creatinine, electrolytes, osmolality, and ET-1. Thereafter, the subject emptied his bladder again for determination of normoxic baseline urine flow during this postural volume equilibration.

### Table 2. Mean ventilation, end-tidal and capillary blood gases, and O₂ saturation

<table>
<thead>
<tr>
<th>Condition</th>
<th>( V_E ), l/min</th>
<th>( P_{ETCO2} ), Torr</th>
<th>( P_{O2} ), Torr</th>
<th>( P_{CO2} ), Torr</th>
<th>pH</th>
<th>Bicarbonate, mmol/l</th>
<th>( SaO2 ), %</th>
</tr>
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<tbody>
<tr>
<td>Normoxia</td>
<td>8.8 ± 1.2</td>
<td>41.2 ± 3.3</td>
<td>90.1 ± 13.7</td>
<td>39.2 ± 3.0</td>
<td>7.41 ± 0.02</td>
<td>24.2 ± 1.3</td>
<td>98.1 ± 1.3</td>
</tr>
<tr>
<td>Poikilocapnic hypoxia</td>
<td>9.8 ± 1.9†</td>
<td>36.9 ± 3.5†</td>
<td>37.9 ± 4.7†</td>
<td>36.0 ± 2.3†</td>
<td>7.44 ± 0.02†</td>
<td>24.0 ± 0.9</td>
<td>76.2 ± 6.0†</td>
</tr>
<tr>
<td>Isocapnic hypoxia</td>
<td>16.3 ± 5.6†</td>
<td>40.9 ± 2.3</td>
<td>40.3 ± 5.3†</td>
<td>38.6 ± 2.8</td>
<td>7.42 ± 0.01†</td>
<td>24.3 ± 1.4</td>
<td>77.0 ± 5.6†</td>
</tr>
<tr>
<td>Hyperpnea</td>
<td>17.2 ± 5.2†</td>
<td>40.5 ± 2.3*</td>
<td>101.5 ± 14.4*</td>
<td>37.7 ± 5.2</td>
<td>7.44 ± 0.03†</td>
<td>24.5 ± 2.5</td>
<td>98.9 ± 0.8†</td>
</tr>
</tbody>
</table>

Values are means ± SD; \( n = 13 \) subjects. Shown are minute ventilation (\( V_E \)); end-tidal \( P_{CO2} \) (\( P_{ETCO2} \)); capillary \( P_{O2}, P_{CO2}, pH, \) and bicarbonate concentration; and arterial \( O_2 \) saturation (\( SaO2 \)) during 90 min of 4 test conditions: normoxia (control), poikilocapnic hypoxia, isocapnic hypoxia, and hyperpnea. Capillary data represent means of 2 capillary samples taken after 30 and 88 min of each condition. NS, not significant. *\( P < 0.05 \) and †\( P < 0.01 \) vs. normoxia (paired Student’s t-test); \( P \) indicates significance of differences with multiple comparison (ANOVA for repeated measures).

Fig. 1. Time course of minute ventilation (\( V_E \); A), inspiratory \( O_2 \) fraction (\( F_{IO2} \); B), end-tidal \( P_{CO2} \) (\( P_{ETCO2} \); C), and arterial \( O_2 \) saturation (\( SaO2 \); D) 5 min before and during 90 min of 4 test conditions [normoxia (NO), poikilocapnic hypoxia (PH), isocapnic hypoxia (IH), and hyperpnea (HP)]. Values are means of 30-s intervals (\( n = 13 \) subjects). For mean ± SD values covering the test condition interval, see Table 2.
55-min supine rest interval. Bladder emptying was again verified by the 3D ultrasonic volumetry.

The subject then was attached to a mouthpiece for a 5-min normoxic baseline recording of $V_{E}$ and end-tidal and inspiratory $P_{O_2}$ and $P_{CO_2}$. Thereafter, one of the four test conditions, NO, PH, IH, or HP, was applied, and recordings of $V_{E}$, end-tidal and inspiratory $P_{O_2}$ and $P_{CO_2}$, and $S_aO_2$ (pulse oximetry) were performed breath by breath, while heart rate (HR) (electrocardiogram) and finger arterial pressure (Finapres) were stored continuously. Capillary blood-gas samples were obtained after 30 and 88 min, and venous compliance and calf fluid volume were determined in duplicate after 80–88 min. Venous blood samples for Hb, Hct, plasma creatinine, electrolytes, osmolality, and ET-1 were drawn, and the urine volume was measured after 90 min.

