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Allopregnanolone-mediated protective effects of progesterone on tributyltin-induced neuronal injury in rat hippocampal slices

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ABSTRACT

Increasing evidence shows that progesterone, a neuroactive steroid, has protective actions in central nervous system, but there is little evidence to show the protective mechanism of progesterone on neurotoxicity induced by environmental chemicals. In this study, we examined the effects of progesterone on neuronal injury induced by tributyltin (TBT) in rat hippocampal slices. Treatment with progesterone dose-dependently suppressed hippocampal neuronal injury induced by TBT. The neuroprotective action of progesterone was completely canceled with pretreatment by finasteride, a 5α-reductase inhibitor, but it was not affected by mifepristone, a progesterone receptor antagonist, or by SU-10603, a cytochrome P450 17α inhibitor. The content of allopregnanolone in the slices was significantly increased by treatment with progesterone, and this increment was greatly suppressed with a pretreatment of finasteride. Treatment with allopregnanolone attenuated neuronal injury induced by TBT in a dose-dependent manner. The neuroprotective effects not only of progesterone but also of allopregnanolone were canceled by bicuculline, a potent gamma-aminobutyric acid A ($GABA_A$) receptor antagonist. Pretreatment with muscimol, a $GABA_A$ receptor agonist, attenuated hippocampal neuronal injury elicited by TBT. Taken together, allopregnanolone converted from progesterone in hippocampal slices could protect neurons from TBT-induced neurotoxicity due to a $GABA_A$ receptor-dependent mechanism. One of the physiological roles of neuroactive steroids might be neuroprotection from environmental chemicals.

1. Introduction

Steroid hormones synthesized in and secreted from peripheral endocrine glands pass through the blood–brain barrier and then perform functions in the central nervous system. In addition, it was observed that the brain possesses an inherent endocrine system and synthesizes some steroid hormones [1]. Recently, increasing evidence has shown that a neuroactive steroid, progesterone, protects neurons. Treatment with progesterone increased the expression of Bcl-2 and decreased the contents of active caspase-3 to suppress apoptosis [2,3]. Progesterone also seems to be beneficial in preventing mitochondrial dysfunction, which results in the loss of hippocampal cells after a controlled cortical contusion [4]. Furthermore, it has been reported that progesterone protects hippocampal slice cultures from cell death following oxygen-glucose deprivation, largely due to its conversion to allopregnanolone via a gamma-aminobutyric acid A ($GABA_A$) receptor-dependent mechanism [5]. Progesterone is readily metabolized, in the brain, to allopregnanolone by activities of 5α-reductase and 3α-hydroxysteroid dehydrogenase [5].

Organotin compounds have long been used as thermal stabilizers, catalytic agents and biocidal compounds for preserving wood, textiles, cordage fibers and electronic equipment. Among them, tributyltin (TBT) has been most widely used in paint formulations to prevent marine fouling on ships, boats and fish-farming nets. Environmental surveying and monitoring of TBT are conducted to prevent the consumption of bioaccumulated TBT by humans. The average intake of TBT by humans from market-bought seafood has been estimated to vary worldwide between 0.18 and 2.6 μg per day per person [6], and the presence of butyltin compounds, including TBT, reportedly exists at concentrations between 50 nM and 400 nM in human blood [7]. Therefore, the effects of TBT on the human brain are now of great concern.

Because many environmental chemicals, including organotin compounds, are lipophilic and have high blood–brain barrier permeability, the central nervous system is exposed to exogenous chemicals. The administration of TBT elicited abnormal behavior and the reduction of brain weight within the cerebellum and decreased synaptogenesis in rats [8–10]. This is evidence

Abbreviations: BDNF, brain-derived neurotrophic factor; GABA, gamma-aminobutyric acid; NGF, nerve growth factor; ROS, reactive oxygen species; PI, propidium iodide; TBT, tributyltin.

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suggesting that the central nervous system is a primary target of TBT. However, the central nervous system is equipped with some endogenous neuroprotectants against harmful chemicals. Some of the most well-known factors are neutrophins such as brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF). BDNF attenuated cellular injury of differentiated neural stem cells induced by trimetyltin by activating the pathways of mitogen-activated protein kinase and phosphoinositide-3-kinase/Akt signals [11]. NGF was up-regulated in murine astrocytes by treatment with trimethyltin and plays an important role to protect neurons [12]. Recently, in addition to neutrophins, neuroactive steroids have been noted as endogenous neuroprotective molecules. However, there is no existing report to show the effects of neuroactive steroids on neurotoxicity induced by organotin compounds.

In this study, we examined the protective effects of progesterone on neuronal injury induced by TBT using rat organotypic hippocampal slice cultures. TBT has been one of the most commonly used organotin compounds and thus accumulates significantly in the environment.

