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Relationship between Local Cerebral Blood Flow and Metabolism during Mild and Moderate Hypothermia in Rats

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Background: **Hypothermia may interfere with the relationship between cerebral blood flow (CBF) and metabolism. Because this conclusion was based on the analysis of global values, the question remains whether hypothermic CBF/metabolism uncoupling exists on a local cerebral level. This study investigated the effects of hypothermic anesthesia on local cerebral blood flow (LCBF) and local cerebral glucose utilization (LCGU).**

Methods: **Thirty-six rats were anesthetized with isoflurane (1 minimum alveolar concentration) and artificially ventilated to maintain normal arterial carbon dioxide partial pressure (*p*H-stat). Pericranial temperature was maintained as normothermic (37.5°C, n = 12) or was reduced to 35°C (n = 12) or 32°C (n = 12). Pericranial temperature was maintained constant for 60 min until LCBF or LCGU were measured by autoradiography. Twelve conscious rats served as normothermic controls.**

Results: **Compared with conscious animals, mean CBF remained unchanged during normothermic anesthesia. Mean CBF significantly increased during mild hypothermia but was unchanged during moderate hypothermia. During normothermic anesthesia, mean CGU was 45% lower than in conscious controls ($P < 0.05$). No further CGU reduction was found during mild hypothermia, whereas CGU further decreased during moderate hypothermia (48%; $P < 0.05$). Local analysis showed a linear LCBF/LCGU relationship in conscious (r = 0.94) and anesthetized (r = 0.94) normothermic animals, as well as in both hypothermic groups (35°C: r = 0.92; 32°C: r = 0.95; $P < 0.05$). The LCBF-to-LCGU ratio increased from 1.4 (conscious controls) to 2.4 (normothermic isoflurane) and 3.6 ml/μmol (mild and moderate hypothermia, $P < 0.05$).**

Conclusions: **Decrease of mean CGU at unchanged or increased mean CBF during hypothermic anesthesia may not indicate uncoupling. Local analysis shows a maintained linear relationship that is reset to a higher CBF/CGU ratio. (Key words: Autoradiography; coupling; local cerebral glucose utilization; neuroprotection; *p*H management; *p*H-stat.)**

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EXPERIMENTAL studies have demonstrated that hypothermia improves the brain's tolerance to ischemia and exerts graded beneficial effects on neurologic outcome after cerebral ischemia.[1,2] In addition, the clinical use of hypothermia has been assumed to be beneficial in the treatment of brain injury,[3–5] although the recent North American multicenter trial on the use of hypothermia in patients with head injury failed to prove this hypothesis (G. L. Clifton, personal communication, May 1999). Furthermore, mechanisms that mediate the brain protection by hypothermia have not been completely elucidated. Hypothermic neuroprotection may be caused by a reduced cerebral metabolism, which could preserve cellular energy stores and aerobic metabolism, or by other mechanisms, such as a reduced release of excitatory neurotransmitters.[6]

In contrast to these beneficial effects, another clinical finding might argue against a protective effect of hypothermia. In cardiac patients undergoing hypothermic cardiopulmonary bypass, an increase of cerebral blood

flow (CBF) along with a decrease of cerebral metabolic rate (CMR) for oxygen and glucose was observed during *p*H-stat management.[7] In addition, Murkin *et al.*[8] reported independent variations of mean CBF and CMR of oxygen in hypothermic cardiac surgery patients that they interpreted as cerebral flow/metabolism uncoupling. Because both studies were based on the analysis of global values only (*e.g.*, using the ^{133}Xenon clearance or argon washin techniques), the question remains whether flow/metabolism relationship is maintained on a local cerebral level. Another factor that could induce discrepancies between CBF and metabolism is anesthesia.[9] Because clinical hypothermia is induced during anesthesia, it is necessary to separate the effects of anesthesia from those of hypothermia.

Our hypothesis was that a linear relationship between local CBF (LCBF) and metabolism is maintained during isoflurane anesthesia, as well as during mild (35°C) or moderate (32°C) hypothermia, and that a major reduction in local cerebral glucose utilization (LCGU) can be achieved by moderate reductions in brain temperature. Therefore, the effects of mild and moderate hypothermia on the relationship between LCBF and LCGU were investigated in anesthetized rats, and the results were compared with those obtained from conscious and anesthetized rats during normothermia.

Materials and Methods

Animals

After obtaining approval from the institutional animal care committee (Regierungspräsidium Karlsruhe, Germany), the experiments were performed on 48 male Sprague-Dawley rats weighing 326 ± 29 g (Charles River Deutschland, Sulzfeld, Germany). Animals were kept under temperature-controlled environmental conditions on a 14:10 light:dark cycle, were fed a standard diet (Altromin C 1000; Altromin, Lage, Germany), and were allowed free access to food and water *ad libitum* until the experiments were started.

Study Groups and Experimental Protocol

Rats were placed in a small box and anesthetized by inhalation of a gas mixture of isoflurane (1 minimum alveolar concentration [MAC]), oxygen (40%), and air (remainder). Afterward, 1 MAC of isoflurane (Forene; Abbott, Wiesbaden, Germany) was administered using a precalibrated vaporizer (Fortec; Cyprane Keighley, United Kingdom) by inhalation *via* a nose cone. MAC values were corrected for reduced actual body temperatures as determined by Vitez *et al.*[10] Isoflurane, 1 MAC, corresponds to 1.2% at 37.5°C, 1.03% at 35°C, and 0.84% at 32°C using a fresh gas flow of 2 l/min. 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). Artificial ventilation was performed using capnometric control of end-tidal carbon dioxide partial pressure (Capnometer, Heyer, Bad Ems, Germany) and continuous pulse oximetry (Onin 8600V Pulse Oximeter; Onin Medical Inc., Plymouth, MN). Arterial blood gases were checked in a pH/blood gas analyzer (AVL Gas Check 939; AVL, Graz, Austria). Pericranial temperature was measured using ultrafast microthermocouple probes (IT-23, diameter 0.3 mm; Almemo 2290-3S Thermometer, Hugo Sachs Elektronic), introduced through the masseter muscle to the outside of the base of the rat skull. Rectal temperature was measured simultaneously, and body temperature was kept constant at 37–37.5°C with a temperature-controlled heating pad during the surgical preparation (Harvard Ltd., Kent, United Kingdom). Physiologic parameters were assessed and recorded just before the determination of CBF or CGU.

