Effect of hypoxia and hypercapnic acidosis on renal autoregulation in the dog: role of renal nerves

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Summary

1. Previous studies suggest that hypoxia and hypercapnic acidosis exert a renal nerve mediated adverse effect on renal haemodynamic function. We therefore examined the effect of hypoxia and hypercapnic acidosis on renal blood flow and glomerular filtration rate responses to lowering renal perfusion pressure from 125 to 75 mmHg in the anaesthetized dog. To study the role of renal nerves in these responses, paired innervated and denervated kidneys were studied in each animal.

2. Hypoxia \((P_{O_2} 43 \pm 3 \, \text{mmHg})\) affected neither renal blood flow nor glomerular filtration rate responses to decreasing renal perfusion pressure.

3. Hypercapnic acidosis \((P_{CO_2} 71 \pm 2 \, \text{mmHg}; \, \text{pH} 7.03 \pm 0.01)\) significantly decreased both renal blood flow and glomerular filtration rate as renal perfusion pressure was lowered. This effect of hypercapnic acidosis could be abolished by renal denervation.

4. These findings suggest that hypercapnic acidosis results in renal nerve stimulation, which prevents the usual decrease in renal afferent arteriolar tone that occurs in response to lowering of renal perfusion pressure.

Key words: glomerular filtration, hypercapnia, hypoxia, renal blood flow.

Introduction

Laboratory studies have indicated that hypoxia and hypercapnic acidosis diminish basal levels of renal blood flow \((RBF)\) and glomerular filtration \((GFR)\). In the anaesthetized dog, hypercapnia \((P_{CO_2} > 60 \, \text{mmHg})\) consistently results in renal vasoconstriction \([1-5]\) and hypoxia \((P_{O_2} < 40 \, \text{mmHg})\) decreases RBF in some \([6, 7]\) but not all studies \([8-10]\). In several studies, renal denervation attenuates the effect of hypoxia and hypercapnic acidosis to decrease RBF \([1, 5-7]\). The present studies were carried out to determine the effect of hypoxia and hypercapnic acidosis on RBF and GFR responses to lowering renal perfusion pressure and to evaluate the role of renal nerves in these responses.

Methods

Studies were performed on 16 mongrel dogs of either sex weighing 20–35 kg. Food was withheld for 18 h before study but all animals had free access to water. All animals were anaesthetized with intravenous pentobarbitol (20–25 mg/kg) and ventilated with a Harvard Respirator (Harvard Apparatus Co., Millis, MA, U.S.A.). Ventilatory tidal volume was 12 ml/kg and respiratory rate was constant at 12–15/min. Additional small, 0.5 mg/kg, non-hypotensive doses of anaesthetic were administered throughout the experiment at 25–30 min intervals.

After induction of anaesthesia, polyethylene catheters were inserted into both ureters and renal veins through bilateral flank incisions by a retroperitoneal approach. The catheters (I-Cath, Delmed Inc., Canton, MA, U.S.A.) were inserted into the renal vein through a direct needle puncture (14 gauge). The vein was punctured through a circular purse-string stitch sutured into the middle third of each renal vein. After puncture, the plastic catheter was advanced into the renal vein and tied in place. A Blalock clamp was inserted through the left flank incision and placed around the abdominal
placed in the femoral and brachial arteries for continuous monitoring of arterial pressure above and below the renal arteries. Arterial pressure was monitored with Statham transducers (Statham Instruments Inc., Oxnard, CA, U.S.A.). The arterial catheters were also utilized for sampling (2 ml) of arterial blood which was obtained at 5-10 min intervals throughout the study. The arterial blood was collected anaerobically into heparinized syringes, kept on ice and oxygen and carbon dioxide tensions were measured on a Corning Blood Gas Analyzer (Corning Scientific Instruments, Medfield, MA, U.S.A.) within 10 min of sampling. In all studies, one kidney was denervated by surgically stripping all visible renal nerves from the renal pedicle and applying 95% ethanol to the pedicle. The side of renal denervation was alternated. We have previously provided biochemical and renal functional data validating this denervation procedure [11].

