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INTRAVENTRICULAR MICROINJECTIONS OF A STABLE ANALOGUE OF PROSTAGLANDIN ENDOPEROXIDE CAUSE FEVER IN RABBITS

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SUMMARY

1. Derivatives of arachidonic acid other than prostaglandin are pyrogenic, the likely candidates being the prostaglandin endoperoxides and/or the thromboxanes.

2. Intraventricular microinjections in rabbits of a stable analogue of prostaglandin endoperoxide resulted in dose-dependent increases of rectal temperature. The pyrexia was delayed in onset; no significant change in body temperature occurred for at least an hour.

3. The pyrexia was unaltered by simultaneous injection of the potent prostaglandin synthetase inhibitor indomethacin.

4. We suggest that both prostaglandins and prostaglandin endoperoxides may be implicated in fever.

INTRODUCTION

There is strong evidence that derivatives of both arachidonic acid and dihomogammalinolenic acid are essential neurochemical mediators of fever. Some members of one class of derivatives, the prostaglandins, are known to be pyrogenic (Feldberg, 1975). However, Laburn, Mitchell & Rosendorff (1977) recently demonstrated that the prostaglandins are not the only derivatives concerned in the febrile response in the rabbit. Intraventricular injections of sodium arachidonate resulted in pyrexia, which was not inhibited by the simultaneous injection of prostaglandin antagonists, although the antagonists inhibited successfully the fever produced by injection of a prostaglandin of the E series. The hyperthermic effects of arachidonic acid are due to its derivatives and not to the precursor substance itself because indomethacin, which prevents breakdown of arachidonic acid, effectively inhibited the sodium arachidonate pyrexia (Laburn *et al.* 1977). These findings extended previous evidence that prostaglandin synthesis is not an essential step in the development of pyrogen fever (Cranston, Hellon & Mitchell, 1975; Cranston, Duff, Hellon, Mitchell & Townsend, 1976).

The prostaglandins are not synthesized directly from their essential fatty acid substrates. Degradation of these fatty acids by prostaglandin synthetase enzymes gives rise to prostaglandin precursors, the prostaglandin endoperoxides. In turn, the endoperoxides give rise to prostaglandins, as well as to a group of potent cellular regulatory agents, the thromboxanes (Hamberg, Svensson & Samuelsson, 1975).

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Investigation into the possible pyrogenicity of prostaglandin endoperoxides or of thromboxanes has not been possible because both these classes of substances are extremely unstable (Hamberg & Samuelsson, 1974). Stable analogues of the prostaglandin endoperoxides have now been synthesized and been shown to be potent constrictors of the bronchi, like the naturally occurring endoperoxides (Wasserman, 1976).

We have investigated the effects of intraventricular microinjections of various doses of one stable prostaglandin endoperoxide analogue on body temperature in rabbits. We suggest on the basis of our findings, that prostaglandin endoperoxides are pyrogenic and constitute an alternative mediator, besides prostaglandin, in the production of fever.

METHODS

New Zealand White rabbits of either sex, weighing between 2·0 and 3·5 kg were used. For the intraventricular microinjections, a Perspex headplate was fixed on to the skull of each rabbit, under general anaesthesia, at least 1 week before any experiments were conducted. The exact positioning of the headplate on the skull allowed the introduction of fine cannulae (o.d. = 0·4mm) into either of the lateral cerebral ventricles (Monnier & Gangloff, 1961).

Experimental groups of rabbits received intraventricular injections of various doses of the prostaglandin endoperoxide analogue or as the control, received injections of the drug vehicle. In another series of experiments intraventricular injections of an intermediate dose of prostaglandin endoperoxide were made in rabbits with or without simultaneous injection of an antipyretic dose of indomethacin. Indomethacin is a potent inhibitor of prostaglandin synthetase (Ziel & Krupp, 1975).

All experiments were carried out at room temperature (20–23 °C) and the rabbits were always conscious, and restrained in conventional stocks throughout the experimental period. Rectal temperature was measured using an indwelling thermistor probe (YSI type 401) inserted into the rabbit's rectum. The thermistors were connected to a YSI scanning telethermometer (Model 47) the outputs of which were continuously recorded on a chart recorder (Kipp BD5).

