Experimental Model of Small Deep Infarcts Involving the Hypothalamus in Rats
Changes in Body Temperature and Postural Reflex

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Background and Purpose—Intraluminal middle cerebral artery (MCA) occlusion in rats has been reported to cause hyperthermia assumed to be caused by hypothalamic damage. To clarify the effects of hypothalamic ischemia on body temperature and to obtain a model simulating lacunar infarction, we attempted to produce small infarcts in deep structures (including the hypothalamus).

Methods—A surgical suture was advanced to occlude the origin of the hypothalamic (HTA) and/or anterior choroidal arteries (ACHA) without compromise of the anterior or middle cerebral artery origins. After treatment, rectal temperature and postural reflex were examined repeatedly for 3 days under nonanesthetic conditions. The AChA and HTA and their link with small deep infarction were then confirmed by TTC, hematoxylin and eosin, and TUNEL stains and by microsurgical dissection after colored silicone perfusion into the cerebral arteries.

Results—Advancement of the suture near to but not occluding the MCA origin (0.5 to 1.9 mm proximal) produced small, deep, nonneocortical stokes in 25 of 36 animals without producing MCA ischemic changes. These infarctions mainly affected the hypothalamus in 13 animals (HTA area: infarct volume 6±1 mm³) and involved both the internal capsule and hypothalamus in 12 animals (HTA+ACHA area infarct volume 48±10 mm³). Rats with HTA infarction alone exhibited persistent hyperthermia for 72 hours; some also had transient mild postural abnormality. The AChA+HTA infarct group showed a transient elevation of body temperature for 24 hours and definitive postural abnormality. In the remaining 11 animals, the suture was inadvertently advanced across the MCA origin, producing a large infarct that affected both the neocortex (MCA territory) and nonneocortical structures (volume 381±30 mm³, n=11). The MCA infarct group displayed a transient hyperthermia and severe postural abnormality.

Conclusions—When properly positioned, the intraluminal suture method permits selective AChA and/or HTA obstruction without inducing MCA territory ischemia. This model confirms that selective hypothalamic infarction produces significant and sustained temperature regulation abnormalities. The model also may be useful in investigating the pathophysiology of small, deep, end-vessel infarction. (Stroke. 1999;30:2743-2751.)

Key Words: cerebral ischemia, focal choroid plexus hypothalamus, internal capsule middle cerebral artery

Fever is a common accompaniment of stroke and is more common in the acute stage and in patients with larger lesions.1,2 Because even mild changes in body temperature can influence the extent of ischemic cerebral injury, most experimental stroke studies are performed under strict thermal control. This elevation of body temperature may be the result of an associated infection, but the possibility that the cerebral damage from the stroke itself causes the hyperthermia has not been excluded. To our knowledge, selective hypothalamic ischemia has never been intentionally produced to test this hypothesis experimentally.

Intraluminal middle cerebral artery (MCA) occlusion can cause persistent fever in rats lasting for at least 24 hours, and

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the hyperthermia can be correlated with ischemia in the hypothalamus and preoptic area.3–6 The intraluminal thread has heretofore been thought to obstruct the anterior cerebral and/or anterior choroidal artery (ACHA), a branch of the internal carotid artery (ICA) that arises just proximal to the MCA bifurcation, thereby reducing flow within the arteries that supply the hypothalamus.7,8 In contrast, the transorbital MCA occlusion method obstructs the MCA without compromise of these proximal branches, thereby avoiding the hypothalamic damage and making body temperature elevations less likely.9

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In rats, 2 small arteries arise from the distal ICA proximal to the MCA bifurcation: the hypothalamic artery (HTA) and the AChA. Intraluminal thread advanced toward the MCA bifurcation probably causes the obstruction of these 2 small arteries concomitant with MCA trunk obstruction. Studies in humans or dogs indicate that AChA occlusion does not cause hypothalamic damage in these species, but whether the AChA supplies the hypothalamic in rats is still unclear. This study was designed to test whether one or both of these arteries could be selectively obstructed to produce focal hypothalamic ischemia and whether the production of an isolated lesion could help clarify the anatomic substrate and mechanisms of ischemia-induced hyperthermia. Additionally, the development of a small, penetrating, artery infarction model could be useful in studying lacunar infarction.

