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THE INTERACTION BETWEEN ANTHELMINTIC DRUGS AND HISTAMINE IN *Ascaris suum*

JEAN L. PHILLIPS, GILLIAN STURMAN & G.B. WEST

Department of Applied Biology, North East London Polytechnic, Romford Road, London E15 4LZ

1 Piperazine reduced the histamine content of *Ascaris suum*, yet it greatly increased the uptake of histamine from the surrounding medium, the neuromuscular structures of the nematode preferentially increasing in amount.

2 Bephenium reduced the histamine content of *Ascaris* and the uptake of histamine from the surrounding medium. However, the relative amount in the neuromuscular structures increased.

3 The flaccid paralysing action of piperazine may thus involve increased histamine absorption whereas the spastic paralysing action of bephenium may be independent of histamine.

Introduction

The spindle-shaped, longitudinal muscles of the body wall of *Ascaris suum* consist of cells which are divided into two portions, contractile and non-contractile. Processes pass from the non-contractile portions to the lateral nerves and the circumpharyngeal nerve ganglion where they form a complex syncytium. Two types of junction are apparent at this level, one between muscle arms and one between muscle arms and nerve cords (de Bell, 1965). When the nerve cords are stimulated, both inhibitory and excitatory effects on the muscle can be identified. The natural excitatory transmitter may be acetylcholine (Gerschenfeld, 1973) but the inhibitory transmitter is still not known. Histamine has been proposed as an inhibitor for it occurs in the tissues of *Ascaris* and is concentrated in the neuromuscular structures (Miyagawi, 1961; Phillips, Sturman & West, 1975*a*). Furthermore, histamine inhibits acetylcholine-induced contractures of muscle preparations of *Ascaris*.

Piperazine, an anthelmintic agent used in the treatment of Ascariasis, causes flaccid paralysis of the parasites and may be acting via an inhibitory system, either by direct stimulation or by interference with the natural inhibitory transmitter. The results of preliminary experiments (Phillips, Sturman & West, 1975*b*), showed that piperazine reduced the histamine content of *Ascaris suum* but it greatly increased the uptake of histamine from the incubation fluid. In fact, the parasites always exhibited a greater degree of paralysis when they were treated with piperazine and histamine together than with either compound alone. As histamine is a natural constituent of the intestines of the host, it was decided to investigate further the interaction between histamine and piperazine in *Ascaris* and compare it with that between histamine and bephenium, an anthelmintic drug producing spastic paralysis.

Methods

Mature, female *Ascaris suum* were collected from the intestines of freshly slaughtered pigs and transported to the laboratory in modified Baldwin-Ringer solution (Baldwin & Moyle, 1947) containing antibiotics (penicillin G, 10,000 i.u./ml; streptomycin, 1.5 mg/ml; tetracycline, 0.5 mg/ml; sulphanilamide, 1.2 mg/ml) and an antimycotic (Nystatin, 1.5 mg/ml) to prevent contaminating micro-organisms contributing to the histamine content of the *Ascaris*. Groups of five parasites were incubated in 1 litre quantities of the Ringer solution at 38°C for 24 h, the medium being changed twice during this period. Drugs were added to the Ringer solution at the start of the incubation. The *Ascaris* were then washed 6 times with drug-free Ringer solution, dried, stunned, weighed and the histamine extracted into *n*-butanol prior to assay fluorimetrically (Shore, Burkhalter & Cohn, 1959) and biologically (Phillips *et al.*, 1975*a*). In other experiments, whole *Ascaris*, after incubation and stunning, were carefully dissected into head and body wall, intestines, reproductive tract and perienteric fluid before being weighed, extracted and assayed.

In a few experiments with the anthelmintic drugs, [ring ^{14}C]-histamine was used to study uptake and distribution of histamine after incubation.

Histidine decarboxylase activity

This was determined by a modification of the method of Telford & West (1961). The incubation mixtures

consisted of homogenized *Ascaris* (4.9 ml of 5 ml/g extract in 0.9% w/v NaCl solution), phosphate buffer (pH 6.0, 4.9 ml) or phosphate buffer containing piperazine or bephenium (final concentration 1 mg/ml), aminoguanidine (0.1 ml of 10 mg/ml) and histidine (0.1 ml of 100 mg/ml). The solutions were thoroughly mixed and incubated with shaking for 3 h at 38°C. Then, 4 ml aliquots were added to 4 ml 0.4 M perchloric acid and taken through *n*-butanol extraction for fluorimetric as well as biological assay for histamine. Blanks contained phosphate buffer instead of histidine.

