The contribution of carotid rete variability to brain temperature variability in sheep in a thermoneutral environment

Shane K. Maloney, Duncan Mitchell, and Dominique Blache


department of Physiology: Biomedical and Chemical Science and Animal Biology, The University of Western Australia, Crawley, Australia; and School of Physiology, University of the Witwatersrand, Parktown, South Africa

Submitted 24 April 2006; accepted in final form 24 October 2006

Maloney SK, Mitchell D, Blache D. The contribution of carotid rete variability to brain temperature variability in sheep in a thermoneutral environment. Am J Physiol Regul Integr Comp Physiol 292: R1298–R1305, 2007. First published November 2, 2006; doi:10.1152/ajpregu.00275.2006.—The degree of variability in the temperature difference between the brain and carotid arterial blood is greater than expected from the presumed tight coupling between brain heat production and brain blood flow. In animals with a carotid rete, some of that variability arises in the rete. Using thermometric data loggers in five sheep, we have measured the temperature of arterial blood before it enters the carotid rete and after it has perfused the carotid rete, as well as hypothalamic temperature, every 2 min for between 6 and 12 days. The sheep were conscious, unrestrained, and maintained at an ambient temperature of 20–22°C. On average, carotid arterial blood and brain temperatures were the same, with a decrease in blood temperature of 0.35°C across the rete and then an increase in temperature of the same magnitude between blood leaving the rete and the brain. Rete cooling of arterial blood took place at temperatures below the threshold for selective brain cooling. All of the variability in the temperature difference between carotid artery and brain was attributable statistically to variability in the temperature difference across the rete. The temperature difference between arterial blood leaving the rete and the brain varied from —0.1 to 0.9°C. Some of this variability was related to a thermal inertia of the brain, but the majority we attribute to instability in the relationship between brain blood flow and brain heat production.

SELECTIVE BRAIN COOLING, the lowering of the temperature of part or all of the brain below aortic (arterial blood) temperature (14), is well described in animals possessing a carotid rete (22). During heat exposure, selective brain cooling contributes to brain homeothermy by preventing brain temperature from rising as much as trunk temperature does (16). However, it has also been observed in sheep exposed to thermoneutral conditions, and even during cold conditions (1, 20). Thus selective brain cooling is not restricted to hyperthermia but appears to be a part of normothermic thermoregulation, where its utility remains enigmatic. The mechanism by which artiodactyl mammals achieve selective brain cooling involves arterial blood in the carotid rete losing heat to venous blood in the cavernous sinus, resulting in a decrease in temperature of arterial blood leaving the rete compared with that of arterial blood entering the rete (22). After leaving the rete, arterial blood enters the Circle of Willis and then perfuses the brain. This blood perfusion is the main route of removal of heat produced by brain metabolism, and so venous blood leaving the brain should be warmer than arterial blood entering it. Indeed venous blood from the brain is significantly warmer than arterial blood in humans (L. Nybo, personal communication with analysis of data in Refs. 25 and 26). Brain tissue is warmer than the arterial blood entering the brain by a margin that depends on local brain heat production and removal of that heat by brain blood flow (24). Selective brain cooling reduces the temperature of blood entering the brain tissue but ought not change the temperature difference between that blood and brain tissue.

The relationship between brain metabolic rate (and therefore brain heat production) and brain blood flow is supposed to be tightly coupled. A recent study showed that, in rats, cerebral blood flow was the major determinant of the temperature difference between the brain and body (28). It is thought that the local partial pressure of CO2 acts as the signal mediating the relationship (3). Because the brain metabolizes glucose aerobically with a respiratory quotient of 1, when metabolic rate increases, CO2 production increases. Via the vasodilator effect of local CO2, the increased CO2 production causes an increase in blood flow. Thus, if brain heat production increases, local PCO2 increases, and the removal of extra heat is facilitated by increased blood flow. Indeed, hypocapnia and hypercapnia increase and decrease, respectively, the temperature difference between increased blood flow and cerebral arterial blood (12). At isocapnia, the temperature margin between perfusing blood and brain should be constant. However, several research articles have presented evidence that it is not; brain temperatures occasionally may be cooler than arterial blood in nonrete mammals (2, 5, 12, 21), and, even when the brain is warmer than arterial blood temperature, the margin shows considerable variability (9). It is not known whether there is similar variability in rete mammals in the margin between the temperature of arterial blood leaving the rete and brain temperature.

