The effects of specific metabolic inhibitors on the energy metabolism of *Globodera rostochiensis* and *Panagrellus redivivus*

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SUMMARY

Three indicators of metabolic activity, oxygen uptake, ATP levels and heat output, have been used to study the effects of specific metabolic inhibitors on second stage juveniles of *Globodera rostochiensis* and adults of *Panagrellus redivivus*. The results indicate that carbohydrates, lipids and amino acids can be used as energy substrates and a glyoxylate cycle is present. There is a major aerobic component in the metabolism of these species although both have alternative anaerobic pathways. Heat output is probably the best indicator of metabolic rate and has the advantage of being non-destructive.

RÉSUMÉ

Action d'inhibiteurs métaboliques spécifiques sur le métabolisme énergétique
de Globodera rostochiensis *et* Panagrellus redivivus

Trois indicateurs de l'activité métabolique, la consommation d'oxygène, la teneur en ATP et le dégagement de chaleur, ont été utilisés pour étudier l'action d'inhibiteurs métaboliques spécifiques sur les juvéniles de deuxième stade de *Globodera rostochiensis* et les adultes de *Panagrellus redivivus*. Les résultats indiquent que les hydrates de carbone, les lipides et les acides aminés peuvent être utilisés comme substrat énergétique, et qu'un cycle glyoxylate est présent. Le composant aérobie est prépondérant dans le matébolisme de ces deux espèces, encore que l'une et l'autre présentent des filières anaérobies de remplacement. Le dégagement de chaleur est probablement le meilleur indicateur de niveau de métabolisme; il présente de plus l'avantage de ne pas être destructif.

Chemical control of plant parasitic nematodes currently centres on the use of highly toxic compounds which act principally by inhibition of acetylcholinesterase (Wright, 1981). The development of acceptable replacement compounds requires a more rational approach to chemical control including the evaluation of alternative target sites. Energy metabolism is a possible target, but the identification of such areas for nematicide development requires a detailed understanding of the basic biochemistry of nematodes.

Information on the biochemistry of plant parasitic nematodes, especially energy metabolism, is scarce or absent. Unlike animal parasitic nematodes, which have an absolute dependency on carbohydrate for their energy source, free-living and plant parasitic species also catabolize lipids and amino acids (Van Gundy, Bird & Wallace, 1967; Barrett, Ward & Fairbairn, 1971; Reversat, 1980). In addition, plant parasitic species have a much greater aerobic requirement than animal parasitic species (Atkinson, 1980).

The small biomass available from most plant parasitic nematodes makes conventional biochemical approaches extremely difficult. However, by investigating the effects of specific inhibitors on metabolism it may be possible to identify the different pathways present in the nematode and to gain some idea of their relative importance.

In this paper, three metabolic parameters have been used to assess the effects of inhibitors on the second stage juveniles of the plant parasitic nematode *Globodera rostochiensis* and on adults of the free-living nematode *Panagrellus redivivus*. The three parameters chosen were oxygen uptake, ATP level and heat output.

Materials and methods

Cysts of *G. rostochiensis* Ro 1, grown on potato cv. Arran Banner in pots, were taken from a single generation and stored dry at room temperature (20°) for six months after extraction from the soil. Potato root diffusate (PRD) was obtained by the method of Fenwick (1949) and diluted with distilled water 1 in 4 by volume. Batches of cysts were soaked for 1 wk in distilled water and then placed in PRD; hatched juveniles were collected after nine to eleven days in PRD, most having

hatched after five days. *P. redivivus* were maintained on autoclaved oats and the worms harvested and cleaned by sedimentation and migration through filter paper as described by Barrett and Butterworth (1984).

Oxygen uptake measurements were made in a Clark type oxygen monitor (Yellowsprings Oxygen Monitor) at 25, with a chamber volume of 2 ml and 2-5 mg fresh weight of nematodes.

Heat output measurements were made at 25° in an LKB ampoule calorimeter. This is a heat flow calorimeter and the worms (0.5 - 2 mg fresh weight) were placed in a stainless steel ampoule, internal volume approximately 1.5 ml. The worms were supported in the ampoule on glass fibre paper discs. This prevented the nematodes sinking to the bottom of the ampoule and becoming anaerobic.

