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

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SUMMARY The effect of hypoxia (reduction of arterial Po2 to 26-28 mm Hg) on cerebral blood flow (CBF) and cerebral oxygen consumption (CMRO2) 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% N2O unexpectedly showed that hypoxia of this severity is accompanied by an increase in CMRO2, 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 CMRO2 to 180% of control. Prior removal of the adrenal glands curtailed the increase in CBF (400% of control) and CMRO2 (125% of control). The excessive increase in CMRO2 (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 CMRO2 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 CMRO2 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 CMR02 occurs in hypercapnia, which is blocked both by diazepam and propranolol.

RECENT RESULTS from this laboratory showed that when the nitrous oxide supply was withdrawn from paralyzed and artificially ventilated rats there was a pronounced increase in cerebral metabolic rate for oxygen (CMRO2) and cerebral blood flow (CBF), which could be blocked by previous adrenalectomy, or by administration of propranolol. It was tentatively concluded that the increase in CMRO2 (and CBF) was a response to immobilization stress, mediated by circulating catecholamines.

In the course of a study of cerebral metabolic responses to hypoxia (reduction of arterial Po2 to 25-30 mm Hg) we unexpectedly found that CMRO2 rose. Assuming that circulatory catecholamines were responsible, we undertook an extensive study of the effects of hypoxia on CBF and CMRO2 in non-adrenalectomized and adrenalectomized rats, using a CBF method of sufficient accuracy to resolve differences in CBF even at high flow rates. It will be shown that in one breed of rats studied, reduction of Po2 was accompanied by an increase in CMRO2 to almost 200 percent of control, most of which was prevented by prior removal of the adrenal glands. However, in another breed of hypoxic rats maintained on 70% N2O, whether adrenalectomized or not, there was a 20-30% increase in CMRO2. This increase was prevented by administration of diazepam. It is tentatively concluded that, during hypoxia, cerebral metabolic rate may be increased by catecholaminergic mechanisms.

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% \text{N}_2\text{O} and 30% \text{O}_2. 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% \text{N}_2\text{O}. 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\(^{-1}\) \text{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 desaturation period (1 min) the nitrous oxide supply was discontinued and the animals were ventilated with 30% \text{O}_2 and 70% \text{N}_2.

### 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 Pao2 of 26-28 mm Hg. In animals maintained on 70% \text{N}_2\text{O}, oxygen was replaced by nitrogen gas to maintain \text{N}_2\text{O} 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,\(^6\) using \text{133Xenon} and measurements of arterial and cerebral venous activities during the desaturation phase.\(^6\) 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,\(^4\) a 5 sec period was allowed to pass, following discontinuation of \text{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 \text{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 trapezoidal rule, using a \text{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 Pao2, Pco2, and pH were measured using microelectrodes, with due correction for any deviation in body temperature from 37°C. Blood oxygen content (\text{CO}_2) was measured in 25 \mu l samples using a polarographic technique.\(^6\) \text{133Xenon} was measured as previously described.\(^4\)

### 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.\(^2\) 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 \(1.12 \pm 0.13\) and with that previously used \(1.20 \pm 0.15\) ml g\(^{-1}\) min\(^{-1}\) (means \pm 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% \text{N}_2\text{O}, while those belonging to series C were given sedative or anesthetic doses of diazepam.\(^6\) 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,\(^6\) 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.

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

\[
\text{\thead{TABLE 1}} \quad \text{Body Temperature, Mean Arterial Blood Pressure (MABP), Arterial Blood Gases and pH, in Rats Hypoxic for 5 and 30 Min, Anesthetized with Either 70% N₂O in Oxygen (Series A and B) or Diazepam (Series C)}
\]

<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>
</tr>
</thead>
<tbody>
<tr>
<td>Series A (70% N₂O)</td>
<td>Control (n = 15)</td>
<td>37.0</td>
<td>142</td>
<td>125</td>
<td>38.8</td>
<td>7.375</td>
</tr>
<tr>
<td></td>
<td>Hypoxia 30 min</td>
<td>36.7</td>
<td>120***</td>
<td>27.7***</td>
<td>36.1***</td>
<td>7.113***</td>
</tr>
<tr>
<td></td>
<td>Hypoxia 30 min</td>
<td>36.2**</td>
<td>115***</td>
<td>27.6***</td>
<td>35.3***</td>
<td>7.072***</td>
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<td>Adrenalect. (n = 6)</td>
<td>36.2</td>
<td>±2</td>
<td>±0.6</td>
<td>±0.9</td>
<td>±0.026</td>
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<tr>
<td></td>
<td>Hypoxia 5 min</td>
<td>36.8</td>
<td>±3</td>
<td>±1.2</td>
<td>±0.5</td>
<td>±0.051</td>
</tr>
<tr>
<td>Series B (70% N₂O)</td>
<td>Hypoxia 30 min</td>
<td>36.4***</td>
<td>109***</td>
<td>26.1***</td>
<td>37.8</td>
<td>7.222***</td>
</tr>
<tr>
<td></td>
<td>Hypoxia 30 min</td>
<td>36.7</td>
<td>±5</td>
<td>±1.0</td>
<td>±1.1</td>
<td>±0.020</td>
</tr>
<tr>
<td></td>
<td>Hypoxia 30 min</td>
<td>36.9</td>
<td>±4</td>
<td>±1.5</td>
<td>±1.1</td>
<td>±0.027</td>
</tr>
<tr>
<td></td>
<td>Hypoxia 30 min</td>
<td>36.6</td>
<td>±7</td>
<td>±0.6</td>
<td>±0.7</td>
<td>±0.010</td>
</tr>
<tr>
<td>Series C (diazepam)</td>
<td>Control (n = 12)</td>
<td>36.8</td>
<td>±6</td>
<td>±3</td>
<td>±0.9</td>
<td>±0.010</td>
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<tr>
<td></td>
<td>Hypoxia 30 min</td>
<td>36.7</td>
<td>±5</td>
<td>±1.0</td>
<td>±1.0</td>
<td>±0.015</td>
</tr>
<tr>
<td></td>
<td>Hypoxia 30 min</td>
<td>36.9</td>
<td>±4</td>
<td>±1.5</td>
<td>±1.1</td>
<td>±0.027</td>
</tr>
</tbody>
</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 Total Oxygen Content in Arterial Blood (CaO₂), Arterio-Venous Difference for Oxygen (AVDO₂), Cerebral Blood Flow (CBF) and Metabolic Rate for Oxygen in Rats, Hypoxic for 5 or 30 Min, Anesthetized with Either 70% N₂O (Series A and B) or Diazepam (Series C)**

