Heat-stress-induced hyperthermia alters CSF osmolality and composition in conscious rabbits

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Received 8 June 2000; accepted in final form 7 August 2000

Amino acids have received increased attention with regard to their thermoregulatory effects and possible role as neurotransmitters within the thermoregulatory system. Overall temperature in mammals is regulated by the hypothalamus (17), which contains high concentrations of taurine (4). When administered centrally, taurine induces dose-related hypothermia accompanied by reduction in vasomotor tone and peripheral vasodilatation (35), whereas central administration of the taurine antagonist 6-aminomethyl-3-methyl-4H-1,2,4-benzothiadiazine-1,1-dioxide increases core temperature of the body (36). GABA, like taurine, is present in relatively high concentrations in various hypothalamic nuclei, the highest contents being achieved in preoptic and anterior hypothalamic areas (43). Short-axon GABA-ergic neurons form local networks that may modulate afferent temperature signals in the hypothalamus (7). Central or systemic injection of either GABA or GABA_A as well as GABA_B agonists usually causes a fall in core temperature, whereas that of both GABA_A and GABA_B antagonists induces hyperthermia (34). GABA-induced hypothermia is thought to depend on direct modulation of the hypothalamic temperature-sensitive network operated by GABA itself (43). Aspartate and glutamate are excitatory neurotransmitters in the cerebral cortex, the area of the CNS that is involved in the control of heat production (25). It has been demonstrated that during PGE_1-induced hyperthermia, these amino acids are released in high amounts in the extracellular fluid of the rat frontal cortex (25). Moreover, centrally injected aspartate and glutamate causes hyperthermia in the rat (8, 11).

The purpose of the present work was to evaluate the changes in CSF concentration of taurine, GABA, aspartate, and glutamate during the exposure to high Ta and further investigate their physiological role in HS. To assess this hypothesis, conscious rabbits, with cannulas chronically implanted into the cisterna magna, were exposed to 40°C for 50 min, during which CSF taurine, GABA, aspartate, and glutamate concentrations were determined. The hypothalamus represents
the integrative center for the perception and the processing of both thermo- and osmoregulatory afferent signals (17a). Because osmoregulation can modulate thermoregulation and vice versa during hypothalamic thermal stimulation, CSF and plasma osmolality were monitored. The levels of some CSF and plasma cations were determined in view of their purported importance, sodium and calcium in particular, in the establishment of the thermal set point in the hypothalamus (27). CSF proteins were also determined, as an index of integrity of the blood-brain barrier.

METHODS

Animals. Adult male New Zealand albino rabbits (Charles River, Calco, Como, Italy) weighing 2.0–2.5 kg were kept in large individual cages under a 12:12-h dark-light cycle at 20°C Ta. Drinking water and conventional laboratory rabbit food were available ad libitum. Before the experimental session, the animals were habituated to restraint and to the rectal probe to minimize the stress response.

Surgery. The animal protocols used were reviewed and approved by the Animal Care and Ethics Committee of the University of Siena, Italy. The rabbits were anesthetized with a mixture of xylazine chloride (Rompun, 10 mg/kg im, Bayer) and ketamine hydrochloride (Ketavet, 35 mg/kg im, Parke Davis/ Warner-Lambert) and implanted with cannulas in the cisterna magna according to the method described by Palmi et al. (29). After surgery, rabbits were injected for at least 5 days with the following drugs: prednisolone acetate (Novosterol, 10 mg/day im, Vetem) and enrofloxacin (Baytril, 25 mg/day im, Bayer). The animals were allowed to recover for at least 15 days.

