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Effects of triphenyltin on glycinergic transmission on rat spinal neurons

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ABSTRACT

Glycine is a fast inhibitory transmitter like γ-aminobutyric acid in the mammalian spinal cord and brainstem, and it is involved in motor reflex, nociception, and neuronal development. Triphenyltin (TPT) is an organometallic compound causing environmental hazard to many wild creatures. Our previous findings show that TPT ultimately induces a drain and/or exhaustion of glutamate in excitatory presynaptic nerve terminals, resulted in blockage of neurotransmission as well as methylmercury. Therefore, we have investigated the neurotoxic mechanism how TPT modulates inhibitory glycinergic transmission in the synaptic bouton preparation of rat isolated spinal neurons using a patch clamp technique. TPT at environmentally relevant concentrations (3–300 nM) significantly increased the number of frequency of glycinergic spontaneous and miniature inhibitory postsynaptic currents (sIPSC and mIPSC) without affecting the current amplitude and decay time. The TPT effects were also observed in external Ca^{2+}-free solution containing tetrodotoxin (TTX) but removed in Ca^{2+}-free solution with both TTX and BAPTA-AM (Ca^{2+} chelator). On the other hand, the amplitude of glycinergic evoked inhibitory postsynaptic currents (eIPSCs) increased with decreasing failure rate (Rf) and paired pulse ratio (PPR) in the presence of 300 nM TPT. At a high concentration (1 μM), TPT completely blocked eIPSCs after a transient facilitation. Overall, these results suggest that TPT directly acts transmitter-releasing machinery in glycinergic nerve terminals. Effects of TPT on the nerve terminals releasing fast transmitters were greater in the order of glycinergic > glutamatergic > GABAergic ones. Thus, TPT is supposed to cause a strong synaptic modulations on glycinergic neurotransmission in wild creatures.

1. Introduction

Triphenyltin (TPT) has been applied as a biocide for agricultural and industrial purposes such as fungicide and antifoulant, respectively (Yi et al., 2012). Even though the use of TPT has been prohibited, some of farmers illegally used this compound as a fungicide (Wu et al., 2010). Increasing trend of pollution of TPT as an antifoulant was also reported in coasts of Taiwan (Meng et al., 2005) and Hong Kong (Ho and Leung, 2014). Because health impacts of TPT are still concerned in these countries, the risk assessment of intake of organotin compounds from fish and seafood has been conducted in Taiwanese population (Lee et al., 2016). Levels of phenyltins in fish and seafood are higher than those of butyltins (Lee et al., 2016). TPT is supposed to be less neurotoxic than trialkyltins (Snoeij et al., 1985; Besser et al., 1987). However, diplopia, drowsiness, giddiness, vertigo, bidirectional nystagmus, impairment of calculation ability, as well as disorientation to time, people and place were developed without significant findings by magnetic resonance imaging and single-photon emission computed tomography in TPT poisoning patient (Lin et al., 1998), suggesting a possibility that TPT causes cellular dysfunction of brain without structural damage, which results in variable CNS clinical presentations. In fact, TPT at nanomolar concentrations facilitates voltage-dependent Na^+ current and decreases voltage-dependent K^+ current of rat brain neurons (Oyama and Akaike, 1990). Furthermore, glutamatergic spontaneous and action potential-evoked excitatory postsynaptic currents (sEPSC and eEPSC) in rat hippocampal CA3 neurons were also promoted by TPT at similar concentrations (Wakita et al., 2015b). Thus, TPT is considered to be a neurotoxicant.

Neuronal excitability is influenced by relative distribution (balance) of excitatory and inhibitory synaptic inputs. Therefore, it is also important to examine the effects of TPT on inhibitory synaptic transmission in order to reveal the cellular basis of neurotoxic actions of TPT. In this study, we examined the effects of TPT on glycinergic and GABAergic inhibitory synaptic transmissions using well established

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'synaptic bouton' preparation (Akaike and Moorhouse, 2003) that allows spontaneous and evoked inhibitory synaptic activities such as spontaneous and evoked inhibitory postsynaptic currents (sIPSC, mIPSC and eIPSC) from isolated brain cells. Glycine is an important fast inhibitory transmitter in vital spinal and supraspinal (brainstem) interneurons of mammalian nervous system, especially in the ventral horn where glycinergic transmission is involved in motor reflex and nociception as well as fast GABAergic transmission (Bowery and Smart, 2006; Harvey et al., 2004). In addition, extrasynaptic (non-synaptic) glycine receptors on nerve cell bodies (soma membrane) are important for neuronal development (Flint et al., 1998) and for tonically active state of neurons by locally released inhibitory substances (Mori et al., 2002). Therefore, it is valuable to study the TPT-induced modulation on the inhibitory glycinergic transmissions (sIPSC and eIPSC) and extrasynaptic glycine receptor-mediated current (I_{Gly}) in mammalian spinal cord. The obtained data are compared GABAergic and glutamatergic ones.

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.

Details of the "synaptic bouton" preparation were described previously (Murakami et al., 2002; Akaike and Moorhouse, 2003). Briefly, Wistar rats (11–23 days old, either sex) were decapitated under pentobarbital (50 mg/kg, i.p.) anesthesia. The brain was removed and immersed in ice-cold oxygenated incubation medium. The ionic composition of the incubation medium is shown in Table 1.

