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Feline endotoxin shock: effects on tissue histamine and histidine decarboxylase activity

James R. Parratt, Samira Saleh[1] & N. Graham Waton

Department of Physiology & Pharmacology, Royal College, University of Strathclyde, Glasgow G1 1XW

1 Tissue histamine levels as well as specific and non-specific histidine decarboxylase were examined before and at various times (5 and 10 min) after the intravenous injection of a lethal dose (2 mg kg^{-1}) of *E. coli* endotoxin in anaesthetized cats.

2 Histamine levels were increased 5 min after endotoxin, especially in the skin, liver, lung and stomach.

3 There was evidence, in most of the cats, for a rapid and substantial activation of specific histidine decarboxylase especially in the lungs, liver, heart and gastrointestinal tract 5–10 min after endotoxin administration.

4 It is suggested that endotoxin induces the local formation of histamine and that this formation and *local* release may contribute to the pathophysiology of endotoxin shock in this species.

Introduction

Histamine has long been implicated in the circulatory disturbances that result from various forms of acute tissue injury and shock, including that induced by bacterial endotoxins. Thus, Hinshaw's group have drawn attention to the similarities in many species between the systemic effects of injected endotoxin and those of released histamine (Hinshaw *et al.*, 1962; Hinshaw, 1964). Endotoxin-induced histamine release has also been demonstrated by a number of workers (Vick, 1960; 1965; Hinshaw *et al.*, 1961; Spink *et al.*, 1964; Vick *et al.*, 1971; Caldwell *et al.*, 1975) and the evidence recently reviewed (Parratt, 1983). There is some evidence that the degree of histamine release relates to survival (Spink *et al.*, 1964). The antihistamines promethazine and diphenhydramine have been shown to prevent the acute cardiovascular effects of endotoxin in dogs (Anderson *et al.*, 1963; Krause & Hess, 1979) and, in the same species, the protective effects of WR-149, 024, have also, at least partly, been attributed to stabilisation of histamine-containing cells (Caldwell *et al.*, 1975).

Further, in a conscious, unrestrained rat model of endotoxin shock, pretreatment with diphenhydramine and the selective H_2-receptor antagonist cimetidine (alone or in combination) prevented endotoxin-induced systemic hypotension, tachycardia and hypoglycaemia and improved survival assessed at 24 h (Brackett *et al.*, 1985).

However, not all the effects of endotoxin can be accounted for by histamine release. Thus, histamine is not responsible for the acute pulmonary effects of endotoxin in the cat (Parratt & Sturgess, 1977). Neither does it mediate endotoxin-induced reticuloruminal stasis in goats (Van Miert *et al.*, 1976). Histamine release may be both endotoxin and species variable; no release for example, occurs in sheep given endotoxin derived from *Pasteurella hemolytica* (Emau *et al.*, 1984).

The purpose of the present studies was to determine whether there was any evidence for the participation of histamine in the acute (early) haemodynamic effects of endotoxin in the cat. This was studied by determining tissue and blood histamine levels after endotoxin administration and also by examining whether endotoxin was able to stimulate histidine decarboxylase in this species. Schayer's early studies (1960a; 1960b) have already provided evidence for the activation of this enzyme by various forms of trauma and shock, including that induced by *E. coli* endotoxin in anaesthetized mice. A preliminary account of these studies was presented to a meeting of the British Pharmacological Society (Saleh *et al.*, 1980).

[1] Present address: Department of Pharmacology, University of Cairo, Kasr El Eini, Cairo, Egypt.

Methods

Cats of either sex, weighing between 1.5 and 3.5 kg, were anaesthetized with a chloralose, urethane mixture (1.67 g chloralose + 16.7 g urethane in 100 ml of water) or with sodium pentobarbitone (40 mg kg^{-1} body weight). Food was withheld for 18 h before the experiments. The carotid artery was cannulated and connected to a mercury manometer for the recording of mean systemic arterial blood pressure. After a period of stabilisation, *E. coli* endotoxin (Difco laboratories, 055:B5), in a dose of 2 mg kg^{-1} suspended in 0.9% saline solution, was injected slowly, over a period of 40–50 s, into a femoral venous catheter.

At specified times (5 and 10 min) after the injection of endotoxin, the animals were killed, various tissues were removed and, with the minimum of delay, dropped into ice-cold saline. The tissues studied were the following: (1) Abdominal skin, removed from the midline, and freed from subcutaneous fat. (2) The stomach, this was opened and washed by hard agitation in saline several times, care being taken to prevent contamination of the mucosa with histamine derived from gastro-intestinal contents. (3) A piece of intestine, 10 cm long, taken from the second loop of the upper jejunum, which was opened along the midline and washed by hard agitation in saline. (4) One whole decapsulated kidney. (5) Pieces cut from each lobe of the liver. (6) The lung. (7) The heart.

