Effects of hypoxia on renal hormonal balance in normal subjects and in patients with COPD

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There is a complex interaction between pulmonary haemodynamics, hormonal, and salt and water balance in patients with chronic obstructive pulmonary disease (COPD) and in normal subjects exposed to hypoxia or high altitude. This study aims to investigate the effects of hypoxia on renal hormonal balance in normal subjects and patients with COPD, particularly the role of urinary dopamine and atrial natriuretic peptide (ANP).

Urinary dopamine output, ANP, and plasma renin activity (PRA) were measured in 12 normal subjects exposed to hypoxia (12% O₂) and hyperoxia (40% O₂) for 1 h and in 15 patients with exacerbations of COPD while breathing air or O₂. These measurements were repeated in six of the patients with exacerbations of COPD when they were clinically stable.

Hypoxia caused an increase in ANP levels (49 ± 6–62 ± 6 pg ml⁻¹, P < 0.05) and a fall in urinary dopamine output (277 ± 39–203 ± 33 ng h⁻¹, P < 0.002) in normal subjects. Hyperoxia was associated with a return of plasma ANP to the baseline values. In patients with exacerbations of COPD plasma ANP levels were higher (181 ± 36 pg ml⁻¹) than in normal subjects (19 ± 5 ± 6 pg ml⁻¹, P < 0.001). Urinary dopamine output breathing air (175 ± 34 ng h⁻¹) was similar to the levels when normal subjects were made hypoxaemic and PRA was elevated in comparison to normal values. There was no change in their levels following the acute administration of oxygen in patients presenting with exacerbations of COPD, but oxygen improved urinary sodium excretion (P < 0.05). In six patients re-studied when clinically stable there was a fall in urinary dopamine output, plasma ANP and PRA when breathing air in comparison to the acute stage of the disease (P < 0.05).

These data suggest presence of renal hormonal imbalance including endogenous urinary dopamine output during hypoxic exacerbation of COPD and in normal subjects exposed to hypoxia.

Introduction

Recurrent exacerbations of chronic obstructive pulmonary disease (COPD), lead to development of cor pulmonale. However, the exact pathogenesis of this syndrome remains unclear (1–4). The view that the oedema in cor pulmonale is due to right heart failure is the subject of much debate (1,3,5,6). There is evidence that complex interactions occur between pulmonary haemodynamics and hormonal and salt and water balance in patients with hypoxic COPD (4,7–9) and in normal subjects exposed to hypoxia or living at high altitude (10–13). High plasma renin activity (PRA) and aldosterone levels are present in patients with respiratory failure secondary to COPD (8,14,15). Pulmonary hypertension which develops as a result of hypoxaemia leads to enlargement and dilatation of the right ventricle and atrium. Atrial and ventricular stretch appears to be the major stimulus to the secretion of atrial natriuretic peptide (ANP) (4,7,14) which could potentially counteract the activation of the renin–angiotensin–aldosterone system (RAA). However, there is no general agreement on how the renal and endocrine systems are affected by hypoxaemia.

In recent years it has become apparent that dopamine has an important regulatory role in renal function (16–19). The main source of renal dopamine appears to be circulating L-dopa (16,18,20). Dopamine is formed in the proximal convoluted tubule under the action of L-dopa decarboxylase (L-amino acid decarboxylase LAAD). Dopamine produced in this way is then free to act on renal dopamine receptors leading to vasodilatation and natriuresis (16–18, 20,71). Whether dopamine has a role in the pathogenesis of the oedema in hypoxic COPD has not been established.

In this study our aim was to examine plasma renin, renal dopamine output and plasma ANP levels during acute normocapnic hypoxia and hyperoxia in normal subjects. We also wished to examine the same factors in patients with acute and stable COPD, before and after the administration of oxygen.
Methods

The study had ethical approval by the regional ethics committee. It was conducted in the Department of Medicine, Unit of Respiratory Medicine and in the Rayne Laboratory, University of Edinburgh.

STUDY POPULATIONS

Normal subjects

Twelve normal subjects (all male), mean age 27 years (range 23–39 years) were studied. None had respiratory, cardiac, hepatic or renal disease, as assessed by clinical examination and standard laboratory tests. None were receiving any medication. Blood samples were obtained from a peripheral venous catheter. Arterial oxygen saturation (SaO₂) was monitored continuously using a pulse oximeter (Hewlett-Packard). Breath-by-breath end-tidal CO₂ and O₂ were measured using a mass spectrometer. Hypoxia was induced by breathing a gas mixture containing 12% O₂. Isocapnic hypoxia was maintained by the addition of CO₂ using a rotameter system which maintained end-tidal PCO₂ constant. SaO₂ was maintained at 80% during hypoxia and at 98–100% during hyperoxia while breathing 40% O₂.

