Rapid Changes in Pial Arterial Diameter and Cerebral Blood Flow Caused by Ipsilateral Carotid Artery Occlusion in Rats

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Abstract. We investigated rapid changes in pial arterial diameter and in cerebral blood flow (CBF) caused by transient ipsilateral common carotid artery occlusion (CCA-O) in anesthetized rats in order to elucidate how the cerebral circulation reacts to acute stem artery occlusion. In separate groups of rats, pial arterial diameter was recorded through a closed cranial window and CBF was recorded by laser-Doppler flowmetry. CCA-O was performed for 5 minutes under normotension and normocapnia (control) and under graded hypotension, hypercapnia and hypocapnia. In the control condition, pial arterial diameter increased rapidly, triggered by CCA-O. It took 12 ± 3 s to reach the maximum of 204 ± 42% of the value before CCA-O, and 60 ± 24 s to become stable at 131 ± 11%. CBF decreased rapidly to 66 ± 11%, then increased reactively to 135 ± 9%, and again decreased to 91 ± 3%. The reactive increase in CBF caused by CCA-O decreased in parallel with the degree of hypotension, and also became barely detectable under hypercapnia. Our data suggest that active vascular dilation in the territory of the occluded artery is important for inducing collateral circulation. (Keio J Med 46 (3): 120-127, September 1997)

Key words: pial artery, cerebral blood flow, carotid occlusion, collateral circulation, reactive hyperemia

Introduction

We closely investigated rapid changes in both pial arterial diameter and cerebral blood flow (CBF) during and after transient occlusion of the ipsilateral common carotid artery (CCA-O) in rats. The aim of this study was to elucidate how rapidly the resistant arteries react to acute occlusion of the stem artery and how CBF follows the arterial responses in the process of induction of collateral circulation.

The initial change occurring in the territory of the occluded artery is a reduction in perfusion pressure, which may stimulate autoregulatory mechanisms to maintain local CBF. It has not been fully investigated, however, in what manner and by what mechanisms both the cerebral vessels and CBF react to a sudden pressure reduction. While myogenic, metabolic, neurogenic, and endothelial factors have been extensively discussed concerning cerebral autoregulation,1 different factors might be dominant in different situations, and it has not been clarified how those factors work against acute change of local perfusion pressure.

On the other hand, since laser-Doppler flowmetry (LDF) has allowed noninvasive real-time monitoring of blood flow in the cerebral microcirculation, it has been demonstrated that local CBF varies from moment to moment, showing rhythmical flow motion in the physiological condition.2,3 Such flow motion is considered to reflect vasomotion in the cerebral arteries.4,5 This suggests that we need to focus on the time course of changes in the order of seconds, when we try to analyze dynamic changes in the cerebral circulation.

It has been observed that CCA-O does not always produce cerebral infarction owing to collateral circu-
lation, though CCA-O decreases CBF in the ipsilateral hemisphere.6-9 Collateral circulation also contributes to the survival of brain tissue around the area of infarction following occlusion of a cerebral artery. Hemodynamics in such a border zone area is an important subject to be studied, with relevance to clinical treatment. However, the process of induction of collateral flow immediately after CCA-O or in the acute phase of focal ischemia has not been analyzed clearly. Our simple model is useful for studying the process of induction of collateral blood supply.

In this study we found a discrepancy between the changes in arterial diameter and those in blood flow before a new stable hemodynamic condition was produced. Our results obtained in the control condition suggested that active vascular dilation contributes to the changes in CBF. We also evaluated the effects of systemic blood pressure and arterial blood gases on such changes, because these factors are known to be closely related to the regulation of CBF. We induced stepwise hypotension to observe the relationships among perfusion pressure, arterial reactivity and CBF. CO2 inhalation was applied to monitor the changes in CBF under the condition where additional vasodilation is diminished.

Materials and Methods

Animal preparation

A total of 24 male Sprague-Dawley rats weighing 300–400 g were used. The animals were anesthetized with a-chloralose (50 mg/kg, i.p.) and urethane (500 mg/kg, i.p.), immobilized with alcuronium chloride and mechanically ventilated with room air. The body temperature was kept between 37.0° and 38.0° using a heating blanket. The head was also covered except for the surgical field, in order to maintain the temperature. Both femoral arteries and one femoral vein were catheterized; systemic arterial blood pressure was monitored and arterial blood samples were periodically collected for blood gas analysis. The head of the animal was fixed in a stereotaxic frame. The right common carotid artery was exposed by the dorsal approach and accompanying autonomic nerve fibers were carefully divided from the arterial wall.