In rete mammals, the temperature margin between carotid arterial blood and brain depends not only on the interaction of brain heat production and brain blood flow but also on heat removal from arterial blood in the carotid rete. Because selective brain cooling is defined as a brain cooler than arterial blood (14), distinguishing the source of cooling between either cooling of blood entering the brain or changes in the relationship between brain heat production and brain blood flow is important in discussing the mechanisms of selective brain cooling. Distinguishing between the two sources of variability in a rete mammal requires the measurement of the temperature of blood leaving the rete and entering brain tissue. We make

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Address for reprint requests and other correspondence: S. Maloney, Physiology M311, Univ. of Western Australia, 35 Stirling Highway, Crawley 6009, Australia (e-mail: shanem@cyllene.uwa.edu.au).
these measurements here. That measurement is technically difficult, especially because proper investigation of rete function requires animals to be unrestrained (22). Baker and Hayward (1) reported that temperatures measured in the Circle of Willis provided an accurate measure of the temperature of arterial blood leaving the rete in the sheep.

We therefore have set out to measure, analyze, and identify the source of variability in the margin between brain temperature and carotid arterial blood temperature in sheep, an archetypical artiodactyl with a carotid rete, while the sheep were normothermic. The measurements also allowed us to explore rete function in the thermoregulation of normothermic animals. Using thermometric data loggers, we obtained continuous measures, over several days, of temperature in the hypothalamus, temperature just proximal to the Circle of Willis as a measure of the temperature of arterial blood leaving the rete and about to perfuse the brain, and temperature of blood in the common carotid artery, which is arterial blood temperature before that blood enters the rete. The sheep were unrestrained and remained at a constant ambient temperature of 20 – 22°C. We have asked several questions as follows: 1) How variable (or stable) is deep brain (hypothalamic) temperature in relation to the temperature of its blood supply? Is it as fixed as suggested from the data of Baker and Hayward (1), or is it similar to that reported in nonrete mammals? (9, 23) 2) How much does variability in heat transfer in the rete contribute to variability in the temperature difference between arterial blood in the common carotid artery and the brain?

MATERIALS AND METHODS

Five adult merino ewes with body mass between 45 and 60 kg were used in these experiments. The Animal Ethics and Care Committee of the University of Western Australia approved all experimental procedures.

Each animal was implanted with three blind-ended catheters, for the subsequent measurement of temperature, in the left common carotid artery (Tcar), in the left hypothalamus (Thyp), and proximal to the Circle of Willis on the right of the midline (TCoW). For implantation, animals were anesthetized with sodium thiopentone (15 mg/kg, Jurox P/L; Rutherford, New South Wales, Australia), intubated, and maintained in areflexia with halothane in oxygen (2 – 4%). The head was fixed in a stereotoxic apparatus (7), and the scalp was opened. A 3-mm-diameter hole was drilled through the skull 7 mm to the right of the bregma, a 2.1-mm (OD) stainless steel tube attached to a stereotoxic manipulator was advanced in the lateral ventricle, 1 ml of contrast medium (Ultravist; Schering, AG Pharmaceutical Division) was injected, and lateral and dorsofrontal X-ray images were obtained. The hypothalamus and the carotid rete/cavernous sinus complex were identified on these images, and their coordinates were measured. The steel tube then was swapped for a steel-tipped cellulose acetate butyrate guide tube (3.2 mm OD, 1.6 mm ID; World Precision Instruments, Sarasota, FL) that was reintroduced through the skull with the manipulator and lowered so that the tip just pierced the floor of the cranium, lateral to the sella turcica. Thus the stainless steel point of the guide tube lay within the top of the rete, with the thermistor, lying 2 mm above the tip, just proximal to the Circle of Willis.

