The Onset of Postischemic Hypoperfusion in Rats Is Precipitous and May Be Controlled by Local Neurons

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Background and Purpose: Reperfusion following transient global cerebral ischemia is characterized by an initial hyperemic phase, which precedes hypoperfusion. The pathogenesis of these flow derangements remains obscure. Our study investigates the dynamics of postischemic cerebral blood flow changes, with particular attention to the role of local neurons.

Methods: We assessed local cortical blood flow continuously by laser Doppler flowmetry to permit observation of any rapid flow changes after forebrain ischemia induced by four-vessel occlusion for 20 minutes in rats. To investigate the role of local cortical neurons in the regulation of any blood flow fluctuations, five rats received intracortical microinjections of a neurotoxin (10 μg ibotenic acid in 1 μl; 1.5-mm-depth parietal cortex) 24 hours before ischemia to induce selective and localized neuronal depletion in an area corresponding to the sample volume of the laser Doppler probe (1 mm^3). Local cerebral blood flow was measured within the injection site and at an adjacent control site.

Results: Ischemia was followed by marked hyperemia (235±23% of control, $n=7$), followed by secondary hypoperfusion (45±3% of control, $n=7$). The transition from hyperemia to hypoperfusion occurred not gradually but precipitously (maximal slope of flow decay: 66±6%/min; $n=7$). In ibotenic acid–injected rats, hyperemia was preserved at the injection site, but the sudden decline of blood flow was abolished (maximal slope of flow decay: 5±3%/min compared with 53±8%/min at the control site; $n=5$, $p<0.001$) and no significant hypoperfusion developed (103±20% of control at 60 minutes).

Conclusions: These data suggest that the rapid transition to cortical hypoperfusion after forebrain ischemia may be triggered locally by a neuronal mechanism but that this mechanism does not underlie the initial hyperemia. (*Stroke* 1992;23:399–406)

KEY WORDS • cerebral blood flow • cerebral ischemia • reperfusion • rats

A cute postischemic cerebral blood flow (CBF) is characterized by an initial hyperemia with subsequent hypoperfusion, which has been recognized in various animal models of transient global cerebral ischemia, in anesthetized and conscious states.[1-8] Hyperemia has been attributed to vasoparalysis due to lactic acidosis or the accumulation of vasoactive substances (adenosine diphosphate [ADP], adenosine monophosphate [AMP], potassium) during ischemia.[9-11]

The mechanisms underlying delayed hypoperfusion remain obscure. The imbalance between oxygen demands and oxygen availability during hypoperfusion may lead to secondary postischemic hypoxia as a potentially damaging consequence of transient ischemia.[3,9] This relationship, however, remains an issue of contro-

versy. In contrast to the substantial amount of data describing absolute postischemic CBF levels in different brain regions at distinct time points, not much is known about the dynamics of postischemic flow derangements. Nevertheless, the transition from hyperemia to hypoperfusion after a transient period of global ischemia has been considered to be a gradual process. Precise knowledge of the existence of any consistent postischemic flow transitions and the presence of any rapid changes would be helpful to address the underlying mechanism more specifically. A variety of mechanisms could be involved, including rheological changes and microvascular obstruction due to the aggregation of blood elements[12] and hemoconcentration, capillary obstruction by endothelial microvilli formation[13] or by neuronal, glial, and endothelial swelling,[1,14,15] all of which would be expected to result in a gradual decline of blood flow. Vasomotion due to the accumulation of vasoconstrictive substances, such as the products of arachidonic acid metabolism,[11] could also be involved in the onset and maintenance of secondary hypoperfusion. Not much attention has been paid so far to the possible contribution of neurogenic control mechanisms.

We investigated the dynamics of the local cortical CBF fluctuations after forebrain ischemia, induced by four-vessel occlusion for 20 minutes, with laser Doppler

flowmetry, which is currently the only technique to permit on-line and continuous monitoring of local CBF in a noninvasive manner. To investigate the contributions of local cortical neurons to the regulation of postischemic CBF, we have used intracortical microinjections of ibotenic acid to eliminate any local neuronal influences on CBF in the injected area without affecting other cell elements. In the current report, the extent of the injected area was gauged to correspond to the spatial resolution of the laser Doppler flow probe to allow intraindividual control laser Doppler flowmetry measurements.

Materials and Methods

The experiments reported herein were conducted according to the principles set forth in the "Guide for the Care and Use of Laboratory Animals," from the Institute of Laboratory Animal Resources, National Research Council (publication No. NIH 85-23, 1985).

