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Early Effects of Acid–Base Management during Hypothermia on Cerebral Infarct Volume, Edema, and Cerebral Blood Flow in Acute Focal Cerebral Ischemia in Rats

Rainer Kollmar, M.D., Thomas Frietsch, M.D.,† Dimitrios Georgiadis, M.D.,* Wolf-Rüdiger Schäbitz, M.D.,* Klaus F. Waschke, M.D.,‡ Wolfgang Kuschinsky, M.D.,§ Stefan Schwab, M.D.‖*

Background: **Although the frequency for the use of moderate hypothermia in acute ischemic stroke is increasing, the optimal acid–base management during hypothermia remains unclear. This study investigates the effect of pH- and α-stat acid–base management on cerebral blood flow (CBF), infarct volume, and cerebral edema in a model of transient focal cerebral ischemia in rats.**

Methods: **Twenty Sprague-Dawley rats were subjected to transient middle cerebral artery occlusion (MCAO) for 2 h during normothermic conditions followed by 5 h of reperfusion during hypothermia (33°C). Animals were artificially ventilated with either α- (n = 10) or pH-stat management (n = 10). CBF was analyzed 7 h after induction of MCAO by iodo[^{14}C]antipyrine autoradiography. Cerebral infarct volume and cerebral edema were measured by high-contrast silver infarct staining (SIS).**

Results: **Compared with the α-stat regimen, pH-stat management reduced cerebral infarct volume (98.3 ± 33.2 mm^3 *vs.* 53.6 ± 21.6 mm^3; $P \geq 0.05$ mean ± SD) and cerebral edema (10.6 ± 4.0% *vs.* 3.1 ± 2.4%; $P \geq 0.05$). Global CBF during pH-stat management exceeded that of α-stat animals (69.5 ± 12.3 ml · 100 g^{-1} · min^{-1} *vs.* 54.7 ± 13.3 ml · 100 g^{-1} · min^{-1}; $P \geq 0.05$). The regional CBF of the ischemic hemisphere was 62.1 ± 11.2 ml · 100 g^{-1} · min^{-1} in the pH-stat group *versus* 48.2 ± 7.2 ml · 100 g^{-1} · min^{-1} in the α-stat group ($P \geq 0.05$).**

Conclusions: **In the very early reperfusion period (5 h), pH-stat management significantly decreases cerebral infarct volume and edema as compared with α-stat during moderate hypothermia, probably by increasing CBF.**

IN the past decade, prolonged moderate hypothermia has been shown to be neuroprotective in animal models of focal cerebral ischemia.[1,2] Potential underlying neuroprotective mechanisms include reduction of excitatory amino acids levels, stabilization of the blood-brain barrier and cell membranes,[3,4] and a reduction of cerebral metabolism.[5] In addition, alteration of cerebral blood flow (CBF) during hypothermia may contribute to its neuroprotective effects because neurologic outcome is associated to CBF.[6,7] However, the optimal acid–base management during hypothermia is still not addressed for cases of ischemic stroke.

Two ventilation strategies for hypothermic acid–base management have been suggested: during α-stat management, arterial CO_2 tension ($Paco_2$) is maintained at 40 mmHg when measured at 37°C. The dissociation fraction of the imidazole moiety of histidine is thereby constant, whereas pH changes parallel to the neutral point of water. In contrast, during pH-stat management, $Paco_2$ is corrected to the patient's actual body temperature.[5] Because of the increased gas solubility during hypothermia, the α-stat strategy results in relative hyperventilation and a decrease in CBF. The effects of the acid–base management are closely dependent on the degree of hypothermia.[5,8] We hypothesized that the regulation of the CBF in the penumbra is unimpaired, and, therefore, the ischemic area and brain edema is reduced by a higher CBF.

In the present study, the effects of pH-stat and α-stat management on CBF, volume of cerebral infarction, and cerebral edema were examined during a 5-h period of hypothermia after transient cerebral ischemia. CBF was investigated by the iodo[^{14}C]antipyrine autoradiographic method.[9] Cerebral infarct volume and edema were measured by high-contrast silver infarct staining (SIS) 7 h after middle cerebral artery occlusion (MCAO), including 2 h of MCAO and 5 h of reperfusion.