Spinal cord slices (400 μm thick) were prepared using a vibrating microtome (VR 1200S; Leica, Nussloch, Germany). The tip of the glass pipette coupled to a vibration device (S1-10 Cell Isolator; K.T. Labs, Tokyo, Japan) was placed on the surface of the slice containing spinal sacral commissural nucleus (SDCN) and was horizontally vibrated at 50 Hz. After mechanical dissociation, the neurons adhered to the bottom of the culture dish.

2.2. Electrophysiological measurements

All recordings were obtained from the 'synaptic bouton' preparation of SDCN neurons receiving multiple inputs from many boutons using conventional whole-cell patch-clamp recordings in voltage-clamp mode. Glycinergic spontaneous, miniature and action potential-evoked inhibitory postsynaptic currents (sIPSCs, mIPSCs, eIPSCs), and glycine or GABA receptor-mediated extrasynaptic currents (I_{Gly} or I_{GABA}) were recorded at a holding potential (V_H) of 0 mV. Extrasynaptic glutamate response (I_{Glu}) and NMDA response (I_{NMDA}) were recorded at a V_H of −65 mV and −40 mV, respectively. 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. All membrane currents were acquired with 20 kHz sampling rate and stored on a computer using pCLAMP 10.2 (Axon Instruments, CA, USA). Compositions of external and internal pipette solutions for recording the receptor-activated currents are listed in Table 2. Voltage-dependent Na^+ channel currents (I_{Na}) and Ba^{2+}-permeable high-threshold Ca^{2+} channel currents (I_{Ba}) were recorded at V_H of −70 mV and −60 mV, respectively. The solutions for recording voltage-gated currents are shown in Table 3.

Table 1
Incubation medium.

124 mM NaCl
24 mM $NaHCO_3$
5 mM KCl
1.2 mM KH_2PO_4
2.4 mM $CaCl_2$
1.3 mM $MgSO_4$
10 mM Glucose
Saturated with 95% O_2 and 5% CO_2 to adjust the pH to 7.45

Table 2
Solutions for recording currents elicited by glycine, GABA, and glutamate.

Recording Currents	Glycinergic and GABAergic Currents	
	External Solution	Internal Pipette Solution
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 Adjusted with Tris base	pH 7.2 Adjusted with Tris base
Recording Current	Glutamatergic Current	
	External Solution	Internal Pipette Solution
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 Adjusted with Tris base	pH 7.2 Adjusted with Tris base

ATP-Mg; adenosine 5′-triphosphate magnesium salt.
EGTA; ethyleneglycol-bis-(α-aminoethylether)-N,N,N′,N′-tetraacetic acid.
HEPES; N-2-hydroxyethylpiperazine-N′−2-ethanesulphonic acid.
QX-314; 2-((2,6-dimethylphenyl)amino)-N,N,N-triethyl-2-oxoethanaminium.
TEA-Cl; tetraethylammonium chloride.
Tris base; tris(hydroxymethyl)aminomethane.

Table 3
Solutions for recording voltage-dependent currents.

Recording Current	Voltage-Dependent Na^+ Current (I_{Na})	
	External Solution	Internal Pipette Solution
Composition	60 mM NaCl	105 mM CsF
	100 mM Choline-Cl	30 mM NaF
	10 mM CsCl	5 mM CsCl
	10 mM glucose	5 mM TEA-Cl
	0.01 mM $LaCl_3$	2 mM EGTA
	5 mM TEA-Cl	10 mM HEPES
	10 mM HEPES	2 mM ATP-Mg
	pH 7.4 Adjusted with Tris base	pH 7.2 Adjusted with Tris base
Recording Current	Ba^{2+} Current (I_{Ba}) Through Voltage-Dependent Ca^{2+} Channels	
	External Solution	Internal Pipette Solution
Composition	145 mM Choline-Cl	80 mM Cs-methanesulfonate
	5 mM CsCl	60 mM CsCl
	5 mM $BaCl_2$	5 mM TEA-Cl
	1 mM $MgCl_2$	2 mM EGTA
	10 mM glucose	10 mM HEPES
	10 mM HEPES	2 mM ATP-Mg
	pH 7.4 Adjusted with Tris base	pH 7.2 Adjusted with Tris base

ATP-Mg; adenosine 5′-triphosphate magnesium salt.
EGTA; ethyleneglycol-bis-(α-aminoethylether)-N,N,N′,N′-tetraaceti acid.
HEPES; N-2-hydroxyethylpiperazine-N′−2-ethanesulphonic acid.
TEA-Cl; tetraethylammonium chloride.
Tris base; tris(hydroxymethyl)aminomethane.

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

Focal electrical stimulation of a single bouton adherent to dissociated CNS neurons has been described previously (Akaike and Moorhouse, 2003). Focal electric stimuli using a bipolar pipette (theta glass) were employed to activate a single glycinergic nerve terminal synapsing an isolated SDCN neuron to measure eIPSCs. Stimulus shocks were delivered for 100 μs at intensities of 0.06–0.1 mA with inter-