A second 3-mm-diameter hole was drilled through the skull 7 mm to the left of the bregma, and a second, identical, guide tube was advanced in the hypothalamus with the manipulator. Finally, four stainless steel screws were anchored in the skull, and the guide tubes were fixed with dental acrylate. The Luer tops of the guide tubes were sealed within a Teflon cap. The skin was sutured closed around the Teflon cap and sprayed with topical antisepsic. The position of the guide tubes was confirmed with further lateral and dorsofrontal X-ray images. For the measurement of temperatures, thermistors (ruggedized glass-coated bead thermistor with insulated extension leads, diameter 0.3 mm; AB0E3B11KA103 N; Thermometrics, Edison, NJ) were advanced in each of the blind-ended catheters. Leads from the thermistors were conveyed externally over the top of the skull and down the neck.

The left common carotid artery then was isolated, and a 70-mm catheter was inserted, containing a thermistor (27 – 10K4A801, 2 mm diameter; Onset Computer, Pocasset, MA) sealed inside silicone rubber tubing (PAT04, 0.75 mm ID, 1.7 mm OD; Allied Biomedical, Pasco Robles, CA) at the end of a thin-walled polytetrafluoroethylene (PTFE) tube (0.9 mm OD; Straight Aortic Flush 4F Catheter, Cordis, The Netherlands). Outside the artery, the PTFE tube was connected to a silicone rubber tube (length 400 mm, 3 mm OD) that covered the leads from the thermistor, exited the neck, and terminated at a stereo plug.

After surgery (1 wk), the thermistor leads were connected to thermometric data loggers housed on a collar on the neck. The loggers (StowAway XTI; Onset Computer) had external dimensions of 50 mm × 45 mm × 20 mm. They were custom modified to have a storage capacity of 32 kb, a measurement range from +34 to +46°C, and a resolution of −0.05°C, depending on the characteristics of individual thermistors. The loggers with their thermistors were calibrated in an insulated water bath at five points over the range 36 – 42°C before implantation, and again at the end of experimenta-

tion, against a mercury-in-glass thermometer certified by the National Association of Testing Authorities, Australia, which was readable to 0.05°C. All of the cranial equipment remained accurate to within the resolution of the calibration thermometer over the course of the experiment. The carotid loggers all read low at the second calibration (between 0.05 and 0.3°C). We assumed the error resulted from constant drift and corrected data points by interpolation. The scan interval of the loggers was set at 2 min. On some days the plugs failed, causing a loss of data. On any day when any data were lost in this way, all data from that animal for that day were ignored.

Temperatures were recorded for several days while the sheep remained indoors in their home pens (2 m × 1 m with wooden slatted floor) at a controlled ambient temperature of 20 – 22°C. At the end of the experimentation, the animals were killed with an overdose of pentobarbital sodium, and the carotid artery was checked for probe placement and patency. In all animals, the probe tip remained in the artery and the probe was patent, confirming that the thermistor had measured the temperature of free-flowing arterial blood. The heads were removed, frozen, and later sectioned to check placement of catheters. All hypothalamic catheters were in the hypothalamus, and Circle of Willis catheters were positioned so that the thermistor measured temperature between the top of the rete and the Circle of Willis.

Because part of our analysis turned to the effects of time and time lags on the temperatures we measured, we determined the thermal inertia of our measuring equipment. Three of each of the steel-tipped cellulose acetate butyrate catheters (used to measure Thyp and TCoW) and three of the silicone-covered PTFE catheters (used to measure Tcar) with their data loggers were set to sample at the maximum rate possible (0.5-s interval) and allowed several minutes to equilibrate in a water bath at 36.1°C. At known times, each probe was transferred as quickly as possible to a different bath at 39.0°C. The inertia of the measuring equipment can be described as the thermal time constant (τ) of the equipment, which is described by the equation

\[ T = T_1 + [(T_2 - T_1) \times (1 - e^{-t/\tau})] \]

where T is the temperature of the probe at time t, T1 and T2 are the initial and final temperatures, respectively, and τ is the thermal time constant.
The thermal time constant of the steel-tipped cellulose acetate butyrate catheters (1.8 s) was slightly longer than that of the silicon-covered PTFE blood catheters (1.3 s). However, there was an error in these calculations introduced by the finite amount of time it took to transfer the probe between baths, as indicated by a lack of change in temperature (and slight decrease in many tests) at the instant of transfer. Measuring the time constant from the time at which the temperature began to increase (at time 0 + 0.5 s) results in time constants of 1.3 and 0.8 s. Even with a time constant of 1.8 s, when the temperature surrounding the probe changed, the probe would register 63% of that change in 1.8 s (τ) and take only 9 s (5 τ) to register 99.4% of the change. Thus we conclude that, in experiments where the logging interval was set at 2 min, the time constant of the equipment was short enough so that temperature differences measured 2 min apart were real differences and not confounded by the inherent inertia of the measuring equipment.

