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THE POSSIBLE ROLES OF HISTAMINE, 5-HYDROXYTRYPTAMINE AND PROSTAGLANDIN $F_{2\alpha}$ AS MEDIATORS OF THE ACUTE PULMONARY EFFECTS OF ENDOTOXIN

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1 In an attempt to investigate the possible role of released vasoactive substances in mediating the pulmonary pressor responses to *E. coli* endotoxin, cats were pretreated with histamine, 5-hydroxytryptamine (5-HT) or prostaglandin antagonists, with a histamine depleting agent (compound 48/80) or with an inhibitor of prostaglandin synthetase (sodium meclofenamate).

2 The administration of endotoxin (2 mg/kg) resulted in a rapidly developing pulmonary hypertension (pressure twice normal after 2–3 min), increases in right atrial and intratracheal pressures, systemic hypotension and bradycardia. These effects were unaffected by methysergide in a dose sufficient to prevent the effects of intravenously administered 5-HT.

3 Endotoxin responses were also unaffected by a combination of mepyramine and burimamide in doses sufficient to reduce markedly the effects of intravenously-administered histamine. In cats pretreated (chronically or acutely) with compound 48/80, endotoxin induced a transient pulmonary pressor response which was not maintained.

4 The pulmonary and systemic responses to endotoxin were prevented by the prior administration of the prostaglandin antagonist, polyphloretin phosphate and by pretreatment with the prostaglandin synthetase inhibitor, sodium meclofenamate.

5 It is concluded that a pulmonary vasoconstrictor prostaglandin is involved in the acute response to endotoxin in the cat.

Introduction

A large number of vasoactive agents are released, in a number of species, following the intravenous administration of endotoxin. These include histamine (Weil & Spink, 1957; Hinshaw, Jordan & Vick, 1961; Hinshaw, 1964), 5-hydroxytryptamine (5-HT) (Armin & Grant, 1957; Davies, McQuarrie & Meeker, 1959), angiotensin (Hall & Hodge, 1971), adrenaline and/or noradrenaline (Nykiel & Glaviano, 1961; Hökfelt, Bygdeman & Sekkenes, 1962; Hall & Hodge, 1971) and 'a cholinergic substance' (Vick, 1965). There is also early activation of the kallikrein-kinin system (Erdös & Miwa, 1968; Nies, Forsyth, Williams & Melmon, 1968; Al-Kaisi, Parratt, Siddiqui & Zeitlin, 1976). More recently, there has been increasing evidence, in a number of species for the release by endotoxin of both E and F prostaglandins (Collier, Herman & Vane, 1973; Kessler, Hughes, Bennett & Nadela, 1973; Herman & Vane, 1974; 1976; Anderson, Jubiz, Tsagaris & Kuida, 1975a; Anderson, Tsagaris, Jubiz & Kuida, 1975b; Herman & Moncada, 1975; Korbut, Ocetkiewicz & Gryglewski, 1975).

The relevance of the release of so many highly active substances to the pathophysiology of endotoxin shock is still not clear despite attempts (Vick, 1960; 1965; Vick, Mehlman & Heiffer, 1971) to correlate early cardiovascular changes with the release of one or other of these agents. There are at least two possible approaches to the problem: (1) the detection of vasoactive agents in blood following endotoxin administration; (2) the classical pharmacological approach, of prevention of synthesis or release, and antagonism of effects of released vasoactive agents. The latter is the approach used in the present experiments. Particular attention has been paid to the initial pulmonary effects of endotoxin in the cat (hypertension, oedema and the reduction in pulmonary compliance) because of the possible relevance to the marked pulmonary changes that occur in patients with septic shock (Pontoppidan, Geffin & Laver, 1971), a condition in which circulating endotoxin levels are often high (Ledingham & McCartney, personal communication).

Three possible vasoactive agents have been examined, histamine, 5-HT and a prostaglandin of the F series. Each has pulmonary effects similar to

endotoxin and there is evidence that each is released, at least in some species, following endotoxin administration. A preliminary account of some of the experiments described in this paper was given to a meeting of the Physiological Society (Parratt & Sturgess, 1975a).

Methods

Cats, of either sex, were anaesthetized with an intraperitoneal injection of sodium pentobarbitone (35 mg/kg), were subjected to thoracotomy and ventilated with room air by means of a Palmer positive-pressure pump. Systemic (carotid) arterial pressure and d*P*/d*t*, pulmonary arterial pressure, right atrial pressure, cardiac output and stroke volume, systolic ejection time, intratracheal pressure, arterial blood gases and pH and arterial lactate and glucose concentrations were measured as described previously (Parratt, 1973; Parratt & Sturgess, 1976).

Escherichia coli endotoxin (Difco Laboratories 055:B5) in a dose of 2 mg/kg, suspended in 0.9% w/v NaCl solution (saline), was injected slowly, over a period of 40–45 s, into a catheter inserted in a femoral vein. Twenty-three cats were given endotoxin only and served as the control group. Particular attention was paid to the initial haemodynamic response and a continuous record was obtained of effects occurring in the first 10 min; thereafter recordings were made every 5 min up to 1 h and then every 30 min until the animal died.