Closed cranial window technique

In 12 rats, a small burr hole was made in the right parietal bone and the dura was opened. The exposed brain surface was immediately flooded with artificial cerebrospinal fluid and covered with a crystal glass window with a metal frame. The frame was fastened to surrounding bone using dental acrylic. The pial vessels were observed through a video camera system developed in our laboratory, and vessel diameters in the region of the middle cerebral artery (MCA) were continuously recorded using a width analyzer. For the analysis, we chose arteries with a diameter of 10 ~ 40 μm.

Laser-Doppler flowmetry

In the other 12 rats, a small hole was made in the right parietal bone using a dental drill, but the dura and a thin inner layer of bone were kept intact. The measuring probe (1.0 mm diameter) was placed in the hole and the microvascular blood flow in the parietal cortex was continuously recorded by laser-Doppler flowmeter (ALF21, Advance, Japan). The position of the probe was carefully chosen to avoid large dural and pial vessels.

Experimental protocol

At the beginning of the experiment the mean arterial blood pressure (MABP) of the rats was set at 90–105 mmHg (control condition), by blood withdrawal from the femoral artery if necessary. The right common carotid artery was occluded by an arterial clip for 5 minutes.

In animals that underwent closed cranial window preparation or LDF, one of the following procedures was performed in separate groups of animals. In 6 rats, MABP was reduced stepwise by blood withdrawal to about 80, 60 and below 45 mmHg. After MABP became stable under each experimental condition, CCA-O was repeated in the same way as in the control condition. In the other 6 rats, moderate and then severe hypercapnia was achieved by inhalation of 5 or 10% CO2, respectively. Hypocapnia was induced by increasing the inspiratory air flow by 78%. In 3 rats, hypercapnia was induced before hypocapnia, while in the other 3 rats hypocapnia preceded hypercapnia.

All experimental procedures were performed according to the Guidelines of the Animal Experiment Committee of Keio University.

Data analysis

Since pial arteries showed spontaneous rhythmical vasomotion, the mean value of the oscillations of the diameter was considered as the arterial diameter. Since CBF expressed as LDF signals also showed rhythmical oscillations, i.e. flow motion, the mean value of these oscillations was considered as CBF.

Arterial diameter and CBF under various experimental conditions were expressed as the percent ratio to those in the resting state before CCA-O in the control condition. Changes in CBF caused by CCA-O were also expressed as the percent of the value before CCA-O at each blood pressure or respiratory condition.
The frequency of vasomotion or flow motion was determined by counting the number of rhythmic oscillations during a period of 2 min and expressed as cycles per min (cpm). The amplitude of these oscillations was measured during the same period and the mean amplitude was calculated. The ratio of the amplitude to the mean arterial diameter or CBF was also calculated.

In statistical presentations, data were expressed as mean ± SD. Statistical significance was determined using analysis of variance (ANOVA) followed by modified t-test according to the Bonferroni method.

**Results**

**Control condition**

Typical recordings of the changes in pial arterial diameter and in CBF caused by CCA-O are shown in Fig 1a and 1b. Representative points, especially the maximal and minimal points, in the initial changes are shown in Fig 2.

In the control condition, the pial arteries started to dilate (initial dilation) at the moment of CCA-O, and arterial diameter reached a maximum of 204 ± 42% (n = 12) of the value before CCA-O at 12 ± 3 s after the onset of CCA-O. While spontaneous vasomotion was observed before CCA-O, the initial dilation was significantly larger than the spontaneous dilation; i.e. half of the amplitude of vasomotion. Then the arterial diameter gradually decreased and became 131 ± 11% of the value before CCA-O at 60 ± 24 s after the onset of CCA-O. Thereafter it remained constant during the remaining period of CCA-O. After reopening of the carotid artery, the pial arterial diameter soon recovered to the control level.