Data analysis. From the original recordings of temperature taken every 2 min, daily means and daily SD of the three temperatures, and a grand mean and SD of each temperature for each animal, were calculated for between 6 and 12 days (6, 6, 11, 11, and 12 days for the 5 animals). The mean temperatures and SDs of all animals were compared with a repeated-measures ANOVA with three levels followed by a Student-Newman-Keuls test when significance was indicated by the ANOVA. We first compared the mean for each animal of carotid arterial blood temperature, Circle of Willis temperature, and hypothalamic temperature. We then used the same method to compare the SD of these temperatures within animals. We then used the same method to compare the mean for each animal of the differences between $T_{CoW}$ and $T_{car}$, $T_{hyp}$ and $T_{CoW}$, and between $T_{hyp}$ and $T_{car}$. Finally, we used the same method to compare the SD of the differences within animals between $T_{CoW}$ and $T_{car}$, $T_{hyp}$ and $T_{CoW}$, and between $T_{hyp}$ and $T_{car}$.

We assessed the contribution of thermal events in the rete to variation in the temperature difference between $T_{hyp}$ and $T_{car}$ by regressing the difference between $T_{CoW}$ and $T_{car}$ and the difference between $T_{hyp}$ and $T_{CoW}$ on the difference between $T_{hyp}$ and $T_{car}$. The relationship between temperatures was analyzed further by comparison, for each animal, of the mean, SD, minimum and maximum brain temperature for each 0.1°C class of $T_{car}$, and also by calculating the proportion of time that $T_{hyp}$ was cooler than $T_{car}$, that is, the proportion of the time the animals were selectively brain cooling. The same procedure was done to compare $T_{CoW}$ with $T_{car}$ and $T_{hyp}$ with $T_{CoW}$.

**RESULTS**

Mean hypothalamic temperature was not significantly different from mean carotid arterial blood temperature, but the Circle of Willis was significantly cooler than both ($F(2,8) = 14.3, P = 0.002$; Fig. 1A). The mean variability within individuals in hypothalamic temperature was less than the variability of the other two temperatures, but the mean variability did not reach significance ($F(2,8) = 3.8, P = 0.07$; Fig. 1B). On average, there was a decrease in blood temperature from the carotid artery to the Circle of Willis of 0.35°C ($T_{CoW} - T_{car}$) and then an increase in temperature from the Circle of Willis to the hypothalamus ($T_{hyp} - T_{CoW}$) of the same magnitude ($F(2,8) = 64, P < 10^{-4}$; Fig. 1C). There was significantly less variation within individuals in $T_{hyp}$ than in $T_{CoW}$ in the other temperature differences, and more variability in $T_{CoW} - T_{car}$ than in the other differences ($F(2,8) = 9.2, P < 0.008$; Fig. 1D).

The sheep spent 50 ± 36% (range 8–100% for all animals) of the time with the hypothalamus cooler than carotid arterial blood, that is, selectively brain cooling. They spent 90 ± 12% (range 73–100%) of the time with the Circle of Willis cooler than carotid arterial blood, that is, with heat being removed from arterial blood in the rete. The hypothalamus was warmer than the Circle of Willis 99.7 ± 0.3% (range 99.4–100%) of the time.