After surgery, 2.5% glucose was administered at 10-15 ml/min for 60-90 min to ensure adequate urine flow throughout the duration of the study. Subsequently, 2.5% glucose was infused at a rate 1-2 ml/min greater than urine flow. No animals demonstrated glycosuria. After completion of surgery, an intravenous infusion of 0.9% sodium chloride solution at 0.5 ml/min was started; this contained sufficient insulin (4.0 g/dl) and p-aminohippuric acid (0.6 g/dl) to maintain plasma levels between 15-20 and 1-3 mg/dl respectively. Both the 2.5% glucose and the 0.9% sodium chloride solution (saline) were infused into a single forepaw vein.

Experiments were begun 1-2 h after completion of surgery and stabilization of urine flow. During the experiments, three timed urine collections of 5-10 min duration were obtained and arterial (7 ml) and renal venous samples (2 ml) were collected at the midpoint of alternate urine samples. All blood removed from the animals was replaced with saline and mean brachial arterial pressure remained constant throughout the protocol. Hypoxia was induced by changing the inspired air mixture from room air to a gas mixture of oxygen/nitrogen (10:90). Hypercapnic acidosis was induced by addition of 100% carbon dioxide at 1-2 ml/min to the inhalation valve of the ventilator. This method of induction and maintenance of hypercapnia resulted in stable Pco₂ values equivalent to those obtained during preliminary experiments, a Beckman Instrument LB2 capnograph being used to provide a constant monitor of end-tidal Pco₂. Since preliminary experiments demonstrated that hypercapnia was associated with marked increases in the rate and depth of breath-

ing, all group 2 animals were paralysed with intravenous succinylcholine (0.5-1.0 mg/min) shortly after induction of anaesthesia and remained paralysed throughout the experiment. Two groups of experiments were performed.

Group 1: effect of hypoxia on autoregulation of RBF and GFR. In these animals (n = 8), blood and urine collections were started 15 min after adjustment of the Blalock clamp to give renal perfusion pressures of 125, 100 and 75 mmHg. These renal perfusion pressures were selected because GFR and RBF remain relatively constant within this range in the anaesthetized dog [12]. In each animal, three collections were made at each level of renal perfusion pressure during both room air (normoxic) and experimental (hypoxic) conditions. Four animals were studied, first breathing room air, then breathing 10% oxygen in nitrogen, and four were studied first breathing 10% oxygen and then room air. The order of study was alternated. The order of study did not influence RBF and GFR as renal perfusion was lowered from 125 to 75 mmHg during both normoxic and hypoxic conditions in the same animal.

Group 2: effect of hypercapnic acidosis on autoregulation of RBF and GFR. The protocol used in these animals (n = 8) was identical with that in group 1 animals with the exception that clearance determinations at renal perfusion pressures of 125, 100 and 75 mmHg were obtained while animals were breathing room air (normocapnic) or excess carbon dioxide. As in group 1, four animals were studied first breathing room air (normocapnia) followed by excess carbon dioxide and four were studied first while breathing excess carbon dioxide and then room air. As in group 1 studies, the order of study did not influence renal responses to lowering renal perfusion pressure.

The analytical procedures and calculations used in these experiments have been described elsewhere [12]. Mean GFR and RBF obtained from each kidney at each level of renal perfusion pressure in control conditions and in either hypercapnia or hypoxia were compared by using the two-tailed paired Student’s t-test. P < 0.05 was considered significant. All values are expressed as the means ± SEM.

