Journal of Physiology (1988), **397**, *pp.* 281–289
With 4 *text-figures*
Printed in Great Britain

IS THE CENTRAL ARACHIDONIC ACID CASCADE SYSTEM INVOLVED IN THE DEVELOPMENT OF ACUTE-PHASE RESPONSE IN RABBITS?

BY AKIO MORIMOTO, NAOTOSHI MURAKAMI AND TATSUO WATANABE
From the Department of Physiology, Yamaguchi University School of Medicine, Ube, Yamaguchi 755, Japan

(*Received 24 April 1987*)

SUMMARY

1. In the present study, endogenous pyrogen (EP), prostaglandin E_2 or arachidonic acid was injected into the cerebral ventricle to investigate whether central arachidonic acid metabolites are involved in the development of the acute-phase response. The central effects of a cyclo-oxygenase inhibitor, indomethacin, and of a lipoxygenase inhibitor, nordihydroguairetic acid (NDGA), on the acute-phase response induced by an intracerebroventricular injection of EP were also examined.

2. The ventricular injection of EP decreased the plasma concentrations of iron and zinc, while increasing those of copper and fibrinogen and the circulating leucocyte count. However, ventricular injection of prostaglandin E_2 affected neither of them, indicating that prostaglandin E_2 does not contribute to the acute-phase response production by itself.

3. Both the ventricular injections of indomethacin and NDGA had no effect on the changes in the plasma concentrations of iron, copper and fibrinogen which were induced by ventricular injection of EP. In addition, when arachidonic acid was administered into the cerebral ventricle, the changes in the plasma levels of iron, copper and fibrinogen were not induced.

4. In contrast, EP-induced hypozincaemia was observed upon pre-treatment with NDGA, but not upon pre-treatment with indomethacin. However, plasma zinc increased after the ventricular injection of arachidonic acid. Ventricular injection of EP alone and of EP with NDGA increased the number of circulating leucocytes 8 and 24 h after the ventricular injection, while ventricular injections of arachidonic acid, and of EP with administration of indomethacin induced leucocytosis 8 h after injections.

5. These results suggest that arachidonic acid metabolites do not participate in the genesis of the acute-phase response. However, there remains a possibility that central arachidonic acid metabolites slightly modulate the induction of the acute-phase response.

INTRODUCTION

Under infectious or inflammatory conditions, a host reacts with fever and various metabolic changes including changes in the plasma concentrations of certain trace metals, hepatic proteins and the count of circulating leucocytes. These metabolic

responses are termed the acute-phase response (APR) (Gordon & Koj, 1985), which has recently been recognized to have survival value for the host (Kluger, Ringler & Anver, 1975; Kluger & Vaughn, 1978; Kluger & Rothenberg, 1979). Fever has generally been thought to be caused by a small mediator protein, called endogenous pyrogen (EP), which is synthesized and released from monocytes activated by pathogenic stimuli such as bacterial endotoxin (Atkins, 1960). Subsequently EP acts on structures both outside and inside the blood–brain barrier to release arachidonic acid (AA) metabolites (Morimoto, Murakami, Nakamori & Watanabe, 1987*b*), which have been thought to be the final mediators in the central nervous system (CNS) for fever production. Among AA metabolites, the prostaglandin E series, induced by a cyclo-oxygenase in the AA cascade system, might produce fever, as EP-induced fever is prevented by inhibitors of cyclo-oxygenase such as indomethacin (Stitt & Bernheim, 1985; Morimoto *et al.* 1987*b*).

The APR has also been demonstrated to be induced also by EP, which is indistinguishable from leucocytic endogenous mediator (Kampschmidt, 1980). Recently, we have shown that EP also acts on structures both outside and inside the blood–brain barrier to produce APR (Morimoto, Murakami, Myogin, Takada, Teshirogi & Watanabe, 1987*a*). However, we considered that AA metabolites do not contribute to production of APR, because systemic injections of indomethacin suppressed the fever but had no effect on APR induced by intravenous injections of endotoxin (Tocco, Kahn, Kluger & Vander, 1983). These results certainly exclude the possibility of the involvement of AA metabolites induced by cyclo-oxygenase outside the blood–brain barrier in the development of APR. However, since indomethacin is resistant to passage through the blood–brain barrier (Hucker, Zacchei, Cox, Brodie & Cantwell, 1966), the involvement of central AA metabolites in APR production is unclear. In addition, it has been reported that the prostaglandin E series (Merriman, Upchurch & Kampschmidt, 1974; Blatteis, Hunter, Llanos, Ahokas & Mashburn, 1984) or several kinds of leukotrienes (Mashburn, Llanos, Ahokas & Blatteis, 1986) injected into the cerebral ventricle or the hypothalamus did not cause APR, but it is still unknown whether AA or AA metabolites in the CNS other than prostaglandin E or leukotrienes are involved in the APR production.