On the other hand, CBF decreased rapidly (initial reduction) at the onset of CCA-O and reached a minimum of 66 ± 11% of the value before CCA-O at 4 ± 1 s after the onset of CCA-O. Then CBF increased reactively (reactive increase) to a maximum of 135 ± 9% at 15 ± 3 s after the onset of CCA-O. After reaching this peak, CBF decreased gradually and remained at 91 ± 3% during the remaining period of CCA-O. While spontaneous flow motion was observed before CCA-O, the initial reduction and the reactive increase were
significantly larger than half of the amplitude of flow motion. When the carotid artery was reopened, CBF increased rapidly (post-occlusive increase) to $117 \pm 2\%$, then gradually returned to the control level.

There were no significant changes in either the systemic blood pressure or blood gas parameters after the onset of CCA-O.

**Hypotension**

Following the degree of hypotension, pial arterial diameter increased progressively (Fig 3 upper panel), and CBF was autoregulated when MABP was above 50 mmHg (Fig 4 upper panel).

During CCA-O, the initial dilation of the pial arteries decreased MABP dependently, and when MABP was below 45 mmHg the diameter did not exceed the value before CCA-O (Fig 3 lower panel). For CBF, the maximum value of the reactive increase showed a linear MABP-dependent decrease, while no significant change was detected in the initial reduction in CBF (Fig 4 lower panel).

In the stable period during CCA-O, the pial arterial diameter increased according to the degree of hypotension, and CBF remained at the control level when MABP was above 50 mmHg, whereas CBF decreased significantly when MABP was below 45 mmHg (Fig 4).

There were no differences in the post-occlusive increase in CBF among the MABP levels.

**Hypercapnia and hypocapnia**

The values of blood gas analysis under hypercapnia and hypocapnia are summarized in Table 1.

Under hypercapnia, arterial diameter and CBF increased markedly before and during CCA-O (Figs 5, 6).
under upper panels). Under hypocapnia, neither the diameter nor CBF changed significantly.

Under hypercapnia, CCA-O did not cause a significant change in arterial diameter (Fig 5 lower panel). The diameter during CCA-O, 203 ± 44% of the control level in moderate hypercapnia or 227 ± 96% in severe hypercapnia, was almost the same as the maximum of the initial dilation in the control condition, 193 ± 36% (n = 6).

While there was no significant difference in the percent change in CBF at the initial reduction, the reactive increase became very slight and CBF did not overshoot the value before CCA-O under hypercapnia (Fig 6 lower panel). The reactive increase also declined under hypocapnia, though less markedly.

There were no significant differences in the post-occlusive increase among the different PaCO2 levels.

**Table 1 Physiological Parameters under Various Experimental Conditions**

<table>
<thead>
<tr>
<th>MABP Level (mmHg)</th>
<th>90 ~ 105</th>
<th>70 ~ 80</th>
<th>50 ~ 60</th>
<th>&lt;45</th>
</tr>
</thead>
<tbody>
<tr>
<td>MABP (mmHg)</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Blood Gas Analysis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PaO2 (torr)</td>
<td>96.3 ± 4.5</td>
<td>113.0 ± 9.7</td>
<td>112.6 ± 14.8</td>
<td>111.6 ± 10.7</td>
</tr>
<tr>
<td>PaCO2 (torr)</td>
<td>35.9 ± 3.7</td>
<td>30.0 ± 1.3</td>
<td>29.7 ± 2.0</td>
<td>35.7 ± 3.0</td>
</tr>
<tr>
<td>pH</td>
<td>7.432 ± 0.032</td>
<td>7.448 ± 0.041</td>
<td>7.438 ± 0.031</td>
<td>7.385 ± 0.013</td>
</tr>
<tr>
<td>PaCO2 Level</td>
<td>Hypocapnia</td>
<td>Normocapnia</td>
<td>Moderate Hypercapnia</td>
<td>Severe Hypercapnia</td>
</tr>
<tr>
<td>MABP (mmHg)</td>
<td>97.9 ± 6.4</td>
<td>98.9 ± 5.8</td>
<td>100.6 ± 8.2</td>
<td>94.5 ± 13.3</td>
</tr>
<tr>
<td>Blood Gas Analysis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PaO2 (torr)</td>
<td>103.4 ± 13.5</td>
<td>96.3 ± 8.8</td>
<td>108.8 ± 13.5</td>
<td>107.9 ± 10.3</td>
</tr>
<tr>
<td>PaCO2 (torr)</td>
<td>18.8 ± 3.2</td>
<td>35.5 ± 2.7</td>
<td>60.7 ± 7.5</td>
<td>93.0 ± 5.4</td>
</tr>
<tr>
<td>pH</td>
<td>7.613 ± 0.037</td>
<td>7.440 ± 0.075</td>
<td>7.240 ± 0.084</td>
<td>7.145 ± 0.032</td>
</tr>
</tbody>
</table>