Experimental protocol. Rabbits (n = 12) restrained in a stainless steel cage were individually housed in a chamber maintained at a neutral temperature (20°C) for 100 min. After four CSF samples had been collected to determine basal osmolality values and basal concentrations of cations and amino acids, and two plasma samples to measure cation concentrations and osmolality, a group of five rabbits was exposed to high Ta. The temperature of the chamber was raised up to 40°C in 50 min (150 min after the beginning of the experimental session), kept at that value for 50 min, and then reduced to 20°C over 50 min. This neutral temperature was then maintained up to the end of the experimental session (375 min). A second group of seven rabbits (controls) was kept at neutral Ta (20°C) for the entire observation period (375 min). CSF and plasma samples were collected from the animals of both groups at 25- and 50-min intervals, respectively, for the entire observation period. To obviate possible, superimposed effects of circadian rhythms, the experimental session started at between 0900 and 0930. At the end, the clinical status of the animals was recorded for the 3 subsequent days.

It was assumed that changes in amino acid and cation concentrations in CSF from the cisterna magna reflected changes in the brain extracellular milieu.

CSF and blood sampling. CSF samples were obtained from the cisterna magna of conscious animals restrained in stainless steel cages by a procedure described elsewhere (29). CSF was drawn through a polyethylene tube connected to a peristaltic pump (LKB, Bromma, Sweden) at a constant flow rate of 5 μl/min; 25-min fractions (125 μl) were collected with a Redirac 2112 fraction collector (LKB). Blood samples were withdrawn from an incision in the vein of the ear, and then plasma was separated as reported previously (16).

Temperature recording. Rabbit rectal and ear skin temperatures (Ta and Tes) were recorded every 5 min by a thermocouple thermometer connected to a personal computer with an Isothermex program (Columbus Instrument, Columbus, OH). Ta and Tes were monitored for at least 1 h before the experimental session.

CSF analysis for amino acids and cations. CSF samples were randomly analyzed for amino acid concentrations (taurine, GABA, aspartate, and glutamate) by reverse-phase HPLC with O-phthalaldehyde precolumn derivatization (5). CSF sodium, potassium, magnesium, and calcium concentrations were determined by an HPLC-conductimetric detection method (16).

Determination of CSF and plasma osmolality and CSF protein. CSF and plasma osmolality were determined with a vapor pressure osmometer (Wescor, Logan, UT). Protein in CSF was determined by the Coomassie blue binding method (10). To assess whether the increased CSF osmolality observed in heat-stressed rabbits could depend on the increase in CSF calcium, protein, or taurine levels, artificial CSF (ACSF) with the following composition was prepared: (in mM) 120 NaCl, 2.9 KCl, 23.3 NaHCO3, 1 MgCl2, and 0.2 Na2HPO4, as well as 0.6 mg/ml glucose. Different amounts of βSA (1, 2, 3, and 4 mg/ml), CaCl2 (1.0, 1.5, 2.0, and 2.5 mM), or taurine (0.1, 0.5, 1.0, 5.0, and 10.0 mM) were added separately to ACSF (3–7 replicates), and the osmolality was measured.

Statistical analysis. Values were expressed as means ± SE. The significance of the difference among ACSF osmolality measurements was checked by ANOVA followed by Dunnett’s post hoc test. The statistical significance of differences between values relative to HS (125–250 min) and post-HS (275–375 min) vs. data observed during the same period in control rabbits was checked by ANOVA. P < 0.05 was considered significant.

RESULTS

Effect of HS on Ta and Tes. To isolate any experimental artifacts arising from restraint, manipulation, or withdrawal of CSF for a long period, a group of seven rabbits was kept at 20°C for 375 min. Basal Ta did not change significantly during the observation period (Fig. 1). A second group of five rabbits, after a 100-min period of adaptation, was exposed to 40°C according to the experimental protocol reported in METHODS. Ta (mean value of 0–100 min: 39.0 ± 0.1°C) began to rise after a 50-min lag period and increased regularly, peaking with a maximum increment of 3.1 ± 0.2°C at 225 min (Fig. 1). As reported in Fig. 1, Tes underwent wide oscillations in control animals. On the contrary, in HS rabbits, it markedly increased with a slope paralleling the thermal ramp of the chamber and then plateaued at 40°C value, the same of Ta. When Ta was again set at 20°C, the thermal ramp of the chamber started with an opposite sign and Tes started to decrease with a 25-min delay, however, and paralleling, in the first phase, Ta slope. This was coincident with the peak of Tes. Body temperature of HS rabbits returned to basal values within 1 h. None of the animals exposed to HS died during the experiment or within the following 3 days of clinical observation.

