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MECHANISM OF ACTION OF PYROGEN

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SUMMARY

1. In unanaesthetized rabbits the cerebral ventricles were perfused for 30–75 min from left lateral ventricle to cisterna magna with solutions of different composition, whilst rectal temperature was continuously recorded.

2. Temperature did not rise during the perfusion when the perfusing fluid consisted of artificial c.s.f.; it did rise, however, when the perfusing fluid consisted merely of a 0·9 % sodium chloride solution.

3. Temperature fell, though not in all rabbits, during the perfusion when the calcium in the perfusing fluid was increased from 1·25 mM, the concentration in c.s.f. to 5 mM.

4. Magnesium chloride had only a weak action, in comparison to calcium, in preventing the rise produced during perfusion with 0·9 % sodium chloride solution. In a concentration of 1·25 mM it had no effect, but in a concentration of 5 mM it delayed and greatly reduced the rise.

5. Temperature did not rise during perfusion with an isotonic sucrose solution.

6. The rise in temperature produced by an intravenous injection of leucocyte pyrogen was not prevented when the injection was made during a perfusion with artificial c.s.f., but it was prevented when the calcium concentration in the perfusing fluid was raised to 5 mM or when the perfusing fluid consisted of isotonic sucrose solution. Again, magnesium had only a weak action in comparison to calcium.

7. These results support the theory put forward recently (Feldberg, Myers & Veale, 1970) that the constancy of temperature depends upon the physiological balance of sodium and calcium ions in the anterior hypothalamus, that the calcium ions act as a kind of 'brake' preventing the sodium ions from exerting their temperature raising effect, and that pyrogen acts by removing the 'calcium brake', the pyrogen fever thus being a sodium fever.

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INTRODUCTION

In unanaesthetized cats in which the cerebral ventricles were perfused from a lateral ventricle to the cisterna magna, it was found that body temperature did not change during a perfusion if the perfusing fluid was artificial c.s.f., but that temperature rose steeply during the perfusion when calcium chloride had been omitted from the artificial c.s.f. or when the perfusing fluid was merely a 0·9 % sodium chloride solution (Feldberg *et al.* 1970). The same results were obtained in anaesthetized cats with the perfusion restricted from lateral ventricle to aqueduct. In these experiments it was further found that temperature did not rise when the sodium had been replaced by sucrose in isotonic concentration (W. Feldberg, R. D. Myers & W. L. Veale, unpublished experiments). These observations are in accord with the view expressed by Feldberg *et al.* that in homoeothermic animals the constancy of temperature may depend on the balance of sodium and calcium ions in the anterior hypothalamus, that the calcium ions may act as a kind of 'brake' and prevent the sodium ions from exerting their hyperthermic effect, and, further, that pyrogens may act by removing the 'calcium brake'. This view, however, would be tenable only if these effects were not confined to cats, but could be obtained in other species as well.

The experiments of perfusing the cerebral ventricles with solutions of different composition were therefore repeated in unanaesthetized rabbits. In addition, pyrogen was injected during such perfusions to find out if its hyperthermic effect could be prevented when either the calcium content of the perfusing fluid was increased or its sodium ions were replaced by sucrose.

The rabbit was chosen because in this species the monoamines, present in the hypothalamus as transmitter substances, have effects opposite from those in cats when acting on the anterior hypothalamus. If calcium lack were to act through the release of the monoamines in the hypothalamus, the effect should be different in the two species. On the other hand, if calcium lack, or, rather, the sodium ions, were to raise temperature in cats as well as in rabbits, a relatively simple mechanism would be unmasked for raising temperature independent of the response of a species to the monoamines, and this would strengthen the view that suppression of this mechanism is the cause of the constancy of temperature in homeothermic animals, and release of this mechanism the cause of the pyrogen fever, particularly if in addition it could be shown that the fever is prevented when the 'calcium brake' is either increased by excess of calcium or made unnecessary by replacement of the sodium ions by inert sucrose.

METHODS

In male albino New Zealand rabbits weighing between 2·5 and 4 kg, the cerebral ventricles were perfused from the left lateral ventricle to cisterna magna without anaesthesia. The perfusion method was essentially the same as that described recently for the cat (Feldberg *et al.* 1970), and is illustrated diagrammatically in Fig. 1.

