Pharmacology Biochemistry and Behavior, Vol. 43, pp. 1253–1262, 1992
Printed in the U.S.A.

0091-3057/92 $5.00 + .00

Calcium Changes in Rabbit CSF During Endotoxin, IL-1β, and PGE_2 Fever

MITRI PALMI,[1] MARIA FROSINI AND GIAMPIETRO SGARAGLI

Centro Interdipartimentale di Ricerca sul Metabolismo dei Farmaci Psicotropi
Istituto di Scienze Farmacologiche, Via E.S. Piccolomini 170, Villa Betlem, 53100 Siena, Italy

Received 16 December 1991

PALMI, M., M. FROSINI AND G. P. SGARAGLI. *Calcium changes in rabbit CSF during endotoxin, IL-1β, and PGE_2 fever.* PHARMACOL BIOCHEM BEHAV 43(4) 1253–1262, 1992.—New Zealand rabbits were chronically incannulated in the lateral ventricle and cisterna magna to assess the hypothesis that calcium concentration (Ca) of cerebrospinal fluid (CSF) varies during fevers of diverse origin. In normothermic and febrile animals recovering from surgery, CSF Ca was positively and significantly correlated to rectal temperature (TR). IV injection of *E. coli* endotoxin and ICV injection of human recombinant interleukin 1β (hrIL-1β) induced a TR rise of 1.7 ± 0.3°C (mean ± SEM) and 1.45 ± 0.25°C, respectively, accompanied by significant increases in CSF Ca. After endotoxin administration, maximal Ca increases ranged between 0.21 and 0.48 mM above basal values in individual animals ($p < 0.01$), whereas after administration of hrIL-1β increases were 0.17 and 0.25 mM ($p < 0.05$). Acetylsalicylic acid (ASA) countered the fever induced by both endotoxin and hrIL-1β administrations and concomitantly antagonized the Ca increase in CSF. HrIL-1β-derived nonapeptide was characteristically devoid of pyrogenic effect and did not modify CSF Ca. Although ICV injection of prostaglandin E_2 (PGE_2) increased TR by 2.1 ± 0.77°C, it failed to have any effect on CSF Ca. Differently from the other Ca enhancers, PGE_2, however, increased CSF protein concentration (protein). These findings suggest that brain calcium metabolism plays a role in fever development and that prostaglandin involvement is only engaged once changes in CSF calcium concentration have taken place.

CSF calcium Pyrogens Fever

REGULATION of body temperature involves a delicate balance between the production and loss of heat. The hypothalamus regulates the set point at which body temperature is maintained.

In fever, this set point is raised, and aspirin-like drugs bring it back to normal. Fever is caused by various conditions that have as a common feature the enhanced formation of cytokines such as interleukin 1 (IL-1) or tumor necrosis factor (14), which in turn induces the synthesis of prostaglandin E_2 (PGE_2) in vascular organs of the preoptic hypothalamic area (10).

Prostaglandins (PGs) act within the hypothalamus to raise body temperature by processes that are still unclear. Aspirin-like drugs reduce fevers caused by IL-1 and other cytokine enhancers but do not reduce fever caused by PGs (17), thus suggesting that these compounds act by inhibiting PGE_2 synthesis. Although there is agreement over the nature of fever mediators, the precise neurochemical mechanisms are not yet understood. Early experimental evidence of Myers et al. states that the set point for body temperature in mammals is regulated by the antagonistic action of Ca and Na in the extracellular fluid within the posterior hypothalamus (22,23).

Evidence of the importance of calcium and sodium in thermoregulation was provided by Myers et al., who showed that abnormally high concentrations of calcium perfusing the posterior hypothalamus caused prolonged hypothermia in cats, whereas the absence of calcium evoked a sharp rise in temperature. Further, in other experiments perfusion of the hypothalamus with excess calcium counteracted hyperthermia in animals during physical exercise (25), whereas in resting mammals the decrease in body temperature was linearly correlated with the increase in calcium dosage (14). Increased efflux of ^{45}Ca from the diencephalon and other brain regions has also been observed in monkeys (12), rats, and mice (36) exposed to low T_a.

In a subsequent study, it was shown that during fever elicited by bacterial endotoxin, where cat diencephalic tissue had been preloaded with either ^{45}Ca or ^{22}Na, there was a sharp increase in ^{45}Ca efflux in cerebrospinal fluid (CSF) of the third ventricle, whereas the efflux of ^{22}Na diminished (21).

Pharmacological experiments also confirm the role of calcium on spontaneous and evoked thermoregulatory response. Direct intracerebral injections of a calcium channel inhibitor such as verapamil can differentially alter temperature in cats depending upon the locus of injection. Thus, hypothermia was elicited from the anterior hypothalamus and hyperthermia from the posterior hypothalamus (5,29). The authors of this article showed that ICV infusion of verapamil, nifedipine,

[1] To whom requests for reprints should be addressed.

and cinnarizine evoked hyperthermia in rabbits, whereas Bay K-8644, a dihydropyridine calcium channel agonist, elicited a dose-related hypothermic response (28).

In light of recent knowledge regarding the pathways and sequences of fever genesis, our present study tests the hypothesis that calcium concentration in CSF varies during fevers of different origins.

METHOD

Chemicals and Animals

Acetylsalicylic acid (ASA) was purchased from Farmitalia-Carlo Erba, Analytical Division (Milano, Italy). *E. coli* endotoxin was obtained from Difco Laboratories (Detroit, MI). PGE_2 was obtained from Sigma Chemical Co. (St. Louis, MO). Calcium test reagent [a solid mixture of orthocresolphtalein complexone (OCPC), 8-hydroxyquinoline-5-sulfonic acid, and Tris buffer], hrIL-1β (specific activity 1.0×10 U/mg protein), and hrIL-1β-derived nonapeptide HCl corresponding to the 163-171 portion of the entire molecule were obtained from Sclavo S.p.A. (Siena, Italy). Lipopolysaccharide (LPS) contamination of hrIL-1β and IL-1β-derived nonapeptide was respectively <1.2 pg/μg and <0.02 pg/μg as measured by the limulus amebocyte lysate chromogenic assay (Centro Ricerche Sclavo, Siena, Italy). "Pyrogen-free" water was obtained from sterile water prepared for clinical use after filtration through a column (10 cm height; 1 cm, i.d.) packed with detoxigel (Pierce Chemical Co., Rockford, IL). Stock solutions of hrIL-1β and hrIL-1β-derived nonapeptide, prepared by dissolving the compounds in double-distilled pyrogen-free water, were distributed in small volumes into microtubes and stored in liquid nitrogen. Each solution was thawed only once prior to use and diluted appropriately.

