EFFECTS OF 2, 3, 7, 8-TETRACHLORODIBENZO-*P*-DIOXIN TREATMENT ON METHIONINE-ENKEPHALIN IMMUNOREACTIVITY IN THE BRAIN OF THE LONG-EVANS RAT

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Introduction

Our recent studies indicated that in utero and lactational exposure to 2, 3, 7, 8-tetrachlorodibenzo-*p*-dioxin (TCDD) decreased serotonin-like immunoreactivity in the mouse offspring, and that acute TCDD administration to adult Long-Evans rats also decreased content of neuronal nitric oxide synthase in the brain, and elicited c-Fos-like protein expression in the distinct brain areas [1, 2, 3]. Previous studies showed effects of TCDD exposure on brain neurotransmitters and hormones, e.g. endorphin, dopamine, histamine, noradrenaline and corticotrophin-releasing hormone (CRH) [4, 5]. Enkephalin plays roles in the regulation of the secretion of hormones, food intake, body temperature, and stress [6, 7]. The aim of this study is to examine the effect of TCDD administration on methionine-enkephalin-like immunoreactivity (MEK-I) in the brain of Long-Evans rat, a species strain considered to be the most TCDD-susceptible.

Methods and Materials

All experimental procedures were conducted in accordance with the Guidelines of Animal Experimentation of Kagoshima University. Animals (male, weighting 300-450g) were administered a single lethal dose of TCDD (dissolved in olive oil, 50 μg/kg) or an equivalent volume of vehicle (olive oil) by gavage. After TCDD treatment, animals were allowed to survive for 1 day (n=2), 2 days (n=1), 3 days (n=3), 1 week (n=2), or 2 weeks (n=5). For the control experiment, animals were allowed to survive for 2 days (n=1), 3 days (n=2), 1 week (n=1), or 2 weeks (n=5). Then, animals were anesthetized, and perfused as described before[1, 2]. Compared sections from the TCDD-treated and vehicle-treated rats were simultaneously processed for enkephalin immunohistochemistry under the same conditions using PAP method as described in our previous study [1, 2]. Photographs of two sections of each examined nucleus at the different levels were digitally taken. The relative density of MEK-I cell bodies, fibers and terminals (RDEK) was measured using the Scion Image program. RDEK was defined as the percentage of the area of MEK-I perikarya, fibers and terminals (pixels per μm^2) among the area of this nucleus (pixels per μm^2). The results were analyzed using a student's *t*-test. Significance was set at $p<0.05$.

Results and Discussion

After a single lethal dose of TCDD administration, all the animals suffered the acute intoxication symptoms as our recent studies reported[1, 2, 3]. A marked increase in the RDEK was initially observed at three days after TCDD treatment, and reached the maximum at two weeks after TCDD treatment in