In an aseptic operation under pentobarbitone sodium (30 mg/kg intravenously) anaesthesia, one Collison cannula without shaft, the ventricular cannula, was implanted above the left lateral ventricle, and another, the cisternal cannula, with a 10 mm stainless-steel shaft, was fixed to the back of the skull so that the opening of its shaft rested above the atlanto-occipital membrane. The ventricular cannula was screwed into the skull at a point 7 mm lateral to the midpoint of the sagittal suture

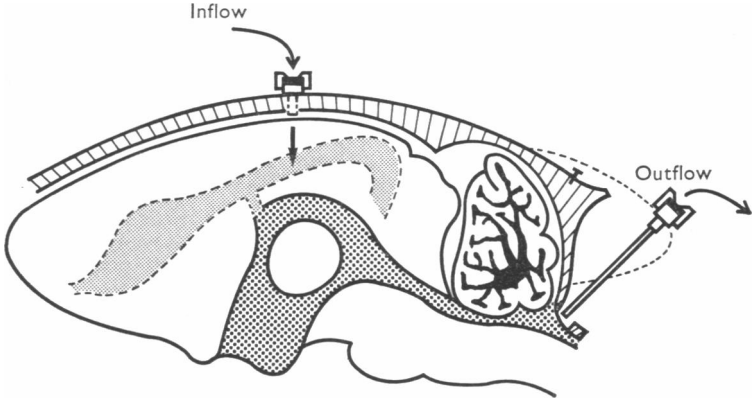

Fig. 1. Diagram of rabbit's brain with the cerebral ventricles showing the two permanently implanted Collison cannulae used for perfusion: the inflow cannula, without shaft, screwed into the skull above the lateral ventricle and the outflow cannula, the cisternal cannula, positioned so that the opening of its shaft lies about 1 mm above the atlanto-occipital membrane. The area between the skull and the interrupted line represents the acrylic cement by which the cisternal cannula is fixed and anchored by two small screws (one being shown) to the back of the skull.

and then further secured by applying some acrylic dental cement around the cannula. To fix the cisternal cannula, the total length of which was 22 mm, to the back of the skull a midline incision was made at the back of the neck, and the underlying muscles were separated in the mid line and retracted. The interparietal and the supra-occipital bones were scraped clean and, on either side of the mid line of the interparietal bone, a small stainless-steel screw was inserted. The cannula was then fixed to the bone, in the mid line position shown in Fig. 1, with acrylic dental cement anchored by the two screws. Penicillin was sprinkled into the wound, and the neck muscles and the skin were sutured. At least three days were allowed to elapse before the ventricles were perfused, and the perfusions were carried out not more than twice a week.

A few hours before each perfusion, a thermistor probe was inserted about 10 cm into the rectum and held in position by adhesive tape affixed to the protruding end

of the probe and wrapped around the base of the tail. The rabbits were then placed into a 16 cm wide, 40 cm long and 10 cm high open box, but otherwise were restrained as little as possible, and temperature was continuously monitored by a Kent multi-channel recorder. The temperature records reproduced in Figs. 2–8 of this paper were plotted directly from tracings obtained in this way.

Immediately before each perfusion the caps of the Collison cannulae were removed and the lumina of the cannulae were washed out with artificial c.s.f. or 0·9 % sodium chloride solution. A needle of 26 gauge with its hub removed was pierced through the rubber diaphragm of each cap.

The free end of the needle to be inserted into the ventricular cannulae was connected by a length of 80–90 cm polyethylene tubing to the syringe of a slow infusion pump. After needle, tubing and syringe had been filled with the perfusion fluid the cap was screwed on again and, without starting perfusion, the needle was lowered to a depth of 15·5–16 mm when its tip rested in the lateral ventricle. Heat from the rabbit's own ear was used to warm the perfusion fluid. For this purpose about 40 cm of the tubing, rolled into a coil of about 3 cm diameter was placed within the pinna which was then folded over it and secured in position by holding the two edges of the pinna together with adhesive tape.

The free end of the needle to be inserted into the cisternal cannula was connected by a length of 40–50 cm polyethylene tubing to a 1 ml. syringe, and with the system filled with the perfusion fluid the cap was screwed on again. To find out the depth to which the cisternal needle had to be lowered a hypodermic needle was inserted through the rubber diaphragm into the uppermost part of the cannula. When fluid was injected into the cisternal needle it escaped into the hub of the hypodermic needle until the tip of the cisternal needle had reached the atlanto-occipital membrane. When this occurred some resistance was usually encountered on trying to inject fluid through the cisternal needle. On then lowering the needle another 2 mm and disconnecting the syringe, drops of c.s.f. flowed out from the free end of the tubing which was held a few cm. lower than the head of the rabbit. Inflow was started a few minutes before or after piercing the atlanto-occipital membrane. The rate of inflow was 0·1 ml./min and the outflow was adjusted to about the same rate by lowering or raising the level of the free end of the tubing.

