SODIUM AND CALCIUM IONS IN THE CONTROL OF TEMPERATURE SET-POINT IN THE PIGEON

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1   The effect of altering the ionic balance of the cerebrospinal fluid (CSF) on cloacal temperature of unanaesthetized pigeons kept at room temperature (20–25°C) was examined by injection or infusion of solutions of different ionic composition into a cannulated lateral cerebral ventricle.

2   An increase in the concentration of calcium ions caused a fall in temperature and behavioural sedation. The effects were the same whether the calcium was present as calcium chloride or as the calcium disodium salt of ethylenediamine tetra-acetic acid ($CaNa_2EDTA$).

3   When the concentration of sodium ions in the CSF perfusate was increased by addition of NaCl or that of calcium ions was decreased by addition of Na_2EDTA a rise in temperature was often produced but this was not consistent. NaCl sometimes had either no effect or lowered the temperature. Na_2EDTA while producing a rise when first injected failed to do so when repeated a few hours, 24 h and often 72 h later. Prolonged infusion of either agent caused intense behavioural excitement leading to death.

4   Potassium ions, like sodium ions, caused a rise in temperature but only when infused continuously. Behavioural excitement was only rarely observed.

5   Magnesium produced a fall in temperature. The concentration required was much higher than that of calcium but the hypothermia was more prolonged suggesting a slower elimination of the magnesium ions from the CSF. Magnesium ions caused tremors, nystagmus and ataxia as opposed to sedation caused by calcium.

6   All these were central effects as they were not obtained when the substances were injected intravenously.

7   Since changes in body temperature of the pigeon produced by injection of calcium or sodium ions into the CSF were similar to those seen in various species of mammal, it is concluded that the relative concentration of these ions within the brain plays an important role in establishing the temperature set-point in both birds and mammals.

Introduction

A change in the ratio of sodium to calcium ions in the artificial cerebrospinal fluid (CSF) perfused through the cerebral ventricles produces consistent changes in body temperature in the same direction in the cat (Feldberg, Myers & Veale, 1970; Myers & Veale, 1971), rabbit (Feldberg & Saxena, 1970a, b), monkey (Myers, Veale & Yaksh, 1971), rat (Myers & Brophy, 1971) and sheep (Myers, 1974). Feldberg *et al.* (1970) suggested that in homoeothermic animals the constancy of temperature depends on the balance of sodium and calcium ions in the anterior hypothalamus and that calcium ions may act as a kind of 'brake' and prevent the sodium ions from exerting their hyperthermic effect. According to Myers & Yaksh (1971), the animal retains its capacity to thermoregulate normally in either direction by mobilizing all the physiological systems for heat production or heat loss after a new set-point is reached following alteration of the sodium-calcium ratio. The set-point around which the body temperature is regulated depends on structures within the posterior hypothalamus in the cat (Myers & Veale, 1971) and the monkey (Myers & Yaksh, 1971) and can be reached by ions present in solutions perfused through the ventricles or microinfused into discrete areas in the posterior hypothalamus. The present study shows that the effects of sodium and calcium ions in determining set-point which have been observed in several species of mammal, also obtain in another class of homoeothermic animals, the birds.

