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Small Differences in Intraischemic Brain Temperature Critically Determine the Extent of Ischemic Neuronal Injury

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Summary: We have tested whether small intraischemic variations in brain temperature influence the outcome of transient ischemia. To measure brain temperature, a thermocouple probe was placed stereotaxically into the left dorsolateral striatum of rats prior to 20 min of four-vessel occlusion. Rectal temperature was maintained at 36–37°C by a heating lamp, and striatal temperature prior to ischemia was 36°C in all animals. Six animal subgroups were investigated, including rats whose intraischemic striatal brain temperature was not regulated, or was maintained at 33, 34, 36, or 39°C. Postischemic brain temperature was regulated at 36°C, except for one group in which brain temperature was lowered from 36°C to 33°C during the first hour of recirculation. Energy metabolites were measured at the end of the ischemic insult, and histopathological evaluation was carried out at 3 days after ischemia. Intraischemic variations in brain temperature had no significant influence on energy metabolite levels measured at the conclusion of ischemia: Severe depletion of brain ATP, phosphocreatine, glucose, and glycogen and elevation of lactate were observed to a similar degree in all experimental groups. The histopathological consequences of ischemia, however, were markedly influenced by variations in intraischemic brain temperature. In the hippocampus, CA1 neurons were consistently damaged at 36°C, but not at 34°C. Within the dorsolateral striatum, ischemic cell change was present in 100% of the hemispheres at 36°C, but in only 50% at 34°C. Ischemic neurons within the central zone of striatum were not observed in any rats at 34°C, but in all rats at 36°C. In rats whose striatal temperature was not controlled, brain temperature fell from 36 to 30–31°C during the ischemic insult. In this group, no ischemic cell change was seen within striatal areas and was only inconsistently documented within the CA1 hippocampal region. These results demonstrate that (a) rectal temperature unreliably reflects brain temperature during ischemia; (b) despite severe depletion of brain energy metabolites during ischemia at all temperatures, small increments of intraischemic brain temperature markedly accentuate histopathological changes following 3-day survival; and (c) brain temperature must be controlled above 33°C in order to ensure a consistent histopathological outcome. Lowering of the brain temperature by only a few degrees during ischemia confers a marked protective effect. **Key Words:** Ischemia—Brain temperature—Rats—Hypothermia.

Despite the utility of small animal models of global ischemia, several groups have noted variability of outcome from animal to animal (Payan et al., 1965; Furlow, 1982; Blomqvist et al., 1984; Harrison et al., 1985; Vibulsresth et al., 1987). Although these variations, in part, may be the consequence of differences in the severity of ischemia itself, other factors not directly related to the primary insult may also play an important role in determining the ultimate histopathological outcome.

Recent studies in our laboratory using the four-vessel model of global ischemia in restrained or anesthetized rats have demonstrated that very different histopathological consequences could be obtained, depending upon whether a heating lamp was positioned over the rat's head or body. Additional pilot observations suggested that the extent of histopathological outcome was less severe when the

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calvarium was exposed (for procedures such as EEG recording) than when the scalp was intact. These histopathological variations occurred in rats whose body temperature was maintained at 36–37°C. We therefore suspected that these manipulations might have influenced brain temperature and thereby may have altered the pathological outcome.

The beneficial effects of *moderate* degrees of systemic hypothermia have been consistently demonstrated during surgical procedures and in the postneurosurgical patient (Lougheed et al., 1955; Little, 1959; Rosomoff, 1959; Negrin, 1961; Connolly et al., 1962; Venugopal et al., 1973). Moderate decreases of body temperature have also been shown to have a protective effect in experimental brain ischemia (Marshall et al., 1956; Rosomoff, 1957; Kramer et al., 1968; Michenfelder and Theye, 1970; Kopf et al., 1975; Berntman et al., 1981; Young et al., 1983). To date, however, no investigations have documented the effects of *small* differences in intraischemic brain temperature on final histopathological outcome. The present study was therefore initiated to define the consequences of small alterations of brain temperature during an ischemic insult. Metabolic studies were carried out at the end of the ischemic period to document the severity of the insult itself, and histopathological sequelae were assessed following a 3-day survival period. As sizable temperature gradients between brain and body may develop during periods of decreased cerebral blood flow, brain temperature was monitored directly in the present study by means of an implanted intrastriatal thermocouple probe.

MATERIALS AND METHODS

Animal model

Male Wistar rats, weighing between 275 and 325 g, were kept in a 12-hour light-cycle environment and had free access to both food and water. Four-vessel occlusion (Pulsinelli and Brierley, 1979) was carried out in a two-stage procedure. On the first day, the two vertebral arteries were cauterized under 3% halothane anesthesia by means of a modified conical probe. Following an overnight fast, they were again anesthetized with 3% halothane and maintained on 1.5–2% halothane during operative procedures. The head was secured in a stereotaxic apparatus (David Kopf). The coordinates for the placement of the temperature probe were then marked (1 mm anterior and 2.5 mm lateral to the bregma), and a small burr hole was drilled at this site. A stainless-steel guide for the thermocouple probe was then introduced into the left striatum and glued to the surface of the skull.