Effect of HS on CSF and plasma osmolality. In control rabbits, CSF and plasma osmolality did not vary...
Heat stress and CSF composition

Fig. 1. Effect of heat stress (HS) on rectal (T<sub>r</sub>) and ear skin temperature (T<sub>es</sub>). Time course of means ± SE of T<sub>r</sub> and T<sub>es</sub> in HS (●) or control (○) rabbits is shown. Dotted line (―) indicates changes in ambient temperature (T<sub>a</sub>) during HS. After 100 min from the beginning of the experimental session, the temperature of the chamber was raised to 40°C in 50 min, and kept at that value for 50 min. T<sub>a</sub> was then reduced to 20°C over the following 50 min and then was maintained around this value up until the end of the experimental session (375 min). Controls were kept at 20°C T<sub>r</sub> for 375 min (dashed line, [ ]). Group data of T<sub>r</sub> or T<sub>es</sub> were compared statistically by ANOVA. HS period (125–250 min) T<sub>r</sub> and T<sub>es</sub>: control vs. HS, P < 0.001. Post-HS period (275–375 min) T<sub>r</sub> and T<sub>es</sub>: control vs. HS, P < 0.01.

The hyperthermia elicited by HS (dotted line) was accompanied by an increase in CSF osmolality, which was maintained throughout the experiment. CSF osmolality (basal value 293.5 ± 2.4 mosmol/kgH<sub>2</sub>O) began to rise after a lag of 75 min, reaching a maximum of 312.3 ± 5.1 mosmol/kgH<sub>2</sub>O after 325 min and then decreasing to 304.0 ± 5.68 mosmol/kgH<sub>2</sub>O at the end of the experimental session. CSF osmolality detected during a 125- to 250-min period in HS rabbits, however, did not differ statistically from that observed in control rabbits during the same period, whereas that detected during 275–375 min was significantly higher (P < 0.01).

Plasma osmolality (basal value 298.7 ± 2.7 mosmol/kgH<sub>2</sub>O) remained constant during HS treatment (125–250 min), whereas it was significantly increased afterward during 275–325 min (P < 0.05), reaching a peak of 308.0 ± 2.1 mosmol/kgH<sub>2</sub>O at 300 min.

Effect of HS on CSF protein content. In control animals, there was a progressive and significant increase in CSF proteins with respect to basal concentrations (95.5 ± 16.7 mg/100 ml) (Fig. 3). The percent increments were 23.4 ± 2.8% (P < 0.01) between 125 and 250 min and 29.4 ± 3.1% (P < 0.01) between 275 and 375 min.

In HS rabbits, CSF protein rose progressively from the basal values of 78.1 ± 16.3 to that of 165.0 ± 15.49 mg/100 ml at 250 min. The increase observed during the 125- to 250-min period, however, did not differ statistically from that observed in control rabbits during the same period. After HS, CSF protein continued to rise, peaking with a value of 182.6 ± 9.2 mg/100 ml at 325 min and then decreased to 147.0 ± 25.3 mg/100 ml at the end of the experimental session. CSF protein of HS rabbits during the 225- to 375-min period was significantly higher (P < 0.01) than that detected in controls during the same period.

Effect of HS on CSF and plasma concentrations of calcium, sodium, potassium, and magnesium. In control animals, CSF and plasma calcium concentrations were constant, oscillating around basal values of 1.37 ± 0.07 mM in CSF and 3.8 ± 0.2 mM in plasma (Fig. 4, A and B). HS, however, after a lag period of 50 min, induced a significant and steady rise in CSF calcium concentration from basal values of 1.34 ± 0.1 up to 1.60 ± 0.07 mM at 225 min. It is noticeable that T<sub>r</sub> and CSF calcium concentration began to increase at the same time. At the end of the experiment, however, CSF calcium concentration was still above basal concentrations, although T<sub>r</sub> had returned to baseline values. Plasma calcium concentration in HS rabbits, on the contrary, did not change significantly over the entire observation period.