Results

Effect of hypoxia on autoregulation of RBF and GFR (group 1, Table I and Figs. 1 and 2)

Values (the means of the mean from each animal) for all arterial blood determinations
TABLE 1. Mean values for all arterial blood gas samples

<table>
<thead>
<tr>
<th>Group</th>
<th>Experimental condition</th>
<th>$P_{O_2}$ (mmHg)</th>
<th>$P_{CO_2}$ (mmHg)</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 ($n = 8$)</td>
<td>Normoxic</td>
<td>81.7 ± 4.2</td>
<td>29.9 ± 1.4</td>
<td>7.37 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>Hypoxic</td>
<td>42.6 ± 3.1*</td>
<td>28.4 ± 1.3</td>
<td>7.39 ± 0.01</td>
</tr>
<tr>
<td>2 ($n = 8$)</td>
<td>Normocapnic</td>
<td>84.5 ± 3.7</td>
<td>26.0 ± 2.0</td>
<td>7.38 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>Hypercapnic</td>
<td>79.6 ± 3.3</td>
<td>71.2 ± 2.2*</td>
<td>7.03 ± 0.01*</td>
</tr>
</tbody>
</table>

* $P < 0.001$.

FIG. 1. Values for glomerular filtration rate in (a) denervated and (b) innervated kidneys at renal perfusion pressures of 125, 100 and 75 mmHg. ○, Values obtained during normoxia; ●, values obtained during hypoxia.

FIG. 2. Values for renal blood flow in (a) denervated and (b) innervated kidneys at renal perfusion pressures of 125, 100 and 75 mmHg. ○, Values obtained during normoxia; ●, values obtained during hypoxia.
during control and hypoxic periods are given in Table 1. The effect of lowering $P_{O_2}$ from $81.7 \pm 4.2$ to $42.6 \pm 3.1$ mmHg on RBF, and GFR responses to decreasing renal perfusion pressure, are given in Figs. 1 and 2. Glomerular filtration rate and RBF values at each level of renal perfusion pressure were comparable during hypoxia and normoxia and innervated and denervated kidney autoregulatory responses were similar. Hypoxia did not significantly alter $p$-aminohippurate extraction in either innervated $(0.78 \pm 0.03$ to $0.76 \pm 0.04)$ or denervated $(0.80 \pm 0.02$ to $0.76 \pm 0.03)$ kidneys. 

**Effect of hypercapnic acidosis on autoregulation of RBF and GFR (group 2, Table 1 and Figs. 3 and 4)**

The arterial blood gas alterations induced in these animals are given in Table 1 (means of the mean value for each animal). The effects of increasing $P_{CO_2}$ ($26.0 \pm 2.0$ to $71.2 \pm 2.2$ mmHg) and decreasing pH $(7.38 \pm 0.01$ to $7.03 \pm 0.01)$ on RBF and GFR at each level of renal perfusion pressure are shown in Figs. 3 and 4. In contrast to group 1 animals, a difference between innervated

![Fig. 3. Values for glomerular filtration rate in (a) denervated and (b) innervated kidneys at renal perfusion pressures of 125, 100 and 75 mmHg. ○, Values obtained during normocapnia; ●, values obtained during hypercapnic acidosis. * Significant ($P < 0.05$) difference between normocapnic and hypercapnic values.](image)

![Fig. 4. Values for renal blood flow in (a) denervated and (b) innervated kidneys at renal perfusion pressures of 125, 100 and 75 mmHg. ○, Values obtained during normocapnia; ●, values obtained during hypercapnia. * Significant ($P < 0.05$) difference between normocapnic and hypercapnic values.](image)
and denervated kidney autoregulatory responses was obtained. Hypercapnic acidosis resulted in significant decreases in both RBF and GFR at renal perfusion pressures of 100 and 75 mmHg in innervated kidneys, an effect not observed in paired denervated kidneys. Innervated and denervated kidney autoregulatory responses were comparable during normocapnia. Hypercapnia decreased p-aminohippurate extraction in both innervated (0.76 ± 0.04 to 0.70 ± 0.04, P < 0.02) and denervated (0.76 ± 0.04 to 0.69 ± 0.05, P < 0.02) kidneys.

Discussion

Previous laboratory studies have suggested that decreases in $P_{O2}$ and increases in $P_{CO2}$ impair basal renal haemodynamic function [1-10]. Moreover, in perfused organ systems, hypoxia and hypercapnia have been demonstrated to exert vasoactive influences in several vascular beds, including the kidney [13]. Thus, the present studies were undertaken to evaluate the effect of hypoxia and hypercapnic acidosis on RBF and GFR as renal perfusion pressure was decreased.