In the present study, to investigate the involvement of central AA metabolites in APR production, we examined whether AA injected into the cerebral ventricle induces APR. The central effects of a cyclo-oxygenase inhibitor, indomethacin (Krupp & Ziel, 1979), and a lipoxygenase inhibitor, nordihydroguairetic acid (Kunkel, Chensue, Mouton & Higashi, 1984), on the APR induced by EP injected into the cerebral ventricle were also examined.

METHODS

The animals used in this study were male New Zealand white rabbits, weighing 3·0–4·0 kg. A total of twenty-one rabbits had been implanted previously with a stainless-steel cannula (1·0 mm o.d.) in the third ventricle. This implantation was done at least 2 weeks before the start of the experiment under general anaesthesia (sodium pentobarbitone, 20 mg/kg, I.V.). The present study consisted of two experimental groups. In Expt 1, twenty-one rabbits were divided into three groups and rabbits ($n = 7$) in each group were intraventricularly injected with EP, prostaglandin E_2 or saline. In Expt 2, twenty-one rabbits were divided into three groups of seven rabbits each; one

group was administered indomethacin (INDO, 400 μg) 15 min before ventricular injection of EP, another group was administered nordihydroguairetic acid (NDGA, 400 μg) 15 min before ventricular injection of EP, and the third group was injected with AA only. In Expt 2, the group which received EP with pre-treatment of INDO was called the EP + INDO injection group and the group which received EP with pre-treatment of NDGA was called the EP + NDGA injection group. Similarly, the group which received AA was called the AA injection group. EP, prostaglandin E_2, INDO, NDGA, AA or saline were directly injected through the cannula into the third ventricle.

The EP was prepared from white blood cells of male rabbit (New Zealand white strain). The white blood cells were stimulated by lipopolysaccharide of *Salmonella typhosa* endotoxin (Difco). The general procedures for preparing EP have been described in detail elsewhere (Morimoto, Watanabe, Ono, Sakata & Murakami, 1986). Partial purification was achieved by ultrafiltration using two types of membranes (10YM10, 10XM50, Amicon), which removed all substances of molecular weight outside the range of 10000–50000. Consequently, 1·0 ml of this partially purified EP solution was derived from approximately $1{\cdot}5 \times 10^7$ white blood cells. Intravenous injections of 0·2 ml/kg of this EP solution produced a monophasic fever (> 1 °C) in rabbits ($n = 4$). Furthermore, we confirmed that an intravenous injection of heat-treated EP (0·2 ml/kg), which had been inactivated by heating in a hot water bath of 60 °C for 30 min, did not cause fever in rabbits. This demonstrated that this partially purified EP had no contamination with endotoxin. Prostaglandin E_2 was dissolved in saline containing 2 % ethanol at a concentration of 0·5 mg/ml. Indomethacin (INDO) or nordihydroguairetic acid (NDGA) was dissolved in ethanol (99 %) at a concentration of 20 mg/ml. Sodium arachidonic acid (AA) was dissolved in saline at a concentration of 3 mg/ml.

On the day of the experiment, animals were minimally restrained in the conventional stocks at an ambient temperature of 21 ± 1 °C between 08.30 and 18.00 h. To avoid the effect of stress due to restraint, all had been well trained to adapt to the stock for 6 h every other day, for at least 10 days before the start of experiment. Throughout the experiment, the rectal temperature was measured every minute with a copper–constantan thermocouple. All injections of pyrogenic substances or saline control were performed at the time of 12.00 h. INDO or NDGA was injected intraventricularly 15 min before the administration of EP. Intraventricular injections were made through a stainless-steel needle (0·6 mm, o.d.) attached to a polyethylene tube, and the volume infused was always 20 μl. Intraventricular injections were performed with a microsyringe pump (Infors AG. CH-4015) for a period of 5 min.