The stable endoperoxide analogue (15*S*)-hydroxy-11α,9α-(epoxymethano) prosta-5*Z*, 13*E*-dienoic acid (U-46619, Upjohn) was prepared as a stock solution in absolute alcohol. Doses of 14, 28 and 43 n-mole were injected unilaterally into a lateral cerebral ventricle. Each dose was injected in 10 μl. of absolute ethanol made up to a volume of 20 μl. with sterile saline, and was flushed through the injection cannula with 30 μl. sterile rabbit artificial cerebrospinal fluid (Cameron & Semple, 1968). Control experiments consisted of identical injections except that absolute ethanol alone was substituted for the solution containing the endoperoxide analogue. Six rabbits were used for the control injections as well as for injections of each dose of endoperoxide analogue.

In a further series of experiments, 28 n-mole of the prostaglandin endoperoxide was injected together with an antipyretic dose (28 n-mole) of indomethacin (Sigma) dissolved in 10 μl. ethanol (Clark & Cumby, 1975). The solutions were flushed through the injection cannula with 30 μl. sterile distilled water. Control experiments consisted of similar injections of 28 n-mole of the prostaglandin endoperoxide analogue with 10 μl. ethanol. Intraventricular injections of indomethacin alone have been shown previously to have no effect on the rectal temperatures of rabbits for 2·5 h following injection, and thereafter a slight hyperthermic effect (Laburn *et al.* 1977).

All data were subjected to the Student's t test and values for P equal to or less than 0·05 were considered to be significant.

RESULTS

Changes in rectal temperatures are expressed as the deviation from the temperature at the time of injection. The actual rectal temperatures of the groups of rabbits at this time are shown in Table 1.

TABLE 1. Rectal temperatures of groups of rabbits at the time of injection

Experimental series	No. of animals	Rectal temp. in °C (mean ± S.E.)
Control	6	39·1 ± 0·3
14 n-mole endoperoxide	6	39·2 ± 0·1
28 n-mole endoperoxide	6	38·9 ± 0·3
43 n-mole endoperoxide	6	38·4 ± 0·2
28 n-mole + indomethacin vehicle	5	39·2 ± 0·1
28 n-mole + indomethacin	5	39·0 ± 0·2

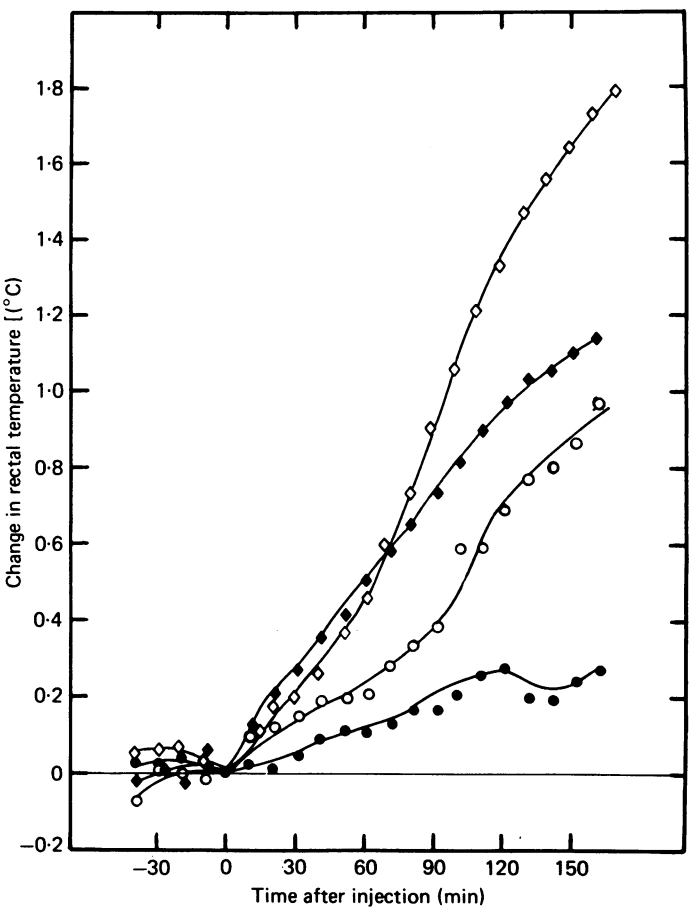

Fig. 1. Change in rectal temperature of rabbits after intraventricular injection of prostaglandin endoperoxide analogue 14 n-mole (○—○), 28 n-mole (◆—◆), 43 n-mole (◇—◇) or of injection of drug vehicle alone (●—●). Each point represents the mean response for six rabbits.

