A role of the central catecholamine neuron in cerebral circulation

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The effect of the central catecholaminergic neurons on the cerebral microcirculation was investigated by means of a unilateral intracerebral injection of 6-hydroxydopamine (6-OHDA) which produced the degeneration of catecholamine (CA) nerve terminals. Subsequent observation with CA histofluorescence revealed an absence of CA fibers in the vicinity of the 6-OHDA injection site. A significant increase in regional cerebral blood flow (rCBF), measured by the hydrogen clearance method, was demonstrated in the CA-depleted cortex under normocapnia as compared with rCBF in the control cortex (CA-depleted cortex 47.0 ± 2.8 ml/100 gm/min; control cortex 38.5 ± 3.5 ml/100 gm/min; p < 0.005). The increased rCBF in the cortex treated with 6-OHDA was suppressed by the iontophoretic replacement of noradrenaline (NA) to the CA-depleted cortex. An iontophoretic replacement of 10⁻⁵ M dopamine (DA) mildly suppressed the increased rCBF in the 6-OHDA-treated cortex. The CO₂ reactivity in the CA-depleted cortex was significantly lower than that of the control cortex (CA-depleted cortex 2.13% ± 0.67%/mm Hg; control cortex 3.53% ± 0.70%/mm Hg). No change was noticeable in the cerebral glucose metabolism in the CA-depleted cortex in an investigation based on tritiated (3H)-deoxyglucose uptake. It is suggested that the 6-OHDA-induced change in cerebral blood flow (CBF) is not secondary to alterations in cerebral metabolic rate, and that the central NA neuron system innervating intraparenchymal blood vessels regulates CBF through a direct vasoconstrictive effect on the cerebral blood vessels. The central DA neuron system may modulate the cerebral circulation as a mild vasoconstrictor.

KEY WORDS • catecholamine neuron • 6-hydroxydopamine • cerebral circulation • cerebral metabolism

It is well established that the cerebral pial blood vessels are innervated by sympathetic nerve fibers (peripheral noradrenergic), parasympathetic nerve fibers (peripheral cholinergic), and the other fibers containing serotonin, substance P, vasoactive intestinal peptide, pancreatic polypeptide, and gastrin-releasing peptide. It has also been demonstrated that the intraparenchymal small blood vessels are innervated by central noradrenergic, serotonergic, and vasoactive intestinal polypeptide nerve fibers. Although central catecholaminergic nerve terminals abut on the intraparenchymal small blood vessels, the role of the catecholamine (CA) neuron with regard to cerebral microcirculation remains controversial. For example, physiological studies on the effects of the central noradrenaline (NA) neurons on cerebral blood flow (CBF) have shown a reduction in CBF during stimulation of the locus ceruleus. On the other hand, it has been found that the noradrenergic system can induce vasodilation via β-adrenergic receptors localized on the microvessels. As for the function of the dopamine (DA) neurons, stimulation of the substantia nigra or the lesion of the nigrostriatal dopaminergic system has shown that the dopaminergic neurons may provide vasoconstrictive tone.

The aim of the present investigation was to study the role of the central NA neurons accompanying the cerebral microvessels with regard to the control of the cerebral microcirculation in vivo.

Materials and Methods

Twenty-five Sprague-Dawley rats, each weighing between 250 and 320 gm, were used for these studies. With the rats under intraperitoneal pentobarbital anesthesia (Nembutal, 50 mg/kg), the heads were fixed in a stereotaxic apparatus. To produce degeneration of CA nerve terminals, 6-hydroxydopamine (6-OHDA), a...
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neurotoxin specific to CA nerve terminals) was injected into the rat cerebral cortex. Just before the injection, 5 µg of 6-OHDA was dissolved in 5 µl of saline containing 4 mg/ml ascorbic acid. In 18 rats, the 6-OHDA solution was stereotactically injected into the left parietal cortex (AP: 2 mm, L: 2 mm, D: 2 mm) with a fine glass cannula (10 µm in inner diameter). Five sham-treated animals were given 5 µl of the vehicle solution.