The thermocouple used in these studies (HYP-O, Omega Corporation) was a rapidly responding probe. Each thermocouple probe was calibrated periodically against a calibrated mercury thermometer in a water bath, as temperatures were increased in 0.4°C increments from 35°C to 38°C. In initial animal studies (four rats), an Omega model 650 thermocouple meter was employed, having a resolution of 1.0°C. In subsequent studies, a more sensitive meter (Omega model 670T) was employed, with a 0.1°C resolution. Calibration was shown to be linear throughout the range tested. A daily 1-point calibration was performed at 37°C prior to each animal studied.

The rats were removed from the stereotaxic apparatus, and both femoral arteries and one femoral vein were cannulated with PE-50 polyethylene tubing. Special care was taken not to damage the femoral nerves. Ligatures consisting of a loop of PE-10 polyethylene tubing contained within a dual-bore Silastic tubing were then gently placed around both common carotid arteries. Rats underwent endotracheal intubation with the help of a metal guide. They were immobilized with pancuronium bromide, 0.6 mg/kg intravenously, and were connected to a small-animal respirator; additional doses of 0.2 mg/kg were administered at half-hourly intervals. Animals were placed in a prone position and the head secured with earbars and noseholder. The thermocouple needle-probe was next inserted through the previously implanted guide into the left striatum (5 mm ventral to the dura). Halothane anesthesia was then discontinued, and anesthesia was maintained with 70% nitrous oxide and 30% oxygen delivered via a Mucomyst-containing humidifier. Respiratory adjustments were made to maintain normal arterial blood gases. Following a 45-min waiting period, the ischemic insult was initiated. Before the ischemic insult, blood was withdrawn to adjust the preischemic blood pressure to 90 mm Hg. Both carotid arteries were then occluded, and blood pressure was maintained at 80 mm Hg by withdrawing additional blood or by reinjecting blood (maintained at 36–37°C) during the 20-min period of carotid occlusion.

Rectal temperature was maintained at 37°C throughout all portions of the experiment by means of a rectal thermistor probe and a thermostatically regulated heating lamp placed above the body of the animal. Intrastriatal temperature was monitored in all animals and was adjusted by manipulating the height of a small high-intensity lamp placed above the head. Prior to the ischemic insult, intrastriatal temperature was held at 36°C. In one animal subgroup, brain temperature was allowed to fall spontaneously during the ischemic insult whereas, in the other experimental groups, brain temperature was maintained at either 33, 34, 36, or 39°C during the insult by appropriate adjustments of the heating lamp. In all of the above subgroups, brain temperature was maintained at 36°C for the first hour of the postischemic recirculation period. In one other animal subgroup, however, brain temperature was maintained at a 36°C during the insult, but was lowered to 33°C during the first hour of recirculation by a small high-speed fan placed near the head, to test whether this early postischemic decrement of brain temperature would influence the final pathological outcome.

In four animals, an additional thermocouple was placed in the temporalis muscle in order to assess the nature of the relationship between brain temperature and externally monitored head temperature during and following ischemia.

Regional metabolic analysis

Metabolite analysis was carried out at the end of the ischemic period in rats with brain temperatures of 30, 33,

and 36°C in order to determine whether variations in brain temperature during ischemia altered the severity of the ischemic insult. In these animals, the brain was frozen transcalvarially with liquid nitrogen over a 5-min interval while mechanical ventilation was continued (Pontén et al., 1973). Arterial blood pressure during the freezing process was maintained at 80 mm Hg. The frozen head was then trimmed under continuous irrigation with liquid nitrogen on a band saw to remove extracranial tissue, and coronal brain slices were stored under liquid nitrogen. For regional sampling, coronal sections were transferred to a glove-box refrigerated at −30°C. After a 15-min temperature equilibration period, an 18-gauge needle was used to punch cores of tissue orientated perpendicularly to the plane of the section. Regions of the cerebral cortex as well as striatum were taken for analysis. Weighing was carried out to within 0.001 mg in a Cahn electrobalance contained within the glove-box. Each tissue core weighed 5–10 mg.

Tissue cores were prepared for analysis as described previously (Busto and Ginsberg, 1985). Assays for phosphocreatine (PCr), ATP, ADP, AMP, lactate, and glucose were performed by direct fluorometric techniques (Lowry and Passonneau, 1972), and pyruvate was measured by cycling (Kato and Lowry, 1973). Glycogen was measured using amylo-α-1,4-α-1,6-glucosidase (Passonneau and Lauderdale, 1974).

Histopathological evaluation

In rats designated for histopathological examination, the ligatures were removed following 20 min of ischemia, and the shed blood, which had been maintained at 36–37°C, was slowly reinfused. During the next hour, blood gases and mean arterial pressure were repeatedly monitored. At this time, the vascular catheters and thermocouple probe were removed and the hole in the skull filled with bone wax. The soft tissues were then reapposed over the skull and wounds closed. Animals were extubated and were given 50% O_2 by mask for 2 h. At the end of this period, the animals were returned to their cage and kept in a dark, quiet environment.