The tissues were transferred to small beakers and finely chopped with scissors. These minced tissues were then assayed for histamine and for specific and non-specific histidine decarboxylase.

Histamine content

Tissue samples, except the skin, were dropped into 4 volumes of a 10% solution of trichloracetic acid (TCA); for skin samples 9 volumes were used. The extracts were allowed to stand for about 18 h, filtered and extracted four times with 4 volumes of anaesthetic ether to remove the acid. Excess ether was removed by gentle heating. After neutralisation with sodium bicarbonate, the extracts were assayed for their histamine content on the guinea-pig isolated ileum suspended in a 6 ml organ bath containing oxygenated Tyrode solution at 32°C. Mepyramine maleate was used to establish that histamine was the active substance present in the extract. Histidine, in concentrations up to 5 mg ml^{-1} base had no effect on the guinea-pig isolated ileum.

Non-specific amino acid decarboxylase

To determine the non-specific enzyme, histamine formation of each tissue was determined after incubating the minced tissue at pH 8.2 with histidine. The method used was essentially that of Waton (1956). The incubation mixture was as follows: 4.0 ml 0.2 M sodium phosphate buffer pH 8.2, 0.4 ml 200 μg ml^{-1} pyridoxal-5-phosphate (co-enzyme), 0.1 ml 0.001 M aminoguanidine bicarbonate and 1.0 ml neutralized (–)-histidine monohydrochloride (15 mg (as base) ml^{-1}).

For samples of kidney and intestine 0.4 ml aminoguanidine solution was used. The enzyme was allowed to react with the co-enzyme for 10 min before the addition of the substrate and one drop of benzene (roughly about 20 mg) was added, to each flask, just before stoppering. After incubation for 3 h at 37°C, the pH was brought to 5.5–6 with 1N HCl, and the mixtures boiled and filtered. The possibility that histidine might be decarboxylated spontaneously during incubation with the reagent mixture was checked by incubating this mixture with histidine for 3 h in the absence of tissue before acidification and boiling (reagent blank). After neutralization with sodium bicarbonate, the filtrates were assayed for their histamine content on the guinea-pig isolated ileum by the bracketing technique. Specificity of the responses was checked by the addition of mepyramine maleate.

The amount of histamine formed was calculated by subtracting the histamine content of extracts incubated in the absence of histidine (i.e. the endogenous histamine content of the tissues) from the histamine content of extracts incubated in the presence of histidine. The results are expressed as ng of histamine base formed over a 3 h period per g wet tissue (Waton 1956).

Specific histidine decarboxylase

The specific enzyme was determined in each tissue by incubating the minced tissue for 3 h at pH 6.5 (see Schayer, 1956), with 25 μg [^{14}C]-histidine (0.1 μCi radioactivity). Controls (including reagent blanks) were included in all experiments, to measure the amount of histamine already present in the histidine sample, as well as any histamine which was formed non-enzymatically during the incubation period.

After incubation, 1 ml of the histamine/histidine carrier (66.4 mg histamine dihydrochloride + 40 mg histidine monohydrochloride) and 10 ml cold 10% TCA were added and the mixtures allowed to stand for not less than 2 h.

The separation of [^{14}C]-histamine from [^{14}C]-histidine was achieved by an ion exchange technique, the histamine was converted into its dibenzenesulphonyl derivative, purified and counted in a liquid scintillation spectrometer (Waton, 1964). The efficiency of counting was estimated for each sample and varied between 66–75%. Background counts varied from 18–33 c.p.m. The results are expressed as ng histamine formed per 3 h and per g wet tissue (ng $3h^{-1}$ g^{-1} wet tissue). This is more meaningful than

Table 1 Blood pressure and the histamine contents of plasma or cells at various times after the intravenous injection of *E. coli* endotoxin (2 mg kg^{-1}).

Time after endotoxin	*n*	*Blood pressure (mmHg)*	*Histamine content (μg ml^{-1})*	
			Plasma	*Cells*
Control	15	120 ± 6	0.83 ± 0.20	0.64 ± 0.30
1 min	13	97 ± 11	0.56 ± 0.21	0.66 ± 0.33
3 min	12	58 ± 12*	0.67 ± 0.30	0.92 ± 0.44
5 min	13	71 ± 9*	0.60 ± 0.25	0.96 ± 0.50
10 min	6	68 ± 6*	0.54 ± 0.13	0.32 ± 0.14

Values are mean ± s.e. mean
*$P < 0.01$ compared with control blood pressure

expressing the results, as others have done, in terms of counts per minute or disintegrations per minute.