mean ± SD

**Vasomotion and flow motion**

In the control condition, the frequencies of vasomotion and flow motion tended to decrease during CCA-O (Fig 7), whereas no significant changes were detected in their amplitudes.

Following the degree of hypotension, the frequencies of vasomotion and flow motion decreased before CCA-O and during CCA-O. When MABP was 50~60 mmHg, vasomotion disappeared during CCA-O in 4 of 6 rats. When MABP was below 45 mmHg, both vasomotion and flow motion disappeared (Fig 7).

Vasomotion and flow motion disappeared under hypercapnia. Under hypocapnia, the frequencies of vasomotion and flow motion increased both before and during CCA-O, whereas their amplitudes did not show significant changes.

**Discussion**

Although previous clinical and experimental studies have assessed the hemodynamics in the brain following unilateral carotid artery occlusion, how the cerebral vessels react to acute stem artery occlusion and how they produce a new hemodynamic state through the development of collateral circulation have not been fully investigated. Continuous measurement of the pial vessel diameter using the video camera method and monitoring the real-time course of CBF using LDF have made it possible to study rapid vascular responses to CCA-O.

The changes in pial arterial diameter and CBF during CCA-O in our present study can be divided conveniently into the initial phase and the later phase, which refer to before and after reaching a new steady state, respectively.

During the new steady state, i.e. the later phase, the pial arteries were dilated and CBF was maintained at around 90% of control when MABP was above 60 mmHg. Clinical studies revealed that the blood volume increased in the brain tissue distal to the occluded or narrowed artery, even if CBF was not significantly reduced. The increase in blood volume was considered to reflect dilation of the arteries. It is well known that pial arteries dilate following systemic hypotension, reflecting autoregulation in the brain. Vascular changes caused by CCA-O may be mediated by similar autoregulatory mechanisms against a reduction in perfusion pressure. The integrated mechanism of autoregulation in the brain, however, is still under debate. Myogenic, neurogenic, metabolic, and endothelial mechanisms have been proposed, and all these factors could be involved in the later phase of CCA-O.
The mechanism of the initial phase soon after CCA-O seems more controversial. The pial arteries started to dilate as soon as the ipsilateral carotid artery was occluded. In spite of this instant dilation of arteries, CBF initially fell markedly. In this period, there was a discrepancy between pial arterial diameter and CBF. Florence and Seylaz showed a transient fall in CBF after very rapid induction of systemic hypotension, using LDF. It seems that during the initial period of acute reduction of perfusion pressure the arteries did not dilate enough to maintain CBF, and such a time delay causes a latency of the autoregulatory response. However, it may not be hypoxia or other metabolic changes caused by hypoperfusion, but the intravascular pressure change itself that triggered arterial dilation, because the onset of arterial dilation was almost simultaneous with the CCA-O. It has been shown that contractility of vascular smooth muscle changes depending on intraluminal pressure through alteration of the frequency of action potentials or of static membrane potential. Recently, it has been suggested that endothelial cells transduce mechanical conditions, such as shear stress or transmural pressure. Such intrinsic mechanisms in the vascular wall may contribute to the above rapid vascular response. The role of the nervous network innervating the vascular wall, especially vasodilatory nerves releasing VIP or nitric oxide, awaits further investigation. Metabolic factors might
affect the later component of the changes.