The experiments were carried out on eighteen barrier-reared male Sprague-Dawley rats (Taconic Farms, Germantown, N.Y.) weighing 320–380g, housed at controlled temperature (22°C) and light/dark (12 hr/12 hr) cycle with free access to food and water. On day 1 of the experiments, anesthesia was induced by intraperitoneal injection of 12 mg ketamine and 0.12 mg acepromazine per 100 g body weight. Transient forebrain ischemia was induced according to the four-vessel occlusion technique.[16] After implantation of a femoral arterial catheter for monitoring systemic mean arterial pressure (MAP) and arterial blood gas analysis, the common carotid arteries were exposed through a midline neck incision and carefully isolated with heat-blunted glass pipettes. Nontraumatic snare loops (Silastic, outer diameter 0.63 mm; Dow Corning, Midland, Mich.) were placed loosely around the common carotid arteries. The Silastic snares were attached to cleaning wires (diameter 0.02 mm; Hamilton, Reno, Nev.) and tunneled subcutaneously to be exteriorized on the back of the animals through Silastic guide tubing (inner diameter 0.75 mm, outer diameter 1.63 mm). The guide tubing was extended at the carotid end by a piece of polyethylene tubing (PE-240) that stiffened the guide tubing tip and allowed it to be sutured to the adjacent sternocleidomastoid muscle. Skin incisions were closed by suture. The rats were then placed in a stereotaxic instrument to expose the body of the atlas by a midline posterior neck incision. The vertebral arteries were permanently occluded by monopolar cautery through the alar foramina.[16] Additionally, a neck tourniquet (2-0 silk) was implanted to encompass the prevertebral and posterior neck muscles and exteriorized on the posterior neck, and the wounds were closed by suture.

In eight rats, the calvaria were exposed and a rectangular craniectomy (2×4 mm) was performed above the right parietal cortex, using a high-speed drill under the operating microscope, leaving the dura mater intact and avoiding heat-damage by frequent saline irrigation. Glass micropipettes (tip diameter 75–100 μm) were filled with either ibotenic acid ($n=5$) in a dose of 10 μg in 1 μl of phosphate-buffered saline (pH 7.4) or vehicle alone ($n=3$) and attached to a micromanipulator. Loading and discharging of the micropipettes were achieved by a microinjection pump (CMA/100, Carnegie Medicine, West Lafayette, Ind.). The micropipettes were
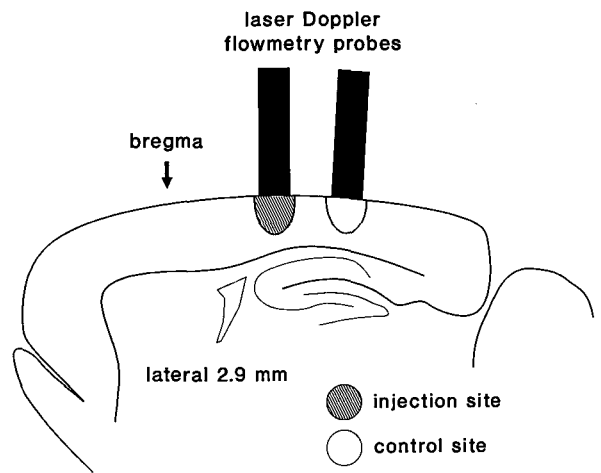

FIGURE 1. *Schematic view of lateral section of rat brain 2.9 mm lateral to bregma. Positions of adjacent laser Doppler flowmetry probes are indicated. There was no overlap of local CBF sampling between injected and noninjected control site.*

aimed perpendicularly at a zone of the parietal cortex devoid of major pial vessels, located approximately 3 mm lateral and 3 mm caudal to the bregma. The dura was pierced and the micropipette lowered 1.5 mm below the dural surface. Ibotenic acid or the vehicle was delivered at a rate of 0.2 μl/min over 5 minutes.[17] At the end of the injection, the micropipettes were left in place for 5 minutes and then withdrawn, the bone flap replaced, and the wounds closed by suture. After these preparations, all animals were returned to their cages and allowed to recover for 24 hours with free access to water only.