Materials and Methods

The experimental protocols were approved by the Institutional Animal Care and Use Committee of National Cardiovascular Center, Osaka, Japan, and the University of Florida, Gainesville. Eighty 10-week-old rats, purchased from Oriental Yeast Co (Kyoto, Japan) and Charles Rivers Laboratories (Wilmington, Mass), were caged in groups of 5 and acclimatized with a laboratory diet and tap water ad libitum under a fixed light-dark cycle for 2 to 3 weeks before experimentation. Sixty rats were then randomly divided into 3 groups according to the type of intraluminal procedure, including (1) proximal suture placement attempting to produce deep infarction without MCA involvement (n=36), (2) sham-operated control (no occlusion of the HTA, AChA, or MCA, n=5), and (3) MCA occlusion (n=13). As described later, the 3 groups were partially intermixed during later portions of the experiment. The remaining 20 rats were used to measure arterial blood gases and blood pressure for any changes after isolated HTA and/or AChA territory infarctions and for perfusion studies.

Surgical Procedure and Infarction Techniques

All operations were performed between 3:30 and 6:00 pm to control for any diurnal variations in temperature. Rats were anesthetized with 2% isoflurane in a mixture of 30% oxygen and 70% nitrous oxide delivered by face mask. During the operation, rats were warmed by a heating pad set at 37.0°C. Introduction of cerebral ischemia was initiated within 25 minutes after anesthetic induction. Each rat was kept in a separate cage after surgery and allowed to recover from anesthesia at room temperature (21 ± 1°C).

In an 11 mm deep infarction (proximal) group, the protocol was designed to induce an infarct in the HTA or AChA territories without affecting the MCA territory. The occluder tip was aimed to reach a position 1 to 2 mm proximal to the MCA bifurcation, a point chosen because the distance between the AChA and the MCA was >1 mm in female rats, whereas the distance between the HTA and the MCA was not always clear. Briefly, with the use of an operating microscope, a 3-0 nylon suture coated with 0.1% poly-L-lysine was introduced through the right common carotid artery (CCA) into the right ICA and advanced intracranially until resistance was encountered. When the advanced distance from the CCA bifurcation was shorter than 18 mm, the occluder tip was presumed to have reached the MCA bifurcation. The suture was then withdrawn 2 mm to allow the MCA origin to remain unobstructed and free to receive blood retrograde from the anterior cerebral artery. If the suture could be advanced ≥18 mm from the CCA bifurcation without feeling resistance, it was assumed that the occluder tip had crossed beyond the MCA origin from the ICA and was now within the anterior cerebral artery. The suture was then withdrawn to a position 15 to 16 mm from the CCA bifurcation.

In the sham-operated control group, the occluder was advanced 13 mm from the CCA bifurcation, thereby avoiding compromise of none of the deep penetrating vessels or the MCA. In the MCA-infarction group, the occluder was advanced until resistance was felt or >18 mm from the CCA bifurcation. Once proper placement was attained, the suture was secured in this position and left in place for the remainder of the experiment. At 72 hours, the animals were euthanized and investigated for catheter position, infarct size and location, and associated microscopic changes.

Since the induction of isolated deep cerebral ischemia in the proximal occlusion group was done blindly, at least 4 results could be predicted: no infarction or strokes in the HTA, AChA, or MCA territories. If rectal temperature elevation by >0.3°C and/or abnormal postural reflexes did not appear within 1 hour after surgery (supposing that the manifestations indicate HTA or AChA territory ischemia), the rat was subjected to a second operation, advancing the occluder 0.5 to 1 mm further. Six of 36 rats received the second operation.

In a parallel experiment, 3 groups of 5 rats each (proximal, sham-operated, and MCA occlusion) were randomly selected for determination of arterial blood gases and blood pressure. In these rats, the femoral artery was cannulated and used to continuously record arterial blood pressure until 10 minutes after the onset of cerebral ischemia. Arterial blood sampling for measurements of pH, PaCO₂, and PaO₂ was performed twice, once at rest before surgery and the other at 10 minutes after the onset of ischemia.