Methods

Healthy pigeons of either sex and weighing 200–300 g were used. The right lateral ventricle was aseptically cannulated under intravenous pentobarbitone sodium anaesthesia according to the method described earlier
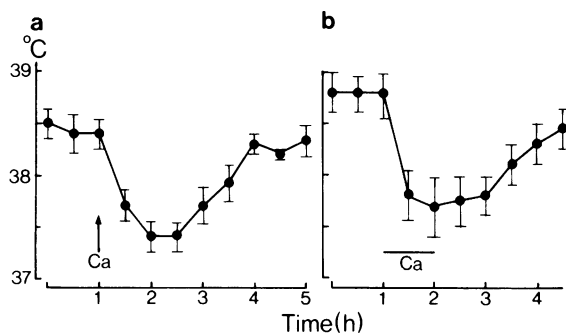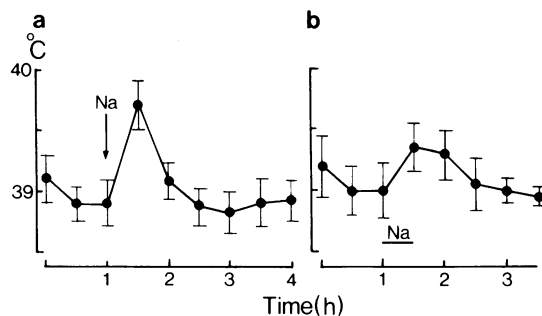
Figure 1 Mean cloacal temperature in un-anaesthetized pigeons. At the arrow in (a) 0.02 ml of 1.04% CaCl$_2$ solution was injected into a lateral ventricle. The horizontal bar in (b) indicates a 60 min infusion of 0.2 ml of the same solution. The vertical bars indicate s.e. means of eight experiments in each of the two records.

Figure 2 Mean cloacal temperature in un-anaesthetized pigeons. At the arrow in (a) 0.02 ml of 0.9% NaCl solution was injected into a lateral cerebral ventricle. The horizontal bar in the right record indicates a 30 min infusion of 0.1 ml of the same solution. The vertical bars indicate s.e. means of 15 experiments in each group.

(Chawla, Johri, Saxena & Singhal, 1974). The pigeon was allowed two days to recover from the operation and thereafter used not more than twice a week for temperature study. The placement of the cannula, the zone of access of the injected solution and the ventricular contours were studied macroscopically at the end of a series of experiments in every pigeon, using bromophenol blue for vital staining.

Food and water were withheld during the period of experiment. The room temperature was maintained between 20–25°C. The pigeon's temperature was noted every 15 min from a multichannel tele-thermometer with the thermistor probe placed in the cloaca. The figures in this paper are based on these records.

The following solutions were employed for in-traventricular or intravenous administration: pyrogen-free distilled water; artificial CSF of Merlis (1940) (composition: NaCl 8.1 g, 10% KCl 2.5 ml, 1 M CaCl$_2$ 1.27 ml, 1 M MgCl$_2$ 1.3 ml, 25% glucose 2.44 ml, urea 0.13 g, Na$_2$HPO$_4$ 0.07 g, NaHCO$_3$ 1.76 g, water to 1000 ml); NaCl 0.9 and 1.5%; CaCl$_2$ 0.72, 1.04 and 1.45% (equivalent to 35, 50 and 70 times the concentration present in the artificial CSF); KCl 1.15% (equivalent to 46 times the con-centration present in the artificial CSF); MgCl$_2$ 0.62 and 3.1% (equivalent to 50 and 250 times the con-centration present in the artificial CSF); disodium salt of ethylenediamine tetra-acetic acid (Na$_2$EDTA) and calcium disodium salt of ethylenediamine tetra-acetic acid (Calcium Triplex-Merck; CaNa$_2$EDTA) 0.25 and 0.5%.

Glass articles, needles and tubes were made pyrogen-free before use by autoclaving and/or boiling. Pyrogen-free distilled water was used for preparation of the solutions.

Injections were made into the cannulated lateral ventricle by the technique described earlier (Chawla et al., 1974). The volume of injection was 0.02 ml. For infusion of the solution into the lateral ventricle, a continuous slow injector was used in which was fitted a tuberculin syringe filled with the solution to be infused and connected to the protruding end of the in-traventricular cannula by means of a 40 cm long polyethylene tube. The rate of infusion was 0.2 ml per hour. Intravenous injections were made into the pectoral vein and the volume of each injection was 0.1 ml.

Results

Injection or a 60 min infusion of distilled water or artificial CSF into a lateral cerebral ventricle did not influence the cloacal temperature of the pigeon. Thus, any dilatation of the ventricles caused by the injection procedure was of no consequence. Intravenous injections of 0.1 ml of these same solutions and of CaCl$_2$ (1.45%), NaCl (0.9%), KCl (1.15%), MgCl$_2$ (3.1%), Na$_2$EDTA (0.5%) and CaNa$_2$EDTA (0.5%) were also without effect.