At day 2, the animals were first anesthetized with 4% halothane in an induction chamber and then transferred to a stereotaxic frame. Anesthesia was maintained with 1% halothane in nitrous oxide (70%) and oxygen (30%), delivered by a closed face mask. Rectal temperature was monitored and maintained at 37°C by a heating pad. In the seven noninjected animals, a rectangular craniectomy was performed in the same location and in the same fashion as in the injected animals described above. In the noninjected group, one laser Doppler flow probe (P435, Vasamedics) was placed above an area that corresponded to the injected site of the injected group and was devoid of major pial vessels. The laser Doppler flow probe was held by a stereotaxic arm and allowed to hang freely for approximately 10 cm before it made contact with the dura (without exerting pressure) to dampen vibration-related artifacts. Normal saline was applied to prevent drying of the dura. The probe was connected to a perfusion monitor (LaserFlo BPM403, Vasamedics) for continuous measurements of local CBF. Output from this device was directed as a voltage signal to a pen chart recorder. When the laser Doppler flow probe was positioned, external conditions were kept constant, particularly the level of ambient light.

In the injected groups, two laser Doppler flowmetry probes were placed in the same fashion, one superimposed on the injected site and the other at an adjacent control site (distance approximately 2 mm) of the ipsilateral hemisphere (Figure 1). The position of the

TABLE 1. Physiological Variables Before (Baseline), During (5 Minutes Before Reperfusion), and 30 Minutes After Forebrain Ischemia and Body-Weight Loss (7-Day Survival) in Noninjected, Ibotenic Acid–Injected, and Vehicle (Phosphate Buffered Saline)-Injected Animals

Variable	Noninjected (n=7)	Ibotenic acid–injected (n=5)	Phosphate buffered saline–injected (n=3)
MAP (mm Hg)			
Baseline	98±4	101±4	95±3
Ischemia	126±5*	129±7*	120±3*
30 minutes	110±3	106±4	103±5
Po₂ (mm Hg)			
Baseline	149±8	149±5	151±5
Ischemia	132±9	136±5	137±6
30 minutes	126±5*	130±3*	129±6*
Pco₂ (mm Hg)			
Baseline	31±3	30±2	29±3
Ischemia	30±2	32±2	28±2
30 minutes	36±3	36±3	33±4
pH			
Baseline	7.42±0.02	7.43±0.02	7.41±0.01
Ischemia	7.44±0.01	7.40±0.01	7.42±0.01
30 minutes	7.39±0.01	7.37±0.02	7.39±0.01
Body weight loss (%)	−18±2	−19±2	−20±2

Data are mean±SEM. MAP, mean arterial pressure.
*$p<0.05$ difference between experimental and baseline values within a group.

probes ensured that no overlap sampling of local CBF occurred between the injected site and the control site.

Forebrain ischemia was induced by temporary occlusion of the common carotid arteries and tightening of the neck tourniquet for 20 minutes. During ischemia, halothane was reduced to 0.5% because higher halothane concentrations can impair autoregulation and alter CBF.[18] Reperfusion was initiated by release of the carotid occluders and removal of the neck tourniquet. With the onset of reperfusion, halothane was discontinued for 30 minutes to exclude any side effects from anesthesia. Local CBF and MAP were monitored before, during, and for 60 minutes after ischemia. Arterial blood gas samples were taken before ischemia, 5 minutes before the onset of reperfusion, and at 30 minutes after ischemia. At the end of the observation period, the wound was closed by suture, and the animals were returned to their cages and allowed to survive for 7 days with free access to food and water. At the end of the experiment, the animals were anesthetized with an overdose of pentobarbital and perfused through the left side of the heart with 150 ml normal saline, containing 10 IU heparin/ml, followed by 150 ml 10% buffered formalin (pH 7.2). The brains were kept in fresh fixative overnight and then embedded in paraffin for coronal cutting. Serial sections (6 μm) were stained with hematoxylin/eosin for assessment of ischemic damage and quantification of the ibotenic acid–induced neuronal depletion. Lesion dimensions in the cortical zone exposed to ibotenic acid or vehicle in the injected group were assessed by an eyepiece micrometer disk in the section with the widest lesion extension.

In three additional rats, forebrain ischemia was induced by bilateral carotid occlusion combined with systemic hypotension[19] by controlled arterial hemorrhage. Therefore, two arterial femoral catheters were implanted on day 1 of the experiment. Otherwise, the experimental animals were subjected to the same surgical procedures as described above except permanent occlusion of the vertebral arteries and cortical microinjection. Forebrain ischemia was induced on the second experimental day by lowering the MAP to 50 mm Hg combined with bilateral carotid occlusion for 20 minutes under ketamine/acepromazine anesthesia. Laser Doppler flowmetry was monitored throughout the experiment as described above.

All data are mean±SEM of the indicated number of rats, if not otherwise stated. Statistical analysis was done by Wilcoxon matched pairs test or Kruskal-Wallis analysis of variance followed by Mann-Whitney U test. Values of $p<0.05$ were accepted as statistically significant.