The solutions used for perfusion were artificial c.s.f. of Merlis (1940) and 0·9 % sodium chloride solution, with or without the additions of calcium chloride or magnesium chloride, or isotonic sucrose solution. The artificial c.s.f. was made pyrogen-free by filtering; the 0·9 % sodium chloride solution and the glass distilled water used for dissolving the sucrose by twice autoclaving them at 121° C. All glassware used was sterile and pyrogen-free.

The leucocyte pyrogen was kindly given to us by Professor Cranston, St Thomas's Hospital, and stored at +4° C. It was prepared by incubation of rabbit blood with 'E' pyrogen (0·3 μg/100 ml.) and spinning down the corpuscles. Of this pyrogen containing plasma, 2·5 ml. was injected, over 60–90 sec, into an ear vein.

RESULTS

Artificial c.s.f.

During the perfusion of the cerebral ventricles with artificial c.s.f. for periods varying between 30 and 75 min, there were no obvious effects on the behaviour of the animal, and temperature did not change. In about two thirds of the experiments, however, temperature began to rise 30–150 min after the perfusion and then continued to rise for several hours.

The upper two temperature records of Fig. 2 were obtained from different rabbits, in neither did the temperature rise during the 1 hr perfusion, but in one it began to rise 70 min later and rose from 39·2 to 40·8° C during the following 3 hr. With repeated perfusions in the same rabbit there was sometimes an after-rise, sometimes not.

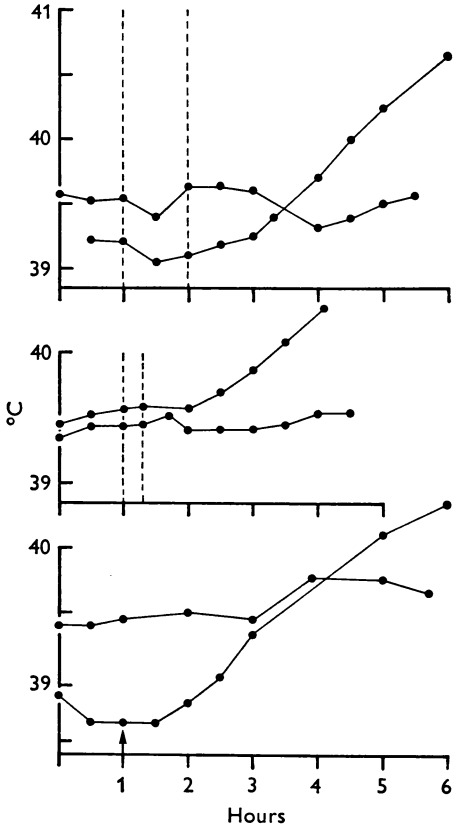

Fig. 2. Records of rectal temperature from six rabbits. The interval between the interrupted vertical lines indicates for the two uppermost records 1 hr perfusion of artificial c.s.f. from left lateral ventricle to cisterna, for the middle two records 20 min infusion of 2 ml. artificial c.s.f. into left lateral ventricle. In the bottom two records the arrow indicates injection of 0·2 ml. artificial c.s.f. into left lateral ventricle.

The after-rise cannot be attributed to changes in the intracranial pressure or to accumulation of fluid in the liquor spaces because it was found that an infusion of 2 ml. artificial c.s.f. into a lateral ventricle over a period of 20 min, without cannulating the cisterna for collection of the outflow produced an after-rise in some but not in other experiments. The two middle records of Fig. 2 illustrate one experiment with and one without an after-

rise. Again, during the infusion period itself, temperature did not change in either experiment.

Even after single injections of 0·2 ml. artificial c.s.f. a late rise in temperature which began after a latency of 30–150 min occurred in some but not in all experiments. In the two experiments shown in the bottom records of Fig. 2 it occurred in one but not in the other.

The after-rise was obtained not only following a perfusion with or an injection of artificial c.s.f. but also following the perfusion with the other fluids used.

Sodium chloride

During perfusion of the cerebral ventricles with a 0·9 % sodium chloride solution, temperature rose as illustrated in Fig. 3. The upper record shows again that the perfusion with artificial c.s.f., this time for 30 min only, did not affect temperature, the initial transient small rise was due to some struggling which occurred on insertion of the perfusion needles. The middle curve, which was obtained from the same rabbit shows a steep rise of nearly 2° C during a perfusion with 0·9 % sodium chloride solution. The rise varied in magnitude in different experiments. At the end of the perfusion temperature soon began to fall; it usually did not return to the pre-injection level and was often followed by a secondary after-rise.

