

of a single altered behaviour, are pleiotropic and therefore defective in other chemical sensitivities.

4. The Dyf phenotype is associated with the loss of sensitivity to many different chemicals. Alterations in the shape or matrix content of the amphidial channel also result in a Dyf phenotype.

5. While water soluble chemicals seem to have receptors on the neurons whose cilia are exposed to the outside (ASH and ADL), attraction to and repulsion by volatile chemicals are mediated by receptors present on the amphidial wing cells AWA, AWB and AWC.

REFERENCES

- BARGMANN C.I., THOMAS J.H. and HORVITZ H.R. : Chemosensory cell function in the behavior and development of *Caenorhabditis elegans*. *Cold Spring Harbor Symp. Quant. Biol.*, 1990, LV, 529-538.
- BARGMANN C.I., HARTWEIG E. and HORVITZ H.R. : Odorant-selective genes and neurons mediate olfaction in *C. elegans*. *Cell*, 1993, 74, 515-527.
- CHALFIE M. and WHITE J.-G. : The nervous system in The Nematode *Caenorhabditis elegans*. Wood W.B. (ed.), Cold Spring Harbor Lab. C.S.H., New York, 1988, 337-391.
- WHITE J.G., SOUTHGATE E., THOMSON J.N. and BRENNER S. : The structure of the nervous system of the nematode *Caenorhabditis elegans*. *Philos. Trans. R. Soc. London, Ser. B.*, 1986, 314, 1-340.

N-ACETYLATION OF POLYAMINES AND BIOGENIC AMINES IN PARASITIC NEMATODES

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KEYWORDS : Polyamines, biogenic amines, N-acetylase, nematodes.

The previously described polyamine N-acetylase from *Fasciola hepatica* has been observed to have an additional function, the acetylation of biogenic amines (Aisien and Walter, 1992, 1993). It was concluded that N-acetylation plays a major role in the amine metabolism of trematodes. In continuation of these ongoing studies on the process of N-acetylation in parasitic helminths, we have detected biogenic amine acetylation in the tissue dwelling filaria *Onchocerca volvulus* and the intestinal parasite *Ascaris suum*. The aim of our study was to ascertain if our previous finding that a single enzyme is responsible for diamine, polyamine and biogenic amine acetylation in trematodes, is a feature common to all helminths. Results from investigation using *A. suum* indicate that two independent enzymes are respectively responsible for polyamine and biogenic amine acetylation. Chromatography of the 100,000 g supernatant on DEAE-cellulose revealed two enzyme activity peaks, both of which have activity for histamine. The first peak was observed to catalyse only biogenic amine acetylation while the second acetylated putrescine and other diamines. On the basis of the K_m -values obtained with both enzymes for histamine, peak II seems to be more specific for this substrate

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with a K_m -value of 37 μ M compared to peak I which had a K_m -value of 500 μ M. In contrast the K_m -values obtained for biogenic amines with peak I were as follows : Tyramine, 0.9 μ M, tryptamine, 1.7 μ M, octopamine, 29 μ M, serotonin, 9.5 μ M and β -phenylethylamine, 1.5 μ M. Epinephrine, norepinephrine and dopamine were not physiological substrates for the N-acetylase. The enzyme has a molecular mass of approximately 30 kDa and was slightly inhibited by coenzyme A, a product of the acetylation process. The specificity of peak II for diamines and histamine and its lack of activity for polyamines indicates that this enzyme is most probably the novel putrescine acetylase previously reported by Wittich and Walter (1990, 1991) from *O. volvulus* and *A. suum*.

ASSESSMENT OF THE POLYAMINE METABOLISM OF FILARIAL WORMS AS A TARGET FOR CHEMOTHERAPY

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KEYWORDS : Polyamines, metabolism, chemotherapy, filaria, N-acetyltransferase, polyamine oxidase, S-adenosylmethionine decarboxylase.

Polyamines are essential for the proliferation and differentiation of cells and organisms. The presence of polyamines has been demonstrated in *Onchocerca volvulus* and allied parasites and the investigation of the polyamine metabolism has identified unusual pathways which are potential chemotherapeutic targets. These pathways are crucial for parasite survival and differ in some biochemical aspects from the host counterparts, thus allowing for the rational design of drugs for a parasite-specific chemotherapeutic attack. A proposed scheme of the polyamine metabolism in filarial worms is shown in Figure 1.

Since the presence of ornithine decarboxylase (ODC) activity is questionable, the initial step in the biosynthesis of polyamines appears to be lacking and thus the parasite depends on uptake from the host for its polyamine supply. An interconversion pathway for polyamines has been demonstrated which does not involve the polyamine N-acetyltransferase, the rate limiting step in the interconversion of polyamines in mammals. A novel type of polyamine oxidase has been identified which is solely responsible for the interconversion and degradation of polyamines. In addition, a parasite-specific N-acetyltransferase for putrescine was found which is involved in the degradation and excretion of excess polyamines.