Experiments with antagonists of vasoactive agents released by endotoxin

The following drugs were administered intravenously prior to endotoxin: (1) A combination of mepyramine (2 mg/kg) and burimamide (2 mg/kg), to antagonize the effects of released histamine (6 cats); or (2) methysergide (1 mg/kg), to antagonize the effects of released 5-HT (6 cats) or (3) polyphloretin phosphate, in doses up to 200 mg/kg to antagonize some of the cardiovascular effects of released prostaglandins (6 cats).

Polyphloretin phosphate (a generous gift from Dr B. Hogberg of A.B. Leo, Halsingborg, Sweden) was dissolved in 0.1 N NaOH and injected slowly intravenously over 5 to 10 minutes.

Experiments with compound 48/80

Two groups of cats were administered compound 48/80: (i) the drug was administered acutely to 7 anaesthetized cats in gradually increasing doses (1, 2, 5, 10, 20 and 50 μg/kg by intravenous injection) until the typical haemodynamic responses (hypotension, and increases in pulmonary artery pressure, heart rate and carotid d*P*/d*t max;* Table 4) were abolished or markedly attenuated (i.e. until the animals became refractory to further injections of the drug); (ii) four cats were treated with compound 48/80, by intraperitoneal injection over four days, according to the schedule described by Waton & West (1966). This procedure reduced abdominal skin (mast cell) histamine by about 90% (see Results section) but had little effect on the histamine content of the intestine or lung.

Twenty-four hours after the last injection the cats were anaesthetized and prepared as described above. Biopsies taken from the skin of the abdomen, the lung and from the first part of the jejunum, were extracted and assayed for histamine as outlined by Waton & West (1966). At the end of the experiments (1 to 2 h after the administration of endotoxin) small pieces of abdominal skin, lung and small intestine were again removed and their histamine content determined, in order to see whether endotoxin itself released histamine.

Experiments with sodium meclofenamate

Sodium meclofenamate was given to 7 cats, in an intravenous dose of 2 mg/kg, 30 min before the intravenous administration of endotoxin. This dose was chosen because in preliminary experiments it had been found (Parratt & Sturgess, 1975b) to abolish the initial effects of endotoxin even when given up to 5 h beforehand.

Results

Haemodynamic effects of E. coli *endotoxin*

The intravenous administration of endotoxin resulted in marked haemodynamic changes within 1 min of the injection and indeed, often whilst the injection was still in progress. The main effects were increases in pulmonary artery and intratracheal pressures, a transient bradycardia and a systemic hypotension. The results from the 23 cats are summarized in Table 1. They were compared with those obtained when the same dose (and batch) of endotoxin was administered to cats pretreated with drugs which would either block the effects of released vasoactive substances or which would interfere with their storage or synthesis.

The possible role of released histamine

(a) *Evidence from experiments using a combination of H_1 and H_2-receptor blocking agents:*

(i) *Responses to histamine infusions, and to injections of compound 48/80.* Black, Owen & Parsons (1975),

Owen (1975) and Flynn & Owen (1975), have clearly demonstrated that histamine-induced vasodepression is only partially inhibited by H_1 and H_2-receptor antagonists given separately. We therefore gave a combination of mepyramine and burimamide. When given together, these drugs had no significant effect on diastolic pressure (74 ± 8 mmHg before the injection and 76 ± 11 mmHg 1 min afterwards; 1 mmHg $\simeq$133Pa), on mean pulmonary artery pressure (12.4 ± 0.5 and 12.4 ± 1.1 mmHg) or on systolic ejection time (118 ± 6 and 127 ± 7 milliseconds). Heart rate was slightly, although not significantly, reduced (from 226 ± 14 to 192 ± 15 beats/minute). The haemodynamic responses to intravenous infusions of histamine (5 μg kg^{-1} min^{-1}) were almost completely abolished by this combination of antagonists (Table 2). The haemodynamic effects of released histamine were also markedly reduced. For example, compound 48/80 (10 μg/kg) lowered diastolic blood pressure by a mean of 34 mmHg before the administration of the two blocking drugs and by only 7 mmHg afterwards; the corresponding figures for carotid dP/dt *max* were $^{+}578$ $mmHgs^{-1}$ and $^{+}139$ $mmHgs^{-1}$ and, for pulmonary artery pressure, $^{+}1.8$ mmHg and 0.3 mmHg. We expected on the basis of these results that this combination of antagonists would greatly reduce responses to *E. coli* endotoxin if these were in fact due to histamine release.

(ii) *Responses to* E. coli *endotoxin.* The initial pulmonary hypertension and systemic vasodepression that resulted from the administration of endotoxin were in no way modified by the combination of mepyramine and burimamide (Table 3) and the time course of the effects was similar to that found in cats administered endotoxin alone (Figure 1). For example, the systemic and pulmonary pressures 1 h after endotoxin in the cats pretreated with mepyramine and burimamide (74 ± 4 mmHg and 13 ± 1.2 mmHg respectively) were similar to those obtained following endotoxin in the control group of cats (75 ± 8 mmHg and 15.6 ± 2.0 mmHg). The 'delayed' effects of endotoxin (reduced cardiac output, lactate production, hypoglycaemia) were also apparent in the cats treated with the blocking drugs. Thus after 3 h the cardiac output was reduced from 119 ± 16 to 70 ± 8 ml min^{-1} kg^{-1} ($P < 0.05$), the stroke volume from 1.61 ± 0.24 to 0.93 ± 0.18 ml/beat, and the arterial glucose from 144 ± 28 to 66 ± 25 mg/100 ml; arterial lactate had risen from 11.7 ± 2.7 to 30 ± 6.8 mg/100 ml. These changes were similar to those observed in cats administered *E. coli* endotoxin alone. However, there was an indication that survival rate was increased by the combination of mepyramine and burimamide; 2 of the 6 cats were alive at 6 h compared to 2 out of the 23 administered endotoxin only.

