EFFECT OF ACUTE HYPOCAPNIA ON SOME ASPECTS OF RENAL
FUNCTION IN ANAESTHETIZED DOGS

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SUMMARY

Hypocapnia was induced in dogs lightly anaesthetized with nitrous oxide and fentanyl. Measurements were made of estimated renal plasma flow (ERPF), glomerular filtration rate, vascular resistance and urine production. During the (short) duration of the experiments hypocapnia was found to be associated with ERPF twice that during normocapnia. Glomerular filtration rate and urine output were increased by hypocapnia in an approximately similar proportion, whilst renal vascular resistance halved. Though some of the experimental animals had a degree of metabolic acidosis this was thought not to have greatly influenced the results.

The most important physiological components of the renal vasculature, the afferent and efferent glomerular arterioles and vasa recta, have a muscular component in their walls with sympathetic, and probably also parasympathetic, innervation. Nearly all the renal blood flow passes through these vessels and their calibre, therefore, is a major determinant of renal blood flow (RBF). Moreover, the calibre of the afferent and the efferent arterioles, both in absolute terms and relative to one another, is an important determinant of the glomerular filtration rate (GFR).

In general terms, carbon dioxide has a direct effect on blood vessels, producing vasodilatation, and an indirect effect, mediated by the sympathetic nervous system, causing vaso-constriction. It would be expected, therefore, that changes in carbon dioxide tension would have a considerable effect on renal function. It is also clearly not possible to predict the effect of hypocapnia on such variables as RBF and GFR without experimental investigation.

Acute changes in \( P_{CO_2} \) are common in anaesthetic practice and a survey of the literature revealed no adequate indication of the effect of hypocapnia on renal function. In an attempt to answer this question a series of experiments was conducted on dogs. An acute change in \( P_{CO_2} \) was imposed on lightly anaesthetized animals during controlled ventilation of the lungs, whilst at the same time renal blood flow, glomerular filtration rate and renal vascular resistance were measured. The duration of each period of hypocapnia and normocapnia was 90 min.

METHODS

Fourteen experiments were conducted on five healthy mature Labrador dogs, weighing 14–25 kg. In half of the experiments \( P_{CO_2} \) was maintained initially at a normal value and hypocapnia induced later, whilst in the other half the order was reversed. RBF and GFR were determined throughout the course of the experiment.

Preparation before operation

The dogs were fed a normal, commercial diet, but were starved overnight and water was withheld on the days of the experiments. Exteriorization of the carotid artery was performed at least 3 weeks before the initial experiments to provide access for repeated arterial cannulation.

At least 3 weeks before each experiment, blood (500 ml) was removed and stored in acid–citrate–dextrose solution. This was used as an autotransfusion to replace blood lost by sampling.

Anaesthesia

No premedication was given. Anaesthesia was induced with thiopentone 250 mg i.v. and fentanyl 200 \( \mu \)g, preceded by atropine 0.6 \( \mu \)g; alcuronium 2.5 mg was administered to provide neuromuscular blockade. Following tracheal intubation, anaesthesia was maintained with a mixture of fentanyl at the rate of 0.4 \( \mu \)g min\(^{-1}\). Incremental doses of alcuronium 1.25–2.5 mg were given when indicated.

Intermittent positive pressure ventilation was effected with a Beaver ventilator (Mark II) attached...
to the expiratory limb of a Mapleson E type circuit via a Ruben non-return valve. The frequency of ventilation was 20 cycles min\(^{-1}\) and the tidal volume approximately 40 ml kg\(^{-1}\) body weight. End-expiratory carbon dioxide tension (\(P_{E'CO_2}\)) could be changed from normocapnia to hypocapnia or vice versa by increasing or decreasing the fresh gas flow supplied to the circuit, without changing appreciably the intra-thoracic pressure.