For measuring the blood cell counts and the plasma concentration of iron, zinc, copper and fibrinogen, about 5 ml of blood was withdrawn through the marginal ear vein. The blood samplings were made three times: 1 h before, and 8 and 24 h after injections of EP, prostaglandin E_2, AA or saline. Immediately after collecting the blood, both the white blood cell count and red blood cell count were taken with an automatic cell counter (Coulter, Model S plus II). By preparing Wright-stained smears of the blood, the differential leucocyte count was microscopically measured, and the percentage composition of neutrophils, lymphocytes, monocytes, basophils and eosinophils were calculated. The remaining blood was collected into heparinized polyethylene tubes. It was centrifuged at 2000 r.p.m. for 15 min at 4 °C, and the plasma was collected and stored at −20 °C until the measurements of iron, zinc, copper and fibrinogen concentration were made. The procedures for measuring the plasma concentrations of iron, zinc and copper are described in detail elsewhere (Morimoto *et al.* 1987*a*). Fibrinogen concentration was measured by the method of von Clauss (1957). Data was analysed for statistical significance using Student's *t* test.

RESULTS

Figure 1 shows the changes in the rectal temperature (ΔT_{re}) of rabbits after intracerebroventricular injection of EP and prostaglandin E_2. About 30 min after ventricular injection of EP (20 μl), the rectal temperature gradually started to rise. In contrast, prostaglandin E_2 (10 μg) produced a monophasic fever with a rapid onset and the time to peak fever was 80–90 min. Saline (20 μl), as a control, did not change the rectal temperature (not illustrated in Fig. 1).

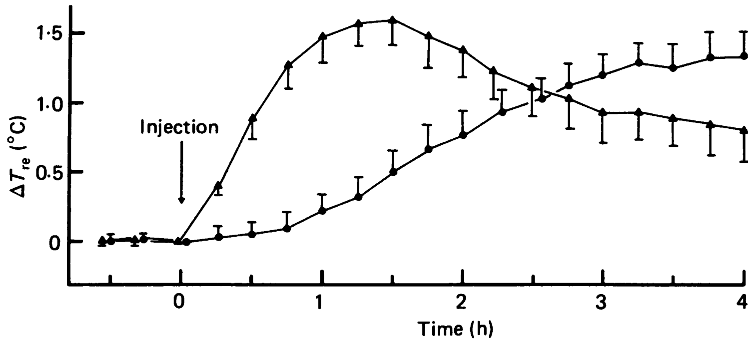

Fig. 1. Mean changes (mean ± S.E.M.) in the rectal temperature (ΔT_{re}) of rabbits ($n = 7$) after intracerebroventricular injections of endogenous pyrogen (●; 20 μl) or prostaglandin E_2 (▲; 10 μg).

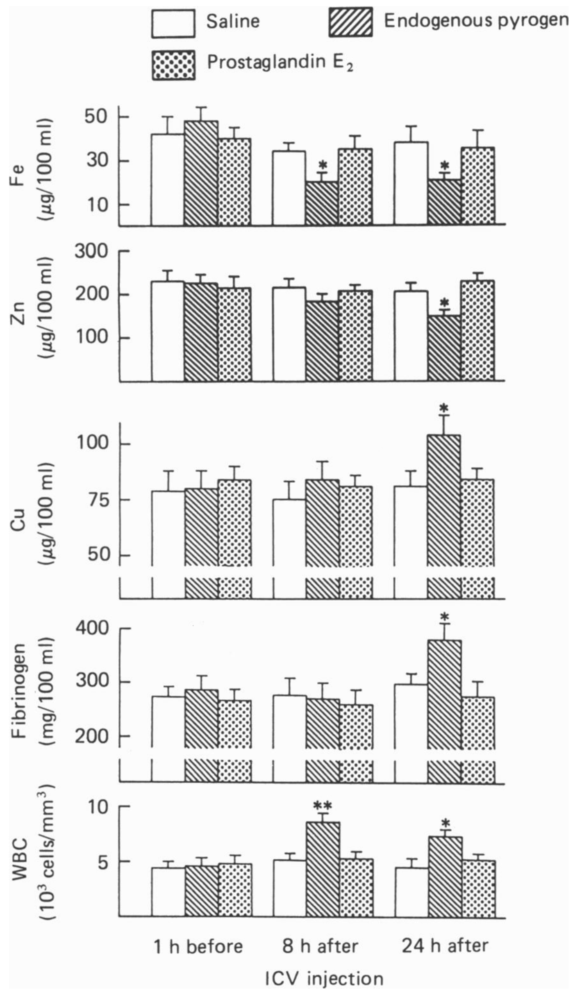

Fig. 2. Changes in plasma concentrations of iron, zinc, copper and fibrinogen, and the changes in the circulating leucocyte count (WBC), 1 h before, and 8 and 24 h after intracerebroventricular (ICV) injection of saline, endogenous pyrogen or prostaglandin E_2. $*P < 0.05$, $**P < 0.01$.