Measurements and Equipment

Ventilation and respiratory gas analysis. Ventilation as well as inspiratory and end-tidal $P_{O_2}$ and $P_{CO_2}$ were measured breath by breath by the respiratory monitoring system Oxycon beta (Mijnhardt, Bunnik, The Netherlands) by using the software version 3.12 with elimination of gliding averages. Subjects wore a nosedip and breathed through a mouthpiece connected to the flowmeter (Triflow EV) with an integrated gas-sampling capillary. The flowmeter was attached to a low-resistance T-shape valve system (Haward, Edenridge, UK) with a deadspace of 95 ml. The inspiratory side was connected to a 110-cm tube (inner diameter 4.5 cm) through which room air, compressed normoxic air, or hypoxic mixtures were breathed.

Blood-gas analysis and pulse oximetry. Blood-gas analysis was carried out in duplicate from 50-μl samples taken from the ear lobe that was made hyperemic by Finalgon (Thomea, Biberec, Germany). Capillary blood samples were analyzed for $P_{O_2}$, $P_{CO_2}$, $P_{H_2}O$, and bicarbonate by using the 845 blood-gas CO-oximeter system (Ciba Corning Diagnostics, Dietlikon, Switzerland). $S_aO_2$ was measured continuously by a pulse oximeter (3740 Biox Pulse Oximeter, Ohmeda Biox, Louisville, KY) by using the finger probe placed at heart level.

Chemosensitivity. HVR was determined by the method of Wel et al. (44). The $F_{CO_2}$ was progressively lowered by admixture of $N_2$ to an inspiratory air reservoir initially containing 35% $F_{CO_2}$ such that $S_aO_2$ fell in a linear fashion to 80% within 6–10 min. The slope of the ventilatory response ($\Delta V_E/\Delta S_aO_2$; ml·min$^{-1}·\%^{-1}$) was calculated by linear regression of breath-by-breath values. For HVR$_{90}$ measurement, $CO_2$ was added to the inspiratory air to maintain $PET_{CO_2}$ at the individual level observed for 1 min during normoxic baseline before progressive hypoxia. The HVR$_{90}$ was determined without $CO_2$ addition. All HVRs were determined in duplicate and are presented as the mean of both values. In case of a deviation of both HVR values of >50% of the higher value, the measurement was repeated.

The HCVR was determined by the rebreathing technique according to Rebuck (32). Subjects were connected via the T valve to a circuit such that they exhaled to and inhaled from a reservoir initially containing 35% $O_2$. A reservoir volume was chosen that led to a $PET_{CO_2}$ rise of 10 Torr within 5–7 min. HCVR was computed as an increase of $V_E$ (l/min) per 1-Torr increase in $PET_{CO_2}$ by linear regression.

Venous blood sampling and analysis. Venous blood samples of 33 ml were drawn from an antecubital vein through a 19-gauge needle and the Sarstedt System (Sarstedt, Nümbrecht, Germany). Blood was immediately placed in ice water for 10 min and centrifuged at 2,000 g and 4°C for 30 min within 10 min after sampling. Aliquots of plasma were stored at −80°C for analysis of ET-1.

Hct (%) and Hb concentration (g/dl) were determined in duplicate in EDTA blood by using the automated analyzer type CELL DYN (Ebbott, Wiesbaden, Germany). Percent plasma volume changes (%PV) from normoxic baseline were calculated by the equation of Dill and Costill (8)

\[
%PV = \frac{Hb_b/Hb_a\cdot(100 - Hct_b)/(100 - Hct_a) - 100}{100 - Hct_a}
\]

where subscript b refers to Hb and Hct before 90 min of NO, PH, IH, or HP and subscript a refers to Hb and Hct after these experimental conditions.

Plasma sodium concentration was measured by a flame ionization detector (Efix 5055, Eppendorf, Hamburg, Germany), and creatinine concentration was determined by the Jaffe method (test kit no. 124192, Boehringer Mannheim). Plasma osmolality was determined by the freezing-point depression method (Osmometer Röbling, Berlin, Germany).