\(P_{E'CO_2}\) was monitored throughout the experiment using a Beckman (LB2) infra-red analyser which had been calibrated previously with two appropriate gas mixtures the composition of which had been determined with a Haldane apparatus: this measurement was used merely as an approximate and rapid guide to \(P_{aCO_2}\).

Throughout anaesthesia the dog lay on a warming blanket and was covered to minimize changes in body temperature: rectal temperature was monitored throughout.

Residual effects of alcuronium were antagonized at the end of anaesthesia with neostigmine 5 mg (given with atropine 1.2 mg). Following the return of spontaneous ventilation the endotracheal tube was removed and the animal was allowed to recover in a quiet environment.

**Further experimental preparation**

After induction of anaesthesia a cannula was inserted into the carotid loop to monitor arterial pressure and to facilitate blood sampling. A second cannula was introduced to the inferior vena cava for the preliminary infusion of hypotonic saline and for administering para-aminohippuric acid (PAH), inulin and fentanyl (see below). A third cannula was inserted into a peripheral vein for autotransfusion of previously stored blood in quantities equal to that of the blood lost during sampling.

The bladder was catheterized, emptied, and subsequently allowed to drain freely into a measuring cylinder; urinary volume was measured after manual expression of the bladder. To produce an adequate flow of urine for the determination of RBF and GFR (that is at least 0.5 ml min\(^{-1}\)) hypotonic saline (0.45%) 100 ml was administered over the hour following induction of anaesthesia.

The animal was considered to have attained a steady physiological state about 1 h after induction of anaesthesia: at this point the loading doses of PAH and inulin were commenced.

**Monitoring**

Arterial and venous pressures were measured using suitable transducers (Bell and Howell type 4/327/L221) and displayed on a Devices recorder (MX2R); the transducers were calibrated against columns of mercury and water respectively in the usual way.

Mean arterial pressure was taken as the sum of the diastolic pressure and one-third of the pulse pressure. This was considered to be equal to the pressure in the renal artery when determinations of renal vascular resistance were made. The mean pressure in the IVC was estimated from the pressure trace: this was assumed to be equal to the pressure in the renal vein.

**Determination of RBF and GFR**

The methods used were similar to those described by Smith (1956). The loading doses of PAH (10 mg kg\(^{-1}\)) and of inulin (60 mg kg\(^{-1}\)) were given over a 10-min period 1 h or more after anaesthesia had been induced: immediately before starting this infusion a sample of blood was withdrawn to provide "blank" values for calibration purposes. Thereafter, plasma concentrations of both substances were maintained as constant as possible using an infusion of 2 ml min\(^{-1}\) of a solution containing PAH (2.5 mg ml\(^{-1}\)) and inulin (5 mg ml\(^{-1}\)) in hypotonic saline (0.45%); to this infusion was added fentanyl 0.2 \(\mu\)g ml\(^{-1}\). A constant infusion pump was used for this purpose (Watson-Marlow, Mark 3).

Arterial blood samples (12 ml) were withdrawn for the estimation of PAH, inulin, haematocrit (Hct) and blood-gas estimation at intervals of 30 min during the experiment; there were thus three samples taken during the periods of normocapnia and three during hypocapnia.

Before blood samples were taken urine was collected for a period of 5 min; the rate of formation was noted and the sample was subsequently analysed for PAH and inulin concentration.

Estimated renal plasma flow (ERPF) was measured (ml min\(^{-1}\)) according to the clearance equation:

\[
C_{PAH} = ERPF = \frac{U_{PAH}}{P_{PAH}} \times V
\]

where \(C_{PAH}\) is the renal clearance of PAH, \(U_{PAH}\) and \(P_{PAH}\) are, respectively, the concentrations of PAH in urine and plasma and \(V\) is the volume of urine produced per minute.

The equation

\[
RBF = ERPF \times \frac{100}{100 - Hct}
\]
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gives a correct value for RBF only if the extraction of PAH (E_{PAH}) is 100%. The actual extraction was tested during hypocapnia on three occasions by placing a catheter in the renal vein and withdrawing samples from it. Subsequently the experimental animal was sacrificed.