Several factors contributed to the rise which occurred during a perfusion with 0·9 % sodium chloride solution, and not all of them constitute effector mechanisms originating in the anterior hypothalamus. There was skin vasoconstriction, increased muscle tone and shivering or tremor. But there was also restlessness, and particularly at the end of a 30 min perfusion period, there were short periods of struggling, and sometimes of convulsive activity, facial twitching, chewing movements, and even generalized convulsions lasting a few seconds. Therefore, the perfusions were usually terminated after 30 min. Although these motor effects contributed to the rise, temperature began to rise before they appeared. In addition, there was nystagmus and tachypnoea, later followed by laboured respiration. None of these effects occurred on perfusion with artificial c.s.f.

Calcium chloride

The rise in temperature produced during a perfusion with 0·9 % sodium chloride solution was due to lack of calcium. This became evident when calcium chloride was added to the sodium chloride solution in the concentration present in artificial c.s.f. This prevented the rise in temperature as well as the behavioural effects. In Fig. 3 the bottom record was obtained from the same rabbit as the other two records and shows that

when 1·25 mM calcium chloride was added to the sodium chloride solution, temperature no longer rose during the perfusion.

It was not even necessary to add calcium in physiological concentration to the perfusion fluid to prevent the rise. For instance, in two experiments in which perfusion was with a solution made up of four parts of artificial

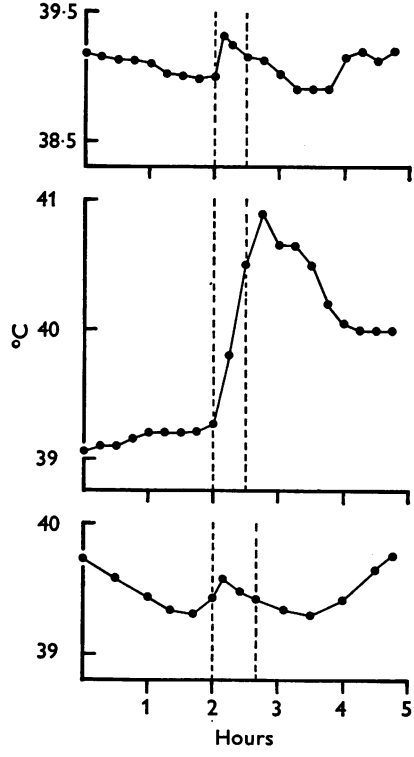

Fig. 3. Three records of rectal temperature obtained from the same rabbit on different days. The interval between the interrupted vertical lines indicates perfusion from left lateral ventricle to cisterna, in the upper record for 30 min with artificial c.s.f., in the middle record for 30 min with 0·9 % sodium chloride solution, and in the bottom record for 40 min with 0·9 % sodium chloride solution to which 1·25 mM calcium chloride had been added.

c.s.f. and six parts of 0·9 % sodium chloride solution, therefore containing only about 40 % of the calcium in c.s.f., temperature did not rise during the 75 min perfusion.

When the calcium concentration in the perfusion fluid was increased to twice that in c.s.f., i.e. to 2·5 mM, temperature did not change during the perfusion. Such a result is shown in the bottom record of Fig. 8. However,

with an increase of the calcium chloride to 5 mM, i.e. to about four times the concentration in c.s.f., a hypothermic effect was obtained though not in all rabbits; for the same rabbit, the response was relatively constant on repeated perfusions. Fig. 4 illustrates results obtained from four rabbits; in each the cerebral ventricles were perfused for 60–75 min with a 0·9 %

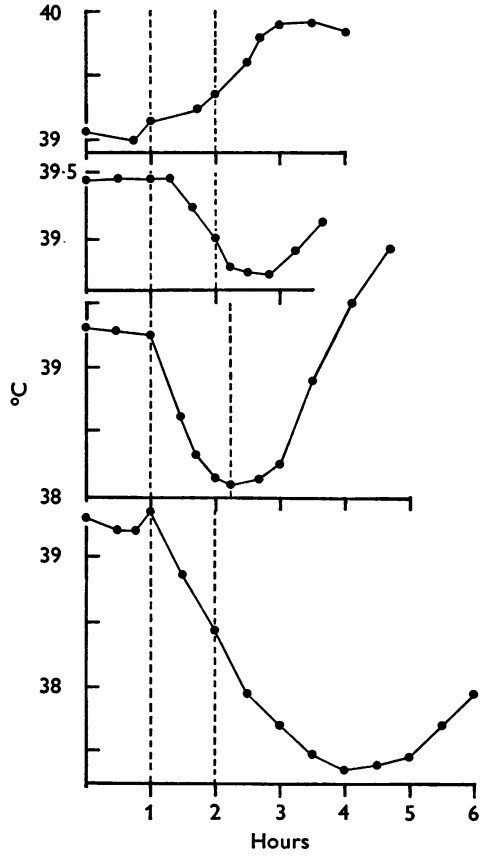

Fig. 4. Records of rectal temperature from four rabbits. The interval between the vertical interrupted lines indicates perfusion from left lateral ventricle to cisterna with a 0·9 % sodium chloride solution containing 5 mM calcium chloride.

sodium chloride solution containing 5 mM calcium chloride; similar results were obtained when the perfusion fluid consisted of artificial c.s.f. containing 5 mM calcium chloride.