This article is featured in "This Month in Anesthesiology." Please see this issue of ANESTHESIOLOGY, page 5A.

Methods

The animal experiments were performed after approval of the animal care committee (Regierungspräsidium Karlsruhe, Germany). Twenty male Sprague-Dawley rats weighing 280–320 g (Charles-River Deutschland, Sulzfeld, Germany) were kept under temperature-controlled environmental conditions on a 14:10 h, light-to-dark cycle, fed a standard diet (Altromin C 1000; Altromin, Lage, Germany), and allowed free access to food and water until the experiments started. Before surgery, animals were randomly assigned to either pH-stat or α-stat management.

* Resident, ‖ Research Coordinator and Attending Physician, Department of Neurology, § Professor and Chair, Department of Physiology and Pathophysiology, University of Heidelberg, † Resident, ‡ Attending Physician, Department of Anesthesiology and Critical Care Medicine, University of Heidelberg, and Faculty of Clinical Medicine, Theodor-Kutzer-Ufer, Mannheim.

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Address reprint requests to Dr. Schwab: Department of Neurology, University of Heidelberg, Im Neuenheimer Feld 400, 69120 Heidelberg, Germany. Address electronic mail to: Stefan_Schwab@med.uni-heidelberg.de.

Experimental Protocol

Surgery. Anesthesia was induced and maintained by inhalation of a gas mixture of isoflurane (Forene; Abott, Wiesbaden, Germany), oxygen (40%), and air (remainder) administered using a precalibrated vaporizer (Fortec; Cyprane Keighley, UK). Minimum alveolar concentrations (MAC) were corrected for the actual body temperature.[10] One MAC isoflurane corresponds to 1.2% at 37.5°C, and 0.87% at 33°C using a fresh gas flow of 2 l/min.[10,11] Tracheostomy and cannulation of the right femoral artery and vein were performed using polyethylene catheters (PE-160 and PE-50; Labokron, Sinsheim, Germany). Mean arterial blood pressure and heart rate were registered continuously by a quartz pressure transducer (Hewlett-Packard, Palo Alto, CA). Animals were mechanically ventilated (Small animal ventilator, KTR4; Hugo Sachs Electronic, March, Germany). Arterial blood gases were examined in a pH–blood gas analyzer (AVL Gas Check 939; AVL, Graz, Austria). Various parameters like blood glucose, hematocrit, and hemoglobin were analyzed during the experiment. Pericranial temperature was measured using ultrafast microthermocouple probes (IT-23, diameter 0.3 mm; Almemo 2290–3S Thermometer, Hugo Sachs Elektronik, March-Hugstetten, Germany), which were introduced to the outside of the base of the rat skull through the masseter muscle on each side of the skull.[10] Rectal temperature was measured simultaneously. Body temperature was kept constant at 37–37.5°C with a temperature-controlled heating pad during the surgical preparation (Harvard Ltd., Kent, UK). Physiologic parameters were recorded over the whole experimental time.

Middle Cerebral Artery Occlusion

Transient focal cerebral ischemia was induced using the suture occlusion technique[12] as previously described.[13] The right common carotid artery (CCA), internal carotid artery (ICA), and external carotid artery (ECA) were exposed throughout a midline incision of the neck. After further dissection, the origin of the pterygopalatine artery was ligated by a 6–0 silk suture. Following careful separation from the adjacent vagus nerve, the proximal parts of the right CCA and ECA were ligated with 5–0 surgical sutures. A 4–0 monofilament silicone-coated nylon suture was inserted into the distal right CCA and gently advanced into the ICA until the tip occluded the origin of the MCA, approximately 18 or 19 mm from the carotic bifurcation. The occluding suture was removed from the MCA after 120 min in all animals.