In one animal on a randomly chosen day (Fig. 2A), temperatures at the hypothalamus and the Circle of Willis tracked each other closely, with an almost invariant 0.4°C difference between them. The Circle of Willis generally was cooler than carotid arterial blood. Selective brain cooling predominated after 1200, except for short periods when both the Circle of Willis and the hypothalamus warmed rapidly and transiently. After 1814 and after 1904 (Fig. 2B), there were increases in the temperature at the Circle of Willis, but the hypothalamus remained stable until the next 2-min sample when it also increased in temperature. In neither case had the temperature of carotid arterial blood reaching the rete increased. These examples illustrate a general picture that emerged from all animals,

**Fig. 1.** A: mean temperature of carotid arterial blood in the Circle of Willis (CoW) and in the hypothalamus over the course of the study. Data are means ± SD. B: mean ± SD of each temperature within animals. C: mean ± SD differences between the three temperatures over the course of the study. D: mean ± SD of each temperature difference within animals. ANOVA revealed significant differences between means in A, C, and D. Means that were significantly different by post hoc comparisons have different superscript letters ($P < 0.05$). Mean ± SD difference between animals shown on left, and means for individuals shown on right, each with a unique symbol. $T_{car}$, $T_{hyp}$, and $T_{CoW}$, temperature in left carotid artery, left hypothalamus, and proximal to CoW on right of midline, respectively.
that the larger changes in hypothalamic temperature invariably were preceded by a change in temperature at the Circle of Willis, with a slight inertial delay in hypothalamic temperature.

As a consequence of that close tracking, most of the variability in the temperature difference between the hypothalamus and the carotid arterial blood resulted from variability in the amount of cooling experienced by arterial blood traversing the rete/sinus. For one of the sheep, this phenomenon is illustrated in Fig. 3, showing a linear regression relating $T_{\text{hyp}} - T_{\text{CoW}}$ to $T_{\text{hyp}} - T_{\text{car}}$ with the relation $y = -0.05x + 0.33$ ($r^2 = 0.01$) and a linear regression relating $T_{\text{CoW}} - T_{\text{car}}$ to $T_{\text{hyp}} - T_{\text{car}}$ with the relation $y = 1.05x - 0.33$ ($r^2 = 0.84$). For all animals, each of the regressions of $T_{\text{CoW}} - T_{\text{car}}$ on $T_{\text{hyp}} - T_{\text{car}}$ was significant (average $P < 10^{-6}$) and the average slope of the lines was 1.08 ± 0.13, the average intercept was $-0.34 \pm 0.19$, and the average $r^2$ was 0.72 ± 0.2. For each animal, the regression of $T_{\text{hyp}} - T_{\text{CoW}}$ on $T_{\text{hyp}} - T_{\text{car}}$ was also significant (average $P < 10^{-6}$), and the average slope of the lines was 0.08 ± 0.13, the average intercept was $0.34 \pm 0.19$, but the average $r^2$ was only 0.07 ± 0.12. Figure 3B is discussed below.

Temperature at the hypothalamus was only very rarely lower than that measured at the Circle of Willis (Fig. 4A), and hypothalamic temperature for this animal was consistently 0.3°C higher than each category of temperature at the Circle of Willis, unless that temperature decreased below 38.5°C. The temperature at the Circle of Willis varied widely within most categories of carotid arterial blood temperature (Fig. 4C) but was cooler than carotid arterial blood temperature more often than warmer; mean temperature at the Circle of Willis was below carotid arterial blood temperature at all categories above 38.5°C for this animal, and above 38.6 ± 0.1°C for all five animals. Selective brain cooling, that is, hypothalamic temperature cooler than carotid arterial blood temperature, occurred on average in this animal only at carotid arterial blood temperatures above 39.1°C (Fig. 4E) and above 39.1 ± 0.5°C for all five animals. Thus, over the range of carotid arterial blood temperatures experienced most frequently by the sheep (Fig. 4D), arterial blood traversing the rete was cooled without hypothalamic temperature dropping to values that would qualify as selective brain cooling.

Although very little of the variability in the relationship between hypothalamic temperature and carotid arterial blood temperature arose after the rete, compared with within the rete (Fig. 3), our data showed that there was some variability in hypothalamic temperature at any temperature of blood leaving the rete (Fig. 4A). One explanation for some of this variability might be thermal inertia of the brain. We tested for such an effect by regressing the difference between hypothalamic and Circle of Willis temperatures, measured at each sample, on the rate of change in temperature at the Circle of Willis since the previous sample (that is, in the previous 2 min). The relationship proved to be statistically significant for each animal (all $P < 10^{-6}$) and is shown for one animal in Fig. 5. The $r^2$ for the regression shown is 0.13 (0.13 ± 0.02 for all animals). The mean slope of the regression was $-0.96 \pm 0.22$, and the intercept was 0.35 ± 0.16.