Results

The induction of forebrain ischemia was followed by a significant increase in the MAP resulting from the baroreflex inhibition upon occlusion of the common carotid arteries (Table 1) throughout ischemia. The MAP returned to baseline after the onset of reperfusion. The arterial blood gases remained in the normal range throughout the experiment and showed no statistical differences between the groups (Table 1). None of the animals of either group showed any signs of distress before ischemia.

Neuropathology at day 7 following 20 minutes of forebrain ischemia revealed bilateral hippocampal degeneration in the CA-1 region (Figure 2A). Scattered neuronal necrosis was found in cortical layers 3, 5, and 6, as well as in the striatum. The changes were similar in the noninjected as well as both injected groups (ibotenic acid or phosphate buffered saline).

In the ibotenic acid–injected animals, a well-demarcated, hemispheroid-shaped area of cortical neuronal
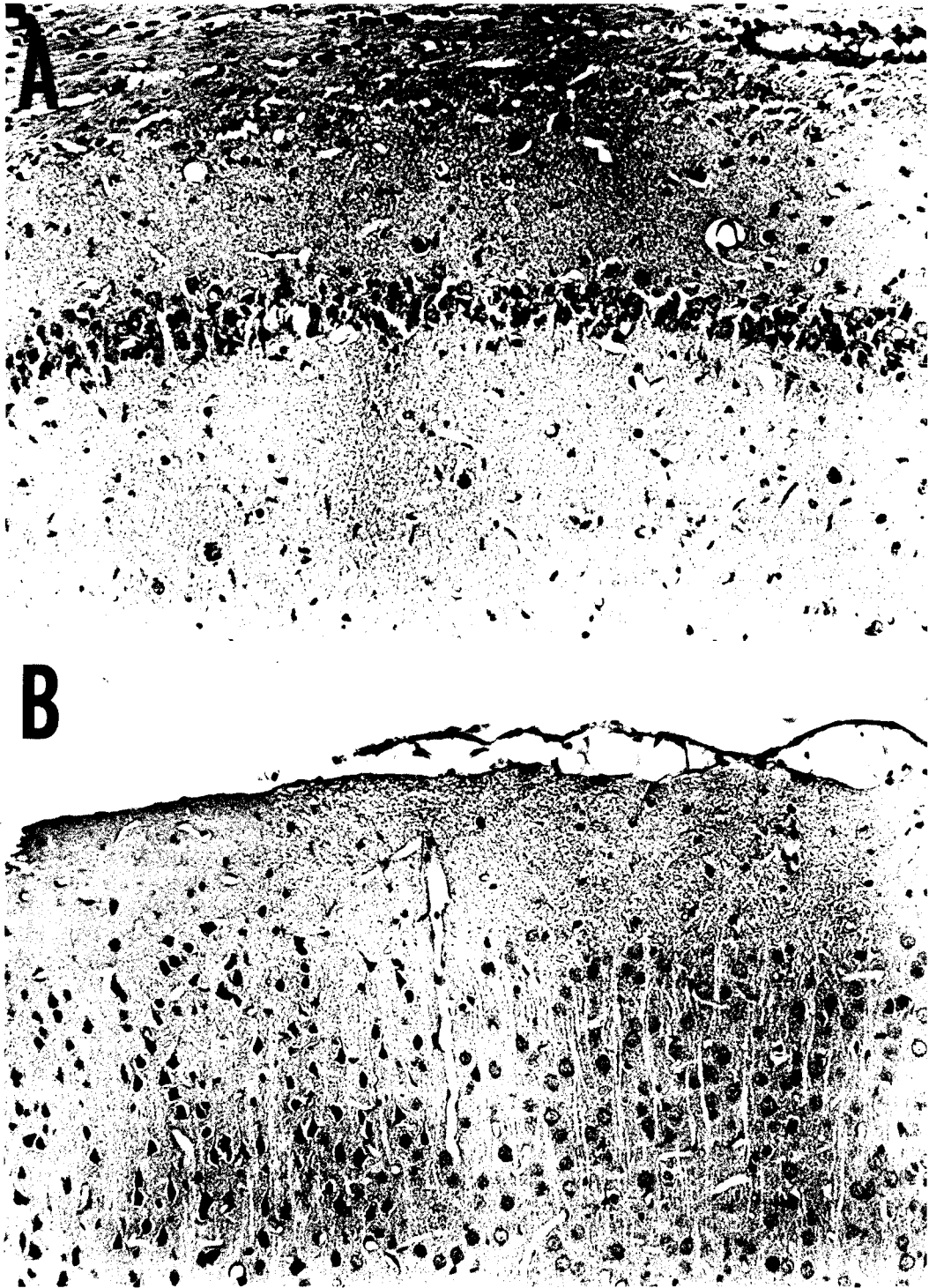
FIGURE 2.   Panel A: *Histological appearance of the hippocampal CA-1 subfield at 7 days after 20 minutes of forebrain ischemia, showing a reduction of viable neurons to approximately one third. Hematoxylin and eosin stain. Magnification ×160. Panel B: Histological aspect of sharply demarcated border zone between ibotenic acid–induced lesion and adjacent cortical tissue 7 days after forebrain ischemia (8 days after microinjection). Ibotenic acid–induced lesion on left side of photomicrograph is characterized by complete neuronal necrosis with a sharp transition to normally appearing cortical tissue on right. Hematoxylin and eosin stain. Magnification ×160.*