Figure 2 summarizes the changes in the plasma levels of iron, zinc, copper and fibrinogen concentrations, and the changes in the white blood cell count, 1 h before, and 8 and 24 h after injection of EP, prostaglandin E_2 or saline. The changes in each parameter represent the mean ± S.E.M. of the same group of rabbits ($n = 7$).

Ventricular injection of EP significantly decreased the plasma concentrations of iron (8 and 24 h after injection) and zinc (24 h after injection), compared with the concentrations measured 1 h before injections. In contrast, ventricular injection of EP produced significant increases in the plasma concentrations of copper and fibrinogen (24 h after injection), and in the white blood cell count (8 and 24 h after injection). The elevation of the white cell count was largely due to neutrophils. Neither of the injections affected the number of red blood cells. Prostaglandin E_2 and saline had no effect on the plasma concentrations of iron, zinc, copper and fibrinogen, and the white blood cell count.

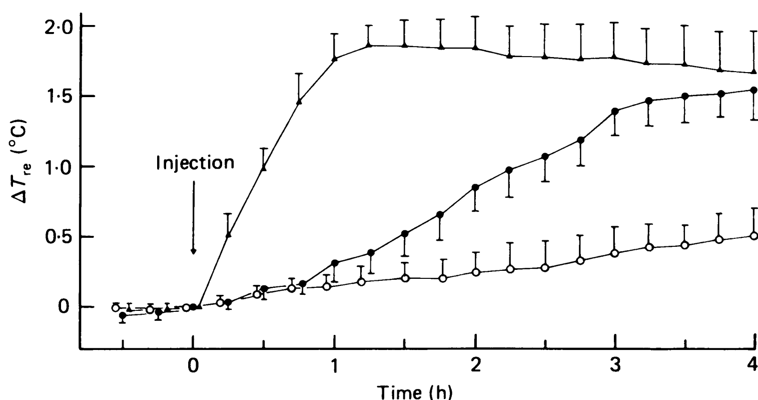

Fig. 3. Mean changes (mean ± S.E.M.) in the rectal temperature (ΔT_{re}) of rabbits ($n = 7$) after intracerebroventricular injection of endogenous pyrogen (20 μl) with administration of indomethacin (○; 400 μg) or nordihydroguairetic acid (●; 400 μg) 15 min before ventricular injection of EP, and after ventricular injection of arachidonic acid (▲; 60 μg).

Figure 3 shows the changes in the rectal temperature (ΔT_{re}) of rabbits in the EP + INDO (EP, 20 μl; INDO, 400 μg), EP + NDGA (EP, 20 μl; NDGA, 400 μg) and AA (60 μg) injection groups. The EP + INDO injection group did not show any changes in the rectal temperature. However, a gradual rise in the rectal temperature, which was almost identical to EP-induced fever (see Fig. 1), was observed in the EP + NDGA injection group. In contrast, a fever with a rapid onset was produced in the AA injection group. The time to peak fever was between 60 and 90 min and the fever persisted over a period of 4 h.

Figure 4 summarizes the changes in the plasma levels of iron, zinc, copper, and fibrinogen concentration, and the changes in the white blood cell count, 1 h before, and 8 and 24 h after injection in the EP + INDO, EP + NDGA or the AA injection group. The changes in each parameter represent the mean ± S.E.M. of the same group of rabbits ($n = 7$). The plasma concentration of iron significantly decreased 8 and 24 h after injection both in the EP + INDO and EP + NDGA injection groups. Both the EP + INDO and the EP + NDGA injection groups showed increases in the plasma