After completion of the surgical preparation, animals were randomly assigned to one of the following four groups:

1. Normothermic, conscious control animals (n = 12). These animals were allowed to recover from anesthesia after instrumentation of the femoral vessels. Thereafter, the animals were placed in a rat restrainer and were studied approximately 60 min after recovery from anesthesia. The temperature-controlled heating pad was only used during the surgical preparation, and physiologic temperature regulation was not influenced after recovery from anesthesia.
2. Normothermic, anesthetized animals (n = 12). In these rats, anesthesia was maintained at 1.2% isoflurane (1 MAC), and body temperature was kept constant at 37.5°C using a hollow plastic spiral surrounding the entire animal body, which was continuously perfused with water of target temperature. Animals were mechanically ventilated to maintain an arterial carbon dioxide partial pressure (Pa_{CO_2}) of 40 mmHg,

and experiments were started after a 60-min stabilization period.

3. Mildly hypothermic, anesthetized, animals (n = 12). Animals assigned to this group were first allowed to cool down passively and then were actively cooled until the target temperature was reached (approximately 30 min). Active cooling was also performed using the hollow plastic spiral, continuously perfused with water of target temperature. After achieving the target temperature of 35°C, pericranial temperature was maintained constant for 60 min until the radioactive tracer was administered. Blood gases were corrected for actual body temperature, and ventilatory support was adjusted to maintain a Pa_{CO_2} of 40 mmHg (after correction for individual core temperature, *i.e.*, *p*H-stat management).
4. Moderately hypothermic, anesthetized animals (n = 12). Animals assigned to this group were treated as described for the mildly hypothermic group, with the exception that body temperature was decreased to 32°C.

Measurement of LCBF and LCGU

In each group, the 12 rats were randomized to be used either for the autoradiographic determination of LCBF (n = 6) or for the measurement of LCGU (n = 6) according to the methods described by Sokoloff *et al.*[11] and Sakurada *et al.*[12] Previous studies have validated these autoradiographic methods for a wide range of body and brain temperatures down to a body temperature of 9°C.[13–15]

For the measurement of LCGU, 125 μCi/kg body weight of 2-[1-^{14}C]deoxy-D-glucose (specific activity, 50–56 mCi/mmol; New England Nuclear, Dreieich, Germany) was injected as a pulse *via* the femoral venous catheter over 20 s, and timed arterial blood samples of 80 μl were collected through the arterial catheter at 15, 30, and 45 s and at 1, 2, 3, 5, 7.5, 10, 15, 25, 35, and 45 min. The blood samples were immediately centrifuged and stored on ice until assays for plasma 2-[1-^{14}C]deoxy-D-glucose and glucose concentrations were performed. Immediately after the final arterial blood sample was collected, the animal was decapitated and the brain was rapidly removed and frozen in isopentane chilled to −60°C.

For the measurement of LCBF, 100 μCi/kg body weight of 4-iodo[*N*-methyl-^{14}C]antipyrine (specific activity, 54 mCi/mmol; Amersham-Buchler, Braunschweig, Germany) dissolved in 1 ml saline was infused continuously at a progressively increasing infusion rate for 1 min *via* the femoral venous catheter. The progressively increasing infusion rate, a modification of the method described previously,[12] was chosen to minimize equilibration of rapidly perfused tissues with arterial blood during the period of measurement. During the 1-min infusion period, 14–20 timed blood samples were collected in drops from the free-flowing arterial catheter directly onto filter paper disks (1.3 cm in diameter) that had been prepared in small plastic beakers and weighed. The samples were weighed, and radioactivity was estimated 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 animal was decapitated and the brain was removed as quickly as possible and frozen in isopentane chilled to −60°C. In both the 2-[1-^{14}C]deoxy-D-glucose and 4-iodo[*N*-methyl-^{14}C]antipyrine experiments, the frozen brains were coated with 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 autoradiographed along with precalibrated [^{14}C]methyl methacrylate standards.

Local tissue concentrations of [^{14}C] were determined from the autoradiographs by densitometric analysis. LCGU and LCBF were calculated from the local concentrations of [^{14}C] and the time courses of the plasma [^{14}C]deoxyglucose and iodo[^{14}C]antipyrine concentrations, including corrections for the lag and washout in the arterial catheter.[11] The washout correction rate constant was 100/min, and the brain–blood partition coefficient for iodo[^{14}C]antipyrine was 0.9 in our rats.[16]

Autoradiographic images were converted to digitized optical density images by an image processing system (MCID; Imaging Research, St. Catharines, Canada). For measurements of separate brain structures, an ellipsoid cursor was used and adjusted to the size of the individual region. For measurement of mean global CBF or mean global CGU (referred to hereafter as mean CBF and mean CGU), coronal sections were analyzed as a whole at distances of 200 μm, and the values were summarized to obtain the area-weighted means of all measured sections.[17]

Statistical Analysis

Differences between the experimental groups were evaluated by analysis of variance, and Bonferroni correction was used when multiple comparisons were performed. Data are presented as mean ± SD, and a P value < 0.05 was considered statistically significant. The