Sodium, potassium, and magnesium concentrations in CSF and plasma of rabbits either kept for 375 min at 20°C or heat stressed did not change significantly during the entire experimental session (data not shown).

Fig. 2. Effect of HS on cerebrospinal fluid (CSF) and plasma osmolality. Time course (means ± SE) of CSF (A) and plasma (B) osmolality in HS (●) or control (○) rabbits is shown. Dotted line, effect of the exposure to 40°C on T<sub>r</sub> in HS rabbits (basal values 39.0 ± 0.1°C); horizontal bar, changes in T<sub>a</sub> during HS. After 100 min from the beginning of the experimental session, the temperature of the chamber was raised to 40°C in 50 min (left gray bar section), kept at that value for 50 min (black bar), reduced to 20°C over 50 min (right gray bar section), and then maintained at that value up to the end of the experimental session (375 min). Controls were kept at 20°C for 375 min. Group data of CSF or plasma osmolality were compared statistically by ANOVA. HS period (125–250 min) CSF and plasma: control vs. HS, not significant (NS). Post-HS period (275–375 min) CSF: control vs. HS, P < 0.01; plasma: control vs. HS, P < 0.05.
Effect of HS on CSF concentrations of taurine, GABA, aspartate, and glutamate. In rabbits kept at 20°C, there was a progressive and significant reduction in CSF concentrations of taurine from the basal value of 4.2 ± 0.4 to 2.7 ± 0.2 μM between 125 and 250 min (P < 0.01 vs. basal value), with a subsequent increase to 3.7 ± 0.4 μM between 275 and 375 min (P < 0.05 vs. basal value) (Fig. 5). Remarkably, in HS rabbits, CSF taurine concentration (basal value 4.5 ± 0.4 μM) remained constant during the exposure to 40°C (4.9 ± 0.5 μM mean value between 125 and 250 min) and rose progressively thereafter, with a peak value of 5.4 ± 1.0 μM between 275 and 375 min. The statistical analysis showed that during the exposure to 40°C (125–250 min) and during the post-HS period (275–375 min), the amount of CSF taurine concentration in HS rabbits was significantly higher (P < 0.01) than that detected in control rabbits in the same period of time. It is worth noticing that T_r increased, whereas CSF taurine concentration levels were constant. Subsequently, they began to decrease when CSF taurine concentration rose progressively. At the end of the experiment, T_r had returned to baseline values, whereas CSF taurine concentration was still above basal values.

Among the other amino acids analyzed, only GABA had a pattern similar to taurine. As shown in Fig. 6, in control rabbits CSF GABA contents decreased from the basal value of 2.1 ± 0.5 to 1.2 ± 0.5 μM (mean value between 125 and 250 min, P < 0.05 vs. basal value) and remained stable around this value thereafter. During HS, CSF GABA slightly rose from basal levels of 2.1 ± 0.6 up to 2.5 ± 1.2 μM at 150 min and then decreased regularly, reaching the value of 0.5 ± 0.4 μM at the end of the experimental session. CSF GABA levels between 125 and 250 min were significantly higher than those observed in control rabbits during the same period (P < 0.05). On the contrary, during the post-HS period (275–375 min), CSF GABA did not differ statistically from the amount detected in controls.

Aspartate and glutamate CSF levels were similar in both HS and control animals during the entire observation period (Fig. 7). In both groups, values exhibited a trend with time to decrease that, however, did not reach statistical significance.