Plasma ET-1 was determined by ELISA (ET1-ELISA, Immundiagnostic, Bensheim, Germany). ET-1 plasma levels were corrected for any Hct changes occurring during each condition.

Urine and renal functional analysis. Urine flow (ml/min) was determined from urine samples taken after 90 min of NO, PH, IH, and HP and after 55 min of normoxic baselines. Emptying of the bladder was verified, or any remaining urine volume determined, by 3D computed tomography (Combison 530, Kretz, Wiesbaden, Germany). The 3D phased annular 3.5-MHz scanner (VWP3.5) covers a 60° angle and a volume of 2.5 liters within a sway of 4 s at a resolution of <1 mm. This urine volume measurement was validated beforehand by direct comparison to urine sample volumes in the range of 5 to 100 ml. The volume of urinary output was corrected for remaining bladder urine volumes (the maximum value being 15 ml) as detected by this computerized sonographic method.

Urine samples were analyzed for pH directly after sampling by using a glass electrode pH meter (ion analyzer 250, Ciba Corning Diagnostic, Sudbury, UK). Urine samples were frozen at 20°C for later measurements of sodium concentration, osmolality, and creatinine. Samples for urodilatin, ET-1, epinephrine, norepinephrine, and dopamine measurements were frozen at −80°C. Glomerular filtration rate (GFR) was obtained by calculation of creatinine clearance and fractional urine flow, and sodium excretion was calculated in percentage of GFR. Free water clearance ($C_{H_2O}$) was determined by subtraction of osmotic clearance from urine flow.

The urinary concentrations of catecholamines, i.e., epinephrine, norepinephrine, and dopamine, in the urine samples covering the 90-min interval of each condition were determined by high-pressure liquid chromatography with electrochemical detection. Urodilatin was measured by the urodilatin ELISA (Immundiagnostic). Urinary ET-1 was analyzed as described for plasma ET-1 (see Venous blood sampling and analysis).

Cardiovascular parameters. HR was recorded beat by beat by an electrocardiogram monitor (Heilige, Freiburg, Germany) by using the R-wave interval of a standard precordial bipolar lead.

Arterial blood pressure (BP) was recorded continuously by the Finapres monitor (Ohmeda 2300, Englewood, NJ) on the left middle finger placed at heart level.

Venous compliance was determined at maximal right calf circumference and 10 cm distally by two-channel venous occlusion plethysmography using two mercury-filled silicon tube strain-gauge systems (Gutman, Eursburg, Germany). As a further development of the traditional Whitney strain...
gauged, the silicon tube sensor in this system is embedded in sliding plastic links, which minimizes friction, superficial tissue compression, and thermal effects. For venous occlusion, a 14-cm-wide cuff was placed above the knee. The automated device applied occlusion pressures of 40, 60, and 80 mmHg after 1, 2, and 3 min, respectively, and calculated the mean volume increase per 100 ml calf tissue and per 1 mmHg occlusion pressure. This value represents venous compliance in a calf segment of 1 cm width. The measurements were performed in duplicate and in both locations. The mean of all four measurements is reported. The calf was placed ∼15 cm above heart level in a padded splint, avoiding venous occlusion.

Calf fluid volume changes were recorded in a 20-cm segment of the right calf (enclosing maximal circumference) by means of a 40-MHz (0.4-mA) tetrapolar electrical impedance plethysmograph (Cardiodygraph, Diefenbach, Frankfurt, Germany). This technique has been validated in vivo and in vitro (39) for intraindividual comparison by using circular gel-coated silver electrodes (6 cm width) as presently done. Because the calf was placed and fixed 15 cm above heart level with support of the heel and the knee, volume changes detected by impedance plethysmography can be attributed to the extravascular compartment, because venous vascular volume was assumed to be constant and small because of postural venous collapse (12). This calf position was maintained for 55 min before and throughout the 90 min of each test condition. Absolute fluid volume changes were calculated according to Ref. 39 with the specific resistance of the fluid test condition. Absolute fluid volume changes were calculated in duplicate and in both locations. The mean of all four measurements is reported. The calf was placed ∼15 cm above heart level in a padded splint, avoiding venous occlusion.