The ATP content of the different samples was determined using a luciferin/luciferase system (Packard, Picozyme F) in a Lumac 1070 luminometer. Following incubation in the different inhibitors, the nematodes (0.3 - 1.5 mg fresh weight) were rapidly washed and the ATP extracted by diluting the sample 1 : 4 with boiling buffer (Tris-HCl, 50 mM, pH 7.4; EDTA, 4 mM; $MgCl_2$, 2.5 mM). The mixture was boiled for 3 min, then quenched in ice and aliquots taken for ATP determination. A standard curve was prepared from ATP samples treated in the same way, the limit of detection of the method being 50 pg of ATP (500 femtomoles). Controls were also run with the inhibitors to check that they did not interfere with the luciferin/luciferase system.

The nematodes were exposed to the different inhibitors (see Tab. 1) for a standard 30 min at room temperature before oxygen uptake, heat output or ATP measurements were made. The data were analysed by the methods of Dean and Dixon (1951) after arcsine transformation.

Results

The results of oxygen uptake, heat output and ATP levels all showed considerable variability, despite efforts to standardize the methods as far as possible. Much of this error was probably due to variations in the different batches of nematodes and to the small biomass available. Since in this study whole organisms were being used, much higher levels of inhibitors had to be employed to produce an effect than would be necessary with homogenates or sub-cellular fractions.

Tables 1 and 2 show the effects of inhibitors on metabolic activity of *G. rostochiensis* and *P. redivivus* respectively. The inhibitors used fall into three groups : plant nematicides, animal anthelmintics and specific metabolic inhibitors. All of the known nematicides and anthelmintics tested had an effect on oxygen uptake and heat output; none had a marked effect on ATP levels.

Aldicarb, the most effective of the four nematicides used, reduced heat output considerably in both species while oxamyl had virtually no effect. Fenamiphos and carbofuran caused enhanced oxygen uptake and heat output in *P. redivivus* but caused inhibition of these two metabolic indicators in *G. rostochiensis*. Although differences between species and life cycle stages in relative rates of uptake, metabolism and elimination of the pesticides may have contributed to the variation, the enhanced metabolism of *P. redivivus* is unexpected.

Of the anthelmintics tested, the greatest reduction in heat output and oxygen uptake in both species occurred with bunamidine, ivermectin, piperazine and pyrantel. The site of action of bunamidine is unknown; ivermectin, piperazine and pyrantel affect neuromuscular systems. Of the biochemical inhibitors, the cytochrome chain inhibitors had the most dramatic effects. This suggests that free-living and plant parasitic nematodes, unlike animal parasitic nematodes, rely heavily on aerobic metabolism.

Discussion

All three methods of measuring metabolic rate — oxygen uptake, heat output and ATP levels — showed considerable variability and, although there was general agreement, they did not give comparable percentage reductions. This could be due in part to the time constants of the different methods; ATP measurements reflect instantaneous levels, oxygen uptake was averaged over 10 min whilst heat output was recorded for approximately 1 hour. The variability in the results, however, means that the conclusions must remain speculative.

The lack of any effect on ATP levels by nematicides and anthelmintics might be expected since tissues will, as far as possible, maintain their ATP levels at the expense of other processes. Muscle paralysing agents such as levamisole, piperazine, pyrantel and ivermectin, in particular, would not be expected to have much effect on ATP content. Measurement of the ATP level does not of course give any indication of the rate of ATP turnover. In this respect heat output may be a better indicator of metabolic rate. Praziquantel, which had a negligible effect on metabolism, is an anti-cestode drug and is inactive against nematodes.

