Cerebral Oxygen Consumption and Blood Flow in Hypoxia: Influence of Sympathoadrenal Activation

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SUMMARY The effect of hypoxia (reduction of arterial Po$_2$ to 26-28 mm Hg) on cerebral blood flow (CBF) and cerebral oxygen consumption (CMRO$_2$) was studied in paralyzed and artificially ventilated rats, using a CBF technique of improved accuracy at high flow rates. Results obtained on animals maintained on 70% N$_2$O unexpectedly showed that hypoxia of this severity is accompanied by an increase in CMRO$_2$, and they indicated that 2 different mechanisms are involved, both related to catecholamine metabolism. In one breed of Wistar rats studied, hypoxia was accompanied by a 6-fold increase in CBF and by an increase in CMRO$_2$ to 180% of control. Prior removal of the adrenal glands curtailed the increase in CBF (400% of control) and CMRO$_2$ (125% of control). The excessive increase in CMRO$_2$ (to 180% of control) did not occur in another breed of Wistar rats. However, since infusion of adrenaline in normoxic animals gave rise to a doubling of CMRO$_2$ it is concluded that, at least under some circumstances, circulating catecholamines can increase oxygen consumption in the hypoxic brain. In the second breed of rats studied, hypoxia was consistently accompanied by a 20-30% increase in CMRO$_2$ which was unaffected by prior adrenalectomy. Since the increase was prevented by sedative and anesthetic doses of diazepam, it is tentatively concluded that the increase was elicited by increased activity in cerebral catecholaminergic pathways. The conclusion is supported by parallel studies showing that a similar increase in CMRO$_2$ occurs in hypercapnia, which is blocked both by diazepam and propranolol.

Methods

All experiments were performed on male S.P.F. Wistar rats (290-375 g). During the course of the study, the supplier (Møllegaard, Copenhagen) started breeding a new colony of Wistar rats. Since the results obtained on these breeds differed, they will be described separately. Rats from the original breed constitute series A, from the second, series B and C.

Operative, Anesthetic and Sampling Techniques

Series A. Anesthesia was induced with halothane (2-3%). After they became unresponsive, the animals were tracheotomized and connected to a respirator...
delivering 70% N₂O and 30% O₂. Both femoral arteries were cannulated for blood pressure recording and anaerobic sampling of blood, and one femoral vein for slow infusion of donor blood during CBF measurement. In one group, the adrenal glands were removed by a dorsal approach. The posterior part of the superior sagittal sinus was exposed by a small burr hole for sampling of cerebral venous blood. Body temperature was maintained close to 37°C, and ventilation was adjusted to give an arterial Pco₂ of 35-40 mm Hg.

Series B. Procedures were similar to those in series A except that in 3 of 4 groups 0.7-0.8% halothane was delivered during the operative procedures. When these were completed, halothane supply was discontinued and the animals were ventilated on 70% N₂O. In one group, the adrenal glands were removed. In another, the animals were sham-operated (with gentle handling of the adrenal glands).

Series C. Initial anesthetic and operative procedures were the same as in series A. Following completion of operative procedures, rats were either infused with 2.25 mg-kg⁻¹ of diazepam during 1 min, followed by a constant infusion of 5 mg-kg⁻¹-hr⁻¹, or with 7.5 mg-kg⁻¹, followed by 15 mg-kg⁻¹-hr⁻¹. At the end of the initial infusion period (1 min) the nitrous oxide supply was discontinued and the animals were ventilated with 30% O₂ and 70% N₂.

Induction of Hypoxia

Irrespective of the previous handling of the animals and the anesthesia used, the animals were allowed a 15-20 min steady state period before hypoxia was induced. In all animals, the inspired oxygen concentration was then lowered to give an arterial Po₂ of 26-28 mm Hg. In animals maintained on 70% N₂O, oxygen was replaced by nitrogen gas to maintain N₂O concentration constant. In animals given diazepam, O₂ was replaced by N₂. In all animals, a small amount of carbon dioxide was added to the insufflated gas mixture at the time of induction of hypoxia to prevent a marked fall in arterial Pco₂. In each animal included, Pao₂ remained constant between 25 and 30 mm Hg during the last 25 of the 30 min hypoxic period.

