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4,5-Dichloro-2-octyl-4-isothiazolin-3-one (DCOIT) modifies synaptic transmission in hippocampal CA3 neurons of rats

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HIGHLIGHTS

- 4,5-Dichloro-2-octyl-4-isothiazolin-3-one (DCOIT) is an antifoulant.
- DCOIT increased the frequency of synaptic currents mediated by GABA and glutamate.
- The increase was mainly due to Ca^{2+} release from intracellular Ca^{2+} stores by DCOIT.
- DCOIT-induced excess facilitation of neurotransmission may result in neurotoxicity.

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ABSTRACT

4,5-Dichloro-2-octyl-4-isothiazolin-3-one (DCOIT) is an alternative to organotin antifoulants, such as tributyltin and triphenyltin. Since DCOIT is found in harbors, bays, and coastal areas worldwide, this chemical compound may have some impacts on ecosystems. To determine whether DCOIT possesses neurotoxic activity by modifying synaptic transmission, we examined the effects of DCOIT on synaptic transmission in a 'synaptic bouton' preparation of rat brain. DCOIT at concentrations of 0.03–1 μM increased the amplitudes of evoked synaptic currents mediated by GABA and glutamate, while it reduced the amplitudes of these currents at 3–10 μM. However, the currents elicited by exogenous applications of GABA and glutamate were not affected by DCOIT. DCOIT at 1–10 μM increased the frequency of spontaneous synaptic currents mediated by GABA. It also increased the frequency of glutamate-mediated spontaneous currents at0.3–10 μM. The frequencies of miniature synaptic currents mediated by GABA and glutamate, observed in the presence of tetrodotoxin under external Ca^{2+}-free conditions, were increased by 10 μM DCOIT. With the repetitive applications of DCOIT, the frequency of miniature synaptic currents mediated by glutamate was not increased by the second and third applications of DCOIT. Voltage-dependent Ca^{2+} channels were not affected by DCOIT, but DCOIT slowed the inactivation of voltage-dependent Na^{+} channels. These results suggest that DCOIT increases Ca^{2+} release from intracellular Ca^{2+} stores, resulting in the facilitation of both action potential-dependent and spontaneous neurotransmission, possibly leading to neurotoxicity.

1. Introduction

In response to the banning of organotin antifoulants, such as tributyltin and triphenyltin, chemical companies have actively developed alternative biocides (Qian et al., 2013). 4,5-Dichloro-2-octyl-4-isothiazolin-3-one (DCOIT) is supposed to be an environmentally acceptable alternative to organotin antifoulants (Jacobson and Willingham, 2000). DCOIT is the active ingredient in a series of biocide formulations marketed by the Dow Chemical Company and its global affiliates (The Dow Chemical Company, 2012). DCOIT was found in sediments of port, harbor, and coastal areas in Asian countries (Harino et al., 2007; Tsunemasa and Yamazaki, 2014; Kim et al., 2015a,b; Mochida et al., 2015). The cctanol-water partition

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coefficient of DCOIT is reported to be 4.5 (Shade et al., 1993) or 2.85 (Willingham and Jacobson, 1996). Thus, since there is a possibility that DCOIT accumulates in organisms, this compound may have impacts on ecosystems (Guardiola et al., 2012). The toxicity of DCOIT in wild animals is not reported. However, DCOIT is found to possess adverse actions in in vivo studies (Ito et al., 2013; Chen et al., 2014a,b, 2015, 2016). DCOIT induced apoptosis of testicular germ cells in mummichog (Ito et al., 2013). In marine Medaka, DCOIT altered oxidative stress and neurotransmission, caused endocrine disruption, and induced proteomic changes in brain and liver tissues (Chen et al., 2014a,b, 2015, 2016). DCOIT significantly inhibited acetylcholinesterase activity in the brain of marine Medaka (Chen et al., 2014a). The inhibition of acetylcholinesterase produces effects reminiscent of neurotoxicity, as described for some insecticides (Veronesi et al., 1990; Russom et al., 2014). Therefore, the aim of this study is to examine whether DCOIT has adverse actions on neuronal transmission, especially in mammalian CNS. Synaptic transmission is one of main targets for pathological and toxicological studies because it is the most vulnerable step in neuronal transmission (or signaling). Parkinson's disease, schizophrenia, dementia, and depression are related to malfunction of synaptic transmission. Therefore, this study possesses toxicological and pathological (probably biological) implications. Methyl mercury (MetHg) is one of environmental pollutants. This compound is known to cause 'Minamata Disease'. MetHg acts on neuronal functions including synaptic transmission in various brain regions. Thus, it is important to conduct the experiment to reveal the effects of environmental pollutants on the neuronal transmission. In this study, we examined the effects of DCOIT on synaptic transmission using a 'synaptic bouton' preparation (Akaike et al., 2002; Akaike and Moorhouse, 2003) isolated from hippocampus of rat brain. Hippocampal neurons are a sensitive, well-established assay for synaptic transmission function. Here, we describe the actions of DCOIT on synaptic transmission mediated by GABA and glutamate in rat brain. These actions are mediated mainly via the mobilization of intracellular Ca^{2+}. The results suggest that DCOIT at its present environmentally relevant concentrations in sea sediments may be neurotoxic in wild mammals.

