

Stress-induced fever after postischemic rectal temperature measurements in the gerbil

Darren L. Clark, Suzanne B. DeBow, Melanie D. Iseke, and Frederick Colbourne

Abstract: Postischemic temperature, which modulates brain injury, is commonly determined via a rectal temperature (T_{rec}) probe. This procedure causes a stress-induced fever (SIF) in rodents that may aggravate injury or diminish the efficacy of a neuroprotectant. We continually measured core temperature (T_{core}) via an implanted telemetry probe and made 16 T_{rec} measurements over 4 days in sham and ischemic gerbils (5 min bilateral carotid artery occlusion). Controls did not have T_{rec} sampled, but T_{core} was measured. Rectal temperature measurements predicted T_{core} in sham and ischemic gerbils. The T_{rec} measurements caused a SIF (1°C peak) in shams that did not habituate, whereas the SIF was initially absent and then increased over days in ischemic gerbils. Ischemic groups had similar CA1 injury (~32% remaining), presumably because T_{rec} measurements only resulted in a significant SIF starting on day 2 postischemia, when cell death is less sensitive to hyperthermia. Caution is warranted with T_{rec} measurements, since the resultant SIF occurs to different extents in normal and ischemic rodents. Furthermore, the SIF could vary according to many other factors, such as the type and severity of insult, the time and frequency of measurement, and drug treatment. Accordingly, postischemic T_{rec} measurements should be replaced with telemetry probes.

Key words: ischemia, stress-induced fever, hyperthermia, gerbil, rectal temperature.

Résumé : La température postischémique, modulatrice de lésions cérébrales, est couramment évaluée à l'aide d'une sonde rectale. Cette procédure entraîne cependant une fièvre provoquée par le stress (FPS) chez les rongeurs, ce qui est susceptible d'aggraver les lésions ou de diminuer l'efficacité d'un neuroprotecteur. Nous avons mesuré, chez les gerbilles ischémiques (à la suite d'une occlusion bilatérale de la carotide d'une durée de 5 min) et chez les gerbilles témoins, la température centrale de façon continue à l'aide d'une sonde télémétrique implantée, et nous avons fait la saisie de 16 températures rectales (T_{rec}). Ces mesures se sont effectuées sur une période de 4 jours. Nous avons uniquement mesuré la température centrale chez les groupes témoins. Les mesures de température rectale ont été utilisées pour prédire la température centrale chez les gerbilles ischémiques et les gerbilles témoins. La prise de température rectale a entraîné une FPS (hausse maximale de 1 °C) chez les groupes témoins et les gerbilles de ce groupe ne se sont jamais accoutumées à cette manipulation. Par contre, cette fièvre, quoiqu'initialement absente, a augmenté graduellement chez les gerbilles ischémiques au cours de la période expérimentale. Les groupes de gerbilles ischémiques souffraient vraisemblablement du même type de lésion CA1 (~32 % du reste) puisque les mesures de T_{rec} ont seulement démontré une FPS à la deuxième journée de postischémie, période à laquelle l'hypothermie est moins susceptible de provoquer la mort cellulaire. Il faut quand même être prudent en ce qui concerne l'utilisation de mesures de T_{rec} puisque la FPS qui en résulte n'a pas la même ampleur chez les rongeurs ischémiques que chez les rongeurs normaux. De plus, la FPS est possiblement affectée par des facteurs additionnels, tels que le type et la gravité de la lésion, le temps et la fréquence de la prise des mesures et le médicament utilisé. Par conséquent, les mesures de T_{rec} postischémiques devraient être remplacées par des mesures par sonde télémétrique.

Mots clés : ischémie, fièvre provoquée par le stress, hyperthermie, gerbille, température rectale.

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Introduction

Global (e.g., cardiac arrest) and focal (e.g., embolic stroke) cerebral ischemia is a leading cause of morbidity and death. Of many factors affecting outcome, temperature is

one of the most important determinants (Colbourne et al. 1997; Ginsberg and Busto 1998). Intra- and postischemic hypothermia improves outcome whereas hyperthermia worsens it. Accordingly, intra- and postoperative rectal temperature (T_{rec}) measurements are commonly used in rodent ischemia studies (DeBow et al. 2003).

