Rectal temperature measurement results in artifactual evidence of selective brain cooling

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SOME MAMMALS CAN MAINTAIN the brain, or at least parts of it, at a temperature cooler than that of the arterial blood supply from the trunk (1, 20). This phenomenon has been termed selective brain cooling (SBC). It is most conspicuous in mammals possessing a carotid rete. In these mammals, arterial blood on its way to the brain is cooled in the carotid rete via heat exchange with cool venous blood returning from the respiratory surfaces of the nasal cavity (1). It is thus postrete arterial blood temperature that is affected by SBC with the cooled arterial blood lowering the temperature of perfused regions of the brain. Quantification of SBC therefore requires knowledge of prerete blood temperature and brain temperature. Hayward and Baker (18) found that in monkeys, no difference in temperature existed between arterial blood in the aorta and at other sites as distant as the anterior cerebral artery, basilar artery, or abdominal aorta. On that basis, Baker and Hayward (3) proposed that carotid blood temperature provides a reliable measure of prerete arterial blood temperature.

Measurement of carotid blood temperature is not a simple task, and many investigators have relied on other measures of trunk temperature as a surrogate for prerete arterial blood temperature. If these surrogate temperatures were the same as arterial blood temperature, then using them to decide the degree to which an animal was using SBC would entail no error. However, temperatures measured at different trunk sites are not identical (5, 6, 19, 30, 40), so using surrogate temperatures may confound the determination of SBC. For example, there is a definite threshold of arterial blood temperature for onset of SBC in goats (27), but using rectal and brain temperature to estimate SBC, Mitchell et al. (33) concluded that a related artiodactyl, the sheep, always used SBC even during cold exposure. Rectal temperature (Tre) often exceeds arterial blood temperature in sheep (19, 40, personal observation), so the results of studies using Tre to estimate SBC require reinterpretation. Most of the studies that report apparent SBC in mammals that do not possess a rete, and in birds, have used some surrogate trunk temperature rather than arterial blood temperature to estimate SBC and so may be similarly confounded.

The main aim of our study was to evaluate the utility of Tre in estimating whether an animal is using SBC. We have used the adult sheep as the model for this study, because all published studies reporting SBC in sheep have relied on Tre as a surrogate for arterial blood temperature. We have measured brain temperature in the hypothalamus (Thyp), arterial blood temperature in a common carotid artery (Tcar), and Tre in sheep exposed to 40, 22, and 5°C. We also report observations on some nonthermal factors that influence SBC in the laboratory.

MATERIALS AND METHODS

Animals. Four Dorper-cross ewes (average mass ± SD = 54.9 ± 8.4 kg) were used. The sheep were housed individually in connecting indoor pens where ambient temperature varied...
between 21 and 25°C and a natural light-dark cycle was maintained. Water was provided ad libitum, and commercial feed (Epol, Johannesburg, South Africa) and lucerne chaff were provided in the morning. Fresh straw was provided daily. The procedures were approved by the Animal Ethics Screening Committee of the University of the Witwatersrand (protocol 95/14/5).

Temperature measurements. Under halothane general anesthesia (8% for induction, 2% for maintenance) and in sterile conditions, each animal was fitted with blind-ended thermocouple guide tubes for measurement of carotid blood and brain temperature. The carotid guide tube consisting of a polythene core ensheathed in silicone rubber (1.4 mm internal diameter, 1.9 mm outer diameter) was inserted into the left common carotid artery through a puncture made with a hypodermic needle. The puncture was sealed, and the guide tube was secured in place, with a purse string suture around the tube that did not occlude the artery. Outside the artery, the guide tube was enclosed in a thicker silicon rubber tube that was secured to muscle near the site of insertion, extended from the skin on the neck, and was attached to a cloth collar. The brain guide tube consisted of a length of acetate tubing (1.6 mm internal diameter, 3 mm outer diameter) through a nylon head plate. Anatomical markers were used to direct the guide tube toward the hypothalamus via a hole drilled in the skull 3 mm left of the midline. The nylon head plate was secured to the skull with bone screws. Correct positioning in the hypothalamus was confirmed post mortem.

