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THE EFFECT OF A NEW ANTI-INFLAMMATORY DRUG, FLURBIPROFEN, ON THE RESPIRATORY, HAEMODYNAMIC AND METABOLIC RESPONSES TO *E. coli* ENDOTOXIN SHOCK IN THE CAT

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1 The intravenous administration of *E. coli* endotoxin (2.0 mg/kg) in cats anaesthetized with sodium pentobarbitone resulted in immediate pulmonary hypertension and reductions in lung compliance and systemic arterial P_{O_2}. These effects were abolished, or greatly reduced, by the prior intravenous administration of flurbiprofen in doses (100 and 250 μg/kg and 1.0 mg/kg) which were devoid of cardiovascular or metabolic effects. Flurbiprofen is thus the most active antipyretic-analgesic drug so far examined in this experimental model.

2 Production of lactate, characteristic of the severe, secondary endotoxin shock phase, was delayed only by the highest dose of flurbiprofen; hypotension, hypoglycaemia and the reduction in cardiac output which occurs during this phase, were unaffected.

3 These findings are discussed with reference to the treatment of the 'shocked lung' syndrome of human septicaemia.

Introduction

Marked pulmonary changes occur in patients with septic shock; there is pulmonary vascular obstruction (perhaps due, in part, to emboli, intravascular coagulation and platelet aggregation), interstitial pulmonary oedema, a reduction in lung compliance and disturbances in the ventilation-perfusion relationship (Pontoppidan, Geffin & Laver, 1971). These changes give rise to the so-called 'shock lung' syndrome. Similar changes have been described in rabbits following the administration of *Salmonella typhi* endotoxin (Gemer, Hayes, Ishikawa, Cuevas & Hirsch, 1973).

In a previous study (Parratt & Sturgess, 1974) we showed that indomethacin, in a single intravenous dose of 10 mg/kg, prevented the pulmonary hypertension and oedema that follows the administration of *E. coli* endotoxin in the cat. It also delayed the onset of the secondary shock phase, which is characterized in this species by systemic hypotension, a reduction in cardiac output and an increasingly severe metabolic acidosis (Parratt, 1973). However, one problem with using indomethacin in this experimental model is that it can cause substantial myocardial depression (Parratt & Sturgess, 1974). Although it is not known whether the pulmonary changes that occur in septic shock are modified by drugs like indomethacin it seemed worthwhile exploring the effect of other antipyretic-analgesic drugs on the pulmonary effects of endotoxin in the cat; such drugs might be more effective than indomethacin and cause less myocardial depression, an important consideration since myocardial function is often depressed in septic shock (Siegel & Fabian, 1967). This paper describes the effects, in experimental endotoxin shock, of flurbiprofen (2-(2-fluoro-4-biphenylyl)-propionic acid) a potent, orally active non-steroidal anti-inflammatory drug (Glenn, Rohloff, Bowman & Lyster, 1973; Adams, McCullough & Nicholson, 1975) which recently has been shown to be extremely active in inhibiting human platelet aggregation (Nishizawa, Wynalda, Suydam & Molony, 1973; Davies, Lederer, Spencer & McNicol, 1974; Sim, McCraw & Sim, 1975).

Methods

Cats of either sex were anaesthetized with sodium pentobarbitone (36 mg/kg body weight, intraperitoneally), with additional 6 mg doses intravenously given as required throughout the course of the experiment to maintain anaesthesia. A femoral vein was cannulated for the administration of endotoxin and drugs, and 300 units/kg of heparin was given intravenously before proceeding with further surgery. Systemic arterial pressure was recorded with

a capacitance transducer (Elema-Schönander EMT 35) from a catheter inserted, by way of the right carotid artery, such that the tip lay in the aortic arch. Mean pressure was obtained by electronic integration and aortic dP/dt was continuously determined with an Elema-Schönander differentiator. Pulmonary artery ('downstream') pressure was recorded, following a left thoracotomy, via a needle-tipped catheter inserted directly through the wall of the artery, and connected to a Statham P23Db transducer.