Core temperature (T_{core}) should not be used to estimate brain temperature (T_{brain}) during anesthesia and global ischemia since they can dissociate by 5–6°C (Busto et al. 1987). In nonanesthetized rodents, T_{core} accurately reflects T_{brain} (Dilsaver et al. 1992; DeBow and Colbourne 2003). However, obtaining T_{rec} , which estimates T_{core} in conscious rodents, causes a stress-induced fever (SIF), which is due to an elevated temperature set-point resulting in a controlled

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rise in temperature (Kluger et al. 1987; Briese and Cabanac 1991; Cabanac and Briese 1992). This SIF has not been examined in the gerbil, which is commonly used to model global ischemia (DeBow et al. 2003). Furthermore, the pattern of SIF in ischemic versus nonischemic gerbils is unknown. We hypothesized that T_{rec} measurements would cause a SIF in all gerbils, but that the SIF would be attenuated in ischemic gerbils because of the presence of spontaneous postischemic hyperthermia (Colbourne and Corbett 1994). The SIF resulting from T_{rec} measurements may worsen histological outcome, as fever aggravates injury (Ginsberg and Busto 1998).

Materials and methods

Subjects

Forty-five female Mongolian gerbils (60–90 g, Charles River, St. Constant, Que.) were used. All procedures were in accordance with the Canadian Council for Animal Care Guidelines. Four of these gerbils were excluded because of surgical error.

Implantation procedure

Animals were anaesthetized with sodium pentobarbital (65 mg/kg i.p.) for implantation of a telemetry probe (Model TA10TA-F20, Data Sciences International, St. Paul, Minn.) into the abdominal cavity. Additionally, a 5-mm guide cannula was fixed to the surface of the skull at 1 mm anterior and 1 mm lateral to bregma, as previously described (DeBow and Colbourne 2003).

Ischemia and temperature measurement

Between 3 and 7 days following implant surgery, gerbils were anesthetized with isoflurane (~1.5% maintenance in 70% N₂O and 30% O₂) and randomly assigned to receive ischemia (ISC) (5 min bilateral common carotid artery occlusion) or sham surgery (SHAM) (carotid isolation only) as previously described (Colbourne and Corbett 1994). Brain temperature was maintained at ~36.0°C (via overhead infrared lamp) using a thermocouple needle probe (model HYP1-30-1/2-T-G-60-SMP-M, OMEGA Engineering, Inc., Stamford, Conn.) inserted into the dorsal lateral striatum (via previously implanted cannula).

A lubricated thermocouple probe (model RET-3, Physitemp Instruments, Inc., Clifton, N.J.) was used to measure T_{rec} at 1, 2, 3, and 4 h postischemia (ISC- T_{rec}) or sham occlusion (SHAM- T_{rec}), and at the same times on the following 3 days (i.e., 16 samples total over 4 days; ~30 s procedure). The T_{rec} samples were obtained in gently restrained, conscious gerbils. Control sham (SHAM-C) and ischemic (ISC-C) animals did not have T_{rec} measured. The T_{core} was sampled and recorded every 30 s (2-s averages) with an Advanced Research Technology (ART) data acquisition system (Data Sciences, International) for several days prior to and 96 h following surgery in all animals. The peak T_{core} change (SIF) in the SHAM- T_{rec} and ISC- T_{rec} groups was determined by averaging the measurements collected during the 15–30 min following the T_{rec} measurement, corresponding to the maximal temperature rise, and subtracting from this value the average temperature for the same period in the respective control group. The peak change in T_{core} following the four

T_{rec} measurements was averaged for each day, since there were no notable differences with each day (i.e., no time effect within a day) (repeated measures ANOVA). Accordingly, an ANOVA on the peak SIF was done on the T_{rec} groups over 4 days (mixed ANOVA).

Histology

Seven days after ischemia or sham occlusion, gerbils received an overdose of sodium pentobarbital (~80 mg/kg i.p.) and were transcardially perfused with 10% formalin. Brains were frozen, sectioned coronally (10 µm), and stained with cresyl violet. CA1 sector cells were counted and summated from the medial (adjacent to subiculum), middle (apex), and lateral (next to CA2) sections of CA1 at –1.7 mm to bregma. Data were analyzed with ANOVA with two factors (SHAM vs. ISC and T_{rec} measurements vs. none). Data are reported as means ± SD.