Lowering $P_{O2}$ from 82 to 43 mmHg did not result in significant impairment of either RBF or GFR autoregulation over renal perfusion pressures ranging from 125 to 75 mmHg. These results are compatible with earlier studies which examined the effect of hypoxia on autoregulation of the perfused dog kidney [13-15]. In these latter studies, RBF autoregulation of the perfused dog kidney remained intact despite perfusate $P_{O2}$ as low as 3-7 mmHg. It should be noted, however, that interruption of renal oxidative metabolism and induction of histotoxic hypoxia by large doses of intrarenal cyanide may interfere with RBF autoregulation [16]. Together the results of the present studies in vivo and previous perfused kidney studies suggest that RBF and GFR autoregulation occurs despite moderate to profound decreases in arterial or perfusate $P_{O2}$.

Studies have demonstrated that the degree of hypercapnic acidosis induced in the present study results in renal nerve mediated increases in renin secretion [17], renal vascular resistance [1, 2, 5] and tubular sodium reabsorption [18]. In the present study, hypercapnic acidosis impaired autoregulatory responses of both RBF and GFR and this impairment was confined to innervated kidneys. Thus increased renal nerve activity induced by hypercapnic acidosis appears to prevent the usual diminution in renal afferent arteriolar tone that occurs in response to lowering renal perfusion pressure.

Previous studies on the effect of renal nerves on renal autoregulation report conflicting results. It is known that autoregulation of RBF and GFR occurs in the denervated kidney perfused in vitro [14]. Moreover, Kiil et al. [19] found that electrical renal nerve stimulation sufficient to reduce basal RBF did not affect RBF autoregulation in four anaesthetized dogs and a preliminary report supports this observation [20]. In contrast, more recent studies by Kiil and collaborators [21] show that electrical nerve stimulation impairs RBF autoregulation in the dog and this impaired autoregulation can be restored by $\alpha$-but not $\beta$-adrenergic blockade. In studies by Folkow & Langston [22] induction of endogenous stimulation of renal nerves by increasing intracranial pressure abolished RBF autoregulation in the anaesthetized cat and RBF autoregulation could be normalized by renal denervation. Finally, preliminary studies by Kelleher et al. [23] found impaired RBF autoregulation 1 week after intrarenal administration of noradrenaline in the rat. This impaired RBF autoregulation could be normalized by renal denervation. Together, the results of the present and previous studies support a role for renal nerves to mediate an impaired renal autoregulatory response to lowering renal perfusion pressure in several settings.

Although the present studies suggest a role for renal nerves to impair autoregulation in the anaesthetized hypercapnic dog, the results do not exclude entirely a role for humoral factors, such as angiotensin II. Thus we have demonstrated that hypercapnia induces renin secretion [17] and that the combination of intact renal nerves and angiotensin II may be synergistic in lowering RBF and GFR in response to other systemic stimuli such as hypotensive haemorrhage [11, 24]. In addition, the present results do not clarify whether the increase in $P_{CO2}$ or the decrease in pH is responsible for the diminished renal autoregulation. This is of importance since lowering pH per se may affect adversely basal levels of RBF and GFR in the anaesthetized dog [3]. Finally, the extreme degree of hypercapnia induced in the present studies and the anaesthetized state of the animals may have contributed to the diminished autoregulation. Thus studies with lesser degrees of hypercapnia in conscious animals will be required to determine the physiological significance of the present observations.

In summary, the present studies indicate that autoregulation of RBF and GFR is not altered in the dog kidney in situ by induction of moderate hypoxia. In contrast, hypercapnic acidosis resulted in impaired RBF and GFR autoregulation. This impaired autoregulation could be abolished by
renal denervation. The present study suggests that renal nerve stimulation induced by hypercapnic acidosis prevents the usual diminution in renal afferent arteriolar tone that normally occurs in response to lowering renal perfusion pressure.

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References