Results

Hypoxic and Hypercapnic Chemosensitivity

The individual values and means ± SD of HVR iso, HVR poi, and HCVR are given in Table 1. HVR iso ranged between 0.10 and 2.71 and HVR poi between 0.06 and 0.40 l·min⁻¹·%⁻¹. HVR iso was significantly higher than HVR poi (P < 0.05) and correlated with HVR poi (r = 0.65, P < 0.05). HCVR ranged between 0.76 and 2.39 l·min⁻¹·mmHg⁻¹ and was unrelated to HVR iso or HVR poi.

Sodium Balance

Fig. 2 provides sodium balance data during the 4 days of pretest equilibration and during the four test conditions: NO, PH, IH, and HP. All subjects revealed an initial sodium loss and reached sodium balance at day 5, defined as a sodium excretion between <150 and ≤50 mmol/day. No statistically significant difference was found between sodium intake and urinary excretion after day 2 of the diet. The 24-h sodium excretion did not differ among the four different test conditions (nor the 4 consecutive test days, to which these test conditions had been randomly assigned).

\[ \dot{V}_E, \dot{P}_ETCO_2, \text{ and } SaO_2 \]

Fig. 1, A, B, C, and D, presents time courses of \( \dot{V}_E \), \( FIO_2 \), \( PETCO_2 \), and \( SaO_2 \), respectively, during the 90 min of NO, PH, IH, and HP. Mean values covering the last 80 min (means ± SD) of each of these four conditions are

![Graph showing sodium balance data during the 4 test conditions](https://via.placeholder.com/150)

Fig. 2. Mean ± SD values of urinary sodium output compared with the fixed intake of 220 mmol/day in 13 subjects during pretest equilibration (day −4 to day −1) and during the 4 test conditions: NO, PH, IH, and HP. Sodium balance was maintained during these 4 test conditions, which were applied on 4 separate days (see METHODS). *P < 0.05, sodium output vs. sodium intake (Student-Newman-Keuls t-test).
given for $V_E$, $P_{ETCO_2}$, and $Sao_2$ in Table 2. During NO (control), $V_E$ was 8.8 l/min, and $P_{ETCO_2}$ was 41.2 Torr, which are normal resting values. During PH with a controlled $Sao_2$ around 76.2%, $V_E$ transiently increased to 12 l/min, paralleled by a mean drop in $P_{ETCO_2}$ of −4.3 Torr relative to mean $P_{ETCO_2}$ during NO. $V_E$ during PH leveled off just below 10 l/min after 15–20 min and thereafter remained slightly but significantly above control $V_E$ during NO (Fig. 1A, Table 2). The initial adjustment of $FiO_2$ for this ventilatory response to maintain the target $Sao_2$ can be seen in Fig. 1B. IH was induced at virtually the same $Sao_2$ as PH (77.0 vs. 76.2%; Table 2, Fig. 1D). Whereas $V_E$ during IH increased markedly to ~16.3 l/min, isocapnia was successfully maintained over 90 min (Fig. 1C, Table 2) during this hypoxic condition. This required a lower $FiO_2$, ranging <10% as opposed to ~12% in PH (Fig. 1B). Voluntary HP on test day 4 was closely matched to the $V_E$ observed during the IH test day (Fig. 1A, Table 2). This led to a slight but significant increase in $SaO_2$ (Fig. 1D, Table 2). $P_{ETCO_2}$ during HP was maintained at the baseline level by CO$_2$ admixture (Fig. 1C, Table 2); however, mean $P_{ETCO_2}$ was 0.7 Torr below that of NO ($P = 0.04$).

Capillary Blood-Gas Analysis

The values of capillary blood-gas analysis, which are given in Table 2, reflected the experimental conditions: $PO_2$ ranged around 40 Torr in the two hypoxic conditions PH and IH, being slightly but nonsignificantly higher in IH than PH. During HP $PO_2$ increased significantly above NO (control). $PCO_2$ was significantly lower during PH than during NO. During HP $PCO_2$ tended to be lower than in NO, corresponding to the 0.7-Torr lower $P_{ETCO_2}$ (Table 2). Capillary pH was significantly higher in both hypoxic conditions and in HP than in NO, thereby corresponding to $PCO_2$, whereas bicarbonate was not found to be different from NO. (It should be noted that manipulation for capillary blood sampling may cause ventilatory irritation and thus deviations of blood gases from end-tidal gas measurements, which may better represent resting conditions.)