Table 1 The acute haemodynamic effects of *E. coli* endotoxin in anaesthetized cats

	Time after endotoxin administration (min)											
	0		*1*		*3*		*4*		*5*		*10*	
Carotid artery pressure:												
systolic (mmHg)	110 ± 5	(23)	73 ± 10	(17)*	98 ± 10	(17)	110 ± 11	(15)	112 ± 8	(22)	102 ± 5	(22)
diastolic	83 ± 5	(23)	50 ± 8	(17)**	61 ± 9	(17)**	73 ± 8	(15)	77 ± 6	(22)	71 ± 6	(22)
Pulmonary artery pressure:												
systolic (mmHg)	18.8 ± 0.9	(12)	26.1 ± 2.2	(11)*	36.9 ± 3.8	(10)**	41.3 ± 4.5	(8)**	36.3 ± 4.5	(8)**	30.8 ± 2.6	(10)
diastolic (mmHg)	9.4 ± 0.4	(12)	17.8 ± 2.4	(11)*	23.2 ± 2.7	(10)**	26.0 ± 2.9	(8)**	23.1 ± 2.1	(12)**	18.6 ± 1.7	(10)
Right atrial pressure (mean, mmHg)	0.9 ± 0.3	(11)	1.7 ± 0.2	(5)**	2.7 ± 1.3	(5)	2.7 ± 1.2	(8)	1.2 ± 1.1	(8)	–	
Heart rate (beats/min)	196 ± 8	(23)	174 ± 15	(17)	160 ± 16	(17)**	199 ± 17	(14)	196 ± 11	(20)	201 ± 9	(22)
Intratracheal pressure (% pre-endotoxin level)	100	(5)	191 ± 26	(5)**	180 ± 20	(5)**	172 ± 21	(5)**	164 ± 9	(5)*	174 ± 12	(5)*

Values are mean ± s.e. mean. Number of experiments in parentheses.
* $P < 0.001$; ** $P < 0.01$.

(b) *Evidence from the use of compound 48/80*

(i) *Results from cats given cumulative single injections.* Compound 48/80 was given in increasing doses by intravenous injection until the haemodynamic effects were abolished or markedly attenuated. Small doses (1–5 μg/kg) usually slightly elevated both systemic and pulmonary arterial pressures; higher doses (10–50 μg/kg) reduced systemic arterial pressure but still induced pulmonary hypertension (Table 4). Carotid d*P*/d*t max* and heart rate were always increased. The final effects of the cumulative intravenous administration of the drug were a significantly reduced systemic arterial pressure (Table 4), a reduced cardiac output (from 244 ± 17 to 127 ± 11 ml/min) and stroke volume (from 2.8 ± 0.4 to 1.8 ± 0.15 ml/beat); there were increases in arterial lactate (from 7.0 ± 1.3 to 32.6 ± 7.9 mg/100 ml) and glucose (from 106 ± 23 to 224 ± 67 mg/100 ml).

The effect of administering endotoxin to these cats is shown in Figure 2. Systemic hypotension and bradycardia still occurred and, in the first few minutes, there was an elevation of pulmonary artery pressure (from 13.0 ± 1.4 mmHg to 22.4 ± 4.8 mmHg after 1 min, 19.8 ± 5.0 mmHg after 2 min and 18.6 ± 4.9 mmHg after 3 minutes). However, the pulmonary artery pressure 5 min after endotoxin (13.9 ± 2.0 mmHg) was not significantly different from the pre-endotoxin level. The transient nature of this pulmonary hypertensive response was in contrast to the response in the control animals (Table 1). All the cats pretreated with compound 48/80 exhibited a raised arterial lactate 1 h after endotoxin (48.1 ± 10.3 mg/100 ml) and none of the 6 animals were alive at 4 hours.

(ii) *Results from cats pretreated chronically with compound 48/80.* Only 3 of the 4 cats survived the dosage schedule outlined in the Methods section. Biopsies taken from 3 of the animals before the administration of endotoxin showed that skin histamine was greatly depleted (values of 0.47, 0.23 and 1.25 μg/g). The histamine content of the lung (24,

Table 2 Haemodynamic effects of intravenous infusions of histamine (5 μg kg^{-1} min^{-1}) before, and after, the administration of mepyramine (2 mg/kg) plus burimamide (2 mg/kg)

	Before		*After*	
	Control	*Change*	*Control*	*Change*
Carotid artery pressure:				
systolic (mmHg)	102 ± 11	−24 ± 5 (6)	103 ± 8	−3 ± 1 (6)*
diastolic (mmHg)	75 ± 10	−37 ± 8 (6)	71 ± 8	−5 ± 1 (6)*
Pulmonary artery pressure:				
systolic (mmHg)	18.1 ± 0.8	+3.7 ± 1.5 (6)	19.0 ± 1.0	+0.8 ± 0.2 (6)**
diastolic (mmHg)	9.5 ± 0.4	+1.0 ± 0.5 (6)	9.7 ± 0.4	+0.2 ± 0.2 (6)†
Heart rate (beats/min)	219 ± 17	+21 ± 6 (6)	206 ± 13	+2 ± 5 (6)**

* $P < 0.001$; ** $P < 0.02$; † $P < 0.05$ compared with change induced before the histamine antagonists.