Measurement of CBF and CMRO₂

Cerebral (cortical) blood flow and oxygen consumption were estimated by a modification of the Kety and Schmidt technique, using ¹³³Xenon and measurements of arterial and cerebral venous activities during the desaturation phase. In each animal, the saturation period was 15-20 min. In order to allow more accurate determinations at the high flow rates encountered in hypoxia, the procedures were slightly modified. Previously, a 5 sec period was allowed to pass, following discontinuation of ¹³³Xenon supply, before the first arterial and cerebral venous samples were collected. Presently, this 5 sec lag was omitted and, following the disconnection of the ¹³³Xenon-containing bag from the respirator, 2 operators sampled arterial and cerebral venous blood in rapid succession while a third recorded the time of each sample. In this way, the arterial and cerebral venous desaturation curves could be accurately resolved (see below).

As previously, CBF was calculated by the trapezoid rule, using a ¹³³Xenon partition coefficient of 0.83. CMRO₂ was calculated by multiplying CBF with the arteriovenous difference in oxygen content (AVDO₂). The latter was determined at least twice. If AVDO₂ differed by more than 10% between two consecutive samples, the experiment was discarded.

Analytical Techniques

Arterial Po₂, Pco₂, and pH were measured using microelectrodes, with due correction for any deviation in body temperature from 37°C. Blood oxygen content (CO₂) was measured in 25 μl samples using a polarographic technique. ¹³³Xenon was measured as previously described.

Statistics

Since only one CBF measurement was performed in each animal, statistical differences between control and hypoxic rats were calculated using the unpaired Student’s t-test.

Results

As figure 1 shows, the modifications allowed accurate assessment of the arterial and cerebral venous desaturation curves even when CBF exceeded 5 ml-g⁻¹-min⁻¹. With the new procedure, control values for CBF (and CMRO₂) were slightly lower than those previously published. In 9 control experiments CBF was calculated both with the new procedure and with that previously used. In the latter calculation, samples drawn during the first 5 sec of desaturation were disregarded. With the present procedure, calculated CBF was 1.12 ± 0.13 and with that previously used 1.20 ± 0.15 ml-g⁻¹-min⁻¹ (means ± SEM). The results indicate that our previous procedure for calculating CBF slightly overestimates CBF (the mean difference was 6% with a SEM of 1%), probably because the area between the arterial and cerebral venous curves during the initial desaturation curve is not accurately defined unless the 5 sec lag is omitted.

Results obtained in control and hypoxic groups are given in tables 1 and 2. Animals in series A and B were maintained on 70% N₂O, while those belonging to series C were given sedative or anesthetic doses of diazepam. Series A and B differ in that animals of series A were obtained from the original breed of Wistar rats, those of series B from the new breed. Of the nitrous oxide controls, 6 of 15 were studied before the CBF technique was modified and the values were therefore corrected, using the 6% figure described above. The results obtained on hypoxic, diazepam-injected animals were compared to previous control material, corrected for a 6% overestimation of CBF.

Table 1 shows physiological variables measured in
Area 85.2
CBF 0.97 ml·g⁻¹·min⁻¹
AVDO₂ 4.03-4.06 µmol·g⁻¹·min⁻¹
CMRO₂ 3.92 µmol·g⁻¹·min⁻¹

FIGURE 1. Modifications permitted assessment of desaturation curves even when CBF (and CMRO₂) were markedly increased.

FIGURE 2. Area 12.7
CBF 6.54 ml·g⁻¹·min⁻¹
AVDO₂ 1.14-1.04 µmol·g⁻¹·min⁻¹
CMRO₂ 7.13 µmol·g⁻¹·min⁻¹

control and hypoxic animals. In all hypoxic groups, mean PaO₂ was reduced to between 25.7 and 28.2 mm Hg, demonstrating that the hypoxic insults were of similar severity. Body temperature was within 1°C of control and PacO₂ was sufficiently similar to exclude any significant effect on variables measured. Mean arterial blood pressure fell during hypoxia, and there was a relatively marked plasma acidosis. After 5 min of hypoxia, changes in blood pressure and arterial pH were less marked.

Values for CO₂, AVDO₂, CBF and CMRO₂ are given in table 2. In all animals exposed to hypoxia of 30 min duration, arterial oxygen content was reduced to about 25% of control. After 5 min of hypoxia the reduction was somewhat less pronounced, probably reflecting the absence of a marked fall in plasma pH (cf. table 1). In all groups, AVDO₂ was markedly reduced.