Changes in osmolality of ACSF after the addition of varying amounts of BSA, CaCl₂, or taurine. Increasing amounts of BSA (from 1 up to 4 mg/ml) did not affect ACSF osmolality. On the contrary, when calcium contents were changed by adding to ACSF without calcium (270.0 ± 2.1 mosmol/kgH₂O), CaCl₂ from 1 to 2.5 mM, ACSF osmolality significantly rose to 295.0 ± 1.1 mosmol/kgH₂O, with an increment of ≈10 mosmol·kgH₂O⁻¹·1 mM CaCl₂⁻¹. Thus the increase in CSF calcium concentration observed in vivo in HS rabbits (≈0.26 mmol) can contribute only in part (≈2.5 mosmol/kgH₂O) to the total increase in CSF osmolality (≈15–19 mosmol/kgH₂O) observed. Taurine was able to significantly modify the ACSF osmolality only when the temperature of the chamber was raised to 40°C in 50 min (left gray bar section), kept at that value for 50 min (black bar), reduced to 20°C over 50 min (right gray bar section), and then maintained at that value up to the end of the experimental session (375 min). Controls were kept at 20°C for 375 min. Group data of CSF proteins were compared statistically by ANOVA. HS period (125–250 min): control vs. HS, NS. Post-HS period (275–375 min): control vs. HS, P < 0.01.

Fig. 3. Effect of HS on CSF protein content. Time course (means ± SE) of CSF protein in HS (●) or control (○) rabbits is shown. Dotted line, effect of the exposure to 40°C on T_r in HS rabbits; horizontal bar, changes in T_r during HS. After 100 min from the beginning of the experimental session, the temperature of the chamber was raised to 40°C in 50 min (left gray bar section), kept at that value for 50 min (black bar), reduced to 20°C over 50 min (right gray bar section), and then maintained at that value up to the end of the experimental session (375 min). Controls were kept at 20°C for 375 min. Group data of CSF proteins were compared statistically by ANOVA. HS period (125–250 min): control vs. HS, NS. Post-HS period (275–375 min): control vs. HS, P < 0.01.

Fig. 4. Effect of HS on CSF and plasma calcium concentration. Time course (means ± SE) of CSF (A) and plasma (B) calcium concentrations in HS (●) or control (○) rabbits is shown. Dotted line, effect of the exposure to 40°C on T_r in HS rabbits; horizontal bar, changes in T_r during HS. After 100 min from the beginning of the experimental session, the temperature of the chamber was raised to 40°C in 50 min (left gray bar section), kept at that value for 50 min (black bar), reduced to 20°C over 50 min (right gray bar section), and then maintained at that value up to the end of the experimental session (375 min). Controls were kept at 20°C for 375 min. Group data of CSF or plasma calcium contents were compared statistically by ANOVA. HS period (125–250 min) CSF: control vs. HS, P < 0.01. Post-HS period (275–375 min) CSF: control vs. HS, P < 0.001. HS period (125–250 min) and post-HS period (275–375 min) plasma: control vs. HS, NS.
added in millimolar (>1) amounts. ACSF osmolality, in fact, did not change significantly when 0.1, 0.5, or 1 mM taurine was added to the solution, whereas it rose progressively with the addition of higher amounts. ACSF osmolality rose from 277.8 ± 0.7 (ACSF 1 mM taurine) to 282.1 ± 0.7 (ACSF 1.5 mM taurine) and to 287.3 ± 0.6 (ACSF 1.10 mM taurine) mosmol/kg H2O with an increase of ≈5 mmol·kg−1·5 mM taurine−1. This suggests that taurine is not involved in the changes in CSF osmolality observed in HS rabbits.

**DISCUSSION**

In the present study, conscious rabbits, without access to water, were exposed to an T_a of 40°C for 50 min, a period sufficient to elicit a significant increase in T_r without induction of heat stroke. In fact, heat stroke in rabbits is characterized by deep hyperthermia, with T_r ≥43°C, loss of sensation, decreased muscle tone, unconsciousness, and coma (37). It has been reported that cerebral ischemia is the main cause for the onset of the heat stroke syndrome (20). The mean exposure time at 40°C T_a for the onset of heat stroke in rabbits was found to be ~90 min (20). In the present investigation, none of the rabbits exposed to the same T_a for 50 min
underwent heat stroke. Nevertheless, these animals had their thermoregulatory systems overridden because body temperature overcame $T_a$ despite the activation of thermoeffectors (panting rate and vasodilation) toward heat dissipation.