Of the specific inhibitors, the amino-transferase inhibitor amino-oxyacetate, the glycolytic inhibitor iodoacetamide and the beta-oxidation inhibitor 4-pentenoic acid all gave marked inhibition of oxygen uptake and heat output. This indicates that carbohydrate, lipids and amino acids can all be used as substrates. The inhibitory effect of itaconic acid suggests the presence of a glyoxylate cycle. Nematodes are the sole metazoan group possessing the complete glyoxylate bypass, a tricarboxylic acid cycle variant (Barrett, Ward & Fairbairn, 1970). The cytochrome chain inhibitors cyanide,

Table 1

Effect of inhibitors on indicators of metabolic activity in *Globodera rostochiensis*
(Nematodes were exposed to inhibitors for a standard 30 min unless otherwise stated)

Inhibitor	Concentration (M)	% inhibition over untreated controls Mean (range) n = 4		
		Oxygen uptake	Heat output	ATP level
NEMATICIDES				
Aldicarb	10^{-3}	n.d.	66* (36-85)	10 (+ 5-27)
Fenamiphos	10^{-3}	3 (0-5)	23* (10-30)	+ 3 (+12-1)
Carbofuran	10^{-3}	12* (4-20)	28* (10-40)	0 (+16-13)
Oxamyl	10^{-3}	+ 20* (0-+ 30)	3* (0-5)	10* (12-9)
ANTHELMINTICS				
Bunamidine	10^{-3}	10* (5-20)	40* (30-59)	0 (+11-7)
Dichlorvos	10^{-3}	14 (0-28)	0 (+ 5-9)	15 (+ 1-28)
Ivermectin	10^{-3}	30* (17-43)	44* (12-66)	+ 20 (+ 60-16)
	10^{-6}	7 (0-17)	19* (0-25)	0 (+ 5-10)
Levamisole	10^{-3}	27* (11-50)	30* (20-50)	5 (+ 32-19.5)
Nitroxynil	10^{-3}	+ 9 (+ 15-11)	+ 8 (+ 10-4)	4 (+ 2-7)
Piperazine	10^{-3}	6* (0-9)	25* (16-33)	23 (7-42)
Pyrantel	10^{-3}	12 (0-25)	33* (22-45)	6 (+ 16-20)
Vyprinium	10^{-3}	2 (0-7)	0 (+ 20-19)	3 (+ 10-4)
Cambendazole	10^{-3}	2 (0-5)	0	(inhibits assay)
24 h	10^{-3}	14 (0-33)	41* (34-49)	(inhibits assay)
Parbendazole	10^{-3}	0	0	+ 13 (+ 28-5)
24 h	10^{-3}	10* (6-14)	0	0 (+ 7-6)
Praziquantel	10^{-3}	3 (0-9)	0	+ 17 (+ 8-+ 32)
METABOLIC INHIBITORS				
Amino-oxyacetate	10^{-3}	43* (20-55)	54* (40-69)	6 (1-15)
Itaconic acid	10^{-3}	16* (0-29)	21* (15-27)	+ 5 (+ 20-10)
Iodoacetamide	10^{-3}	30* (20-39)	25* (10-48)	30* (21-42)
4-Pentenoic acid	10^{-3}	21* (10-37)	34* (17-50)	6 (0-15)
CYTOCHROME CHAIN INHIBITORS				
Antimycin	4×10^{-6}	0 (+ 5-2)	0 (+ 14-11)	0 (+ 2-1)
Cyanide	10^{-4}	21* (12-30)	32* (10-54)	27* (17-31)
2,4 dinitrophenol	5×10^{-3}	2* (+ 10-2)	15* (10-28)	26* (4-39)
	5×10^{-4}	+ 7* (+ 14-30)	+ 4 (+ 9-0)	5 (0-10)
Rotenone	10^{-3}	69* (66-72)	50* (28-80)	0 (+ 26-19)
Salicylhydroxamic acid	10^{-3}	2 (0-10)	10* (4-15)	9 (+ 10-30)

+ Indicates stimulation; n.d. = no data; * significantly different from controls at the 95 % confidence level.

2,4 dinitrophenol and rotenone all had a marked effect on oxygen uptake and heat output showing that there was a significant aerobic component in the metabolism of these nematodes. The relative lack of effect of cytochrome chain inhibitors on the ATP levels also indicates that these organisms have alternative anaerobic pathways. The relative lack of effect by salicylhydroxamic acid and antimycin compared to rotenone may be due to failure of these compounds to penetrate the cuticle.