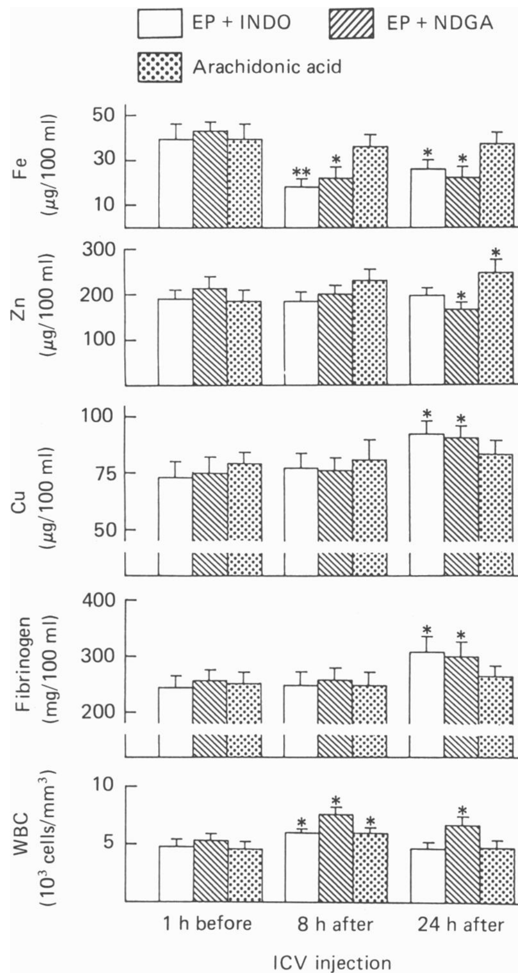

Fig. 4. Changes in plasma concentrations of iron, zinc, copper and fibrinogen, and changes in the circulating leucocyte count (WBC), 1 h before, and 8 and 24 h after intracerebroventricular (ICV) injection of endogenous pyrogen (EP) with administration of indomethacin (INDO) or nordihydroguairetic acid (NDGA) 15 min before ventricular injection of EP, and after ventricular injection of arachidonic acid. $*P < 0.05$, $**P < 0.01$.

concentrations of copper and fibrinogen 24 h after injection. The AA injection group produced no changes in the plasma concentrations of iron, copper and fibrinogen. The plasma concentration of zinc was significantly decreased 24 h after injection in the EP+NDGA injection group, while the AA injection group showed hyperzincaemia 24 h after injection. In contrast, no changes in the plasma zinc concentration were observed in the EP+INDO injection group. The number of circulating leucocytes was increased in the EP+INDO, EP+NDGA and the AA injection group 8 h after injection. In the EP+NDGA injection group, leucocytosis was also observed 24 h after injection.

DISCUSSION

According to recent results (Morimoto *et al.* 1987*b*), EP raises body temperature by its action on structures both inside and outside the blood–brain barrier, which subsequently synthesize and release AA metabolites as the final mediators in the CNS for producing fever. Especially, the prostaglandin E series is believed to play a major role in producing fever (Milton & Wendlandt, 1970). However, some reports (Cranston, Duff, Hellon, Mitchell & Townsend, 1976; Cranston, Hellon, Mitchell & Townsend, 1983) indicate that not only the prostaglandin E series but also other AA metabolites are involved in the development of fever, although the febrile response induced by ventricular injection of prostaglandin E is quite different from that induced by AA injection. In the present results, fever induced by AA was significantly prolonged over 4 h, while the prostaglandin E_2-induced rise in body temperature rapidly fell to the pre-injection level within 3 h. Therefore, it is likely that several metabolites of the AA cascade might be involved in the development of fever, and, in the CNS, these metabolites may produce fever in different ways.

We have already shown that the APR is also produced through the action of EP on structures both inside and outside the blood–brain barrier (Morimoto *et al.* 1987*a*). As for the contribution of the AA cascade system to APR causation, previous results indicate that AA metabolites induced by cyclo-oxygenase do not activate the peripheral mechanisms existing outside the blood–brain barrier to produce the APR, as the APR induced by systemic injections of EP or endotoxin was not affected by pre-treatment with systemic injections of inhibitors of cyclo-oxygenase such as indomethacin (Tocco *et al.* 1983) or ibuprofen (Sobrado, Moldawer, Bistrian, Dinarello & Blackburn, 1983). However, from the previous studies, it is uncertain whether EP actually enters the CNS to trigger the AA cascade system; because a small concentration of circulating EP does not pass through the blood–brain barrier (Morimoto *et al.* 1987*b*). Moreover, since indomethacin seems to be relatively resistant to passage through the blood–brain barrier (Hucker *et al.* 1966), it is unclear that systemic injection of indomethacin inhibits the cyclo-oxygenase in the CNS. Therefore, the previous results have not shown clearly whether the AA cascade system in the CNS is involved in the development of APR.

