Presentation 5 – Carrolee Barlow

NTE and identification of possible molecular targets of neurotoxic exposures in Gulf War Veterans

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Oct 27, 28 2003 Meeting of the Research Advisory Committee on Gulf War Veterans' Illnesses
US Department of Veteran Affairs

Drosophila *Swiss Cheese* (SWS) Gene

- Use information from model organisms.
- Progressive glial hyperwrapping and apoptosis of both neurons and glia.
- Mechanism unknown.

Environmental Toxins and Neurodegeneration

- Drosophila swiss cheese (*sws*)
- Biochemically identified Neuropathy Target Esterase (NTE) as the mammalian *sws (Glynn and others)*
- Thought to be targeted by a class of organophosphates (OPs) that cause progressive neurological symptoms

Chris Winrow

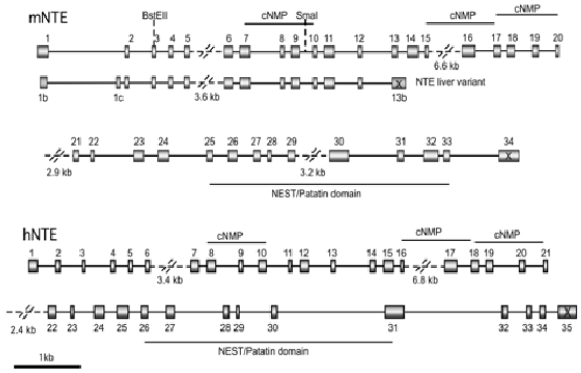
Neuropathy Target Esterase (NTE)

Esterase activity inhibited by a subset of organophosphates (OPs) responsible for neuropathies (paraoxan-resistant, mipafox sensitive).

Two functional domains identified:
- Regulatory N-terminal domain (cyclic nucleotide binding region/PKA regulatory subunit)
- Catalytic C-terminal domain (serine esterase)

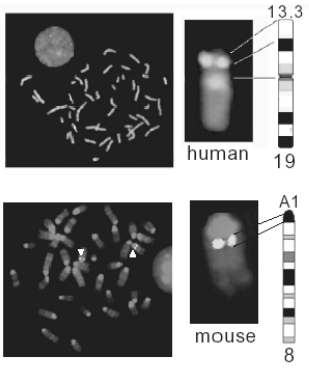
Natural substrate and function not clear.