ASA was dissolved in previously sterilized 0.1 N NaOH so that final pH was 7.0. PGs were dissolved directly prior to use in a 1 : 1 (v/v) mixture of ethanol : H_2O.

Adult, male New Zealand albino rabbits weighing 2.0–2.5 kg were supplied with food and water ad lib and individually caged at a T_a of 15–20°C with natural night and day cycles.

Implantation of Outflow and Inflow Cannulae

Modifying a cerebroventricular perfusion procedure described by Barkai et al. (3), a cannula guide was chronically implanted into the cisterna magna to allow for withdrawal of CSF. Under sodium pentobarbitone (Nembutal) anesthesia (30 mg/kg body weight IV), a cannula guide was placed by means of a stereotaxic apparatus (Stoelting Instruments, Chicago, IL) 1–1.5 mm posterior to lambda by using a simple "planilabe" device to establish the horizontal zeroplane (8). Modifications concerned the incannulating device: The cannula guide was adapted from an 18-ga disposable hypodermic needle cut off 16 mm from the base to accommodate a 50-mm long, 22-ga outflow cannula with a side orifice about 0.5 mm above the rounded tip. A polystyrene bur, 10 mm long and 3.5 mm in diameter, was threaded on the outside and tight fitted around the outflow needle about 34 mm above the side orifice. The hub of the guide cannula, made of a polystyrene bur, was drilled out and threaded so that the outflow cannula could be screwed into the guide and precisely calibrated to different depths. The free flow of CSF revealed that the cannula had entered the cisterna magna. For ICV drug administration, an inflow cannula guide was inserted stereotaxically into a lateral cerebral ventricle according to the coordinates described by Sawyer and coworkers (32).

Collection of CSF Samples

Samples of CSF drawn from the cisterna magna through a polyethylene tube connected to a peristaltic pump (LKB, Bromma, Sweden) at a constant flow rate of 5 μl/min were collected into 3-ml plastic tubes in 25-min fractions (125 μl) by a Redirac 2112 collector (LKB, Bromma, Sweden).

Fractions were refrigerated at 4°C and analyzed the same day as the experimental session. The rate of CSF withdrawal was well below 9.6 ± 0.9 (SD) μl/min, the value reported for CSF formation in conscious, restrained rabbits (3).

ICV Injections

HrIL-1β, hrIL-1β-derived nonapeptide, and PGE_2 were administered ICV in 10 μl volume by an Agla micrometer syringe (Burroughs Wellcome and Co., London, UK).

Temperature Recording

Conscious rabbits were individually housed in a thermostated chamber (Soc. K.W., Siena, Italy) and restrained in stainless steel cages. T_a was set at 20°C.

Core temperature was measured every 5 min by a Thermocouple thermometer (Columbus Instruments) inserted 10 cm in the rectum and connected to a personal computer with an isothermex (Columbus Instruments) program. Temperature was monitored for at least 1 h before the experimental session.

Chemical Assays

Calcium and protein assay. Calcium in CSF was determined colorimetrically at 573 nm by the method of Stern and Lewis (35) using a "calcium test reagent" specific for Ca ion, as no other cations have been shown to cross react (13,31). The method uses OCPC as Ca ion complexing agent and 8-hydroxyquinoline-5-sulfonic acid to eliminate Mg ion interference. By this method, the low existing CSF bound calcium (15%) is converted into ionic form so that calcium determined by this procedure represents the total calcium concentration. To evaluate experimental reproducibility and accuracy of the method, the interassay deviation and percent recovery were calculated respectively. Methodological sensitivity was calculated by the molar extinction coefficient of calcium with the dye in the reaction conditions described above.

The interassay deviations ranged between 2.3 and 4.3% ($n = 250$). Average recovery was $96.67 \pm 0.966\%$ ($n = 52$) while molar extinction coefficient was $1.9 \cdot 10 \cdot M^{-1}cm^{-1}$.

Protein in CSF was determined by the coomassie blue binding method (7).

Statistical Analysis

Values are expressed as means ± SEM. The significance of differences between pre- and posttreatment data was analyzed by analysis of variance (ANOVA).

Linear regression analysis was used in plotting the single values of each parameter (i.e., Ca, protein, and rectal temperature) against the others. A p value less than 0.05 was considered significant.

Control Experiments

Reference values for CSF Ca, protein, and TR were collected in the first 100 min of each experimental session. To avoid any possible effects of the physical manipulation itself, a control group of six animals was kept at neutral T_a and

liquor withdrawn for a period of 6 h without showing any statistically significant differences.

RESULTS

CSF Calcium and Protein Concentration in Animals Recovering From Surgery and Kept at Neutral T_a

Animals submitted to surgery 2–3 days before, exhibited a wide range of TR, from normothermia to fever (38.4–40.9°C). Calcium concentration in CSF (average of five fractions) varied between 1.01 and 1.70 mM. Protein concentration in CSF (average of five fractions) oscillated between 28.6 and 94.7 mg/100 ml. The relationship between these parameters is shown in Fig. 1. CSF Ca was found to be linearly and positively correlated to TR in a highly statistically significant way ($p < 0.01$). On the contrary, CSF protein was not significantly correlated to TR or Ca.

CSF Calcium and Protein Concentration and TR After IV Injection of E. coli *Endotoxin*

Four rabbits were injected IV with two successive doses of *E. coli* endotoxin (0.15 μg/kg body weight, in 1 ml sterile saline) 2.5 h apart. As shown in Fig. 2, TR rose shortly after the endotoxin injection and increased regularly from 39.1 ± 0.4°C to 40.8 ± 0.2°C, at which point the second dose was given. This was followed by a modest decrease in TR that leveled off to 40.6 ± 0.1°C for a period of 2 h. TR values after endotoxin treatment were significantly higher ($p < 0.01$) than basal values.

