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

LEIF BERNTMAN, M.D., CHRISTER CARLSSON, M.D., PH.D., AND BO K. SIJESJÖ, M.D., PH.D.

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 CMRO2 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 administered 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, Pma 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 trapezoidal 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 Pao₂, 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
control and hypoxic animals. In all hypoxic groups, mean Pao$_2$ 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 Paco$_2$ 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 CO$_2$, AVDO$_2$, CBF and CMRO$_2$ 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, AVDO$_2$ 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 CMRO$_2$ to 180% of control. Prior removal of the adrenal glands considerably curtailed, but did not completely prevent, the increase in CMRO$_2$. Additional experiments showed that, in non-adrenalectomized animals, the excessive increase in CMRO$_2$ occurred after 5 min of hypoxia.

When the experiments were repeated on animals of the new breed (Series B), CMRO$_2$ 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 CMRO$_2$ 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 CMRO$_2$ 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 Po$_2$, following administration of propranolol, resulted in cardiovascular failure. Animals were therefore pretreated with diazepam, a drug that has

**Table 1** Body Temperature, Mean Arterial Blood Pressure (MABP), Arterial Blood Gases and pH, in Rats Hypoxic for 5 and 30 Min, Anesthetized with Either 70% N$_2$O 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>PacO$_2$ (mm Hg)</th>
<th>PacO$_2$/CMRO$_2$</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Series A (70% N$_2$O)</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>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>Hypoxia 5 min (n = 8)</td>
<td>36.2**</td>
<td>115***</td>
<td>26.7***</td>
<td>35.3***</td>
<td>7.072***</td>
<td></td>
</tr>
<tr>
<td>Series B (70% N$_2$O)</td>
<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>
</tr>
<tr>
<td>Series C (diazepam)</td>
<td>Control (n = 12)</td>
<td>36.8</td>
<td>145</td>
<td>128</td>
<td>37.3</td>
<td>7.381</td>
</tr>
<tr>
<td>Hypoxia 30 min (n = 8)</td>
<td>36.7</td>
<td>103**</td>
<td>25.7***</td>
<td>32.4***</td>
<td>7.155***</td>
<td></td>
</tr>
<tr>
<td>Hypoxia 5 min (n = 8)</td>
<td>36.9</td>
<td>104***</td>
<td>28.2***</td>
<td>33.9***</td>
<td>7.129***</td>
<td></td>
</tr>
</tbody>
</table>

Animals given diazepam are ventilated with 70% N$_2$O 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 (CaO$_2$), Arterio-Venous Difference for Oxygen (AVDO$_2$), Cerebral Blood Flow (CBF) and Metabolic Rate for Oxygen in Rats, Hypoxic for 5 or 30 Min, Anesthetized with Either 70% N$_2$O (Series A and B) or Diazepam (Series C)

<table>
<thead>
<tr>
<th>Experimental series</th>
<th>Experimental group</th>
<th>CaO$_2$ (µmol·ml$^{-1}$)</th>
<th>AVDO$_2$ (µmol·l$^{-1}$)</th>
<th>CBF (ml·g$^{-1}$·min$^{-1}$)</th>
<th>CMRO$_2$ (µmol·g$^{-1}$·min$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Series A (70% N$_2$O)</td>
<td>Control (n = 15)</td>
<td>9.83</td>
<td>3.76</td>
<td>1.11</td>
<td>4.00</td>
</tr>
<tr>
<td>Hypoxia 30 min (n = 8)</td>
<td>2.14***</td>
<td>1.18***</td>
<td>6.52***</td>
<td>7.72***</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% N$_2$O)</td>
<td>Hypoxia 30 min (n = 8)</td>
<td>2.17***</td>
<td>0.97***</td>
<td>5.26***</td>
<td>5.20***</td>
</tr>
<tr>
<td>Hypoxia 5 min (n = 8)</td>
<td>2.06***</td>
<td>1.12***</td>
<td>4.38***</td>
<td>4.85*</td>
<td></td>
</tr>
<tr>
<td>Series C (diazepam)</td>
<td>Control (n = 12)</td>
<td>10.37</td>
<td>5.70</td>
<td>0.74</td>
<td>4.18</td>
</tr>
<tr>
<td>Hypoxia 30 min (n = 8)</td>
<td>2.13***</td>
<td>1.36***</td>
<td>2.98***</td>
<td>4.08</td>
<td></td>
</tr>
<tr>
<td>Hypoxia 30 min (n = 4)</td>
<td>2.13***</td>
<td>1.45***</td>
<td>2.91***</td>
<td>4.03</td>
<td></td>
</tr>
<tr>
<td>7.5 mg·kg$^{-1}$ (n = 7)</td>
<td>0.15</td>
<td>0.14</td>
<td>0.02</td>
<td>0.14</td>
<td></td>
</tr>
</tbody>
</table>

Animals given diazepam are ventilated with 70% N$_2$O 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 PO₂ to below 50 mm Hg leads to an increase in CBF, there is no change in CMRO₂ unless PO₂ 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.⁹⁻¹¹ Similar results have been reported for dogs.¹² In the rat, more severe degrees of hypoxia have been studied.¹³ 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.,¹⁴ 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.¹⁵-¹⁶ Probably, failure to observe an increase in CMRO₂ in our previous study on rats¹⁷ was due to difficulties of accurately resolving arterial and cerebral venous¹⁸⁻²³ Xenon desaturation curves at the high flow rates encountered in hypoxia.

