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The Effect of Calcium Ions Chelation and Sodium Ions Excess in the Cerebrospinal Fluid on Body Temperature in Conscious Dogs

Bogdan Sadowski and Ewa Szczepańska-Sadowska

Departments of Applied Physiology of the Polish Academy of Sciences Medical Research Centre and of the Institute of Physiological Sciences, School of Medicine Warsaw, Poland

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Summary. The effects of chelation of calcium ions and elevation of sodium ions concentration in cerebrospinal fluid on body temperature was examined in conscious dogs. Decrease of calcium ions concentration brought about by an infusion of disodium edetate (Na_2EDTA) into the lateral ventricle elicited an increase in body temperature in 8 out of 10 experiments by $1.49 \pm 0.36°C$. Huddling, piloerection, lowering of respiratory rate, vasoconstriction and shivering occurring in the course and after Na_2EDTA infusion argue that hyperthermia resulted both from excess production of heat and activation of heat conservation mechanisms. Two intraventricular infusions of artificial CSF containing 88 mM Na^+ in excess of its physiological concentration did not produce any effect on body temperature.

The role of calcium ions concentration in brain centres regulating body temperature is discussed.

Key words: Body Temperature Regulation — CSF Calcium — CSF Sodium.

There is accumulating evidence indicating that the ionic composition of the cerebrospinal fluid and of the extracellular fluid in the brain tissue plays an important role in determining the animal's behaviour. It was recently suggested that the inherent ratio between the concentrations of two cations Na^+ and Ca^{++} may be a physiological basis of the set-point for body temperature [7]. Body temperature was found to increase when the cerebral ventricles were perfused with a fluid containing sodium ions in excess of their physiological concentration, and to decrease when calcium ions concentration was augmented [6]. The shift in the intrinsic ratio between sodium and calcium was proposed to exert a selective action upon the posterior hypothalamus, as changes of this ratio in other hypothalamic areas did not produce any evident effects on body temperature [8].

The fact that alterations of these two ions concentrations exerted the same effect on body temperature in several species such as rabbit, rat, cat and monkey [3, 6, 8, 9] made this hypothesis particularly attractive. How-

ever, Seoane and Baile [12] failed to find any effect of calcium injected in excess into the third ventricle on body temperature in the sheep, and Hanegan and Williams [4] reported that alterations in Ca^{++} concentration within the preoptic area and anterior hypothalamus influenced body temperature of ground squirrels. These two findings justify the assumption that the effect of calcium ions in the cerebrospinal fluid on deep body temperature may in fact depend on species differences.

The present study was aimed to examine the effects of lowered calcium and increased sodium concentrations in the ventricles on body temperature in the conscious dog.

Materials and Methods

The experiments were performed on three male conscious mongrel dogs weighing 16—22 kg accustomed to the experimental situation requiring partial restraint on the Pavlov stand.

Surgery. Under hexobarbital anaesthesia the animals were aseptically implanted with guide tubes leading to the lateral cerebral ventricle and with thermistors aimed at the anterior hypothalamus. Coordinates taken from the stereotaxic atlas of Lim *et al.* [5] were corrected for individual variations in the dimensions of the dog's skull using APO-infraorbital coefficient [11]. The procedure described by Traczyk [13] was adopted to approach the lateral ventricle. A plexiglas socket was screwed into the parietal bone. The guide tube cut from a stainless steel needle (1.25 mm o.d.) was placed in a holder of the stereotaxic instrument and lowered slowly at 13—16 mm anteriorly to the interaural plane, 11—13 mm from the sagittal plane and at an angle of 75° to the horizontal plane through a hole in the socket until cerebrospinal fluid be could aspirated from its outer end, and was kept secure in this position with acrylic cement. The thermistor was placed in a 0.6 mm (o.d.) glass tube, with one of its platinum-iridium wires passed through a thin glass capillary. The two wires were soldered to copper teflon-coated leads. The place of soldering was carefully insulated using Araldite (Ciba-Geigy). The thermistor was implanted at A—25 mm, L—3 mm and V—7 to 9 mm, and fastened to the skull with the cement. The leads were placed in the socket and wound freely around the guide tube. The guide tube was closed with an obturator and a plexiglas cap was screwed onto the socket. Prophylactic doses of penicillin and streptomycin were injected during 3 days following the surgery. A period of at least 2 weeks was allowed for recovery.