Body Temperature Measurement and Postural Reflex Test

Body temperature was measured by a probe temporarily inserted 2 cm into the rectum. Measurements were recorded before and 1, 3, 24, 48, and 72 hours after surgery in hand-hanled, nonanesthetized rats. The resting level temperature before surgery was measured at 3:00 to 3:30 pm in all animals.

To evaluate sensorimotor function, the postural reflex test developed by Bederson et al was carried out at the same time as body temperature determination. The degree of abnormal posture was estimated by suspending rats with their tails 20 cm above the floor. Intact rats extended both forelimbs toward the floor. Rats displaying this behavior were recorded as a score 0. Abnormal posture included flexing the contralateral limb toward the body and/or rotating the contralateral shoulder and limb medially. If the abnormal posture was observed, the rat was placed on a sheet of soft plastic-backed paper that could be gripped by its claws. Lateral pressure was applied from behind the shoulders so that the forelimbs slid gently to the left and then to the right. Rats that resisted sliding in both directions were graded as 1, more severely affected animals exhibiting a decreased resistance to the lateral push were scored as 2, and those that circled toward the paretic side consistently were graded as 3.

Confirmation of Occluder Tip Location

Seventy-two hours after ischemia onset, each animal was reanesthetized with pentobarbital (50 mg/kg body wt) and killed by decapitation; changes in the catheter position were carefully avoided. An ipsilateral craniectomy was performed, and the tip of the occluder was exposed by slightly elevating the temporal cortex with fine forceps. The tip of occluder relative to the MCA was confirmed in most rats with photography. Establishment of the catheter relation to the MCA was handicapped in MCA occlusion animals because of difficulties in retracting infarcted neocortical tissue. In sham controls, photography was not done because the occluder tip was located deep under the brain base and was not visible. Instead, the distance between the tip of the occluder and the coronal bone crevice of the cranial base underneath the optic chiasm was measured after brain removal.

To further clarify the relation between HTA and/or AChA occlusion and small deep infarcts, 5 rats subjected to each type of surgical procedure were perfused with colored silicone after perfusion of 100 mL of saline and 10% phosphate-buffered formalin just before decapitation. The circle of Willis was exposed by use of microsurgical technique and photographed for evidence that the occluder was obstructing the HTA and/or AChA proximal to the...
MCA. Brain samples were then submitted for histological analysis with hematoxylin and eosin and TUNEL staining methods.

**Histological Assessment**

**TTC Staining**

Each brain was coronally sectioned into seven 2-mm-thick slices starting from the frontal pole and stained with TTC. Areas ipsilateral to the occlusion not stained red with TTC were recorded as infarcted. All samples \( n = 13 \) with an infarct limited to the hypothalamus or those with AChA infarct without showing TTC lesion in the hypothalamus \( n = 5 \) were further analyzed by hematoxylin and eosin staining, and some were corroborated by the TUNEL method.

**Hematoxylin and Eosin Staining and In Situ Detection of TUNEL-Positive Cells**

Hematoxylin and eosin staining and the terminal deoxynucleotidyl transferase (TdT)-mediated deoxyuridine triphosphate–biotin nick labeling (TUNEL) method were used to define the ischemic area more precisely and to prevent a potential underestimation of infarction size and distribution. All coronal sections in the HTA infarct group, the 3 slices located 4 to 6, 6 to 8, and 8 to 10 mm from the frontal pole in the AChA infarct group (which were used for evaluating ischemic lesion by TTC), and those samples perfused with colored silicone were paraffin-embedded and cut into 5-μm slices. Adjacent 5-μm slices were evaluated for morphological changes with the use of hematoxylin and eosin staining and the TUNEL method.