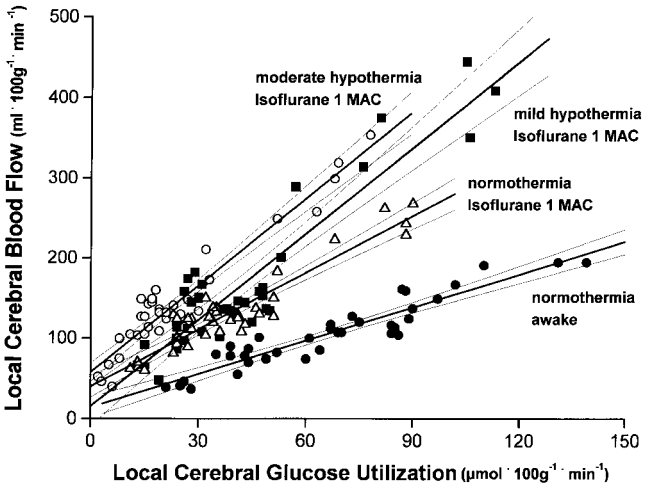

Fig. 1. Relationship between local cerebral glucose utilization (LCGU) and local cerebral blood flow (LCBF) in normothermic, conscious (filled circles), normothermic, anesthetized (triangles), mildly hypothermic, anesthetized (35°C; squares), and moderately hypothermic, anesthetized (32°C, open circles) animals. For each of the examined 37 brain structures, the mean of the values of LCBF is plotted against the mean of the values of LCGU. The regression lines were calculated according to y = ax + b, with y being LCBF and x being LCGU. Normothermic conscious control group: y = 1.4x + 14, r = 0.94; normothermic anesthetized group: y = 2.4x + 40, r = 0.94; mildly hypothermic, anesthetized group: y = 3.6x + 16, r = 0.92; and moderately hypothermic, anesthetized group: y = 3.6x + 58, r = 0.95 ($P < 0.05$ between the slopes of all groups except for mild *vs.* moderate hypothermia).

overall relationship between LCGU and LCBF in the examined structures of the brain (fig. 1) was assessed by the least-squares fit of the data to y = ax + b, where x is the mean LCGU in a given region and y is the mean LCBF in that same area. Contrasts of slopes of the LCBF/LCGU regression lines were tested by common *t* test statistics with Bonferroni correction for multiple comparisons. Because of the limitations of this kind of analysis, an additional, more rigorous statistical approach using log-transformed data were applied, examining the relationship of LCBF and LCGU by a repeated measure of the analysis of variance according to McCulloch *et al.*[18] and Ford *et al.*[19] For this analysis, a computer software package (BMDP2v; BMDP Statistical Software Inc., Los Angeles, CA) considering interanimal variability and enabling the detection of heterogeneities in the relationship between LCGU and LCBF was used.

Results

All animals survived the surgical procedure. Physiologic variables of conscious, anesthetized, and hypothermic rats are given in table 1. No statistically significant differences in baseline values were observed between all four groups. Compared with normothermic conscious rats, mildly hypothermic animals showed slightly, although significantly reduced, heart rate and pH, whereas arterial oxygen tension and plasma glucose concentrations increased. In the moderately hypothermic group, mean arterial pressure reached baseline values of conscious controls, and hematocrit increased slightly compared with the normothermic conscious rats (table 1).

Cerebral blood flow was measured for the entire brain, as well as regionally for 37 different brain structures. The results are shown in table 2. Compared with normothermic conscious animals, mean CBF was not significantly altered during isoflurane anesthesia (1 MAC). Mean CBF was significantly increased during mild hypothermia ($P < 0.05$) but did not differ from normothermic conscious controls in the moderately hypothermic group. On a local level, no significant reduction in LCBF was observed during mild or moderate hypothermia in any structure compared with normothermic anesthetized animals. LCBF significantly increased in 9 of 37 structures during mild hypothermia and in 1 structure during moderate hypothermia compared with normothermic anesthetized animals ($P < 0.05$).

In addition to blood flow, mean CGU and LCGU were measured in the four different groups of rats. The results are shown in table 3. During isoflurane anesthesia (1 MAC), mean CGU was reduced from 56 ± 3 to 31 ± 5 $\mu mol \cdot 100\ g^{-1} \cdot min^{-1}$ compared with the conscious group (45%; $P < 0.05$). No further reduction in mean CGU was achieved by cooling to 35°C (32 ± 3 $\mu mol \cdot 100\ g^{-1} \cdot min^{-1}$; P = nonsignificant). Moderate hypothermia resulted in an additional significant reduction of mean CGU to 16 ± 3 $\mu mol \cdot 100\ g^{-1} \cdot min^{-1}$ (48% *vs.* isoflurane and 71% *vs.* conscious group; $P < 0.05$). The local changes observed in the 37 brain structures are also shown in table 3. Compared with the normothermic anesthetized group, mild hypothermia did not induce significant changes in LCGU within any individual structure investigated. This result is congruent with the lack of change in mean CGU in this group. Moderate hypothermia induced a significant reduction in LCGU compared with the normothermic anesthetized group in 32 of 37 brain regions examined ($P < 0.05$). LCGU was reduced by > 50% in 15 of these 32 brain structures and by 20–50% in 17 structures.

The relationship between LCBF and LCGU is plotted in figure 1. A strong linear relationship was found between LCBF and LCGU in the conscious and anesthetized nor-

Table 1. Physiologic Variables of the Experimental Groups

	Normothermia Conscious	Normothermia 1 MAC Isoflurane	Mild Hypothermia 1 MAC Isoflurane	Moderate Hypothermia 1 MAC Isoflurane
Pericranial temperature (°C)	36.7 ± 0.7	37.1 ± 0.2	34.9 ± 0.3*†	32.0 ± 0.1*†‡
Arterial *p*H	7.40 ± 0.02	7.42 ± 0.04	7.30 ± 0.04*†	7.35 ± 0.04*†‡
Pa_{O_2} (mmHg)	93 ± 4	114 ± 28*	215 ± 46*†	160 ± 55*†‡
Pa_{CO_2} (mmHg)	41 ± 2	41 ± 4	41 ± 2	43 ± 11
Plasma glucose concentration (mg/dl)	153 ± 15	195 ± 30*	207 ± 53*	243 ± 75*
Hematocrit (%)	43 ± 3	41 ± 3	43 ± 3	46 ± 3*†‡
MAP (mmHg)	134 ± 17	114 ± 18*	110 ± 11*	134 ± 16†‡
Heart rate (beats/min)	427 ± 47	363 ± 41*	381 ± 53*	363 ± 33*

Values are mean ± SD. MAP = mean arterial blood pressure; MAC = minimum alveolar concentration.