Fever was accompanied by a significant increase ($p < 0.05$) in CSF Ca that peaked after 1.25–2.5 h with 0.1–0.35 mM increases above basal values. The average increase of Ca was 0.15 ± 0.03 mM and was reached 1.7 h after the first injection. After the second injection, Ca further rose with overall increases ranging between 0.21 and 0.48 mM ($p < 0.01$). The maximal increase of Ca was reached 0.42 h after

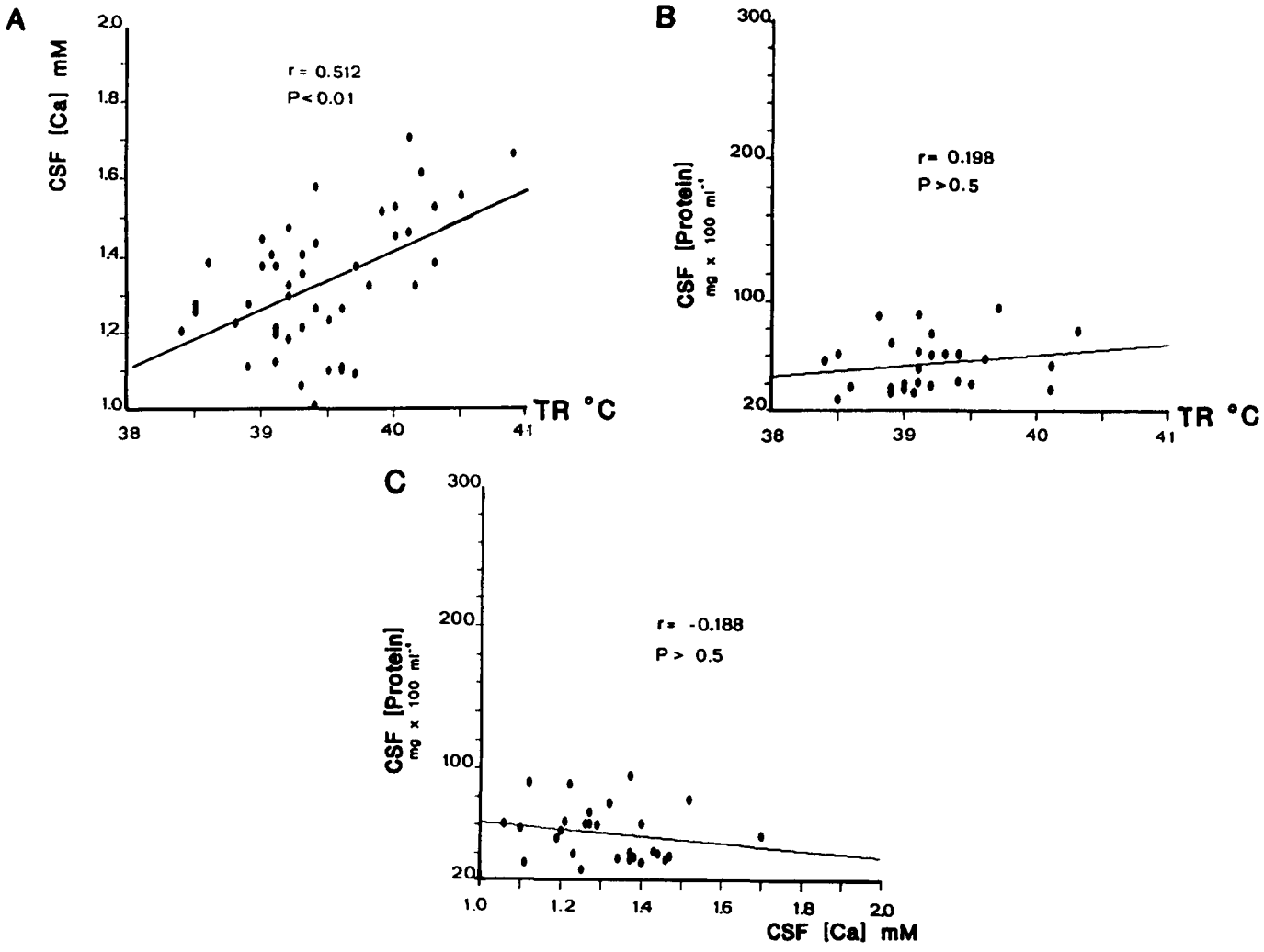

FIG. 1. Correlation between rectal temperature (TR), calcium concentration (Ca), and protein concentration in post surgical cerebrospinal fluid (CSF) of rabbits kept at 20°C T_a for 2 h. Each CSF Ca + protein curve point represents the individual's mean value from 5 CSF fractions collected at 25-min intervals and analyzed in quadruplicate. The TR value represents the average of five measurements 25 min apart. Each parameter was plotted against all others. (A). CSF Ca from 45 animals against TR, (B) and (C). Protein from 27 animals against TR and CSF Ca. The regression lines were fitted by the least-square deviation method; r is the correlation coefficient and p the significance of r.