Renal vascular resistance (RVR) was derived from:

\[ RVR = \frac{\text{mean arterial pressure} - \text{mean pressure IVC}}{\text{RBF}} \]

This was expressed per 100 g kidney weight, the weight of the kidney being taken as 0.03% of total body weight.

GFR was determined in a similar way using the inulin clearance equation:

\[ \text{GFR} = C_\text{IN} = \frac{U_\text{IN}}{P_\text{IN}} \times V \]

Where \( C_\text{IN} \) is the renal clearance of inulin, \( U_\text{IN} \) and \( P_\text{IN} \) are respectively the concentrations of inulin in urine and plasma and \( V \) is the volume of urine produced per minute.

**Biochemical estimations**

PAH was determined in plasma and urine by the method of Dick and Davies (1949) and inulin by the method of Bacon and Bell (1947) using a double-beam spectrophotometer (Shimadsu UV-140).

Most of the plasma concentrations of PAH were 3–5 mg dl\(^{-1}\) and of inulin 20–50 mg dl\(^{-1}\); most of the urine concentrations (after dilution to 1/100) were in the same range. Recovery experiments were performed between the ranges of PAH 0.3–9 mg dl\(^{-1}\) (86 individual recoveries) and inulin 3.3–90 mg dl\(^{-1}\) (98 individual recoveries). In these recovery experiments the standard deviation of the PAH measurements was \( \pm 0.08 \) mg dl\(^{-1}\) and inulin \( \pm 1.04 \) mg dl\(^{-1}\).

\( P_{\text{CO}_2} \), \( pH \) and \( P_{O_2} \) were determined using appropriate electrodes (IL LH) and blood-gas analyser (IL 213–227).

**RESULTS**

**Acid-base state**

Mean \( P_{\text{CO}_2} \) during "normocapnia" was 5.4 kPa (range 4.1–6.4 kPa); during hypocapnia the mean was 2.4 kPa (range 1.4–3.4 kPa).

Mean arterial pH during normocapnia was 7.28 (range 7.13–7.49) and during hypocapnia 7.46 (range 7.30–7.63). Using values for \( pK' \) and solubility constant for carbon dioxide obtained from human blood and corrected for pH and temperature (Severinghaus, 1965) the mean actual bicarbonate concentration in hypocapnia was 13.5 mmol litre\(^{-1}\) and during normocapnia 19 mmol litre\(^{-1}\) (\( P = 0.000043; \) two-sided probability). Thus some of the experimental animals had a metabolic acidosis before the experimental period commenced and this became accentuated during hypocapnia (see below). It should be noted that all the samples were obtained after induction of anaesthesia.

**Renal function**

In 13 of the 14 experiments an increase occurred in the ERPF and GFR during hypocapnia, associated with a reduction in RVR (figs 1 and 2, table I). ERPF, GFR and \( V \) increased approximately two-fold in hypocapnia, while renal vascular resistance decreased by approximately half.

The differences between ERPF, GFR and \( V \) in hypocapnia and normocapnia were statistically significant (table II) (paired \( t \) test).

Applying the Sign test to the values for ERPF, GFR and RVR, it was found that the effect produced
by a change in $P_{a\text{CO}_2}$ in any one dog was of greater significance than any individual variation in the values between dogs (table II).

In the three experiments in which $E_{PAH}$ was tested during hypocapnia the value obtained was at least 90%. The mean arterial pressures during the phases of the study are shown in table III.

**DISCUSSION**

There have been many studies on the effects of changes in $PCO_2$ on the renal aspects of acid-base state. Usually these studies have been on relatively long-term changes (Gennari, Goldstein and Schwartz, 1972), but some have been short-term (Simmons and Olver, 1965). There was a relatively recent study of the effect of hypercapnia on renal blood flow and other variables in the dog (Norman et al., 1970). There has, however, been no satisfactory study on the effects of low $PCO_2$ on renal blood flow and glomerular filtration rate.