In all cats the trachea was cannulated with a glass T-piece cannula and the animals respired with room air using a Palmer positive pressure ventilation pump at a rate of 20 per minute. The stroke volume was adjusted to approximately 20 ml/kg body weight, in order to give an arterial P_{O_2} of over 80 mmHg, and was usually within the range 40–60 ml. In order to measure the intratracheal pressure, a polythene catheter was sealed into the side arm of the cannula, and connected to a Statham P23Db transducer. At various times during these experiments, in order to assess changes in airway dynamic compliance, the respiratory stroke volume was readjusted to different levels, and the corresponding change in intratracheal pressure measured; at least 20–30 min were allowed to elapse after this procedure before any attempt was made to record cardiac output or to sample blood.

Cardiac output was measured by thermodilution as previously described (Parratt, 1973).

Systemic arterial pressure, pulmonary artery pressure, aortic dP/dt, and the electrocardiogram (standard lead II) were recorded simultaneously on a display oscilloscope (Racal Instruments) and on an ink-jet writing recorder (Elema-Schönander mingograph 81).

Blood samples (1.0–2.0 ml) were withdrawn from the arterial catheter to be analysed for O_2 and CO_2 tensions, and pH, using appropriately calibrated systems (Radiometer, Copenhagen). The pH electrode was calibrated by means of standard buffers, and the O_2 and CO_2 electrodes with gas mixtures, the concentrations of which had been measured with a modified Lloyd-Haldane apparatus. O_2 and CO_2 tensions, and pH, were corrected for any temperature difference between the animals' mid-oesphageal temperature and the electrode temperature (usually 37.2°C), by means of the blood gas calculator described by Severinghaus (1966). Arterial lactate and glucose were measured as described previously (Parratt & Sturgess, 1974).

After a period of stabilization, the cats were given *E. coli* endotoxin (Difco Laboratories 055:B5) suspended in 0.9% w/v sodium chloride solution (saline), and administered slowly by intravenous injection in a dose of 2 mg/kg. This dose kills 92% of the animals within 6 h (Parratt & Sturgess, unpublished results). Three groups, each of five cats, were administered sodium flurbiprofen, in doses of 100 μg/kg, 250 μg/kg, and 1 mg/kg respectively, 30 min before the endotoxin; the responses were compared with those of a group of eight cats given endotoxin alone. Measurements were made of the haemodynamic and metabolic parameters until either the animals died, or the experiments were terminated 6 h post-endotoxin.

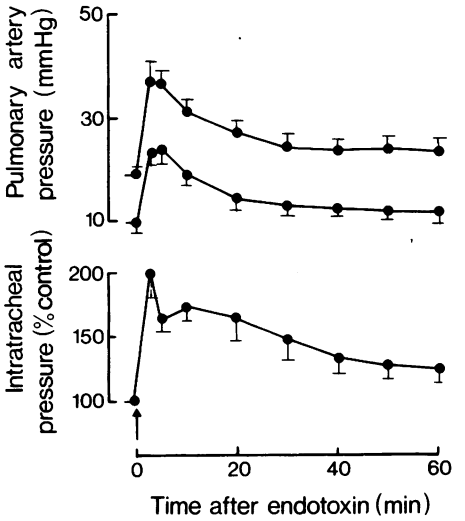

Figure 1 The effects of *E. coli* endotoxin (2 mg/kg) on pulmonary arterial and intratracheal pressures in the cat. The initial response to endotoxin consisted of marked pulmonary hypertension, and an increase in intratracheal pressure at a constant volume lung inflation (i.e. a decreased compliance). Values are means, vertical lines show s.e. means.