During experiments, copper/constantan thermocouples were inserted into each guide tube to measure T hyp and T car. An additional thermocouple, ensheathed in vinyl tubing, was used to measure T re 100 mm beyond the anal sphincter. The reference junction of each thermocouple was maintained at 0°C (Omega Ice-Point, Omega), and thermocouple voltage was measured by a Hewlett-Packard model 8410 voltmeter to microvolt resolution. The voltmeter was interfaced to a personal computer that was programmed to scan the relevant channels on the voltmeter multiplexer every 30 s to convert voltage to temperature using calibration equations obtained for each thermocouple against a certified quartz thermometer (Quat 100, Heraeus, Germany) and to record temperatures to file. Resolution of thermocouple measurement was 0.025°C. In comparisons of thermocouple temperature against known (Quat 100) temperatures in a water bath, maximum error never exceeded 0.07°C.

Procedures. Beginning 10 days after surgery, several sets of measurements were made on each animal. Sheep were placed individually into cage trolleys, transported to a temperature-controlled room, and had thermocouples inserted by 0730 local time. There were always two animals in separate trolleys in the climate chamber during an experiment. Initial ambient temperature was maintained at 22–23°C. On different days, the animals were exposed in random order to different ambient temperatures after 0900. The animals either remained in the same climate room where ambient temperature was maintained at 22°C until 1500, remained in the same climate room where ambient temperature was increased to 40°C over ~1 h and then maintained at 40°C until 1500, or wheeled into a cool room maintained at 5°C until 1500. Every 30 min, an investigator entered the climate chamber to measure dry and wet-bulb temperatures with a psychrometer and to time 30 respiratory movements.

After completion of the experiments, the sheep were killed with an overdose of pentobarbital sodium and guide tubes were checked. All common carotid arteries containing guide tubes were patent.

Statistical analysis. Two-way, repeated-measures ANOVA, followed by Student-Newman-Keuls tests where appropriate, was used to compare hourly means of T hyp, T car, and T re during 40, 22, and 5°C exposures. Trunk – brain temperature differences (T car – T hyp and T re – T hyp) were compared in the same manner. One-tailed t-tests were performed on the hourly means of T car – T hyp and T re – T hyp to test the null hypothesis that they were greater than zero. Breath frequency during heat exposure was tested with a repeated-measures ANOVA. Probabilities <0.05 were taken to indicate that effects were greater than could be accounted for by random variation. All data reported are means ± SD, as stated.

The SBC threshold was calculated by regressing T hyp on T car recorded during heat exposure for each animal and by determining where that regression line crossed the line of identity. For presentation of the relationship between T car and T hyp during each exposure, T car was sorted into 0.1°C classes and average, SD, minimum and maximum of T hyp at each class of T car were calculated.

RESULTS

During 40°C exposure (Fig. 1), temperatures varied among the three sites of measurement [F(2,6) = 13.78, P = 0.006] with T re always being significantly higher than T car and T hyp. The temperature at all three sites increased with duration of the exposure [F(6,18) = 29.5, P < 10^{-6}]. There was a significant interaction between duration of exposure and measurement site.
Breath frequency during heat exposure increased significantly at 1000 when all three body temperatures began to rise, and then again at 1030, when body temperatures had risen by ~0.4°C, wherein it remained stable until 1500 (Fig. 2). During 5°C exposure (Fig. 1), temperatures also varied between measurement sites \( F(2,6) = 15.6, P = 0.004 \) with \( T_{re} \) always being significantly greater than \( T_{car} \) and \( T_{hyp} \), whereas the latter two temperatures were not significantly different at any time. The temperature at all three sites decreased with duration of the exposure \( F(6,18) = 10.5, P < 10^{-4} \). There was no significant interaction \( F(12,36) = 0.8 \).

During 22°C exposure (Fig. 1), there were no changes in temperatures over time \( F(6,18) = 0.14, P = 0.98 \), and the difference between measurement sites approached significance \( F(2,6) = 3.5, P = 0.09 \). The interaction was not significant \( F(12,36) = 0.88, P = 0.57 \). Three of the animals maintained \( T_{re} \) on average 0.3°C greater than \( T_{car} \), whereas in the other animal, the two temperatures were similar.

During 40°C exposure (Fig. 3), \( T_{re} - T_{hyp} \) was always significantly greater than \( T_{car} - T_{hyp} \) \( F(1,3) = 40.0, P = 0.008 \). Time had a significant statistical effect, with \( T_{re} - T_{hyp} \) and \( T_{car} - T_{hyp} \) increasing during the last 2 h of the exposure when \( T_{car} \) exceeded 39.5°C \( F(6,18) = 7.9, P < 0.001 \). The interaction was not significant \( F(6,18) = 1.6, P = 0.19 \). During 5°C exposure (Fig. 3), \( T_{re} - T_{hyp} \) was always significantly greater than \( T_{car} - T_{hyp} \) \( F(1,3) = 553.7, P = 0.0001 \). The effect of time was not significant \( F(6,18) = 0.22, P = 0.96 \) nor was the interaction \( F(6,18) = 1.5, P = 0.22 \). During 22°C exposure (Fig. 3), the effect of time and the interaction were not significant \( \text{time} F(6,18) = 0.9, P = 0.51 \); interaction \( F(6,18) = 0.86, P = 0.54 \). Mean \( T_{re} - T_{hyp} \) was always greater than \( T_{car} - T_{hyp} \), but the difference did not quite reach significance \( F(1,3) = 7.5, P = 0.07 \).