The hypoxic groups of series A were studied consecutively. When animals were rendered hypoxic for 30 min CBF increased 6-fold and there was an unexpected increase in CMRO₂ to 180% of control. Prior removal of the adrenal glands considerably curtailed, but did not completely prevent, the increase in CMRO₂. Additional experiments showed that, in non-adrenalectomized animals, the excessive increase in CMRO₂ occurred after 5 min of hypoxia.

When the experiments were repeated on animals of the new breed (Series B), CMRO₂ only increased to about 125% of control. In order to study whether or not this increase was due to circulating catecholamines, 2 more groups were included, 1 in which animals were adrenalectomized and another in which a sham operation was performed. In both groups, 0.7-0.8% halothane was given during the operative procedures to minimize pain and pressor effects. The results confirmed that hypoxia is accompanied by a 20-30% increase in CMRO₂ and showed that this increase was not prevented by prior removal of the adrenal glands.

The results obtained indicated that circulating catecholamines could have been responsible for the ex-
cessive increase in CMRO₂ observed in series A but that the moderate increase observed in series B was due to other factors. Since it could be suspected that an increased activity of intrinsic catecholaminergic pathways was responsible, attempts were made to pretreat animals with a β-adrenoceptor blocker (propranolol). This invariably failed since reduction of arterial Po₂, following administration of propranolol, resulted in cardiovascular failure. Animals were therefore pretreated with diazepam, a drug that has

### Table 1

<table>
<thead>
<tr>
<th>Experimental series</th>
<th>Experimental group</th>
<th>Body temp (°C)</th>
<th>MABP (mm Hg)</th>
<th>Paco₂ (mm Hg)</th>
<th>Paco₂ (mm Hg)</th>
<th>pH</th>
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</thead>
<tbody>
<tr>
<td>Control (n = 15)</td>
<td>37.0</td>
<td>142</td>
<td>125</td>
<td>38.8</td>
<td>7.375</td>
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<tr>
<td>Hypoxia 30 min (n = 8)</td>
<td>36.7</td>
<td>120***</td>
<td>27.7***</td>
<td>36.1***</td>
<td>7.113***</td>
<td></td>
</tr>
<tr>
<td>Adrenalect. (n = 6)</td>
<td>36.2**</td>
<td>115***</td>
<td>27.6***</td>
<td>35.3***</td>
<td>7.072***</td>
<td></td>
</tr>
<tr>
<td>Hypoxia 5 min (n = 8)</td>
<td>36.8</td>
<td>131*</td>
<td>27.3***</td>
<td>35.0**</td>
<td>7.321**</td>
<td></td>
</tr>
<tr>
<td>Series B (70% N₂O)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypoxia 30 min (n = 8)</td>
<td>36.4***</td>
<td>109***</td>
<td>26.1***</td>
<td>37.8</td>
<td>7.222***</td>
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<tr>
<td>Shamop. (n = 6)</td>
<td>36.7</td>
<td>112***</td>
<td>25.7***</td>
<td>32.4***</td>
<td>7.155***</td>
<td></td>
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<tr>
<td>Hypoxia 30 min</td>
<td>36.9</td>
<td>104***</td>
<td>28.2***</td>
<td>33.9***</td>
<td>7.129***</td>
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<tr>
<td>Adrenalect. (n = 7)</td>
<td>0.1</td>
<td>±0.2</td>
<td>±1.0</td>
<td>±1.1</td>
<td>±0.020</td>
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<tr>
<td>Series C (diazepam)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (n = 12)</td>
<td>36.8</td>
<td>145</td>
<td>128</td>
<td>37.3</td>
<td>7.381</td>
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</tr>
<tr>
<td>Hypoxia 30 min (n = 4)</td>
<td>36.7</td>
<td>103**</td>
<td>25.7***</td>
<td>34.4</td>
<td>7.188***</td>
<td></td>
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<tr>
<td>Hypoxia 30 min</td>
<td>36.6</td>
<td>101***</td>
<td>25.7***</td>
<td>35.3</td>
<td>7.187***</td>
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<tr>
<td>7.5 mg·kg⁻¹ (n = 7)</td>
<td>0.2</td>
<td>±0.7</td>
<td>±0.6</td>
<td>±0.7</td>
<td>±0.010</td>
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</table>

Animals given diazepam are ventilated with 70% N₂ in oxygen. Two experimental groups are adrenalectomized, one is sham-operated. n is number of animals in each group. *p < 0.05, **p < 0.01, ***p < 0.001.