loss with a diameter of 1.5±0.2 mm (n=5) was observed at day 7 after ischemia (Figure 2B).

The injection site of the phosphate buffered saline–injected animals did not show any changes to discriminate it from the adjacent cortical areas.

Induction of forebrain ischemia was followed by an immediate drop in local cortical CBF (Figure 3, insert). The flow reduction was calculated as percentage of baseline, which was established 20 minutes before ischemia. The average blood flow reduction during ischemia
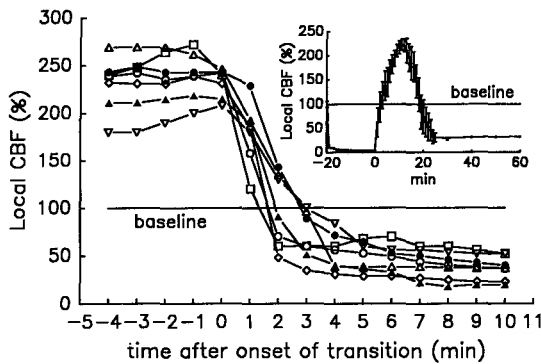
FIGURE 3. *Graph of local cerebral blood flow (CBF) after 20-minute forebrain ischemia in noninjected rats. Graph emphasizes transition period from hyperemia to hypoperfusion to highlight the suddenness of changes in individual courses. Time point zero indicates onset of transition. Insert graph demonstrates the average changes of local cortical CBF during and for 60 minutes after 20 minutes of transient forebrain ischemia, showing the familiar pattern of an initial hyperemic phase followed by delayed hypoperfusion.*
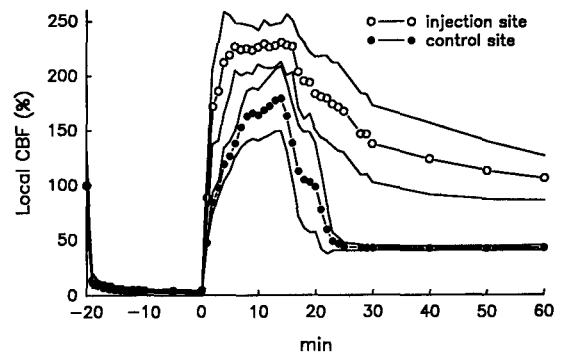

FIGURE 4. *Graph of local cerebral blood flow (CBF) after 20-minute forebrain ischemia in ibotenic acid–injected rats. Laser Doppler flowmetry was assessed at injection site (○) (data are mean+SEM) and adjacent control site (●) (data are mean−SEM). Pattern of postischemic blood flow changes in control sites was similar to findings in noninjected group. At injected site hyperemia was preserved, but sudden decline in local CBF did not occur and state of hypoperfusion did not develop.*

was similar in all groups and at all sites (Table 2). Reperfusion was characterized by a hyperemic response at all measured sites, including the ibotenic acid–injected site (Table 2 and Figures 3 and 4). Maximal hyperemic CBF values in noninjected control rats were 235±23% (*n*=7). The magnitude of hyperemia, calculated as the area under the curve between 0 and 20 minutes after ischemia, was not significantly different among the groups or sites (Table 2), although we observed a tendency for exaggerated hyperemia in the ibotenic acid-injected site. In the noninjected group, hyperemia continued for 17±1 minutes (Figure 3, insert). Then, local CBF precipitously decreased to approximately one third of baseline (Figure 3) within a few minutes without any indication of a gradual decline in blood flow in any of the individual experiments. The drop in flow was unrelated to the MAP and showed no tendency for recovery within the observation period of 60 minutes after ischemia (Figure 3, insert). The precipitous decline of local CBF occurred at distinctly different time points up to minutes apart in adjacent laser Doppler flowmetry monitoring sites within the same experimental animal.