Table 3 The acute haemodynamic effects of endotoxin in anaesthetized cats when administered after a combination of mepyramine (2 mg/kg) plus burimamide (2 mg/kg)

	Time after endotoxin administration (min)					
	0	*1*	*3*	*4*	*5*	*10*
Carotid artery pressure:						
systolic (mmHg)	98 ± 7	73 ± 16	91 ± 12	113 ± 13	106 ± 9	90 ± 6
diastolic (mmHg)	66 ± 6	46 ± 14	53 ± 13	74 ± 13	70 ± 10	53 ± 4
Pulmonary artery pressure:						
systolic (mmHg)	18.8 ± 1.3	32.3 ± 3.0**	36.2 ± 3.9**	32.2 ± 3.3**	30.6 ± 3.4**	29.7 ± 4.2†
diastolic (mmHg)	9.2 ± 0.4	23.4 ± 3.3**	23.5 ± 2.3*	21.4 ± 2.7*	20.6 ± 3.2**	18.0 ± 2.8†
Heart rate (beats/min)	215 ± 15	175 ± 25	201 ± 12	236 ± 30	226 ± 26	223 ± 26

Results are mean of 6 experiments ± s.e. mean.
*$P < 0.001$; ** $P < 0.01$; † $P < 0.02$.

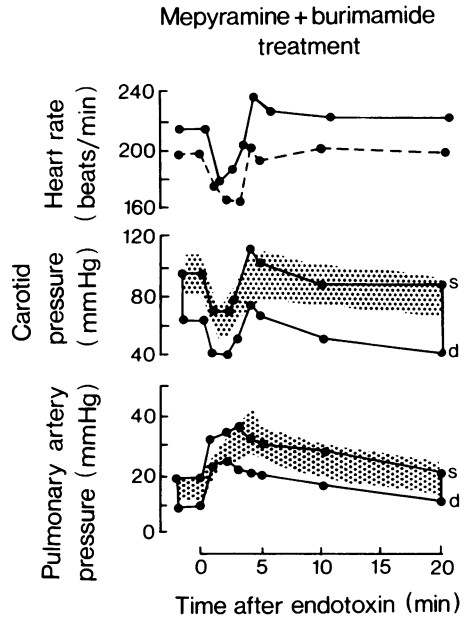

Figure 1 The effects of *E. coli* endotoxin (2 mg/kg; at time zero) on heart rate, carotid artery and pulmonary artery pressures (s = systolic; d = diastolic) when administered after a combination of mepyramine and burimamide (2 mg/kg of each; ●—●). The values are the means obtained from 6 animals; standard errors have been omitted for clarity. The shaded areas and points joined with broken lines (●- -●) in this and in Figures 2–6 represent the effects of endotoxin in 11–22 control (untreated) cats. It is clear that a combination of H_1 and H_2-receptor blocking agents does not modify the haemodynamic effects of endotoxin.

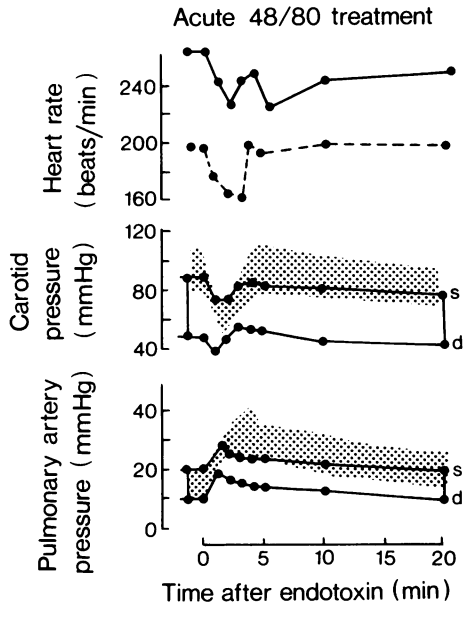

Figure 2 The haemodynamic effects of endotoxin (2 mg/kg at time zero) in cats treated acutely with compound 48/80 to deplete mast cells of histamine (●—●). The values are the means obtained from 7 experiments; standard errors have been omitted for clarity. The effects of endotoxin are reduced and the rise in pulmonary artery pressure is not sustained compared with control cats administered endotoxin (shaded areas).