### Table 2

<table>
<thead>
<tr>
<th>Experimental series</th>
<th>Experimental group</th>
<th>CaO₂ (µmol·ml⁻¹)</th>
<th>AVDO₂ (µmol·ml⁻¹)</th>
<th>CBF (ml·g⁻¹·min⁻¹)</th>
<th>CMRO₂ 37°C (µmol·g⁻¹·min⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n = 15)</td>
<td>9.83</td>
<td>3.76</td>
<td>1.11</td>
<td>4.00</td>
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<tr>
<td>Hypoxia 30 min (n = 8)</td>
<td>2.14***</td>
<td>1.18***</td>
<td>6.52***</td>
<td>7.72***</td>
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</tr>
<tr>
<td>Adrenalect. (n = 6)</td>
<td>2.17***</td>
<td>1.16***</td>
<td>4.14***</td>
<td>4.91*</td>
<td></td>
</tr>
<tr>
<td>Hypoxia 5 min (n = 8)</td>
<td>2.99***</td>
<td>1.17***</td>
<td>6.30***</td>
<td>7.80***</td>
<td></td>
</tr>
<tr>
<td>Series B (70% N₂O)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypoxia 30 min (n = 8)</td>
<td>2.17***</td>
<td>0.97***</td>
<td>5.26***</td>
<td>5.20***</td>
<td></td>
</tr>
<tr>
<td>Shamop. (n = 6)</td>
<td>2.06***</td>
<td>1.12***</td>
<td>4.38***</td>
<td>4.85*</td>
<td></td>
</tr>
<tr>
<td>Hypoxia 30 min</td>
<td>2.30***</td>
<td>1.20***</td>
<td>4.23***</td>
<td>4.80**</td>
<td></td>
</tr>
<tr>
<td>Adrenalect. (n = 7)</td>
<td>0.17</td>
<td>±0.11</td>
<td>±0.57</td>
<td>±0.67</td>
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<tr>
<td>Series C (diazepam)</td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>Control (n = 12)</td>
<td>10.37</td>
<td>5.70</td>
<td>0.74</td>
<td>4.18</td>
<td></td>
</tr>
<tr>
<td>Hypoxia 30 min (n = 4)</td>
<td>2.13***</td>
<td>1.36***</td>
<td>2.98***</td>
<td>4.08</td>
<td></td>
</tr>
<tr>
<td>2.25 mg·kg⁻¹ (n = 4)</td>
<td>0.29</td>
<td>±0.09</td>
<td>±0.16</td>
<td>±0.08</td>
<td></td>
</tr>
<tr>
<td>Hypoxia 30 min</td>
<td>2.13***</td>
<td>1.45***</td>
<td>2.91***</td>
<td>4.03</td>
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</tr>
<tr>
<td>7.5 mg·kg⁻¹ (n = 7)</td>
<td>0.15</td>
<td>±0.14</td>
<td>±0.44</td>
<td>±0.14</td>
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</tbody>
</table>

Animals given diazepam are ventilated with 70% N₂ in oxygen. Two experimental groups were adrenalectomized, one is sham-operated. n is number of animals in each group. *p < 0.05, **p < 0.01, ***p < 0.001.
been assumed to block a stress-induced increase in cerebral noradrenergic neurons (see Discussion). As the results show (series C), diazepam completely prevented the increase in CMRO\textsubscript{2} during hypoxia. Furthermore, although CBF increased about 4-fold, the absolute CBF values were less than those observed in nitrous oxide-anesthetized animals.

**Discussion**

Current information suggests that although a reduction in arterial PO\textsubscript{2} to below 50 mm Hg leads to an increase in CBF, there is no change in CMRO\textsubscript{2} unless PO\textsubscript{2} falls to excessively low values. This information is partly based on studies in man, in whom the PO\textsubscript{2} was reduced to 35-40 mm Hg, either at decreased or normal CO\textsubscript{2} tensions.\textsuperscript{9,10} Similar results have been reported for dogs.\textsuperscript{11} In the rat, more severe degrees of hypoxia have been studied.\textsuperscript{12} The results showed that reduction of PO\textsubscript{2} to 25 mm Hg, or lower (leading to a fall in arterial oxygen content to less than 25\% of control), induced a 4- to 5-fold increase in CBF at an unchanged CMRO\textsubscript{2}.