* $P < 0.05$ *versus* normothermic, conscious animals.

† $P < 0.05$ *versus* normothermic, anesthetized animals.

‡ $P < 0.05$ *versus* mildly hypothermic, anesthetized animals.

mothermic groups, as well as in both hypothermic groups. This is indicated by the correlation coefficients between LCBF and LCGU for normothermic conscious ($r = 0.94$), normothermic anesthetized ($r = 0.94$), and hypothermic rats (35°C: $r = 0.92$; 32°C: $r = 0.95$). These correlation coefficients were significantly different from zero ($P < 0.05$).

The ratio between LCBF and LCGU is reflected by the slope of the individual LCBF–LCGU regression line (fig. 1). Isoflurane anesthesia caused a significant increase in the slope of the regression line from 1.4 in the normothermic conscious animals to 2.4 ml/μmol after anesthesia induction ($P < 0.05$). Mild and moderate hypothermia further reset the ratio of LCBF to LCGU, and the slope of the regression line increased significantly to 3.6 ml/μmol ($P < 0.05$). No significant difference was found between the slopes of the regression lines of mildly and moderately hypothermic rats.

Discussion

The main finding of the present study was that a strong linear relationship between LCBF and metabolism is maintained during normothermic isoflurane anesthesia (1 MAC), as well as during mild and moderate hypothermic isoflurane anesthesia. Anesthesia resulted in a significant resetting of the slope of the LCBF-to-LCGU regression line to a higher level. During mild hypothermia, a further significant increase in the LCBF-to-LCGU slope was observed when compared with normothermic anesthesia. No additional increase in the slope of the LCBF-to-LCGU regression line was found during moderate hypothermia compared with mild hypothermia. Therefore, a marked reduction in mean CGU at unchanged or even increased mean CBF does not necessarily indicate flow/metabolism uncoupling, because a strong linear relationship is maintained on a local level, suggesting intact coupling.

In this context, the following issues need consideration: (1) relationship between CBF and metabolism as reported in previous studies; (2) influence of acid-base management during hypothermia on CBF; (3) applicability of autoradiographic methods during hypothermia; (4) role of the flow–metabolism relationship in the neuroprotection induced by hypothermia; and (5) limitations of the study.

Relationship between CBF and Metabolism during Hypothermia

In the present study, using *p*H-stat management, mean CBF was unchanged during anesthesia (26%) and was significantly increased (42%; $P < 0.05$) or remained stable during mild or moderate hypothermia, respectively, compared with conscious controls. No reductions in mean CBF or LCBF were observed during mild or moderate hypothermia compared with conscious controls. The response of mean CBF to hypothermia in the normal brain is still a matter of a controversial discussion. It seems to depend on the acid-base management strategy used and the presence or absence of anesthesia.[9] Although several investigators have reported hypothermia-induced reductions in CBF,[20–22] Verhaegen *et al.*[23] demonstrated in anesthetized rats that hypothermia reduces CBF mainly during α-stat management, whereas CBF remains similar to normothermic controls during *p*H-stat management. This is in accordance with the clinical observation of a higher CBF in patients undergoing hypothermic cardiopulmonary bypass using *p*H-stat

Table 2. Local Cerebral Blood Flow of the Experimental Groups

	Local Cerebral Blood Flow (ml · 100 g^{-1} · min^{-1})			
	Normothermia Conscious	Normothermia 1 MAC Isoflurane	Mild Hypothermia 1 MAC Isoflurane	Moderate Hypothermia 1 MAC Isoflurane
Cerebellum				
Cerebellar cortex	87 ± 6	124 ± 30	158 ± 37*	127 ± 29
Dentate nuclei	159 ± 15	223 ± 57	375 ± 93*†	258 ± 55*‡
Medulla-pons				
Vestibular nucleus	149 ± 20	229 ± 48	409 ± 66*†	354 ± 83*†
Cochlear nucleus	161 ± 20	262 ± 74	351 ± 102*	299 ± 68*
Superior olive	191 ± 26	268 ± 55	445 ± 68*†	319 ± 93*‡
Pontine gray	101 ± 11	129 ± 31	174 ± 33*†	149 ± 21*
Lateral lemniscus	158 ± 19	183 ± 47	289 ± 50*†	211 ± 15‡
Mesencephalon				
Inferior colliculus	195 ± 26	243 ± 45	314 ± 52*†	249 ± 26‡
Superior colliculus	117 ± 14	138 ± 26	167 ± 34*	160 ± 37
Substantia nigra, c.p.	100 ± 15	137 ± 24	163 ± 45*	148 ± 43
Substantia nigra, r.p.	90 ± 17	132 ± 22*	150 ± 33*	114 ± 22
Diencephalon				
Medial geniculate body	167 ± 20	150 ± 27	182 ± 47	149 ± 54
Lateral geniculate body	107 ± 18	124 ± 27	151 ± 28*	145 ± 35
Mammillary body	127 ± 22	150 ± 21	201 ± 40*†	173 ± 39
Hypothalamus	78 ± 9	93 ± 8	100 ± 17	105 ± 22*
Ventral thalamus	112 ± 10	124 ± 26	145 ± 46	127 ± 24
Lateral thalamus	120 ± 20	138 ± 21	136 ± 34	143 ± 57
Telencephalon				
Hippocampus CA1	74 ± 6	108 ± 22	102 ± 19	109 ± 43
Hippocampus CA2	70 ± 11	112 ± 31*	112 ± 20*	104 ± 40
Hippocampus CA3	82 ± 8	117 ± 23	137 ± 28*	119 ± 40
Hippocampus CA4	85 ± 7	127 ± 33	120 ± 22	117 ± 46
Dentate gyrus	74 ± 7	104 ± 25	108 ± 20	105 ± 35
Amygdaloid complex	80 ± 13	89 ± 9	97 ± 26	100 ± 29
Globus pallidus	55 ± 8	81 ± 17	87 ± 25	75 ± 38
Caudate nucleus	104 ± 16	129 ± 16	146 ± 31	132 ± 47
Nucleus accumbens	107 ± 10	128 ± 25	133 ± 33	136 ± 49
Visual cortex	116 ± 14	125 ± 26	144 ± 35	130 ± 40
Auditory cortex	195 ± 25	130 ± 24*	153 ± 33	134 ± 39
Parietal cortex	113 ± 10	122 ± 14	134 ± 41	114 ± 42
Sensory motor cortex	137 ± 14	126 ± 24	137 ± 40	132 ± 41
Frontal cortex	124 ± 21	120 ± 18	134 ± 31	124 ± 38
Pyriform cortex	106 ± 18	108 ± 15	158 ± 29*†	141 ± 35
Lateral septal nuclei	78 ± 6	101 ± 13	114 ± 25*	99 ± 35
Myelinated fiber tracts				
Internal capsule	41 ± 7	62 ± 15*	66 ± 10*	46 ± 19
Corpus callosum	37 ± 6	60 ± 16*	48 ± 4	40 ± 8
Genu of corpus callosum	39 ± 6	63 ± 9*	64 ± 12*	52 ± 19
Cerebellar white matter	46 ± 5	70 ± 15*	92 ± 15*†	67 ± 7*‡
Mean CBF	90 ± 8	113 ± 18	128 ± 26*	109 ± 20