Before surgery, animals were assigned to one of the following two groups:

1. α-stat Group (n = 10): MCAO was performed during normothermic conditions (body temperature = 37.0°C). The removal of the occlusion suture 120 min later marked the beginning of reperfusion. Animals were artificially ventilated and cooled to 33°C body temperature by a water-perfused temperature coil. Target temperature was reached within 15 min in all animals. Hypothermia was maintained at 33°C body temperature for the next 4.75 h. The respiratory rate was adjusted to a $Paco_2$ of 40 mmHg **not** corrected for the body temperature (α-stat).
2. pH-stat Group (n = 11): All procedures were done as described above, but pH-stat acid–base management was used. Thus, the respiratory rate was adjusted to a $Paco_2$ of 40 mmHg corrected for the body temperature.

Measurement of Local Cerebral Blood Flow

All animals were subjected to autoradiographic determination of CBF of various brain regions after 2 h of normothermic MCAO followed by 5 h of hypothermic reperfusion, according to the method of Sakadura *et al.*[9] One hundred μCi/kg body weight of 4-iodo[M-methyl-^{14}C]antipyrine (specific activity, 54 mCi/mmol; Amersham-Buchler, Braunschweig, Germany), dissolved in 1 ml saline, was continuously infused at a progressively increasing infusion rate for 1 min *via* the femoral venous catheter. During the 1-min infusion period, 14–20 blood samples were collected in drops from the free-flowing arterial catheter directly onto filter paper disks that had been prepared in small plastic beakers and weighed. The samples were weighed, and radioactivity was measured with a liquid scintillation counter (TriCarb 4000 series; Canberra Packard, Frankfurt, Germany) after extraction of the radioactive compound with ethanol. After the 1-min infusion and sampling period, the animals were decapitated, and their brains removed as quickly as possible and frozen in isopentane chilled to −60°C. The frozen brains were coated in chilled embedding medium (Lipshaw, Detroit, MI), stored at −80°C in plastic bags, divided in 20-μm sections at −20°C in a cryostat, and subjected to autoradiography, along with precalibrated [^{14}C] methyl methacrylate standards. Details of this technique were extensively described elsewhere.[5,9,10]

Local tissue concentration of [^{14}C] was determined from autoradiographs using densitometric analysis. Local CBF (LCBF) was calculated from the local concentrations of [^{14}C] and the time course of the plasma iodo[^{14}C]antipyrine concentrations.[5,9,10]

Autoradiographic images were converted to digitized optical density images by an image processing system (MCID 4; Imaging Research, St. Catharines, Canada). Measurement of separate brain structures was performed with an ellipsoid cursor and adjusted to the size of the individual region (*e.g.*, parietal cortex, thalamus, basal ganglia). CBF was measured for the entire brain, each hemisphere, and for 23 different brain regions in each hemisphere with blood supply from the MCA. For measurement of the mean global CBF, coronal sections were analyzed as a whole at distances of 200 μm, and the

Table 1. Physiological Variables

	Baseline		60 min		120 min		180 min	
	*p*H-stat	Alpha-stat	*p*H-stat	Alpha-stat	*p*H-stat	Alpha-stat	*p*H-stat	Alpha-stat
Rectal temperature (°C)	37.1 ± 0.2	37.1 ± 0.1	37.2 ± 0.2	37.2 ± 0.1	37.2 ± 0.2	37.1 ± 0.2	32.8 ± 0.3	32.9 ± 0.1
Pericranial temperature								
Nonischemic hemisphere (°C)	37.0 ± 0.1	36.9 ± 0.2	36.8 ± 0.3	36.7 ± 0.4	36.8 ± 0.4	36.8 ± 0.2	32.7 ± 0.3	32.9 ± 0.1
Ischemic hemisphere (°C)	36.9 ± 0.2	36.6 ± 0.3	36.5 ± 0.4	36.4 ± 0.3	36.5 ± 0.3	36.4 ± 0.2	32.4 ± 0.2	32.4 ± 0.2
BGA uncorrected								
*p*H	7.40 ± 0.04	7.39 ± 0.05	7.39 ± 0.05	7.38 ± 0.04	7.40 ± 0.03	7.38 ± 0.07	7.29 ± 0.03	7.32 ± 0.04
$Paco_2$ (mmHg)	36.7 ± 3.5	35.9 ± 6.4	36.6 ± 3.7	37.3 ± 3.4	37.1 ± 2.8	38.3 ± 4.8	47.7 ± 3.6*	39.8 ± 1.7
Pao_2 (mmHg)	151.5 ± 21.1	149.2 ± 27.5	162.0 ± 24.8	162.9 ± 16.5	164.8 ± 19.2	172.2 ± 8.7	149.8 ± 26.7	168.6 ± 14.2
Base excess	3.2 ± 2	3.9 ± 1.9	2.7 ± 3.2	4.3 ± 3.3	2.3 ± 3.1	2.9 ± 3.6	5.6 ± 2.8	3.9 ± 2.1
Plasma glucose concentration (mg/dl)	134.5 ± 12.3	131.2 ± 14.7	148.5 ± 15.6	151.3 ± 11.0	173.4 ± 18.5	170.9 ± 12.9	153.9 ± 11.0	150.6 ± 12.1
MAP (mmHg)	97 ± 9	99 ± 9	92 ± 9	97 ± 15	93 ± 10	97 ± 16	104 ± 10	110 ± 17
Heart rate (beats/min)	412 ± 12	408 ± 13	389 ± 36	395 ± 29	406 ± 32	394 ± 37	392 ± 15	396 ± 11

