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Plenary article

Embryonic exposure to thimerosal, an organomercury compound, causes abnormal early development of serotonergic neurons

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ABSTRACT

Even though neuronal toxicity due to organomercury compounds is well known, thimerosal, an organomercury compound, is widely used in pediatric vaccine preservation. In the present study, we examined whether embryonic exposure to thimerosal affects early development of serotonergic neurons. Thimerosal (1 mg Hg/kg) was intramuscularly administered to pregnant rats on gestational day 9 (susceptible time window for development of fetal serotonergic system), and fetal serotonergic neurons were assessed at embryonic day 15 using anti-serotonin antibodies. A dramatic increase in the number of serotonergic neurons localized to the lateral portion of the caudal raphe was observed in thimerosal group (1.9-fold increase, $p < 0.01$ compared to control). These results indicate that embryonic exposure to thimerosal affects early development of serotonergic neurons.

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Thimerosal, an organomercury compound, is known for its preservative effects on pediatric vaccines [2,12]. Thimerosal biotransforms *in vivo* to ethylmercury and subsequently into inorganic forms of mercury [19,20], which are toxic to animals [4,7]. Therefore, accumulation of mercury through frequent vaccine administration is a concern [2,26].

The adverse effects of thimerosal have been studied extensively; neonatal administration of thimerosal induces impairment of sensitivity to pain [16] and neurodegeneration of hippocampus [17]. Although fetal organomercury poisoning (fetal Minamata disease) is known to exhibit systemic effects on fetus [5,6], little is known regarding the mechanism of action of thimerosal during the embryonic period.

Serotonergic neurons are one of the earliest neurotransmitter phenotypes to appear during the development of the nervous system [1,8,10]. In the fetal rat, serotonergic neurons were identified at around embryonic day (E) 13 (day of insemination = E1) [1,18]. However, precursor cells that were fated to serotonergic neurons are known to appear, at the latest, by E9 [25]. We previously reported that E9 is the most critical time window for early development of serotonergic neurons [15], because exposure of pregnant rats to thalidomide resulted in caudal shift of serotonergic neurons in the dorsal raphe, suggestive of perturbed neuronal migration [13]. The effect of thalidomide was specific for the day of thalidomide administration, demonstrating that embryonic exposure at E9 is specifically crucial in the normal development of serotonergic neurons.

Since the early development of serotonergic neurons is time specific and three-dimensional [1,8,10], precise evaluation of serotonergic neuronal development by conventional immunohistochemical methods is difficult. In the present study, we utilized whole-mount preparation method for embryonic brain [1,9], which facilitates assessment of spatiotemporal data on the development of neurotransmitter system. Using this technique, we investigated whether exposure to thimerosal at E9 affects early development of serotonergic neurons.

Thimerosal administration: Pregnant Wistar rats were purchased by CLEA Japan, Inc. (Tokyo, Japan). Thimerosal (Sigma–Aldrich, St. Louis, MO) dissolved in saline (1 mg Hg/kg) was administrated into pregnant rats on gestational day 9 in volume of 50 μl, by intramuscular injection into *glutei maximi*. For control group, saline was administrated in the same way. Three dams for each group (thimerosal vs control) were examined. All animal experiments were authorized by the Animal Research Committee in Mie University.

Flat whole-mount preparation of rat brain: The procedure for preparing flat whole-mount hindbrain has been described previously [1,9,21,22]. In brief, E15 fetuses were dissected out and the

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