In the present results, the magnitudes of febrile response induced by ventricular injection of EP and prostaglandin E_2 were almost identical. With regard to the pattern of febrile response, however, EP caused the gradual rise in rectal temperature with a latency of 30 min, while prostaglandin E_2 produced a monophasic fever with a rapid onset. As for the APR, the ventricular injection of EP decreased the plasma concentrations of iron and zinc, while increasing those of copper, fibrinogen, and the circulating leucocyte count. In contrast, prostaglandin E_2 affected none of them. The inability of prostaglandin E_2 to induce the APR has been reported previously (Merriman, *et al.* 1974; Blatteis, *et al.* 1984). Therefore it appears that central prostaglandin E_2 and subsequently occurring hyperthermia do not contribute directly to the APR.

Fever induced by the ventricular injection of EP was significantly suppressed by a pre-treatment of ventricular injection with inhibitor of cyclo-oxygenase, indomethacin, but not with inhibitor of lipoxygenase, NDGA. AA produced a fever

with rapid onset which continued over 4 h. However, both indomethacin and NDGA had no effect on the changes in the plasma concentration of iron, copper and fibrinogen which were induced by the ventricular injection of EP. In addition, AA did not change the plasma levels of iron, copper and fibrinogen. These results indicate that AA metabolites, metabolic products induced by both cyclo-oxygenase and lipoxygenase, do not participate in the genesis of the changes in the plasma level of iron, copper and fibrinogen concentrations. Hypozincaemia was observed in the EP + NDGA injection group, indicating that the EP-induced decrease in the plasma zinc does not result from the action of AA metabolites in the pathway induced by lipoxygenase. In contrast, hypozincaemia was not induced in the EP + INDO injection group, and the plasma concentration of zinc increased 24 h after the ventricular injection of AA. From this result, it is inferred that AA metabolites induced by lipoxygenase, which are derived from the large dose of AA used in the present study, essentially induce a hyperzincaemia, but EP has strong potency to decrease the plasma level of zinc. In other words, EP causes hypozincaemia by itself, in spite of its capability of releasing AA metabolites which might increase the plasma concentration of zinc. Furthermore, AA metabolites induced by cyclo-oxgenase might contribute to causation of hypozincaemia. Concerning the white blood cell count, the ventricular injections of EP + INDO and AA induced leucocytosis 8 h after injections. In the EP + NDGA injection group the circulating leucocyte count was increased 8 and 24 h after injection. These results suggest that EP and AA metabolites induced by cyclo-oxygenase both mediate the increase in the leucocyte count. Therefore, it is likely that leucocytosis observed 8 and 24 h after venticular injection in the EP + NDGA injection group was caused by the action of both EP and AA metabolites induced by cyclo-oxygenase. The observation that the EP + INDO and AA injection groups showed leucocytosis only 8 h after injection suggests that leucocytosis results from the action of either EP or AA metabolites induced by cyclo-oxygenase.

The present study was carried out to investigate whether AA or certain AA metabolites in the CNS are involved in the central mechanisms of APR production. Our results suggest that AA metabolites do not participate in the genesis of the APR because plasma levels of iron, copper and fibrinogen were shown not to be affected by AA metabolites. However, in the light of the capability of AA metabolites to cause certain changes in the plasma level of zinc and the circulating leucocyte count, there remains a possibility that central AA metabolites slightly modulate the induction of APR.

REFERENCES

ATKINS, E. (1960). Pathogenesis of fever. *Physiological Reviews* **40**, 580–646.

BLATTEIS, C. M., HUNTER, W. S., LLANOS-Q, J., AHOKAS, R. A. & MASHBURN JR, T. A. (1984). Activation of acute-phase responses by intrapreoptic injections of endogenous pyrogen in guinea pigs. *Brain Research Bulletin* **12**, 689–695.

CRANSTON, W. I., DUFF, G. W., HELLON, R. F., MITCHELL, D. & TOWNSEND, Y. (1976). Evidence that brain prostaglandin synthesis is not essential in fever. *Journal of Physiology* **259**, 239–249.