Fig. 1 shows the effects on rectal temperature of the rabbits of intraventricular injections of the control solution, and the solutions containing various doses of prostaglandin endoperoxide analogue.

Injection of the control solution produced a slow, slight rise in rectal temperature which averaged less than 0·3 °C after 160 min.

Intraventricular injection of all the doses of the prostaglandin endoperoxide analogue resulted in elevations of rectal temperature, which were significantly

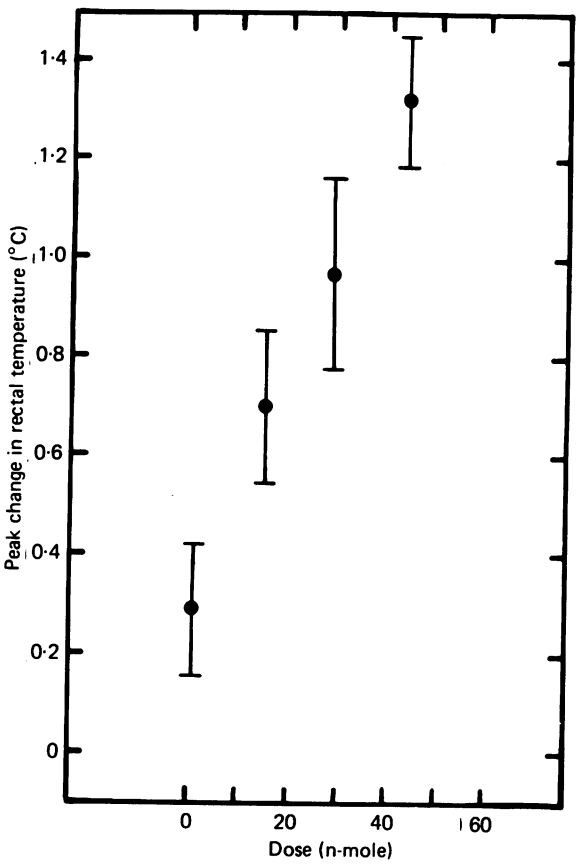

Fig. 2. Mean elevations in rectal temperature of rabbits, as measured 120 min after injection, induced by the various doses of prostaglandin endoperoxide analogue and by injection of drug vehicle alone (at zero dose). Each point represents the mean ± S.E. of six observations.

greater than the rise in temperature following injection of the control solution after 130 min for the 14 n-mole dose, after 70 min for the 28 n-mole dose, and after 60 min for the 43 n-mole dose, and in each case, for the remainder of the experimental period. It appears that for all doses, including the highest dose of prostaglandin endoperoxide analogue tested (43 n-mole), no significant elevations in rectal temperature occurred within the first 60 min after injection.

Fig. 2 illustrates the dose-related effects on rectal temperature of the endoperoxide analogue, prevailing 120 min after injection.

Fig. 3 shows the effect of injection of 28 n-mole of the prostaglandin endoperoxide analogue with or without a simultaneous injection of an antipyretic dose of indomethacin. Indomethacin did not diminish the elevation in rectal temperature caused by the prostaglandin endoperoxide analogue.

