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PARTIAL CHARACTERIZATION OF NEUROTOXIC ESTERASE OF HUMAN PLACENTA

(Organophosphorus; delayed neurotoxicity; axonopathy)

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SUMMARY

Neurotoxic esterase (NTE), the putative target for organophosphorus-induced delayed axonopathy, has been found in preparations of human placenta. The activity was primarily found in membrane-enriched fractions rather than high-speed supernatant. NTE was solubilized from a mixture of mitochondrial and microsomal membranes with Triton X-100. The crude and solubilized activities had inhibitor characteristics similar to preparations from avian brain. Because of the similarities to NTE from brain and ready availability, human placenta may be an ideal source for the bulk purification of a human form of the enzyme.

INTRODUCTION

Delayed, distal axonopathy produced by organosphosphorus compounds such as tri-*o*-cresyl phosphate, diisopropylphosphofluoridate, and *N,N'*-diisopropylphosphorodiamidofluoridate (mipafox) is believed to be initiated by the inhibition and subsequent aging of a membrane protein called NTE [1]. Although initial studies of NTE have been conducted with chicken brain [1], NTE has been found in the neural tissue of a large number of different vertebrates [2]. Recently NTE has been found in non-neural tissues such as avian splenic and circulating lymphocytes [3], and human leukocytes [4].

To better understand the function of NTE and its relationship with the delayed neuropathy, we have been engaged in subcellular fractionation [5] and solubilization

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Abbreviations: BSA, bovine serum albumin; NTE, neurotoxic esterase; PV, phenyl valerate.

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[6] of the enzyme activity from avian brain. In an effort to find a convenient source of human enzyme to purify and characterize, we decided to determine if full-term human placenta contained NTE activity similar to the activity found in avian [3] and human [7] brain.

MATERIALS AND METHODS

Studies were conducted with full-term human placenta obtained from the delivery room from the University of Michigan Hospital, Ann Arbor, MI, or St. Joseph Mercy Hospital, Ann Arbor, MI. The donors were all normal, and reportedly did not smoke tobacco or consume alcohol during pregnancy. Placentas were obtained within 2 h of delivery and immediately chilled by sealing the tissue in a polyvinyl chloride bag and immersing it in crushed ice. Further processing was carried out after returning to our lab.

Approx. 50 g of the placental lobules were teased apart, freed from any visible blood clots, and suspended in about 1 liter of cold (4°C) buffer consisting of 0.25 M sucrose, 0.05 M Tris, and 0.1 mM EDTA, pH 8.0 at 25°C. After suspending the tissue in the buffer, the tissue was allowed to settle, and the buffer was decanted and discarded. The tissue was washed three times with the above buffer and an additional three times with unbuffered 0.25 M sucrose. The tissue was finally suspended in about 200 ml of 0.25 M sucrose, homogenized in a Waring blender at 4°C for 3 min, and adjusted to 250 ml with 0.25 M sucrose. The homogenate was then assayed after dilution with buffer.

Crude subcellular fractions were prepared by centrifugation in 0.25 M sucrose at 4°C [5]. The fractions were diluted with buffer before measurement of NTE activity or protein.

For solubilization with Triton X-100, the crude homogenate was centrifuged at 800 × *g* for 10 min, and the P_1 fraction (nuclei + debris) was discarded. The remaining supernatant was centrifuged at 100000 × *g* for 60 min to give a combined P_2P_3 fraction (mitochondria + microsomes). Pellets (P_2P_3 fraction) were thoroughly drained, and a single pellet was suspended in about 10.0 ml of 0.25 M sucrose with the aid of a Ten Broeck homogenizer. Protein was immediately determined by the biuret method [8] using BSA as the standard. For each 6.6 mg of biuret protein, 1.0 ml of aqueous Triton X-100 of varying concentrations was added to the remaining pellets. The pellets were then suspended in the detergent solution with 15 passes of a Ten Broeck homogenizer. The solubilized material was centrifuged at 100000 × *g* for 60 min and the supernatant saved for further analyses.

Assays of NTE activity and titrations of total esterase activity were done with PV as the substrate and diethyl *p*-nitrophenyl phosphate (paraoxon) and mipafox as the inhibitors [2, 3]. NTE activity was always measured at 37°C in buffer composed of 0.1 mM EDTA and 0.05 M Tris, pH 8.0. Specific activity is always expressed as nmol phenol released/min/mg of Lowry protein [9] with BSA used as the standard.