Because we had established that the lags evident in Fig. 5 were not the consequence of instrument inertia, and so were presumably of anatomic or physiological origin, we removed the effects of inertia by analyzing data points collected only when temperatures were stable. The data in Fig. 3B are from the same animal as Fig. 3A, but for analysis of temperature differences across the rete ($T_{\text{CoW}} - T_{\text{car}}$), data points were removed if carotid arterial blood temperature had changed.
0.35°C in arterial blood traversing the rete and then an increase of the same magnitude in the blood after it left the rete. Mean hypothalamic temperature became lower than that of carotid arterial blood at a hypothalamic temperature of 39.1°C, similar to the threshold reported previously for Dorper sheep (20) and for goats (16). However, arterial blood was losing heat in the rete/sinus at carotid arterial blood temperatures >38.6°C. That means there is a mismatch between the mathematical definition of selective brain cooling and the operation of the mechanism that produces selective brain cooling. Caputa et al. (5) argued that, because the temperature increment from cerebral arterial blood to brain was determined by the relationship between cerebral heat production and blood flow and because the normal situation in mammals is for the brain to be 0.3–0.4°C warmer than the cerebral arterial blood, any measurements showing a brain temperature < 0.4°C higher than carotid arterial blood temperature were indicative of selective brain cooling. That proposal contravenes the current IUPS (14) definition of selective brain cooling, but it is obvious that cooling of arterial blood destined for the brain can occur below the threshold for selective brain cooling.

We do not, however, propose a revision of the definition of the selective brain cooling threshold to “brain temperature less than carotid arterial blood temperature plus 0.4°C.” Given the variability we report in the extent to which arterial blood is warmed after leaving the rete, independent of any heat removal from arterial blood in the carotid rete or by any other means, it would be difficult to defend a threshold defined as carotid arterial blood temperature plus a fixed amount. Kuhn and Jessen (16) recognized the functional limitations of defining selective brain cooling mathematically and proposed that the true onset of selective brain cooling occurred when the stable difference between brain and carotid blood temperature began to decrease. However, they acknowledged the difficulty of defining the “stable” difference. Similarly, Kuhnen and Mercer (17) suggested using the inflection point on a plot of brain vs. carotid arterial blood temperature. Still, such functional definitions become difficult to implement when patterns are not standard, such as in ostrich, where there was no stable difference across the full range of body temperature (8), or pigs, in which control of selective brain cooling was unrelated to brain or arterial blood temperature (10).

Given the problems inherent in a functional definition of selective brain cooling, as discussed above, it seems unlikely that there is merit in changing the definition, but the functional limitations of the definition should be considered in any study of selective brain cooling. The data we present here show that inertia effects should also be considered, and the studies purporting to show selective brain cooling in nonrete animals, such as the rabbit (5) and the horse (21), when body temperatures were changing, must be interpreted with caution.

In all animals, the relationship between brain temperature and the temperature of blood in the common carotid arteries can change if the relationship between cerebral blood flow and metabolic heat production in the brain changes (12). In mammals with a carotid rete, the relationship also can change if heat transfer in the rete changes. We have shown that, in sheep, which have a carotid rete, variation in heat exchange in the rete accounts for most of the variation in the temperature difference between the hypothalamus and carotid arterial blood. Figure 3 indicates that, as hypothalamic temperature changed from...
0.5°C below carotid arterial blood temperature (a value typical for selective brain cooling) to equal to carotid arterial blood temperature (absence of selective brain cooling), the difference between hypothalamic temperature and temperature at the Circle of Willis decreased by an average of only 0.03°C, whereas the average temperature change in arterial blood traversing the rete decreased by 0.53°C. The average intercepts of 0.34°C for this group of sheep indicates that, when hypothalamic temperature was equal to carotid arterial blood temperature, there had been a decrease in temperature of 0.34°C in the blood traversing the rete/sinus and then an increase of the same magnitude between blood leaving the rete/sinus and the hypothalamus.