Bohlen and Harper\textsuperscript{18} reported that the arteriolar response to pressure change reached a new steady state within 10–15 s. Kontos \textit{et al}\textsuperscript{19} showed that when sinusoidal variations in blood pressure were induced, pial vessel diameter followed the change in blood pressure with a lag of about 10 s. Our present study showed a similar time course of vascular reactivity; that is, it took 12 ± 3 s until pial arteries achieved maximum dilation.

In the control condition, CBF started to recover a few seconds after CCA-O, and overshot the value before CCA-O, then gradually decreased to a level lower than that before CCA-O. This indicates a rapid blood supply through collateral pathways. Previous reports have not given proper consideration to this overshoot phenomenon, which might be similar to reactive hyperemia\textsuperscript{20,21} in some aspects. In our experimental model, however, the phenomenon occurred without reopening of the occluded artery, and the ischemic period was extremely short. The peak of the overshoot corresponded to the peak of rapid dilation of the pial arteries. Kontos \textit{et al}\textsuperscript{19} reported that hypotension of only a few seconds induced dilation of the pial arteries. Our study showed that, once triggered, the pial arteries continued to dilate toward the maximum during 10 s, even if the blood flow was restored. These phenomena involving pial arterial diameter and CBF suggest that vascular wall tension is controlled by changes in intraluminal pressure through a feedback mechanism which works within the order of seconds. It seems that it takes a few seconds for arteries to fully dilate in response to the initial pressure fall, and that an inappropriate reduction in vascular resistance allows CBF to become higher than normal before the arteries switch to constriction stimulated by high blood flow. Bevan and Joyce\textsuperscript{22} suggested that the blood flow per se possesses an ability to change the vessel wall tension. Thus, fluctuations in CBF occur before the vascular wall tension is readjusted to maintain a stable CBF. Such a feedback mechanism might also contribute to vasomotion or flow motion.

During stepwise hypotension, the degree of reactive increase decreased almost linearly in a MABP-dependent manner. When MABP was 55–60 mmHg, that is, near the lower limit of autoregulation, CBF did not overshoot, and when MABP was below the limit of autoregulation, CBF did not reach the pre-occlusive level. These findings suggest that the arteries in the hemisphere ipsilateral to CCA-O always dilate to nearly maximum extent as a reaction to the sudden fall of perfusion pressure, then the ischemic area is flushed dependent on a new perfusion pressure through collateral pathways. This new perfusion pressure may relate to the systemic blood pressure. Therefore, the reactive increase would be dependent on MABP.

Under hypercapnia, CBF markedly increased, while the reactive increase decreased significantly. Under severe hypercapnia, when arteries in the whole brain are maximally dilated, sufficient collateral blood supply could not be expected. These findings suggest that active arterial dilation in the ipsilateral hemisphere and production of a difference in vascular resistance between the hemispheres is important for induction of collateral circulation.

Although it is well known that vasomotion and flow motion exist in the cerebral circulation,\textsuperscript{2–4} neither their physiological roles nor the mechanisms producing them have been clarified. Osol and Halpern\textsuperscript{23} postulated that intrinsic oscillation in membrane calcium and potassium conductance may underlie the rhythmic contractile activity of the arteries. Endothelial factors such as nitric oxide\textsuperscript{24,25} may also be involved. Although CBF was maintained in the steady state during CCA-O, vasomotion and flow motion patterns were altered. Considering the results of our previous experiment, the changes in vasomotion during CCA-O in our present study can be explained by a reduction in perfusion pressure in the ipsilateral hemisphere. Under certain

![Figure 7 Relationships between MABP and the properties of vasomotion and flow motion, before and during CCA-O. No marks are indicated when vasomotion or flow motion became obscure during hypotension.](image-url)
levels of systemic hypotension, the flow motion persists despite the fact that the pial arteries are fully dilated and have lost vasomotion. This suggests that the intraparenchymal arteries retain vasomotion even at low pressure levels at which it has been lost by the pial arteries.

In conclusion, the cerebral arteries dilate very rapidly when triggered by a sudden reduction in perfusion pressure, but it still takes about 10 s for them to fully dilate and about 50 s for CBF to reach a new steady state. The initial dynamic changes in CBF after CCA-O reflect the expression of intrinsic feedback mechanisms in the vascular wall. Active vascular dilation in the territory of the occluded artery is important in the development of collateral circulation.

References