Three days after the ischemic insult, rats were transcardially perfused with a mixture of formaldehyde–glacial acetic acid–methanol as previously described (Pulsinelli and Brierley, 1979). Heads were then immersed in the fixative overnight prior to brain removal. Brains were next paraffin embedded, and 6-μm coronal sections of the forebrain were prepared and stained with hematoxylin–eosin. Microscopic sections for each animal were analyzed by an investigator (W.D.D.) who was blinded to the experimental conditions. At the coronal levels of the anterior commissure (striatum, frontal cortex) and of the dorsal hippocampus (thalamus, parietal cortex), the severity of the ischemic cell change was evaluated on a semiquantitative scale: 0 = normal; 1 = few affected neurons; 2 = many affected neurons; and 3 = most neurons affected. In order to characterize more completely the effect of brain temperature on the production of ischemic cell change, quantitative counting of ischemic neurons was carried out in the striatum. Separate quantitation was carried out for the central and dorsolateral portions of the striatum. For each region, six fields (500 × 500 μm area) were selected at random. Ischemic neurons were characterized by eosinophilic cytoplasm and pyknotic nuclei.

RESULTS

Physiological variables

These are summarized in Table 1. No significant differences were observed among temperature subgroups.

Metabolite data

Profound degrees of energy metabolite depletion and energy charge decline were observed at the end of 20 min of ischemia in all subgroups studied (Table 2). Phosphocreatine, glucose, and pyruvate were significantly decreased in all groups to a similar degree, and lactate increased to 11–12 μmol/g in all groups. The similarity of these changes among animals with intraischemic brain temperatures of 30, 33, and 36°C confirms that the severity of the ischemic insult itself was uniform among subgroups. Furthermore, no significant side-to-side differences in metabolite levels were observed in the striatum or cortex. Thus, the presence of the thermosensor probe in the striatum produced no important asymmetries in degree of ischemia. For this reason, Table 2 depicts only those changes oc-

TABLE 1. *Physiological variables*

Intra-ischemic temp.	Before ischemia				During ischemia				After ischemia			
	36°C	33°C	30°C	36°C[a]	36°C	33°C	30°C	36°C[a]	36°C	33°C	30°C	36°C[a]
Pco_2 (mm Hg)	39.0 ± 1.1	38.3 ± 1.4	41.6 ± 0.9	37.5 ± 1.6	40.6 ± 2.6	42.0 ± 3.5	43.1 ± 1.0	34.8 ± 3.2	37.9 ± 1.4	39.4 ± 0.9	37.5 ± 0.7	40.5 ± 1.4
Po_2 (mm Hg)	140.2 ± 18.9	155.0 ± 13.5	148.9 ± 7.6	126.7 ± 6.3	131.1 ± 10.6	133.7 ± 11.0	139.6 ± 8.5	126.5 ± 8.4	134.7 ± 3.8	159.6 ± 14.0	155.7 ± 5.7	138.4 ± 16.8
pH	7.42 ± 0.01	7.45 ± 0.02	7.41 ± 0.01	7.42 ± 0.02	7.31 ± 0.03	7.34 ± 0.04	7.33 ± 0.01	7.36 ± 0.01	7.38 ± 0.03	7.36 ± 0.01	7.41 ± 0.01	7.31 ± 0.06
MAP (mm Hg)	120.0 ± 14.7	125.0 ± 8.6	119.3 ± 7.2	124.0 ± 9.3	81.3[b] ± 1.3	80.0[b] ± 0.0	85.0[b] ± 1.1	80.0[b] ± 0.0	133.8 ± 3.8	135.0 ± 9.4	125.0 ± 5.0	110.0 ± 4.5

Mean values ± SEM, n = 4–7 rats. MAP, mean arterial pressure.
[a] Brain temperature during recirculation reduced to 33°C for 1 h.
[b] Different from respective pre- and postischemic value, by one-way analysis of variance; $p < 0.01$.