Values are mean ± SD. c.p. = compact part; r.p. = reticular part; MAC = minimum alveolar concentration; CBF = cerebral blood flow.

* $P < 0.05$ *versus* normothermic, conscious animals.

† $P < 0.05$ *versus* normothermic, anesthetized animals.

‡ $P < 0.05$ *versus* mildly hypothermic, anesthetized animals.

compared with α-stat management.[7,24] Therefore, the major reason for the increased CBF in the present study seems to be the use of *p*H-stat management in anesthetized rats.

In the present study, mean CGU was reduced to approximately one half during normothermic isoflurane anesthesia. No further reduction was observed during mild hypothermia, whereas moderate hypothermia resulted in a further significant depression of CGU to approximately one half of the normothermic anesthe-

Table 3. Local Cerebral Glucose Utilization of the Experimental Groups

	Local Cerebral Glucose Utilization (μmol · 100 g^{-1} · min^{-1})			
	Normothermia Conscious	Normothermia 1 MAC Isoflurane	Mild Hypothermia 1 MAC Isoflurane	Moderate Hypothermia 1 MAC Isoflurane
Cerebellum				
Cerebellar cortex	44 ± 3	24 ± 5*	26 ± 3*	15 ± 4*†‡
Dentate nuclei	88 ± 13	68 ± 32	81 ± 20	63 ± 26
Medulla-pons				
Vestibular nucleus	97 ± 5	88 ± 14	113 ± 25	78 ± 19‡
Cochlear nucleus	87 ± 26	82 ± 19	106 ± 11	68 ± 21‡
Superior olive	110 ± 23	90 ± 28	105 ± 19	69 ± 12*‡
Pontine gray	47 ± 5	23 ± 2*	27 ± 3*	14 ± 4*†‡
Lateral lemniscus	72 ± 5	52 ± 8*	57 ± 7*	32 ± 10*†‡
Mesencephalon				
Inferior colliculus	139 ± 33	88 ± 13*	76 ± 8*	52 ± 8*†
Superior colliculus	67 ± 9	34 ± 4*	31 ± 3*	18 ± 5*†‡
Substantia nigra c.p.	61 ± 7	46 ± 4*	48 ± 10*	27 ± 7*†‡
Substantia nigra r.p.	39 ± 6	35 ± 4	30 ± 8	16 ± 8*†‡
Diencephalon				
Medial geniculate body	102 ± 16	32 ± 6*	29 ± 5*	17 ± 4*†
Lateral geniculate body	70 ± 10	27 ± 5*	30 ± 5*	17 ± 4*†‡
Mammillary body	73 ± 7	51 ± 8*	53 ± 10*	33 ± 8*†‡
Hypothalamus	39 ± 6	24 ± 4*	24 ± 2*	11 ± 4*†‡
Ventral thalamus	67 ± 5	28 ± 5*	28 ± 4*	14 ± 3*†‡
Lateral thalamus	75 ± 6	35 ± 8*	37 ± 5*	16 ± 4*†‡
Telencephalon				
Hippocampus CA1	60 ± 7	36 ± 6*	36 ± 6*	19 ± 4*†‡
Hippocampus CA2	44 ± 6	28 ± 7*	27 ± 5*	13 ± 3*†‡
Hippocampus CA3	52 ± 5	43 ± 7	49 ± 10	24 ± 5*†‡
Hippocampus CA4	64 ± 9	51 ± 9*	45 ± 10*	24 ± 5*†‡
Dentate gyrus	49 ± 11	32 ± 7*	31 ± 6*	15 ± 4*†‡
Amygdaloid complex	35 ± 4	27 ± 8	26 ± 2*	8 ± 4*†‡
Globus pallidus	41 ± 7	23 ± 4*	24 ± 2*	8 ± 4*†‡
Caudate nucleus	86 ± 6	41 ± 7*	41 ± 4*	19 ± 5*†‡
Nucleus accumbens	69 ± 5	41 ± 7*	40 ± 6*	19 ± 6*†‡
Visual cortex	84 ± 12	41 ± 9*	43 ± 5*	25 ± 5*†‡
Auditory cortex	131 ± 22	47 ± 6*	48 ± 7*	30 ± 6*
Parietal cortex	85 ± 8	39 ± 7*	50 ± 8*	26 ± 4*†‡
Sensory motor cortex	90 ± 6	33 ± 4*	38 ± 5*	21 ± 3*†‡
Frontal cortex	89 ± 6	34 ± 5*	39 ± 4*	21 ± 3*†‡
Pyriform cortex	84 ± 6	42 ± 4*	47 ± 6*	23 ± 7*†‡
Lateral septal nuclei	43 ± 6	24 ± 4*	24 ± 3*	8 ± 3*†‡
Myelinated fiber tracts				
Internal capsule	25 ± 5	11 ± 5*	13 ± 1*	3 ± 2*†‡
Corpus callosum	28 ± 4	15 ± 5*	19 ± 2*	6 ± 3*†‡
Genu of corpus callosum	21 ± 4	13 ± 4*	15 ± 4*	2 ± 2*†‡
Cerebellar white matter	26 ± 6	13 ± 4*	15 ± 1*	5 ± 3*†‡
Mean CGU	56 ± 3	31 ± 5*	32 ± 3*	16 ± 3*†‡