The presence of TUNEL-positive cells was assessed in situ by direct immunoperoxidase detection of digoxigenin-labeled 3′-OH DNA stand breaks with the TUNEL method\(^{15,16}\) (the Apop Tag in Situ Apoptosis Detection Kit–Peroxidase). The 5-μm slices were washed twice with xylene for 5 minutes, 95% and 70% ethanol for 3 minutes each wash, and double-distilled water. They were then treated with 20 μg/mL proteinase K (Sigma Chemical Co) for 15 minutes at room temperature and washed 4 times with distilled water for 2 minutes. Endogenous peroxidase was inactivated by covering the sections with 2% \( \text{H}_2\text{O}_2 \) for 5 minutes at room temperature. The sections were rinsed with PBS and immersed in TdT buffer for 15 minutes at room temperature. TdT enzyme was then added to incubate at 37°C for 60 minutes. The reaction was terminated by stopping buffer for 30 minutes at 37°C. After washing 3 times with PBS for 5 minutes, the sections were treated by anti-digoxigenin-peroxidase for 30 minutes at room temperature, washed again with PBS, and visualized by DAB Chromagen. After methyl green staining, the sections were washed again, dried, and mounted.

Figure 1. Schematic maps showing cerebral infarcts (left side) in 3 different territories of cerebral arteries, the MCA, AChA, and HTA, correlated to 3 different locations (right side) of the occluder tip. MCAI indicates MCA infarct; AChAI, AChA infarct; HTAI, HTA infarct; CUP, caudatoputamen; IC, internal capsule; LH, lateral hypothalamus; and MH, medial hypothalamus.

Figure 2. a, The HTA and AChA. Five rats subjected to the experimental surgery (see text for details) were perfused with colored silicone after perfusion with 100 mL of saline and 10% phosphate-buffered formalin, respectively, after onset of cerebral ischemia. The circle of Willis was exposed by microsurgical technique. The HTA or at least 1 branch to the hypothalamus and the AChA stemming from ICA proximal to the MCA was identified in the nonischemic side. ACA indicates anterior cerebral artery; Br. to optic n., branch to optic nerve; Brs. to periam. c., branches to periamygdaloid cortex; PCA, posterior cerebral artery; PS, pituitary stalk. b and c, Occluder tip and the MCA. The occluder was advanced proximal to the MCA, resulting in obstruction of both the AChA and HTA. CUOC indicates coronal bone crevice of the cranial base underneath the optic chiasm; OC, optic chiasm.
contralateral areas to the occluded vascular territory (HTA or AChA) as internal controls for each brain slice.

**Infarct Distribution and Measurement**

Infarctions were classified into MCA, AChA, and HTA subgroups. For convenience of analysis, the cerebral cortex above the rhinal fissure was considered as neocortex, whereas the piriform cortex was defined as a non-neocortical structure. As shown in Figure 1, we defined a TTC stainless lesion involving the caudatoputamen and adjacent neocortex as an MCA infarct even though the lesion might cover some non-MCA territory structures such as those supplied by the AChA. Similarly, we named lesions encompassing a core of the internal capsule without affecting the MCA territory as an AChA infarct. An infarct fundamentally limited to the hypothalamus including the preoptic area, anterior hypothalamus, and medial and lateral hypothalamus was designated as a HTA infarct.

Infarct size was determined by Epscan Mac 1.40 with the use of the NIH Image 1.54 computer program. The total infarct volume was calculated by the numerical integration of infarct volume from all 2-mm thick brain slices, based on TTC staining in all rats. The infarct volume in the HTA infarct group was calculated again by use of hematoxylin and eosin staining. Similarly, the calculation of the infarct volume in the non-neocortical structures was derived by subtraction of the value of the neocortex from the total area.

**Statistical Analysis**

Fisher’s ANOVA and paired t test were used for comparison of blood physiological parameters, blood pressure, and body temperature between groups and between those before and after ischemia in the same group. The Mann-Whitney test was used for comparison of the infarct size and postural reflex scores between the groups. The statistical software Statview-J 4.1 (Abacus Concepts, Inc) was used. The value of P<0.05 was considered significant.