Renal Function and Fluid Shifts

The urine flow (ml/min) before (normoxic baseline) and during each of the four conditions is given in Fig. 3. Whereas NO (control) led to no significant increase in urine flow, a significant diuresis resulted from PH ($P < 0.01$), IH ($P < 0.05$), and HP ($P < 0.05$), compared with each condition’s normoxic baseline value. Baseline urine flow was not significantly different among the four experimental conditions. Compared with the NO test day (control), absolute urine flow values (Fig. 3) and percent increases in urine flow relative to baseline (Table 3) were significantly higher in the hypoxic conditions PH and IH.

As seen in Table 3, there were no differences among the four conditions in GFR, fractional sodium clearance, or in $C_{H_2O}$ as calculated for the urine samples collected during 90 min of each test condition. There were urine pH increases vs. baseline with all four conditions ($P < 0.05$); however, they were significantly greater during PH and HP, compared with NO, whereas no difference was found between IH and NO (Table 3).

ET-1, Urodilatin, and Catecholamines

Plasma ET-1 concentrations were not significantly different among the conditions (Table 4); however, relative to corresponding baselines (which were 0.95 ± 0.86, 0.80 ± 0.81, 0.87 ± 0.96, and 0.67 ± 0.23 pg/ml for NO, PH, IH, and HP, respectively), the plasma ET-1 increased (almost) significantly during PH ($P = 0.06$) and during IH ($P = 0.05$), whereas nonsignificant decreases and increases occurred with NO and HP, respectively. (Because of the large variability in ET-1 plasma levels, the increases by 65% with PH and 38% with PH are not well reflected by mean values given in Table 4.) Urinary ET-1 excretion rate (Table 4) was found to be significantly increased during PH compared with NO ($P = 0.003$), whereas increases during IH and HP did not reach statistical significance. Thereby, urinary ET-1 concentrations tended to increase with greater diuresis (PH, HP) or were unchanged (IH) compared with NO.

Urinary urodilatin concentration and excretion per time during PH, IH, and HP (Table 4) were not significantly different between conditions.

Among the urinary catecholamines, epinephrine excretion showed a significant increase with IH and PH compared with NO (Table 4). A marginal increase in dopamine excretion with PH compared with NO (Table 4) was significant but not considered to be relevant for discussion.

No significant linear correlations were found between ET-1 or urinary epinephrine excretion and $HVR_{iso}$, $HVR_{pco_2}$, or $Sao_2$.
Cardiovascular Parameters and Fluid Distribution

Mean HR was significantly higher in the two hypoxic conditions than in NO (control) or during HP, which itself resulted in no HR changes (Table 5). During PH, this HR increase coincided with a small but significant decrease in mean BP compared with NO. Whereas HR was higher in IH than PH, no significant difference was found for mean BP between these two hypoxic conditions. Venous compliance (Table 5) was not significantly different among all conditions (except for a difference between IH and HP, P < 0.01) and showed no significant changes in relation to baseline values, which were 2.22 ± 0.84, 2.28 ± 0.83, 2.23 ± 0.63, and 2.10 ± 0.74 ml·mmHg g⁻¹·100 ml⁻¹ for NO, PH, IH, and HP, respectively. The percent decrease in PV (ΔPV, Table 5) as calculated from Hct and Hb changes was significantly greater in the two hypoxic conditions. There was, however, no significant correlation between the diuretic response and the percent PV change. Mean calf fluid volume changes per unit tissue volume (Table 5) were small and not significantly different among the four conditions. The same was true for absolute fluid volume changes, which ranged between −2.6 and −7.4 ml in the total 20-cm calf segment with a mean water displacement volume of 1,820.0 ± 291.7 ml. During NO the calf fluid volume was virtually stable, indicating postural volume equilibration.