2. Materials and methods

2.1. Materials

Tributyltin chloride was obtained from Tokyo Chemical Industry (Tokyo, Japan). Progesterone was obtained from Sigma–Aldrich (St. Louis, MO, USA). Finasteride and (−)-bicuculline methiodide were purchased from Enzo Life Science International (Farmingdale, NY, USA). Allopregnanolone was obtained from Agrisera (Vannas, Sweden). Mifepristone and muscimol were purchased from Cayman Chemical (Ann Arbor, MI, USA). SU-10603 was a kind gift from Dr. C.R. Jefcoate, University of Wisconsin. All other chemicals were obtained from Nacalai Tesque (Kyoto, Japan) or Sigma–Aldrich and were of reagent grade.

2.2. Animals

All procedures performed on animals were in accordance with the Fundamental Guidelines for Proper Conduct of Animal Experiments and Related Activities in Academic Research Institutions under the jurisdiction of the Ministry of Education, Culture, Sports, Science and Technology, Japan and the Animal Care and Use Committee of Hiroshima University, Hiroshima, Japan. Postnatal 7-day-old Wistar rats were obtained from CLEA Japan (Tokyo, Japan) and were maintained in a temperature-controlled animal facility with 12 h light–dark cycles.

2.3. Organotypic hippocampal slice cultures

Rat organotypic hippocampal slices were prepared according to our previous report [13]. Briefly, hippocampi were dissected from postnatal 9- to 11-day-old Wistar rats and then cut transversely into 300 μm slices. Slice culture of hippocampus from older than 15-day rat was not succeeded in our experimental condition. Tissue sections were plated on Millicell or Omnipore membranes (Millipore, Bedford, MA) that were then inserted into culture plates filled with the culture medium. The slices were maintained in a humidified CO_2 incubator at 37 °C. The culture medium was changed every 2 days, and sections were cultured for 6 or 7 days. One day before any treatment, the slices were transferred to serum-free medium and then used in the experiments.

2.4. Drug treatment

Stock solutions of TBT (3 mM), progesterone (10 mM), allopregnanolone (10 mM) and finasteride (100 mM) were prepared with ethanol. Stock solutions of mifepristone (20 mM) and SU-10603 (10 mM) were prepared with dimethyl sulfoxide. Bicuculline (10 mM) and muscimol (10 mM) were dissolved in phosphate-buffered saline. Final concentrations of these reagents for treatment of the slices were decided by preliminary experiments to fulfill the following criteria; (i) the reagents alone did not induce neuronal toxicity and (ii) the concentrations of the reagents were not largely different from those in previous studies.

Progesterone or allopregnanolone was added to the serum-free culture medium 2 h before treatment with TBT. The pre-treatment period was decided by preliminary experiments. Mifepristone, finasteride, SU-10603 or bicuculline was added 20 min before treatment with progesterone or allopregnanolone. Muscimol was added 20 min before treatment with TBT.

2.5. Measurement of cell death

To visualize neuronal cell death in three different regions of the slice (the CA1, CA3 and dentate gyrus (DG)), hippocampal slices were stained by adding propidium iodide (PI) into the culture medium at a concentration of 1 μg/mL throughout the TBT or vehicle treatment, according to the method reported previously [13]. Slices were excited with a 540 ± 25 nm light, and the emitted fluorescence was acquired at 605 ± 55 nm on an inverted fluorescent microscope (BZ-9000, Keyence, Osaka, Japan) to determine the cellular PI uptake. All cells were killed after the experiment by keeping the cultures at 4 °C for 48 h, and the value of the maximum PI uptake for each slice was obtained by microscopic observation and subsequent analysis. The cell injury was expressed as the percentage of cell death, which was calculated by the formula: (PI uptake/maximum PI uptake) × 100. Five to seven slices were used for each of three to five separate experiments.

2.6. Quantification of allopregnanolone

Hippocampal slices were washed with ice-cold phosphate-buffered saline and then collected. The allopregnanolone concentration was determined by Asuka Pharmamedical Co. Ltd. (Kawasaki, Japan) using liquid chromatography–tandem mass spectrometry methods.

2.7. Statistical analyses

All data are expressed as the mean ± standard error (S.E.). Data were statistically analyzed by one way analysis of variance (ANOVA) followed by Bonferroni post hoc analysis. Probability (P) values <0.05 were considered to be statistically significant.

3. Results

3.1. Suppressive effects of progesterone on neuronal cell death induced by TBT

The treatment of rat hippocampal slices with various concentrations of TBT (0.1, 0.3, 1, 3 and 10 μM) for 24 h increased the PI-derived fluorescence in a dose-dependent manner, indicating that dose-dependent cell death was elicited by TBT (Fig. 1), as reported previously [13]. Hippocampal neurons in CA1, CA3 and DG were similarly injured by treatment with TBT (Fig. 1). Because approximately 50% of the hippocampal cells were dyed by treatment with 3 μM TBT, this condition was suitable for analyzing the mechanism of TBT-induced neurotoxicity.