Solutions Used for Intraventricular Infusions. 1. Control artificial cerebrospinal fluid (CSF) was similar to that used by Myers and Yaksh [10] and contained: Na^+ 142.5 mM, K^+ 2.6 mM, Ca^{++} 1.3 mM, Mg^{++} 0.9 mM, Cl^- 134.5 mM, HCO_3^- 10.0 mM, HPO_4^{--} 2.5 mM and glucose 3.4 mM. 2. EDTA-CSF used in experiments with Ca^{++} chelation had the same composition as the control one, but Ca ions were omitted and ethylene-diamine-tetraacetic acid disodium salt (Na_2 EDTA) was added in a concentration of 3.6 mM. 3. Na-CSF had the same composition as the control CSF, except that it contained 88 mM of Na^+ and Cl^- in excess of their physiological concentration. All three solutions were titrated to a final pH of 7.3 with 0.1 N HCl. Control and EDTA-CSF were brought into isoosmolarity (300 mOsm/l) with sodium chloride or water, whereas NaCSF had a higher osmolarity (415 mOsm/l). The syringes, inflow cannula and polyethylene tubing were stored in 70% ethanol, and flushed with a commercial pyrogen-free saline and finally with the solution used for intraventricular infusion just before each experiment.

Experimental Design. The experiments were performed at ambient temperatures ranging 18—23° C and relative air humidity of 40—50%. Three variants were carried out depending on the kind of solution used for intraventricular infusion. The infusions were given alternately and an interval of at least 48 hrs was allowed between them. At the beginning of each experiment a semiconductor thermosensitive probe was placed 10 cm beyond the anus to measure rectal temperature (T_{re}), and leads of the thermistor were connected to a thermistor bridge to measure hypothalamic temperature (T_{hy}), skin temperature (T_{ear}), respiratory movements, electrocardiogram and electromyogram were recorded continuously. Skin temperature was measured using copper-constantan thermocouple attached to the dorsal surface of the ear. Respiratory movements were recorded using a resistance transducer placed around the dog's chest. Electromyogram was recorded from the gluteal muscle.

An inflow cannula was introduced into the lateral ventricle through the guide tube. It was connected by means of a polyethylene tube to a syringe placed in a Palmer slow injection apparatus. The intraventricular infusion started when all measured parameters stabilized approximately at the same level for a period of 20 min. The rate of infusion was 75 µl/min and the time varied from 18—90 min. The experiment was finished when deep body temperature went down to the preinfusion level.

Histology. At the conclusion of the experiments the animals were sacrificed to verify histologically the placements of the guide tubes and the thermistors. A routine procedure of fixation, sectioning and staining after Weil was applied.

Results

Calcium Chelation in the Cerebrospinal Fluid. The effects of ten intraventricular infusions of EDTA-CSF together with corresponding infusions of control CSF on body temperature are shown in Table 1. Control infusions produced a slight decrease in deep body temperature by 0.1—0.35° C, whereas an increase in body temperature was observed in 8 out of 10 EDTA-CSF experiments. The mean increase in T_{re} in 8 experiments with hyperthermia equalled to 1.49 ± 0.36° C.

Despite the same rate of intraventricular infusion in each experiment the time course and the range of body temperature increase was different in individual experiments on the same dogs. Both T_{re} and T_{hy} started to rise within 6 to 30 min after the onset of the infusion and continued to increase for some time after the infusion was stopped.