Results

Responses to E. coli *endotoxin*

Within 30 s of the completion of the injection, and often whilst it was still in progress, pulmonary artery pressure rose, attaining a maximum after 3–5 min (Figure 1). Thereafter, pulmonary artery pressure tended to return slowly towards normal, although it remained slightly, but significantly, elevated up to 2 h after endotoxin administration. The rise in pulmonary pressure preceded by a few seconds a parallel increase in intratracheal pressure (Figure 1). The maximum, however, occurred somewhat earlier than that of the pulmonary pressure; expressing intratracheal pressure as a percentage of the pre-endotoxin value, it was $199 \pm 23\%$ 2 min after endotoxin, and $172 \pm 21\%$ 4 min afterwards. Endotoxin greatly reduced pulmonary dynamic compliance (as assessed 30 and 60 min after the injection from curves relating intratracheal pressure to respiratory stroke volume) and this is illustrated in Figure 2. These pulmonary

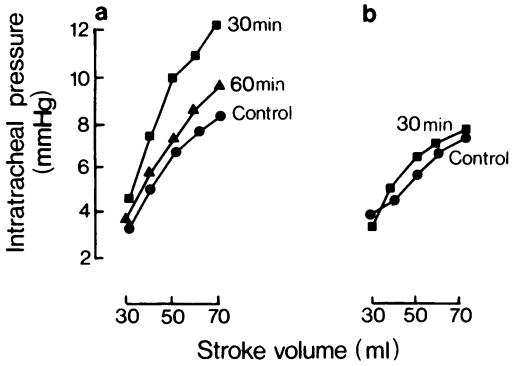

Figure 2 (a) Pulmonary compliance curves before (0), and 30 and 60 min after, the administration of endotoxin. Each curve is the mean of from 3 to 5 experiments in different animals. An increase in intratracheal pressure at given fixed respiratory stroke volumes indicates a reduction in pulmonary compliance. (b) The effect of endotoxin in 5 cats pretreated with sodium flurbiprofen (100 μg/kg); the endotoxin-induced reduction in compliance is almost completely prevented.

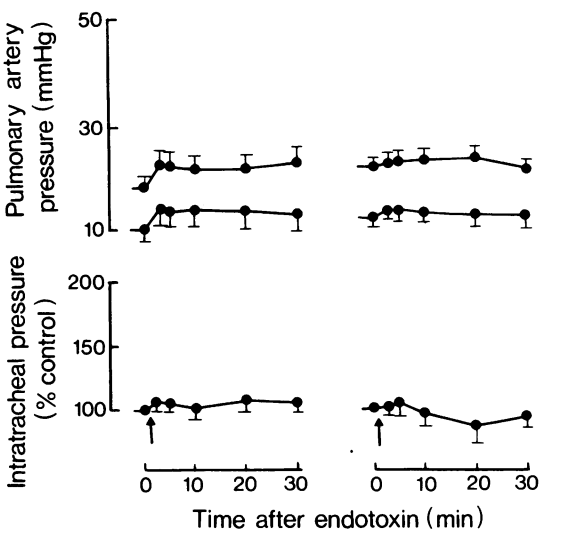

Figure 3 Modification by pretreatment with sodium flurbiprofen (100 μg/kg on the left and 250 μg/kg on the right) of the responses to endotoxin (at the arrow). There is an almost complete abolition of the pulmonary effects (compare endotoxin-alone responses in Figure 1).

changes also affected gas exchange; during the initial hour following endotoxin administration the systemic arterial Po_2 was reduced by 22 ± 5.7 mmHg.

Beginning a few seconds after the start of the rise in pulmonary artery pressure, and usually after that of the intratracheal pressure, carotid artery pressure fell rapidly, often to levels below 50 mmHg. This was only a transient effect, much shorter in duration than that on the pulmonary circulation. Carotid artery dP/dt *max* showed a similar transient decrease. Heart rate decreased with the fall in systemic arterial pressure, and ventricular extrasystoles were observed in a number of experiments. These parameters returned to control values within 3–4 min, but there was a decline in cardiac output and in systemic pressure over the next 3–5 h, results similar to those described in detail in a previous publication (Parratt & Sturgess, 1974).