Results

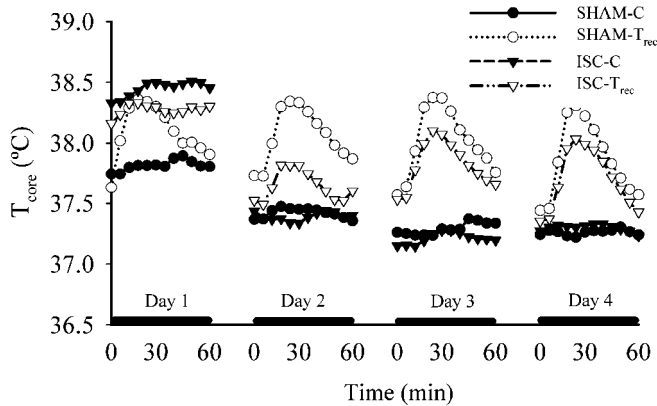
The average baseline T_{core} (24-h average) for all animals was $37.5 \pm 0.2^\circ\text{C}$ and there were no significant main effects or interactions ($p \geq 0.498$). The T_{brain} during surgery, which was regulated, was also not significantly different ($p \geq 0.073$) among groups, with an overall average temperature of $36.6 \pm 0.09^\circ\text{C}$. As expected, the ISC- T_{rec} ($38.3 \pm 0.4^\circ\text{C}$; average temperature during the first 6 h after ischemia) and ISC-C ($38.2 \pm 0.6^\circ\text{C}$) groups experienced significant ($p = 0.004$) spontaneous postischemic hyperthermia (T_{core}) compared with the SHAM- T_{rec} ($37.9 \pm 0.5^\circ\text{C}$) and SHAM-C groups ($37.6 \pm 0.5^\circ\text{C}$). The T_{core} was similar among groups on subsequent days (data not shown).

The T_{rec} measurements caused a SIF (T_{core}) that was greater in the SHAM- T_{rec} than ISC- T_{rec} group ($p = 0.030$; Fig. 1). There was a significant day main effect ($p < 0.001$), indicating that the SIF differed among the 4 days. For example, a simple effects analysis showed that the SIF on day 1 was less than on other days ($p < 0.001$). This effect appears to be largely due to the ISC- T_{rec} group, which had an increasing SIF over days (Fig. 1), although the day × group interaction only approached significance ($p = 0.081$). At its maximum, the SIF response peaked at approximately 1°C with a length of about 1 h. The SIF response did not change over the four samples taken within each day.

A two-way ANOVA on hippocampal CA1 cell counts revealed a significant main effect for insult (SHAM vs. ISC; $p < 0.001$), but no significant T_{rec} measurement effect ($p = 0.349$) or interaction ($p = 0.905$). Thus, the ISC- T_{rec} (28.2% of normal CA1 cells remaining) and ISC-C (36.8%) groups were not significantly different.

The difference between the T_{core} and T_{rec} measurements (i.e., $T_{\text{core}} - T_{\text{rec}}$) was 0.2 ± 0.3 and $0.2 \pm 0.3^\circ\text{C}$ in the SHAM- T_{rec} and ISC- T_{rec} groups, respectively ($p = 0.709$). Additionally, the T_{rec} measurements correlated well with the T_{core} (Fig. 2) taken immediately before the rectal probe insertion in the SHAM- T_{rec} ($R = 0.904$, $p < 0.001$) and ISC- T_{rec} ($R = 0.880$, $p < 0.001$) groups. The slopes and intercepts of the regression lines did not differ significantly between these groups ($p = 0.155$).

Fig. 1. Mean core temperature (T_{core}) ($^{\circ}\text{C}$) for 1 h (5 min averages) following the rectal temperature (T_{rec}) measurements on days 1–4 following ischemia or sham occlusion surgery. Data were averaged over the four T_{rec} measurements taken each day. SHAM-C, control sham; SHAM- T_{rec} , sham occlusion with T_{rec} measurement; ISC-C, control ischemic; ISC- T_{rec} , ischemia with T_{rec} measurement.