Values are mean ± SD. c.p. = compact part; r.p. = reticular part; MAC = minimum alveolar concentration; CGU = cerebral glucose utilization.

* $P < 0.05$ *versus* normothermic, conscious animals.

† $P < 0.05$ *versus* normothermic, anesthetized animals.

‡ $P < 0.05$ *versus* mildly hypothermic, anesthetized animals.

tized values. These findings are in accordance with the literature. Previous studies in rats reported that isoflurane (1 MAC) induces CGU reductions of approximately 40%,[9,17] whereas moderate hypothermia alone reduces LCGU by 13–42% in conscious rats.[14] Furthermore, the magnitude of LCGU suppression by hypothermia has been reported to be relatively independent of the acid-base management strategy used.[25]

By combining LCBF and LCGU data, regression lines were constructed, demonstrating a maintained strong

relationship between LCBF and LCGU during normothermic anesthesia and mild or moderate hypothermic anesthesia. During normothermic isoflurane anesthesia, the LCBF-to-LCGU ratio increased. This relative hyperperfusion was further augmented by hypothermia. No differences were observed in the LCBF-to-LCGU ratio between mild and moderate hypothermia. Despite this relative hyperperfusion, the physiologic flow/metabolism relationship was maintained on a local level during normothermic and hypothermic isoflurane anesthesia. In two clinical studies, an uncoupling of CBF and metabolism has been reported during hypothermia and *p*H-stat management.[7,8] Both investigations[7,8] were performed in patients during hypothermic cardiopulmonary bypass and showed a marked increase in CBF (argon washin technique or ^{133}Xenon clearance) together with a marked decrease in CMR of oxygen and cerebral glucose uptake,[7] which has been interpreted as uncoupling. This conclusion was based on the analysis of global values of CBF and CMR of oxygen. However, the present study shows that a marked decrease of mean CGU together with an unchanged or even increased mean CBF does not necessarily indicate uncoupling, because a strong linear relationship between CBF and CGU is observed on a local level.

Influence of Acid-base Management during Hypothermia on CBF

Due to the interdependence of pH, Pa_{CO_2}, and CBF, the method chosen for acid-base management during hypothermia is of crucial importance for the interpretation of CBF measurements. At a reduced blood temperature, the solubility of carbon dioxide is markedly increased. If the total carbon dioxide content of the blood remains constant, the Pa_{CO_2} is reduced during hypothermia. Two different strategies for the acid-base management during hypothermia have been proposed. In α-stat management, Pa_{CO_2} is maintained at 40 mmHg when measured at 37°C. The dissociation fraction of the imidazole moiety of histidine is kept constant, and the pH is changed in parallel to the changes in the neutral pH of water. In *p*H-stat management, Pa_{CO_2} is maintained at 40 mmHg when corrected to the patient's actual body temperature. Because temperature correction results in reduced Pa_{CO_2} (because of increased gas solubility at the lower temperature), the *p*H-stat strategy requires controlled hypoventilation or addition of carbon dioxide to the inspired gas. As a consequence, the net effect of *p*H-stat management is an increase in CBF.[26] Therefore, *p*H-stat management used in the present study may have caused the significantly higher CBF values of the anesthetized mildly hypothermic rats compared with the anesthetized normothermic rats. There is considerable debate concerning the choice of α-stat or *p*H-stat management for the prevention and amelioration of ischemia, *e.g.*, during hypothermic cardiopulmonary bypass. *p*H-stat management is associated with higher CBF, resulting in an increased intensity of brain cooling to the desired level of cerebral hypothermia.[27] Furthermore, during *p*H-stat management, cortical deoxygenation[27] and oxyhemoglobin affinity[28] are decreased compared with α-stat management. Disadvantages of *p*H-stat management are an actually decreased *p*H and increased intracranial blood volume. *p*H-stat management was chosen for the present study to achieve a more rapid and homogeneous temperature profile throughout the entire rat brain. By the increased values of CBF, *p*H-stat management might reduce the risk of temperature gradients within the brain, which could result in gradients of LCBF and LCGU.

Applicability of Autoradiographic Methods during Hypothermia

In the present study, autoradiographic methods were used for the quantification of CBF[12] and CGU.[11] These techniques are based on kinetic models that may need modification during hypothermia. For the autoradiographic determination of LCGU, a model is used in which the major part of radiolabeled 2-deoxyglucose has to be phosphorylated after the experimental period of 45 min. The enzymatic process of phosphorylation may be slowed down by hypothermia, which could result in an overestimation of CGU. However, Nakashima *et al.*[15] have demonstrated during hypothermia (25°C) that all of the labeled 2-deoxyglucose in brain is phosphorylated after the 45-min waiting period. The investigators concluded that no adaptations in the method of Sokoloff *et al.*[11] are necessary to autoradiographically assess LCGU in hypothermic rats. The [^{14}C]iodoantipyrine was used for the measurement of LCBF in the present study. The distribution of [^{14}C]iodoantipyrine is mainly diffusion-dependent. With the relatively small changes in body temperature, negligible changes in [^{14}C]iodoantipyrine diffusion can be expected. Consequently, the [^{14}C]-iodoantipyrine method has been used for determination of CBF even in hibernating squirrels at a core temperature of 9°C.[13] Autoradiographic techniques have previously been used in several studies of hypothermia.[14,15,29]

