Role of cyclic nucleotide phosphodiesterases in chemosensation of the nematode *C. elegans*

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Abstract

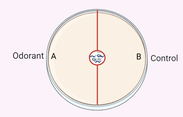
Over 4,000 species of plant parasitic nematodes have a serious impact on global food security, with ~$100B in crop damages annually. Nematode behavior and lifecycle depend on cyclic nucleotide signaling. Phosphodiesterases (PDEs) are major determinants of cellular levels of cyclic nucleotides because they are solely responsible for lowering intracellular levels of the cyclic nucleotide second messengers, cAMP and cGMP. However, there is limited knowledge of the role of individual PDEs in mediating nematode signalling pathways. Mammals possess 11 PDE families (PDE1-PDE11), whereas nematodes have PDE genes representing six families. Using the free-living nematode *C. elegans* to generate transgenic strains lacking individual PDE genes, we show that ablating *pde-1* abolished chemosensation to two attractant compounds (2-butanone and isoamyl alcohol) and a repellent compound (1-octanol). To mimic the phenotype observed in this *pde-1* "knock-out" strain, we found that exposure of wild-type *C. elegans* (N2 strain) to a human PDE1-specific inhibitor also reduced the chemotactic response to the 2-butanone. This nematode PDE family may be a future target for the development of chemical nematicides that disrupt the ability of parasitic nematodes to identify and invade their host. This work supports the feasibility of designing nematicidal compounds targeting specific parasitic nematode PDEs lacking adverse effects on vertebrate animals or agricultural crops.

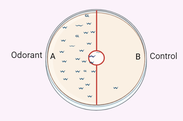
C. elegans PDE gene	Canonical sequence	Vertebrate ortholog	Name	PDE inhibitors	Substrate specificity
pde-1	T04D3.3a.1	PDE1	Ca^{2+}-calmodulin-dependent PDE	PF-04471141	cAMP, cGMP
pde-2	R08D7.6a	PDE2	cGMP-stimulated PDE	PF-05085727	cAMP, cGMP
pde-3	E01F3.1e.1	PDE3	cGMP-inhibited PDE	Cilostamide	cAMP > cGMP
pde-4	R153.1d.1	PDE4	cAMP-specific PDE	Rolipram	cAMP
pde-5	C32E12.2.1	PDE10	cAMP-inhibited PDE	MP-10	cAMP > cGMP
pde-6	Y95B8A.10	PDE8	high affinity cAMP-specific PDE	PF-4957325	cAMP

Methodology

Chemotaxis assay:

- Young adults (L3-L4) nematodes were exposed to PDE inhibitor in liquid culture for 1 h.
- 2 µL of (attractant or repellant: A) or ethanol (control: B) were spotted on the A and B spots
- ~ 25 nematodes were transferred to the center circle of the chemotaxis plate
- Nematodes in each hemisphere were counted and the chemotactic index (C.I) was calculated C.I = (A-B)/(A+B)
  - Positive CI = attraction
  - Negative CI = avoidance/repulsion





References

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Results

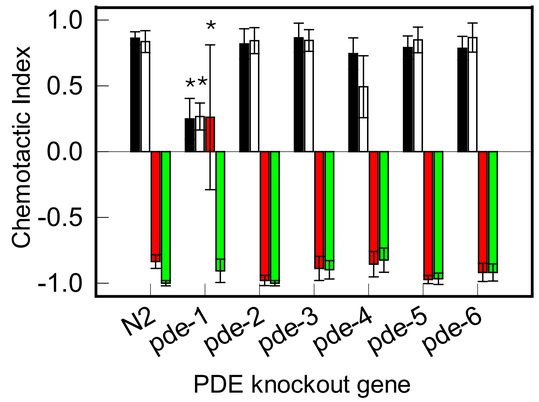

Fig. 1. Chemosensation in the *pde-1* "knock-out" strain is disrupted. Juvenile *pde-1* knockout nematodes were exposed to 2-butanone (*black*), isoamyl alcohol (*white*), 1-octanol (*red*), or nonanone (*green*) and allowed to move freely for 30 min. The chemotactic index (*see Methods*) was calculated by counting nematodes in the A and B hemispheres; * denotes $p < 0.05$ (n=2) compared to the N2 control.

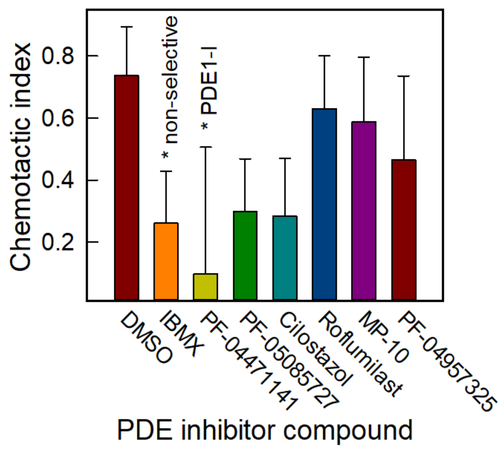

Fig. 2. Chemoattraction to 2-butanone is abolished in wild-type nematodes exposed to a human PDE1-selective inhibitor: Juvenile wild-type (N2) *C. elegans* were exposed for 1 h to a pan-specific inhibitor (IBMX) or human PDE family-selective compounds (500 µM for IBMX, 125 µM for most compounds and 25 µM for PF-04471141) prior to initiating the chemotaxis assay. * denotes $p < 0.05$ (Holm-Siak pairwise test; n = 3).

Conclusions

- Ablation of the nematode *pde-1* gene disrupts chemosensation to several attractant and repulsive chemical compounds.
- Exposure of wild-type *C. elegans* to a human PDE1-selective inhibitor mimics the effect of gene ablation in abolishing chemoattraction to 2- butanone.
- Targeting nematode PDE1 with chemical or genetic controls holds promise for disrupting the ability of plant parasitic nematodes to identify and invade plant hosts of agricultural importance, thereby enhancing crop production.

Acknowledgments

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