Comparison of the trunk and brain temperature differences with zero revealed that during 40°C exposure, \( T_{re} - T_{hyp} \) was always greater than zero, except between 1000 and 1100 when all temperatures were rising rapidly, whereas \( T_{car} - T_{hyp} \) was greater than zero only for the final 2 h of the heat exposure. During 5°C exposure, \( T_{re} - T_{hyp} \) was greater than zero for the last 5 h of exposure, whereas at no time was \( T_{car} - T_{hyp} \) greater than zero. During exposure to 22°C, neither \( T_{re} - T_{hyp} \) nor \( T_{car} - T_{hyp} \) was significantly greater than zero at any time.

Figure 4 shows for one animal the average \( T_{hyp} \) at each 0.1°C category of \( T_{car} \) during 40, 22, and 5°C exposure. The intersection of the regression line fitted to the 40°C data and the line of identity (i.e., the threshold for SBC) occurred at 39.0°C. The threshold for SBC varied between animals, with the values for the other three animals being 38.7, 39.4 (this animal depicted in Fig. 5), and 39.7°C, but the shape of the relationship was the same in all four animals. The four animals entered the chamber with similar body temperatures \( (T_{car} = 38.6 - 38.8°C) \), but \( T_{car} \) at the end of the 6-h exposure to 40°C varied from 39.6 to 40.5°C. There was a tendency for the animals with the lowest SBC threshold to experience the larger increases in \( T_{car} \) during 40°C exposure, but there was no significant relationship between SBC threshold and final \( T_{car} \) \( F(1,2) = 3.7, P = 0.19 \), change in \( T_{car} \) \( F(1,2) = 1.29 \), and the interaction were not significant \( \text{time} F(2,6) = 0.57 \). Three of the animals maintained \( T_{re} \) on average 0.3°C greater than \( T_{car} \), whereas in the other animal, the two temperatures were similar.

\( \text{Fig. 3. Thirty-minute averages of } T_{re} - T_{hyp} \text{ and } T_{car} - T_{hyp} \text{ of sheep exposed to 40, 5, and 22°C. Ambient temperature was 22°C before 0900. Points show means } \pm \text{ SE, } n = 4. \)
P = 0.37], or body mass [F(1,2) = 5.1, P = 0.15] during 40°C exposure.

The plots showing 30-min averages for each body temperature (Fig. 1) mask variability at a fine time scale, especially the variability in T hyp reflected in the SD error bars and range of T hyp in Fig. 4. As an example of the short-term variability in T hyp, Fig. 5 shows the 30-s records of T hyp, T car, and T re in one animal during the final 4 h of heat exposure (top) and the calculated SBC over this time (T car - T hyp; bottom). The figure shows regular oscillations in T hyp over a 0.5°C range resulting in periods where the animal was using SBC of up to 0.3°C, interspersed with periods where SBC was abolished and T hyp exceeded T car by 0.2°C. The other three animals showed similar variability. The arrows on the abscissa indicate the times when an investigator entered the climate chamber to measure psychometric data and breath frequency, which took on average 5 min. In every instance, the entry of the investigator into the climate chamber led to a decrease in SBC and a consequent increase in T hyp.

DISCUSSION

Our results are the first reports of carotid blood and brain temperature in sheep and show that this species can use SBC as part of the response to thermal stress. The occurrence and magnitude of SBC depend on body temperature but also on nonthermal factors such as the presence of an investigator. The study has highlighted the consequences of using T re as a surrogate for arterial blood temperature, and we concur with the advice originally offered by Baker and Hayward (3) that because it is postrete arterial blood temperature that is altered by the SBC effector mechanism, T car is the temperature that should be used to calculate SBC. As judged by the difference between T hyp and T car, the sheep selectively brain-cooled only during the final few hours of 40°C exposure and not at all during exposure to 22 or 5°C. If we had used T re to estimate SBC, we would have erroneously concluded that the animals always were using SBC even after 6 h exposed to 5°C.