NTE is highly conserved

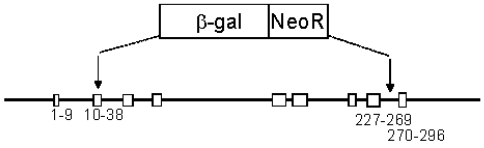


Human and Mouse NTE mapping

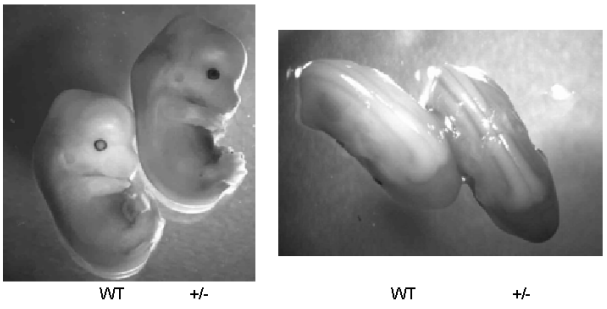
a

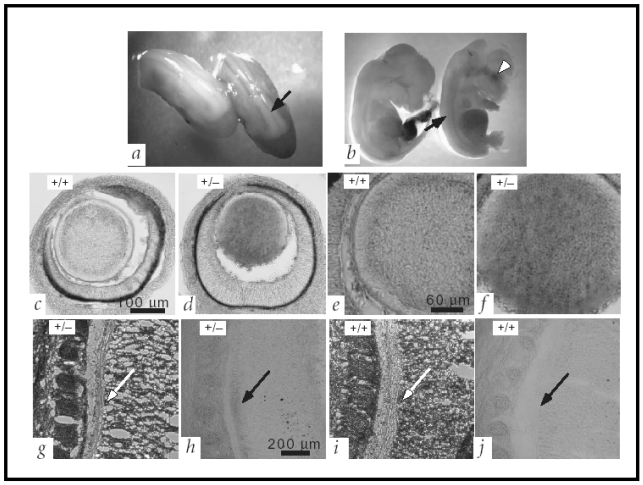


mNTE knock-out insertion sites

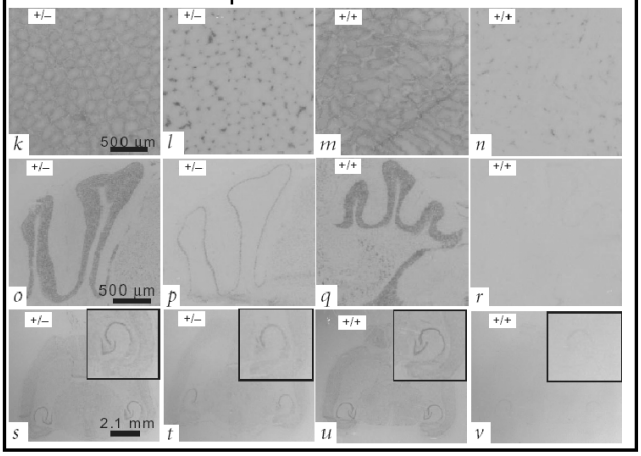


X-gal staining enables localization of NTE in heterozygous mice

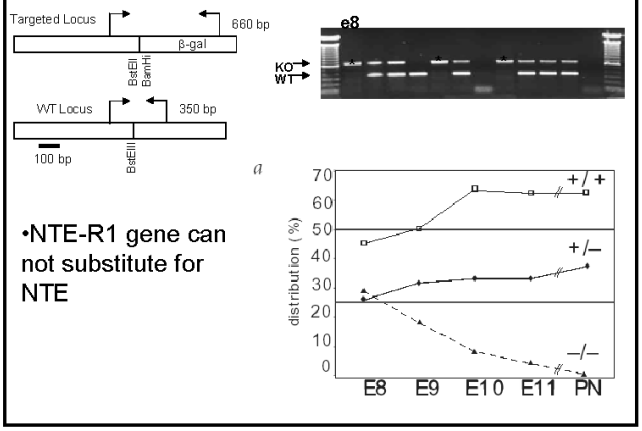




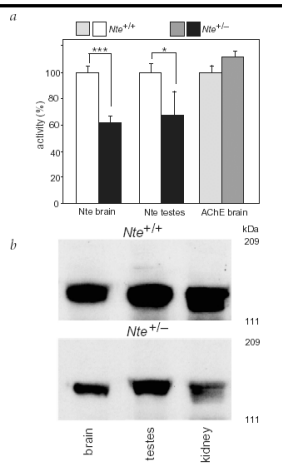
Expression in adult



NTE -/- Embryos Are Not Viable Beyond e8-e9



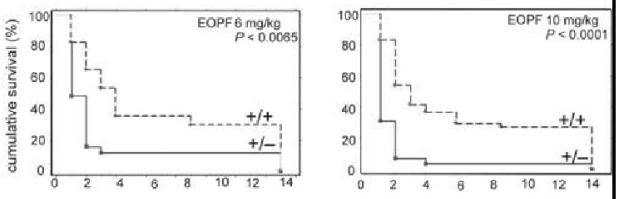
•NTE-R1 gene can not substitute for NTE



WT NTE heterozygous mice appear grossly normal but have reduced levels of NTE activity and protein compared to wildtype littermates (30-50%)

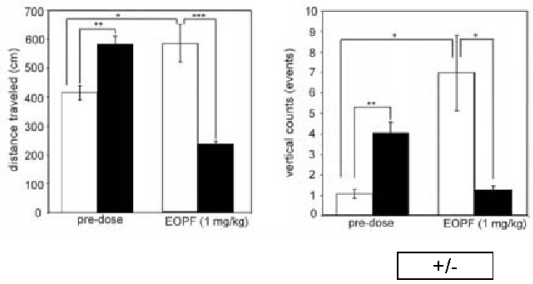
John Casida

Heterozygous mutants have +/- increased sensitivity to OPs WT.



Matt Hemming, Rob Helton

Partial inhibition of NTE (chemical OR genetic) leads to hyperactivity



Establishes that NTE is a target of OPs that cause neurological symptoms in mammals

news & views

Neurotoxic esterase: not so toxic?

James P. O'Callaghan

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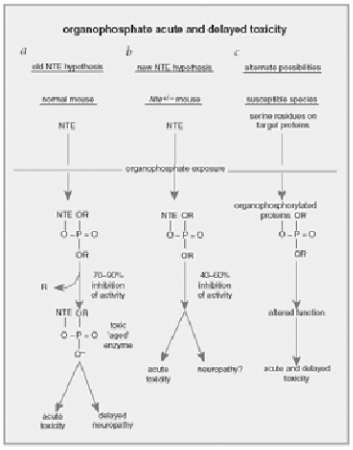
Published online 17 March 2003; doi:10.1038/ntg1135

An altered form of an esterase has been implicated in the development of neurotoxicity after exposure to organophosphates. Mice deficient in this enzyme should be less susceptible to toxicity, but the opposite turns out to be the case.