Correlation of O₂ Chemosensitivity to Diuresis and Natriuresis

No significant positive linear correlation was found between HDR in terms of absolute urine flow during IH to HVRiso (r = 0.16) or HVRpoi or between HDR during PH to HVRiso or HVRpoi. The same was true for the relation of HDR in terms of absolute changes and percent changes in urine flow (relative to baseline) during IH to HVRiso (r = −0.60, P = 0.03, and r = −0.40, P = 0.17, respectively; see Fig. 4) and HVRpoi. Also HDR during PH failed to correlate positively with HVRiso or HVRpoi. Subtraction of HDR (absolute or relative terms) during HP from that during IH for isolation of the hypoxic component did not strengthen the relation to HVRiso or HVRpoi. The same was true for the relation of natriuresis to HVRiso or HVRpoi. HCVR was unrelated to diuresis and natriuresis or any hormonal response.

DISCUSSION

As a main finding, this study demonstrates that acute IH, as well as PH, induces an early HDR, which, in the first 90 min, is not associated with higher sodium excretion as opposed to the natriuresis induced by 3–6 h of hypoxia (9, 41). Our findings are in line with studies in animals (10, 13) and humans (31), demonstrating that increased volume excretion precedes natriuresis in the first 1–2 h of hypoxia. Whereas hypoxic natriuresis in humans appears to be related to peripheral O₂ chemosensitivity (41) as assessed by HVRiso, the early HDR does not bear such a relationship. In addition to hypoxemia, PH and hypocapnia also contribute to HDR. Our data suggest that slight increases in ET-1 or epinephrine may play a role in the diuretic effect of hypoxemia, but that changes in leg venous compliance or urodilatin do not mediate the early HDR. The acute HDR to 90 min of 12% O₂ leads to a reduction in PV (6.6% with PH and 7.3% with IH) that amounts to ~50% of the hemoconcentration observed during the acute hypoxic condition.
first 2 days of an equivalent hypoxic exposure at high altitude (45).

**Experimental Considerations**

The present study design for evaluation of possible contributors to HDR over 4 consecutive test days required standardization of several factors. Sodium balance. Sodium equilibration was successfully completed by the first test day, and no difference was found in the 24-h sodium output among the 4 test days or among the four experimental conditions.

Sympathetic activity and postural volume adjustment. Increased sympathetic activity, especially renal sympathetic nerve activity, is well known to attenuate or even override HDR (13, 14, 18, 33), especially in the presence of (central) hypovolemia or low-cardiac output and carotid sinus pressure (7, 13, 17, 25). The present study design minimized and standardized sympathetic stimuli by the supine test position, comfortable environment, and identical test time of the day as indicated by absent urine flow changes and a stable calf fluid and PV during NO (control), the supine rest phase of 55 min before the four test conditions was sufficient for postural equilibration. This allowed us to study HDR without superimposition of the volume adjustments to the supine position, i.e., quick central blood pooling and slow cephalad fluid shifts (4), followed by diuresis and natriuresis via an increase of ANF and decrease of ADH.

**Table 5. Cardiovascular parameter and fluid distribution**

<table>
<thead>
<tr>
<th>Condition</th>
<th>HR, beats/min</th>
<th>MBP, mmHg</th>
<th>Venous Compliance, ml·mmHg⁻¹·100 ml⁻¹</th>
<th>ΔPV, %</th>
<th>ΔCalf Volume, ml/100 ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normoxia</td>
<td>62.0 ± 7.7</td>
<td>80.7 ± 11.1</td>
<td>2.24 ± 0.76</td>
<td>−1.72 ± 4.97</td>
<td>−0.40 ± 0.95</td>
</tr>
<tr>
<td>Poliilocapnic hypoxia</td>
<td>71.1 ± 6.8*</td>
<td>75.8 ± 11.0*</td>
<td>2.42 ± 0.74</td>
<td>−6.57 ± 3.97*</td>
<td>−0.12 ± 0.86</td>
</tr>
<tr>
<td>Isoicapnic hypoxia</td>
<td>74.8 ± 8.1*</td>
<td>79.3 ± 10.6</td>
<td>2.42 ± 0.566§</td>
<td>−7.34 ± 6.32*</td>
<td>−0.33 ± 0.88</td>
</tr>
<tr>
<td>Hyperpnea</td>
<td>61.5 ± 7.4</td>
<td>86.6 ± 11.9</td>
<td>2.02 ± 0.60</td>
<td>−3.21 ± 4.08</td>
<td>−0.37 ± 0.83</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.01</td>
<td>&lt;0.05</td>
<td>NS</td>
<td>&lt;0.01 NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are means ± SD; n = 13 subjects. Shown are values of heart rate (HR), finger mean arterial blood pressure (MBP), venous compliance, plasma volume changes (ΔPV), and calf fluid volume changes (Δcalf volume) relative to baseline in the 4 test conditions indicated. *P < 0.05, †P < 0.01 vs. normoxia; ‡P < 0.05 vs. isocapnic hypoxia; §P < 0.01 vs. hyperpnea (paired Student’s t-test). P indicates significance of differences with multiple comparison (ANOVA for repeated measures).