Diamine oxidase activity

The incubation mixtures consisted of hog kidney diamine oxidase (0.1 ml of 0.5 i.u./ml), phosphate buffer (pH 6.3, 4.8 ml) or phosphate buffer containing piperazine or bephenium (final concentration 1 mg/ml) and histamine (0.1 ml of 5 mg/ml). The solutions were thoroughly mixed and incubated for 1 h at 38°C. The reaction was then stopped by the careful addition of 0.1M HCl and the histamine was assayed fluorimetrically and biologically. Suitable blanks were prepared in which diamine oxidase, histamine or the anthelmintics were omitted.

Drugs

The drugs used were as follows: histamine acid phosphate (Sigma), histidine hydrochloride (Sigma), piperazine hexahydrate (Sigma), bephenium hydroxynapthoate (The Wellcome Foundation Ltd.), diamine oxidase (Sigma) and [ring-2-^{14}C]-histamine dihydrochloride (Radiochemical Centre, Amersham).

Results

Effect of anthelmintics on histamine content

The mean (± s.e. mean) histamine content of 10 control *Ascaris* after incubation for 24 h at 38°C was 93 ± 6 mg/gram. Both piperazine and bephenium significantly reduced the histamine content of *Ascaris* by 19% and 28% respectively (Table 1). Some inhibition of movement of the nematodes was noted when each drug was present.

Effect of anthelmintics on histamine uptake

In the presence of histamine (10 µg/ml), the histamine content increased about three-fold (Table 2), but, when piperazine was included in the incubation fluid together with the histamine, a seven-fold increase in content was obtained. On the other hand, bephenium greatly reduced histamine uptake. These changes in the histamine content of *Ascaris* in the presence of piperazine and bephenium are illustrated in Figure 1. Similar results were obtained when the uptake of radioactive histamine was studied.

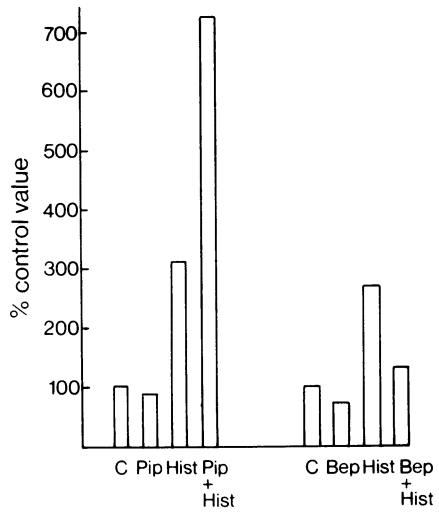

Figure 1 Changes in the histamine content of *Ascaris suum* after incubation with piperazine or bephenium in the absence or presence of histamine. Pip = piperazine 1 mg/ml: Hist = histamine 10 µg/ml: Bep = bephenium 1 mg/ml: C = control value without drugs. Histamine contents are percentages of control values taken as 100%. Note that piperazine increases uptake of histamine whereas bephenium decreases uptake.

Distribution of histamine after incubation

Table 3 shows the relative histamine content of parts of the nematode after incubation with Ringer alone. More than half of the total histamine was found to be present in the head and body wall (where the muscle and nerve processes are located). When histamine was included in the incubation mixture, this percentage

Table 1 The histamine content (ng/g) of *Ascaris suum* after incubation at 38°C for 24 h with piperazine or bephenium

Anthelmintic	*Piperazine (1 mg/ml)*	*Bephenium (1 mg/ml)*
Absent	82 ± 5	104 ± 7
Present	67 ± 6	75 ± 5
% Decrease	19	28
Significance	$P < 0.05$	$P < 0.05$

Histamine contents are expressed as mean ± s.e. mean of 5 experiments.

figure (55%) did not change although the total histamine increased some three-fold, but in the presence of piperazine or bephenium relatively more was found in this part of the nematode. For example, 73% of the total histamine was in the head and body wall when piperazine and histamine were in the incubation mixture, whereas with bephenium it was 63% of the total.

Histidine decarboxylase

The activity of this enzyme from *Ascaris* was inhibited 55% by piperazine (1 mg/ml) yet bephenium (1 mg/ml) had no effect.