Hypocapnia is induced frequently during anaesthesia and is common in patients undergoing artificial pulmonary ventilation in the intensive therapy unit. Thus, in two situations in which renal function

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**TABLE I.** Mean values for estimated renal plasma flow (ERPF), glomerular filtration rate (GFR), renal vascular resistance (RVR) and urine volume ($V$) for dogs during normocapnia and hypocapnia. Each mean is of three values in each of the 14 experiments. Note that hypocapnia preceded normocapnia in half the experiments. Units of renal vascular resistance are mm Hg per ml per min per 100 g kidney weight.

<table>
<thead>
<tr>
<th></th>
<th>ERPF ($ml \text{ min}^{-1} \text{ kg}^{-1}$)</th>
<th>GFR ($ml \text{ min}^{-1} \text{ kg}^{-1}$)</th>
<th>RVR (mm Hg $ml^{-1} \text{ min}^{-1}$ per 100 g kidney weight)</th>
<th>$V$ ($ml \text{ min}^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normocapnia ($P_{a\text{CO}_2}$ 5-5.5 kPa)</td>
<td>15.5</td>
<td>5.8</td>
<td>0.39</td>
<td>1.4</td>
</tr>
<tr>
<td></td>
<td>SEM 2.4</td>
<td>SD 9.2</td>
<td>$t = 3.841$</td>
<td>$P = 0.0022^{**}$</td>
</tr>
<tr>
<td></td>
<td>SD 9.2</td>
<td>SD 4.2</td>
<td>$t = 3.926$</td>
<td>$P = 0.0017^{**}$</td>
</tr>
<tr>
<td>Hypocapnia ($P_{a\text{CO}_2}$ 2-2.5 kPa)</td>
<td>36.7</td>
<td>14.5</td>
<td>0.17</td>
<td>3.2</td>
</tr>
<tr>
<td></td>
<td>SEM 8.0</td>
<td>SD 29.7</td>
<td>$t = 5.528$</td>
<td>$P = 0.0001^{***}$</td>
</tr>
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**TABLE II.** Significance values obtained when the paired $t$ test and Sign test were applied to the values for ERPF, GFR and RVR during hypocapnia and normocapnia. $t = \text{ratio of the mean of the sample to the standard error of the sample}$

|                | ERPF ($ml \text{ min}^{-1} \text{ kg}^{-1}$) | GFR ($ml \text{ min}^{-1} \text{ kg}^{-1}$) | RVR (mm Hg $ml^{-1} \text{ min}^{-1}$ per 100 g kidney weight) |
|----------------|------------------------------------------|------------------------------------------|----------------------------------------------------------------|-----------------|
| Paired $t$ test | $P = 0.0022^{**}$                       | $P = 0.0017^{**}$                       | $P = 0.0001^{***}$                                               |
|                | ($t = 3.841$)                            | ($t = 3.926$)                            | ($t = 5.528$)                                                    |
| Sign test      | $P = 0.013^{*}$                         | $P = 0.057$                              | $P = 0.00024^{***}$                                             |
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Table III. Values for mean arterial pressure (mm Hg) in each of the 14 experiments. The values given were obtained over a 2-min period around the time of blood sampling for PAH and inulin. In experiments 1–7 the change imposed was from hypocapnia to normocapnia and in 8–14 from normocapnia to hypocapnia