Each study was performed in the morning. The subjects had a light breakfast, 2 h prior to the study. They were studied semi-recumbent. An intravenous cannula was inserted into an antecubital vein. The subjects breathed room-air through a mouthpiece for 2 h. Saline 0.9% was infused at a rate of 150 ml h⁻¹ throughout the study to maintain adequate urinary output. SaO₂ was measured continuously using a pulse oximeter and heart rate and rhythm using a cardiac monitor.

After the first hour of baseline measurements the subjects emptied their bladder and the urine sample was discarded. At the end of the second hour of baseline measurements plasma ANP and renin activity, electrolytes, urea and creatinine were measured. Urine was collected to measure dopamine output and urinary sodium excretion. An identical set of measurements was obtained at the end of each subsequent 1-h period, when the subjects had breathed 12% and 40% O₂, respectively. Plasma renin activity was measured in only six of the subjects.

Patients with COPD

We studied 15 patients presenting with an exacerbation of COPD (Table 1). These patients presented with worsening blood gas values and increasing dyspnoea, but without acute acidosis. Patients were studied within an hour of admission, when breathing room-air. Repeated measurements were made breathing O₂ (2 l min⁻¹ via nasal prongs) for 1 h. Measurements were repeated again breathing air and O₂, when the patients condition was stable, at least 6 weeks after the exacerbation (n=6) (Table 1). Drug therapy in these patients during their acute episode included, oxygen 2 l min⁻¹ by nasal prongs, nebulized bronchodilators, antibiotics as appropriate and diuretics (40–80 mg frusenide) in those with oedema (n=7).

<table>
<thead>
<tr>
<th>Acute exacerbations of:</th>
<th>COPD</th>
<th>Stable COPD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>68 ± 5</td>
<td>66 ± 4</td>
</tr>
<tr>
<td>FEV₁ (l)</td>
<td>0.6 ± 0.2</td>
<td>—</td>
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<tr>
<td>FEV₁ (% pred)</td>
<td>27 ± 34</td>
<td>—</td>
</tr>
<tr>
<td>PaO₂ (air) (kPa)</td>
<td>6.9 ± 1.1</td>
<td>7.9 ± 1.0*</td>
</tr>
<tr>
<td>PaO₂ (O₂) (kPa)</td>
<td>10.1 ± 2.1</td>
<td>10.9 ± 1.9</td>
</tr>
<tr>
<td>PaCO₂ (air) (kPa)</td>
<td>6.2 ± 1.1</td>
<td>5.8 ± 0.6</td>
</tr>
<tr>
<td>PaCO₂ (O₂) (kPa)</td>
<td>6.6 ± 1.3</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>H⁺ (air) (mmol l⁻¹)</td>
<td>36.9 ± 3.9</td>
<td>35.8 ± 2.1</td>
</tr>
<tr>
<td>H⁺ (O₂) (mmol l⁻¹)</td>
<td>38 ± 4.2</td>
<td>37 ± 3.5</td>
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</table>

*P<0.05.

HORMONAL ASSAYS

Plasma ANP

Ten ml of venous blood were withdrawn into chilled EDTA tubes. Plasma was rapidly separated by centrifugation, stored at −70°C and assessed in batches within 2 months. ANP was measured in these samples by a specific radioimmunoassay using commercial kit. ANP antibody was raised in rabbit by conjugation of synthetic ANP 1-28 with bovine thyroglobulin, using carbodimide as the coupling agent. ANP was extracted from plasma using Sep-Pak C18 cartridges (Waters Ass, U.K.) and diluted with a mixture of ethanol, water and acetic acid. The lower limit of detection for the assay was 10 pg ml⁻¹. The level of ANP was measured using 125I-ANP as the radioactive tracer (Amersham International, U.K.). The range of plasma ANP in our laboratory in normal subjects is 10–55 pg ml⁻¹.

Plasma renin activity and aldosterone levels

Plasma renin activity was determined indirectly by the generation of angiotensin I and then by radioimmunoassay of this peptide. Plasma aldosterone was determined by a double antibody technique (Coat a Count U.K., commercial kit).

Urinary dopamine (in-house assay)

Urine samples were collected into 5 M HCl as a preservative. Dopamine was measured after extraction from urine by a modification of the method of Anton and Sayre (20). In brief, dopamine was measured by high-performance liquid chromatography (HPLC) using a Millipore 4u ‘Novapack’ column under radial compression. Eluates from the column were recorded using a Shimadzu computing integrator. Epinephrine was added to all samples in standard concentrations as an internal standard. The standard response was calibrated by division of the area of the
internal standard into five known concentrations and unknowns were derived from this. Intra- and inter-assay coefficients of variation were 1.0% and 2.2%, respectively.