Calcium

Intraventricular injections of CaCl$_2$ solutions consistently produced a fall in cloacal temperature, the magnitude of which was in direct proportion to the concentration of the substance. Figure 1a shows the average effect in a group of pigeons of an injection of a 1.04% solution. In Figure 1b the effect of a 60 min infusion of the same solution is shown. The fall in
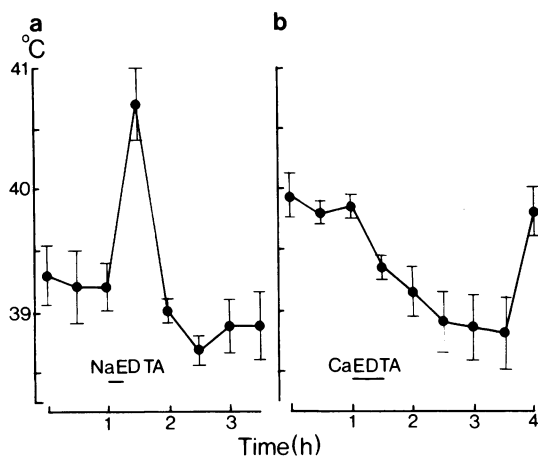
Figure 3 Mean cloacal temperature in unanaesthetized pigeons. The horizontal bars indicate 0.2 ml per hour infusions of a 0.5% solution of Na$_2$EDTA (NaEDTA) (15 min) in (a) and of CaNa$_2$EDTA (CaEDTA) (30 min) in (b). The vertical bars indicate s.e. means of eight experiments in each group. These were the first infusions of Na$_2$EDTA the pigeons had received.

Figure 4 Mean cloacal temperature in unanaesthetized pigeons. At the arrow in (a) 0.02 ml of 1.15% KCl solution was injected into a lateral ventricle. The horizontal bar in the right record indicates a 30 min infusion of 0.1 ml of the same solution. The vertical bars indicate the s.e. means of 10 experiments in each group.
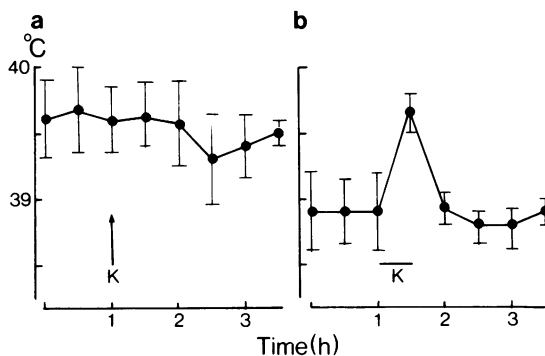
temperature was about 1°C in each case and the time courses of the two responses were similar.

Infusion of a 1.45% CaCl$_2$ solution for the same length of time was followed by a profound fall in temperature and death several hours later in the three pigeons tested. However, pigeons receiving injections of the same solution survived.

All the pigeons were behaviourally sedated following administration of CaCl$_2$.

Sodium

Intraventricular injections or infusions of NaCl solutions produced only a mild effect on temperature but in the opposite direction to that of calcium. The record in Figure 2a shows the average rise in a group of pigeons following injection of 0.9% solution. The temperature rose by about 1.5°C following the injection and returned to normal within an hour. The pigeon became restless. It was not possible to sustain the rise even when the solution was infused for up to 30 minutes. On the other hand, such infusions produced a smaller rise. This is shown in Figure 2b. The large standard errors of the means (vertical bars) indicate that the rise was not a constant feature of intraventricular injections or infusions of NaCl, the temperature sometimes falling slightly below the original level. Infusion of a more concentrated solution (1.5%) over 30 min caused excessive motor excitement and resulted in death in 2 out of 3 pigeons.