CRANSTON, W. I., HELLON, R. F., MITCHELL, D. & TOWNSEND, Y. (1983). Intraventricular injections of drugs which inhibit phospholipase A_2 suppress fever in rabbits. *Journal of Physiology* **339**, 97–105.

GORDON, A. H. & KOJ, A. (1985). The acute-phase response to injury and infection. *Research Monographs in Cell and Tissue Physiology*, vol. 10. New York: Elsevier.

HUCKER, H. B., ZACCHEI, A. G., COX, S. V., BRODIE, D. A. & CANTWELL, N. H. R. (1966). Studies on the absorption, distribution and excretion of indomethacin in various species. *Journal of Pharmacology and Experimental Therapeutics* **153**, 237–240.

KAMPSCHMIDT, R. F. (1980). Metabolic alterations elicited by endogenous pyrogens. In *Fever*, ed. LIPTON, J. M., pp. 49–56. New York: Raven.

KLUGER, M. J., RINGLER, D. H. & ANVER, M R. (1975). Fever and survival. *Science* **188**, 166–168.

KLUGER, M. J. & ROTHENBERG, B. A. (1979). Fever and reduced iron: their interaction as a host defense response to bacterial infection. *Science* **203**, 374–376.

KLUGER, M. J. & VAUGHN, L. K. (1978). Fever and survival in rabbits infected with *Pasteurella multocida*. *Journal of Physiology* **282**, 243–251.

KRUPP, P. J. & ZIEL, R. (1979). Antipyretics. In *The Body Temperature*, ed. LOMAX, P. & SCHONBAUM, E., pp. 381–401. New York: Dekker.

KUNKEL, S. L., CHENSUE, S. W., MOUTON, C. & HIGASHI, G. I. (1984). Role of lipoxygenase products in murine pulmonary granuloma formation. *Journal of Clinical Investigation* **74**, 514–524.

MASHBURN, JR., T. A., LLANOS-Q, J., AHOKAS, R. A. & BLATTEIS, C. M. (1986). Thermal and acute phase protein responses of guinea pigs to intrapreoptic injection of leukotrienes. *Brain Research* **376**, 285–291.

MERRIMAN, C. R., UPCHURCH, H. F. & KAMPSCHMIDT, R. F. (1974). Prostaglandin E_1, aspirin and the action of leukocytic endogenous mediator. *Journal of Pharmacology and Experimental Therapeutics* **188**, 516–519.

MILTON, A. S. & WENDLANDT, S. (1970). A possible role for prostaglandin E_1 as a modulator for temperature regulation in the central nervous system of the cat. *Journal of Physiology* **207**, 76–77*P*.

MORIMOTO, A., MURAKAMI, N., MYOGIN, T., TAKADA, M., TESHIROGI, S. & WATANABE, T. (1987*a*). Separate mechanisms inside and outside the blood–brain barrier, inducing metabolic changes in febrile rabbits. *Journal of Physiology* **392**, 637–649.

MORIMOTO, A., MURAKAMI, N., NAKAMORI, T. & WATANABE, T. (1987*b*). Evidence for separate mechanisms of induction of biphasic fever inside and outside the blood–brain barrier in rabbits. *Journal of Physiology* **383**, 629–637.

MORIMOTO, A., WATANABE, T., ONO, T., SAKATA, Y. & MURAKAMI, N. (1986). Rat endogenous pyrogen and fever. *American Journal of Physiology* **250**, R776–782.

SOBRADO, J., MOLDAWER, L. L., BISTRIAN, B. R., DINARELLO, C. A. & BLACKBURN, G. L. (1983). Effect of ibuprofen on fever and metabolic changes induced by continuous infusion of leukocytic pyrogen (interleukin 1) or endotoxin. *Infection and Immunity* **42** (3), 997–1005.

STITT, J. T. & BERNHEIM, H. A. (1985). Differences in endogenous pyrogen fevers induced by iv and icv routes in rabbits. *Journal of Applied Physiology* **59**(2), 342–347.

TOCCO, R. J., KAHN, L. L., KLUGER, M. J. & VANDER, A. J. (1983). Relationship of trace metals to fever during infection: are prostaglandins involved? *American Journal of Physiology* **244**, R368–373.

VON CLAUSS, A. (1957). Gerinnungsphysiologische Schnellmethode zur Bestimmung des Fibrinogens. (1957). *Acuta haematologica* **17**, 237–246.