In the one experiment of Fig. 4 (upper record) there was no fall in temperature during the perfusion; in the second, temperature began to fall about 20 min after the beginning of the perfusion and continued to fall not only during the remainder of the perfusion period but for about one

hour afterwards. In the other two rabbits, temperature fell from the beginning and during the whole perfusion period; the fall came to an end shortly after the end of the perfusion in the one, but continued for another 2 hr in the other of these two rabbits, and the fall amounted to 1·2 and 2° C respectively. In the rabbit in which the perfusion had caused a fall of 1·2° C a perfusion with a sodium chloride solution containing only 1·25 mM calcium chloride was carried out on another day. There was no fall during the 75 min perfusion and afterwards temperature rose in the same way as after the perfusion with 5 mM calcium chloride.

During the perfusions with the high calcium in the perfusion fluid, the rabbits became quiet and ataxia developed, but the tone of the leg muscles did not appear to be diminished. During the latter part of the perfusions and for some time afterwards, the rabbits appeared to have difficulty in keeping their heads erect; in consequence their heads came to rest on the rim of the restraining box.

Magnesium chloride

Magnesium ions had a much weaker effect than calcium ions in preventing the hyperthermia which occurred during perfusion with a 0·9% sodium chloride solution. The addition to this solution of 1·25 mM magnesium chloride, about the concentration in c.s.f., did not prevent the hyperthermia, whereas the addition of 5 mM magnesium chloride delayed and greatly reduced the rise but did not fully prevent it. As illustrated by the uppermost record of Fig. 5, temperature did not change during the first half hour of such a perfusion, and during the second half hour temperature rose by only 0·35° C, whilst the rabbit became restless.

Sucrose

Perfusion of the cerebral ventricles with isotonic sucrose solution had no effect on temperature. In the experiment illustrated by the bottom record of Fig. 5 such a perfusion was continued for 30 min before pyrogen was given and during these 30 min temperature did not change. The perfusions with sucrose were associated first with tachypnoea and later with laboured respiration; the rabbits became apathetic and weak and tended to lie on their sides. Perfusions lasting longer than 40 min were not always well tolerated, sometimes the rabbits remained apathetic and weak for a day or two.

Intravenous injection of leucocyte pyrogen during perfusion of the cerebral ventricles

Following a single intravenous injection of the leucocyte pyrogen temperature began to rise within 10–15 min, usually rose 1–1·3° C during the following 30–50 min, then fell and, within 2–3 hr, returned to the pre-

injection level, as illustrated in the uppermost record of Fig. 7, or fell half a degree or more below the pre-injection level. The response to pyrogen was not much affected when the injection was made during perfusion of the cerebral ventricles with artificial c.s.f. or with a 0·9% sodium chloride solution containing 1·25 mM calcium chloride. Temperature began to rise

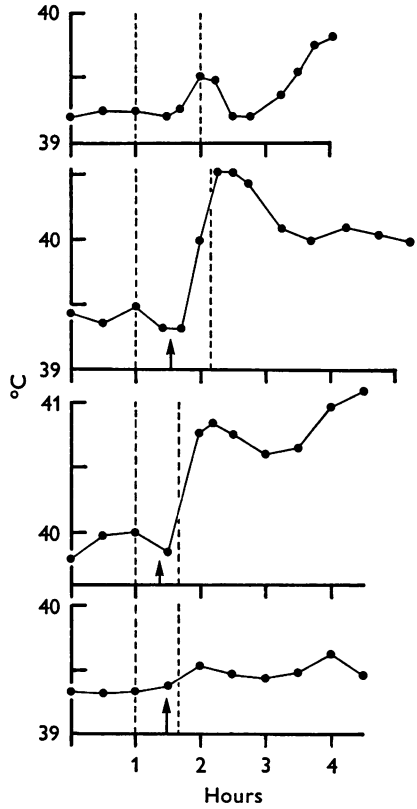

Fig. 5. Four records of rectal temperature from the same rabbit obtained at different days. The interval between the interrupted vertical lines indicates perfusion for 30–70 min from left lateral ventricle to cisterna with fluid of the following compositions: for the two upper records, 0·9% sodium chloride solution containing 5 mM magnesium sulphate; for lower middle record, artificial c.s.f.; for bottom record, isotonic sucrose solution. The arrows in each of the three lower records indicate intravenous injection of 2·5 ml. leucocyte pyrogen containing plasma.

after the same latency and the maximum rise was reached after about the same time, but it was usually 0·1–0·2° C less. The main difference concerned the subsequent fall. Temperature did not always return to the pre-injection level but after an initial fall began to rise again, probably representing the after-rise following perfusion without a pyrogen injection.