EDTA

The effects of Na$_2$EDTA resembled those of NaCl presumably because it altered the sodium-calcium ratio in favour of sodium by chelating calcium ions. The first intraventricular injection of a 0.25 or 0.5% solution produced a rise in cloacal temperature which subsided within an hour. A second injection into the same pigeon on the same day or up to three days later often failed to produce the response. The rise in temperature could also not be sustained by infusing the solution. Figure 3a shows the average effect of a 0.5% solution infused for 15 min into a group of pigeons. In each case, this was the first infusion of Na$_2$EDTA that the pigeons had received. The sharp rise in temperature was followed by a slight short-lasting fall. When the infusion was prolonged to 25 min and beyond, intense motor excitement was produced and the pigeon died.

Intraventricular injections or infusions of CaNa$_2$EDTA produced essentially similar effects on temperature to those obtained with CaCl$_2$ solution. Injections caused a dose-dependent fall in cloacal temperature. An infusion produced a larger and more sustained fall. Figure 3b illustrates the average fall in temperature in a group of pigeons following a 30 min infusion of a 0.5% solution of CaNa$_2$EDTA. The pigeons became sedated.

Potassium

Figure 4 illustrates the average effect on temperature of intraventricular injections (Figure 4a) and infusions (Figure 4b) of a 1.15% solution of KCl in two groups of pigeons. The injections were ineffective but the
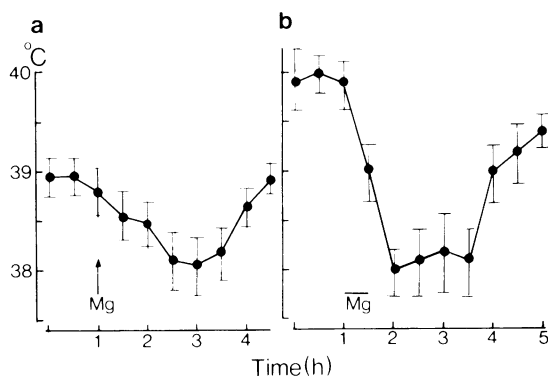
Figure 5 Mean cloacal temperature in un-anaesthetized pigeons. At the arrow in (a) 0.02 ml of 3.1% MgCl₂ solution was injected into a lateral ventricle. The horizontal bar in (b) indicates a 30 min infusion of 0.1 ml of the same solution. The vertical bars indicate the s.e. means of 10 experiments in each group.

30 min infusions produced a rise which resembled that produced by injections of NaCl solutions (see Figure 2a). However, behavioural excitement was observed only in one out of ten experiments.

Magnesium

The effect on temperature of MgCl₂ resembled that of CaCl₂. Single intraventricular injections of MgCl₂ were relatively less effective than a continuous infusion (Figure 5). The record in Figure 5a shows the average effect in a group of pigeons of injections of a 3.1% solution of MgCl₂. The fall in temperature is somewhat smaller than that produced by injections of a 1.04% solution of CaCl₂ (Figure 1a). Upon infusion of these same solutions, however, it was observed that a 30 min infusion of MgCl₂ produced a more profound fall (2°C) than a 60 min infusion of CaCl₂ (1°C) (Figures 5b and 1b).

The behavioural effects were different for the two substances. While there was sedation following intraventricular administration of calcium, similar administration of magnesium produced trembling, ataxia and nystagmus. The pigeon fell on to one side and remained in that state for 30 min or more.

Discussion

The results of the present investigation support the concept that the constancy in the ratio between sodium and calcium ions is the inherent mechanism by which the set-point of body temperature is determined.

Excess sodium ions shift the set-point to a higher level and excess calcium ions to a lower level. This ionic mechanism, which is common to many species of mammal (Myers, 1974), thus appears to obtain in another class of homoeothermic animals, the birds. As in the mammals, the neurones controlling the set-point can be reached by ions present in solutions injected or infused into a lateral cerebral ventricle of the pigeon.