Previous reports have claimed that sheep used SBC of up to 0.8°C during heat exposure and 1.3°C during fever (29, 33, 35, 36), a much larger magnitude than we observed for T car - T hyp in this study. In all of these previous studies, SBC was estimated using T re. Because T re always exceeded T car (under the conditions of our study), the use of T re to estimate SBC will result in an overestimate of SBC. The magnitude of SBC we observed in sheep is consistent with that reported in other ungulates where T car has been used to calculate SBC (11, 15, 21, 23, 27, 28, 34).

Not only will using T re to estimate SBC lead to false conclusions regarding the magnitude of SBC, but it could also lead to the conclusion that the SBC effector mechanism was activated when it was not. Nijland et al. (35) concluded that because angularis oculi vein occlusion in heat-stressed sheep did not alter T re - T hyp, this vessel was not a major source of cool blood to the cavernous sinus. We later reported that in anesthetized sheep, blood flow in the angularis oculi vein accounted for over 80% of the cooling responsible for SBC (31). We can now explain the discrepancy. Figure 1 shows that after a 3-h exposure to 40°C, T hyp and T car were only beginning to diverge after becoming equal during the preceding hour. It was after 3 h of 40°C exposure that Nijland et al. (35) occluded the vein. Thus there was no SBC to be inhibited. But T re was 0.4°C greater than T hyp, leading to the false conclusion that SBC was present at the time of occlusion. A similar explanation presumably accounts for the conclusion reached by Mitchell et al. (33). With the use of T re to estimate SBC in cold-exposed sheep, they concluded that sheep always selectively brain-cooled in the cold. Proper estimates using T car show that SBC
did not occur in our animals exposed to 5°C (Fig. 3). Indeed, SBC in the sheep has a threshold temperature in the upper normothermic range as is observed in other species.

Nijland et al. (36) showed that superior cervical sympathectomy in the sheep led to a decrease in $T_{\text{hyp}}$, which they attributed to an increase in SBC under conditions of heat exposure, cold exposure, and fever. Our present results raise doubts about their estimated magnitude of the SBC but do not affect the conclusion that sympathectomy led to a decrease in $T_{\text{hyp}}$. Sympathetic mechanisms could explain the phenomena illustrated in Fig. 5, a rapid increase in $T_{\text{hyp}}$ independent of any change in $T_{\text{car}}$ subsequent to an investigator entering the climate chamber. Sympathetic activity is an important nonthermal input to the SBC controller (12, 28, 34) with increased activity suppressing SBC even at high body temperatures. It appears that entry of an investigator caused activation of the sympathetic nervous system and suppression of SBC in our experimental animals. Sympathetically mediated attenuation of SBC may account for the observed increase in SBC threshold in some species during exercise (12, 28, 34). Two scenarios have been proposed to explain the effect of sympathetic stimulation on SBC: constriction of either the angularis oculi vessels or arteriovenous anastomotic shunts in the nasal mucosa (3, 24, 31).

An implication of suppression of SBC by the presence of an investigator is that the magnitude of SBC indicated by Fig. 4 underestimates the true nature of the thermally driven relationship between $T_{\text{car}}$ and $T_{\text{hyp}}$ in sheep. A similar implication may apply to other laboratory studies so that the “thresholds” for SBC we and others have calculated should be viewed with caution. The magnitude of SBC was periodically suppressed by the experimental protocol that influenced the mean $T_{\text{hyp}}$ at each category of $T_{\text{car}}$. For example, the calculated SBC threshold for the animal depicted in Fig. 5 was 39.4°C, but the animal was selectively brain-cooling with a $T_{\text{car}}$ of 39.2°C at the start of the trace.

The effect of sympathetic activity on SBC onset was proposed by Jessen et al. (23) to account for the absence of SBC in free-ranging black wildebeest during intense exercise. Such an effect may also underlie differences in SBC patterns between field and laboratory studies. The SBC threshold is approximately 38.8–38.9°C in tame goats in the laboratory (26, 27). However, when measurements were made on goats in outdoor enclosures, the threshold was less predictable and there was a range of $T_{\text{car}}$ from 38.6 to 39.3°C over which the animals may or may not have been employing SBC (21, 22). Similarly, laboratory studies on reindeer reveal a precise threshold of 38.7°C (28), whereas free-ranging wildebeest and springbok have ranges for SBC onset between 38.7–39.3 and 39.0–39.3°C, respectively (23, 34). Presumably, this discrepancy reflects the additional stressors that free-ranging animals experience. As a corollary, determining the true nature of the thermally driven relationship between central blood and brain temperature requires elimination of the stress on experimental animals.