Regional cerebral blood flow (rCBF) was measured by the hydrogen clearance method, the details of which have been published elsewhere. 17 Briefly, 1 week after injection of either 6-OHDA or the sham solution, the animal's head was fixed in the stereotaxic apparatus under intravenous alpha-chloralose (60 mg/kg) anesthesia, and hydrogen electrodes (Teflon-insulated platinum wires with bare conical tips 1 mm in length and 100 µm in diameter) were introduced stereotactically into both parietal cortices (AP: 4 mm, L: 2 mm, D: 1 mm). In the left parietal cortex, the hydrogen electrode was placed just 2 mm anterior to the previous injection site of 6-OHDA or the vehicle solution. An Ag/AgCl electrode was set in the subcutaneous region of the occipital scalp to serve as a reference electrode. All electrodes were allowed to stabilize for 60 minutes after placement in the cortices. Arterial blood pressure, PaCO2, PaO2, and pH were monitored through a catheter placed in the iliac artery. The animals breathed 2 liters/min room air by means of spontaneous respiration through a mask. Body temperature measured in the rectum was kept close to 37°C by use of a heating pad.

The rCBF was measured simultaneously at the control and experimental sites in both parietal cortices by the initial-slope method after 90 seconds inhalation of air containing 10% hydrogen gas. 26 Each rCBF measurement was performed three times under normocapnia and hypercapnia, the latter of which was produced by inhalation of 10% to 13% CO2 so that PaCO2 reached 56.0 to 66.3 mm Hg. Carbon dioxide reactivity was defined as the percent change in flow (relative to rCBF of normocapnia)/mm Hg change in PaCO2 and was expressed as %/mm Hg. After measurement of rCBF, five rats (three from the 6-OHDA-injected group and two from the solution-injected group) were investigated by CA histofluorescence to determine the effect of intracerebral injection of 6-OHDA on noradrenergic terminal fibers. Details of the CA histofluorescence method have been published elsewhere. 14

One week after 6-OHDA injection into the left parietal cortex, 10 rats were used for microiontophoretic application of catecholamines. Five were given NA and five DA. For application of NA, two barrel-shaped glass micropipettes (tip diameter 10 µm) filled with 10-5 M or 10-7 M NA bitartrate in saline were attached to the hydrogen electrode (AP: 4 mm, L: 2 mm, D: 1 mm). First, 10-7 M NA bitartrate was iontophoretically injected into the CA-depleted cortex for 30 minutes with a 5-A ejection direct current. 12 Fifteen minutes after application, rCBF was simultaneously measured at both the experimental and the control sites. One hour after rCBF measurement, 10-5 M NA bitartrate was applied and rCBF was measured again in the same manner. In the DA application group, 10-7 M or 10-5 M DA hydrochloride was iontophoretically injected into the CA-depleted cortex and rCBF was measured.

Cerebral metabolism was investigated by means of tritiated 2-[5,6-3H] deoxyglucose uptake. 35 One week after 6-OHDA pretreatment, 125 µCi deoxy-2-fluoro-d-glucose 2-[5,6-3H] in 0.5 ml saline was intravenously injected into two rats under alpha-chloralose (60 mg/kg) anesthesia. The animals were quickly sacrificed with an overdose of Nembutal 60 minutes after the injection. Their brains were removed, mounted onto cryostat chucks, and immediately frozen in powdered dry ice. The frozen brains were cut in coronal sections (15 µm thick) at −15°C on a cryostat. The sections were thaw-mounted onto microscope slides previously coated with chrome-alum gelatin and rapidly dried on a slide warmer at 60°C. These slides were applied against LKB Ulrofilm† to generate autoradiograms, and exposed at −20°C for 2 weeks. The films were developed in Dektol developer and fixed in Super Fuji Fixer.‡ Slide-mounted autoradiographs were examined with a microscope and grain density was quantified in slides of both the control and 6-OHDA-injected cortices.

Comparison of mean rCBF between the control site and the 6-OHDA-treated site with NA or DA application was performed using the Student paired t-test.

Results

The CA histofluorescence study confirmed that a denervated area of CA fibers extended for a radius of about 2.5 mm centered on the injection site on the sagittal section 1 week after 6-OHDA injection (Fig. 1).

FIG. 1. Camera lucida drawing of histofluorescence of catecholamine (CA) nerve fibers in the 6-hydroxydopamine (6-OHDA)-injected cortex (sagittal section). The 6-OHDA injection site and the placement site of the H2 electrode are indicated (arrows). A CA-depleted area of about 2.5 mm in radius is visible around the injection site.

† Ulrofilm manufactured by LKB, Inc., Bromma, Sweden.
‡ Dektol developer manufactured by Eastman Kodak Co., Rochester, New York; and Super Fuji Fixer manufactured by Fuji Photo Film Co., Ltd., Tokyo, Japan.