**Results**

In the proximal occlusion group, the mortality rate during 72 hours of permanent focal cerebral ischemia was 8% (3 of 36). Autopsy showed that the rats died from pressure effects associated with MCA territory infarction. In the MCA occlusion group, 5 (38%) of 13 rats died before evaluation of ischemic outcome at 72 hours. No death occurred in the sham-operated controls.
No significant differences were noted between rats representing the proximal, sham-operated, and MCA occlusion groups in arterial pH, Pao₂, and Paco₂. Mean values were 7.36 to 7.41, 189 to 214 mm Hg, and 36 to 45 mm Hg, respectively, at resting and 10 minutes after sham-operation or ischemia. The blood pressure in all groups increased 10 minutes after sham operation or ischemia (systolic pressure mean 140 to 145 mm Hg; diastolic pressure mean 92 to 97 mm Hg) versus resting state (systolic/diastolic pressure means 122 to 129/81 to 88 mm Hg), but the rise did not reach statistical significance. Animals used for blood gas and blood pressure evaluation were not used for infarct volume assessment.

Infarct Distribution and Suture Position
Positioning the suture proximal to the MCA origin produced an HTA infarct in 13 rats and an AChA infarct in 12 rats, findings confirmed by the occluder tip location relative to the MCA bifurcation and the distribution of ischemic changes with TTC and hematoxylin and eosin staining. Of the remaining 11 rats, 3 had a large (but survivable) MCA infarct, 5 had a smaller MCA infarct involving the caudatoputamen with or without partial neocortical infarction, and 3 died. The absolute success rate for production of isolated deep cerebral infarcts was 69% (25 of 36). The data from rats with MCA infarction resulting from erroneous MCA occlusion in the proximal occlusion group (n=3) and survivable infarction...
animals in the MCA occlusion group (n=8) were pooled as one group for statistical analysis because there were no structural differences between the two. All sham controls showed normal TTC and hematoxylin and eosin staining in their brains. Data from dead rats or those with partial MCA infarction were excluded from the study analysis.

The occluder tip and the MCA bifurcation were usually not on the same plane, and the arteries were not fully filled with blood after the rats were killed, making adequate well-focused photography showing both areas simultaneously technically difficult. Colored silicone perfusion facilitated demonstration of the relation much better (Figure 2, b and c) and defined the AChA and HTA quite well (Figure 2a). Linking AChA occlusion with a small deep infarct was established by histological identification of the ischemic lesion after perfusion of colored silicone into cerebral arteries.

The cerebral histology stained by TTC and hematoxylin and eosin correlated well with occluder position. When the suture tip was located >3 mm proximal to the MCA bifurcation (sham-operated control), the histology was normal in all animals. In those animals exhibiting HTA and AChA infarctions (proximal occlusion group), the distance between occluder and the MCA bifurcation was 0.7 to 1.9 mm (mean±SEM 1.4±0.1 mm, n=12) in the AChA infarct group and 1.5±0.2 mm (n=9) in the HTA infarct group. Those with MCA infarctions had suture tips 0.5 to 1 mm across the MCA origin.

The distance between the occluder tip and the coronal bone crevice of the cranial base beneath the optic chiasm (refer to Figure 2) was longer in the sham-operated control group (4.3±0.2 mm) than in the others (P<0.01, respectively), whereas no significant difference between the AChA (2.5±0.2 mm) and the HTA infarct groups (2.7±0.2 mm) was detected. The distance in the MCA infarct group was the shortest among groups (0.6±0.2 mm, P<0.01, respectively).

**Body Temperature**

Body temperature was identical (37.6°C to 37.8°C) in all groups before surgery (Figure 3). In the sham group, the temperature diminished at 3 hours after surgery (P<0.05) and returned to the baseline level during 24 to 72 hours after surgery. In the HTA infarct group, the temperature increased significantly to 38.1°C at 1 hour (P<0.05) and remained elevated thereafter and constantly higher than that in the sham-operated group throughout the duration of ischemia. In the AChA infarct group, the temperature also increased significantly at 1 hour after ischemia (P<0.05), remained elevated until 24 hours after ischemia, and then returned to normal. In the MCA infarct group, the temperature showed a similar significant increase from 1 to 24 hours (P<0.05), and then normalized.