In the control sampling site of the ibotenic acid–injected rats, the same pattern could be reproduced (Figure 4). In the ibotenic acid–injected site, however,

the sudden decline of blood flow after hyperemia was completely abolished (Figure 4). Hypoperfusion was absent, and local CBF returned gradually and asymptotically to baseline by 60 minutes of reperfusion (Table 2). The slope of the blood flow changes during the transition phase in noninjected animals (Figure 3 and Table 2) was substantially steeper than in the ibotenic acid–injected site (Figure 4 and Table 2). The slope at the control site of the ibotenic acid–injected rats was not different from the noninjected animals (Figure 4 and Table 2).

The changes of local CBF in the phosphate buffered saline–injected animals were similar to those observed in the noninjected group at both the injected and the control site (Table 2).

Figure 5 shows an exemplary course of changes of local CBF and MAP in an animal subjected to forebrain ischemia by two-vessel occlusion and systemic hypotension under ketamine/acepromazine anesthesia. The postischemic flow changes, particularly the abrupt transition from hyperemia to hypoperfusion, were similar to those observed in the four-vessel occlusion model. This finding could be reproduced in two additional animals. The slope of the blood flow changes during the transition phase in this additional group of animals was not

TABLE 2. Local CBF Expressed As Average Area/Min Under Laser Doppler Flowmetry Curve During Ischemic (−20 to 0 Minutes), Hyperemic (0–20 Minutes), and Hypoperfusion (30–60 Minutes) Periods in Percentage of Baseline

Period	Noninjected (*n*=7)	Ibotenic acid–injected (*n*=5)		Phosphate buffered saline–injected (*n*=3)	
		Control site	Injection site	Control site	Injection site
Ischemia (%)	5.1±0.7	3.3±1.3	5.9±1.6	4.9±0.7	4.8±0.2
Hyperemia (%)	154±10	140±30	187±33	154±40	164±10
Hypoperfusion (%)	45±3	44±4	113±30*	42±1	45±4
Transition–slope (%/min)	66±6	53±8	5.9±3.2*	59±19	62±7

Data are mean±SEM. CBF, cerebral blood flow.
    *$p<0.05$ with respect to areas of ischemia, hypoperfusion, and hyperemia and rate of transition from hyperemia to hypoperfusion.
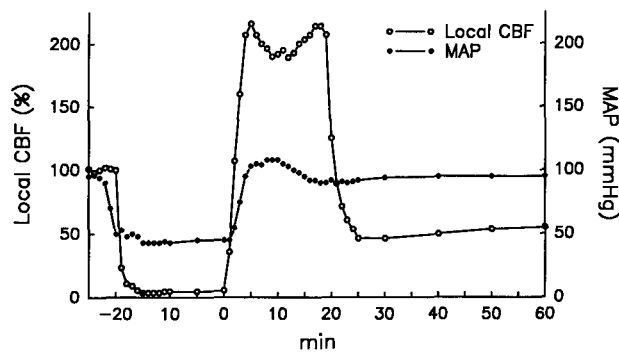
FIGURE 5. *Local cerebral blood flow (CBF) and mean arterial pressure after 20-minute forebrain ischemia by bilateral carotid occlusion combined with systemic hypotension by controlled arterial hemorrhage under ketamine/acepromazine anesthesia. Reperfusion is characterized by hyperemia followed by precipitous decline of local CBF to delayed hypoperfusion, similar to flow changes observed in the four-vessel occlusion model using halothane/nitrous oxide anesthesia.*

different ($69 \pm 11\%$/min, $n=3$) from the control findings in the animals subjected to four-vessel occlusion.

Discussion

We have studied the dynamics of postischemic CBF changes by laser Doppler flowmetry. It could be demonstrated that the transition from hyperemia to delayed hypoperfusion after transient forebrain ischemia does not occur in a gradual manner but is, instead, precipitous. The time point of transition to cortical hypoperfusion showed regional variability being up to minutes apart in adjacent cortical areas. Elimination of the local neuronal activity in a circumscribed cortical area abolished the rapid CBF decline but did not affect the initial hyperemia. Taken together, these data suggest that the transition to postischemic cortical hypoperfusion following transient forebrain ischemia is regulated by a locally integrated mechanism, which requires viable neuronal cells within a cortical region to be operable. The presence of local cortical neurons appears not to be essential for the development of the initial transient hyperemia. These findings could be reproduced in two different models of transient forebrain ischemia, the four-vessel occlusion and the two-vessel occlusion models, and was independent from the anesthetic agent, halothane or ketamine/acepromazine. This suggests that the pathological mechanisms involved in the regulation of the observed postischemic CBF responses are likely to be identical and not an artifact of the specific ischemia model or the type of anesthesia.