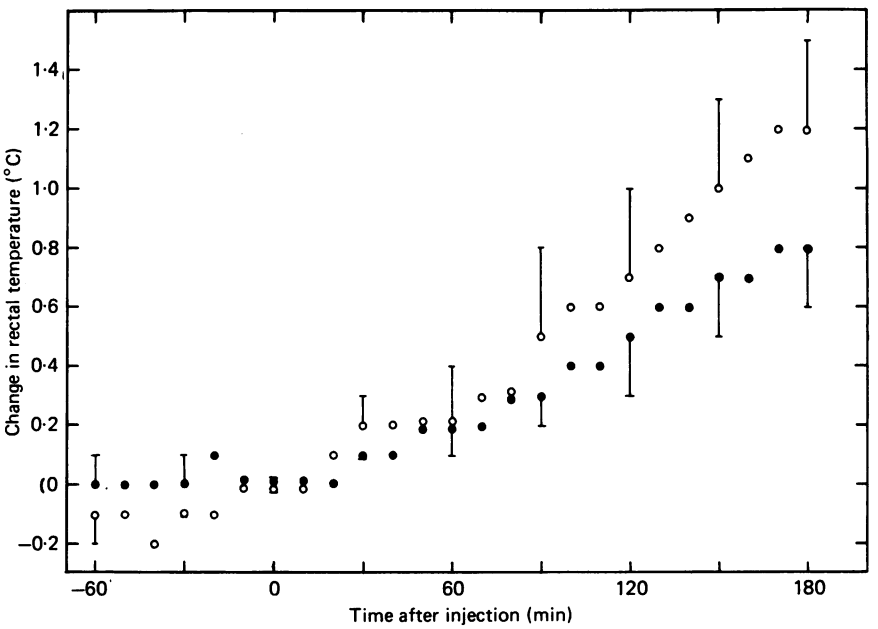

Fig. 3. Changes in rectal temperatures of rabbits after intraventricular injection of 28 n-mole prostaglandin endoperoxide with (○) and without (●) simultaneous injection of 28 n-mole of indomethacin. Each point represents the mean response in five rabbits, and the bars represent 1 S.E.

DISCUSSION

Two important findings emerge from our results. The first is that the stable endoperoxide analogue U-46619 produces dose-dependent elevations in rectal temperature of rabbits after intraventricular injection. However, intraventricular injections even of sterile saline solution may result in a non-specific pyrexia, through the release of prostaglandin from the surrounding tissue (Wendlandt, 1972; Feldberg, 1975). That this is not the explanation for the pyrexia following prostaglandin endoperoxide analogue injection is demonstrated by the observation that this pyrexia is unaffected by the potent prostaglandin synthetase inhibitor, indomethacin. Thus the endoperoxide pyrexia is due either to the analogue itself, or to the triggering off of a secondary response which does not involve prostaglandin synthetase.

That an analogue of prostaglandin endoperoxide is intrinsically pyrogenic is consistent with the recently formulated idea that prostaglandin is not an essential intermediate in fever and that other pyrogenic derivatives of arachidonic acid must exist. Cranston *et al.* (1975) showed that raised concentrations of prostaglandin E in the cerebrospinal fluid of rabbits was not a necessary accompaniment to fever, and more recent work (Cranston *et al.* 1976) has shown that leucocyte pyrogen fever is

unaffected by the simultaneous intraventricular injection of either of two prostaglandin antagonists, but that prostaglandin E_2 fever is effectively attenuated by these antagonists. Laburn *et al.* (1977) have shown that the pyrexia following sodium arachidonate injection is also unaffected by simultaneous administration of prostaglandin antagonists. Moreover the sodium arachidonate pyrexia was brought about by derivatives of arachidonic acid which are pyrogenic. One such derivative is prostaglandin. We now suggest that prostaglandin endoperoxide is also pyrogenic.

The second finding in our experiments is that there is an apparent delay of at least an hour in the onset of prostaglandin endoperoxide pyrexia, even at the highest dose. This is consistent with the idea that prostaglandin is the faster acting pyrogenic derivative of arachidonic acid (Laburn *et al.* 1977). The origin of the latency of response to endoperoxide remains unknown.

The results of our experiments are in conflict with the recent work of Hawkins & Lipton (1977), who concluded that injections of endoperoxide analogues into the anterior/preoptic hypothalamic region and into a presumed secondary control centre in the medulla oblongata of rats had no effect on rectal temperature. The doses per kilogram body weight of endoperoxide analogue used by Hawkins & Lipton were similar to those used in our experiments, and were in fact effective in causing a large rise in body temperature of the rats. Their results were not significant, however, because control injections of drug vehicle were equally hyperthermic.

In conclusion we suggest that the natural endoperoxides may be implicated in the production of fever in their own right. This suggestion would explain the findings that prostaglandin synthesis (and release into the cerebrospinal fluid) is not essential for fever and that antagonism of prostaglandin action does not reduce the febrile response to leucocyte pyrogen nor sodium arachidonate. It is possible that a third class of derivative, namely the thromboxanes, is pyrogenic as well. However, these compounds still elude study as they have a very short half-life in aqueous solution (Hamberg *et al.* 1975). The implication of the natural endoperoxide precursors in the production of fever rests on the assumption that the endoperoxide analogues have the same range of biological activity and mode of action as the naturally occurring endoperoxides. The validity of this assumption has not yet been shown conclusively, although there is evidence to suggest that it is probably the case (Wasserman, 1976).

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