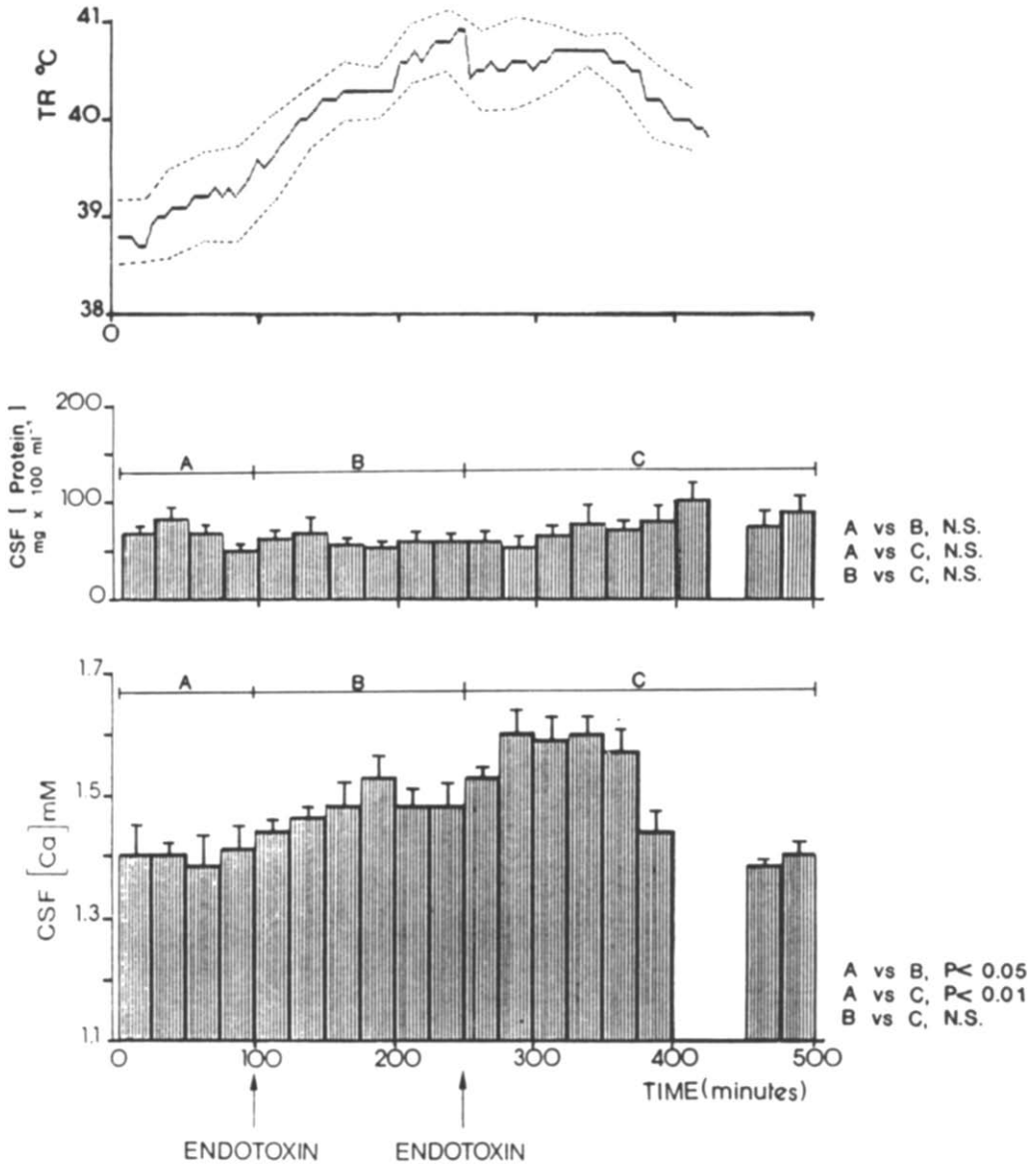

FIG. 2. Effect of *E. coli* endotoxin on rectal temperature (TR), cerebrospinal fluid (CSF) calcium concentration (Ca), and protein concentration of rabbits kept at 20°C T_a. Two equivalent doses of endotoxin (0.15 μg/kg body weight) were administered IV 2.5 h apart. Arrows show injection times. Ca and protein values were determined in quadruplicate and represented as the mean of four animals. Bars and dotted lines indicate SEM. Individual basal and treatment values were gathered into three groups and compared for statistical significance by analysis of variance. A value of $p < 0.05$ was considered significant. Group A, pretreatment; group B, first treatment; group C, second treatment.

treatment and averaged 0.21 ± 0.04 mM and was stable for the next 1.7 h. As soon as TR declined, CSF Ca fell to basal values.

CSF protein, on the contrary, did not vary significantly during the whole experimental session.

Effect of ASA on E. coli *Endotoxin-Induced Changes in TR and CSF Calcium Concentration*

Three rabbits were injected IV with ASA at a dose of 80 mg/kg body weight, sufficient to counter the pyrogenic effect of *E. coli* endotoxin (35). Two consecutive doses of ASA were given 1.7 h apart. The injection of *E. coli* endotoxin (0.15 μg/kg body weight in 1 ml sterile saline) followed the second ASA administration. As shown in Fig. 3, the first ASA injection had no effect on TR, CSF Ca, and protein. The complete ASA treatment, however, almost totally neutralized the effects of the endotoxin both on TR and CSF Ca. TR, in fact, significantly increased only by 0.5 ± 0.1°C, while CSF Ca did not change. CSF protein showed no significant changes.

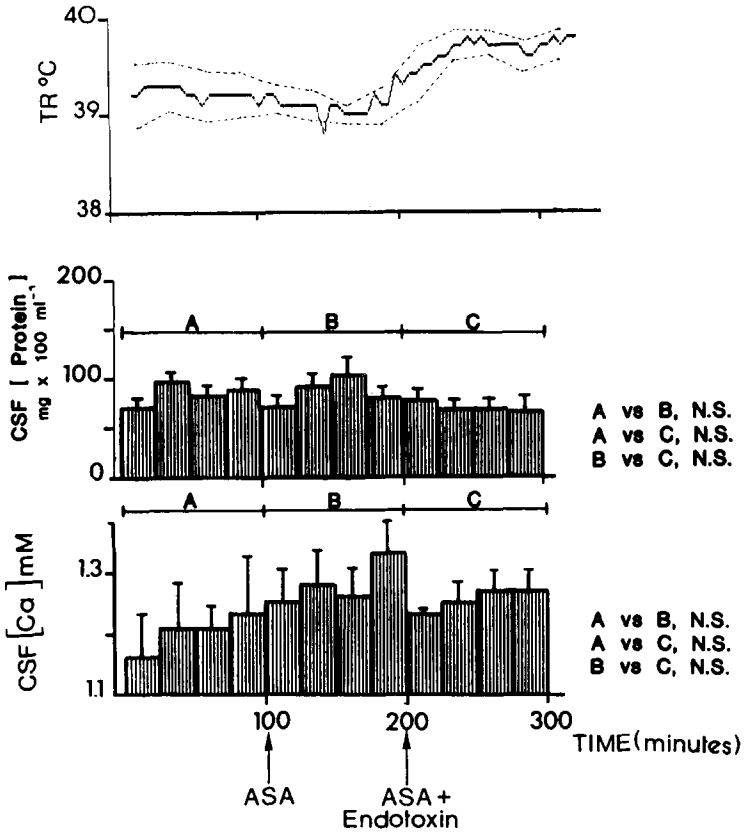

FIG. 3. Effect of acetylsalicylic acid (ASA) on rectal temperature (TR) and cerebrospinal fluid (CSF) calcium concentration (Ca) variations induced by *E. coli* endotoxin. Two equivalent ASA doses (80 mg/kg body weight) were injected IV 1.7 h apart. *E. coli* endotoxin was injected IV (0.15 μg/kg body weight) immediately after the second ASA injection. Values are given as means of three animals. Bars and dotted lines indicate SEM. Arrows show injection. For other details, see Fig. 2.

CSF Calcium and Protein Concentration and TR After ICV Injection of hrIL-1β or hrIL-1β-Derived Nonapeptide

Five rabbits were treated ICV with a single dose (25 ng) of hrIL-1β. As shown in Fig. 4, shortly after injection TR rose progressively and significantly ($p < 0.01$) above basal values, leveling off to 1.45 ± 0.25°C. This effect was accompanied by a consistent increase of CSF Ca by amounts comprised between 0.17 and 0.25 mM ($p < 0.05$). This increase averaged 0.14 ± 0.02 mM and was reached 25 min after treatment. On the contrary, CSF protein did not change significantly. hr-IL-1β-Derived nonapeptide when injected ICV at the dose of 25 ng modified neither TR nor CSF Ca nor protein (data not shown).