Laser Doppler flowmetry has introduced the opportunity to measure local CBF on-line and continuously without affecting the state of circulation by the measurement. The method has been validated for measurement of cortical CBF changes in rats.[20] Based on theoretical estimates, it is generally believed that the spatial resolution of the laser Doppler flow probe used in the present report approximates 1 mm^3.[21] The area of neuronal depletion by ibotenic acid microinjections resembled a hemispheroid and was approximately 1.5 mm in diameter and depth, with a resulting volume of approximately 0.9 mm^3. Therefore, it might be assumed

that the region of local CBF sampling by laser Doppler flowmetry and the zone of neuronal depletion were nearly congruent if the probe was positioned above the injection site.

Cortical ibotenic acid microinjections have been used previously to study the role of localized intrinsic neuronal populations in the regulation of local CBF.[17,22–24] Ibotenic acid, a conformationally restricted analog of glutamic acid, causes acute neuronal degeneration, which is thought to be mediated by a glutamatergic excitatory mechanism.[22] Neuronal depletion is complete by 24 hours after injection and strictly confined to the injected area, possibly due to a low diffusion rate and rapid metabolism.[17,22] Nonneuronal cells (glia, endothelium) and extrinsic nerve terminals and fibers on passage are initially not affected. Glial fibrillary acidic protein immunoreactivity remains unchanged acutely after ibotenic acid injection but has increased 3 days later at the borderzone of the lesion, reaching maximum reactivity between 5 and 7 days.[17] At this time point, other cellular elements, including macrophages and endothelium, have also proliferated.[17] This prompted us to choose the 24-hour time point for induction of ischemia to avoid the possibility that proliferating non-neuronal elements could affect postischemic CBF. Retrograde and anterograde axonal transport is maintained within an ibotenic acid–injected cortical area. This was tested by microinjections of wheat germ agglutinin conjugated to horseradish peroxidase either into the lesion or into the homotopic area of the contralateral hemisphere.[17] The lack of an effect on neurotransmitter levels such as norepinephrine, dopamine, or choline acetyltransferase after striatal or hippocampal ibotenic acid injections further supports the view that terminals of extrinsic origin to the injected area are not affected.[22] Resting cortical CBF remains within the normal range during the first days after elimination of the intrinsic cortical neuronal population by ibotenic acid.[17] The vasculature in the ibotenic acid–injected region remains reactive to certain physiological stimuli that produce vasodilation such as hypercapnia.[23] Conversely, the injected area is also responsive to vasoconstrictive stimuli: endothelin-1, microinjected (200 fmol/200 nl; unpublished observation from this laboratory) into the lesion, caused a massive and precipitous decrease in local cortical perfusion monitored by laser Doppler flowmetry upon endothelin injection, which was virtually identical to previous findings in normal cortical tissue.[25]

Under physiological conditions, CBF is thought to be regulated through myogenic, biochemical, and neurogenic mechanisms.[26–29] Myogenic mechanisms operate mainly in muscular arterioles, which constrict in response to distending pressure to maintain capillary perfusion constant. The metabolic activity of neurons and glia may result in the activation of vasodilatory mechanisms such as tissue hypoxia, hypercarbia, and the accumulation of adenosine, lactic acid, potassium, and other factors. The coupling of CBF and cerebral metabolism could thereby function in a highly localized and restricted manner to match neuronal activation with the supply of nutrients. A prime candidate for the metabolic regulation of local CBF has, however, not yet been identified.[30] Extraparenchymal and intraparenchymal cerebral blood vessels are adjoined by nerves at all segments. Extrinsically, the major cerebral vessels re-

ceive an elaborate innervation by sympathetic, parasympathetic, and sensory nerve fibers from cranial ganglia, involving a variety of transmitters such as vasoactive intestinal peptide, acetylcholine, substance P, neuropeptide Y, and calcitonin gene-related peptide.[31] Recent interest has been focused on the role of intrinsic nervous mechanisms, such as innervation of cerebral blood vessels by muscarinic fibers from the cerebellar fastigial nucleus,[32] or by noradrenergic fibers originating in the locus coeruleus of the brain stem,[33] and by other brain stem nuclei. Most of the proposed mechanisms, with the possible exception of the metabolic theories, emphasize the regulation of global and microcirculatory CBF at the level of larger arteries. Not much attention has been paid to the possibility that microcirculatory blood flow may be mainly regulated on a microcirculatory level, although it has been long suspected that periodic constriction and dilatation ("vasomotion") of intrinsic arterioles and venules in the densely vascularized neopallium would serve as regulators of flow in columns of brain cells.[34]

