DOPAMINE ACCUMULATION IN THE MOUSE BRAIN UNDER HYPOXIA

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Abstract—Effects of hypoxia on brain catecholamines were investigated in mice using high-performance liquid chromatography combined with electrochemical detection. When mice were exposed to hypoxic gas mixtures containing 6 or 7% oxygen, dopamine (DA) accumulated in the brain, regardless of the body temperature of the mice. Hypoxia-induced DA accumulation was greater in the norepinephrine (NE) rich areas (cerebellum, cerebral cortex and pons+medulla oblongata) than in the DA rich area (striatum). The DA depletion induced by inhibition of tyrosine hydroxylase with α-methyl-p-tyrosine was markedly inhibited by hypoxia. The brain homovanillic acid (HVA) accumulation induced by inhibiting active transport of organic acid with probenecid was also significantly inhibited by hypoxia while brain HVA levels were unchanged. The brain 3,4-dihydroxyphenylacetic acid (DOPAC) level and probenecid-induced DOPAC accumulation were not significantly affected by hypoxia. These findings indicate that hypoxia inhibits DA-β-hydroxylase in the NE rich areas and results in an accumulation of DA, as the NE precursor, while hypoxia-induced DA accumulation in the DA rich area may be due to an inhibition of monoamine oxidase and/or of DA release.

The effect of hypoxia on synthesis, degradation and endogenous levels of neurotransmitters as well as energy metabolism in the brain has been investigated (1-5). The acute hypoxia was shown to result in an increased brain γ-aminobutyric acid level in various species (6). However, changes in endogenous levels of catecholamines have not been apparent in hypoxic rats, as determined using the fluorescence method for measurement of catecholamines. Nevertheless, there are two oxygen requiring steps in catecholamine synthesis, and one in degradation. A significant decrease of in vivo activity of tyrosine hydroxylase and the partial inhibition of dopamine-β-hydroxylase and monoamine oxidase have been demonstrated in rats inhaling a hypoxic gas mixture (1-5). The development of a sensitive microassay method for measurement of catecholamines has enabled studies on the precise distribution of catecholamines and the localized changes in catecholamine metabolism under physiological manipulations or drug treatments (7, 8).

In view of the paucity of information on the effect of hypoxia on endogenous catecholamine levels, the effect of hypoxia on dopamine and norepinephrine levels in various brain areas was studied using high-performance liquid chromatography with electrochemical detection. We used mice as this species is more vulnerable to hypoxia (6).
MATERIALS AND METHODS

Male ICR mice, weighing 20–30 g were used. For at least 4 days prior to experiments all animals were housed in cages in a temperature controlled room with a fixed lighting schedule and given food and water ad libitum. The mice were killed between 11–12 a.m.

Hypoxic conditions: Mice were exposed to a N₂–O₂ environment in a sealed 10 liter glass dessicator modified to serve as an experimental chamber. The oxygen concentration of the gas mixtures was 6.0 or 7.0%. The mixture was passed through the chamber through inlet and outlet holes at a rate of 4 liters/min. Control animals were kept in an identical open chamber exposed to room air. The room temperature was conditioned at 25°C, and in some experiments mice were kept on a heated plate (38–39°C) to maintain the body temperature at 37–38°C. The rectal temperature of the experimental animals was monitored. At the end of the experiment the mice were decapitated and the brains rapidly removed. In some experiments the brains were dissected into 6 regions; olfactory bulb, striatum, cerebral cortex (without frontal cortex), diencephalon + mesencephalon, pons+medulla oblongata and cerebellum. The samples were weighed and frozen immediately after dissection and pooled for subsequent analyses. The blood samples were withdrawn from the abdominal aorta of mice anesthetized with pentobarbital (50 mg/kg, i.p.) after 60 min exposure to hypoxic gas mixtures or room air and arteral pO₂, pCO₂ and pH were determined using a micro blood gas analyzer (ABL 2 Acid-Base Laboratory, Radiometer, Copenhagen).

Chemical assays for dopamine, norepinephrine, homovanillic acid and 3,4-dihydroxyphenylacetic acid: The samples were homogenized in ice-cold 5 ml of 0.4 N perchloric acid containing 5 mg Na₂S₂O₅ and 20 mg EDTA and centrifuged for 10 min at 10,000 r.p.m. Supernatants were assayed for DA and NE by the method of Keller et al. (7) (recovery: 85% for DA, 93% for NE, sensitivity: 40 pg for DA and NE) and in some experiments for homovanillic acid (HVA) and 3,4-dihydroxyphenylacetic acid (DOPAC) by the method of Hefti (9) (recovery: 60% for HVA, 31% for DOPAC, sensitivity: 100 pg for HVA and DOPAC) using high-performance liquid chromatography with electrochemical detection.

Catecholamine turnover: Effect of hypoxia on brain catecholamine turnover was studied by the method of Brodie et al. (10). Mice were given DL-α-methyl-p-tyrosine (α-MT, 250 mg/kg) i.p. and a immediately placed in an experimental chamber equilibrated with a hypoxic gas mixture containing 6% oxygen, or room air at room temperature (25°C). Control animals were given injections of saline. The mice were killed 60 min after the injection and the DA and NE levels in the whole brain were determined. In addition, probenecid (200 mg/kg) or saline was given i.p. and these mice were subsequently handled as in the α-MT experiments. Here the whole mouse brain was analyzed for HVA and DOPAC.