Effect of ASA on hrIL-1β-Induced Changes in TR and CSF Calcium Concentrations

Five rabbits were injected IV with two consecutive doses of ASA (80 mg/kg body weight, in a 3 ml volume) 0.8 h apart. The second ASA administration was followed by ICV injection of hrIL-1β (25 ng). As shown in Fig. 5, ASA antagonized the pyrogenic effect of hrIL-1β so that animals responded with a mean increase in TR of only 0.4 ± 0.12°C (not statistically significant). Basal values of CSF Ca did not change significantly after either first ASA injection or IL-1β treatment. However, interestingly enough, two animals presented a decrease of 0.18–0.2 mM in CSF Ca and one of them showed a fall in TR of 1°C. CSF protein did not change significantly. Percent variations of CSF Ca and protein over respective baselines were calculated per each fraction following hrIL-1β injection and group comparisons made against ASA + hrIL-1β-treated animals. ANOVA showed that CSF Ca levels of hrIL-1β-treated animals were significantly higher ($p < 0.001$) than those primed with ASA. The same significant difference was found in TR scores. Protein did not vary significantly.

CSF Calcium and Protein Concentrations and TR After ICV Injection of PGE_2

Three animals were injected ICV with a single dose of PGE_2 (25 μg). As shown in Fig. 6, TR started to increase immediately and after injection averaged 1.2 ± 0.77°C without significant variations in CSF Ca. CSF protein, however, increased significantly ($p < 0.01$) by 44 ± 9.8% with respect to basal values. CSF protein values remained unchanged when CSF samples had been centrifuged at 2,000 × *g* for 15 min. The PGE_2 well-documented vasodilating action and its effect

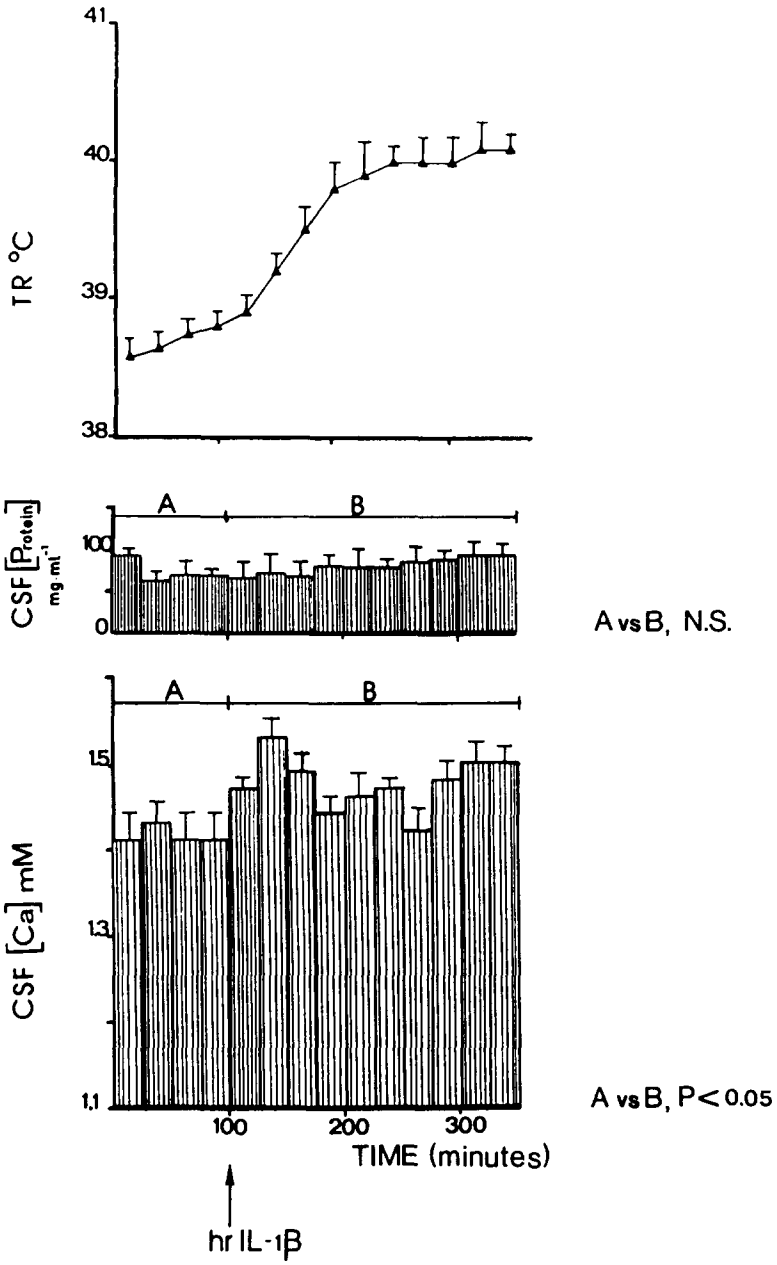

FIG. 4. Effect of human recombinant interleukin 1β (hrIL-1β) on rectal temperature (TR), cerebrospinal fluid (CSF) calcium concentration (Ca) and protein concentration of rabbits kept 20°C T_a. hrIL-1β was administered ICV at a dose of 25 ng at the time arrowed. Values are given as means of five animals. Bars indicate SEM. For other details, see Fig. 2.

as potentiators of microvascular permeability induced by leukotrienes may have resulted in leakage of brain blood capillaries or ependymal cells lining CSF spaces, thus accounting for the increased levels of proteins observed.

DISCUSSION

The blood-brain barrier permits relatively constant levels of calcium within the brain and CSF to be maintained, even during conditions of plasma hyper- and hypocalcemia (4, 19,20). These conditions of ionic balance may, however, abruptly be perturbed during fever states.

The data reported in this article show that when the normothermic conditions of animals were altered, as in postsurgical fever or *E. coli* and IL-1 fever, there was a consistent and highly significant upward shift of CSF Ca concomitant with an increase of TR. A previous study failed to correlate changes in CSF Ca with fever in man (26); however, this study monitored the levels of calcium withdrawn from lumbar fluid, and Ca changes in CSF adjacent to brain tissues may not have

been recognized when diluted over the entire cerebrospinal space.

Our experimental data provide the first direct evidence of a long-lasting increase in CSF Ca in febrile mammals. Increase calcium levels are linearly correlated to degree of temperature gain and are implicated in all fever responses regardless of the endogenous or exogenous origin of the pyrogen.