TABLE 2. *Brain metabolites at end of 20 min of ischemia*

	Right neocortex				Right striatum			
		Ischemia[a]				Ischemia[a]		
	Controls	36°C	33°C	30°C	Controls	36°C	33°C	30°C
Phosphocreatine	5.77 ± 0.07	0.03 ± 0.02	0.04 ± 0.03	0.04 ± 0.04	5.74 ± 0.14	0.06 ± 0.06	0.02 ± 0.02	0.06 ± 0.05
Creatine	5.21 ± 0.09	11.27 ± 0.74	11.26 ± 0.78	11.73 ± 0.72	5.61 ± 0.06	11.79 ± 0.88	12.32 ± 0.54	11.99 ± 0.49
ATP	2.85 ± 0.03	0.03 ± 0.02	0.00 ± 0.00	0.06 ± 0.03	2.99 ± 0.12	0.09 ± 0.06	0.03 ± 0.03	0.13 ± 0.09
ADP[b]	0.24 ± 0.01	0.23 ± 0.02	0.22 ± 0.01	0.30 ± 0.03	0.23 ± 0.03	0.40 ± 0.07	0.30 ± 0.03	0.38 ± 0.06
AMP	0.02 ± 0.01	1.72 ± 0.28	1.44 ± 0.24	1.20 ± 0.19	0.02 ± 0.01	1.74 ± 0.25	1.47 ± 0.20	1.48 ± 0.13
Ad	3.10 ± 0.02	1.99 ± 0.30	1.63 ± 0.25	1.57 ± 0.16	3.24 ± 0.05	2.32 ± 0.20	1.81 ± 0.19	2.02 ± 0.13
ECh	0.95 ± 0.00	0.12 ± 0.02	0.07 ± 0.02	0.15 ± 0.04	0.96 ± 0.01	0.12 ± 0.02	0.11 ± 0.03	0.21 ± 0.06
Glucose	1.30 ± 0.19	0.13 ± 0.03	0.18 ± 0.06	0.09 ± 0.03	1.09 ± 0.10	0.04 ± 0.01	0.05 ± 0.03	0.13 ± 0.08
Glycogen	1.32 ± 0.30	0.02 ± 0.02	0.07 ± 0.02	0.12 ± 0.05	1.29 ± 0.14	0.03 ± 0.03	0.07 ± 0.03	0.13 ± 0.03
Lactate	1.04 ± 0.11	12.10 ± 0.74	11.16 ± 0.25	10.80 ± 0.30	1.38 ± 0.09	11.89 ± 1.27	11.65 ± 0.38	12.19 ± 0.05
Pyruvate	0.100 ± 0.004	0.033 ± 0.009	0.019 ± 0.003	0.019 ± 0.006	0.096 ± 0.006	0.027 ± 0.005	0.013 ± 0.005	0.015 ± 0.004

Mean values ± SEM, n = 4–6 rats. Values are in μmol/g. One-way analysis of variance revealed no significant intergroup differences among the three ischemic data sets.

[a] Intraischemic striatal temperature.

[b] All ischemic values differ significantly from control by one-way analysis of variance ($p < 0.005$) except for ADP.

curring on the right side of the cortex or striatum, which did not contain the temperature probe.

Local cerebral blood flow

In order to confirm the impression gained from metabolite analysis, that the ischemic insult was severe in all temperature groups, local cerebral blood flow (lCBF) was assessed autoradiographically at the end of 20 min of ischemia in representative rats with intraischemic brain temperatures of 30°C (n = 2), 33°C (n = 1), and 36°C (n = 1). These methods have been published previously (Sakurada et al., 1978). In all cases, lCBF was virtually absent in both cerebral cortex (range 0.01–0.03 ml/g/min) and striatum (range 0.02–0.04 ml/g/min).

Neuropathological findings

When brain temperature was allowed to drop spontaneously to 30–31°C during the ischemic insult, ischemic cell damage was only infrequently observed (Table 3). The single structure that showed a consistent degree of involvement was the subiculum (CA1a) of the hippocampus, which was damaged in three of seven rats. In contrast, the CA1 sector of the hippocampus showed no histopathological damage in this group.

When brain temperature was held at 33°C during the ischemic insult, ischemic cell change was present in the dorsolateral sector of the striatum in only one of four rats (Table 3). Ischemic damage within the hippocampus was selective to the subiculum and occurred in only one of four rats.

The major difference between the 34°C and the 30–31°C groups was the presence, in the former, of ischemic neurons within the dorsolateral segment of the striatum (Tables 3 and 4). Three of five rats demonstrated large numbers of ischemic neurons in this location. Additionally, one rat showed involvement of the CA1 sector of hippocampus while only the subiculum was affected in a second rat. Focal ischemic cell change in the thalamus was present in three of five rats.

When brain temperature had been maintained at 36°C during ischemia, striatal pallor was evident macroscopically within both the central and dorsolateral segments of the striatum. Severe histopathological damage was apparent microscopically in both the central and dorsolateral segments of this structure in all rats (Fig. 1). The results of ischemic cell counts in the striatum are shown in Table 4. Ischemic cells were also regularly observed within cortical, hippocampal, and thalamic areas. Within the hippocampus, CA1 neurons were damaged in all five rats (Table 3). When cortical damage was observed, it appeared to be restricted mostly to the superficial cortical layers. In brain regions having a high frequency of ischemic cell change, the neu-

TABLE 3. *Histopathological assessment of ischemic damage*

Experimental groups[b]	Grade of injury	% Hemispheres with grade 0–3 damage[a]				
		Central striatum	Dorsolateral striatum	Cortex	Hippocampus	Thalamus
36–30–36	0	100	100	71	43	100
(n = 14)	1	0	0	29	57	0
	2	0	0	0	0	0
	3	0	0	0	0	0
36–33–36	0	75	75	50	75	75
(n = 8)	1	25	0	50	25	0
	2	0	0	0	0	25
	3	0	25	0	0	0
36–34–36	0	100	50	60	60	40
(n = 10)	1	0	10	40	20	20
	2	0	10	0	0	40
	3	0	30	0	20	0
36–36–36	0	0	0	20	0	20
(n = 10)	1	0	0	40	0	20
	2	0	0	0	20	20
	3	100	100	40	80	40
36–39–36	0	0	0	0	0	0
(n = 4)	1	0	0	0	0	50
	2	0	0	100	0	0
	3	100	100	0	100	50
36–36–33	0	50	21	29	21	43
(n = 14)	1	7	7	57	7	57
	2	0	0	14	0	0
	3	43	72	0	72	0
Statistical significance[c]						
H′ =		32.58	18.53	10.32	20.72	12.34
p <		0.001	0.01	NS	0.001	0.05