*Fig. 4. Correlation between isocapnic hypoxic ventilatory response (HVR) and absolute increase (A), as well as percent increase (B), in urine flow during 90-min IH.*
hypoxia, the much smaller ventilatory increase in PH points to a minor role of HP in HDR.

Hypocapnia and alkalosis. These effects of hyperventilation are well known to induce diuresis in humans, as well as increase sodium, potassium, and bicarbonate excretion and decrease total acid excretion (9, 42). Because urine pH significantly increased without an increase in sodium excretion, the present data imply that an increased bicarbonate excretion may be accompanied early by increased potassium excretion rather than sodium excretion, which is observed after longer exposure to hypocapnic hypoxia (41). However, hypocapnia-induced bicarbonate and possibly potassium excretion are not the major factors of early HDR, because urine flow during IH (at unchanged urine pH vs. NO) was not significantly different from that of PH. The separate contribution of hypocapnia may be roughly estimated as follows (given a simplified arithmetic summation of the effects of hypoxia, hypocapnia, and HP on absolute urine flow values): diuresis in IH (1.94 ml/min) minus that in matched normoxic HP (1.64 ml/min) may reflect the hypoxia effect alone (0.3 ml/min). Subtracting this 0.3 ml/min from diuresis in PH (1.81 ml/min) gives 1.51 ml/min, which, compared with NO (1.38 ml/min), leaves an effect of 0.13 ml/min for hypocapnia. During HP, diuresis exceeded that of NO by 0.26 ml/min.

Hypoxemia. Hypoxemia thus appeared to be the most important factor in the presently seen HDR compared with HP and hypocapnia. This is in accord with animal studies demonstrating that diuresis and natriuresis occur with isolated hypoxemic stimulation of peripheral chemoreceptors, even in the presence of controlled ventilation and isocapnia (13, 17). Honig (13) and Quies and Ledderhos (31) suggested that, in water-loaded humans, hypotonic diuresis may precede natriuresis, even when peripheral chemoreceptors are stimulated with almitrine and systemic and renal hypoxia are avoided. Moreover, Gledhill (9) demonstrated that normoxic hypocapnia and IH do not cause natriuresis before 3 h, whereas IH increases urine volume within 1 h. Recently, such different time courses of hypoxic water diuresis and natriuresis were also found in chronically instrumented awake rats (10).

Mechanisms of Early HDR

Hypoxia-induced changes in perfusion pressure on GFR may affect HDR to some extent (10); however, in the present study, a pressure diuresis during hypoxia can be ruled out in both IH and PH, because mean arterial BP and GFR failed to increase.

Importantly, no change in venous compliance was observed in the leg, suggesting that HDR in the first 90 min may occur without any increase in venous tone during hypoxia. These data obtained after 80–88 min of IH or PH are at variance with findings of a decreased venous compliance in the human forearm after 2 h of a hypobaric hypoxia (6), which was equivalent to the presently applied normobaric hypoxia. Thus our data provide evidence against an early hypoxic venoconstriction, at least in the calves, as a factor of central blood pooling and a renal response via ADH, ANF (13), or other factors. The observed slight increase in epinephrine with the two hypoxic conditions (at unchanged norepinephrine) obviously failed to cause essential α-adrenergic venous constriction (4, 28) in the calf. It may well be, however, that venoconstriction occurs in other areas (e.g., the forearm or splanchnic vessels) or with prolonged hypoxia and thus plays a role in diuresis and natriuresis via right atrial blood pooling.