**Postural Reflex**

All rats showed normal postural reflex (score=0) before intervention. The sham procedure produced no postural reflex test abnormality afterward. In the HTA infarct group, 2 of 13 rats showed early but transient grade 2 abnormality, subsequently followed by the tendency toward recovery. In the AChA infarct group, 5 of 12 rats manifested early grade 2 or 3 abnormalities that tended to decrease with time; all rats in this group exhibited at least grade 1 or 2 abnormality at the end of the experiment. In the MCA infarct group, 8 of 11 rats demonstrated early grade 2 or 3 abnormalities that persisted for 72 hours in the majority of animals.

**Infarct Size**

In the AChA infarct group, an infarct was located only in the nonneocortical structure (Figure 4, upper 2 rows). In 8 of 12 rats, the lesion was limited to 2 slices, that is, 8 and 10 mm from the frontal pole. In the remaining 4 rats, lesions expanded to 2 to 4 mm rostral to and 2 mm caudal to the 2 centrally affected slices. All AChA infarcts had discoloration of TTC-staining in the internal capsule. Additional structures involved in AChA infarction were the rostral caudatoputamen, dorsal hippocampus, and part of the thalamus. In the HTA infarct group, the lesion was fundamentally limited to the medial hypothalamus (Figure 4, lower 2 rows), although the lesion expanded to the preoptic area in 2 rats and part of the internal capsule in 5 rats.

As shown in Figure 5a, infarct area in MCA infarct group extended to all the slices examined and was significantly larger than that in the AChA infarct and HTA infarct groups in most slices (P<0.05). The volume in the HTA infarct group was the smallest among the 3 groups (P<0.05). The
area in the AChA infarct group was significantly larger than that in the HTA infarct group on slices 8 and 10 mm from the frontal pole ($P<0.05$). The infarct volume in the MCA infarct group, AChA infarct group, and HTA infarct group was 381±30 mm$^3$, 48±10 mm$^3$, and 4±1 mm$^3$, respectively, determined on the TTC staining slices. Because infarct area on most slices stained by hematoxylin and eosin was larger than that on their corresponding slices stained by TTC, the infarct area presented on Figure 5a was based on hematoxylin and eosin stainings (data not shown), scattered (Figure 6G) or clustered (Figure 6H) TUNEL-positive cells were detected in the medial hypothalamus, thus showing a discoloration in only 7 of 12 rats. Hematoxylin and eosin staining corroborated with the TUNEL method showed that 11 of 12 rats had the ischemic damage in this region (Figure 6, B, D, and F). Ischemic changes in the medial hypothalamus and/or preoptic area were found by TTC staining in only 7 of 12 rats. The positive frequency in these regions was elevated to 8 of 12 rats by hematoxylin and eosin staining. In 2 of the remaining 4 rats judged normal by TTC and hematoxylin and eosin stainings (data not shown), scattered (Figure 6G) or clustered (Figure 6H) TUNEL-positive cells were detected in the medial hypothalamus, thus increasing the frequency further to 10 of 12 rats.

**Hematoxylin and Eosin and TUNEL Staining**

In the HTA infarct group (n=13), TTC staining delineated the HTA territory lesion in 11 of 13 rats. Combinations of hematoxylin and eosin staining methodology confirmed the lesion in all animals, 4 of which were proved by the TUNEL method only (Figure 6, A, C, and E).

In the AChA infarct group (n=12), infarction was identified by TTC staining in all rats, but the lateral hypothalamus showed a discoloration in only 7 of 12 rats. Hematoxylin and eosin staining corroborated with the TUNEL method showed that 11 of 12 rats had the ischemic damage in this region (Figure 6, B, D, and F). Ischemic changes in the medial hypothalamus and/or preoptic area were found by TTC staining in only 7 of 12 rats. The positive frequency in these regions was elevated to 8 of 12 rats by hematoxylin and eosin staining. In 2 of the remaining 4 rats judged normal by TTC and hematoxylin and eosin stainings (data not shown), scattered (Figure 6G) or clustered (Figure 6H) TUNEL-positive cells were detected in the medial hypothalamus, thus increasing the frequency further to 10 of 12 rats.