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No effect was observed in the control cortex. Sham treatment with injection of vehicle solution resulted in no significant change in CA fibers.

The rCBF was simultaneously measured in the control and the 6-OHDA-treated cortices. Under normocapnia, rCBF in the 6-OHDA-treated cortex was 47.0 ± 2.8 ml/100 gm/min and rCBF in the control cortex was 38.5 ± 3.5 ml/100 gm/min. This difference in rCBF was statistically significant (p < 0.005) (Fig. 2). Under hypercapnia, rCBF in the 6-OHDA-treated cortex was 67.7 ± 6.3 ml/100 gm/min and rCBF in the control cortex was 60.1 ± 5.7 ml/100 gm/min. Thus, the 6-OHDA-treated cortex showed a significant increase in rCBF as compared with the control cortex under hypercapnia (p < 0.05) (Fig. 2). In the sham-treated group, there was no significant change in rCBF between the injected cortex and the control cortex (Fig. 2).

The CO2 reactivity was 3.53% ± 0.70%/mm Hg in the control cortex and 2.13% ± 0.67%/mm Hg in the 6-OHDA-treated cortex. The CO2 reactivity in the 6-OHDA-treated cortex was significantly reduced (p < 0.05). Physiological parameters during measurement of rCBF under normocapnic and hypercapnic conditions are shown in Table 1. There was no significant difference in mean arterial blood pressure with regard to states of normocapnia and hypercapnia.

**Table 1**

<table>
<thead>
<tr>
<th>Animal Group</th>
<th>pH</th>
<th>PaCO2 (mm Hg)</th>
<th>PaO2 (mm Hg)</th>
<th>MABP (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6-OHDA group</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>normocapnia</td>
<td>7.398±0.038</td>
<td>41.2±4.6</td>
<td>91.4±7.6</td>
<td>116.7±7.6</td>
</tr>
<tr>
<td>hypercapnia</td>
<td>7.268±0.060</td>
<td>60.4±4.5</td>
<td>107.9±8.2</td>
<td>117.0±8.2</td>
</tr>
<tr>
<td>sham-treated group</td>
<td>7.369±0.038</td>
<td>39.2±2.0</td>
<td>85.4±4.7</td>
<td>110.0±3.5</td>
</tr>
</tbody>
</table>

*Values are means ± standard deviations in 18 rats that received an injection of 6-hydroxydopamine (6-OHDA) and five rats that received sham treatment with vehicle solution. rCBF = regional cerebral blood flow; MABP = mean arterial blood pressure.

**Discussion**

It is well known that CA nerve fibers are found not only around pial arteries and veins, but also around intraparenchymal blood vessels. Noradrenaline-containing nerve fibers in the walls of pial arteries and veins have been shown to regulate cerebral blood flow, arterial blood pressure, and oxygen uptake. The 3H-2-deoxyglucose uptake autoradiographic investigation showed no significant difference in grain density between the 6-OHDA-treated cortex and the control cortex. This result indicates that a deprivation of central CA fibers does not have a marked influence on cerebral metabolism (Fig. 3).

The effect of NA iontophoretic application on rCBF is shown in Fig. 4 left. The increased rCBF in the 6-OHDA-treated cortex was suppressed by 10^{-7}-M applications of NA and normalized after 10^{-5}-M applications of NA. Figure 4 right shows the effect of DA iontophoretic application on rCBF. Although the 10^{-7}-M applications of DA did not influence the increased rCBF in the 6-OHDA-treated cortex, the 10^{-5}-M applications of DA normalized the increased rCBF in that cortex.

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**Fig. 2.** Bar graph showing the effects of 6-hydroxydopamine (6-OHDA) on regional cerebral blood flow (rCBF). The 6-OHDA-treated cortex showed a significant increase in rCBF compared with the control cortex under normocapnic and hypercapnic conditions. There was no significant change in rCBF in the sham-treated cortex. Values are means ± standard deviations.