[a] Ischemic injury grade: 0 = no injured neurons, 1 = few affected neurons, 2 = many affected neurons, and 3 = all neurons affected.
[b] The three sets of numbers for each experimental group refer to brain temperature in °C, preceding, during, and in the 1-h period following the ischemic insult, respectively; n's refer to hemispheres.
[c] Kruskal-Wallis one-way analysis of variance by ranks, based upon bihemispheral average ischemia grades for each animal.

ropil appeared intact although microglia were occasionally seen. Frank necrosis of neuropil with glial proliferation and severe macrophage infiltration was not encountered.

In brains held at 39°C during ischemia, striatal pallor was again very striking in all specimens. Involvement of the central and dorsolateral segments of the striatum was readily apparent (Table 3 and Fig. 1). Parenchymal vacuoles were present in the most dorsolateral segment of the striatum. Severe damage of the superficial cortical layers was also observed in this group. Focal thalamic damage was present in four of five rats, which again consisted of ischemic cell change. Unique to this animal group was the difficulty in obtaining 3-day animal survival to permit histopathological examination; these animals were therefore perfusion fixed 24 h following the ischemic insult.

Relationship between brain and head temperature

Figure 2 displays intrastriatal, temporalis muscle, and rectal temperatures of rats subjected to reversible cerebral ischemia. In each case, the scalp had been reflected to permit insertion of the striatal thermistor, and no effort was made to regulate brain temperature during ischemia. Intrastriatal temperature in each instance fell to 30°C at the end of the ischemic insult. In Fig. 2A, in the absence of a heating lamp on the head, brain temperature rose to 34–35°C during recirculation while, in Fig. 2B, external warming of the head raised brain tempera-

TABLE 4. *Ischemic cell counts within striatum*[a]

Experimental group[b]	Central striatum[c]	Dorsolateral striatum[c]
36–30–36	0**	0**
36–33–36	0**	12.5 ± 12.5**
36–34–36	0**	11.6 ± 8.0**
36–36–36	54.5 ± 4.8	56.8 ± 4.0
36–39–36	73.0 ± 7.0	71.0 ± 5.0
36–36–33	23.6 ± 10.7*	39.7 ± 8.0

[a] Numbers per 500 × 500 μm high-power field. Values given as means ± SEM.
[b] Temperature designations as described in Table 3.
[c] Significantly different from 36–36–36 temperature group, by one-way analysis of variance; *($p < 0.05$) and **($p < 0.01$).