Our sheep were resting indoors in a 22°C, low-humidity environment. They exhibited selective brain cooling at body temperatures within the normothermic range, as has been reported previously in sheep (1, 18) and goats (16), confirming that selective brain cooling is not a defense mechanism invoked during life-threatening hyperthermia but a component of normothermic body temperature regulation (22). They were not heat stressed but did on occasion become hyperthermic, and, at the upper end of the spectrum of body temperatures, generally employed selective brain cooling. Selective brain cooling is controlled by the manipulation of the flow of venous blood, cooled at the evaporating surfaces of the upper respiratory tract, entering the cavernous sinus in which the rete lies. Classically selective brain cooling is thought to be elicited at a threshold brain temperature by switching that venous blood

---

**Fig. 4.** A: hypothalamic temperature as a function of the temperature at the Circle of Willis. B: frequency plot of the temperature at the Circle of Willis. C: temperature at the Circle of Willis as a function of carotid arterial blood temperature. Points on top, for one sheep, mean ± SD of the temperature on the ordinate at each 0.1°C category of the temperature on the abscissa. Lines above and below the means show maximum and minimum values, respectively, in each category. The solid diagonal line is the line of identity (ordinate = abscissa), shown for reference. Bottom: frequency histograms for the temperature on the abscissa of each panel. D: frequency plot of carotid arterial blood temperature. E: hypothalamic temperature as a function of carotid arterial blood temperature. F: D repeated for easier comparison with the data above.

---

**Fig. 5.** Difference between the temperatures of the hypothalamus and Circle of Willis as a function of the rate of change in temperature at the Circle of Willis over the previous 2 min. Solid line is least squares line of best fit. Note that n for this analysis was 7919, with many points superimposed because of the resolution of the loggers.

---

**Fig. 6.** Number of occurrences of each 0.1°C category of the temperature difference between hypothalamic and Circle of Willis temperatures. Data points when the upstream (Circle of Willis) temperature had changed over the previous 2 min were not included.
from the facial vein toward the sinus via the angularis oculi vein and its collateral veins. That view of the control of selective brain cooling cannot be sustained; our sheep employed graded heat transfer in the carotid rete at brain temperatures below the threshold for selective brain cooling.

An aspect of the control of selective brain cooling emerging from our study, and largely ignored previously, is that, under identical conditions, individual animals of the same breed and gender employed selective brain cooling to vastly different degrees. In our sheep, one animal exhibited brain cooler than carotid arterial blood 100% of the time and another only 11% (Fig. 1). The differences in utilization were not related to heat stress, since all of the animals were constantly exposed to 20–22°C ambient temperature. Maloney et al. (19) found differences in the degree of utilization of selective brain cooling between genders in free-ranging oryx, but all of our sheep were ewes. If the mechanism responsible for inhibition of selective brain cooling is sympathetic stimulation, as has been suggested in several studies (for review, see Ref. 22), then it is possible that an animal’s temperament in responding to external stimuli influences the use of selective brain cooling. In pigs in the laboratory, sympathetic drive unrelated to thermal stress obscured any thermal influence on the use of selective brain cooling (10). Maloney et al. (20) reported that the entry of an experimenter into a climate chamber housing hyperthermic pigs was related to rapid increase in brain temperature, that is, to inhibition of selective brain cooling. It remains to be seen whether the large interindividual variability in the utilization of selective brain cooling occurs in free-living animals, free from disturbance by human observers.

Some effects of disturbance can be seen in Fig. 2A, which shows an animal that often exhibited selective brain cooling. There were several occasions when rapid increases in temperature of blood leaving the rete, and subsequently hypothalamic temperature, occurred such that selective brain cooling was abolished. The largest and most prolonged change occurred just after 0800, when the animals were fed and their pens cleaned, activities that required someone to be in the room for a prolonged period. Other changes were more transient, and some occurred when people entered the holding rooms. Other transients, for example, those evident in the early morning hours, occurred when no people were present at the facility. The stimuli leading to these transients remains enigmatic, but even loud noises appear to result in an inhibition of selective brain cooling (unpublished data).