Role of Flow–Metabolism Relationship in the Neuroprotection Induced by Hypothermia

The aim of clinical hypothermia is the protection of neurons compromised by ischemia.[3–5] Several mechanisms are under discussion that could mediate such a protective effect. One potential mechanism is the reduction of cerebral metabolism. In the present study, mean CGU was reduced by approximately 50% during moderate hypothermia (32°C) when compared with normothermic isoflurane anesthesia. Such a strong reduction of CGU points to reduced cerebral metabolism as an important mechanism of brain protection by moderate hypothermia. On the contrary, mean CGU during mild hypothermia (35°C) remained unchanged when compared with normothermic anesthetized animals. Under these conditions, a reduction of cerebral metabolism cannot be considered as the primary cause of hypothermic neuroprotection. Because several studies have demonstrated that mild hypothermia also reduces cell death or even improves neurologic outcome,[30,31] other mechanisms, such as a reduced release of excitatory neurotransmitters or oxygen free radical or an inhibition of protein kinase C, must be considered.[32–35] Furthermore, the resetting of the ratio of CBF to CGU to higher values observed in the present study might exert beneficial effects by improving oxygen availability within the brain. Therefore, CMR reduction seems to contribute to neuroprotection during moderate hypothermia, but additional mechanisms, such as a reduced release of excitotoxins, seem to be related to the beneficial effects of mild hypothermia.

Limitations of the Study

One limitation of the present study is that, because of animal welfare, the effects of mild hypothermia on LCGU and LCBF were studied during anesthesia. However, the use of conscious animals would result in shivering and stress during cooling, which could induce additional changes in CBF and CGU. Furthermore, anesthesia is also used in the clinical setting when hypothermia is induced for the prevention of brain damage.

A second limitation of the present study is that brain temperature was not measured directly.[36] Direct measurement of brain temperature by implanted probes was avoided because of a potential tissue damage that could result in altered values of CBF and CGU. Tissue damage was avoided in the present study by the use of ultrafast microthermocouple probes that were advanced through the masseter muscle to the outside of the base of the rat skull.[1] By this procedure, the temperature was recorded as close to the brain as possible without potential traumatic damage.

A third possible limitation of the study might be the statistical approach chosen for data analysis. Assessing the relationship between LCBF and LCGU by linear regression analysis and the derived correlation coefficients is open to criticism. A fundamental assumption of this analytical approach is that observations on different regions of the brain are statistically independent. Because observations are repeated on multiple regions of all animals and only the average values for each region are used, the real uncertainty about the relationship between CGU and CBF might be greater than the regression analysis suggests.[18] To exclude erroneous data interpretation by inappropriate statistical analysis, a second approach proposed by McCulloch *et al.*[18] and Ford *et al.*[19] was used in this study. This approach examines the LCBF/LCGU relationship by a repeated-measure analysis of variance using log-transformed data and yielded the same results.

In conclusion, the decrease of mean CGU at unchanged or increased mean CBF observed during normothermic and hypothermic isoflurane anesthesia using *p*H-stat management does not necessarily indicate uncoupling of blood flow and metabolism in the brain. Local analysis shows a strong linear relationship between LCBF and LCGU, suggesting an intact coupling that is reset to a higher blood flow/metabolism ratio. Therefore, mild and moderate hypothermia during *p*H-stat management did not interfere with the local relationship between CBF and metabolism.

References

1. Hoffman WE, Thomas C: Effects of graded hypothermia on outcome from brain ischemia. Neurol Res 1996; 18:185–9

2. Gunn AJ, Gunn TR, de Haan HH, Williams CE, Gluckman PD: Dramatic neurological rescue with prolonged selective head cooling after ischemia in fetal lambs. J Clin Invest 1997; 99:248–56

3. Clifton GL, Jiang JY, Lyeth BG, Jenkins LW, Hamm RJ, Hayes RL: Marked protection by moderate hypothermia after traumatic brain injury. J Cereb Blood Flow Metab 1991; 11:114–21

4. Clifton GL, Allen S, Barrodale P, Plenger P, Berry J, Koch S, Fletcher J, Hayes RL, Choi SC: A phase II study of moderate hypothermia in severe brain injury. J Neurotrauma 1993; 10:263–71

5. Marion DW, Penrod LE, Kelsey SF, Obrist WD, Kochanek PM, Palmer AM, Wisniewski SR, DeKosky ST: Treatment of traumatic brain injury with moderate hypothermia. N Engl J Med 1997; 336:540–6

6. Illievich UM, Zornow MH, Choi KT, Scheller MS, Strnat MAP: Effects of hypothermic metabolic suppression on hippocampal glutamate concentrations after transient global cerebral ischemia. Anesth Analg 1994; 78:905–11

7. Stephan H, Weyland A, Kazmaier S, Henze T, Menck S, Sonntag H: Acid-base management during hypothermic cardiopulmonary bypass does not affect cerebral metabolism but does affect blood flow and neurological outcome. Br J Anaesth 1992; 69:51–7

8. Murkin JM, Farrar JK, Tweed A, McKenzie FN, Guiraudon G: Cerebral autoregulation and flow/metabolism coupling during cardiopulmonary bypass: The influence of $PaCO_2$. Anesth Analg 1987; 66: 825–32

9. Maekawa T, Tommasino C, Shapiro HM, Keifer-Goodman J, Kohlenberger RW: Local cerebral blood flow and glucose utilization during isoflurane anesthesia in the rat. ANESTHESIOLOGY 1986; 65:144–51

10. Vitez TS, White PF, Eger EI: Effects of hypothermia on halothane MAC and isoflurane MAC in the rat. ANESTHESIOLOGY 1974; 41:80–1