The hyperthermia that followed HS in the present study was accompanied by an increase in CSF and plasma osmolality. It is well known that acute exposure of mammals to high $T_a$ elicits a highly coordinated response mediated by osmoreceptors (panting rate and vasodilation) toward heat dissipation. It is probable that the responses mediated by osmoreceptors (panting rate and vasodilation) toward heat dissipation. This is mainly achieved by enhancing the evaporative heat loss by increasing the panting rate (42). Consequently, HS also generates water loss from the cellular and extracellular fluid compartments, as it was the case of HS rabbits in the present study. This results in increased plasma osmolality, which in turn stimulates arginine vasopressin (AVP) secretion by the posterior pituitary into the peripheral circulation, by activation of osmo- and baroreceptor pathways (18). AVP causes vasoconstriction and reduces urinary water loss, thus counteracting water imbalance and contributing to the body fluid homeostasis during HS (18). It has been suggested that AVP regulates CSF composition during brain adaptation to acute increases in plasma osmolality (39). In the present study, the HS-induced increase in plasma osmolality might have stimulated central AVP secretion, with subsequent changes in the composition and osmolality of CSF. Recent studies have demonstrated that AVP released by neurons of the hypothalamo-neurohypophysial tract is markedly and positively dependent on changes in plasma osmolality (12). The osmotic threshold at which AVP release is stimulated is 286 mosmol/kgH$_2$O in rats (12) and 302 mosmol/kgH$_2$O in cats (14). Although it is still undefined in rabbits, it is conceivable that it had been achieved in the first phase of HS in the present study.

Brain osmoreceptors can track the concentration of solutes in the extracellular fluid and activate the mechanisms by which evaporative heat loss and increase in body temperature are inhibited in mammals undergoing dehydration from exposure to high $T_a$ (42). In particular, intracerebroventricular infusion of hypertonic ACSF solutions (1,500 mosmol/kgH$_2$O) in conscious rabbits placed in a warm environment (33°C) led to an increase in cisternal CSF osmolality of ~30.0 mosmol/kgH$_2$O, which was accompanied by a reduction of either panting rate and $T_a$ and by an increase in body temperature. The perfusion with ACSF with an osmolality of 1,000 mosmol/kgH$_2$O, on the contrary, significantly decreases panting rate without affecting $T_a$ and brain temperature. These observations suggest that thermoregulatory responses in dehydrated animals are controlled in part by neuronal or hormonal systems stimulated by activation of cerebral osmoreceptors (42). This is considered to be the physiopathological mechanism underlying the heat stroke syndrome. Because in the present study the HS-induced increase in CSF osmolality was ~19.0 mosmol/kgH$_2$O, it is probable that the responses mediated by osmoreceptors were not activated.

The present results suggest that the increase in CSF osmolality did not depend on the increase in CSF proteins, taurine, and GABA levels but is only partly dependent on the increase in CSF calcium. Although taurine is considered an osmoregulator in the brain, it is imported into neurons and astrocytes bathed by hyperosmotic media to compensate for the osmotic gradient, thereby avoiding cell shrinkage (31); the increase in CSF osmolality could not be ascribed to taurine in consideration of the changes at micromolar concentrations observed here. HS-induced increase in CSF calcium levels deserves special comment. It has been proposed that the set point of body temperature in mammals is regulated by extracellular changes in calcium concentrations within the hypothalamus (27). Previous studies from this laboratory showed that when thermoregulation is set toward the promotion of heat dissipation, as happens when animals become hypothermic after an intracerebroventricular injection of taurine, there is a significant and long-lasting increase in CSF calcium content (36). Consequently, when thermoregulation is set toward the promotion of heat dissipation during the exposure to high $T_a$, changes in brain calcium metabolism may follow, giving rise to an increase in CSF calcium concentrations, as it was observed in the present study. This hypothesis, although attractive, cannot be supported in view of the positive correlation between CSF calcium contents and fever of various origins described in previous studies from this laboratory (28, 29). In those studies, in fact, CSF calcium increase was considered as a trigger for prostaglandin $E_2$ synthesis. This was further supported by the observation that interleukin-1β (IL-1β) was able to promote, in vitro conditions, $^{45}$Ca$^{2+}$ release from brain tissues (30). The fact that an intracerebroventricular injection of PGE$_2$ induced fever without modifying CSF calcium concentrations (28) was coherent with this result. These observations, however, rule out the possibility that CSF calcium concentrations could, in some way, be linked to the activation of thermoeffectors, as advocated by previous studies (27). Consequently, it can be speculated that CSF calcium increases following taurine increase in CSF. No data are available, however, on the cause-effect relationship between calcium increase in CSF and the activation of thermoeffectors, despite the parallelism at the time course of the two phenomena observed in the present study.

