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Research Report

Prenatal exposure to the acetylcholinesterase inhibitor methanesulfonyl fluoride alters forebrain morphology and gene expression

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Abstract

Methanesulfonyl fluoride (MSF) is a CNS-selective acetylcholinesterase (AChE) inhibitor, currently being developed and tested for the treatment of symptoms of Alzheimer's disease [D.E. Moss, P. Berlanga, M.M. Hagan, H. Sandoval, and C. Ishida, Methanesulfonyl fluoride (MSF): a double-blind, placebo-controlled study of safety and efficacy in the treatment of senile dementia of the Alzheimer type, Alzheimer Dis. Assoc. Disord., 13 (1999) 20–25] [43]. We have previously confirmed that a single in utero exposure to MSF at clinically appropriate doses inhibits AChE activity in fetal rat brain by 20%, and when administered throughout gestation, MSF achieves a 40% level of inhibition. Here, we show that rats chronically exposed in utero to MSF display marked sex-specific differences in morphological development of the cerebral cortical layers compared with controls at 7 days of age. Forebrain size and cortical thickness were increased in females and decreased in males. An analysis of gene expression in neonate brain on the day of birth revealed sex-specific differential expression of over 25 genes, including choline acetyltransferase (ChAT), which were affected by prenatal MSF exposure. Many of these genes are associated with sexual differentiation and brain development, while others are involved in more generalized cellular and metabolic processes. The changes observed in cortical morphology and gene expression suggest a critical developmental role for AChE in the fetal nervous system, most likely through its effect on cholinergic neurotransmission.

Theme: Development and regeneration
Topic: Neurotrophic factors: biological effects

Keywords: Methanesulfonyl fluoride; Gene expression; Acetylcholinesterase; Cortical morphogenesis

1. Introduction

The primary function of acetylcholinesterase (AChE) is the normal termination of cholinergic neurotransmission by breaking down acetylcholine (ACh) at the synaptic cleft. Its early expression and distribution during embryogenesis of vertebrates is closely linked to neuronal outgrowth and development of the nervous system itself [31]. It is reasonable to assume that many and possibly all of the developmental effects associated with AChE can be ascribed to the enzyme's effect on cholinergic neuro-

Abbreviations: ACh, acetylcholine; AChE, acetylcholinesterase; ChAT, choline acetyltransferase; CNS, central nervous system; CDK8, cyclin-dependent kinase 8; CGI-110, comparative gene identification, transcript 110; CYP19, cytochrome p450, subfamily XIX (aromatase); EAA, excitatory amino acid; EGFR, epidermal growth factor receptor; ESTs, expressed sequence tags; IGF-2, insulin-like growth factor receptor 2; MSF, methanesulfonyl fluoride; nBM, nucleus basalis magnocellularis (basal forebrain); PKC-γ, protein kinase C, gamma subunit; PND, postnatal day; PO, peanut oil; qPCR, quantitative polymerase chain reaction; SYTX, syntaxin 5A

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transmission [19,20], although AChE could play a morphogenetic role in its own right.

To better understand the influence of the cholinergic system on brain growth and development, we chose to inhibit the activity of AChE during gestation by prenatal exposure to methanesulfonyl fluoride (MSF), an AChE inhibitor that binds irreversibly to the serine residue of AChE, blocking its hydrolase activity. We have previously shown that a single, clinically significant exposure to MSF during gestation results in a 20% reduction of fetal AChE activity when measured at embryonic day 19 [11]. Furthermore, chronic exposure during the latter 2 weeks of gestation results ultimately in a 40% inhibition of AChE.

Both long-acting and highly specific for the central nervous system, MSF has been proposed and recently tested for its potential in treating dementia of the Alzheimer type [43,44,48]. In studies designed to observe the effects of prenatal AChE inhibition on cognitive behavior in the adult rat, significantly impaired performance in the 8-arm radial maze was observed [14], as well as decreased interest in exploring novel objects, among 2-month-old offspring born to MSF-treated females [11]. These behavioral deficits are consistent with alterations induced by perinatal stress [17,36,47,58], other gestational toxins such as Pb [5,6,39,52] and organophosphates which are known AChE inhibitors [37,40,42,51,56].