Increase of body temperature was accompanied by behavioural changes typical of defense against cold. If the dog was panting during the preinfusion period, he closed his mouth and dramatically slowed the respiratory rate. Huddling, piloerection and shivering appeared soon thereafter. Shivering increased as the infusion was continued. Vasoconstriction (estimated from a decrease of T_{ear}) and increase in heart rate were also evident. When the body temperature exceeded some critical level (usually about 41° C) the heat dissipation mechanisms were activated. The respiratory rate suddenly increased and later on panting accompanied by salivation and vasodilatation (estimated from an

Table 1. Effect of infusion of control (CSF) and disodium edetate containing (EDTA-CSF) cerebrospinal fluid into the lateral ventricle on rectal temperature (T_{re}) in dogs

Dog	T_a °C	Solution infused	Time of infusion (min)	max ΔT_{re} °C
1	22	EDTA-CSF		− 0.10
	23	EDTA-CSF		+ 0.80
	18	CSF		+ 0.10
	21	EDTA-CSF		− 0.15
	19	EDTA-CSF	60	+ 0.55
	22	CSF		− 0.10
	23	EDTA-CSF		+ 0.85
	23	CSF		− 0.05
2	23	CSF	90	− 0.20
	21	EDTA-CSF	28	+ 3.20
	22	CSF	28	− 0.10
	23	EDTA-CSF	20	+ 1.80
	20	CSF	20	− 0.15
	22	EDTA-CSF	30	+ 0.30
	22	CSF	25	− 0.15
3	22	CSF	30	− 0.10
	24	EDTA-CSF	20	+ 2.30
	24	CSF	30	− 0.35
	20	EDTA-CSF	18	+ 2.20

T_a = ambient temperature.

increase of T_{ear}) developed. For a short period of time (10—15 min) the dog was shivering and panting at the same time. Thirty to 60 min after the end of the infusion the body temperature decreased to the control value or stabilized for some time at a slightly higher level (Figs.1, 2, and 3).

Other behavioural effects not related directly to the thermoregulatory mechanisms were also noted. They consisted of sniffing, overexcitability and sometimes aggressiveness. In some experiments defecation and epileptic seizures appeared. The latter could be prevented by injection of 10 mg of luminal natrium/kg body weight intramuscularly 30 min before the intraventricular infusion. This dose was found not to abolish or diminish the increase in body temperature. No epileptic seizures were observed in the course of control CSF infusions.

Sodium Excess in Cerebrospinal Fluid. Two infusions of Na-CSF lasting 40 and 90 min did not produce any changes in body temperature as compared to control CSF infusions (Fig.3). The same dog responded with high increase in body temperature to EDTA-CSF infusions. In one

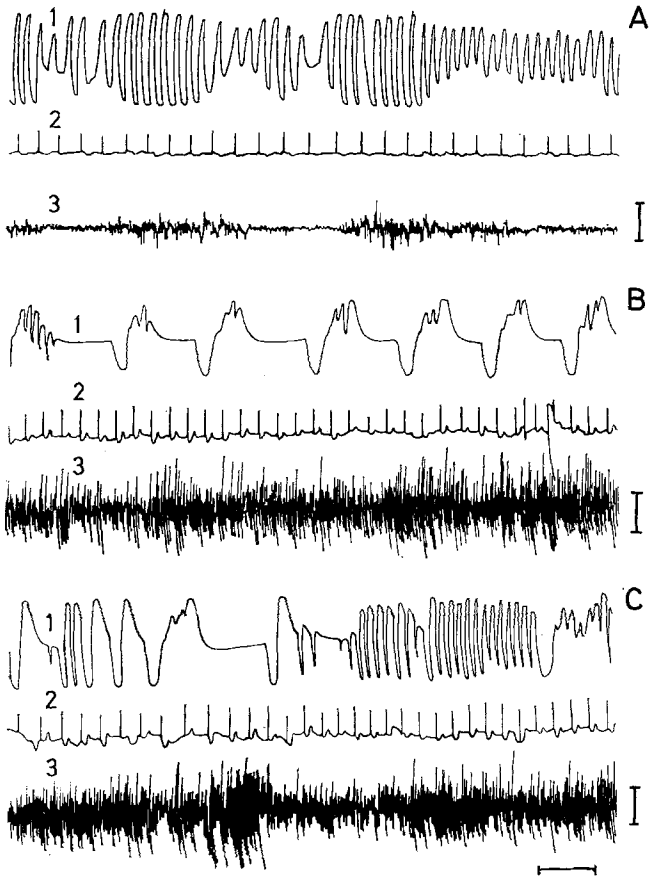

Fig. 1 A—C. Respiratory movements (*1*), electrocardiogram (*2*) and electromyogram (*3*) of a dog during intraventricular infusion of calcium free artificial cerebrospinal fluid containing disodium edetate. (A) Preinfusion control. (B) The 15th min of infusion. (C) 6 min after the end of infusion. Calibration: 200 μV, 1 sec. Note decrease of respiratory rate in B, and the appearance of panting in C while shivering is still present. Details of the record were retouched for photography

of the experiments with Na-CSF the dog had free access to water heated to 38° C and drank 540 ml during 90 min. No drinking was observed with control CSF.