Diamine oxidase

Piperazine (1 mg/ml) had no effect on the activity of this enzyme yet bephenium (1 mg/ml) stimulated the inactivating enzyme by over 30%.

Discussion

The results of the present experiments show that *Ascaris* readily take up histamine. In the presence of piperazine (a drug which by itself reduces the histamine content), the uptake of histamine is further increased. The relative amount of histamine in the neuromuscular structures (that is, head and body wall) increased and it was noted that under these conditions the paralysing action of piperazine was augmented. On the other hand, in the presence of bephenium (a drug which also reduces the histamine content of the worm), the uptake of histamine is decreased. The relative amount of histamine in the neuromuscular structures increased although no change was found in the spastic paralysing action of bephenium.

Piperazine may owe part of its paralyzing action on *Ascaris suum* to its ability to increase the uptake of histamine from the surrounding medium. The pig's intestinal fluid contains about 10 μg/ml histamine (Phillips *et al.*, 1975*b*) and so *in vivo* the uptake may well occur. Histamine is concentrated in the neuromuscular structures and it produces flaccid paralysis of the parasite. However, this effect can only be contributory to the action as piperazine itself produces paralysis and inhibits histidine decarboxylase, the specific enzyme involved in the formation of histamine within the parasite. Such an inhibition may explain the reduction in histamine content when piperazine was used alone.

Table 2 The histamine content (ng/g) of *Ascaris suum* after incubation with histamine or with piperazine or bephenium in the presence of histamine

Anthelmintic	*Histamine (10 μg/ml)*	*Piperazine (1 mg/ml)*	*Bephenium (1 mg/ml)*
Absent	Absent	104 ± 9	137 ± 9
Absent	Present	335 ± 43	366 ± 28
Present	Present	778 ± 133	187 ± 15
% Change		191% increase	78% decrease
Significance		$P<0.05$	$P<0.05$

Histamine contents are expressed as mean ± s.e. mean of 5 experiments.

Table 3 The relative percentage distribution of histamine in the tissue of *Ascaris suum* after incubation alone, with histamine, or with piperazine or bephenium in the presence of histamine

Tissue	*Control*	*Histamine*	*Piperazine and histamine*	*Bephenium and histamine*
Head and body wall	55	54	73	63
Reproductive tract	19	20	14	16
Intestines	15	8	7	8
Perienteric fluid	11	18	6	13

Results are the mean values of 10 experiments.

Bephenium, which produces spastic paralysis of *Ascaris suum*, also lowers the histamine content of the worm but apparently by stimulating its metabolism rather than by inhibiting its synthesis. It also reduces the uptake of histamine from the surrounding medium. However, as with piperazine, these interactions with histamine are probably secondary to a more major mechanism of action of bephenium in producing paralysis of the parasites. The histamine content of *Ascaris* following incubation with histamine and bephenium is not significantly different from that of fully mobile parasites taken straight from the pig's intestine.

Other workers (for example, Jones, Rothwell, Dineen & Griffiths, 1974) have found that during the course of infection of guinea-pigs with another gastrointestinal nematode, *Trichostrongylus colubriformis*, histamine levels in the small intestine increase about three-fold. Furthermore, guinea-pigs immunized by previous infection and challenged with *T. colubriformis* show an additional increase in intestinal histamine levels just prior to worm expulsion while the infusion of histamine together with 5-hydroxytryptamine (5-HT) into the small intestines of animals during the 4th larval stage of a primary infection leads to significant worm expulsion (Rothwell, Pritchard & Love, 1974). Histamine may exert an action on either the parasite or host or on both to assist expulsion of the worms. If an action on the parasite by histamine is considered, then it could possibly be a stimulation of an inhibitory nervous system, as postulated for *Ascaris suum*. Any treatment which increases histamine uptake would theoretically promote worm expulsion. It would therefore be of interest to see if piperazine increases the uptake of histamine by *T. colubriformis*. Drugs which inhibit the action of histamine are known also to inhibit the expulsion of *T. colubriformis* by infected guinea-pigs.

5-HT levels in the intestines also rise during infection with intestinal nematodes and this system may be implicated in the mechanism of action of anthelmintics as peristalsis is controlled, in part, by the formation and release of 5-HT. Further work is now in progress studying the interaction between 5-HT and anthelmintics.

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