Depletion of polyamine levels in filarial worms is thought to have cytostatic if not cytotoxic effects, as has been shown previously in the chemotherapy of cancer and protozoan infections by DL- α -difluoromethylornithine (DFMO). Filarial worms and other helminths lack ornithine decarboxylase, the target for DFMO. Thus, as the parasite depends on the uptake of polyamines from the host tissues, inhibition of transport mechanisms resulting in rapid depletion of polyamines should be exploitable for chemotherapy.

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Polyamine analogues, e.g. *bis*(benzyl)polyamine (MDL 27695), although shown to interfere strongly with both polyamine uptake systems of filarial worms, probably exhibit their filaricidal effects by competing and interacting with polyamine binding sites such as nucleic acids and structural macromolecules.

Inhibition of S-adenosylmethionine decarboxylase, a regulatory and rate-limiting step in the biosynthesis of polyamines which provides the aminopropyl group for the synthesis of spermidine and spermine, results in depletion of polyamines. MDL 73811, an irreversible inhibitor of S-adenosylmethionine decarboxylase, affects both polyamine synthesis and the viability of nematodes maintained *in vitro*.

The strategy proposed here is directed to the interconversion and degradation pathway for polyamines. This is based on the findings that the polyamine oxidase is the rate-limiting step for the back conversion of spermine to spermidine and putrescine and that elevated levels of spermine are lethal for *Brugia* worms maintained *in vitro*. The potential of polyamine oxidase as a target for chemotherapy has been assessed by treatment of *Brugia* and *Ascaris* worms with MDL 72145, an irreversible inhibitor of nematode polyamine oxidase, which resulted in enzyme inactivation and a subsequent accumulation of spermine. By structural analysis of the filarial polyamine oxidase and the isofunctional mammalian enzyme, differences may be identified and exploited for the synthesis of drugs which react selectively with the parasite enzyme.

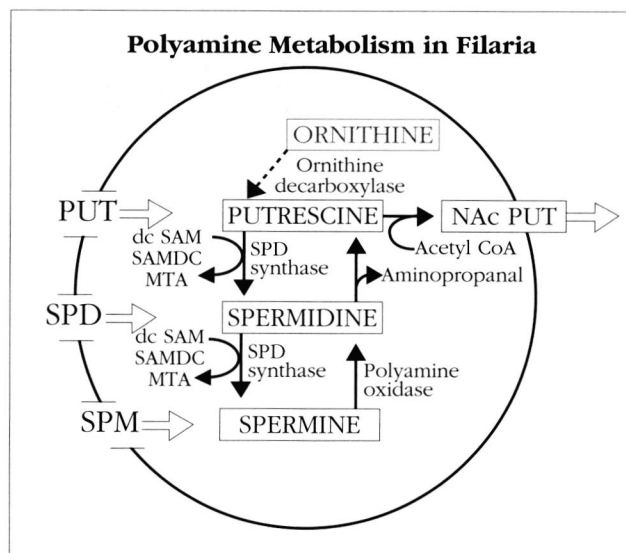


Figure 1.

VECTOR BIOLOGY

HUMORAL DEFENSE MOLECULES IN VECTORS OF FILARIASIS

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KEYWORDS : insect immunity, defense, *Onchocerca*, humoral proteins.

INTRODUCTION

The relationship between the definitive host, man, and filarial parasites, has for long been a subject of intense study, and as this network attests, still is today. However, the interactions between vectors of lymphatic filariasis and onchocerciasis and these nematodes, is less well understood. There is now a considerable body of data describing the innate and acquired resistance of vectors to the parasites they carry (eg. reviewed by Townson and Chaithong, 1993 ; Christensen and Severson 1993 ; Ham, 1992). Blackflies and mosquitoes possess complex immune systems, comprising inter-related cellular and humoral components. Not only is immunity expressed in the haemocoel, but mechanisms of refractoriness also appear to be linked

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to gut lumen components (see review Ham, 1992) and thoracic tissues (Wattam & Christensen, 1992).

RESPONSES TO INFECTION

Three generalised responses appear to take place following infection of insects : (1) an alteration in metabolic activity, including protein synthesis, (2) induction of specific immune peptides and proteins some of which have protective functional antibiotic activity, and (3) enhanced haemocytic proliferation. This proliferation maybe associated with increased secretion of molecules in (2) (Figure 1). As well as responses to infection, insects, including vectors of filariasis may exhibit responses to others forms of stress such as cold, overcrowding and physical trauma such as injury (eg Komano *et al.*, 1980).

HUMORAL DEFENSE MOLECULES

A number of defense peptides have been observed in insects, and many of these are known to be induced by bacterial pathogens such as *Escherichia coli*, as well as filariae (see Figure 2 which shows immune and non immune