Selective permeability to the different inhibitors considerably complicates the interpretation of the re-sults. At least some of the differences between the responses of *P. redivivus* and *G. rostochiensis* to the different inhibitors may be due to the failure of the test compounds to penetrate the cuticle of *G. rostochiensis*. Similarly the slow response to cambendazole and parbendazole could be due either to a slow acting target (such as tubulin binding) or to slow penetration of the cuticle.

This work indicates that heat output is a more sensitive measure of metabolism than ATP levels and it has the advantage over ATP determination that it is non-

Table 2

The effect of inhibitors on indicators of metabolic activity in *Panagrellus redivivus*
(Nematodes were exposed to inhibitors for a standard 30 min unless otherwise stated)

Inhibitor	*Concentration (M)*	*% Inhibition over untreated controls Mean (range) n = 4*		
		Oxygen uptake	*Heat output*	*ATP level*
NEMATICIDES				
Aldicarb	10^{-3}	n.d.	24* (+ 20-67)	n.d.
Fenamiphos	10^{-3}	+ 5* (+ 2-+ 8)	+ 22* (+ 21-+ 26)	+ 5* (+ 2-+ 6)
Carbofuran	10^{-3}	+ 13 (+ 26-0)	+ 25* (+ 19-+ 37)	6 (+ 9-20)
Oxamyl	10^{-3}	+ 2* (+ 4-0)	8* (4-13)	13 (2-25)
ANTHELMINTICS				
Bunamidine	10^{-3}	52* (36-62)	34* (28-40)	0 (+ 15-9)
Dichlorvos	10^{-3}	5* (0-7)	12* (10-14)	0 (+ 9-7)
Ivermectin	10^{-3}	36* (29-47)	62* (50-75)	0 (+ 3-5)
	10^{-6}	9 (0-17)	9* (0-10)	0 (+ 4-10)
Levamisole	10^{-3}	12* (6-18)	55* (30-70)	0 (+ 3-18)
Nitroxynil	10^{-3}	+ 25* (+ 19-+ 30)	+ 14 (+ 26-0)	+ 8 (+ 20-10)
Piperazine	10^{-3}	23* (15-35)	76* (70-80)	0 (+ 2-5)
Pyrantel	10^{-3}	17 (6-45)	63* (50-74)	4 (+ 4-25)
Vyprinium	10^{-3}	10 (0-40)	37* (30-46)	2 (+ 20-10)
Cambendazole	10^{-3}	0	0	0 (0-8)
24 h	10^{-3}	17* (10-25)	32* (15-44)	8 (0-20)
Parbendazole	10^{-3}	0	0	0 (+ 10-5)
24 h	10^{-3}	4 (0-9)	33* (19-55)	14* (7-22)
Praziquantel	10^{-3}	0	0	0 (+ 6-17)
METABOLIC INHIBITORS				
Amino-oxyacetate	10^{-3}	50* (47-54)	21* (8-36)	0 (+ 12-25)
Itaconic acid	10^{-3}	22* (19-24)	22* (4-40)	0 (+ 9-5)
Iodoacetamide	10^{-3}	0	0	0 (+ 5-2)
4-Pentenoic acid	10^{-3}	18* (14-23)	7* (5-10)	7 (+ 14-16)
CYTOCHROME CHAIN INHIBITORS				
Antimycin	4×10^{-6}	33* (22-46)	32* (12-50)	0 (+ 30-25)
Cyanide	10^{-1}	18 (0-40)	28* (19-37)	18* (12-25)
2,4 dinitrophenol	5×10^{-3}	41* (15-64)	12* (6-14)	0 (+ 10-10)
	5×10^{-4}	+ 5 (+ 14-0)	+ 2 (+ 5-2)	0 (+ 15-8)
Rotenone	10^{-3}	76* (73-80)	83* (80-88)	18 (+ 10-30)
Salicylhydroxamic acid	10^{-3}	0	0	0

+ Indicates stimulation; n.d. no data; * significantly different from controls at the 95 % confidence level.

destructive and could be automated. By investigating the effects of specific inhibitors on metabolism it should be possible to pinpoint pathways which are especially sensitive to perturbation and are, therefore, potential targets for novel nematicides.

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