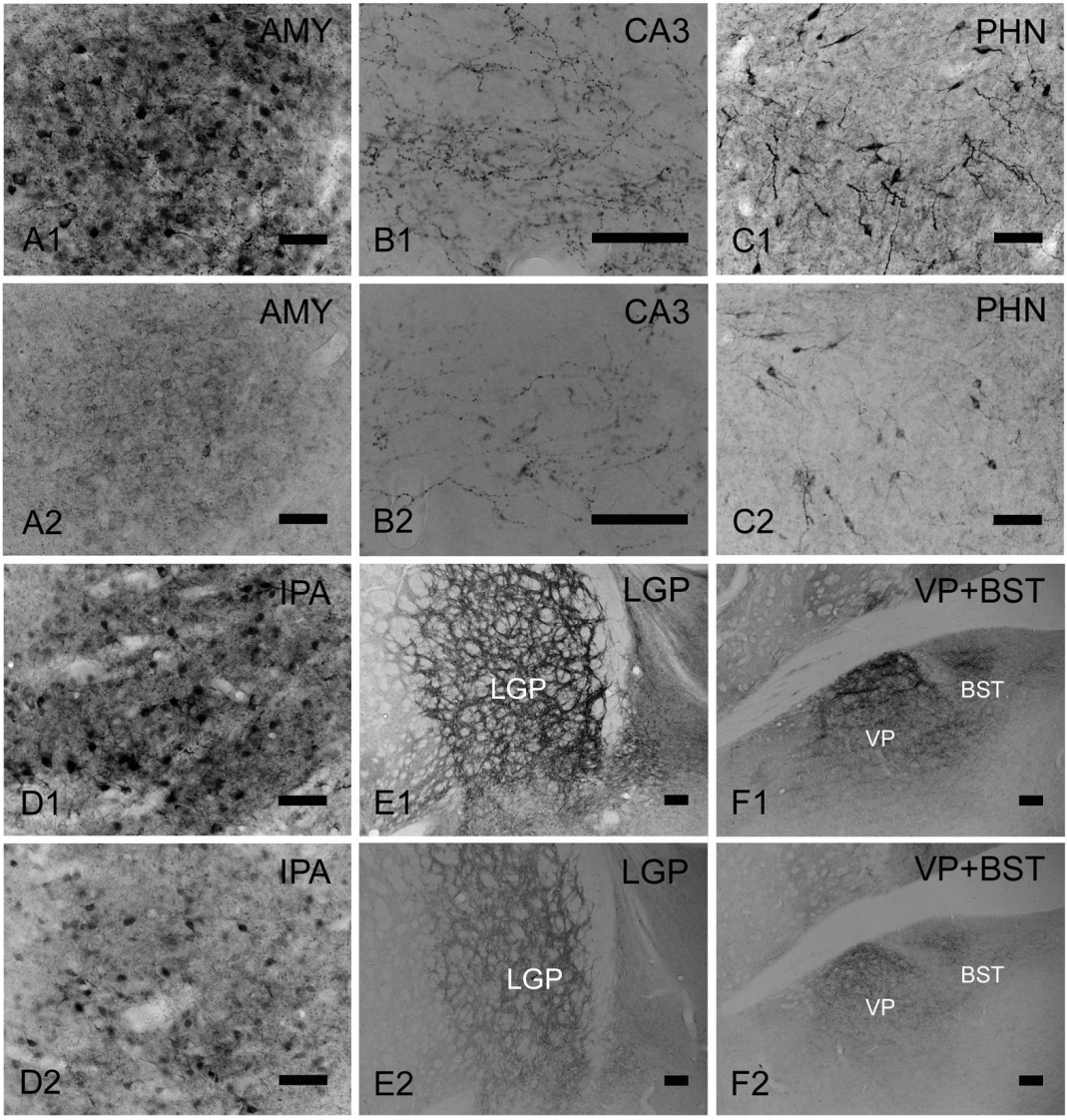

Figure 1. A1, B1, C1, D1, E1 and F1 show MEK-I in the TCDD-treated rats; A2, B2, C2, D2, E2 and F2 show MEK-I in the vehicle-treated rats. AMY: amygdaloid; CA3: field CA3 of hyppocampus; PHN: paraventricular hypothalamic nucleus; IPA: interstitial nucleus of the posterior limb of the anterior commissure; LGP:lateral globus pallidum; VP+BST: ventral pallidum and bed nuclus of the stria terminalis. Scale bars, 50 micrometers.

certain brain regions, i.e. the central amygdaloid nucleus (Control: 20.5±10.1; TCDD exposure: 59.9±13.2; $p<0.05$), field CA3 of hippocampus (Control: 10.5±2.8; TCDD exposure: 23.9±9.2), paraventricular hypothalamic nucleus (Control: 11.7±3.2; TCDD exposure: 37.9±8.8; $p<0.05$), interstitial nucleus of the posterior limb of the anterior commissure (Control: 31.6±11.3; TCDD exposure: 64.7±17.5; $p<0.05$), lateral globus pallidus (Control: 28.4±9.3; TCDD exposure: 79.8±15.2; $p<0.05$), ventral pallidum (Control: 33.6±13.7; TCDD exposure: 67.8±12.4; $p<0.05$), and lateral division of bed nucleus of the stria terminalis (Control: 24.2±12.5; TCDD exposure: 50.3±14.8; $p<0.05$), compared to the vehicle-treated animals (Fig.1).

To date, the mechanisms of TCDD-induced anorexia and hypothermia are still unknown. Due to TCDD-induced progressive body weight loss and low body temperature as observed in this study, it is suggested that the animals may to some extend suffer acute or/and chronic stress. This is supported by the previous findings that TCDD exposure stimulated hypothalamic-pituitary-adrenal axis by increasing CRH mRNA in the hypothalamus and cortisol secretion in blood [5]. Acute or chronic stress has been reported to elicit a marked increase in preproenkephalin mRNA in the brain[8]. In addition, TCDD administration increased the potency of androgens and estrogens in male rats[9], and estrogen treatment increased expression of preproenkephalin mRNA in the brain[8]. Furthermore, low body temperature induced a marked increase in the number of MEK-I fibers in the brain[10]. Our present results indicated an increase in the RDEK in certain brain areas including those involved in the regulation of the food intake, body temperature and stress. Taken together, it is speculated that increased MEK-I may be consequent on TCDD intoxication and act as a compensation for pathophysiological alterations caused by TCDD exposure.

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