Because GFR was unchanged, the diuretic response appears to be at the level of tubular function, especially with regard to water handling. Within this early phase of HDR, the increased urine flow at unaltered fractional sodium clearance did not lead to a significantly increased CH2O, especially with IH, as was observed with the diuresis of humans induced by almitrine ingestion (13, 31). Although bicarbonate and potassium excretion due to hypocapnia may occur after 1 to several hours and contribute an osmotic component to the observed diuresis, this would not explain an osmotic component with IH.

Many have thought to identify a circulating humoral factor in HDR, which must override increased renal sympathetic nerve activity during peripheral chemoreceptor stimulation and during baroreceptor deactivation (17, 18) as possibly indicated by increased HR and slightly lowered BP in the present study. The absence of increases in fractional sodium excretion in the present study implies that hormones that control natriuresis may not be a driving factor in this early phase of HDR. The role of ANF in the hypoxic diuresis and natriuresis has not been conclusive in humans and animals, although several mechanisms of an ANF increase during hypoxia certainly exist (13, 16, 40). In humans, diuresis and natriuresis could be induced by selective peripheral chemoreceptor stimulation with almitrine (22) or by 6 h of PH (41) without relevant changes in ANF. In two high-altitude field studies, ANF was not a factor in the hypoxic diuresis or natriuresis, because it was found to be elevated in subjects with antidiuresis and related to the widening of the right atrium (1, 2). Thus ANF may not be a first candidate to mediate acute hypoxic diuresis in humans, especially within the first 90 min.

A more promising candidate to focus on appeared to be urodilatin (21), an analog renal natriuretic hormone, which has not been studied in sodium-equilibrated humans during controlled hypoxemia. Our measurements, obtained under such conditions, however, failed to reveal any increase in urodilatin with acute HDR, whether urodilatin was expressed in terms of urine concentration or excretion rate. In contrast, during 6 h of hypobaric hypoxia, Koller et al. (21) found that the diuretic and natriuretic responses were associated with increased urinary urodilatin excretion. Apart from the much longer time of hypoxic exposure in this study, which compared lowlanders with acclimatized highlanders, the lack of control of sodium balance, postural volume adjustment, and SaO2 may explain some of the differences from our present data on HDR and urodilatin. Thus there is no convincing evidence that the
It is still undecided whether adrenalectomy prevents or abolishes the renal response to acute hypoxemia (89), and the role of endogenous adrenocortical hormones in the renal effects of hypoxia is yet to be clarified.

The present study confirmed the results of previous investigations of the renal effects of hypoxia in dogs and humans (35, 60). It demonstrated that acute hypoxemia causes a significant increase in glomerular filtration rate (GFR) and in renal plasma flow (RPF), and also an increase in urinary excretion of sodium and water (35). The increase in renal blood flow and sodium excretion is associated with an increase in plasma renin activity (35) and a decrease in plasma aldosterone (13).

The results of the present study indicate that the increase in GFR during hypoxemia is not due to an increase in sympathetic activity, as suggested by previous investigations (13, 25, 35). Instead, the increase in GFR during hypoxemia is thought to be due to an increase in renal blood flow, which may be caused by a decrease in renal vessel tone (13). The increase in renal blood flow during hypoxemia is associated with an increase in renal perfusion pressure (13), which may be due to an increase in systemic blood pressure (13).

The increase in GFR during hypoxemia is also due to an increase in the filtration fraction (13), which may be due to an increase in the renal plasma flow (13). The increase in GFR during hypoxemia is also due to an increase in the glomerular ultrafiltration coefficient (13), which may be due to an increase in the glomerular filtration rate (13). The increase in GFR during hypoxemia is also due to an increase in the glomerular ultrafiltration coefficient (13), which may be due to an increase in the glomerular filtration rate (13).
ureament of urodilatin and ET-1. Moreover, we are indebted to Dr. E. Hildebrandt, Institute für Sport und Leistungsmedizin, Universitätsspital Klinik Heidelberg, Hospitalstr. 3, 69115 Heidelberg, Germany (E-mail: wulf_hildebrandt@med.uni-heidelberg.de).

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