Table 4 Haemodynamic responses to cumulative single intravenous injections of compound 48/80

	Dose of 48/80 (Change from pre-injection level)*				
	Control	*5 μg/kg*	*10 μg/kg*	*20 μg/kg*	*Post-cumulative administration†*
Carotid artery pressure:					
systolic (mmHg)	130 ± 8	+5 ± 3	−8 ± 5	−16 ± 7	89 ± 6**
diatolic (mmHg)	99 ± 7	+5 ± 3	−18 ± 8	−31 ± 11	48 ± 8**
Pulmonary artery pressure:					
systolic (mmHg)	23.7 ± 2.2	+1.5 ± 0.7	+3.8 ± 1.4	+5.2 ± 2.0	21.0 ± 1.6
diastolic (mmHg)	13.0 ± 1.4	+0.8 ± 0.3	+1.1 ± 0.5	+1.5 ± 0.6	10.3 ± 1.5
Heart rate /beats/min)	228 ± 19	+5 ± 4	+14 ± 5	+23 ± 12	264 ± 13

Results are means of 7 experiments ± s.e. mean.
* Apart from the systemic arterial and heart rate responses to a dose of 5 μg/kg all of the changes induced by compound 48/80 were significantly ($P < 0.001$) different from those induced by an equal volume of saline.
† Values are those obtained 30–40 min after the cumulative administration of 200 μg/kg compound 48/80 and immediately before administration of endotoxin. ** $P < 0.01$.

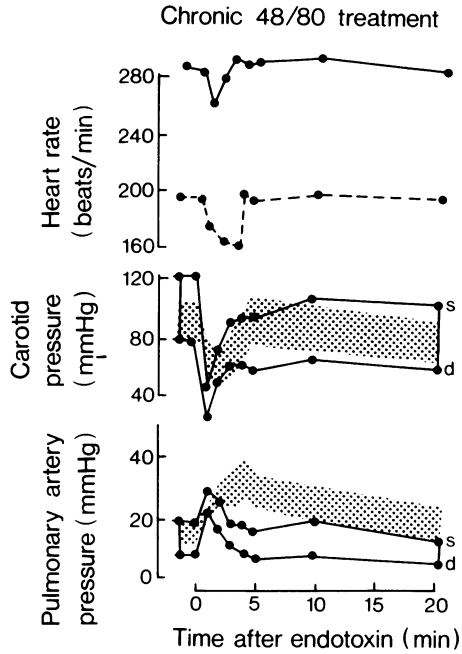

Figure 3 The haemodynamic effects of endotoxin (2 mg/kg at time zero) in 3 cats treated over 4 days with compound 48/80 in order to deplete mast cells of histamine (●——●). The systemic hypotensive effect of endotoxin is unaffected by this treatment but the marked elevation of pulmonary artery pressure observed in control cats (shaded areas) is not sustained.

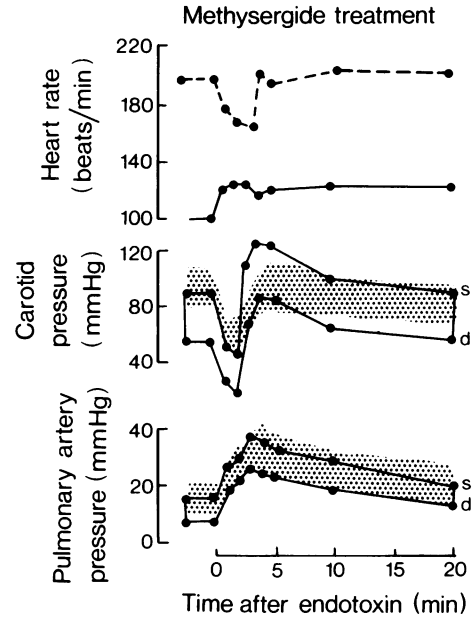

Figure 4 The haemodynamic effects of endotoxin (2 mg/kg at time zero) in 3 cats administered methysergide in a dose (1 mg/kg) that prevented the effects of 5-hydroxytryptamine. The vascular effects of endotoxin were unaffected by this treatment; the bradycardia was abolished (●——●).

23 and 22.5 μg/g) and jejunum (73.8, 71.3 and 45.7 μg/g) were in the normal range for this species (Smith, 1953). These results are in accord with the view that compound 48/80 depletes histamine only from those tissues where it resides in mast cells (Parratt & West, 1957).

The effects of endotoxin in these 'mast cell histamine depleted cats' are illustrated in Figure 3. They were basically similar to those obtained in cats treated acutely with compound 48/80 (Figure 2). There was again a transient pulmonary hypertension (from 13.5 to 26.9 mmHg after 1 min, 21.9 mmHg after 2 min and 16.5 mmHg after 3 minutes). Once again, the pressure after 5 min (12.6 mmHg) was not significantly different from the pre-endotoxin level.

None of these animals survived 3 h and, before death, they all had raised arterial lactate levels and a reduced cardiac output. Samples of intestine were taken at autopsy and there was some evidence that the histamine levels were reduced (46.8 and 58.8 μg/g respectively compared to the pre-endotoxin levels in these same animals of 73.9 and 71.3 μg/gram). In contrast, skin histamine was not further reduced (values of 0.78, 0.78 and 1.26 μg/gram).