2. Materials and methods

2.1. Cell preparation – 'synaptic bouton' preparation

The use of experimental animals was approved by the Ethics Committee of Kumamoto Kinoh Hospital. All experiments were performed in accordance with the Guiding Principles for Care and Use of Animals in the Field of Physiological Sciences of The Physiological Society of Japan.

Details of the "synaptic bouton" preparation were described previously (Akaike et al., 2002; Akaike and Moorhouse, 2003). Briefly, Wistar rats (11–23 days old, either sex) were decapitated under pentobarbital anesthesia. The brain was removed and immersed in ice-cold oxygenated incubation medium. The ionic composition of the incubation medium was 124 mM NaCl, 5 mM KCl, 1.2 mM KH_2PO_4, 24 mM $NaHCO_3$, 2.4 mM $CaCl_2$, 1.3 mM $MgSO_4$ and 10 mM glucose. The medium was saturated with 95% O_2 and 5% CO_2 in order to adjust the pH to 7.45.

Hippocampal slices (400-μm thick) were prepared using a vibrating microtome (VR 1200S; Leica, Nussloch, Germany) and then incubated in the medium at room temperature (21–24 °C) for at least one hour before mechanical dissociation using a fire-polished glass pipette coupled to a vibration device (S1-10 cell isolator; K.T. Labs, Tokyo). The tip of the glass pipette was placed on the surface of the slice in the hippocampal CA3 region and was vibrated horizontally (0.2–2.0 mm displacement) at 50 Hz. After dissociation, the mechanically dissociated neurons were left to settle and adhere to the bottom of the dish for at least 15 min.

2.2. Electrophysiological measurements

All recordings were obtained from the 'synaptic bouton' preparation of CA3 pyramidal neurons using conventional whole-cell patch-clamp recordings in voltage-clamp mode. Tables 1 and 2 show the ionic compositions of the solutions used for the current recordings. Glutamatergic evoked, spontaneous, and miniature excitatory postsynaptic currents (eEPSCs, sEPSCs, mEPSCs), and glutamate receptor-mediated currents (I_{Glu}) were recorded at a holding potential (V_H) of −65 mV. GABAergic evoked, spontaneous, and miniature inhibitory postsynaptic currents (eIPSCs, sIPSCs, mIPSCs) and $GABA_A$ receptor-mediated currents (I_{GABA}) were recorded at a V_H of 0 mV. Voltage-dependent Na^+ channel currents (I_{Na}) and Ba^{2+}-permeable high-threshold Ca^{2+} channel currents (I_{Ba}) were recorded V_Hs of −70 mV and −60 mV, respectively (Multiclamp 700B; Molecular Devices, Sunnyvale, CA) (Wakita et al., 2012). All experiments were performed at room temperature (21–24 °C).

The resistances of the recording pipettes filled with the internal solution were 3–6 MΩ. Neuronal responses were continuously monitored on a computer display and an oscilloscope (DCS-7040; Kenwood, Tokyo, Japan). All membrane currents were acquired with 20 kHz sampling rate, filtered at 3 kHz using a low-pass filter (Multifunction Filter 3611; NF Co., Tokyo, Japan) and stored on a computer using pCLAMP 10.2 (Axon Instruments, CA, USA).

Experiments were performed with wide range (0.03–10 μM) of DCOIT concentration. One may argue the possibility that DCOIT decreases cell viability. In electrophysiological studies, it is impossible to record electrical response from dead cells or the cells with comprised membranes. Thus, electrophysiological recording reveal whether recorded cell is viable.

2.3. Paired-pulse focal electrical stimulation of single boutons using glass pipettes

Focal electric stimuli can be employed to activate a single nerve terminal in order to measure eIPSCs and eEPSCs resulting from a single presynaptic nerve ending rather than a fused event from multiple boutons. This technique offers a unique evaluation of how drugs act on pre- and post-synaptic transmission mechanisms at the single synapse level. Focal electrical stimulation of a single

Table 1
Solutions for recording currents elicited by GABA and glutamate.

Recording Currents	External Solution	Internal Pipette Solution
GABAergic IPSC and I_{GABA}		
Ionic Composition	150 mM NaCl	5 mM CsCl
	5 mM KCl	135 mM Cs-methanesulfonate
	2 mM $CaCl_2$	5 mM TEA-Cl
	1 mM $MgCl_2$	10 mM EGTA
	10 mM Glucose	10 mM HEPES
	10 mM HEPES	4 mM ATP-Mg
	pH 7.4	pH 7.2
	Adjusted with Tris base	Adjusted with Tris base
Glutamatergic EPSC and I_{Glu}		
Ionic Composition	150 mM NaCl	5 mM CsCl
	5 mM KCl	135 mM CsF
	2 mM $CaCl_2$	5 mM TEA-Cl
	1 mM $MgCl_2$	2 mM EGTA
	10 mM Glucose	10 mM HEPES
	10 mM HEPES	5 mM QX-314 bromide
	pH 7.4	pH 7.2
	Adjusted with Tris base	Adjusted with Tris base