Drug: Probenecid was a gift from Nippon Merck-Banyu Co., Ltd. Other chemicals were obtained from commercial sources.

Statistical analysis: Student t-test was used for a statistical comparison between mean values.

RESULTS

Effect of hypoxia on arterial blood pO₂, pCO₂ and pH: All mice exposed to 7% hypoxia were alive and without any remarkable behavioral changes. Some of the mice which had been exposed to 6% hypoxia had convulsions and died under conditions of room temperature (16/55 for 15 min, 20/55 for 30 min and 23/55 for
60 min exposure). All of the mice exposed to 6% hypoxia on the heated plate died during the first 10 min exposure, and these animals were discarded. Arterial pO₂ and pCO₂ were significantly decreased, depending on the oxygen concentration in the hypoxic gas mixture in mice exposed to hypoxia for 60 min. However, arterial blood pH was not altered by hypoxia (Table 1).

**Effect of hypoxia on brain catecholamine levels:** DA and NE were estimated in the whole brain following exposure of the mice to hypoxic gas mixture containing 6% oxygen, for various periods at room temperature. When mice were exposed to hypoxia, there was no statistically significant change in the brain DA and NE levels after 10 min of hypoxia. After this time the DA levels in the whole brain were significantly increased with 30 min of hypoxia and reached 138% of control at 60 min. On the other hand, the NE levels in the brain were decreased to 84% of control by exposure to hypoxia for 60 min (Fig. 1). When mice were exposed to hypoxic gas mixtures for 60 min at room temperature, there was an accumulation of brain DA, and the rectal temperature decreased with decrease in the oxygen concentration. Levels of brain NE also decreased, as shown in Table 2. DA accumulation induced by hypoxia was accelerated when the temperature of the mice was maintained at 37.4±0.1°C (Table 2).

When mice were placed in room air after exposure to the hypoxic gas mixture containing 6% oxygen for 60 min, the increased DA levels were sustained for the first one hour and returned to the control level during the

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**Table 1. Effect of hypoxia on arterial blood pO₂, pCO₂ and pH in mice.**

|            | pO₂ (mm Hg) | pCO₂ (mm Hg) | pH       |
|------------|-------------|--------------|----------|
| Control    | 89.4±5.1    | 40.3±1.5     | 7.30±0.02|
| hypoxia—6% oxygen | 29.1±2.0*   | 14.1±1.4*    | 7.37±0.06|
| hypoxia—7% oxygen | 33.8±1.3*   | 20.1±1.4*    | 7.38±0.03|

Mice were exposed to hypoxic gas mixtures containing 6% or 7% oxygen for 60 min at room temperature (25°C). Blood samples were withdrawn from the abdominal aorta of mice anesthetized with pentobarbital (50 mg/kg, i.p.). Values are means±S.E. of 5 determinations. *Significantly different from each control, p<0.01.

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**Table 2. Relation between hypoxia-induced change in brain catecholamine levels and rectal temperature in mice.**

|                | Dopamine (ng/g wet weight) | Norepinephrine (ng/g wet weight) | Rectal temperature (°C) |
|----------------|---------------------------|----------------------------------|-------------------------|
| Control        | 800.4±12.2                | 364.0±9.8                        | 38.8±0.2                |
| At room temperature (25°C) |                      |                                  |                         |
| hypoxia—6% oxygen | 1107.6±19.8*             | 306.7±7.0*                       | 27.2±1.2                |
| hypoxia—7% oxygen | 982.6±51.6*              | 323.4±16.5*                      | 32.7±0.2                |
| On the heated plate |                      |                                  |                         |
| hypoxia—7% oxygen | 1075.9±61.9*             | 322.8±9.5*                       | 37.4±0.1                |

Mice were exposed to hypoxic gas mixture containing 6 or 7% oxygen at room temperature or on the heated plate for 60 min. Each value is the mean±S.E. of at least 6 determinations. *Significantly different from each control, p<0.01.
second hour. Decrease in the NE levels in the brain was sustained for only 10 min then rapidly recovered (Fig. 1).

Effect of hypoxia on catecholamine levels in various brain regions: DA and NE were measured in the cerebellum, cerebral cortex (without frontal cortex), olfactory bulb, striatum, mesencephalon+diencephalon and pons+medulla oblongata after 60 min exposure of the mice to hypoxic gas mixture containing 6% oxygen at room temperature (Table 3). The NE rich areas such as cerebellum, cerebral cortex and pons+medulla oblongata exhibited a more marked elevation in the DA levels as compared with the striatum, a DA rich area. The hypoxia induced increase in the DA levels in the NE rich areas was in proportion to the ratio of NE to DA, in each control brain area. On the other hand, the NE levels were not significantly changed except in the mesencephalon+diencephalon where a significant decrease in the NE level was observed.