Since the present results demonstrate that CMRO\textsubscript{2} may in fact increase during hypoxia they differ from those previously reported. A direct comparison to results obtained in man is difficult since these pertain to less severe degrees of hypoxia. For example, in the study of Cohen et al.,\textsuperscript{10} CaO\textsubscript{2} was reduced to about 70\% of control, and no plasma acidosis developed. This may be of crucial importance since acidosis is known to release catecholamines from the adrenal glands.\textsuperscript{14,15} Probably, failure to observe an increase in CMRO\textsubscript{2} in our previous study on rats\textsuperscript{13} was due to difficulties of accurately resolving arterial and cerebral venous 133Xenon desaturation curves at the high flow rates encountered in hypoxia.

In order to facilitate discussion of the present results, values for CMRO\textsubscript{2} and CBF in series A, B and C will be considered together with recent data obtained in normoxic animals infused with adrenaline. The results indicate that, at least under some circumstances (series A), CMRO\textsubscript{2} may increase substantially by mechanisms that are related to circulating catecholamines. Although direct proof is lacking, several findings provide circumstantial evidence. First, the increase in CMRO\textsubscript{2} was curtailed by prior removal of the adrenal glands. Second, it is now known that if catecholamines can penetrate the blood-brain barrier they provoke increases in CMRO\textsubscript{2} and CBF.\textsuperscript{16,17} Third, recent experiments have shown that i.v. infusion of adrenaline in a dose of 8 \( \mu \text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1} \) is accompanied by a doubling of CMRO\textsubscript{2} in rats obtained from the same breed as those constituting series B.\textsuperscript{18} Clearly, the effects of adrenaline infusion on CMRO\textsubscript{2} is sufficiently pronounced to explain the excessive rise in CMRO\textsubscript{2} during hypoxia. However, some of the increase in CMRO\textsubscript{2} during hypoxia must have been due to other mechanisms since it persisted following removal of the adrenal glands.

On the basis of the results quoted we tentatively conclude that the excessive increase in CMRO\textsubscript{2} in series A was due to circulatory catecholamines. Obviously, animals of the new breed (series B) could have reacted differently either because less catecholamines were released or because penetration of amines across the blood-brain barrier was different. The results indicate that another mechanism must have been responsible for increasing CMRO\textsubscript{2} by 20–30\% in rats belonging to series B (and in the adrenalectomized animals of series A).

In view of the fact that a variety of stressful situations are associated with signs of an increased activity of cerebral catecholaminergic neurons\textsuperscript{19-22} and that benzodiazepines can block the increase in noradrenaline turnover\textsuperscript{23-25} the animals of series C were given either sedative or anesthetic doses of diazepam before hypoxia was induced. The results show that CMRO\textsubscript{2} did not increase in diazepam-injected animals. These results indicate that the rise of CMRO\textsubscript{2} in nitrous oxide-anesthetized animals is associated with increased activity in cerebral catecholaminergic neurons. At first sight, this conclusion is at variance with results showing that hypoxia leads to a reduction in the hydroxylation of tyrosine, the rate-limiting step in catecholamine synthesis.\textsuperscript{26} However, later results have shown that such a reduction does not occur in paralysed and artificially ventilated animals, possibly due to a stress-induced change in the K\textsubscript{m} value for oxygen of tyrosine hydroxylase.\textsuperscript{27}

In view of the complex effects of diazepam, the results may not provide strong evidence that increased activity in cerebral catecholaminergic neurons was responsible for the 20–30\% rise in CMRO\textsubscript{2}. However, results obtained in hypercapnia provide strong indirect evidence. Thus, when P\textsubscript{ACO\textsubscript{2}} is elevated to about 80 mm Hg CMRO\textsubscript{2} is increased by 20–30\%. This increase persists following removal of the adrenal glands, and following reduction in cerebral venous PO\textsubscript{2} to normal values, but is prevented by pretreatment of animals with either propranolol or diazepam.\textsuperscript{28} Furthermore, it has been shown that even at normal tissue-PO\textsubscript{2} values, hypercapnia is accompanied by increased hydroxylation of tyrosine.\textsuperscript{29} These results indicate that the mechanisms mediating an increase in CMRO\textsubscript{2} may be similar in hypoxia and hypercapnia, possibly involving an effect of tissue acidosis on noradrenaline turnover.

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