In order to facilitate discussion of the present results, values for CMRO₂ 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), CMRO₂ 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 CMRO₂ 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 CMRO₂ and CBF.¹⁸⁻¹⁷ Third, recent experiments have shown that i.v. infusion of adrenaline in a dose of 8 μg · kg⁻¹ · min⁻¹ is accompanied by a doubling of CMRO₂ in rats obtained from the same breed as those constituting series B.¹⁸ Clearly, the effects of adrenaline infusion on CMRO₂ are sufficiently pronounced to explain the excessive rise in CMRO₂ during hypoxia. However, some of the increase in CMRO₂ 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 CMRO₂ 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 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.

**Acknowledgment**

The authors are grateful to Miss Gunilla Gidö for skillful technical assistance. This study was supported by grants from the Swedish Medical Research Council (Project No. 14X-263) and from U.S. PHS Grant No. 5 RO1 NS 07838 from N.I.H.

**References**

2. Kety SS, Schmidt CF: The nitrous oxide method for the quantitative determination of cerebral blood flow in man: theory,
flow and metabolic rate for oxygen in the cerebral cortex of the
4. Norberg K, Siesjö BK: Quantitative measurement of blood flow
and oxygen consumption in the rat brain. Acta Physiol Scand
91: 154-164, 1974
5. Borgström L, Hägerdal M, Lewis L, Pontén U: Polarographic
determination of total oxygen content in small blood samples.
J Clin Lab Invest 34: 375-380, 1974
6. Hägerdal M, Harp J, Nilsson L, Siesjö BK: The effect of in-
duced hypothermia on oxygen consumption in the rat brain. J
Neurochem 24: 311-316, 1975
on oxygen consumption and blood flow in the cerebral cortex of
8. Carlsson C, Hägerdal M, Kaassik AE, Siesjö BK: The effects of
diazepam on cerebral blood flow and oxygen consumption in
rats and its synergistic interaction with nitrous oxide.
Anesthesiology 45: 319-325, 1976
9. Kety SS, Schmidt CF: The effects of altered arterial tensions of
carbon dioxide and oxygen on cerebral blood flow and cerebral
oxygen consumption in normal young men. J Clin Invest 27:
484-491, 1948
hypoxia and normocarbia on cerebral blood flow and
metabolism in conscious man. J Appl Physiol 23: 183-189,
1967
11. Shimozyo S, Scheinberg P, Kogure K, Reinmuth ON: The
effects of graded hypoxia upon transient cerebral blood flow and
oxygen consumption. Neurology (Minneap) 18: 107-112,
1968
flow and oxidative brain metabolism during and after moderate
and profound arterial hypoxemia. Acta Neurochir (Wien)
33: 141-148, 1976
13. Johansson H, Siesjö BK: Cerebral blood flow and oxygen con-
sumption in the rat in hypoxic hypoxia. Acta Physiol Scand
93: 269-276, 1975
14. Morris ME, Millar RA: Blood pH/plasma catecholamine rela-
tionships: respiratory acidosis. Brit J Anaesth 34: 672-681,
1962
15. Nahas GG, Zagury D, Milhaud A, Manger WM, Pappas GD:
Acidemia and catecholamine output of the isolated canine
AM: Cerebral circulation and norepinephrine: Relevance of the
17. MacKenzie ET, McCulloch J, Harper AM: Influence of en-
dogenous norepinephrine on cerebral blood flow and
18. Berntman L, Dahlgren N, Siesjö BK: Influence of intra-
venously administered catecholamines on cerebral oxygen con-
sumption and blood flow in the rat. Acta Physiol Scand 104:
101-108, 1978
19. Bliss EI, Allion J, Zwanziger J: Metabolism of norepi-
 nephrine, serotonin and dopamine in rat brain with stress. J
Pharmacol Exp Ther 164: 122-134, 1968
stress on the activity of central monoamine neurons. Life Sci 7:
107-112, 1968
on the metabolism of norepinephrine, dopamine and serotonin
in the central nervous system of the rat. I. Modifications of
norepinephrine turnover. J Pharmacol Exp Ther 163: 163-171,
1968
22. Korf J, Aghajanian GK, Roth RH: Increased turnover of
norepinephrine in the rat cerebral cortex during stress: Role of
23. Taylor KM, Laverty R: The effect of chlorodiazepoxide,
diazepam and nitrázepam on catecholamine metabolism in
regions of the rat brain. Europ J Pharmacol 8: 296-301, 1969
24. Corrodi H, Fuxe K, Lidsbrink P, Olsson L: Minor tranquilizers,
stress and central catecholamine neurons. Brain Res 29: 1-16,
1971
25. Lidsbrink P, Farnebo LO: Uptake and release of noradrenaline
in rat cerebral cortex in vitro: No effect of benzo diazepines and
26. Davis JN, Carlson A: The effect of hypoxia on monoamine
synthesis, levels and metabolism in the rat brain. J Neurochem
21: 783-790, 1973
27. Davis JN: Brain tyrosine hydroxylase: alteration of oxygen
affinity in vivo by immobilization or electroshock in the rat.
28. Berntman L, Dahlgren N, Siesjö BK: Influence of extreme
hypercapnia on cerebral blood flow and oxygen consumption in
the rat brain. Anesthesiology (in press)
29. Carlsson A, Holmin T, Lindqvist M, Siesjö BK: Effect of
hypercapnia and hypoxia on tryptophan and tyrosine hy-
Cerebral oxygen consumption and blood flow in hypoxia: influence of sympathoadrenal activation.

L Berntman, C Carlsson and B K Siesjö

Stroke. 1979;10:20-25
doi: 10.1161/01.STR.10.1.20

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://stroke.ahajournals.org/content/10/1/20.citation

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Stroke can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at: http://www.lww.com/reprints

Subscriptions: Information about subscribing to Stroke is online at: http://stroke.ahajournals.org/subscriptions/