In view of the evidence cited above for a morphogenetic role for the cholinergic system, and the behavioral consequences of disrupting the normal time course for development of cholinergic pathways, we hypothesized that disrupting cholinergic function with the AChE-inhibiting drug MSF, would alter brain morphogenesis. We also sought to determine if changes in gene expression might underlie the alterations in brain morphology and behavior, as a step toward understanding the mechanism of any effects of prenatal AChE inhibition. We selected the forebrain of the week-old rat for morphological analysis because of its well-defined cytoarchitecture and developmental sensitivity to cholinergic interference at this stage of development [19,20].

2. Materials and methods

2.1. In utero treatment

Thirteen pairs of Sprague–Dawley rats were bred in house and maintained in the animal holding facility of the Department of Biology, University of Texas at El Paso. During breeding and treatment, all animals had access to food and water ad libidum in accordance with institutional animal care requirements and NIH specifications. Female rats were paired with males for a 1-week breeding period and then removed to individual cages during their treatment and gestation period. Twenty-four hours later, the females were started on an injection schedule of either peanut oil (PO) vehicle (n = 6) or MSF (n = 7) (Lancaster Synthesis, Inc., Windham, NH). Backward calculation from the date of birth verified that all pups began their exposure period at gestational day 8 or 9 (GD8–GD9). MSF was dissolved to 1 mg/ml in PO and 0.5 mg/kg body weight was injected subcutaneously at the back of the neck. Doses were calculated from dose/response curves as determined by Moss et al. [44]. They are similar to human clinical doses, and therefore not expected to have obvious teratogenic consequences. Injections continued every other day throughout gestation with all pups receiving a total of 7 in utero exposures. Histological analysis was done on pups collected randomly from PO (n = 3) and MSF (n = 4) litters, and gene expression assays were performed on pups collected from 3 PO and 3 MSF litters.

2.2. Histology

At postnatal day seven (PND 7), 18 MSF-exposed pups (9 male, 9 female) and 16 PO-exposed control pups (8 male, 8 female) were anesthetized with an injection of sodium pentobarbital (65.0 mg/kg body wt) and transcardially perfused with 4% buffered (pH 7.4, PBS) paraformaldehyde solution. Brains were removed from the cranium, postfixed in the perfusion solution for 5 h and cryo-protected overnight (20–24 h) in perfusion solution plus 20% sucrose. Brains were frozen on dry ice and stored at −70 °C until sectioning at 50 μm in the coronal plane, on a frozen stage microtome (Microm). Alternate representative sections through the forebrain were collected for Nissl staining and AChE histochemistry, according to a modification [21,22] of the method previously described by Hardy and Heimer [16].

AChE and Nissl-stained sections were analyzed qualitatively under a light microscope at various magnification levels (2× through 10×) with the observer blind to the condition (MSF or vehicle) of the brain tissue. AChE intensity in cortex and hippocampus was scored on a scale from 1 to 4. Quantitative analysis was done by digitizing Nissl-stained sections into the AIS Image™, image analysis system (St. Catherines, Ontario), and cortical width measurements were taken as previously described in detail [21,23,50]. The width of the total cortex, layer VI, layer V, and the remaining layers identified as cortical plate were assessed separately in three coronal planes: anterior sensory–motor cortex (level 1), anterior barrel field (level 2) area, and posterior somatosensory cortex (level 3). The anterior boundary for level 1 was the first appearance of the anterior commissure and the posterior boundary was the first appearance of the fimbria fornix. Boundaries for levels 2 and 3 were determined using the shape and size of the hippocampus.

All brains were prepared for sectioning with a cut through the center of the cerebellum, perpendicular to the dorsal surface of the neocortex. The resulting large, flat surface of the brain was mounted on the stage of the microtome and cut in sequential coronal sections from