We found residual variation in the temperature difference between the hypothalamus and arterial blood leaving the rete, implying that in this rete species, as has been observed in nonrete species (5, 9, 12, 21, 23), cerebral metabolism and cerebral blood flow are not clamped together rigidly. For our sheep, the average value for the SD of hypothalamic temperature in different temperature categories of blood leaving the rete (confining the analysis to categories where >20 values were obtained) was 0.10 ± 0.03°C. Similar calculations for the difference between the brain and arterial blood temperature (measured in the common carotid artery) for nonrete mammals provide values of 0.09 ± 0.02°C for zebra (9), 0.10 ± 0.02 for horse (23), and 0.07 ± 0.02 for western gray kangaroo (Maloney SK, Fuller A, Meyer LC, Mitchell G, and Mitchell D, unpublished observation). We therefore conclude that the variability in the degree to which arterial blood warms after it enters the brain in nonrete mammals is similar to the variability in the warming of blood leaving the rete in these rete mammals.

Although most of the measured differences between hypothalamic temperature and the temperature of blood leaving the rete were of the order expected (0.3 to 0.5°C), there were enough measurements outside of that range to lead us to consider what factors might affect that difference. Some of the variability might have resulted from transient changes in heat loss directly across the cranium, but variation in transcranial heat exchange would have been minimal in the constant ambient temperature environment. Furthermore, we measured temperature in the hypothalamus and in the deep brain, and direct heat loss across the cranium is thought to affect at most a few millimeters of the subdural cortex (15, 24).

With current knowledge, the most likely explanation for variability in the temperature difference between arterial blood leaving the rete and the brain is variability in the relation between brain heat production and brain blood flow. The coupling of brain blood flow and brain heat production, as first hypothesized >100 years ago (27), may not be as tight as has been thought. Although blood flow velocity is not a direct measure of flow, recent findings show that cerebral blood flow velocity exhibits variability independent of the sleep/wake cycling and body temperature (6) and that fluctuations in regional blood flow seem to be the norm rather than the exception (13). Additionally, transient changes in blood oxygenation levels measured by functional magnetic resonance imaging during brain activation are further evidence for an uncoupling of cerebral blood flow and brain metabolism (4). Any change in the coupling of metabolism to blood flow will change the ratio of heat production to heat removal in the brain and so change the temperature difference between arterial blood leaving the rete and the brain.

It is worth remarking that the reason that Hayward and Baker (see, for example, Ref. 11) were measuring brain temperature, an interest that led to the discovery of selective brain cooling in sheep, was to test whether changes in brain temperature could be used to determine changes in the metabolic activity of specific brain regions. They concluded that there were no major changes in the temperature gradient between brain and arterial blood, which they attributed to tight coupling between regional brain blood flow and metabolic heat production. However, our results, recorded with instruments that now allow long-term monitoring of unencumbered animals, imply that the coupling is not that tight. Indeed, not just short-term but longer-term systematic uncoupling of brain blood flow from brain metabolic heat production seems to occur at low brain temperatures, leading to a plateau in hypothalamic temperature as the temperature of the blood leaving the rete falls (Fig. 4A). Similar plateaus in brain temperature at low arterial blood temperatures have been reported in ostrich (8) and zebra (9).

In conclusion, we have shown that, in the sheep, a mammalian species with a carotid rete, the carotid rete appears to play an active, and graded, role in controlling hypothalamic temperature even at temperatures below those that initiate selective brain cooling. Most of the variability in the relationship between carotid arterial blood temperature and brain temperature results from instability in the amount of heat extracted from arterial blood in the carotid rete. By contrast, what happens to
arterial blood after leaving the rete is more stable but does not conform to a tight coupling of brain metabolic heat production to brain blood flow. Our results provide a different perspective to the conclusion, reached by others measuring brain blood flow directly, that there are as yet unexplained variations in brain blood flow that are not the result of local metabolic demand and so are not proportional to variations in local metabolic heat production.

ACKNOWLEDGMENTS

We thank Kristin Hunt for technical assistance with the experiments and Denise Henry and the staff at the Large Animal Facility at the University of Western Australia for help with animal care. Dr. Lars Nybo kindly provided access to some raw data for reanalysis.

GRANTS

The Australian Research Council funded this research via a Discovery Grant.

REFERENCES