Endotoxin administration resulted in a substantial metabolic acidosis, the arterial pH falling from a pre-endotoxin value of 7.532 ± 0.035 to 7.246 ± 0.034 at 1 h ($P < 0.001$), to 7.135 ± 0.056 at 2 h ($P < 0.001$) and 7.140 ± 0.082 at 3 h ($P < 0.001$). This was associated with a two- to eight-fold increase in arterial blood lactate concentrations; e.g. 5.4 ± 0.5 mg/100 ml pre-endotoxin, to 39.7 ± 4.6 mg/100 ml at 2 hours. Blood glucose progressively declined after endotoxin administration (115 ± 11 mg/100 ml control; 97 ± 20 mg/100 ml at 2 h (NS) and 59 ± 21 mg/100 ml ($P < 0.005$) at 3 hours).

Direct effects of sodium flurbiprofen

Sodium flurbiprofen (0.1, 0.25, and 1.0 mg/kg) had no significant effect on systemic arterial pressure and dP/dt *max*, pulmonary artery pressure, heart rate, cardiac output, intratracheal pressure or lung compliance. Flurbiprofen was also without a significant effect on arterial lactate, glucose, pH, or Po_2. These results are in contrast to those previously obtained with indomethacin (Parratt & Sturgess, 1974), which caused substantial reductions in systemic and pulmonary artery blood pressures, and in cardiac output over a 30 min period.

The effects of sodium flurbiprofen on the responses to endotoxin

The acute response to endotoxin was markedly reduced by all three doses of sodium flurbiprofen. There were only transient decreases of carotid artery pressure and dP/dt, which were much less than in those animals given endotoxin alone. The pulmonary responses to endotoxin were almost completely abolished, even with a dose of sodium flurbiprofen as low as 100 μg/kg (Figure 3). For example, in the endotoxin control animals, increases in pulmonary artery pressure of 20–30 mmHg were observed, whilst in those given flurbiprofen before the endotoxin, the changes were usually less than 5 mmHg. The reduction in pulmonary dynamic compliance, which is a characteristic of the feline response to endotoxin, was abolished by pretreatment with sodium flurbiprofen (Figure 2) and there was no reduction in arterial Po_2 (Table 1).

Although flurbiprofen greatly reduced the initial response of cats to endotoxin, it had little significant effect on the secondary, delayed, shock phase (characterized in this species by systemic hypotension, decreased cardiac output and a pronounced metabolic acidosis; Parratt, 1973; Parratt & Sturgess, 1974). In the control group the carotid blood pressure had fallen from 118 ± 8 mmHg (systolic) and 89 ± 9 mmHg (diastolic) to 89 ± 2 mmHg and 49 ± 10 mmHg 3 h after endotoxin ($P < 0.01$) and the heart rate had increased from 201 ± 16 beats/min to 233 ± 4 beats/minute. The corresponding values 3 h after endotoxin in the flurbiprofen group (1.0 mg/kg) were 85 ± 15 mmHg, 57 ± 15 mmHg and 199 ± 22 beats/minute. However, there was some evidence that the metabolic deterioration induced by endotoxin was slightly delayed in the cats given this dose of flurbiprofen (Table 1). Thus at 1 h the pH in the endotoxin-alone group was 7.246 ± 0.034 (lactate level 24.2 ± 2.2 mg/100 ml) compared with 7.397 ± 0.066 (lactate level 13.1 ± 3.3 mg/100 ml) in the flurbiprofen group ($P < 0.01$). At 3 h there was no significant difference between the two groups and flurbiprofen did not improve survival rate assessed at 4 h or 6 hours. Endotoxin-induced hypoglycaemia which has recently been correlated with lethality in endotoxin shock (Hinshaw, Benjamin, Coalson, Elkins, Taylor, Price, Smith & Greenfield, 1975) was unaffected by pre-treatment with flurbiprofen (Table 1).