Studies on the pathogenesis of fever have established that a cytokine, namely, IL-1, is the primary host mediator of febrile response. Bacterial endotoxin and other fever-inducing agents are thought to stimulate release of IL-1 from many tissues, including the brain (9). There is some consensus that the IL-1 pyrogenic site of action is located primarily in the preoptic, anterior hypothalamus (PO/AH) because microinjections of IL-1 into this area result in fever while delivery into contiguous hypothalamic tissue have little or no effect on

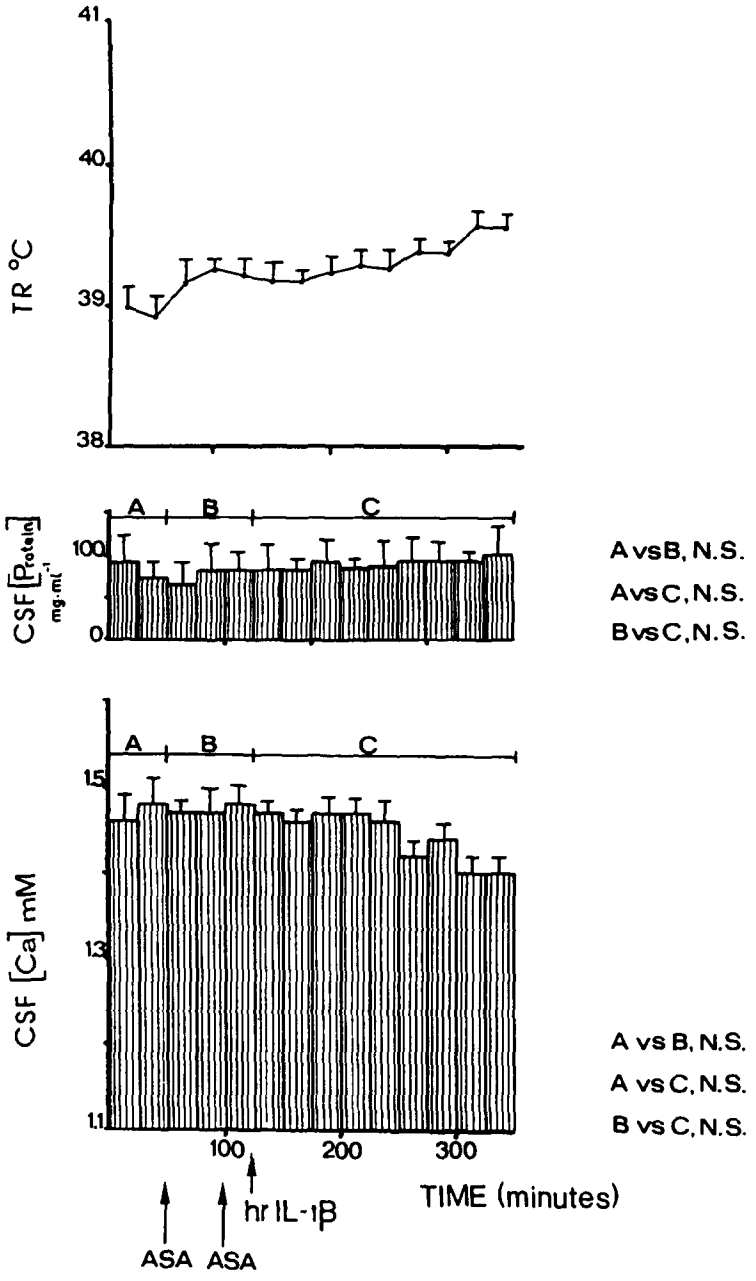

FIG. 5. Effect of acetylsalicylic acid (ASA) on rectal temperature (TR) and calcium concentration (Ca) variations induced by human recombinant interleukin 1β (hrIL-1β). Two equivalent ASA doses were injected IV (80 mg/kg body weight) 0.8 h apart. hrIL-1β was injected ICV (25 ng) 0.4 h after the second ASA injection. Arrows show the injection time. Values are represented as the mean of five animals. Bars indicate SEM. For other details, see Fig. 2.