Values for blood gas analysis are shown uncorrected for the animals' temperature to compare the alpha-stat and *p*H-stat groups. Measurements show the value at the exact time periods. Student *t* test was used for statistical analysis.

* $P \leq 0.05$.

BGA = blood gas analysis; $Paco_2$ = arterial carbon dioxide tension; Pao_2 = arterial oxygen tension; MAP = mean arterial blood pressure. (continues)

values were summarized to obtain the area-weighted means of all measured sections.[5,10]

Measurement of Infarct Volume and Cerebral Edema by the Silver Infarct Staining Method

The SIS method, as described by Vogel *et al.*,[14] was used. In contrast to other staining methods like hematoxylin–eosin, Nissl, or nitroblue tetrazolium staining, SIS allows a reliable delineation of ischemic brain tissue from nonischemic white and gray matter of rat brain cryosection as soon as 2 h after MCAO.[14] Every fourth slide from the cryosection of 20 μm thickness was transferred to glass slides for staining. For SIS, the slides were submerged into a silver impregnation solution, which was shaken vigorously, for 2 min. Slides were then washed in distilled water six times for 1 min before being transferred to a vigorously shaken developer solution for an additional 3 min. After the slides had been washed in distilled water three times for 1 min, they were air-dried. The composition and preparation procedures of the impregnation and developer solution were previously described in detail by Vogel *et al.*[14]

A modified version of the semiautomated method of Swanson *et al.*[15] was used for measurement of the cerebral infarct volume. Briefly, the lowest optical density of the noninfarcted hemisphere was calculated using the MCID and taken as threshold value. The brain area with an optical density equal to or higher than this threshold was considered to be nonischemic, whereas areas with values below threshold values were considered to represent infarcted brain tissue. In addition, the size of the ischemic and nonischemic hemisphere was measured. The areas of the nonischemic hemisphere, ischemic hemisphere, and infarction were multiplied by the slice thickness plus the thickness of the three additional slices taken for the autoradiographic measurements (*e.g.*, volume of the left, nonischemic hemisphere = area of left, nonischemic hemisphere in SIS multiplied by (thickness of SIS slice + the following three autoradiographic slices).

The following formula was used for calculation of the infarct volume corrected for the cerebral edema:[16] corrected infarct volume = volume of the left, nonischemic hemisphere − (volume of the right, ischemic hemisphere − infarct volume). Moreover, the total infarct area was subdivided into cortical and subcortical areas, and a ratio was calculated.

Cerebral edema was determined as the relationship of the volume of the right, infarcted hemisphere to the left, noninfarcted hemisphere.[17]

Statistical Analysis

Data were expressed as mean ± SD and compared with multiple *t* test and analysis of variance (ANOVA) with Bonferroni correction. A P value ≤ 0.05 was considered statistically significant.

Results

Physiologic Parameters

Physiologic variables of both groups were comparable (table 1). As intended, $Paco_2$ differed during hypothermia because of different acid–base managements used ($P \geq 0.05$). Rectal temperature and pericranial temperature showed no significant difference during normothermia and hypothermia. The pericranial temperature of the infarcted hemisphere tended to be lower than the noninfarcted hemisphere during normothermia and hypothermia ($P \leq 0.05$).