Blood histamine

Blood samples (2 ml) were taken from the carotid artery before, and at 1, 3, 5 and 10 min after, the injection of endotoxin. The heparinized blood was centrifuged at 2000 r.p.m. for 10 min for the separation of plasma from cells. To each tube 10 ml 10% TCA was added and mixed well with a glass rod. The extract was allowed to stand overnight, centrifuged at 2000 r.p.m. for 10 min and the supernatant used for the bioassay of histamine after extracting with ether as described above. The results are expressed as μg histamine base per ml of plasma or cells.

Statistics

Significance of differences was estimated by a modified Student's *t* test (Wallenstine *et al.*, 1980).

Results

Immediately after the intravenous injection of endotoxin there was a marked and progressive decrease in the systemic arterial pressure to levels of around 60 mmHg within 3 min. This was followed by a partial recovery over the next 5–7 min (Table 1). Some cats were followed for longer periods and in these, systemic blood pressure continued to fall until death in shock between 3–4 h after endotoxin administration. The detailed haemodynamic changes elecited by the dose and type of endotoxin used in these studies have been described in several previous publications from this laboratory (e.g. Parratt, 1973; Parratt & Sturgess, 1977; Hughes & Parratt, 1985).

The changes in blood histamine levels are also shown in Table 1; there was no evidence for histamine release at any time after endotoxin.

The specific histidine decarboxylase activity in the various tissues of cats before and after endotoxin is shown in Table 2. No demonstrable activity was

Table 2 Specific histidine decarboxylase activity in cat tissues 5 and 10 min after the intravenous administration of *E. coli* endotoxin

Tissue	*5 min after endotoxin*	*10 min after endotoxin*
Skin	0 (0/7)	4.7, 39.3, 20.2 (3/6)
Heart	13.2 (1/7)	0.6, 39.3 (2/6)
Kidney	0 (0/7)	5.0 (1/6)
Lung	13.0, 13.9 (2/7)	5.0, 7.7, 31.9, 58.8 (4/6)
Liver	9:1 (1/7)	6.4,22.0 (2/6)
Stomach	2.5, 6.0, 10.7 (3/7)	11.1, 33.4, 29.6 (3/6)
Intestine	1.6, 21.2 (2/7)	16.9, 42.2, 25.1 (3/6)

Values are expressed as ng histamine formed over a three hour period per g wet tissue, with the number of animals in which the enzyme was present given in parentheses.

Table 3 The histamine content of cat tissues ($\mu g\ g^{-1}$ wet weight) at various times after the intravenous injection of *E. coli* endotoxin ($2\ mg\ kg^{-1}$)

Tissue	*Pre-endotoxin*	*Post-endotoxin*	
		5 min	*10 min*
Stomach	35.5 ± 5.9	58.1 ± 0.2*	17.3 ± 6.5
Skin	28.9 ± 4.9	49.2 ± 8.3*	10.6 ± 4.0*
Intestine	28.3 ± 2.1	55.1 ± 7.1**	16.1 ± 6.1*
Lung	21.6 ± 4.4	59.1 ± 12.0**	19.2 ± 7.3
Liver	1.3 ± 0.3	1.9 ± 0.2*	1.3 ± 0.5
Heart	0.8 ± 0.3	1.8 ± 0.3*	0.6 ± 0.2
Kidney	0.2 ± 0.0	0.7 ± 0.3	0.3 ± 0.1

Values are mean ± s.e. mean; 6–8 cats, two to three tissue samples from each.
* Significantly different from control level at $P < 0.05$ and ** $P < 0.01$ respectively.

present before endotoxin in any of the animals but following its administration there was a progressive rise in enzyme activity to measurable levels in some tissues in all except one of the seven cats. The increase in activity was particularly evident at 10 min after endotoxin and was most pronounced in lung, stomach and intestine (in 6, 6 and 5 of 13 samples respectively). In contrast, activity was demonstrated in only one of 13 kidney samples and in only 3 of 13 heart and skin samples. No formation of histamine was demonstrated in any cat tissue, either before or after the administration of endotoxin, when the non-specific histidine decarboxylase technique was used.

Changes in tissue histamine content are given in Table 3. There was a marked and significant increase in the histamine content of all the tissues examined 5 min after endotoxin administration. These increases were, however, short-lived and normal levels were reached by 10 min post-endotoxin; however, in the intestine and the skin, levels were significantly *below* normal at this time and there was a similar trend in the stomach.

Discussion

Histamine forming capacity in cats

In the present experiments the formation of histamine in cat tissues either by the non-specific aromatic amino acid decarboxylase or by the specific histidine decarboxylase, was investigated under controlled conditions and after endotoxin administration. Histidine decarboxylation was only demonstrated by the specific (radioactive) technique and this only after the administration of endotoxin.