<table>
<thead>
<tr>
<th>Hypocapnia→normocapnia</th>
<th>Normocapnia→hypocapnia</th>
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<tbody>
<tr>
<td>1</td>
<td>8</td>
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<tr>
<td>2</td>
<td>9</td>
</tr>
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<td>3</td>
<td>10</td>
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<td>7</td>
<td>14</td>
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<table>
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<tr>
<th>Hypocapnia</th>
<th>138</th>
<th>144</th>
<th>110</th>
<th>155</th>
<th>82</th>
<th>115</th>
<th>103</th>
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<tr>
<td>Normocapnia</td>
<td>128</td>
<td>143</td>
<td>103</td>
<td>150</td>
<td>72</td>
<td>115</td>
<td>112</td>
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<td></td>
<td>131</td>
<td>150</td>
<td>109</td>
<td>161</td>
<td>73</td>
<td>118</td>
<td>100</td>
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<table>
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<th>Hypocapnia</th>
<th>138</th>
<th>154</th>
<th>110</th>
<th>173</th>
<th>68</th>
<th>114</th>
<th>123</th>
</tr>
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<tbody>
<tr>
<td>Normocapnia</td>
<td>147</td>
<td>165</td>
<td>102</td>
<td>179</td>
<td>79</td>
<td>130</td>
<td>124</td>
</tr>
<tr>
<td></td>
<td>152</td>
<td>166</td>
<td>97</td>
<td>163</td>
<td>79</td>
<td>118</td>
<td>108</td>
</tr>
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</table>

may be important, hypocapnia may occur and yet little is known about its effect on renal physiology.

In this study no observations were made for more than 1.5 h after induction of anaesthesia to ensure that, as far as possible, there was a physiological steady state. Fentanyl was chosen as a supplement to nitrous oxide in maintaining anaesthesia because there is some evidence that in humans (when given together with droperidol) it produces less change in renal blood flow than inhalation agents (Gorman and Craythorne, 1966) which may cause a profound decrease in RBF and GFR (Bastrun and Deutsch, 1976). The technique used here appears to have less effect on ERPF and GFR than some methods of anaesthesia as shown by comparing the results in normocapnia with those of Houck (1948) in 75 conscious dogs. It may be seen (table IV) that the results are comparable, although there was a greater variation in the present study. The mean filtration fraction found in our study is within the normal range for dogs.

A technique of artificial pulmonary ventilation was chosen to maintain intrathoracic pressure more or less constant during normocapnia and hypocapnia. This is important because it has been found that an increase in intrathoracic pressure causes increased secretion of urine (Currie and Ullman, 1961).

Renal plasma flow may be estimated accurately using PAH only if $E_{PAH}$ is 100%. Unfortunately, it was not found possible to estimate $E_{PAH}$ in these experiments: three extraction ratios only were determined in hypocapnia; these were over 90%—that is, within the ratio regarded as normal in unanaesthetized dogs (Smith, 1951). It is known that at high renal blood flows, plasma extraction is decreased (Earley and Friedley, 1965) hence giving smaller plasma values and thus overestimating plasma clearance. These workers' observations, however, were made when saline loading was 50 ml min$^{-1}$; in the experiments reported here only 2 ml min$^{-1}$ was administered (together with blood replacement).

The initial acid–base status of the experimental animals requires comment. Normal acid–base data for unanaesthetized dogs have been summarized (Altman and Dittman, 1964): the normal range of

Table IV. A comparison between the mean, the SEM and SD of those values obtained for ERPF and GFR in the normocapnic anaesthetized dogs and those quoted for conscious dogs (Houck, 1948)

<table>
<thead>
<tr>
<th></th>
<th>ERPF (ml min$^{-1}$)</th>
<th>GFR (ml min$^{-1}$)</th>
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<tbody>
<tr>
<td></td>
<td>Conscious dogs</td>
<td>Anaesthetized normocapnic dogs</td>
</tr>
<tr>
<td>Mean</td>
<td>13.5</td>
<td>15.5</td>
</tr>
<tr>
<td>SEM</td>
<td>0.375</td>
<td>2.4</td>
</tr>
<tr>
<td>SD</td>
<td>3.26</td>
<td>9.2</td>
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</table>
arterial blood pH for dogs has been described as 7.31–7.42 with $P_{CO_2}$ 5.1 kPa (38 mm Hg). Using generally accepted values for $pK'$ and for the solubility constant of carbon dioxide dissolved in human blood (see above) the actual bicarbonate concentration derived from these data would be expected to be between 19 mmol litre$^{-1}$ and 24 mmol litre$^{-1}$. The present results indicate that some of the experimental animals had a degree of metabolic acidosis initially. This is surprising since, although the dogs were kept in cages with a normal diet, they seemed well and gained weight during the duration of the experiments. The cause of this metabolic acidosis is being investigated. However, it may be that the acidosis occurred only during anaesthesia, although samples were not taken until after the animals had been anaesthetized for some time.