Table 1. *(Continued)*

240 min		300 min		360 min		420 min	
*p*H-stat	Alpha-stat	*p*H-stat	Alpha-stat	*p*H-stat	Alpha-stat	*p*H-stat	Alpha-stat
32.8 ± 0.3	33.0 ± 0.1	33.0 ± 0.1	33.1 ± 0.2	33.0 ± 0.1	33.0 ± 0.1	33.1 ± 0.7	33.0 ± 0.1
32.7 ± 0.3	32.8 ± 0.1	32.6 ± 0.3	32.9 ± 0.2	32.7 ± 0.4	32.9 ± 0.1	33.7 ± 0.3	32.9 ± 0.1
32.5 ± 0.2	32.5 ± 0.1	32.9 ± 0.3	32.8 ± 0.1	32.3 ± 0.3	32.5 ± 0.3	32.6 ± 0.2	32.6 ± 0.2
7.29 ± 0.03	7.33 ± 0.03	7.27 ± 0.04	7.32 ± 0.03	7.3 ± 0.03	7.30 ± 0.04	7.29 ± 0.04	7.29 ± 0.03
46.5 ± 3.1*	40.8 ± 1.3	48.4 ± 2.8*	40.1 ± 1.3	47.8 ± 1.3*	41.2 ± 1.1	47.3 ± 1.4*	40.1 ± 0.6
157.1 ± 20.4	172.2 ± 15.4	161.7 ± 18.8	172.0 ± 10.2	164.0 ± 20.2	174.6 ± 12.6	160.3 ± 24.5	164.4 ± 10.4
5.9 ± 3.3	4.5 ± 2.2	5.7 ± 2.5	4.6 ± 1.3	5.3 ± 2.6	4.7 ± 2.2	4.1 ± 2.8	5.7 ± 1.9
135.3 ± 18.9	128.0 ± 12.3	129.0 ± 24.6	133.3 ± 22.4	132.5 ± 21.7	129.0 ± 24.7	137.5 ± 12.2	141.4 ± 15.9
106 ± 11	102 ± 13	103 ± 7	104 ± 9	103 ± 8	99 ± 14	100 ± 12	101 ± 12
394 ± 18	390 ± 35	386 ± 20	400 ± 7	387 ± 22	346 ± 129	395 ± 18	402 ± 16

Cerebral Infarct and Cerebral Edema

Cerebral infarct volume during pH-stat management was nearly half compared with α-stat management. The size of infarct volume in the α-stat group was 98.3 ± 33.2 mm ≤ *versus* 53.6 ± 21.6 mm ≤ in the pH-stat group ($P \geq 0.05$). When the total infarct volume was subdivided, cortical portion in the α-stat group was 10.9 + 6.9% *versus* 6.3 + 4.9% in the pH-stat group (P = NS), respectively, 10.7 mm ≤ *versus* 3.4 mm ≤. The subcortical portion was 89.1 + 6.9% in the α-stat group *versus* 94.7 + 5.2% in the pH-stat group (P = NS), respectively, 87.5 mm ≤ *versus* 50.6 mm ≤.

Cerebral edema was less pronounced in the pH-stat group compared with α-stat management. The size of cerebral edema in the α-stat group was 10.6 ± 4.0% *versus* 3.1 ± 2.4% in the pH-stat group ($P \geq 0.05$).

Global and Local Cerebral Blood Flow

Global CBF of the entire brain (fig. 1) increased by 27% during pH- compared with α-stat management. During pH-stat management, CBF increased by 29% in the ischemic hemisphere and by 27% in the nonischemic hemisphere (fig. 1).

Comparison of 46 brain regions (23 in each hemisphere) revealed 17 regions with a higher CBF in animals of the pH- compared with the α-stat group (table 2). Figure 2 demonstrates the typical autoradiography and high-contrast SIS brain slices of α- and pH-stat management.