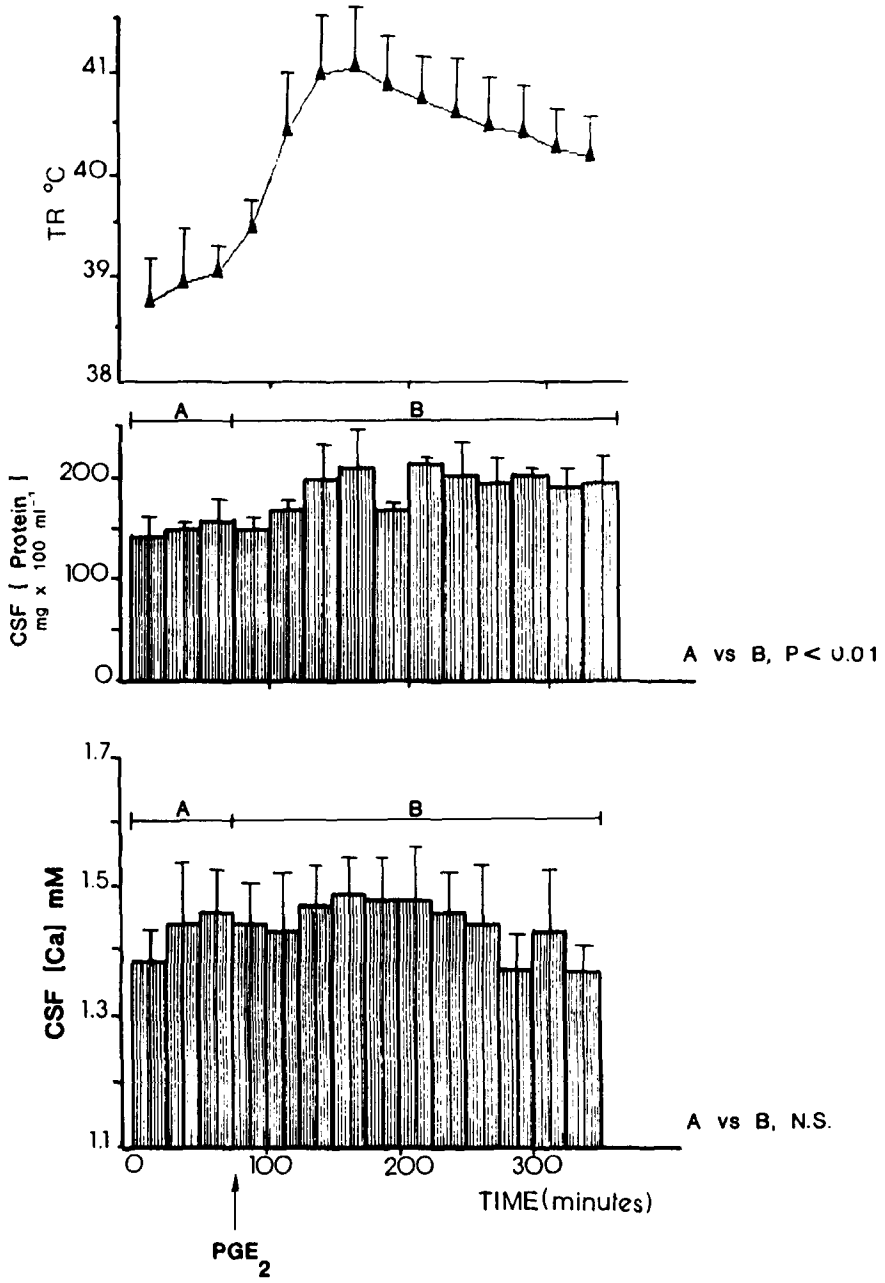

FIG. 6. Effect of prostaglandin E_2 (PGE_2) on rectal temperature (TR), cerebrospinal fluid (CSF) calcium concentration (Ca), and protein concentration of rabbits kept at 20°C T_a. PGE_2 was injected ICV at a dose of 25 μg at the time arrowed. Values are given as means of three animals. Bars indicate SEM. For other details, see Fig. 2.

body temperature (30). Fever conditions induced by central administration of IL-1 were almost indistinguishable from those induced by intravenous endotoxin, and as we observed were inhibited by antipyretics (11).

To assess whether IL-1 was involved in the CSF Ca increase elicited by endotoxin administration, we examined the effect of ICV hrIL-1β on CSF Ca. Indeed, hrIL-1β promoted a significant increase in CSF Ca concurrent with fever production. This effect is unlikely to be the result of endotoxin contamination of IL-1 as the compound used was highly purified. In fact, the LPS content of the hrIL-1β was below 25 fg, much lower than the minimum pyrogenic threshold for rabbits (2).

The effect of hrIL-1β on CSF Ca induced changes seems more likely to be related to the pyrogenic properties of the entire molecule. In fact, the nonpyrogenic 9-residue long synthetic fragment, corresponding to the highly hydrophylic 163-

171 portion of hrIL-1β (1), was neither an effective pyrogen even at much higher molar doses than IL-1β nor indeed did it modify CSF Ca.

The role of IL-1 in boosting CSF Ca receives further support from studies showing that exposure of a pre-β-like cell line to human IL-1β induces changes in intracellular Na and Ca. Exposure led to a marked and prolonged increase in intracellular Na via stimulation of Na^+/H^+ exchange and a transient fall in total intracellular Ca (34).

There is evidence that IL-1 brings about fever by first inducing PG synthesis (6) and our data confirm that PGE_2 induces a pyrogenic response, even though it fails to modify CSF Ca. This might indicate that the increase in PG synthesis during endotoxin- or hrIL-1β-induced fever occurs downstream, as a later step in the sequence following calcium efflux into CSF. Extracellular brain calcium (Ca_o) may play a role in the signal transduction mechanisms evoked therefore by pyrogens. If PGs are the final mediators of febrile response (6), it is legitimate to speculate that Ca_o acts by modulating the synthesis of PGs. Current unpublished work by the present authors bears out this view. Perfusion from cisterna magna to lateral ventricle with artificial, calcium-free CSF containing EGTA antagonizes both TR and PGE_2 increases in rabbit CSF induced by hrIL-1β injection. A possible enzymatic target for calcium activity might be the highly calcium-dependent enzyme phospolipase A_2 (PLA_2) (16). The secretory, extracellular 14-KDa group II form of this enzyme has been found to accumulate at inflammation sites and plays a role in the regulation of eicosanoid synthesis (18). Inflammatory cytokines such as IL-1, IL-6, and tumor necrosis factor induce transcription of the 14-KDa group II PLA_2 gene in various cells, while antiinflammatory glucocorticoids have opposite effects (15,24,33).

Experiments still in progress in the laboratory of the present authors have shown that ICV injection of different glucocorticoids lowers hrIL-1β-induced fever and at the same time decreases calcium and PGE_2 levels in CSF (27). Although our data furnish evidence of the involvement of calcium in the central mechanisms of thermoregulation, they do not address the issue of the source of CSF calcium. To date, experimental findings tend to ascribe a central origin to the increased CSF Ca. The high plasmatic concentration of calcium (twice that of CSF) (4,20), however, does not allow one to exclude the blood as a possible source of calcium. If this were the case, our model would have to anticipate a role for IL-1 in rendering the blood–brain barrier more permeable to calcium.

ACKNOWLEDGEMENTS

This work was supported by MURST (40 + 60%), Roma, Italy. The authors acknowledge Centr Ricerche Sclavo (Siena, Italy) for supplying hrIL-1β and hrIL-1β-derived nonapeptide used in these experiments. The authors thank B. Gorelli and R. Borghi for their technical assistance and Dr. R. Raikes for assistance in improving the manuscript.