Using the biological method of assay for the non-specific enzyme, several attempts to demonstrate histamine formation in cat tissues, *in vitro*, have been unsuccessful (e.g. Kameswaran & West, 1962; Waton, 1963; 1964).

Similarly, in the present studies, no enzyme could be demonstrated with this technique. Although this may be because enzyme activity is low or absent, the formation of very small amounts of histamine may be masked by the efficiency of the catabolic enzymes or by the presence of large amounts of endogenous histamine.

There is controversy regarding the presence of specific histidine decarboxylase activity in cat tissues. For example, Kahlson *et al.* (1964) observed high gastric histidine decarboxylase activity in a number of species including cats ($0.04\ \mu g$ histamine g^{-1} tissue $3\ h^{-1}$), whereas others (Aures *et al.*, 1969), although demonstrating high activities of histidine decarboxylase in the gastric mucosa of rats and mice, failed to demonstrate such in the gastric mucosa of cats. These conflicting results may partly be accounted for by differences in species and experimental techniques. All are radiometric methods, but a low blank would lead to a falsely high level of enzyme being obtained. For example, it has been shown in our experiments that blanks, containing [^{14}C]-histidine and incubated for 3 h, are significantly higher than those containing [^{14}C]-histidine and *not* incubated. This indicates that in reagent blanks histidine gives rise to some histamine non-enzymatically during the incubation time. The reaction causing this phenomenon is unknown. To provide blank values Kahlson *et al.* (1964) added semi-carbazide to the incubation mixture to prevent enzymatic decarboxylation. This might also inhibit non-enzymatic decarboxylation and result in a falsely low blank value (Waton, unpublished). The fact that an enzyme cannot be measured does not of course necessarily mean that it is absent. It has been shown for example that after the intravenous infusion of [^{14}C]-histidine into cats, [^{14}C]-histamine appeared in various tissues (Schayer, 1956; Waton, 1964) indicat-

ing that in this species some tissues are certainly able to decarboxylate histidine.

The role of histamine in endotoxin shock

The aim of the present study was to find out whether or not histamine is involved in the early phase of endotoxin shock. We have therefore restricted our studies to the first 10 min after endotoxin administration. The data presented demonstrate an increase in specific histidine decarboxylase activity to measurable levels in at least some tissues of all but one of the cats examined and an elevation of histamine content in various tissues within 5–10 min of the intravenous injection of a lethal dose of endotoxin.

Earlier work on the role of histamine in the early stages of endotoxin shock has been mainly based on the assumption that it was released from pre-formed tissue stores and that the differences between, for example, the vascular effects of endotoxin and those of the histamine-liberator compound 48/80, was due to release from different sites (Hinshaw, 1971). This would explain the finding that the early haemodynamic effects of endotoxin in anaesthetized cats are relatively unaffected by chronic or acute pretreatment with compound 48/80 (Parratt & Sturgess, 1977). Our present findings do not support the concept of a significant mobilization of histamine from pre-formed storage sites by endotoxin but do suggest that the histamine can be formed locally and rapidly in the tissues by the inducible enzyme, histidine decarboxylase. This is a view originally suggested by Schayer (1960a, b). It is perhaps unlikely that the increased tissue histamine levels are the result of a reduced ability to destroy histamine since it has been reported that endotoxin has no effect on diamine oxidase activity or on histamine methylation (Reilly & Schayer, 1968; 1970).

Clearly then, *E. coli* endotoxin administration results in a rapid and generalized activation of specific histidine decarboxylase. This is true for all the tissues examined (Table 2) although the amounts of histamine formed varied. Particularly high activity was found in the lung (i.e. up to 58 ng g^{-1} tissue), the liver (up to 22 ng g^{-1} tissue), heart (up to 39.3 ng g^{-1} tissue) and gastrointestinal tract. Whether this is a direct action of the lipopolysaccharide is unknown; we believe it more likely that it results from the release of an endogenous activator. Catecholamines would be a possibility (Schayer, 1960b) and indeed, a recent study (published after the present manuscript was originally submitted) on the effects of haemorrhagic shock in dogs on blood and tissue histamine levels would support the possibility that catecholamines are involved. Nagy *et al.*, (1986) found, at all stages of shock induced by haemorrhage, markedly (2–10 fold) increased tissue histamine levels in liver, lungs, heart and in blood vessel walls; these increases occurred at the same time as massive increases in noradrenaline. They concluded that the results of 'sequential measurements of tissue levels during shock suggest that histamine formation was enhanced' and that this enhanced production contributed significantly to the elevated plasma histamine levels found in this model. Their conclusion that early in shock, tissue histamine production is enhanced agrees with the present results and suggests catecholamines as a possible mediator. It would be of interest to repeat these studies after catecholamine depletion or blockade.

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