STATISTICAL ANALYSIS

Non-parametric tests were used in the statistical analysis due to not normal data distribution. Comparisons between mean values were made using the Wilcoxon signed-rank test for the same group and unpaired standard t-test for comparison between groups. A P-value less than 0.05 was considered statistically significant.

Correlations were obtained using linear regression analysis.

Results

NORMAL SUBJECTS

Response to hypoxia and hyperoxia

Following hypoxia for 1 h, when the subjects breathed 12% O₂, their SaO₂ dropped from 98% to 80%. Mean plasma ANP concentration, measured at the end of this period increased from 49 ± 6 to 62 ± 6 pg ml⁻¹ (mean ± SEM), P<0.05. Hypoxia also produced a fall in urinary dopamine output from 277 ± 39 to 175 ± 34 ng h⁻¹, P<0.001. Urinary sodium fell from 124 ± 10 to 63 ± 12 mmol h⁻¹, P<0.001; in addition we observed a trend towards a decrease in renin activity which was not statistically significant.

After hyperoxia the mean SaO₂ rose to 99% and was associated with a return to plasma ANP to control values breathing air (44 ± 5 pg ml⁻¹). Following hyperoxia we did not observe a significant difference in urinary dopamine output in comparison to the period of hypoxia, but the level was still significantly lower than during the control period, breathing air (224 ± 38 ng h⁻¹). However, there was a further fall in plasma renin activity (0.59 ± 0.09 ng ml⁻¹ h⁻¹, P<0.01) during hypoxia. Urinary sodium was higher during hyperoxia in comparison to the hypoxic period (101 ± 28 mmol h⁻¹, P<0.01) and was similar to control values when breathing air. Combined results of the response to hypoxia and hyperoxia in normal subjects are shown in Table 2.

Patients with COPD

Patients studied during acute exacerbations of COPD had higher ANP levels (181 ± 36 pg ml⁻¹) than normal subjects (49.5 ± 6.3 pg ml⁻¹, P<0.001). ANP levels in plasma did not change while breathing O₂. However, administration of O₂ increased urinary sodium significantly (from 31 ± 11 to 39 ± 9 mmol h⁻¹, P<0.005). Plasma ANP breathing either air and O₂ correlated significantly with the PRA (r=0.82) (r=0.68, P<0.001). Combined results of the effect of hypoxia and supplementary O₂ administration during acute exacerbation of COPD are shown in Table 3.

In six patients re-studied 6 weeks after their exacerbation (when clinically stable) there was significant improvement in their partial arterial O₂ pressure while breathing room-air (from 64 ± 1.5 to 7.9 ± 1.2 kPa, P<0.05). There was also a significant fall in urinary dopamine output on air from the exacerbation (178 ± 41 ng l⁻¹) to the time of clinical stability (76 ± 18 ng l⁻¹, P<0.05). We also observed a lower plasma ANP level breathing both air and oxygen at clinical stability compared with corresponding levels during the exacerbation (P<0.005). A similar trend was also shown for PRA. Combined results of this comparison are shown in Table 4.

Discussion

The development of chronic hypoxia (PaO₂<8.0 kPa) in patients with COPD leads to pulmonary vasoconstriction and eventually irreversible changes in the pulmonary vasculature, resulting in turn to chronic pulmonary hypertension and increased right ventricular afterload (22,23). During exacerbations such patients can develop even higher levels of pulmonary arterial pressure, raised jugular venous pressure and oedema (24,25).

Hypoxia has been shown to affect renal function in both normal subjects and in hypoxaemic patients with COPD. Reduced renal blood flow during hypoxia is thought to induce activation of the renin-angiotensin–aldosterone system (11,13,26). In addition plasma ANP is higher in such patients (7,8,14) and also increases in normal volunteers exposed to hypoxia (27). Dopamine also has an important modulating effect on renal function (10,17–19,28). However, the effects of acute or chronic hypoxia on urinary dopamine output in patients with hypoxic COPD and in normal subjects has not been studied. Endogenous renal dopamine produces a natriuresis (16,18) by three mechanisms: (1) by an action on the renal tubules via dopamine receptors, (2) by vasodilatation of the renal vasculature, thus increasing GFR and possibly also (3) by inhibiting renin release.