Typical responses are illustrated by the lower middle record of Fig. 5 and by the upper record of Fig. 6. In those rabbits, however, in which pyrogen produced a rise followed by a fall below the pre-injection level, the fall was also present when the pyrogen was injected during perfusion of the

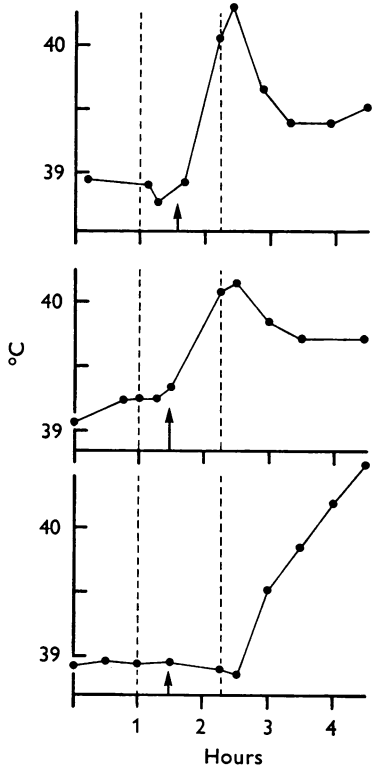

Fig. 6. Three records of rectal temperature obtained from the same rabbit on different days. The interval between the interrupted vertical lines indicates 75 min perfusion from left lateral ventricle to cisterna with artificial c.s.f. containing different concentrations of calcium chloride. Upper record, perfusion with normal artificial c.s.f. containing 1·25 mM calcium chloride; middle record, perfusion with artificial c.s.f. containing 3·75 mM and bottom record, containing 5 mM calcium chloride. The arrow in each of the records indicates an intravenous injection of 2·5 ml. leucocyte pyrogen containing plasma.

cerebral ventricles. For instance, the records of Fig. 8 were obtained from such a rabbit.

Increasing the calcium concentration of the perfusing fluid caused attenuation and finally prevention of the hyperthermic response. Doubling the calcium concentration to 2·5 mM had no effect on the pyrogen response in some rabbits, whereas in others it caused attenuation of different

magnitude although such a perfusion itself had no effect on temperature. This is illustrated by the experiment of Fig. 8. Temperature did not change during perfusion with a 0·9 % sodium chloride solution containing 2·5 mM calcium chloride, as shown in the bottom record, but perfusion with such a solution reduced the hyperthermic response to pyrogen from 1 to 0·5° C, as seen from a comparison of the two upper records. This was the greatest attenuation obtained on doubling the calcium concentration. It was greater than the attenuation produced in the experiment of Fig. 6 by 3·75 mM calcium chloride which reduced the rise from 1·3 to 0·9° C.

Raising the calcium concentration to 5 mM prevented the hyperthermia independent of whether perfusion with this high calcium content did or did not by itself produce a fall in temperature. Fig. 6 was obtained from a rabbit in which perfusion with the high calcium content did not lower temperature. The three records show the effects of pyrogen injected each time 30 min after the beginning of perfusion of the cerebral ventricles which was then continued for another 45 min. The perfusing fluid was artificial c.s.f. with different concentrations of calcium chloride. The upper record shows the typical rise in temperature when the perfusing fluid contained the normal content of calcium, i.e. 1·25 mM. The rise began within 15 min of the pyrogen injection and reached its maximum shortly after the end of the perfusion. The middle record shows the attenuation of the hyperthermic response when the calcium concentration was increased to 3·75 mM, but the time course was not changed. On the other hand, when the perfusion fluid contained 5 mM calcium, the pyrogen injection no longer produced a rise during the perfusion as shown in the bottom record. The later rise which began about 20 min after the end of the perfusion, i.e. at least 1 hr after the pyrogen injection, may simply be the after-rise often following a perfusion, or the rise may have been accentuated by the pyrogen which still had some action after the perfusion with the high calcium concentration had come to an end. This steep after-rise was not obtained in all experiments of this nature. The bottom record of Fig. 7 shows the ineffectiveness of a pyrogen injection to raise temperature in a rabbit in which the high calcium content of 5 mM caused a pronounced fall in temperature. This record has to be compared with the lower middle record of Fig. 4 which was obtained from the same rabbit and shows the fall with after-rise on perfusion with the same concentration of calcium but without injection of pyrogen during the perfusion.