Because our experimental protocol evoked regular unplanned increases in brain temperature, the breath frequencies reported in Fig. 2 probably are overestimated. The rapid rise in $T_{\text{hyp}}$ when the investigator entered the climate chamber would have enhanced heat-loss effectors including panting (26). So, in our study, as in any other study on artiodactyls, attenuation of SBC by experimenter presence would have resulted in an increase in panting rate compared with that which would have prevailed when an experimenter was not present.

If the relationship between $T_{\text{re}}$ and $T_{\text{car}}$ was predictable, then the state of the SBC effector could be deduced using measures of $T_{\text{re}}$. However, in our study, the relationship between the two temperatures was not consistent between treatments. The situation is complicated further when extended to other species. For example, $T_{\text{re}}$ in the horse was similar to or higher than $T_{\text{car}}$ under thermoneutral conditions but consistently lower than $T_{\text{car}}$ during heat exposure and also during exercise (32). We conclude that surmising the state of the SBC effector without actually measuring arterial blood temperature is not possible, and studies that have used surrogate measures of arterial blood temperature are all likely to have reached erroneous conclusions regarding the occurrence and magnitude of SBC.

The process leading to the constant positive temperature difference between the rectum and arterial blood in sheep is not known. The difference could be due to either a heat source in the rectum or a heat sink in the carotid artery. Given the constancy of arterial blood temperature even at sites distant from the heart (18), a source of heat to the rectum is the likely explanation. Heat of microbial activity in the rectum may lead to an increase in rectal relative to other body temperatures (30). Although this hypothesis has been tested and discounted in humans (37), the protocol used can be criticized. The research compared the $T_{\text{re}}$ of people before and after antibiotic treatment and showed no change in $T_{\text{re}}$. However, the relationship between rectal and other body sites was not measured. Another potential heat source to the rectum is venous blood draining from active leg muscles in the standing posture. To our knowledge, the influence of changes in external ileac vein temperature on $T_{\text{re}}$ has not been addressed in any species.

The effect of a surrogate measure for arterial blood temperature is pertinent to the arguments concerning SBC in animals that do not possess a carotid rete. Most studies tendering support for SBC in nonrete mammals have used rectal, abdominal, or interscapular temperature as a surrogate for arterial blood (7–10, 17, 25, 38, 39). If a difference exists in these species between arterial blood and other trunk temperatures, as exists in sheep, then the SBC reported in these studies may be an artifact. The experimental problem of measuring arterial blood temperature in small animals presently precludes investigation of SBC in many nonrete species.
Only four studies have measured arterial blood and brain temperature concurrently in conscious nonrete mammals, two on equids, and one each on the rabbit and a primate. McConaghy et al. (32) found evidence of SBC in the horse during exercise and heat exposure in the laboratory, but Fuller et al. (13) found no evidence of SBC in free-ranging zebra, a close relative of the horse. Selective brain cooling in the horse either is a laboratory phenomenon or the two closely related equids exhibit a very different thermal physiology. In exercising rabbits, brain temperature was lower than Tre but not lower than aortic blood temperature (2). Hayward and Baker (18) measured carotid arterial blood and brain temperature in the rhesus monkey under thermoneutral and hot conditions and found no evidence of SBC. A later study compared rectal and brain temperature in squirrel monkeys and concluded the animals did use SBC in the heat, which was augmented by face fanning (16). That study also measured mixed venous and hypothalamic temperature in anesthetised squirrel monkeys exposed to 25°C, concluding that the animals were capable of SBC. It is not known what influence anesthesia had on these results, but Tm, was more than a degree cooler than the calculated onset for SBC in conscious monkeys (Figs. 1 vs. 4 of Ref. 16). Thus the onset and control of SBC, if present, were distinctly different from those seen in conscious rete mammals. That study also showed that Tm, was consistently higher than mixed venous blood temperature in monkeys. Given that this relationship is similar to that in sheep, the SBC reported in conscious monkeys may be an artifact.

In conclusion, we have shown that sheep in the laboratory exhibit SBC with characteristics very similar to that of laboratory goats. Previous studies describing SBC in sheep generated artifacts, because Tm, was used as a surrogate for arterial blood temperature. Assessing the status of the SBC effector is not possible without a measure of prerete arterial blood temperature. We further show that investigator presence can influence the SBC effector mechanism in experimental animals and possibly lead to erroneous conclusions regarding the magnitude of SBC and even thermal effector responses employed during heat stress.

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REFERENCES