The metabolic acidosis does not seem to have influenced the findings. Changes in RBF, GFR and renal vascular resistance occurred in animals with or without a metabolic acidosis. Nevertheless, the existence of the acidosis in some of these experiments must be kept in mind when interpreting the results.

There is some doubt as to whether a small degree of metabolic acidosis develops during hypocapnia in the human (Utting, 1970). However, in the (extreme) degree of hypocapnia used in the dogs, the evidence is unequivocal. There was a smaller bicarbonate concentration during hypocapnia than during normocapnia.

There is evidence that the decrease in RBF which occurs during anaesthesia depends on the renin–angiotensin mechanism and that the decrease does not occur in dogs which have a large sodium intake or in which the renin–angiotensin system is blocked (Fray et al., 1976). The sodium balance of the experimental animals is, therefore, important. The fact that the sodium intake of the experimental animals was kept on a normal commercial diet would make it unlikely that there was any gross degree of electrolyte imbalance.

The duration of changes noted in this study is not known, since the experiments were relatively short. Preliminary results from current studies suggest that the changes observed here do not depend on the anaesthetic techniques used and occur during light anaesthesia with halothane.

It must be remembered that the studies were performed in animals with moderate diuresis. It is possible that the results may differ in animals with a different rate of urine production.
EFFET DE L'HYPOCAPNIE AIGUE SUR CERTAINS ASPECTS DE LA FONCTION RENALE DE CHIENS ANESTHESIES

RESUME

On a provoqué l'hypocapnie sur des chiens légèrement anesthésiés à l'aide de protoxyde d'azote et de fentanyl. On a pris des mesures du débit estimé de plasma rénal (ERPF), du taux de filtration glomérulaire, de la résistance vasculaire et de la production d'urine. Pendant la (courte) durée de ces expériences on a trouvé que l'on pouvait associer l'hypocapnie à la ERPF laquelle était deux fois plus forte que celle qui se produit pendant la normocapnie, le taux de filtration glomérulaire et la production d'urine se trouvant augmentés par l'hypocapnie dans des proportions à peu près similaires, alors que la résistance vasculaire était réduite de moitié. Bien que certains des animaux servant aux expériences aient eu un certain degré d'acidose métabolique on ne pense pas que cela ait pu grandement influencer les résultats.

DIE WIRKUNG EINES AKUTEN KOHLENSAUREMANGELS IM BLUT AUF VERSCHIEDENE SEITEN DER NIERENFUNKTION IN NARKOTISIERTEN HUNDEN

ZUSAMMENFASSUNG


EFECTO DE LA HIPOCAPNIA AGUDA EN CIERTOS ASPECTOS DE LA FUNCION RENAL EN PERROS ANESTESIADOS

SUMARIO

Se indujo la hipocapnia en perros ligeramente anestesiados mediante fentanilo y óxido nitroso. Se llevaron a cabo mediciones del flujo plasmático renal estimado (ERPF), del ritmo de filtración glomerular, de la resistencia vascular y de la producción de orina. Durante el (corto) período de los experimentos, se comprobó que la hipocapnia se asociaba con un ERPF doble del registrado durante la normocapnia. El ritmo de filtración glomerular y la producción de orina aumentaban por causa de la hipocapnia en una proporción aproximadamente similar, mientras que la resistencia vascular renal se reducía por mitad. Aunque algunos de los animales experimentales presentaran un grado de acidosis metabólica, se estimó que ello no influenciaba mucho los resultados.