Sites of clearance of leucocyte pyrogen in the rabbit

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(Received 3 July 1978; accepted 21 September 1978)

Summary

1. The dose–response curve for sustained infusions of leucocyte pyrogen has been demonstrated, and an optimum dose indicated for leucocyte pyrogen clearance experiments.

2. The lungs, liver and small bowel are not significantly involved in removal of leucocyte pyrogen from the circulation in conscious rabbits.

3. A single circulation through one kidney removes up to half of the infused dose of leucocyte pyrogen.

Key words: clearance sites, dose–response relationship, leucocyte pyrogen.

Introduction

There is a considerable body of evidence to suggest that fever due to inflammatory disease is mediated by a circulating endogenous pyrogen (Cranston, 1966). A pyrogen with similar properties can be produced in vitro by various cells in response to endotoxin, phagocytosis, or appropriate antigenic challenge (Atkins & Bodel, 1971), and is known as leucocyte pyrogen.

Lorber, Tenenbaum, Thurston, Gander & Goodale (1971) suggested that leucocyte pyrogen might be removed from the circulation by the liver; Sokal & Shimaoka (1967) detected a pyrogenic material in the urine of febrile patients with Hodgkin's disease. Apart from this, there has been no other evidence as to the sites of destruction of leucocyte pyrogen.

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We have examined the possible sites of clearance of leucocyte pyrogen by infusing it into different vascular beds. It is reasonable to assume that if any significant loss of leucocyte pyrogen takes place in an organ, a lesser fever will be produced by an infusion which passes through this organ before entering the general circulation, in comparison with an infusion which enters the general circulation directly. As a preliminary, it was necessary to establish the dose–response curve to systemic infusions of leucocyte pyrogen, to establish a dose that was not supramaximal; this has not previously been done.

Methods

Leucocyte pyrogen was obtained from leucocytes in sterile peritoneal exudates produced by the intraperitoneal injection of 200 ml of 3% starch in 155 mmol/l NaCl (Fessler, Cooper, Cranston & Vollum, 1961). At 18 h after injection, cells from the exudates were harvested, centrifuged, washed and suspended in a volume of 155 mmol/l NaCl equal to the volume of the peritoneal exudate. A sample was removed for cell counting, and the suspension incubated at 37°C for 18 h. It was centrifuged at 2000 g for 20 min, and the supernatant fluid containing leucocyte pyrogen was stored at 4°C. The same batch of leucocyte pyrogen was used in all experiments. The dose of leucocyte pyrogen was expressed as the number of leucocytes from which the injection volume was derived.

In preliminary experiments, it was shown that the preparation was free of endotoxin. Identical febrile responses were obtained from normal rabbits, and rabbits made refractory to endotoxin...
by the administration of a large dose of endotoxin 24 h before (Atkins & Snell, 1963). Had a significant amount of endotoxin contributed to the fever, the responses of refractory rabbits would have been smaller than those of normal rabbits.

The leucocyte pyrogen infusion dose–response assays were performed on six rabbits of mixed breeds and sex, weighing 2-4 kg. The rabbits were restrained in stocks in a temperature-controlled laboratory at 19-22°C, and rectal temperatures were measured at 10 min intervals by indwelling thermocouples, recording on a multichannel recorder (Digitec 1268). Infusions were started when temperatures had been stable for 30 min. Different doses were obtained by diluting the stock solution of leucocyte pyrogen; the order of doses was randomized, and experiments separated by at least 48 h. The rabbits were given sustained infusions of leucocyte pyrogen at 0.02 ml/min for 3 h, via a polyethylene cannula introduced into the marginal vein of the ear, the infusions being maintained by a motorized syringe. Each rabbit received six doses; the total doses given over 3 h were equivalent to 3 x 10^7, 1.5 x 10^7, 7.5 x 10^6, 3.75 x 10^6, 1.9 x 10^6 and 5 x 10^6 (10^{7.48}, 10^{7.18}, 10^{6.88}, 10^{6.59}, 10^{6.28} and 10^{6.09}) leucocytes. The temperature at the beginning of the infusion was taken as the reference temperature, and the responses were expressed as the sum of the temperature increments after this time: i.e. the area under the elevated portion of the temperature chart.