An increase in the concentration of the calcium ions is highly effective in lowering temperature (Figure 1). On the other hand, a corresponding increase in the concentration of sodium ions is much less effective in raising it. According to Myers (1974) a set-point temperature coupled with the input from the peripheral and central thermoreceptors determines the load error influencing the thermoregulatory response. The present experiments were performed at ambient temperatures of 20–25°C which is within the thermoneutral range for pigeons (King & Farner, 1961). The low magnitude of the sodium hyperthermia and the inability to sustain it cannot, therefore, be attributed to a lack of thermal load error, which would have been the case at high ambient temperature. It is possible that the capacity to attain or maintain a high sodium-calcium ratio in the fluid bathing the set-point neurones is species-dependent. While intense hyperthermia is produced in the unanaesthetized cat during perfusion of a calcium-free saline solution from lateral ventricle to cisterna magna, a weaker effect is obtained in the unanaesthetized rabbit during a similar perfusion (Feldberg & Saxena, 1970a). The present finding demonstrates that the pigeon resembles the rabbit in this respect.

If the pigeon is unable to attain and maintain a high sodium-calcium ratio in the vicinity of the set-point neurones, this might be attributed to the existence of some mechanism which can rapidly and effectively counteract any tendency towards a rise in the relative concentration of sodium ions. Mobilization of calcium ions may be one such mechanism. The presence of such a compensatory mechanism is suggested by the effects of Na₂EDTA. An intraventricular injection of this substance presumably increases the sodium-calcium ratio by chelating calcium ions, resulting in a rise of temperature. This elevation in the sodium-calcium ratio may be rapidly corrected by mobilization of calcium ions and probably overcorrected as the temperature not only returns but falls slightly below the original level (Figure 3a). Such a mechanism once mobilized would appear to be kept effective for a long time so that a second injection of Na₂EDTA might be ineffective in elevating the sodium-calcium ratio and consequently the temperature up to 72 h after the first injection.

By analogy, the ready availability of calcium from extra-circulatory stores has been demonstrated in man by Spencer, Vankinscott, Lewin & Laszlo (1952). The rapid intravenous administration of Na₂EDTA results in hypocalcaemic tetany. However, a slow infusion

(less than 15 mg/min) into a normocalcaemic individual elicits no hypocalcaemic symptoms.

CaNa₂EDTA was used essentially as a control for Na₂EDTA. It should not elevate the sodium-calcium ratio and so be without effect on temperature. However, in the present study, it produced similar effects on temperature and behaviour to those obtained with CaCl₂. Since CaNa₂EDTA does not dissociate to any significant extent, it seems likely that calcium is acting in its chelated form as certain metals are known to exert their effects on enzymatic processes in this form (Levine, 1970).

Excess of potassium ions in the CSF raised the cloacal temperature but the effect was much weaker than that of sodium ions and an infusion was required to bring about the rise (Figure 4). This finding is in agreement with that of Cooper, Cranston & Honour (1965) who obtained a hyperthermic effect following microinfusion of KCl into the hypothalamus of the rabbit. However, in their experiments isotonic NaCl solution, injected similarly, was without effect on the rectal temperature.

Magnesium resembles calcium in its hypothermic effect but a higher concentration of magnesium was required to produce comparable hypothermia following intraventricular injections. The differences in the time required for the temperature to return to the base line after intraventricular infusion of magnesium and calcium could reflect a difference in the rate at which the two ions are eliminated. The more intense and prolonged effect of magnesium, despite a shorter period of infusion suggests that elimination of magnesium from the CSF is a slower process than that of calcium.

The temperature effects obtained with excess potassium and magnesium in the pigeon were unexpected as neither potassium nor magnesium in concentrations which varied from 2 to 10 times the level in extracellular fluid had any effect on body temperature when these ions were perfused through the cerebral ventricles or microinfused into different regions of the hypothalamus of cat (Myers & Veale, 1971), monkey (Myers et al., 1971), rat (Myers & Brophy, 1971) and golden hamster (Myers & Buckman, 1972). However, the concentrations of these ions employed in the present study are much higher and milligram quantities of magnesium are known to produce a fall and similar amounts of potassium a rise in temperature when injected into the tuberal area of the cat (Hasma, 1930). Alternatively, the CSF-brain barrier permeability to potassium and magnesium ions may be greater in the pigeon than in the mammals and this may account for the more easily obtainable effect of these ions in the pigeon.