Discussion

This study investigated the early effects of pH-stat and α-stat management on cerebral ischemia and edema after prolonged moderate hypothermia induced in the reperfusion period of focal cerebral ischemia. The main results were that (1) pH-stat management increased the LCBF and global CBF during hypothermia in the reperfusion period after MCAO compared with α-stat management, and (2) the increased CBF in pH-stat management was associated with a reduced cerebral infarct volume and cerebral edema.

These results seem to contradict previous comparisons of different acid–base managements during clinical hypothermic cardiac surgery.[6,18,19] Those suggest that α-stat management leads to a better or at least unaltered neurologic and neurophysiologic outcome compared

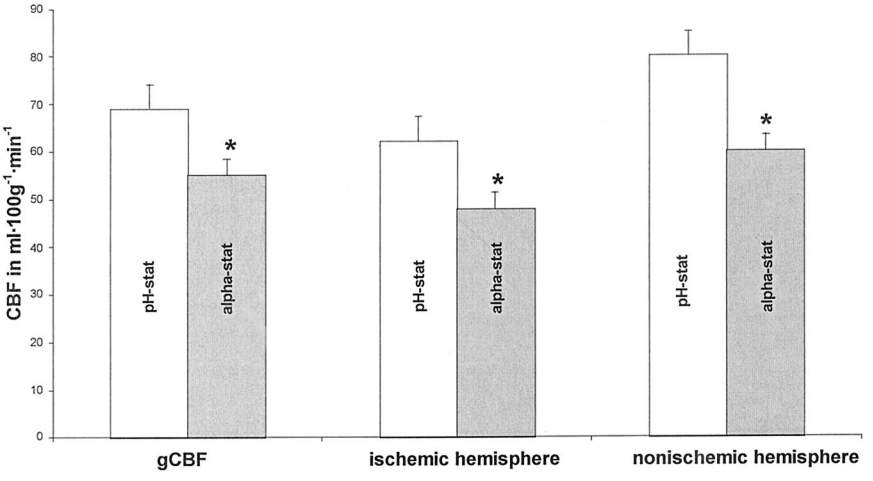

Fig. 1. Cerebral blood flow measured by autoradiography for the entire brain as global cerebral blood flow (GCBF), the nonischemic, and ischemic hemisphere. Values are given in ml · 100 g^{-1} · min^{-1}. For statistical analysis, the Student *t* test was used. Data was expressed as mean ± SD, and a *P* value of < 0.05 was considered statistically significant indicated by asterisks.

Table 2. Regional Cerebral Blood Flow

	Cerebral Blood Flow (ml · 100 g^{-1} · min^{-1})			
	Ischemic, Right Hemisphere		Nonischemic, Left Hemisphere	
	*p*H-stat Group	Alpha-stat Group	*p*H-stat Group	Alpha-stat Group
Telencephalon				
Frontal cortex	59 ± 11	54 ± 14	83 ± 11	73 ± 5
Nucleus accumbens	66 ± 26	37 ± 11	98 ± 24	64 ± 14
Pyriform cortex	82 ± 19	90 ± 16	134 ± 16	122 ± 25
Lateral septal nuclei	55 ± 16	33 ± 10*	66 ± 18	39 ± 7*
Caudate nucleus	52 ± 19	29 ± 17	93 ± 5	62 ± 4*
Parietal cortex	71 ± 8	68 ± 4	81 ± 21	52 ± 21
Sensory motor cortex	60 ± 22	31 ± 17*	78 ± 11	54 ± 15
Globus pallidus	33 ± 8	20 ± 15	52 ± 5	41 ± 9*
Cingulate gyrus	88 ± 11	39 ± 16*	101 ± 16	51 ± 16*
Amygdaloid complex	46 ± 14	56 ± 5	60 ± 37	22 ± 16
Hippocampus CA1	69 ± 28	51 ± 10*	70 ± 28	65 ± 7
Hippocampus CA2	62 ± 29	59 ± 15	71 ± 29	75 ± 6
Hippocampus CA3	61 ± 25	64 ± 16	86 ± 4	81 ± 2
Hippocampus CA4	61 ± 29	54 ± 5	69 ± 22	66 ± 6
Dentate gyrus	65 ± 3	53 ± 9*	68 ± 21	68 ± 4
Diencephalon				
Medial geniculate body	77 ± 15	49 ± 3*	88 ± 14	81 ± 3
Lateral geniculate body	70 ± 14	41 ± 6	83 ± 11	72 ± 7
Hypothalamus	49 ± 24	16 ± 3*	62 ± 32	55 ± 24
Ventral thalamus	95 ± 18	47 ± 13*	103 ± 19	68 ± 10*
Lateral thalamus	67 ± 23	43 ± 13	92 ± 16	64 ± 6*
Myelinated fiber tracts				
Internal capsule	23 ± 4	16 ± 5*	34 ± 3	27 ± 3*
Genu of corpus callosum	26 ± 11	16 ± 14	33 ± 5	23 ± 7*
Cerebellar white matter	40 ± 14	39 ± 11	50 ± 15	36 ± 14