The possible role of 5-hydroxytryptamine

Intravenous injections of 5-HT (10, 20 and 40 μg/kg) reduced systemic (carotid) arterial pressure (by 29 ± 9 mmHg with a dose of 10 μg/kg, 36 ± 12 mmHg with 20 μg/kg and by 54 mmHg after a dose of 40 μg/kg, from the pre-injection diastolic pressure of 95 ± 9 mmHg) and heart rate (-66 ± 7 beats/min after 20 μg/kg and -111 ± 19 beats/min after 40 μg/kg; control level 224 ± 16 beats/minute). There were also increases in pulmonary artery pressure, although these were not dose-related (increases of 3.5 ± 2.0 and 2.9 ± 3.3 mmHg from the control level of 10.1 ± 1.3 mmHg after 10 and 20 μg/kg respectively). Intratracheal pressure was also increased (by 0.3 ± 0.2, 0.7 ± 0.2 and 1.2 mmHg respectively from the resting peak inspiratory pressure of 7.7 ± 0.6 mmHg). These effects were greatly reduced by the prior administration of methysergide (1.0 mg/kg). For example, following a dose of 20 μg/kg of 5-HT, diastolic pressure was reduced by only 6 mmHg (compared to 36 mmHg) heart rate by 18 beats/min (compared to 66 beats/min) and the rise in pulmonary artery pressure which occurred before

methysergide was converted to a slight fall (of 2 mmHg).

Unfortunately methysergide itself had marked effects on the cardiovascular system. There were reductions in systemic pressure (by a mean of 40 mmHg diastolic and 37 mmHg systolic) and in heart rate (63 beats/min), effects rather similar to those of 5-HT itself. Despite these intrinsic effects it was clear that this dose of methysergide greatly modified the responses to injected 5-HT and therefore could be used to examine the possible contribution of released 5-HT to the acute haemodynamic effects of endotoxin. Figure 4 shows that the cardiovascular effects of endotoxin were quite uninfluenced by methysergide.

The possible role of a released (pulmonary vasoconstrictor) prostaglandin

Two possible approaches were investigated; prevention of the effects of released prostaglandins (with polyphloretin phosphate) and prevention of any possible release by inhibition of prostaglandin synthetase (with sodium meclofenamate; Flower, 1974).

(a) *Evidence from the effects of polyphloretin phosphate.* Polyphloretin has been shown to reduce the effects of prostaglandin $F_{2\alpha}$ in cats (Villanueva, Hinds, Katz & Eakins, 1972). Unfortunately, although certainly effective in reducing the pulmonary effects of injected or infused prostaglandin $F_{2\alpha}$, it had considerable inherent depressive effects on the cardiovascular system. Systolic and diastolic carotid pressures were reduced from $128 \pm 12/100 \pm 10$ mmHg (mean pressure 117 ± 11 mmHg) to $67 \pm 10/35 \pm 12$ mmHg (mean pressure 45 ± 11 mmHg; $P < 0.001$), mean pulmonary artery pressure was reduced from 16.5 ± 1.2 mmHg to 11.3 ± 1.5 mmHg ($P < 0.05$), heart rate from 246 ± 10 to 213 ± 24 beats/min ($P > 0.05$) and cardiac output from 373 ± 52 to 110 ± 45 ml/min ($P < 0.01$). Peripheral vascular resistance was unchanged.

After 1 h there was some recovery (carotid artery pressure of $82 \pm 12/50 \pm 10$ mmHg) and at this time the pulmonary hypertensive effects of injected prostaglandin $F_{2\alpha}$ (though not the effects on intratracheal pressure) were greatly reduced (Table 5). There appeared to be some selectively of action because, in three experiments, the hypotensive effect of intravenously administered histamine (1.5 μg/kg) was only slightly reduced by polyphloretin.

The effects of endotoxin were completely prevented by polyphloretin (Figure 5); there was no reduction in carotid artery pressure, no increase in pulmonary artery pressure and only a slight (14–22%) increase in intratracheal pressure.

(b) *Evidence from the administration of sodium meclofenamate.* In doses up to 2 mg/kg (14 experiments), sodium meclofenamate had no effect on systemic or pulmonary artery pressures, on heart rate, systolic ejection time, cardiac output, peripheral vascular resistance or on arterial blood Po_2, Pco_2, pH, lactate or glucose. Detailed results are available from the authors.

When endotoxin was injected 30 min after sodium meclofenamate the initial effects on carotid and pulmonary artery pressures were considerably (and significantly) reduced. This is clear from Figure 6. There was only a slight, transient, pulmonary hypertensive effect in the first 2 min at the injection (an increase in mean pulmonary artery pressure from 15.8 ± 1.0 mmHg before endotoxin to 19.5 ± 1.0 mmHg 1 min afterwards ($P < 0.01$) and to 19.2 ± 0.9 mmHg after 2 min; $P < 0.05$). This effect was in no way comparable to that seen in the cats administered endotoxin only (Table 1 and Figure 6).

Table 5 Pulmonary arterial pressure responses to injections of prostaglandin $F_{2\alpha}$ (PG $F_{2\alpha}$) before, and after, the administration of polyphloretin phosphate (200 mg/kg)

	Change in pressure induced by PG $F_{2\alpha}$ (μg/kg) before (b) and after (a) polyphloretin					
		1.25		*2.5*		*5.0*	
Control	*b*	*a*	*b*	*a*	*b*	*a*
Pulmonary artery pressure:						
systolic (mmHg)	21.8 ± 1.8	+10.3 ± 2.5	+3.4 ± 1.5**	+17.2 ± 2.4	+4.9 ± 0.6*	+24.5 ± 1.4	+6.7 ± 2.0*
diastolic (mmHg)	10.6 ± 0.6	+6.6 ± 1.6	+2.9 ± 1.0**	+10.0 ± 1.6	+4.1 ± 0.5*	+15.3 ± 1.6	+5.6 ± 1.5*

Results are means of 6 experiments ± s.e. mean.
Change after polyphloretin significantly different from change before polyphloretin (*$P < 0.001$; ** $P < 0.01$).