References

CHAWLA, NARESH., JOHRI, M.B.L., SAXENA, P.N. & SINGHAL, K.C. (1974). Effects of catecholamines on thermoregulation in pigeons. Br. J. Pharmac., 51, 497–502.

COOPER, K.E., CRANSTON, W.I. & HONOUR, A.J. (1965). Effects of intraventricular and intrahypothalamic injection of noradrenaline and 5-HT on body temperature in conscious rabbits. J. Physiol., 181, 852–864.

FELDBERG, W., MYERS, R.D. & VEALE, W.L. (1970). Perfusion from cerebral ventricle to cisterna magna in the unanaesthetized cat. Effect of calcium on body temperature. J. Physiol., 207, 403–416.

FELDBERG, W. & SAXENA, P.N. (1970a). Effect on body temperature of perfusing calcium-free saline solution from a lateral ventricle to cisterna magna in the unanaesthetized rabbit. J. Physiol., 207, 52–53P.

FELDBERG, W. & SAXENA, P.N. (1970b). Mechanism of action of pyrogen. J. Physiol., 211, 245–261.

HASAMA, B. (1930). Pharmakologische und physiologische studien uber die Schweisszentren. Arch. exp. Path. Pharmak., 153, 291–308. Quoted in Myers & Veale, 1971.

KING, J.R. & FARNER, D.S. (1961). Energy metabolism, thermoregulation and body temperature. In Biology and Comparative Physiology of Birds, Vol. II, ed. Marshall, A.J., pp. 215–288, New York & London: Academic Press.

LEVINE, W.G. (1970). Heavy-metal Antagonists. In The Pharmacological Basis of Therapeutics, ed. Goodman, L.S. & Gilman, A. pp. 944–957. Fourth Edn. London, Toronto: The MacMillan Co.

MERLIS, J.K. (1940). The effect of changes in the calcium content of the cerebro-spinal fluid on spinal reflex activity in the dog. Am. J. Physiol., 131, 67–72.

MYERS, R. D. (1974). An integrative model of monoamine and ionic mechanisms in the hypothalamic control of body temperature. In Temperature Regulation and Drug Action. Proceedings of a symposium on Temperature Regulation and Drug Action, ed. Lomax, P., Schonbaum, E. & Jacob, J. pp. 32–42, Paris: S. Krager.

MYERS, R.D. & BROPHY, P.D. (1971). Temperature changes in the rat produced by altering the sodium calcium ratio in the cerebral ventricles. Neuropharmacology, 11, 351–361.

MYERS, R.D. & BUCKMAN, J.E. (1972). Deep hypothermia induced in the golden hamster by altering cerebral calcium levels. Am. J. Physiol., 223, 1313–1318.

MYERS, R.D. & VEALE, W.L. (1971). The role of sodium and calcium ions in the hypothalamus in the control of body temperature of the unanaesthetized cat. J. Physiol., 212, 411–430.

MYERS, R.D., VEALE, W.L. & YAKSH, T.L. (1971). Changes in body temperature of the unanaesthetized monkey produced by sodium and calcium perfused through cerebral ventricles. J. Physiol., 217, 381–392.

MYERS, R.D. & YAKSH, T.L. (1971). Thermoregulation around a new 'set-point' established in the monkey by altering the ratio of sodium to calcium ions within the hypothalamus. *J. Physiol.*, **218**, 609–633.

SPENCER, H., VANKINSCOTT, V., LEWIN, I. & LASZLO, D. (1952). Removal of calcium in man by ethylenediamine tetra-acetic acid: a metabolic study. *J. clin., Invest.*, **31**, 1023–1027.