As shown by the upper middle record of Fig. 5, perfusion with a 0·9 % sodium chloride solution containing 5 mM magnesium chloride did not reduce the response to pyrogen. On the contrary, the hyperthermia was, if anything, slightly accentuated. This could readily be explained by the fact that the high magnesium chloride did not fully prevent the rise pro-

duced by sodium ions, and this rise would add to the hyperthermia produced by the pyrogen.

A certain attenuating effect of high magnesium in the perfusion fluid on the hyperthermic response to pyrogen was, however, revealed when the magnesium chloride was added to a perfusion fluid containing double the

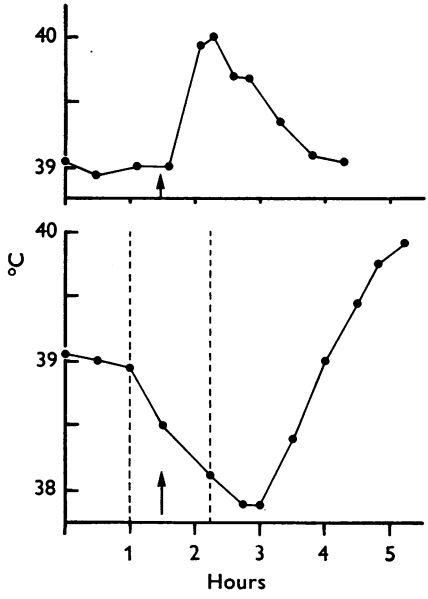

Fig. 7. Two records of rectal temperature obtained from the same rabbit on different days. The arrow in each of the records indicates intravenous injection of 2·5 ml. leucocyte pyrogen containing plasma, and in the bottom record the interval between the interrupted vertical lines indicates 75 min perfusion from left lateral ventricle to cisterna with a 0·9 % sodium chloride solution containing 5 mM calcium chloride. Same rabbit as the one from which the third record from the top in Fig. 4 was obtained.

physiological concentration of calcium chloride. The attenuation of the pyrogen response produced by doubling the calcium concentration became greater when the perfusion fluid contained 5 mM magnesium chloride as well. This is illustrated by the two middle records of Fig. 8.

Perfusion with isotonic sucrose solution prevented the hyperthermic response provided the pyrogen was not injected too early in the perfusion. When perfusion had continued for 30 min, before the pyrogen was injected, the hyperthermia was however fully suppressed even when perfusion was then discontinued 10 min later. The result of such an experiment is illustrated by the bottom record of Fig. 5.

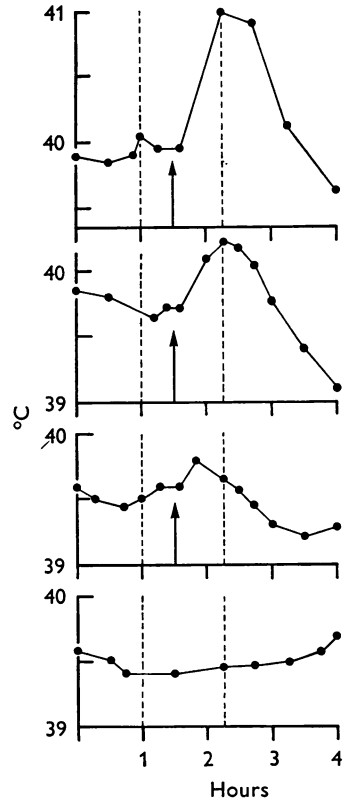

Fig. 8. Four records of rectal temperature from the same rabbit obtained on different days. The interval between the interrupted vertical lines indicates perfusion for 75 min from left lateral ventricle to cisterna with fluid of the following composition. Upper record: 0·9 % sodium chloride solution containing 1·25 mM calcium chloride. Upper middle and bottom records: 0·9 % sodium chloride containing 2·5 mM calcium chloride. Lower middle record: 0·9 % sodium chloride containing 2·5 mM calcium chloride and 5 mM magnesium chloride. The arrows in each of the upper three records indicate intravenous injection of 2·5 ml. leucocyte pyrogen containing plasma.