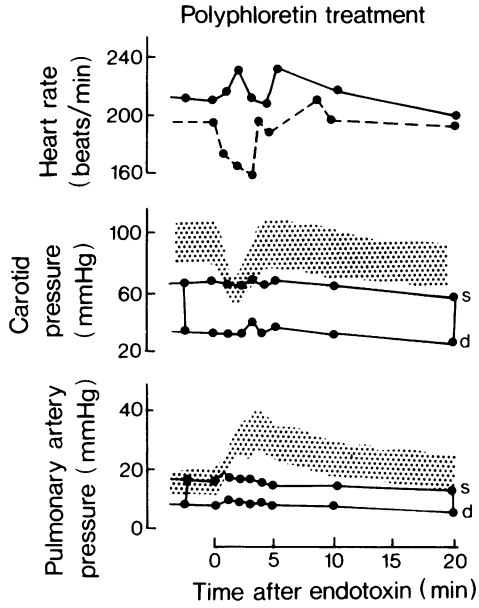

Figure 5 The haemodynamic effects of endotoxin (2 mg/kg at time zero) in 6 cats pretreated with polyphloretin phosphate (200 mg/kg; ●——●). There is an almost complete abolition of the effects of endotoxin normally observed in untreated animals (shaded areas).

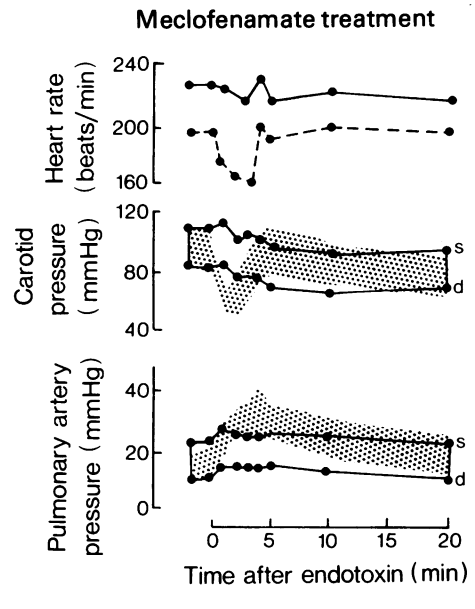

Figure 6 The haemodynamic effects of endotoxin (2 mg/kg at time zero) in 7 cats pretreated with sodium meclofenamate (2 mg/kg; ●——●). The change in systemic and pulmonary arterial pressures (shaded areas) and in heart rate (●- - -●) seen in untreated cats are not observed in cats pretreated with sodium meclofenamate.

There was no acute systemic hypotensive effect (Figure 6) although pressure tended to decrease slightly (by 15–19 mmHg), although not significantly, after 5–10 minutes. The endotoxin-induced bradycardia was absent in cats treated with meclofenamate.

We have not described the results obtained when endotoxin was administered at times other than 30 min after sodium meclofenamate. The acute pulmonary effects of endotoxin were greatly reduced, even when administered up to 5 h after meclofenamate (Sturgess, 1975).

Discussion

One of the principal aims of the present study was to examine which, if any, of the humoral vasoactive mediators released by endotoxin is primarily responsible for the marked pulmonary changes that result from the acute administration of lipopolysaccharide. This may have some relevance to the 'shock lung' syndrome commonly observed in patients with septic shock (Wilson, 1972). These pulmonary changes include oedema, atelectasis, a reduced arterial oxygen tension (often below 60 mmHg), hyperventilation and an abnormal alveolar-arterial oxygen tension gradient (Pontoppidan *et al.*, 1971; Milligan, MacDonald, Mellon & Ledingham, 1974). The mechanisms producing this pulmonary lesion are not known; the most likely appear to be sequestration of platelet aggregates and the release of, as yet unidentified, vasoactive agents (Milligan *et al.*, 1974).

The administration of purified *E. coli* endotoxin in the cat results in pulmonary hypertension, oedema, a reduced arterial oxygen tension and a decreased airways compliance (Kuida, Hinshaw, Gilbert & Visscher, 1958; Kuida, Gilbert, Hinshaw, Brunson & Visscher, 1961; Parratt, 1973; Parratt & Sturgess, 1976). This response begins within 1 min of endotoxin administration and pulmonary artery pressure is more than twice normal 3 to 4 min after the injection (Table 1). There are three possible explanations for this increase in pressure, a direct vasoconstrictor effect of endotoxin on pulmonary vascular smooth muscle, mechanical blockage (by particulate endotoxin or clumped blood cells) or the release of some humoral pulmonary broncho- and vasoconstrictor. The first possibility is unlikely since addition of an endotoxin