Four groups of at least six rabbits were used to determine the ability of the lungs, the liver, the small bowel and the kidney to remove leucocyte pyrogen. To infuse leucocyte pyrogen preferentially through these organs, cannulae were implanted into the jugular vein, the portal vein, the superior mesenteric artery, and the adrenolumbar artery, using techniques described previously (Cranston, Hellon, Riley & Townsend, 1977; Cranston, Hellon & Townsend, 1977; Akinkugbe, Brown & Cranston, 1966). Control infusions were given via cannulae implanted in left atrium (lungs), inferior vena cava (liver), and ear vein (small bowel and kidney). The order of infusions was randomized. At the end of each operation to implant adrenolumbar and superior mesenteric arterial cannulae, the cannulae were injected with Evans Blue, and adequate perfusion of the kidney and small bowel verified. When control infusions were given into the marginal vein of the ear, the dead space of the implanted cannula (into mesenteric or adrenolumbar artery) was filled with leucocyte pyrogen before the infusion was started.

Temperatures were measured for 3 h after the start of the infusion, and paired t-tests were performed on the mean fever heights at 10 min intervals, for each set of test and control infusions.

**Results**

The infusion dose–response curve is shown in Fig. 1. There is a sharp inflexion of the curve between doses obtained from 3.75 x 10^6 and 7.5 x 10^6 (10^{6.59} and 10^{6.88}) leucocyte equivalents. For this reason a standard dose of 7.5 x 10^6 (10^{6.88}) leucocyte equivalents was used.

There was no significant difference between the fevers resulting from test infusions of leucocyte pyrogen into jugular vein, hepatic portal vein or superior mesenteric artery, and their respective controls (Fig. 2). The fever resulting from infusion into the adrenolumbar artery was significantly smaller than that resulting from the control infusion into the ear vein (Fig. 3). The mean response to ear vein infusions was 2.7°C/h, and that to adrenolumbar infusions 1.32°C/h. Comparison with the dose–response curve indicates that at this dose, a single passage through one kidney removes up to half of the infused leucocyte pyrogen.

There was a suggestion that a slightly earlier febrile response was obtained from infusions into the jugular vein, as compared with infusions into the left atrium (Fig. 2c). This might have suggested some enhancement of leucocyte pyrogen activity within the lung in response to leucocyte pyrogen infusion. Further experiments were therefore done, comparing infusions equivalent to 3.75 x 10^6 (10^{6.59}) leucocytes, into the two sites. This dose was used as it should have revealed any increase in febrile response much more clearly than the

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**FIG. 1.** Temperature responses (increase in temperature over the first 3 h) to continuous infusions of leucocyte pyrogen. Abscissa: log numbers of leucocytes from which the infused doses of leucocyte pyrogen were obtained. Ordinate: increase in central temperature over 3 h (°C/h). Bars indicate ±1 SEM.
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Dose equivalent to $7.5 \times 10^6 \ (10^6^{88})$ leucocytes, by virtue of the shape of the dose–response curve. At this dose, there was no significant difference between febrile responses to infusions into the left atrium and jugular vein.

**Discussion**

The reason for the non-linear response of temperature to infusion of increasing concentrations of leucocyte pyrogen is not clear. Presumably it depends on the relationship between infusion rate, removal rate, volume of distribution and any active transport of leucocyte pyrogen from the circulation to the site of action. A dose of leucocyte pyrogen equivalent to $7.5 \times 10^6 \ (10^6^{88})$ leucocytes was chosen as the dose which would show the greatest difference in fever response, when compared with its control, if any clearance of leucocyte pyrogen were to take place.

From our results, we suggest that the lungs, the liver and the small bowel have no significant effect on leucocyte pyrogen, although it is possible that the small intestine removes a small amount (cf. Fig. 2a). The significant results for adrenolumbar artery infusions indicate that the kidney is the principal site of leucocyte pyrogen removal.

No attempt was made to investigate possible clearance by brain. It has previously been shown (King & Wood, 1958) that infusions of leucocyte pyrogen into the carotid artery caused larger febrile responses than intravenous infusions; this suggested that the site of action of leucocyte pyrogen lies within the territory of supply of the carotid artery.

The present observations would be compatible with those of Sokal & Shimaoka (1967), and with the observation of high concentrations of urinary radioactivity after intravenous injection of $^{125}$I-labelled leucocyte pyrogen (C. A. Dinarello, personal communication, 1978). Lorber et al. (1971) suggested that the isolated perfused liver could remove leucocyte pyrogen from the perfusate. Our results do not suggest any important activity of the liver in intact conscious animals.

Whether the kidney removes leucocyte pyrogen by inactivation or excretion is not yet clear.

**Acknowledgment**

We are grateful to Miss Suzanne Cox for secretarial assistance.

**References**