Comparison of the regional cerebral blood flow (rCBF) measured by iodo[^{14}C]antipyrine autoradiography shows a trend toward a higher CBF in the *p*H-stat group. Values of 23 regions in the left or right hemisphere are given in ml · 100 g^{-1} · min^{-1} as mean ± SD.

* $P \leq 0.05$ was considered statistically significant.

with pH-stat.[6,18,19] This effect was attributed to a greater rate of cerebral microemboli because of an increased CBF during pH-stat management.[20–22] However, this mechanism is not relevant in acute stroke. Our MCAO model results in focal cerebral ischemia with an ischemic core and a surrounding penumbra.[14,23] One important factor determining the fate of the penumbral regions is the amount of blood supply[7,14,23]: if the CBF remains below ischemic values, the ischemic core and, consequently, the infarct will increase or *vice versa*. An increased CBF during pH-stat management in the very early reperfusion period may decrease the tissue damage by three factors. (1) An improved oxygen delivery may reduce ischemia. (2) An increased CBF may also remove potentially neurotoxic metabolites like excitatory amino acids, lactate, and oxygen free radicals during cerebral ischemia. In addition, (3) an increased CBF may provide a more homogenous cooling profile of the brain and, therefore, reduce metabolism more effective in all brain regions.

In addition, these results seem to be in contrast to results of a previous experimental study that failed to demonstrate any effects of acid–base management on CBF, cerebral infarct, and cerebral edema.[24] The discrepant CBF results are potentially the result of different CBF measurement techniques and different study designs. Nagai *et al.* used laser Doppler flowmetry (LDF) for measuring flow velocities at one point of the brain, whereas iodo[^{14}C]antipyrine technique was used for CBF evaluation in the present study. This technique allows—in contrast to LDF—a quantitative determination of up to 50 brain regions with a high spatial resolution.[5,9] Because LDF is limited to cortical flow velocity measurement, deeper brain structures dependent on the blood supply from the MCA are missed. Therefore, in congruence with the results of Nagai *et al.*, the difference in local CBF between α- and pH-stat vanished in most cortical regions, but local CBF in deeper brain regions significantly differed in our study, depending on the mode of acid–base management. Further, cerebral infarction size was reported to be not different.[24] However, this discrepancy with the considerable reduction of infarct volume by pH-stat in our study can be ex-