Our hypothesis was that hypoxia would result in a fall in the renal dopamine output followed by a decreased sodium excretion in the urine. Moreover, activation of the renin–angiotensin–aldosterone system by hypoxia would also result in vasoconstriction and fluid overload. In our study hypoxia did result in a fall in urinary dopamine output and a decrease in urinary sodium in normal subjects. In addition we observed a significant fall in PRA during hypoxia, although plasma ANP rose. It has been shown previously in experimental animals that the release of ANP is enhanced during acute or chronic hypoxia (29). In normal human volunteers, as well as in patients with hypoxic COPD, hypoxia induces a decrease in plasma aldosterone despite a
### Table 2. Results

<table>
<thead>
<tr>
<th></th>
<th>Normal subjects</th>
<th></th>
<th>Acute exacerbation of COPD</th>
<th></th>
<th>Patients with COPD</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Air</td>
<td>Hypoxia</td>
<td>40% O₂</td>
<td>Air</td>
<td>O₂</td>
<td>Air</td>
</tr>
<tr>
<td>SaO₂ (%)/PaO₂ (kPa)</td>
<td>98 ± 1</td>
<td>80</td>
<td>99 ± 1</td>
<td>6-9 ± 1-1</td>
<td>10-1 ± 2-1</td>
<td>6-4 ± 1-5</td>
</tr>
<tr>
<td>ANP (pg ml⁻¹)</td>
<td>49 ± 6</td>
<td>62 ± 6*</td>
<td>44 ± 5</td>
<td>181 ± 36</td>
<td>139 ± 34</td>
<td>200 ± 40</td>
</tr>
<tr>
<td>Urinary dopa (ng h⁻¹)</td>
<td>277 ± 39</td>
<td>175 ± 34*</td>
<td>224 ± 38‡</td>
<td>205 ± 33</td>
<td>140 ± 39</td>
<td>178 ± 41</td>
</tr>
<tr>
<td>PRA (ng ml⁻¹ h⁻¹)</td>
<td>1-4 ± 0-25</td>
<td>1-05 ± 0-3</td>
<td>0-6 ± 0-08‡</td>
<td>12 ± 3</td>
<td>11 ± 2</td>
<td>14 ± 5</td>
</tr>
<tr>
<td>Urinary Na (mmol h⁻¹)</td>
<td>124 ± 10</td>
<td>63 ± 12*</td>
<td>101 ± 28†</td>
<td>31 ± 11</td>
<td>39 ± 9§</td>
<td>n.a.</td>
</tr>
</tbody>
</table>

*Comparison between measurement taken on air and during hypoxia.
†Comparison between measurement taken during hypoxic phase and during hyperoxia.
‡Comparison between measurement taken on air and during hyperoxia.
§Comparison between measurement taken on air and on O₂.
*Comparison between measurement taken either on air or on O₂ during acute exacerbation of COPD and when clinically stable, respectively.
rise or no change in PRA. Similar findings were observed in patients with COPD receiving an infusion of ANP which reduced plasma aldosterone levels without influencing PRA.

The observed increase in plasma ANP during hypoxia in our normal volunteers could be in response to the reduced sodium excretion by the hypoxic kidneys and the reduction in endogenous dopamine which would lead to activation of the renin–angiotensin–aldosterone system (13,30,31). Although these subjects were hypoxic, the partial pressures of O₂ in the kidney were not known. By contrast, compared with hypoxia, hyperoxia reduced plasma ANP and returned urinary sodium levels to control values and decreased PRA. However, these changes were not accompanied by significant changes in urinary dopamine output. A further mechanism for the increase in ANP could be as a result of hypoxic pulmonary vasoconstriction, causing the release of ANP due to right atrial stretch (4,7).

These data, from studies in which hypoxia was induced acutely in normal subjects, were compared and contrasted with data obtained in patients presenting with acute exacerbations of COPD. Plasma ANP and PRA levels were higher in patients with an acute exacerbation of COPD than in normal subjects and reverted to normal levels when repeated at a time when the patients were clinically stable. Urinary dopamine output during the acute exacerbation of COPD was lower than in normal subjects breathing air but was similar to the values obtained when normal subjects were made acutely hypoxaemic.

Chronic hypoxaemia in patients with COPD is associated with a fluid and salt retaining state in that there is activation of the renin–angiotensin system, a low urinary sodium and dopamine output but balanced against high plasma ANP. The effects of O₂ on dopamine output could be mediated by a direct effect on the proximal tubules or on the synthesis or degradation of dopamine (32). Dopamine has been shown to act in such a way in numerous situations such as in pre-eclamptic toxemia (33) and in primary aldosteronism (18).

However, in some cases the natriuretic effect of a raised plasma ANP and urinary dopamine are overwhelmed by the salt-retaining effect of the activated renin–angiotensin system and thus these compensatory mechanisms (dopamine and ANP) fail, and oedema develops.

This study of the effects of acute hypoxia supports the hypothesis that changes in plasma ANP level, renal hormonal system and urinary dopamine output are observed in acute exacerbation of COPD and may be important in the pathogenesis of the clinical syndrome of cor pulmonale in patients with hypoxic COPD.

Acknowledgements

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References