Effect of hypoxia on catecholamine turnover: One hour after treatment with α-MT 250 mg/kg i.p., there was a depletion of brain DA and NE contents in mice exposed to room air. The α-MT induced depletion of DA content was inhibited by 60 min hypoxia while that of NE content was not
significantly affected (Table 4). The turnover rate of brain DA in hypoxic mice was decreased to 0.018 μg/g/hr, 6.3% of control (0.286 μg/g/hr). The turnover rate of NE was unaffected by hypoxia (Table 4). As an index of DA turnover, DA metabolites, DOPAC and HVA were estimated in normoxic and hypoxic mice. Sixty min hypoxia did not significantly affect DOPAC and HVA levels in the whole brain. Inhibition of acid-
transport mechanism by treatment with pro-
benecid 200 mg/kg i.p. produced a significant 
accumulation of HVA and DOPAC in the 
brain. Sixty min hypoxia markedly inhibited 
the probenecid-induced accumulation of HVA 
but not that of DOPAC (Table 5).

Table 5. Effect of hypoxia on levels and probenecid induced accumulations of 3,4-dihydroxy-
phenylacetic acid and homovanillic acid in the mouse brain.

| (mean±S.E. (ng/g))                                          |
|-------------------------------------------------------------|
| 3,4-dihydroxyphenyl-                                       |
|      acetic acid                                           |
| Homovanillic acid                                         |
| Control                                                   | 122.9±3.5       | 214.1±  7.7     |
| Hypoxia                                                   | 120.5±6.0       | 233.3±24.5      |
| Probenecid                                                | 156.3±4.1*      | 554.4±31.7*     |
| Hypoxia + Probenecid                                      | 162.7±4.0       | 377.1±19.7**    |

Mice were given probenecid (200 mg/kg, i.p.) or saline and were exposed to hypoxic gas 
mixture or room air. Each value is the mean±S.E. of 5–8 determinations. *Significant 
difference from control, p<0.01. **Statistical difference from probenecid alone, p<0.01.


discussion

Our study provided unequivocal evidence 
that in mice, acute hypoxia produces a 
pronounced elevation in brain DA level and 
a decrease in brain NE level accompanied 
by a decrease in body temperature, arterial 
P02 and pCO2. The accumulation of DA was 
accelerated when the body temperature was 
maintained at 37.4±0.1°C. Thus, it seems 
unlikely that changes in brain catecholamine 
levels may be induced as the result of an 
impaired metabolism and release of catechol-
amines which accompany a fall in body 
temperature.

The newly developed sensitive method 
using high-performance liquid chromato-
graphy combined with electrochemical 
detection made feasible the measurement of 
a small amount of DA, as the NE precursor. 
Lloyd reported that the ratio of NE: DA is 
about 5–10 : 1 in most NE neurons (11). 
We demonstrated in the present study using 
mice that the ratio of NE : DA in NE rich 
areas is 8–20 : 1. These data suggest that 
DA in these NE rich areas may be the NE 
precursor. When assessing the levels of DA 
and NE in 6 brain areas, we found a more 
pronounced accumulation of DA produced 
by hypoxia in the NE rich areas, cerebellum, 
cerebral cortex and pons+medulla oblongata 
than in the DA rich area, striatum and that a 
significant decrease of NE level occurred only 
in the mesencephalon + diencephalon. 
Previous studies (1–5) all have suggested the 
decline in tyrosine hydroxylase activity in 
rat brain by exposure to hypoxia and it is 
considered that this oxygen requiring enzyme 
is inhibited by hypoxia in mouse as well as 
in the rat brain. Therefore, the accumulation 
of DA as NE precursor in the mouse brain 
area rich in noradrenergic innervation may 
be the result of inhibition of the DA-β-
hydroxylase activity by hypoxia, as previously 
suggested by Brown et al. (3) from the 
finding that after monoamine oxidase (MAO) 
inhibition, NE accumulation in the limbic 
forebrain in DOPA-loading rats was decreased 
by hypoxia.

In the mouse brain, NE depletion after 
inhibition of tyrosine hydroxylase with α-
methyl-p-tyrosine was not significantly 
changed by hypoxia, thereby suggesting 
that NE degradation is also inhibited by 
hypoxia, to the same extent as is the inhibition 
of NE synthesis from DA in noradrenergic 
nerve terminals. On the other hand, DA 
depletion after inhibition of tyrosine hydroxy-
lase was completely prevented, under conditions of hypoxia. The first possible mechanism to be considered is the inhibition of oxygen requiring steps in DA degradation. The DOPAC and HVA levels in the mouse brain were not changed, and the HVA accumulation after probenecid was significantly reduced by hypoxia, nevertheless hypoxic states did result in an accumulation of DA in the brain. As the catechol-O-methyltransferase was supposed to be resistant against hypoxia as reported by Faiman et al. (12), our findings indicate that there is a partial inhibition of the brain MAO activity and of the acid transport mechanism in the hypoxic mouse brain. Another possibility is a decrease in DA release due to the suppressed firing of DA neurons, as induced by hypoxia. Further experiments are underway to obtain direct evidence for the inhibition of DA release, under conditions of hypoxia.

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