DISCUSSION

The results obtained in unanaesthetized rabbits on perfusion of the cerebral ventricles from a lateral ventricle to cisterna were the same as those described previously in cats. Again, it was found that body temperature did not change during these perfusions when the perfusing fluid was artificial c.s.f., but that it rose when the perfusing fluid was merely a 0·9 % sodium chloride solution. Again it was shown that the hyperthermia was due to an action of the sodium ions because when they were replaced and the cerebral ventricles were perfused with a calcium-free isotonic

sucrose solution, temperature did not rise during the perfusion. As the sodium ions raise temperature in cats as well as in rabbits, it is unlikely that their effect is mediated by the monoamines released from the monoaminergic fibres ending on the anterior hypothalamus as it would imply that in cats the rise is a 5-HT, in rabbits a noradrenaline response. On the other hand, the finding that sodium ions raise temperature in cats as well as in rabbits, strengthens the view that the function of the 'set point' in the hypothalamus which controls the level at which temperature is to be set and maintained, that is, the constancy of temperature, depends upon the physiological balance of sodium and calcium ions in the anterior hypothalamus. This view is further supported by the finding that excess of calcium in the fluid perfusing the cerebral ventricles leads to a fall in temperature. Although magnesium ions may have some influence as well, this influence can only be small in comparison to that of the sodium and calcium ions. Magnesium had to be perfused in fairly high concentration in comparison to calcium in order to reduce the hyperthermia produced by sodium ions.

It is known that calcium ions reduce the permeability of cell membranes to sodium ions and the constancy of temperature may therefore be the result of the correct permeability of the membranes in the hypothalamus to the sodium ions which is maintained by the calcium ions. Any factor which, like lowering or increasing the calcium concentration, changes this permeability, would result in a rise or a fall in temperature. The effect of calcium on cell permeability is rather a universal phenomenon and reduction of calcium in other parts of the central nervous system is known to increase its excitability. In the present experiment a number of excitatory effects were observed, apart from hyperthermia, on perfusion of the cerebral ventricles with calcium free solution. Similarly, Merlis (1940) has shown in dogs that perfusion of the lower spinal subarachnoid space with calcium-free salt solution resulted in increased reflex excitability. However, for the hypothalamic neurons involved in temperature regulation the correct calcium concentration may be particularly critical.

It is unlikely that pyrogens act, or act mainly, through the release of the monoamines in the anterior hypothalamus (Cooper, Cranston & Honour, 1967; Feldberg, 1968). On the other hand, the theory that pyrogens act by 'removing the calcium brake' thus allowing the sodium ions to exert their effect on the anterior hypothalamus which would result in a higher set-point being maintained, is supported by the present findings. They show that leucocyte pyrogen no longer raised temperature, either when the 'calcium brake' was strengthened, that is, when the pyrogen was injected during perfusion of the cerebral ventricles with a solution containing a high calcium concentration, or when the 'calcium brake' was rendered

unnecessary because the sodium ions in the perfusion fluid were replaced by innert sucrose. And again it was found that magnesium had a weak calcium-like activity revealed only when the pyrogen response was already attenuated as the result of some increase in the calcium concentration of the fluid perfusing the cerebral ventricles.

The theory that pyrogen fever is in fact a sodium fever brought about by removal of a 'calcium brake' poses two pertinent problems that are not answered by the results of the present experiments. First, how does pyrogen produce this action and secondly, is its action confined to the central neuronal mechanisms of temperature regulation, or is it part of a generalized action of pyrogen in the central nervous system but with particular sensitivity of the hypothalamic neurones.

Apart from the changes in temperature produced during the perfusion of the cerebral ventricles with salt solutions of different composition, such perfusions often produced an after-rise of long duration which did not depend on the nature of the perfusion fluid. The same observation was previously made on cats and the rise was attributed mainly to the action of an unknown pyrogen found in the fluid stagnating in the shaft of the Collison cannula implanted into the lateral ventricle (Feldberg *et al.* 1970). Although there is no doubt that long-lasting hyperthermia can be produced in this way, the after-rise observed in the present experiments must be explained differently since stagnation of fluid in the shaft of the Collison cannula was excluded by using a shaftless Collison cannula and washing out its hub before passing the needle connected with the perfusion pump through the cortical tissue into the lateral ventricle. It was also shown that neither the perfusion itself nor pressure changes in the ventricles were responsible for the late rise, since it did not always occur when 2 ml. fluid was infused into the ventricles without establishing an outflow, and on the other hand, it occurred also when merely 0·2 ml. artificial c.s.f. was injected into the ventricles. It would therefore appear that the long lasting after-rise is the result of the small injury produced in the brain tissue by the injection needle. Fever as a result of small brain injuries has long been known, has sometimes been termed 'puncture fever' and has been discussed in the older literature in connexion with the idea of the existence of various 'heat centres' (for references see Moore, 1918). If the after-rise results from the small brain injury the most likely explanation would be that at the site of injury and in response to it, pyrogens are formed and continue to be formed for a long time. They are then either absorbed into the blood stream or they diffuse into the ventricular cavities. The possibility however cannot be completely excluded that, in spite of all precautions, a pyrogenic substance is sometimes introduced into the ventricle with the injection, or by the perfusion.

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