Withdrawal of CSF from the cisterna magna is a nonphysiological procedure that may be responsible for the progressive increase in CSF protein accompanied by a progressive decay in CSF taurine and GABA levels, as shown in control rabbits in the present study. The response of living organisms to a variety of physiological stresses involves the synthesis of HS protein in nerve cells (6). It is generally accepted that at least some of these proteins have a neuroprotective function (6). In rabbits in the present study, the stress arising from handling and restraint may have also contributed to the stimulated synthesis of these proteins and their release into the extracellular space. In control animals,
CSF GABA and taurine decreased slightly during the first 100 min and remained fairly constant thereafter. This phenomenon has been observed also by Singewald et al. (38) during the perfusion of the posterior hypothalamus of conscious rats with a push-pull cannula. Authors have speculated that because CSF samples were collected immediately after the insertion of the needle in the cannula guide used for CSF withdrawal, the tip of the needle could cause little damage to the surrounding tissues responsible for the decline in outflow of amino acids into CSF.

Hyperthermia due to infectious diseases or heat stroke may produce severe CNS dysfunction, including seizures, disorientation, and coma (2, 26, 37). Moreover, it has been shown that hyperthermia potentiates ischemic CNS injury in many experimental animal models, whereas hypothermia attenuates or prevents neurodegeneration (24), thus outlining the importance of brain temperature in pathological and functional outcomes of injured brain. It has been reported that in experimental ischemia, both hyperthermia and mild hypothermia exert their harmful and protective effects, respectively, by impairing or enhancing glutamate uptake (3). On the contrary, hyperthermia or hypothermia per se do not induce significant changes in glutamate extracellular levels in the same experimental model (24). At variance with the previous observation, however, increased extracellular glutamate levels have been shown to accompany damage caused by hyperthermia during experimental seizure (26). In rats, fever induced by injection of PGE$_1$, PGE$_2$, or Escherichia coli is accompanied by an increase in aspartate and glutamate levels in CSF or in microdialysate samples collected from the cortex (22, 25, 32), thus suggesting that these amino acids might be involved in the pathogenesis of fever. However, relatively little is known about the role of aspartate and glutamate in in vivo models of HS or heat stroke.

Cremades and Peñafiel (11) observed that exposure of infant rats (7 or 14 days old) to elevated $T_a$ (40°C for 90 min) induced hyperthermia, accompanied by a significant increase and a significant decrease in glutamate and aspartate levels in brain tissues, respectively. On the contrary, in adult rats (21 days old), HS induced a greater rise in body temperature, which was associated with slight changes in brain content of these amino acids. The authors concluded that the different outcomes to heat exposure in infant or adult rats could be related to different maturation of the blood-brain barrier in the two age groups. Adachi et al. (1) observed that localized brain hyperthermia (41°C) in rats does not cause a change in extracellular glutamate levels, whereas either moderate (43°C) or severe (45°C) hyperthermia increases extracellular glutamate concentrations toward neurotoxic levels. This indicates that glutamate-mediated excitotoxicity might play an important role in hyperthermia-induced cellular injury to CNS. In the present study, hyperthermia was not accompanied by modifications in CSF glutamate and aspartate contents, suggesting that, in HS rabbits, these amino acids do not play a role.

