440 kDa Isoform of Brain Ankyrin as a Sensitive Marker for the Neurotoxicity of Methylmercury

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Selective vulnerability of various parts of the nervous system to injury from methylmercury intoxication is a well-established though as yet unexplained phenomenon in man and experimental animals. One of the areas characteristically altered is the cerebellum both in the adult and during development.¹⁾ This study was carried out in an attempt to investigate the effects of methylmercury on the expression and localization of a neuron-specific protein, 440 kDa isoform of brain ankyrin (440 kDa ankyrin_B),²⁾ in cerebellar neurons of rats. 440 kDa ankyrin_B is localized in the molecular layer of rat cerebellum.

Primary cerebellar neurons prepared from neonatal rats were maintained in a serum-free medium on poly-Lysine coated plates.³⁾ Administration of methylmercury to neonatal rats (5 d old) was performed for 10 consecutive days according to the method of Sakamoto *et al.*⁴⁾ The expression of marker proteins was estimated by immunoblot analysis and their localization was determined by indirect immunofluorescent microscopy. Total mercury determination was performed according to the oxygen combustion-gold amalgamation method.

When primary cerebellar neurons matured for 7 d *in vitro* were exposed to methylmercury at 0.1 μM for 48 h, viability of the cells was unaffected. However, the staining of 440 kDa ankyrin_B, which is localized in axons, almost completely disappeared, while the staining of MAP (microtubule-associated protein) 2, which is localized in dendrites and cell bodies, and of

GFAP (glial fibrillary acidic protein), a marker for astroglial cells coexisting in the culture, remained unchanged. These observations were confirmed by the determination of each protein expressed in the culture by immunoblot analysis.

In cerebella from 16-d old rats treated with methylmercury (10 times at 10 mg Hg/kg body weight) the amount of 440 kDa ankyrin_B was significantly reduced (89% of control), whereas changes in the amount of MAP2 and GFAP were insignificant. Total mercury level in the cerebella was 262 ng Hg/mg protein, while that in cerebellar neurons exposed to 0.1 μM methylmercury for 48 h was 73 ng Hg/mg protein.

These results clearly indicate that selective loss of axonal protein, 440 kDa ankyrin_B, is associated with the degeneration of cerebellar neurons induced by methylmercury both *in vitro* and *in vivo*. 440 kDa ankyrin_B, therefore, should be useful as a specific and sensitive marker for the neurotoxicity of methylmercury, which could partly be ascribed to its selective effects on neuron-specific proteins including 440 kDa ankyrin_B.

- 1) WHO/IPCS (1990) Environmental Health Criteria 101.
- 2) M. Kunimoto, *et al. J. Cell Biol.* **115**, 1319 (1991).
- 3) M. Kunimoto, *et al. Toxic. in Vitro* **4**, 349 (1992).
- 4) M. Sakamoto, *et al. Environ. Res.* **61**, 43 (1993).