Discussion

The experiment with Ca^{++} chelation in the ventricles of the dog gave essentially the same results as those described by Myers and Yaksh [10] in the monkey and by Clark [2] in the cat, i.e. hyperthermia ensued even

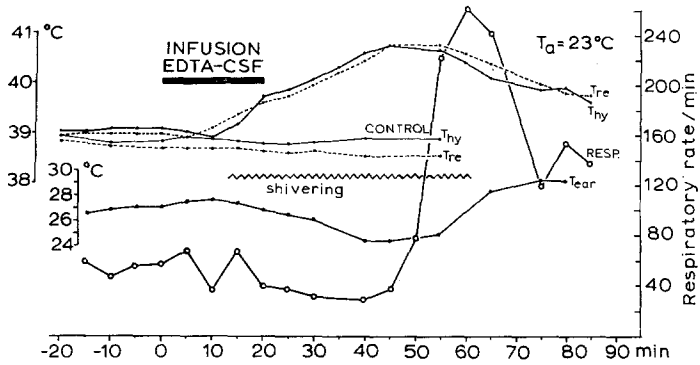

Fig. 2. The effect of infusion of calcium free artificial cerebrospinal fluid containing disodium edetate into the lateral cerebral ventricle on hypothalamic (T_{hy}), rectal (T_{re}) and skin (T_{ear}) temperature, and on respiratory rate (*RESP.*). The time of infusion is indicated by a black bar and the shivering—by a sine line. T_a ambient temperature

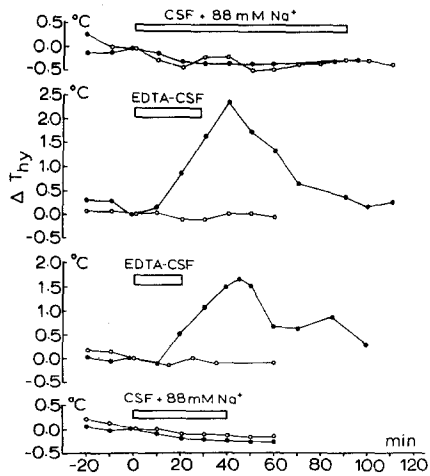

Fig. 3. The effect of infusion of cerebrospinal fluid containing either 3.6 mM disodium edetate (*EDTA-CSF*) or 88 mM of sodium ions in excess (*CSF + 88 mM Na^+*) into the lateral cerebral ventricle on hypothalamic temperature (T_{by}) denoted with black circles. Empty circles—T_{hy} in corresponding experiments with infusions of control *CSF*

though it disappeared more rapidly than in other species. This resulted both from heat overproduction manifested by shivering and from simultaneous activation of heat conservation mechanisms. The latter was demonstrated by huddling, piloerection, decrease of respiratory rate and vasoconstriction.

In most of the experiments body temperature increased nearly linearly until the peak was achieved, and then decreased almost as rapidly. Nevertheless, a kind of plateau lasting 10—15 min usually occurred. During this time the dog was shivering and panting at the same time and thus maintaining his body temperature on the same level. It is not clear why hypocalcium hyperthermia in the dog is so short-lasting. The possibility that the rate of exchange of bound and unbound calcium between the extracellular fluid of the central nervous system and that of the rest of the body may differ among the species cannot be ruled out. The reason for a big variation between hyperthermic effects of EDTA-CSF infusions in consecutive experiments on the same dog is even more obscure. As shown in Table 1 this variability was not related to changes in ambient temperature. It would appear that some other factors are necessary to reveal the hyperthermic effect of Ca^{++} chelation.