REFERENCES

1. Antoni, G.; Presentini, R.; Perin, F.; Tagliabue, A.; Ghiara, P.; Censini, S.; Volpini, G.; Villa, L.; Boraschi, D. A short synthetic peptide fragment of human interleukin-1 with immunostimulatory but not inflammatory activity. J. Immunol. 137:3201–3207; 1986.
2. Atkins, E. Pathogenesis of fever. Physiol. Rev. 40:580–585; 1960.
3. Barkai, A. I.; Budek, M.; Brown, D. L.; Fieve, R. R. Application of an improved cerebroventricular perfusion technique in the rabbit: Effect of pentobarbital or haloperidol on monoamine metabolites and proteins in the perfusate. Neuropharmacology 17:409–414; 1978.
4. Barkai, A. I.; Meltzer, H. L. Regulation of calcium entry in the extracellular environment of the rat brain. J. Neurosci. 2:1322–1328; 1982.
5. Beleslin, D. B.; Rezvani, A. H.; Myers, R. D. Divergent action of verapamil perfused in two hypothalamic areas on body temperature of the cat. Neurosci. Lett. 57:307–312; 1985.
6. Bernheim, H. A. Is prostaglandin E_2 involved in the pathogenesis of fever? Effects of interleukin-1 on the release of prostaglandins. Yale J. Biol. Med. 59:151–158;1986.
7. Bradford, M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding. Anal. Biochem. 72:248–254; 1976.
8. Crowford, I. L.; Kennedy, J. I.; Lipton, J. M. Simple planilabe for rapid establishment of the stereotaxic horizontal zero plane in rabbits. Brain Res. Bull. 2:397–398; 1977.
9. Dinarello, C. A. Induction of acute phase reactants by interleukin-1. In: Weissmann, G., ed. Advances in inflammation research. New York: Raven Press; 1984:203–225.
10. Dinarello, C. A.; Bernheim, H. A. Ability of human leukocyte pyrogen to stimulate brain prostaglandin synthesis in vitro. J. Neurochem. 37:702–708; 1981.
11. Dinarello, C. A.; Marnoy, S. O.; Rosenwasser, L. J. Role of arachidonate metabolism in the immunoregulatory function of human leukocyte pyrogen/lymphocyte activating factor/interleukin-1. J. Immunol. 130:890–895; 1983.
12. Gisolfi, G.; Mira, F.; Myers, R. D. Diencephalic efflux of calcium ions in the monkey during exercise, thermal stress and feeding. J. Physiol. 273:617–630; 1977.
13. Gitelman, H. J. An improved automated procedure for the determination of calcium in biological specimens. Anal. Biochem. 18: 521–531; 1967.
14. Greenleaf, J. E. Thresholds for Na^+ and Ca^{++} effects on thermoregulation. In: Girarder, L.; Geydaux, J., eds. Effectors of thermogenesis. Basel: Birkhauser; 1978:33–43.
15. Kerr, J. S.; Stevens, T. M.; Davis, G. L.; McLaughlin, J. A.; Harris, R. R. Effects of recombinant interleukin-1β on phospholipase A_2 activity, phospholipase A_2 mRNA levels and eicosanoids formation in rabbit chondrocytes. Biochem. Biophys. Res. Comm. 165:1079–1084; 1989.
16. Matsumoto, T.; Tao, W.; Sha'Afi, R. I. Demonstration of calcium dependent phospholipase A_2 activity in membrane preparation of rabbit neutrophils. Biochem. J. 250:343–348; 1988.
17. Milton, A. S. Prostaglandins in fever and the role of action of antipyretic drugs. In: Milton, A. S., ed. Pyretics and antipyretics. Handbook of experimental pharmacology. Berlin: Springer-Verlag; 1982:257–303.
18. Murakami, M.; Kudo, I.; Inoue, K. Eicosanoid generation from antigen-primed mast-cells by extracellular mammalian 14-KDa group II phospholipase A_2. FEBS Lett. 294:247–251; 1990.
19. Murphy, V. A.; Smith, Q. R.; Rapoport, S. I. Homoeostasis of brain and cerebrospinal fluid calcium concentrations during chronic hypo- and hypercalcemia. J. Neurochem. 250:1735–1741; 1986.
20. Murphy, V. A.; Smith, Q. R.; Rapoport, S. I. Uptake and concentrations of calcium in rat choroid plexus during chronic hyper- and hypocalcemia. Brain Res. 484:65–70; 1989.
21. Myers, R. D. Diencephalic efflux of ^{22}Na and ^{45}Ca ions in the febrile cat: Effect of an antipyretic. Brain Res. 103:412–417; 1976.
22. Myers, R. D. Hypothalamic control of thermoregulation: Neurochemical mechanism. In: Morgane, P.; Panksepp, J., eds. Hand-

book of the hypothalamus. New York: Marcel Dekker; 1980:83–210.
23. Myers, R. D.; Veale, W. L. Body temperature: Possible ionic mechanism in the hypothalamus controlling the set-point. Science 170:95–97; 1970.
24. Nakano, T.; Ohara, O.; Terakoa, H.; Arita, H. Glucocorticoids suppress group II phospholypase A_2 production by blocking mRNA synthesis and posttranscriptional expression. J. Biol. Chem. 265:12745–12748; 1990.
25. Nielsen, B.; Greenleaf, J. E. Electrolytes and thermoregulation. In: Cooper, K. E.; Lomax, P.; Shonbaum, E., eds. Drugs, biogenic amines and body temperature. Basel: Karger; 1977:39–47.
26. Nielsen, B. P.; Schwartz, P.; Alhede, J. Is fever in man reflected in changes in cerebrospinal fluid concentrations of sodium and calcium ions? Scand, J. Clin. Lab. Invest. 32:309–310; 1973.
27. Palmi, M.; Frosini, M.; Sgaragli, G. P. Antagonistic effect of glucocorticoids on fever and on increase of cerebrospinal fluid calcium concentration promoted by central administration of interleukin-1β in rabbits. Int. J. Immunopathol. Pharmacol. (in press).
28. Palmi, M.; Sgaragli, G. P. Hyperthermia induced in the rabbit by organic calcium antagonists. Pharmacol. Biochem. Behav. 34: 325–330; 1989.
29. Rezvani, A. H.; Beleslin, D. B.; Myers, R. D. Neuroanatomical mapping of hypothalamic regions mediating verapamil hyper- and hypothermia in the cat. Brain Res. Bull. 17:249–254; 1986.
30. Rosendorff, C.; Mooney, J. J. Central nervous system sites of action of purified leucocytic pyrogen. Am. J. Physiol. 220:597–603; 1971.
31. Sarkar, B. C. R.; Chauhan, U. P. S. A new method for determining microquantities of calcium in biological materials. Anal. Biochem. 20:155–166; 1967.
32. Sawyer, C. H.; Everett, J. W.; Green, J. D. The rabbit diencephalon in stereotaxic coordinates. J. Comp. Neurol. 101:801–824; 1954.
33. Schalkwijk, C.; Pfeilschifter, J.; Marki, F.; Van der Bosch, H. Interleukin-1β, tumor necrosis factor and forskolin stimulate the synthesis and secretion of the group II phospholipase A_2 in rat mesangial cells. Biochem. Biophys. Res. Comm. 174:268–275; 1991.
34. Stanton, T. H.; Maynard, M.; Bomsrtyk, K. Effect of interleukin-1 on intracellular concentration of sodium, calcium and potassium in 702/3 cells. J. Biol. Chem. 261:5699–5701; 1986.
35. Stern, J.; Lewis, W. H. P. The colorimetric estimation of calcium in serum with *o*-cresolphthalein complexone. Clin. Chim. Acta 2: 576–580; 1957.
36. Watanabe, H. K.; Ho, I. K.; Hoskins, B. Effects of cold stress on brain regional calcium content in rats and mice. Brain Res. Bull. 19:407–409; 1986.