The present work shows that HS modifies brain taurine and GABA metabolism, inducing changes in CSF concentrations of these amino acids. The distance between the possible site of taurine and GABA action (brain extracellular space in the hypothalamus) and the CSF where they were monitored (cisterna magna) is so great that an overall diluting effect has to be taken into account. Taurine and GABA increase in CSF cannot be ascribed to blood contamination resulting from a transient opening of the blood-CSF barrier. CSF protein levels, in fact, did not change during HS and were slightly enhanced afterward. Moreover, increased permeability of the blood-CSF barrier seems unlikely, as CSF glutamate remained constant throughout the experiment, despite the high blood-CSF gradient, which approximates the value of 75 (19). It might be speculated that increased output of taurine and GABA from the brain into CSF is aimed at counteracting the hyperthermia promoted by exposure to heat. When the time courses for HS-induced changes in $T_a$ and CSF taurine contents were compared, in fact, it was found that $T_a$ decreased as soon as taurine CSF contents rose. Also, CSF GABA levels were significantly higher during the HS period, just before $T_a$ had regained basal values. However, because compensatory responses in general depend partly on the relative strength of the stimuli, further experiments performed at different $T_a$ with different exposure times might provide more definitive support to a possible role of taurine and GABA as endogenous cryogenic compounds. Still, the difficulty to correlate changes in CSF contents of these amino acids with hypothalamic functions is challenging. Even the use of experimental models based on the positioning of microdialysis probes in the hypothalamus, which seems to be the most profitable approach, is not free from severe limitations, owing to the difficulty in interpreting the meaning of changes in amino acid contents in dialysates (41).

The possible role of taurine and GABA as hypothermic regulators is presently being investigated in several laboratories. Bouchama et al. (9) have shown that heat stroke patients with a $T_a$ of 42°C associated with neurological disorders exhibit a significant increase in plasma and urine concentrations of taurine. These were found to return to basal values within 24 h after the induction of hypothermia. In dorsal horn slices of rat spinal cord, taurine release has been found to depend on temperature. In particular, basal taurine release is depressed at 8°C and enhanced at 37 or 40°C (13). Tigges et al. (40) demonstrated that taurine efflux from the astrocytes and neurons changes in a temperature-dependent fashion. Specifically, taurine efflux from rat hypothalamic astrocytes in culture decreases when temperature is lowered from 37 to 33°C and increases at temperatures above 37°C (37–41°C). This phenomenon was also shown to occur in cerebellar neuron cultures. Furthermore, intracerebroventricular injection of taurine in rabbits was shown to reduce the febrile response caused by intravenous injection of Salmonella typhosa or leukocytic pyrogen or intracerebroventricular injection of endotoxin or prostaglandin.
E₂ (21). Intracisternal injection of E. coli in rabbits, an experimental model of meningitis, caused a significant increase in taurine and GABA concentrations in the extracellular fluid of the posterior frontal cerebral cortex (32). Rats treated with PGE₂, regarded as the final mediator of the febrile response (17), through a microdialysis probe in the lumbar subarachnoid space, showed a marked increase in CSF concentrations of taurine and GABA (22). IL-1 has been shown to stimulate in vitro taurine and GABA release from rat preoptic/mediobasal hypothalamic tissues (15). Experiments performed in this laboratory showed that CSF concentrations of taurine and GABA increase significantly in conscious rabbits during IL-1β-induced fever (unpublished observations).

In conclusion, the results of the present study indicate how levels of protein, calcium, taurine, and GABA are increased in CSF of HS rabbits. These increments cannot be ascribed to a transient failure of the blood-CSF barrier. It is suggested that taurine and GABA are released in the extracellular space of brain tissues during HS, possibly to counteract hyperthermia.

This work was supported by contributions of Ministero degli Affari Esteri (Rome, Italy) under law 212/92 and by Ministero dell’ Università e della Ricerca Scientifica e Tecnologica, Cofin. ‘98.

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