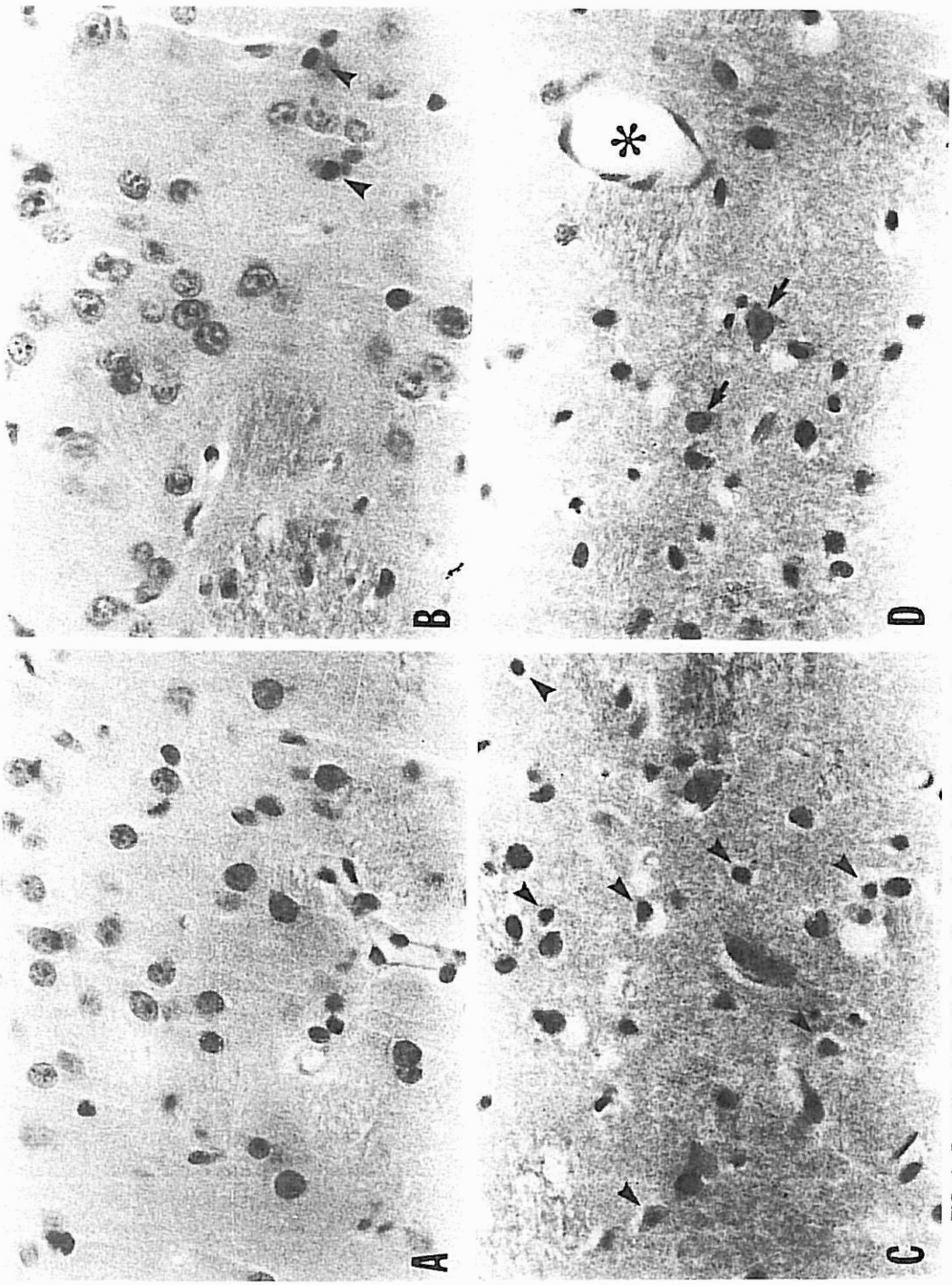

FIG. 1. Paraffin-embedded sections stained with hematoxylin–eosin demonstrating histopathological characteristics of dorsolateral striatum (×800). (In the following, the number sequences refer to striatal temperature, in °C, before, during, and following ischemia. See text for details.) **A:** 36–30–36: normal appearing brain parenchyma. **B:** 36–33–36: two ischemic neurons (arrowheads) are present in this histological field. **C:** 36–36–36: many ischemic neurons (arrowheads) are scattered among normal-appearing neurons. **D:** 36–39–36: the majority of neurons in this field are ischemic. Note the low frequency of normal neurons (arrows). A well-perfused blood vessel (*) within preserved neuropil is seen.