Transient global ischemia sets into motion a complicated pathophysiological sequence, which is only partially understood. Hyperemia upon reperfusion is thought to arise from vasoparalysis, with loss of the ability to autoregulate CBF, possibly due to accumulation of lactic acid and other vasoactive mediators during ischemia. Recent studies indicate that cortical hyperemia might also be mediated by an axon reflex-like mechanism via the trigeminal nerve.[35] Other studies have shown that some central vasodilatory pathways depend on interposed local cortical neurons, such as the vasodilation elicited by stimulation of the cerebellar fastigial nucleus,[23] and others, elicited by stimulation of the basal forebrain do not.[24] Our results suggest that the interposition of local neuronal cells are not essential for the development of the initial hyperemia, which may involve central neurogenic mechanisms with direct effects on cerebral vessels. Up to now it had been generally believed that, after the hyperemic phase, CBF would return to baseline and below gradually. Autoregulation of CBF actually has returned with the onset of hypoperfusion but CO_2 reactivity is lost.[9] Several mechanisms have been proposed to contribute to delayed hypoperfusion, and the mechanism of flow reduction as well as the degree of flow impairment may vary with such factors as duration of ischemia, type of ischemia, and severity of endothelial damage. Edema,[14,15,36] intravascular cell aggregates such as platelets and polymorphonuclear leukocytes,[12,37] rheological changes due to hemoconcentration, endothelial microvilli formation,[13] and vasomotion due to the release of vasoactive mediators have all been postulated to lead to the postischemic decrease in cerebral perfusion. With the exception of vasomotion, the above-mentioned mechanisms would not be expected to result in an abrupt decline of flow, as observed in the present study. Vasomotion could be based on a metabolically or neurogenically mediated process, but it could also result from the accumulation of vasoactive mediators such as arachidonic acid metabolites,[11,38,39] including leukotrienes and thromboxane A_2, as a consequence of reperfusion injury. Neurogenic control of hypoperfusion could involve the local release of vasoactive substances such as neuropeptide Y, norepinephrine, or endothelin. Theoretically, this could be directly modulated by a central, subcortical mechanism, but may or may not require local neurons to operate. The involvement of local neurons would permit the regulation of postischemic CBF on a local level influenced by or even based on local conditions. This hypothesis would be compatible with the observation that cortical hypoperfusion develops precipitously at regionally distinct time points. It has to be emphasized, however, that although it appears that the transition to hypoperfusion in the current study would be triggered by a common and integrated neurogenic mechanism, it is very likely that other pathophysiological events are involved and may supervene to influence postischemic CBF.

It might be speculated, however, that local CBF could be controlled by a subpopulation of intrinsic neurons that serve a dependant group of cortical neurons along with their vascular supply in the meshwork of a functional unit. These regulatory cells could receive innervation from subcortical structures or could react to changes in the local micromilieu. Such a system has been described for the noradrenaline- and vasoactive intestinal peptide–containing neuronal systems in the neocortex,[40] influencing other neurons, glia, and the vasculature by direct innervation or perivascular release of vasoactive mediators. In this scheme, noradrenergic fibers are thought to innervate the cerebral cortex in a global manner. The noradrenergic fibers are organized in a tangential orientation in a plane parallel to the pial surface, with branches to bipolar cortical neurons, containing vasoactive intestinal peptide. These local neurons are oriented in a perpendicular fashion to the cortical surface and receive specific thalamic inputs. Coactivation of both systems would be able to create cortical "hotspots," possibly in response to functional demands. A similar system may regulate blood flow in the postischemic period and account for rapid flow changes. Since the transition from hyperemia to hypoperfusion seems to require viable neurons, it might actually indicate functional recovery and even signal a desirable effect in the sequence of postischemic events. In such a model, endogenous vasoactive mediators such as neuropeptide Y and endothelin are good candidates to act as powerful vasoconstrictors.

Future studies are needed to elaborate on related issues such as the relationship between acute and chronic postischemic perfusion changes and neuronal survival and functional recovery. Therefore, the elucidation of the pathogenesis of the postischemic CBF derangements appears to be important to generate a more comprehensive concept of the pathophysiology of cerebral ischemia and reperfusion.

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