Revisiting the NTE hypothesis. Pathways leading to acute toxicity and delayed neuropathy associated with exposure to organophosphates. According to a long-standing concept in neurotoxicology, the activity of a target esterase (neuropathy esterase or neuropathy target esterase) is inhibited by organophosphorylation at a key serine residue (refs. 4,5, 6). When inhibition of enzyme activity reaches 70–90% and, when phosphorylated NTE is modified by loss of a functional group (R), the enzyme is 'aged' and initiates the steps leading to delayed neuropathy and less prominent acute toxic effects. By generating *Nte*$^{+/-}$ mice, Winrow *et al.*[7] showed that mice with less NTE and lower activity of NTE are more sensitive to the toxic effects of prototypical organophosphate compounds (8), findings that seem to rule out the toxic gain-of-function (aged) phenotype that serves as the key feature of the NTE hypothesis. Alternative possibilities should be considered (2) because organophosphates have the potential to phosphorylate a variety of key serine-containing substrates, leading to altered function of a given protein[9,10] and potentially accounting for acute and delayed neurotoxic effects.



From O'Callahan News and Views

What next?

- Identify in vivo target of NTE
- Better define the biological function of NTE
- Identify individuals at risk?

Evidence that mouse brain neuropathy target esterase is a lysophospholipase

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Contributed by John E. Casida, April 25, 2003

Table 1. Relationship between brain NTE-LysoPLA and NTE activities of NTE-deficient mice

Genotype*	NTE-LysoPLA, mAU/min†	NTE, AU†
Absolute activity		
+/+	4.09 ± 0.22	0.321 ± 0.018
+/−	2.43 ± 0.19‡	0.176 ± 0.045‡
Relative activity, %		
+/− ÷ +/+	59	55

Nte heterozygous 129S6/SvEvTac (*Nte*$^{+/-}$) transgenic mice and their wild-type littermates.
†NTE-LysoPLA and NTE assayed with lysolecithin and phenyl valerate, respectively, $n = 7$ for +/+ and 4 for +/− in each case as the average of four assays for NTE-LysoPLA and two for NTE. Data are mean ± SE.
‡Significant difference ($P < 0.01$) for both NTE-LysoPLA and NTE (comparison of +/+ with +/−).

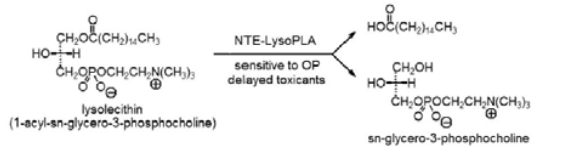


Fig. 2. Proposed biochemical lesion induced by OP delayed toxicants or neurotoxicants involving inhibition of NTE-LysoPLA, which normally hydrolyzes lysolecithin as its physiological substrate. The classical substrate for NTE is phenyl valerate, based on ease of assay (4, 12) rather than mechanistic considerations. NTE-LysoPLA and NTE in brain are resistant to paraoxon but sensitive to mipafox and other delayed toxicants or neurotoxicants.

7984 | www.pnas.org/cgi/doi/10.1073/pnas.1232473100 Quistad *et al.*

Table 2. Relationship between *in vivo* inhibition of brain NTE-LysoPLA and NTE activities and delayed toxicity

Toxicant and dose, mg/kg	Enzyme inhibition, %* NTE-LysoPLA	NTE	Delayed toxicity
EOPF			
1	18 ± 15	0 ± 0	–[†]
2	89 ± 12	89 ± 2	
3	100 ± 0	78 ± 5	+[†]
10	99 ± 1	95 ± 4	+[†]
(*S*)-octyl-BDPO			
5	71 ± 8	92 ± 7	+[†]
(*R*)-octyl-BDPO			
5	7 ± 8	6 ± 7	–[†]
o-Tolyloxy-BDPO			
3	20 ± 17	8 ± 4	–
10	70 ± 4	55 ± 13	–
30	89 ± 7	94 ± 7	–[‡]
100	87 ± 16	100 ± 0	–[‡]
DSF			
100	92 ± 9	100 ± 0	+
Tribufos			
30	7 ± 8	11 ± 9	–[§]
100	85 ± 16	100 ± 0	+[§]

Compounds were administered i.p. to Swiss–Webster mice with determinations of enzyme activities at 4 h and delayed toxicity at 3 days.
*NTE-LysoPLA and NTE were assayed with lysolecithin and phenyl valerate, respectively. $n = 3$ in each case as the average of duplicate assays for NTE-LysoPLA and single determinations for NTE. Data are mean ± SE.
[†]Ref. 14.
[‡]Cholinergic poisoning signs at 100, but not 30, mg/kg.
[§]Ref. 16.