It cannot be definitely stated on the basis of the present results whether changes in calcium concentration exert a selective action upon the posterior hypothalamus and play an essential role in establishing the set-point for body temperature in the dog under physiological conditions, because no data is available on the actual decrease in calcium ion concentration within discrete brain areas during the EDTA-CSF infusions. Neither is the range of changes of this ion concentration known within the extracellular fluid of the central nervous system under physiological and pathological conditions. Numerous side effects appearing during the infusion of EDTA-CSF, not related directly to the defense against cold, but rather to increased arousal or emotional tension, argue for non-specific excitation of various nervous structures, also outside the hypothalamus. So the increase in body temperature would be only one of the symptoms of this state and could result from higher than usual activity of the neurons which control production and conservation of heat in the body.

The fact that infusions of Na-CSF containing as much as 88 mM sodium in excess of its physiological concentration did not produce any changes in body temperature although they exerted the usual dipsogenic effect [1] seems to indicate that neither rise of this ion concentration nor increase in the ratio of sodium to calcium in the central nervous system are essential for regulation of body temperature in the dog. Further experiments with local perfusions of the posterior hypothalamus are needed to elucidate definitely this question, since penetration of ions from the cerebrospinal fluid into the posterior hypothalamus in the dog may be different than in other species.

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References

1. Andersson, B., Jobin, M., Olsson, K.: A study of thirst and other effects of an increased sodium concentration in the 3rd brain ventricle. Acta physiol. scand. **69**, 29—36 (1967)
2. Clark, W. G.: Hyperthermic effect of disodium edetate injected into the lateral ventricle of the unanaesthetized cat. Experientia (Basel) **27**, 1452—1454 (1971)
3. Feldberg, W., Saxena, P.: Effect on body temperature of perfusing calcium free saline solution from a lateral ventricle to cisterna magna in the unanaesthetized rabbit. J. Physiol. (Lond.) **207**, 52—53P (1970)
4. Hanegan, J. L., Williams, B. A.: Brain calcium: role in temperature regulation. Science **181**, 663—664 (1973)
5. Lim, R. K. S., Liu, C. N., Moffitt, R. L.: A stereotaxic atlas of the dog's brain. Springfield, Ill.: Ch. C. Thomas 1960
6. Myers, R. D., Brophy, P. D.: Temperature changes in the rat produced by altering the sodium-calcium ratio in the cerebral ventricles. Neuropharmacology **11**, 351—361 (1972)
7. Myers, R. D., Veale, W. L.: Body temperature: possible ionic mechanism in the hypothalamus controlling the set point. Science **170**, 95—97 (1970)
8. Myers, R. D., Veale, W. L.: The role of sodium and calcium ions in the hypothalamus in the control of body temperature of the unanaesthetized cat. J. Physiol. (Lond.) **212**, 411—430 (1971)
9. Myers, R. D., Veale, W. L., Yaksh, T. L.: Changes in body temperature of the unanaesthetized monkey produced by sodium and calcium ions perfused through the cerebral ventricles. J. Physiol. (Lond.) **217**, 381—392 (1971)
10. Myers, R. D., Yaksh, T. L.: Thermoregulation around a new "set-point" established in the monkey by altering the ratio of sodium to calcium ions within the hypothalamus. J. Physiol. (Lond.) **218**, 609—633 (1971)
11. Sadowski, B.: Negativism to food during self-stimulation in the anterior part of the basal forebrain in dogs. Physiol. Behav. (in press, 1974)
12. Seoane, J. R., Baile, A. C.: Ionic changes in cerebrospinal fluid and feeding, drinking and temperature of sheep. Physiol. Behav. **10**, 915—923 (1973)
13. Traczyk, W.: A stereotaxic method of implanting a polyethylene cannula into the dog's lateral cerebral ventricle. Acta physiol. pol. **8**, 739—744 (1957) (in Polish)

Dr. Bogdan Sadowski
Polish Academy of Sciences Medical
Research Centre
Laboratory of Applied Physiology
Jazgarzewska 1, 00-730 Warsaw, Poland