**Discussion**

We report herein that the advance of a coated 3-0 surgical suture to the position 0.7 to 1.9 mm proximal to the MCA bifurcation could cause infarction in the AChA or HTA territory in female rats. The brain regions affected by this maneuver are fundamentally limited to nonneocortical structures, mainly the medial hypothalamus (HTA infarct) and the internal capsule (AChA infarct). The present protocol provided a 69% rate in successful production of isolated deep infarcts, including 36% in the HTA territory alone and 33% in the AChA distribution. Because the internal capsule was constantly (100%) affected in an AChA infarct, this region appears to be part of the ischemic core in rat AChA occlusion.

That HTA infarct could be produced independent of the AChA area indicate that these 2 regions are supplied by different arteries. An AChA infarct was almost always (91% frequency) accompanied by a HTA infarct. These observations and the results of cerebral artery perfusion with colored silicone (Figure 2) indicate that the HTA or at least 1 branch to the medial hypothalamus stems from the ICA more proximal to the MCA bifurcation than the AChA.

Using intraluminal filament model in rats, hyperthermia was formerly thought to be related to anterior hypothalamus and preoptic area damage associated with obstruction of the anterior cerebral artery and AChA. In this study, however, a small HTA infarct in the medial hypothalamus constantly caused elevation of body temperature. These results indicate that a lesion involving the medial hypothalamus alone can cause hyperthermia irrespective of obstruction of the anterior cerebral artery or the AChA.

The hypothalamus appears to play a pivotal role in body temperature regulation. This region senses changes in local brain temperature, integrates information of temperature from other parts of the body, and sends efferent signals to various regions. Warm-sensitive neurons in the optic area of rats have been recently demonstrated to send excitatory signals to vasodilative neurons in the caudal part of the lateral hypothalamus, ventrolateral periaqueductal gray matter, and the reticular formation and send inhibitory signals to vasoconstrictive neurons in the rostral part of the ventral tegmental area. Damage in the former regions or stimulation in the latter regions probably causes vasoconstriction.

Our observations that hyperthermia follows hypothalamic ischemia is strong evidence that infarction damages those mechanisms that regulate temperature. First, all supratentorial brain regions in the HTA infarct group were thoroughly examined with the use of hematoxylin and eosin staining, and no abnormality was identified, arguing against a possibility that damage in some other cerebral structure had occurred. Second, in a parallel experiment with the use of the transorbital method for MCA occlusion (HTA and AChA are exempt from occlusion), neither proximal (n=4) nor distal (n=10) permanent occlusion of the MCA caused rectal temperature to increase significantly (our unpublished data). Finally, direct injury of the hypothalamus by acute mechanical puncture or the electrolytic method causes hyperthermia. Neurogenic hyperthermia induced by mechanical puncture is hypothesized to affect the pyrogenesis mechanism on the basis of a set point shift, mediated by prostaglandins released from injured tissue and acting on surviving hypothalamic tissue.
We found body temperature to be an accurate indicator predicting deep cerebral infarcts involving the hypothalamus. Special care, however, must be taken to accurately assess body temperature changes because anesthesia induction or a cool environment inhibits body temperature elevation. We used isoflurane at the time of intraluminal arterial occlusion, an anesthetic agent usually accompanied by rapid recovery. Room temperature was maintained constant at 21 ± 1°C. Such management permitted early detection of rises in body temperature in ischemic groups. Hyperthermia persisted for at least 3 days in rats with HTA infarction but lasted for lesser times in those with AChA or MCA territory damage. The mechanisms for this discrepancy are not clear and need further investigation.

The postural reflex test has been used for evaluating sensory-motor integration after focal cerebral ischemia. Striatum and overlying neocortex lesions caused by proximal MCA ligation or rostral forelimb area of cortex damage induced by distal MCA occlusion are both linked with deficits as measured by this test. However, damage involving the striatum and/or overlying neocortex does not necessarily correlate with deficit. Instead, sensory motor dysfunction appears to depend largely on the volume of infarct. In the original report of Bederson et al., the sensitivity of the test was 88%. In their study, rats with small infarction limited to the caudatoputamen and dorsolateral cortex had no abnormality in the test. On the other hand, all rats with AChA infarct involving the internal capsule had a definitive postural deficit irrespective of infarct volume. The results indicate the important role of the internal capsule in the manifestation of postural reflex abnormality. The positive test was a good indicator distinguishing AChA infarct from HTA infarct. Likewise, severe deficit in the early period of ischemia was suggestive of MCA infarct rather than AChA infarct. Thus the HTA, AChA, or MCA pattern can be predicted by the results of early postural reflex testing. (Table 1, 82% of all rats in ischemic groups exhibiting grade ≤2 at 1 hour after ischemia had a deep infarct).