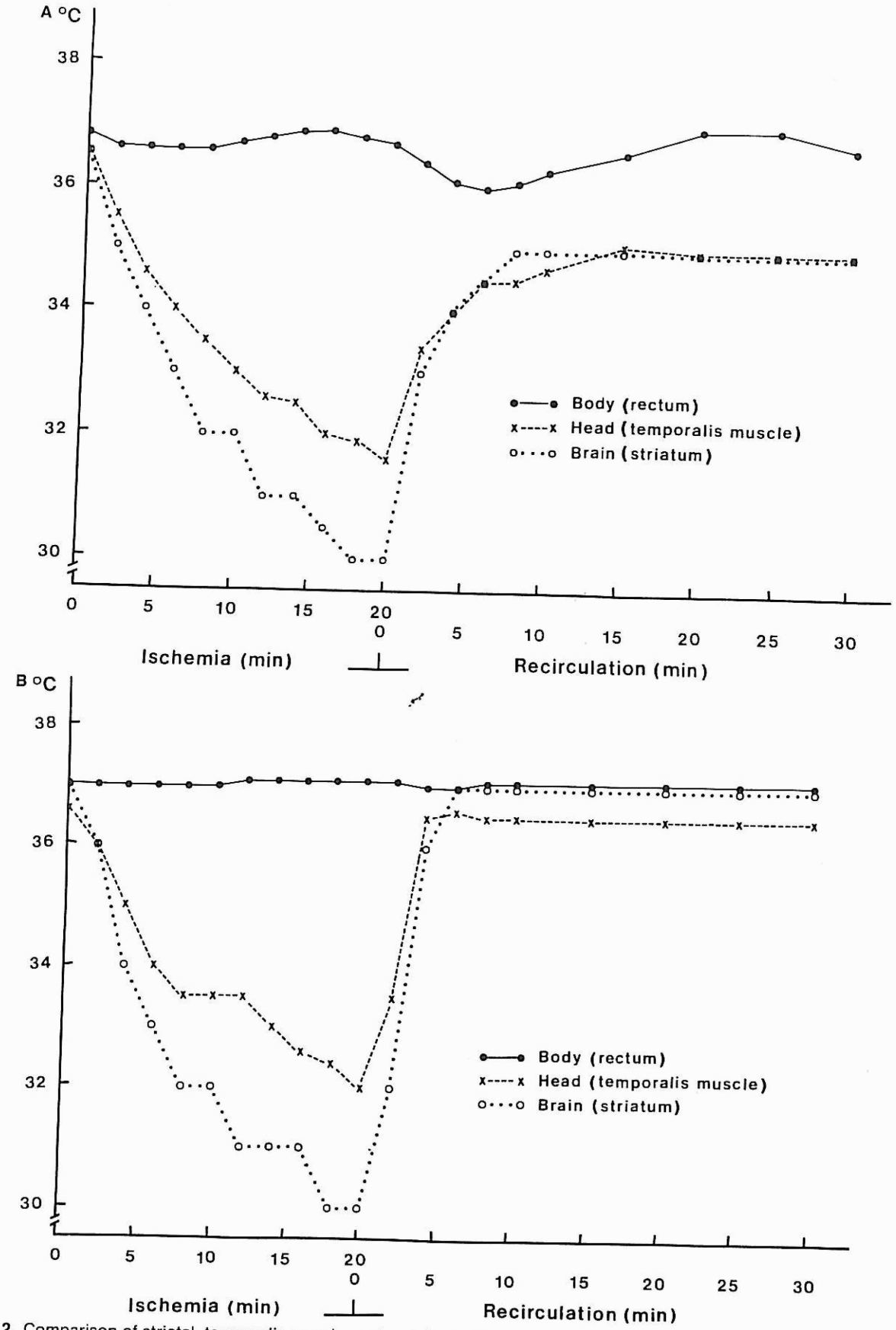

FIG. 2. Comparison of striatal, temporalis muscle, and rectal temperatures in two rats with exposed calvaria subjected to ischemia and postischemic recirculation. **A:** No effort was made to regulate brain temperature during ischemia or recirculation. Striatal temperature declined to 30°C during ischemia and rose to 34–35°C during recirculation. **B:** Brain temperature was allowed to decline spontaneously during ischemia, but was controlled at 36–37°C during the recirculation period by a warming lamp placed near the head.

ture to 36–37°C. Externally monitored head (temporalis muscle) temperature closely reflected brain temperature prior to ischemia and during recirculation, while during ischemia head temperature typically remained 1–2°C above brain temperature. A close linear relationship was apparent (Fig. 3):

$$\text{temporalis temp. (°C)} = 0.703 \times \text{striatal temp. (°C)} + 10.58$$

$$\text{correlation coefficient } r = 0.9998 \ (p < 0.001)$$

Thus, external monitoring of head temperature can provide a reliable indirect estimate of brain temperature in the course of an ischemic insult.

DISCUSSION

In this study, we modified the four-vessel model of global forebrain ischemia in the rat to allow for brain temperature monitoring via an implanted thermosensor probe; rats were anesthetized and intubated to permit respiratory regulation during ischemia and chronic survival. Histopathological damage produced by the temperature probe proved to be well demarcated and restricted to the probe track; this damage did not impede the assessment of pathological outcome.

A marked depletion of forebrain energy metabolites and moderate elevations of tissue lactate were observed in all animal subgroups following a 20-min period of global ischemia, and local blood flow was almost abolished in forebrain structures when studied in representative rats. The general uniformity of metabolite findings among rats with varying intraischemic brain temperatures attests to the uniform severity of the ischemic insult itself across these animal subgroups.

A salient finding of this study is the marked accentuation of ischemic neuronal damage produced by relatively modest (2°C) increments in brain temperature during the ischemic insult. These alterations of pathological outcome were most noticeable between 34 and 36°C. Within the hippocampus, 60% of the hemispheres demonstrated no ischemic damage to CA1 neurons at 34°C whereas all hemispheres demonstrated damage at the 36°C. Striatal neurons also appeared very sensitive to small alterations of intraischemic temperature: the central zone of striatum exhibited no ischemic cell change at 34°C, whereas this region was damaged in all rats at 36°C. Pathological consequences within cortical and thalamic brain regions were less highly correlated with intraischemic variations of brain temperature. These structures continued to demonstrate variable pathological changes although there was a trend toward increased frequency of ischemic cell injury with increasing temperature.

The present results demonstrate that measurements of *body* temperature do not accurately reflect *brain* temperature during an ischemic insult. A large temperature gradient was found to exist between the body and brain during a severe ischemic insult, and this gradient could be easily manipulated by cooling or warming of the head. In rats in whom no effort was made to regulate brain temperature during ischemia, brain temperature was shown to fall below 33°C while body temperature was being maintained at 36–37°C. In the vast majority of previous studies of experimental cerebral hypoxia and ischemia in which hypothermia was employed, only body temperature was monitored and regulated (Marshall et al., 1956; Boyd and Connolly, 1961; Kramer et al., 1968; Michenfelder and Theye, 1970; Hagerdal et al., 1978; Young et al., 1983). Extrapolating from the results of the present study, one would predict considerable variability in brain temperature during such insults. Numerous factors might be expected to influence brain temperature during experimental investigations, including exposure of the cranial vault for brain freezing or EEG recording, positioning of heating lamps in relation to the head, ambient room temperature, species variation, and possibly other factors. Our results indicate that monitoring of temporalis muscle temperature during a cerebral ischemic insult can provide a sensitive indication of brain temperature (Fig. 3).