11. Sokoloff L, Reivich M, Kennedy C, Des Rosiers MH, Patlak CS, Pettigrew KD, Sakurada O, Shinohara M: The [^{14}C]deoxyglucose method for the measurement of local cerebral glucose utilization: Theory, procedure, and normal values in the conscious and anesthetized albino rat. J Neurochem 1977; 28:897–916

12. Sakurada O, Kennedy C, Jehle J, Brown JD, Carbin GL, Sokoloff L: Measurement of local cerebral blood flow with iodo [^{14}C] antipyrine. Am J Physiol 1978; 234:H59–66

13. Frerichs KU, Kennedy C, Sokoloff L, Hallenbeck JM: Local cerebral blood flow during hibernation: A model of natural tolerance to "cerebral ischemia." J Cereb Blood Flow Metab 1994; 14:193–205

14. McCulloch J, Savaki HE, Jehle J, Sokoloff L: Local cerebral glucose utilization in hypothermic and hyperthermic rats. J Neurochem 1982; 39:255–8

15. Nakashima K, Todd MM, Warner DS: The relation between cerebral metabolic rate and ischemic depolarization: A comparison of the effects of hypothermia, pentobarbital, and isoflurane. ANESTHESIOLOGY 1995; 82:1199–208

16. Waschke K, Schröck H, Albrecht DM, van Ackern K, Kuschinsky W: Local cerebral blood flow and glucose utilization after blood exchange with a hemoglobin-based O_2 carrier in conscious rats. Am J Physiol 1993; 265:H1243–8

17. Lenz C, Rebel A, van Ackern K, Kuschinsky W, Waschke KF: Local cerebral blood flow, local cerebral glucose utilization, and flow-metabolism coupling during sevoflurane versus isoflurane anesthesia in rats. ANESTHESIOLOGY 1998; 89:1480–8

18. McCulloch J, Kelly PAT, Ford I: Effect of apomorphine on the relationship between local cerebral glucose utilization and local cerebral blood flow (with an appendix on its statistical analysis). J Cereb Blood Flow Metab 1982; 2:487–99

19. Ford I, McColl JH, McCormack AG, McCrory SJ: Statistical issues in the analysis of neuroimages. J Cereb Blood Flow Metab 1991; 11:A89–95

20. Klementavicius R, Nemoto EM, Yonas H: The Q10 ratio for basal cerebral metabolic rate for oxygen in rats. J Neurosurg 1996; 85:482–7

21. Niwa K, Takizawa S, Takagi S, Shinohara Y: Mild hypothermia disturbs regional cerebrovascular autoregulation in awake rats. Brain Res 1998; 789:68–73

22. Busija DW, Leffler CW: Hypothermia reduces cerebral metabolic rate and cerebral blood flow in newborn pigs. Am J Physiol 1987; 253:H869–73

23. Verhaegen MJJ, Todd MM, Hindman BJ, Warner DS: Cerebral autoregulation during moderate hypothermia in rats. Stroke 1993; 24:407–14

24. Hindman BJ: Choice of α-stat or *p*H-stat management and neurologic outcomes after cardiac surgery: It depends. ANESTHESIOLOGY 1998; 89:5–7

25. Hindman BJ, Dexter F, Cutkomp J, Smith T, Tinker JH: Hypothermic acid-base management does not affect cerebral metabolic rate for oxygen at 27 degrees C: A study during cardiopulmonary bypass in rabbits. ANESTHESIOLOGY 1993; 79:580–7

26. Brian JE: Carbon dioxide and the cerebral circulation. ANESTHESIOLOGY 1998; 88:1365–86

27. Kurth CD, O'Rourke MM, O'Hara IB: Comparison of *p*H-stat and alpha-stat cardiopulmonary bypass on cerebral oxygenation and blood flow in relation to hypothermic circulatory arrest in piglets. ANESTHESIOLOGY 1998; 89:110–8

28. Callaghan PB, Lister MB, Paton BC, Swan H: Effect of varying carbon dioxide tensions on the oxyhemoglobin dissociation curves under hypothermic conditions. Ann Surg 1961; 154:903–10

29. Palmer C, Vannucci RC, Christensen MA, Brucklacher RM: Regional cerebral blood flow and glucose utilization during hypothermia in newborn dogs. ANESTHESIOLOGY 1989; 71:730–7

30. Reith J, Jorgensen HS, Pedersen PM, Nakayama H, Raaschou HO, Jeppesen LL, Olsen TS: Body temperature in acute stroke: Relation to stroke severity, infarct size, mortality, and outcome. Lancet 1996; 347:422–5

31. Sano T, Drummond JC, Patel PM, Grafe MR, Watson JC, Cole DJ: A comparison of the cerebral protective effects of isoflurane and mild hypothermia in a model of incomplete forebrain ischemia in the rat. ANESTHESIOLOGY 1992; 76:221–8

32. Hartung J, Cottrell JE: Mild hypothermia and cerebral metabolism. J Neurosurg Anesthesiol 1994; 6:1–3

33. Thoresen M, Satas S, Puka-Sundvall M, Whitelaw A, Hallström A, Loberg EM, Ungerstedt U, Steen PA, Hagberg H: Post-hypoxic hypothermia reduces cerebrocortical release of NO and excitotoxins. NeuroReport 1997; 8:3359–62

34. Illievich UM, Zornow MH, Choi KT, Strnat MAP, Scheller MS: Effects of hypothermia or anesthetics on hippocampal glutamate and glycine concentrations after repeated transient global cerebral ischemia. ANESTHESIOLOGY 1994; 80:177–86

35. Conroy BP, Lin CY, Jenkins LW, DeWitt DS, Zornow MH, Uchida T, Johnston WE: Hypothermic modulation of cerebral ischemic injury during cardiopulmonary bypass in pigs. ANESTHESIOLOGY 1998; 88:390–402

36. Bacher A, Kwon JY, Zornow MH: Effects of temperature on cerebral tissue oxygen tension, carbon dioxide tension, and pH during transient global ischemia in rabbits. ANESTHESIOLOGY 1998; 88:403–9