With the use of the India ink double-perfusion technique, Ambach et al confirmed that the preoptic area is supplied by arteries emerging from the anterior communicating artery and anterior cerebral artery, whereas the medial hypothalamus is supplied by the anterior, middle, and posterior tuberal arteries, which generally stem from the ICA in rats. The arteries supplying the medial hypothalamus are terminal, with minimal of overlap in their supplying territories. The medial and central parts of the ventromedial nucleus are poorly vascularized. The infarcts localized in the ventral-medial hypothalamus in the current study was consistent with the characteristics of the arterial supply reported by Ambach and Palkovits. In our study, it was unclear whether 1 or more of 3 tuberal arteries were involved in the HTA infarct production. Perfusion with colored silicone delineated arteries to the hypothalamus at the section of the ICA proximal to the AChA (Figure 2). Frequently, a recurrent branch stemming from the branch to the optic nerve arises in addition to the HTA shown in Figure 2, supplying the hypothalamus and being closely adjacent to the AChA (data not shown). An occluder advanced to the position to selectively obstruct the AChA may simultaneously occlude the recurrent branch.

The HTA infarct volume produced by the present protocol was ≈6 mm³. From the viewpoint of small size and deep localization, the HTA infarct is comparable with lacunar infarction. TTC staining of viable 2-mm-thick sections, however, tends to underestimate the extent of infarct, since the diameter of HTA infarct was <2.4 mm in the majority of rats. A lesion confirmed by hematoxylin and eosin and TUNEL method was overlooked by TTC staining in 2 rats with HTA infarct. Similar ischemic changes were also dismissed in the medial hypothalamus (3 rats) and the lateral hypothalamus (4 rats) in animals with AChA infarct. To evaluate the extent of ischemic changes in small infarction, at least hematoxylin and eosin staining should be used concomitantly with TTC staining. Focal permanent and transient cerebral ischemia as well as transient global ischemia can all induce apoptosis, a well-known mechanism of cell death. Reportedly, groups of apoptotic cells were localized in the inner boundary zone of the infarct after transient MCA occlusion in rats. Neuronal apoptosis was present 3 days after mild ischemia in the peri-infarct area, where true infarction might evolve later. Apoptosis could be detected as early as 30 minutes after reperfusion by the TUNEL method, whereas detection of apoptotic body by hematoxylin and eosin staining took 12 hours of reperfusion. Therefore ischemic changes in small deep infarction may be evaluated best by hematoxylin and eosin staining collated with TUNEL method.

In conclusion, a variation of the intraluminal suture MCA occlusion technique can produce ischemia in deep cerebral structures, either a small infarct fundamentally limited in the medial hypothalamus or a larger lesion involving the internal capsule and medial hypothalamus. Both infarcts are accompanied by hyperthermia, suggesting that damage in the medial hypothalamus is responsible for the rise of body temperature. The present model may facilitate research concerning various cerebral functional changes caused by small deep infarction.

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References

The authors present a useful modification of the intraluminal filament technique for producing cerebral infarcts. In this case, the positioning of the filament enables them to produce an infarct restricted to deep structures. Depending on the filament placement, both the hypothalamus and internal capsule are involved or only the medial hypothalamus. By this means the authors were to demonstrate control of body temperature by the medial hypothalamus, with a rise in body temperature being caused by the medial hypothalamic injury. The technique should be useful in enabling the investigator to better correlate functional deficits with discrete brain lesions and hence correlate function with discrete areas in the brain of the experimental animal. This is more difficult to do when the lesion is large and multiple structures are damaged.

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