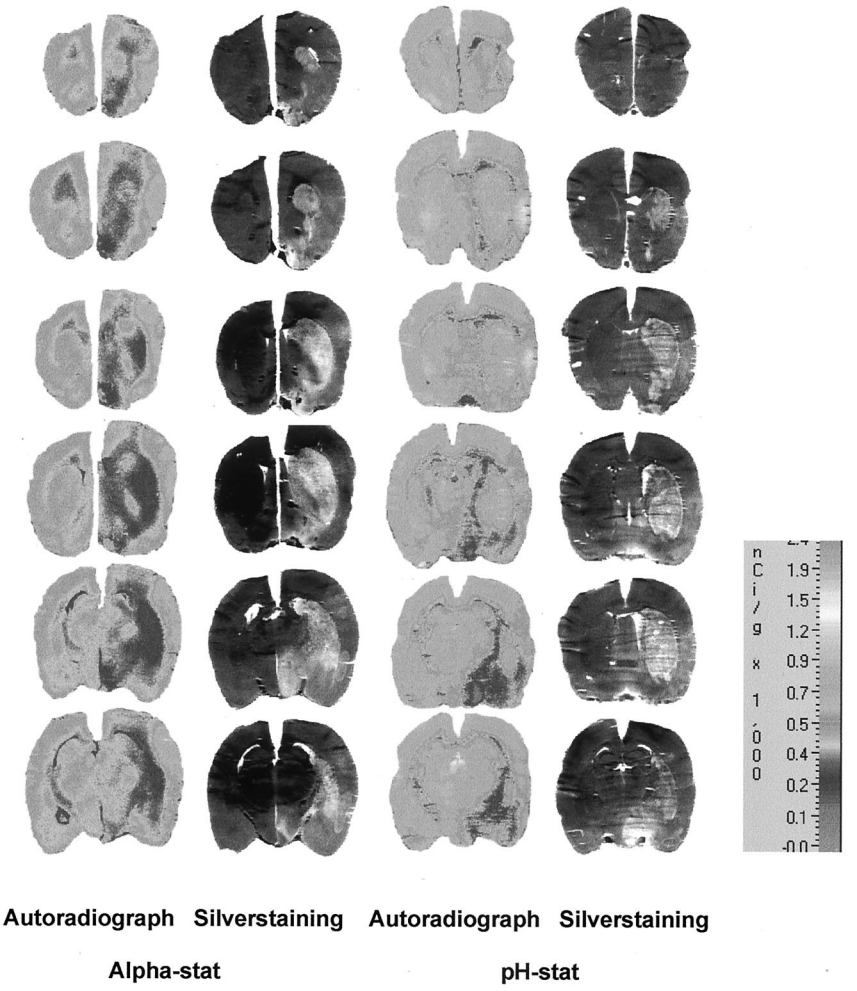

Fig. 2. Examples of autoradiographs and silver infarct staining are shown. Comparison of cerebral blood flow (CBF) measured by 4-iodo[M-methyl-^{14}C] antipyrine and silver infarct staining (SIS) between pH-stat and α-stat. Sample slices from autoradiographs compared with high-contrast silver staining of each group. Color variation indicates different regional blood flow as seen in the scale. In high contrast silver stains, pale areas indicate infarcted regions.

plained by different study designs. Nagai *et al.* used a 2-h hypothermia combining pre-, intra-, and postischemic cooling, whereas our study investigates the effects of delayed hypothermia started in the reperfusion period. In addition, we extended the hypothermic treatment period to 5 h. Consequently, effects of different acid–base managements may be pronounced by a longer application of hypothermia together with a higher CBF in reperfused vessels. Most important, in our study, infarct volume, edema, and CBF were measured during hypothermia and different acid–base management, whereas Nagai *et al.* assessed infarct volume after a 22-h period of normothermia. Consequently, Nagai *et al.* investigated the effects of a relatively short period of hypothermia with acid–base management in combination with an extensively longer normothermic period without control of acid–base status.

However, our study has a major limitation because the effects of acid–base management were investigated after a short time of reperfusion in cerebral ischemia. There was no rewarming, no recovery period, and no time for development of the full tissue reaction known from other studies. Cerebral infarct may still increase after days, and cerebral edema may change after a longer time period[25]. However, the value of our study is the demonstration of very early basic effects of different acid–base managements on CBF, brain edema, and infarction. Because the very early mechanisms are present in the acute phase of tissue reaction, the possibility remains that an increased CBF during pH-stat management may modulate the degree of inflammation, apoptosis, or necrosis. The mechanism by which brain edema decreased is not clear, but it may be related to the decrease in cerebral infarction size. In addition, edema after focal cerebral ischemia is generated more by vasogenic and cytotoxic cascades and not by elevated hydrostatic fluid pressure,[26] which may be the fear about an increased blood volume resulting from pH-stat management.

In conclusion, our study demonstrated that pH-stat management pronounced early neuroprotective effects of hypothermia in experimental focal cerebral ischemia. This finding is of particular importance, if the currently increasing frequency of hypothermia with prolonged use of mechanical ventilation is considered.[9,10] However, because the further destiny of cerebral infarction and outcome in dependency of the acid–base management is unknown, further studies with a more clinically relevant design appear warranted.

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