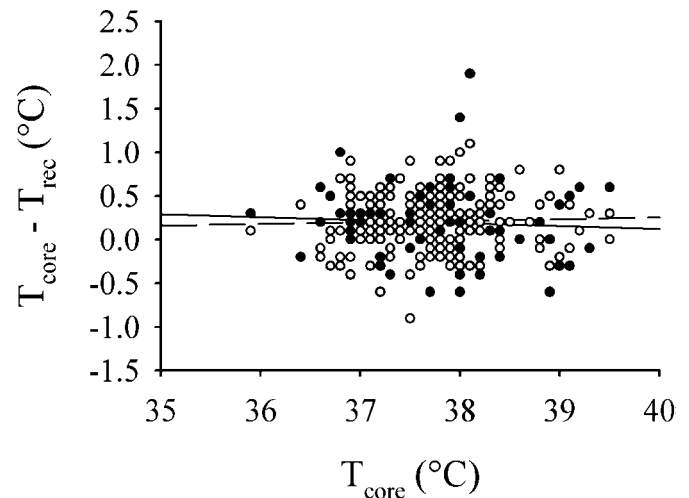
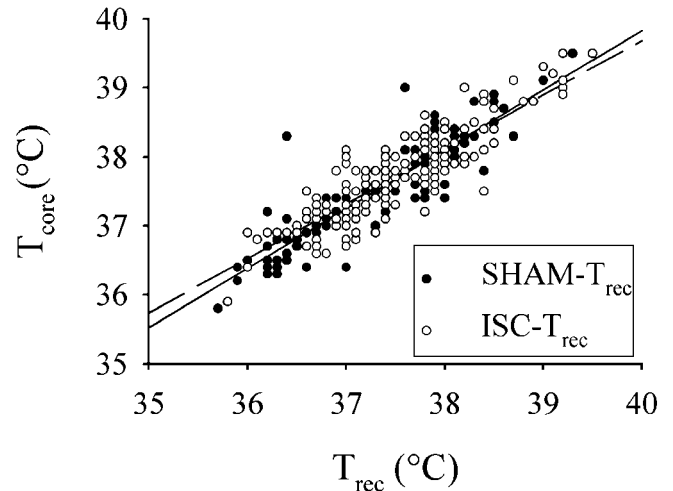
Discussion

The primary finding of this study is that postischemic T_{rec} measurements caused a different SIF profile in SHAM- T_{rec} and ISC- T_{rec} gerbils. The SHAM- T_{rec} gerbils experienced a SIF with a peak of approximately 1°C lasting about one h, a profile that did not habituate even after 16 T_{rec} measurements. The SIF was initially prevented in ISC- T_{rec} gerbils but eventually returned to SHAM- T_{rec} levels. Finally, T_{rec} measurements accurately reflected the concurrent T_{core} in the SHAM- T_{rec} and ISC- T_{rec} groups.

Gerbils frequently experience spontaneous postischemic hyperthermia after forebrain ischemia (Colbourne and Corbett 1994; present data). Perhaps this hyperthermic response resulted in a ceiling effect (Feng et al. 1989), whereby further rises in T_{core} due to T_{rec} measurements were blocked. This, however, cannot explain the attenuated SIF on day 2, since ISC- T_{rec} gerbils were then normothermic. More likely, ISC may have transiently affected molecular mechanisms of antipyresis, such as glucocorticoid release (Kozak et al. 2000), or fever generation. Further studies are needed to determine the mechanisms of spontaneous postischemic hyperthermia and the postischemic SIF response.

The occurrence of a SIF after T_{rec} measurements calls into question the utility of this method in brain injury studies, especially ischemia research. Delayed hyperthermia aggravates ischemic injury (Ginsberg and Busto 1998), and the occurrence of multiple SIF responses due to T_{rec} measurements may, under certain circumstances, aggravate brain damage. Presently, T_{rec} -induced SIF did not occur until the second day, which likely accounts for the lack of effect on CA1 damage. However, T_{rec} measurements at other times (e.g., 12–24 h) may aggravate injury in this model. Furthermore, since the SIF response likely varies with the ischemia model and insult severity, the occurrence of spontaneous temperature changes (i.e., hypothermia or hyperthermia as a result of the insult), and the presence of drugs (e.g., antipyretics) (Briese and Cabanac 1991), it is quite possible that unknown SIF responses may have confounded many studies.