Table 1 Effect of sodium flurbiprofen (1 mg/kg) on the metabolic response to *E. coli* endotoxin

Time after endotoxin	*0 h*	*1 h*	*2 h*	*3 h*	*4 h*
Arterial blood:					
pH (units)	7.538 ± 0.027	7.397 ± 0.066†	7.307 ± 0.094*†	7.215 ± 0.173*	7.075 ± 0.151*
P_{O_2} (mmHg)	91.4 ± 4.0	95.4 ± 6.8**	99.0 ± 8.1	96.7 ± 13.8	103.4 ± 16.7
Lactate (mg/100 ml)	10.30 ± 4.04	13.06 ± 3.29†	29.41 ± 7.84*	31.53 ± 10.24	43.97 ± 9.20*
Glucose (mg/100 ml)	92 ± 17	93 ± 30	72 ± 21	57 ± 11*	33 ± 15*

** Rise in P_{O_2} during first hour = 3.1 ± 3.5 mmHg ($P < 0.01$ *vs.* endotoxin alone where there was a reduction of 22.6 ± 5.7 mmHg).
* $P < 0.05$ difference from pre-endotoxin (0 h) value; † $P < 0.05$ difference between treated and non-treated animals.

Discussion

In the cat the acute administration of *E. coli* endotoxin results in marked pulmonary hypertension (Parratt, 1973 and Figure 1 of the present paper) and this has now been shown to be accompanied by a considerable increase in intratracheal pressure at a fixed respiratory volume (Figure 1). A study of the relationship between intratracheal pressure and respiratory volume 30 min after endotoxin administration (when the end-inspiratory intratracheal pressure was still nearly 150% of control) clearly showed reductions in lung dynamic compliance (Figure 2). There was obvious respiratory distress and pulmonary oedema. This resulted in a decrease in systemic arterial P_{O_2} even at 1 h after endotoxin when pulmonary arterial and intratracheal pressures were only slightly, though significantly, elevated. There is some evidence that these pulmonary changes are mediated partly through the release of a prostaglandin (Parratt & Sturgess, 1975a). Flurbiprofen reduced these endotoxin-induced pulmonary changes in doses as low as 100 μg/kg (Figure 3). This makes the drug several hundred times more active than indomethacin (Parratt & Sturgess, 1974) and about ten times more active than sodium meclofenamate (Parratt & Sturgess, 1975b) in this experimental model. This is in line with the results of

other tests (anti-inflammatory, analgesic and antipyretic; Adams *et al.*, 1975). Furthermore, the protection afforded against the endotoxin-induced pulmonary changes was obtained with doses that had no depressant effects on the cardiovascular system. There appear to be two, possibly related, explanations for this protective effect; inhibition of prostaglandin synthetase (and hence prevention of the synthesis and release of a pulmonary constrictor prostaglandin) and prevention of endotoxin-induced platelet aggregation. It is of interest in this connection that the activity of flurbiprofen in inhibiting collagen-induced platelet aggregation (Nishizawa *et al.*, 1973) approaches that of prostaglandin E_1, the most potent known inhibitor of platelet aggregation.

Despite the protection afforded by flurbiprofen in the initial (pulmonary) phase of endotoxin shock, there appeared to be little modification of the secondary shock phase, apart from a delay in the appearance of the metabolic consequences of endotoxaemia (Table 1). Flurbiprofen did not prevent the onset of the metabolic acidosis nor the pronounced hypoglycaemia, which has been shown by Hinshaw's group to be a relatively early event in the shock process and which is correlated with the progressive development of the irreversible state (Hinshaw *et al.*, 1975). When administered before endotoxin, flurbiprofen clearly had no effect on the severity or development of the delayed shock process. A point of difference with results obtained with indomethacin (Parratt & Sturgess, 1974) and with sodium meclofenamate (Parratt & Sturgess, 1975c) was the absence of any sustained rise in blood pressure following the administration of endotoxin, an effect ascribed to inhibition of prostaglandin synthetase.

Although pulmonary changes are often marked in patients with septic shock (reduction in lung compliance and increase in pulmonary resistance) we know of no pathological studies of the lesions that result from endotoxin administration in the cat. Such a comparative study of the pulmonary lesions in experimental endotoxin shock and in septic shock patients has recently been started (Clements, Parratt & Rodger, unpublished). Clearly, if the lesions are similar and have a common pathogenesis (active airway constriction by a prostaglandin $F_{2\alpha}$-like mediator or mechanical obstruction with aggregating platelets) there is a case for an investigation of flurbiprofen as an adjunct to the therapy of septic shock.

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