**Fig. 3.** Brain studied by 3H-deoxyglucose uptake autoradiography showing no significant difference in grain density between the 6-hydroxydopamine (6-OHDA)-injected and the control cortices. The dotted lines indicate the region of catecholamine depletion by 6-OHDA injection.
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arise from the superior cervical ganglion and those along the intraparenchymal blood vessels arise from brain-stem NA cell nuclei (mainly the locus ceruleus). Although there have been many experimental studies on the neurogenic control of CBF, the role of CA nerve fibers in cerebral circulation has not yet been established. It has been reported that CA-denervated lesions induced by intracisternal or intraventricular injections of a CA-related neurotoxin, 6-OHDA, caused no noticeable change in CBF during normocapnia. In the present study, we used intracortical injection of 6-OHDA and simultaneously measured rCBF in the CA-depleted and the control cortex with the hydrogen clearance method. This study demonstrated a significant increase in rCBF at the CA-depleted region under normocapnia. Two explanations are proposed in interpretation of the increased rCBF demonstrated at the CA-depleted region. One explanation is that the CA denervation by 6-OHDA directly causes a vascular dilatation of the parenchymal blood vessels, and the other is that the CA denervation primarily causes enhancement of the cerebral metabolism, which induces the secondary increases in rCBF. The results of our 1H-deoxyglucose uptake autoradiography studies give little support to the view that the CA denervation has a clear influence on cerebral glucose metabolism. It may be concluded that the CA denervation directly causes the dilatation of intraparenchymal blood vessels and the increase in resting CBF.

During hypercapnia, rCBF in the 6-OHDA-treated cortex was higher than that in the control cortex, but CO2 reactivity of the 6-OHDA-treated cortex was significantly reduced as compared with that of the control cortex. Bates, et al, showed that bilateral electrically induced lesions of the locus ceruleus caused the CBF under normocapnia to increase over control levels, whereas there was lack of CO2 reactivity of CA-denervated areas. Edvinsson, et al, demonstrated a significant increase in CO2 reactivity after intraventricular 6-OHDA administration, and Mendelow, et al, showed that intracisternal 6-OHDA injection reduced CO2 reactivity. There are several points of difference between those studies and the present one; these include the species used, the CBF measurement method, the type of anesthesia employed, and the technique to induce lesions of the central CA fibers. It is suggested that lack of CO2 reactivity after lesion formation in the bilateral locus ceruleus is due not only to the depletion of the central noradrenergic neurons but also to the location of an extensive lesion in the high area of the brain stem. In the cat, since the locus ceruleus CA-containing cells were markedly scattered in the pontine tegmental area, a large electrical lesion in the cat locus ceruleus may involve many other neuron systems. It may be speculated that the lesions induced by intraventricular or intracisternal injections of 6-OHDA affected central CA fibers in the whole brain. In contrast, intracortical 6-OHDA injection can produce a small area of CA-depletion. The involved lesion in the high brain stem and CA fibers in the whole brain could explain the discrepancy in results between the previous studies mentioned above and the present one.

The CO2 reactivity of the control cortex in the present study was 3.50%/mm Hg, which corresponds to the results of Olesen, et al, (2 to 6%/mm Hg). The site of action of increased PaCO2 is probably the intrapar-
encephalmal vessels, and the perivascular pH is considered the main factor in the changes in rCBF. It is proposed that CA nerves prevent the vasoparalysis caused by perivascular tissue acidosis during hypercapnia and provide neurogenic regulation of CBF as a vasoconstrictor.

The effects of NA and DA on the cerebral circulation were studied by measuring rCBF after the iontophoretic application of either of these agents. This study represents a new attempt to demonstrate CBF response to the iontophoretic application of NA or DA. The increased rCBF in the 6-OHDA-treated cortex was suppressed by the iontophoretic replacement of NA to the CA-depleted cortex and was normalized after the replacement of 10⁻⁷ M NA. It is suggested that NA and DA neurons innervating intraparenchymal blood vessels regulate rCBF as a vasoconstrictor and provide the resting tone to cerebral blood vessels.

The present study has demonstrated that application of only 10⁻⁵ M DA significantly reduces the increased rCBF in the 6-OHDA-treated cortex under normocapnia. Dahlgren, et al., found that a central dopaminergic neuron system has no effect on CBF under normocapnia. Lindvall, et al., indicated that a release of endogenous DA following removal of the dopaminergic input produces a reduction in blood flow. McCulloch and Harper suggested that apomorphine (a stimulator of DA receptors) primarily caused the excitation of cerebral metabolism and that dilatation of cerebral vessels occurred secondarily. One possible explanation for this discrepancy is a difference in the method of DA administration. Systemic administration of apomorphine stimulating DA receptors may be an unsuitable experimental model for elucidating the interaction between DA neurons and the cerebral circulation because the site affected by apomorphine is unclear. Thus, the results obtained here suggest that the central DA neuron system may exert a mild vasoconstricting effect on the intraparenchymal blood vessels.

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