TABLE I

SUBCELLULAR DISTRIBUTION OF NTE FROM HUMAN PLACENTA

Fraction	Spec. act.[a]	Total act.[b]	% Recovery of activity	Total protein (mg)	% Recovery of protein
Crude homogenate	16.1	12228	100.0	759.5	100.0
Nuclei + debris	17.1	2545	20.8	148.8	10.0
Mitochondria	38.1	3002	24.6	78.8	10.4
Microsomes	36.6	3221	26.3	88.0	11.6
Supernatant	0.6	259	2.1	431.8	56.9

[a]nmol phenol released/min/mg protein.
[b]nmol phenol released/min.

All results reported below are means of duplicate experiments except for Table I which illustrates a representative experiment.

RESULTS AND DISCUSSION

The amount of NTE found in crude placental homogenate as well as crude subcellular fractions is shown in Table I. The specific activity of NTE in the crude homogenate and the P_1 fraction are similar, but the specific activity is lower than similar fractions from adult hen brain [6]. Crude mitochondrial and microsomal fractions were both enriched in NTE activity. NTE in placenta thus appears to be membrane associated as is the case with the enzymes from avian brain [5].

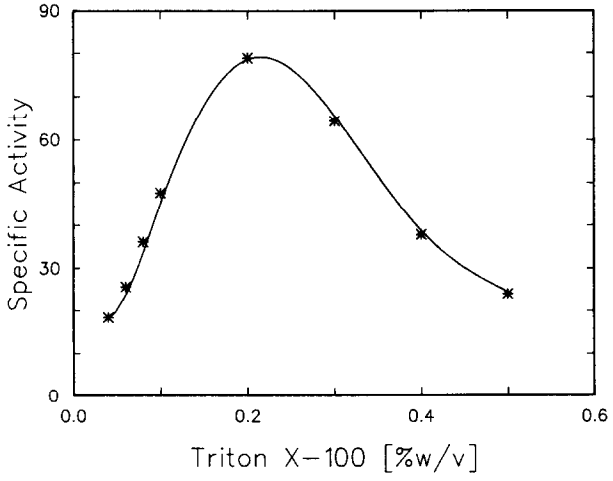

Fig. 1. Solubilization of NTE from P_2P_3 fraction of human placenta with Triton X-100.

Placental NTE activity could be solubilized from membranes with aqueous Triton X-100 as seen in Fig. 1. The most effective concentration of detergent is 0.2% w/v or when the weight ratio of detergent to protein equals 0.30. Solubilization of NTE results in a preparation with a specific activity of 80 nmol/min/mg of protein which compares with 40 to 60 nmol/min/mg of protein for solubilized preparations from hen brain [10].

Fig. 2. shows further characterization of placental PV esterase activity by differential titration in the presence of 50 μM paraoxon. The titration curves are similar to those obtained from preparations of adult chicken brain [1, 3] and human brain [7]. Placental preparations had mipafox pI_{50} values of 4.7–5.0 which were similar to those obtained for hen brain homogenate or solubilized P_3 (5.1) [6] or human brain homogenate (4.8–4.9) [7]. These results indicate that the inhibitor characteristics of NTE are similar in placental and brain preparations both in native and solubilized form, and provide evidence that the brain and placental activities may represent the same enzyme in different tissue sites.

Preincubation of the crude soluble fraction with mercuric acetate gave a pI_{50} of 5.8, consistent with PCMB inhibition obtained by Johnson [14] with hen brain homogenate. Inhibition by the mercuric ion suggests that a sulfhydryl is probably required for esteratic activity in the enzymes of placenta and brain.

Finding NTE in human placenta should not be too surprising since a cholinergic system [11], beta-endorphin [12], and methionine enkephalin, [13] which are associated with neuronal systems, have also been reported. Our results show that human placental NTE is similar to forms found in avian and human brain. Since human placenta is easily obtained compared to other human tissue, it may be an ideal source of material for further purification and characterization of NTE.

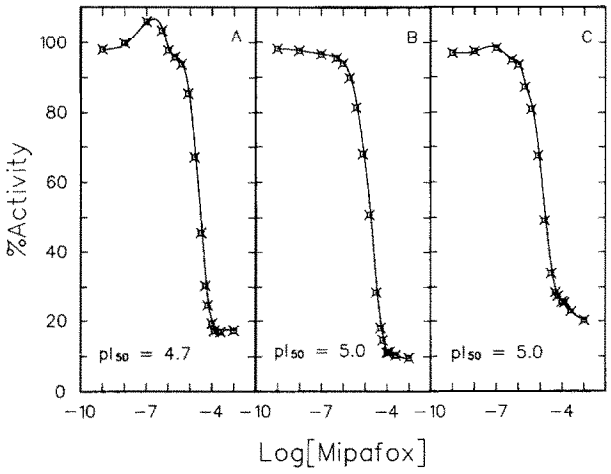

Fig. 2. Titration of placental NTE with mipafox. (A) crude homogenate: (B) P_2P_3 fraction; (C) solubilized P_2P_3 fraction.

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