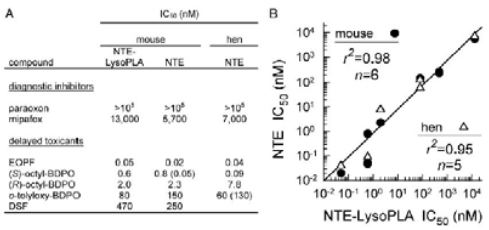
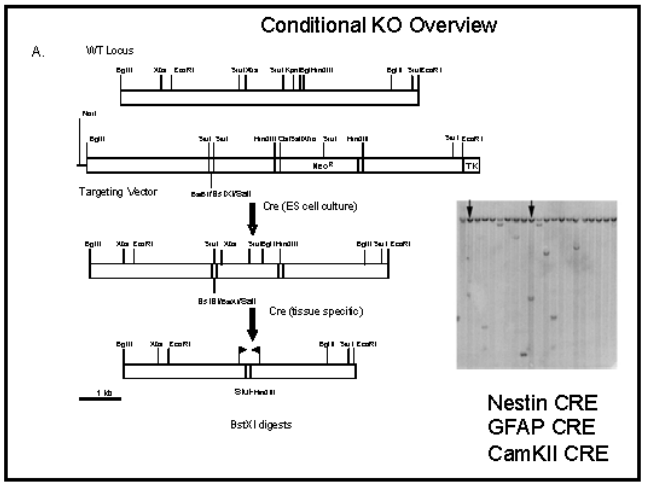


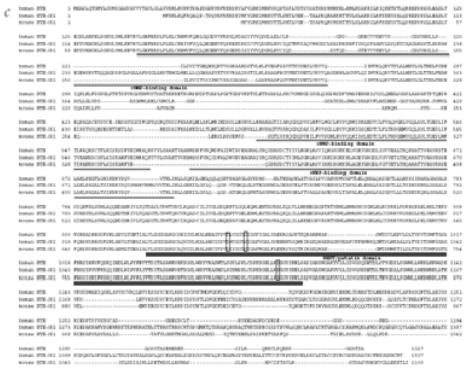
Fig. 3. Relationship between *in vitro* sensitivities of brain NTE-LysoPLA and NTE activities. NTE-LysoPLA and NTE were assayed with lysolecithin and phenyl valerate, respectively. Compound structures are shown in Fig. 1. (*A*) The data for mouse NTE-LysoPLA for all compounds and mouse NTE for paraoxon, (*S*)-octyl-BDPO, and DSF are from this study with Swiss–Webster mice. Other data for mouse NTE are from this laboratory (14, 16) or Veronesi *et al.* (26) for *o*-tolyloxy-BDPO. The hen NTE data are from Wu and Casida (13, 15, 27), Johnson (11), and Veronesi *et al.* (26). (*B*) Correlation for sensitivity of NTE-LysoPLA from mouse brain versus NTE from mouse brain (●) and hen brain (△). Correlation coefficients (r^2) do not include tabulated values for paraoxon (>10^3 nM) or those in parentheses, which were determined by a different investigator (0.05 nM for mouse NTE) or a different laboratory (130 nM for hen NTE).

Generate animals with tissue and time specific complete loss of NTE function



Use advancing genetic/genomic/protein technologies to define populations at increased risk if exposed to OP's

Highly conserved



High throughput genotyping services/products

- Perlegen
- Affymetrix

- Sequenom
- DeCode
- many others

Measuring levels in blood or skin biopsy samples

- Gene expression - Affymetrix
- or TaqMan based probes

- Best to evaluate protein level by Elisa or activity assays

Correlating biochemical and genetic markers with disease

- Clinical databases combining all types of data in high level analytical relational databases- Teradata (NCR)
- Information Management Consultants (IMC, McClain VA)
- Walter Reed/Windber/USUHS

Press Release Source: NCR Corporation

Data Warehousing Used for First Time to Create a Single Database to Help Find the Cause of Breast Cancer
Tuesday September 23, 11:31 am ET Windber Research Institute Determines Teradata as the Only Solution to Aggregate, Seamlessly Integrate and Mine Biological and Clinical Data

SEATTLE--(BUSINESS WIRE)--Sept. 23, 2003-- Windber Research Institute has chosen Teradata, a division of NCR Corporation (NYSE:NCR - News), to create the first and only central data warehouse where molecular and clinical information is being assembled and seamlessly integrated in a single data warehouse to help find the cause of breast and other forms of cancer.

Windber Research Institute (www.wriwindber.org) is an integrated research facility that has the unique ability to simultaneously examine the function of many genes and proteins related to reproductive cancers and heart disease. The Institute is a key component of a multi-institutional coalition consisting of the Clinical Breast Care Project at Walter Reed Army Medical Center, the Joyce Murtha Breast Care Center at the Windber Medical Center, and the Immunology Research Center at the Uniformed Services University of the Health Sciences in Bethesda, Maryland.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