<table>
<thead>
<tr>
<th>Experimental series</th>
<th>Experimental group</th>
<th>CaO₂ (mmol·l⁻¹)</th>
<th>AVDO₂ (mmol·l⁻¹)</th>
<th>CBF (ml·g⁻¹·min⁻¹)</th>
<th>CMRO₂ (mmol·g⁻¹·min⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Series A (70% N₂O)</td>
<td>Control (n = 15)</td>
<td>9.83</td>
<td>±0.14</td>
<td>±0.16</td>
<td>±0.09</td>
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<tr>
<td></td>
<td>Hypoxia 30 min</td>
<td>2.14***</td>
<td>±0.06</td>
<td>±0.09</td>
<td>±0.09</td>
</tr>
<tr>
<td></td>
<td>Hypoxia 30 min</td>
<td>2.17***</td>
<td>±0.09</td>
<td>±0.04</td>
<td>±0.04</td>
</tr>
<tr>
<td></td>
<td>Hypoxia 5 min</td>
<td>2.99***</td>
<td>±0.11</td>
<td>±0.57</td>
<td>±0.57</td>
</tr>
<tr>
<td>Series B (70% N₂O)</td>
<td>Hypoxia 30 min</td>
<td>2.17***</td>
<td>±0.03</td>
<td>±0.23</td>
<td>±0.23</td>
</tr>
<tr>
<td></td>
<td>Hypoxia 30 min</td>
<td>2.06***</td>
<td>±0.08</td>
<td>±0.48</td>
<td>±0.48</td>
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<tr>
<td></td>
<td>Hypoxia 30 min</td>
<td>2.30***</td>
<td>±0.11</td>
<td>±0.56</td>
<td>±0.56</td>
</tr>
<tr>
<td>Series C (diazepam)</td>
<td>Hypoxia 30 min</td>
<td>2.13***</td>
<td>±0.09</td>
<td>±0.16</td>
<td>±0.16</td>
</tr>
<tr>
<td></td>
<td>Hypoxia 30 min</td>
<td>2.13***</td>
<td>±0.14</td>
<td>±0.44</td>
<td>±0.44</td>
</tr>
</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 CMRO₂ 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 PaO₂ to below 50 mm Hg leads to an increase in CBF, there is no change in CMRO₂ unless PaO₂ falls to excessively low values. This information is partly based on studies in man, in whom the PaO₂ was reduced to 35–40 mm Hg, either at decreased or normal CO₂ tensions.¹⁻¹² Similar results have been reported for dogs.¹² In the rat, more severe degrees of hypoxia have been studied.¹¹ The results showed that reduction of PaO₂ 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 CMRO₂.

Since the present results demonstrate that CMRO₂ 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.,¹⁰ Cao₂ 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.¹³,¹⁴ Probably, failure to observe an increase in CMRO₂ in our previous study on rats¹³ was due to circulatory catecholamines. Observe, however, that the increase in CMRO₂ 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 CMRO₂ 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 neurons¹⁹⁻²² and that benzodiazepines can block the increase in noradrenaline turnover²³⁻²⁵ the animals of series C were given either sedative or anesthetic doses of diazepam before hypoxia was induced. The results show that CMRO₂ did not increase in diazepam-injected animals. These results indicate that the rise of CMRO₂ 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.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 Km value for oxygen of tyrosine hydroxylase.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 CMRO₂. However, results obtained in hypercapnia provide strong indirect evidence. Thus, when PaCO₂ is elevated to about 80 mm Hg CMRO₂ is increased by 20–30%. This increase persists following removal of the adrenal glands, and following reduction in cerebral venous Po₂ to normal values, but is prevented by pretreatment of animals with either propranolol or diazepam.²⁸ Furthermore, it has been shown that even at normal tissue-Po₂ values, hypercapnia is accompanied by increased hydroxylation of tyrosine.²⁹ These results indicate that the mechanisms mediating an increase in CMRO₂ may be similar in hypoxia and hypercapnia, possibly involving an effect of tissue acidosis on noradrenaline turnover.

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