suspension to an organ bath containing pulmonary arterial or venous smooth muscle strips does not result in contraction (Parratt & Sturgess, unpublished observations). Mechanical obstruction is more likely, particularly since endotoxin induces platelet clumping and thrombocytopenia (Kitzmiller, Lucas & Yelenosky, 1972; Robb, Margulis & Jabs, 1972; Lucas & Kitzmiller, 1972). However there are a number of observations which argue against small vessel obstruction *per se* being the most significant mechanism: (i) The reduction in platelet count in the present experiments, at the height of the pulmonary pressor response, was not dramatic. Thus 5 min after endotoxin the platelet count had fallen from $324 \pm 91 \times 10^3$ per mm^3 to $268 \pm 77 \times 10^3$ per mm^3 ($P < 0.05$). (ii) Inhibition of platelet aggregation with carbochromen and with dipyridamole does not influence pulmonary hypertension induced by endotoxin (Parratt & Sturgess, 1975a). (iii) In complement-depleted cats the thrombocytopenic response to endotoxin is much reduced, although the pulmonary lesions in these animals (congestion and oedema) are no different from those found in normal cats given endotoxin (Kitzmiller *et al.*, 1972). (iv) Sheep, like cats, respond to endotoxin by a marked increase in pulmonary artery pressure. In pairs of anaesthetized sheep with a connecting unilateral carotid-jugular anastomosis, pulmonary embolism in the donor sheep produces an increase in pulmonary artery pressure (and a reduction in airways compliance) in the recipient animal (Halmagyi, Starzecki & Horner, 1964). This suggests that humoral factors predominate. (v) Protamine chloride, like endotoxin, causes a marked thrombocytopenia (due to platelet aggregation) and pulmonary hypertension when infused into heparinized animals. The pressor response, but not the platelet aggregation, is inhibited by aspirin (Radegran & McAslan, 1972). This again suggests that the release of a smooth muscle contracting substance, and not mechanical (intravascular) obstruction, is the predominant mechanism involved.

The present experiments allow only tentative conclusions to be drawn as to which humoral substance is primarily responsible for mediating the pulmonary responses induced by endotoxin. It is unlikely that 5-HT is involved to any great extent since the endotoxin effects on pulmonary and systemic arterial pressure were unaffected by methysergide in a dose that completely prevented the effects of injected 5-HT (Figure 4). The experiments with the antagonists mepyramine and burimamide suggest that histamine is of relatively minor importance (Figure 1); we have additional evidence that even in huge doses (up to 70 mg/kg) mepyramine alone does not inhibit the pulmonary pressor response to endotoxin (Sturgess, 1975). On the other hand, the experiments with compound 48/80 might suggest that histamine is involved in maintaining, but not initiating, the pulmonary response. The increase in pulmonary artery pressure at 1 min was as marked in the compound 48/80-treated cats as in the control (endotoxin alone) cats; however at 2 min (and thereafter) the response was significantly ($P < 0.01$) less than in the cats administered only endotoxin (Figures 2 and 3). Previous studies (Hinshaw, Emerson, Iampietro & Brake, 1962; Parratt, 1973) have also demonstrated that some of the haemodynamic effects of endotoxin (elevation of portal venous pressure in the dog and of pulmonary pressure in the cat) are greatly attenuated by compound 48/80. Clearly histamine release does occur after endotoxin administration. This release is not primarily from mast cells (Sandusky, Johnson & Moran, 1973). From the present studies it appears that some at least of the released histamine is derived from sites other than mast cells in the gut.

One explanation for the apparent discrepancy between the results obtained with histamine antagonists and with the histamine releaser is that compound 48/80 may have effects other than mast cell disruption. For example Dawson, Delano, Hamilton & Stekiel (1974), having summarized the considerable evidence that histamine is not the main mediator of hypoxia-induced vasoconstriction in cat isolated lungs, concluded that compound 48/80 inhibits this vasoconstriction by 'a mechanism that does not involve histamine release'. The fact that in the present experiments, the endotoxin response was rather less when compound 48/80 was given acutely (Figure 2) than when given by chronic administration (Figure 3) might imply that the presence of the drug in the circulation was of greater importance than histamine depletion *per se*. As far as we are aware there have been no studies to examine whether compound 48/80 inhibits prostaglandin synthetase. There is however, some evidence (Anggard & Strandberg, 1971) that it can release prostaglandin $F_{2\alpha}$ from perfused cat paws. An effect on prostaglandin synthesis or release would adequately explain both our results with endotoxin and the results of Dawson *et al.* (1974) described above. There is now some evidence that hypoxia induces release of prostaglandins from the lung (Said, Yoshida, Kitamura & Vreim, 1974). A common mediator for the pulmonary vasoconstriction induced by hypoxia and by endotoxin is suggested by experiments of Grover & Reeves (1974); after endotoxin administration the pulmonary pressor response to hypoxia was abolished or markedly reduced.

Most of the evidence from these studies suggests that a pulmonary vasoconstrictor prostaglandin is involved in the acute response to endotoxin in the cat. The effects of endotoxin were abolished by polyphloretin phosphate, in a dose that inhibited the response to exogenous prostaglandin $F_{2\alpha}$, and were

completely prevented by the prior administration of sodium meclofenamate. Similar effects were seen with two other inhibitors of prostaglandin synthetase, indomethacin (Parratt & Sturgess, 1974) and sodium flurbiprofen (Parratt & Sturgess, 1976). Furthermore, prostaglandin $F_{2\alpha}$ is known to be present in the lung (Samuelson, 1964) and analysis of pulmonary venous blood of calves administered endotoxin has demonstrated a two-fold increase in prostaglandin $F_{2\alpha}$ (Anderson *et al.*, 1975b). What is still required is a detailed study of the time course and mechanism of this release and its relationship, if any, to endotoxin-induced pulmonary hypertension, increased airways resistance and decreased airways compliance.

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