Fig. 2. Relationship between rectal temperature (T_{rec}) and core temperature (T_{core}) ($^{\circ}\text{C}$) in groups receiving T_{rec} measurements following sham occlusion (SHAM- T_{rec}) or ischemia (ISC- T_{rec}) groups (top panel). The T_{rec} was highly predictive of T_{core} in both SHAM- T_{rec} (dashed regression line) and ISC- T_{rec} (solid line) groups. There are 16 samples per animal (4 per day over 4 days). The bottom panel shows the agreement of T_{rec} and T_{core} measures (i.e., $T_{\text{core}} - T_{\text{rec}}$) over the range of T_{core} values. The T_{rec} value averaged 0.2°C lower than T_{core} .



In summary, T_{rec} measurements accurately predict T_{core} in normal and ischemic gerbils. However, T_{rec} -induced SIF differed between the SHAM- T_{rec} and ISC- T_{rec} groups and over test days. Studies that rely upon multiple T_{rec} measurements may be confounded. Therefore, our findings illustrate the need for nonstressful methods of measuring postoperative temperature, such as the use of telemetry probes, and perhaps the need to avoid early behavioral manipulations that can also cause a SIF (Colbourne et al. 1998).

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References

- Briese, E., and Cabanac, M. 1991. Stress hyperthermia: physiological arguments that it is a fever. *Physiol. Behav.* **49**: 1153–1157.
- Busto, R., Dietrich, W., Globus, M.-T., Valdés, I., Scheinberg, P., and Ginsberg, M. 1987. Small differences in intras ischemic brain temperature critically determine the extent of ischemic neuronal injury. *J. Cereb. Blood Flow Metab.* **7**: 729–738.
- Cabanac, A., and Briese, E. 1992. Handling elevates the colonic temperature of mice. *Physiol. Behav.* **51**: 95–98.
- Colbourne, F., and Corbett, D. 1994. Delayed and prolonged post-ischemic hypothermia is neuroprotective in the gerbil. *Brain Res.* **654**: 265–267.
- Colbourne, F., Sutherland, G., and Corbett, D. 1997. Postischemic hypothermia: a critical appraisal with implications for clinical treatment. *Mol. Neurobiol.* **14**: 171–201.
- Colbourne, F., Auer, R.N., and Sutherland, G.R. 1998. Behavioral testing does not exacerbate ischemic CA1 damage in gerbils. *Stroke*, **29**: 1967–1971.
- DeBow, S., and Colbourne, F. 2003. Brain temperature measurement in awake and freely moving rodents. *Methods*, **30**: 167–17.
- DeBow, S.B., Clark, D.L., MacLellan, C., Colbourne, F. 2003. Incomplete assessment of experimental cytoprotectants in rodent ischemia studies. *Can. J. Neurol. Sci.* **30**. In press.
- Dilsaver, S.C., Overstreet, D.H., and Peck, J.A. 1992. Measurement of temperature in the rat by rectal probe and telemetry yields compatible results. *Pharmacol. Biochem. Behav.* **42**: 549–552.
- Feng, J.D., Price, M., Cohen, J., and Satinoff, E. 1989. Prostaglandin fevers in rats: regulated change in body temperature or change in regulated body temperature? *Am. J. Physiol.* **257**: R695–R699.
- Ginsberg, M.D., and Busto, R. 1998. Combating hyperthermia in acute stroke: a significant clinical concern. *Stroke*, **29**: 529–534.
- Kluger, M.J., O'Reilly, B., Shope, T.R., and Vander, A.J. 1987. Further evidence that stress hyperthermia is a fever. *Physiol. Behav.* **39**: 763–766.
- Kozak, W., Kluger, M.J., Tesfaigzi, J., Kozak, A., Mayfield, K.P., Wachulec, M., and Dokladny, K. 2000. Molecular mechanisms of fever and endogenous antipyresis. *Ann. N.Y. Acad. Sci.* **917**: 121–134.