Previous explanations for the beneficial effects of hypothermia on brain injury have included reduced tissue oxygen consumption, increased arterial oxygen content and depressed metabolic acidosis (Fleming, 1954; Deterling et al., 1955; Rosomoff and Holaday, 1954; Hagerdal et al., 1975). Berntman and colleagues (1981) demonstrated that small decreases in body temperature can minimize brain energy failure during an hypoxic–ischemic insult. In the present study, the degree of high-energy phosphate depletion and lactate accumulation at the end of the ischemic insult did not correlate with brain temperature variations. Thus, there were no differences in ischemic severity among the experimental groups to account for the observed differences in histopathological outcome. Nevertheless, intraischemic changes in brain temperature must have influenced subsequent brain function. Among the consequences of cerebral ischemia which are known to be temperature sensitive, include ion homeostasis, calcium influx into neuronal structures, degradation of membrane lipids, and permeability of the blood–brain barrier (Norwood and Norwood, 1982; Krantis, 1983; Rossi and Britt, 1984; Lantos et al., 1986).

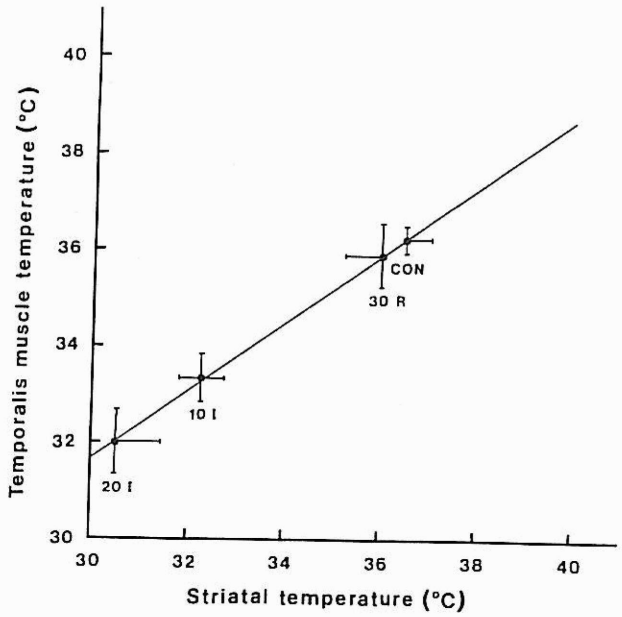

FIG. 3. Correlation between brain (striatal) temperature and head (temporalis muscle) temperature monitored in four rats during ischemia and recirculation. Experimental conditions are as described in Fig. 2B. CON, control period; 10 I, 10 min ischemia; 20 I, 20 min ischemia; and 30 R, 30 min recirculation. Error bars denote standard deviations. The line represents least-squares fit (see text for equation). Correlation coefficient $r = 0.9998$.

Excitatory neurotransmission has been implicated in the pathophysiology of brain damage within hippocampal and striatal areas following ischemia, hypoglycemia, and epilepsy (Jorgensen and Diemer, 1982; Simon et al., 1984; Schwarcz and Meldrum, 1985; Onodera et al., 1986; Rothman and Olney, 1986). Dopamine has been recently found to be an important determinant of ischemic injury within the striatum (Globus et al., 1987*a* and *b*). It is therefore particularly pertinent that these same brain regions were found to be extremely sensitive to relatively small variations in intraischemic brain temperature. Decreases in temperature are known to inhibit the biosynthesis, release, and uptake of various neurotransmitters (Vanhoutte et al., 1981; Boels et al., 1985; Haikala et al., 1986; Okuda et al., 1986). Small decreases in brain temperature may therefore alter neurotransmitter or receptor function and, through such a mechanism, affect the outcome of an ischemic insult.

In animals whose intraischemic brain temperature was maintained at normothermic (36–37°C) levels, lowering of *postischemic* temperature was shown to have a significant beneficial effect on outcome. Mechanisms accounting for this beneficial effect are not clear. Nonetheless, our results suggest that modest reductions of brain temperature during the early recirculation period may be a potentially helpful procedure in limiting the histopathological consequences of a cerebral ischemic insult.

In summary, our results demonstrate that modest differences in ischemic brain temperature are an important factor influencing the genesis of ischemic cell change following periods of transient global ischemia. In experiments in which brain temperature is not monitored, there is a potential for a variable brain temperature response during ischemia, which would be expected to result in variable histopathological consequences. The detrimental effects associated with prolonged body hypothermia have been previously discussed (Michenfelder and Milde, 1977; Steen et al., 1979). The present findings are therefore of clinical importance in that the beneficial effects of hypothermia were seen without lowering overall body temperature. Studies designed to determine the mechanism(s) by which small intraischemic variations in brain temperature exert their effects on final histopathological outcome should contribute to our understanding of the pathophysiology of neuronal injury.

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