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RESEARCH HIGHLIGHT

Human nicotinic acetylcholine receptor is a potential pharmacological target of oseltamivir

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Oseltamivir (Tamiflu) effectively inhibits influenza virus-specific neuraminidase and therefore, is widely prescribed as an anti-influenza medication. Although a wide safety margin of oseltamivir has been reported, the possible neuronal adverse effects of this drug via unknown mechanisms are shown in some studies: dyskinesia, depressive episodes, hypothermia, and other CNS dysfunctions. We therefore, examined effects of oseltamivir on human nicotinic acetylcholine (ACh) receptors (nAChRs) with electrophysiological methods and found that oseltamivir reversely blocks nicotine- and ACh-evoked membrane currents in a concentration dependent manner in neuroblastoma cells derived from human peripheral neurons (IMR32) and in HEK cells expressing recombinant human α3β4 nAChRs. By contrast, the nicotine-evoked membrane currents were not affected by oseltamivir carboxylate (OC), which is an active metabolite of oseltamivir. Moreover, single channel analysis revealed that oseltamivir reduces the channel open time of nAChR without affecting the channel conductance. Our results demonstrate that human α3β4 nAChRs are a potential pharmacological target of oseltamivir, hence explaining a part of the adverse effects after ingestion of oseltamivir.

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Oseltamivir (Tamiflu) is widely prescribed as an anti-influenza medication to relieve influenza symptoms such as cough, myalgia, nasal obstruction, sore throat, fatigue, headache, and/or feverishness. Being shown a wide safety margin of oseltamivir, the possible adverse effects of this drug have been reported. However, the limited information of the molecular target(s) of oseltamivir and/or OC avoids understanding the adverse effects in animal models [1-5] and in patients [6-13].

Nicotinic acetylcholine receptors (nAChRs) are composed of pentamers of homomeric or heteromeric combinations of five types of the subunits [14]. The α subunit is essential to function as a ligand-gated ion channel. Neuronal nAChRs are widely expressed in central and peripheral neuronal tissues and an endogenous ligand, ACh controls the channel opening. It has been proposed that the anti-nicotinic action of oseltamivir causes the hypothermia in mice via reduction in

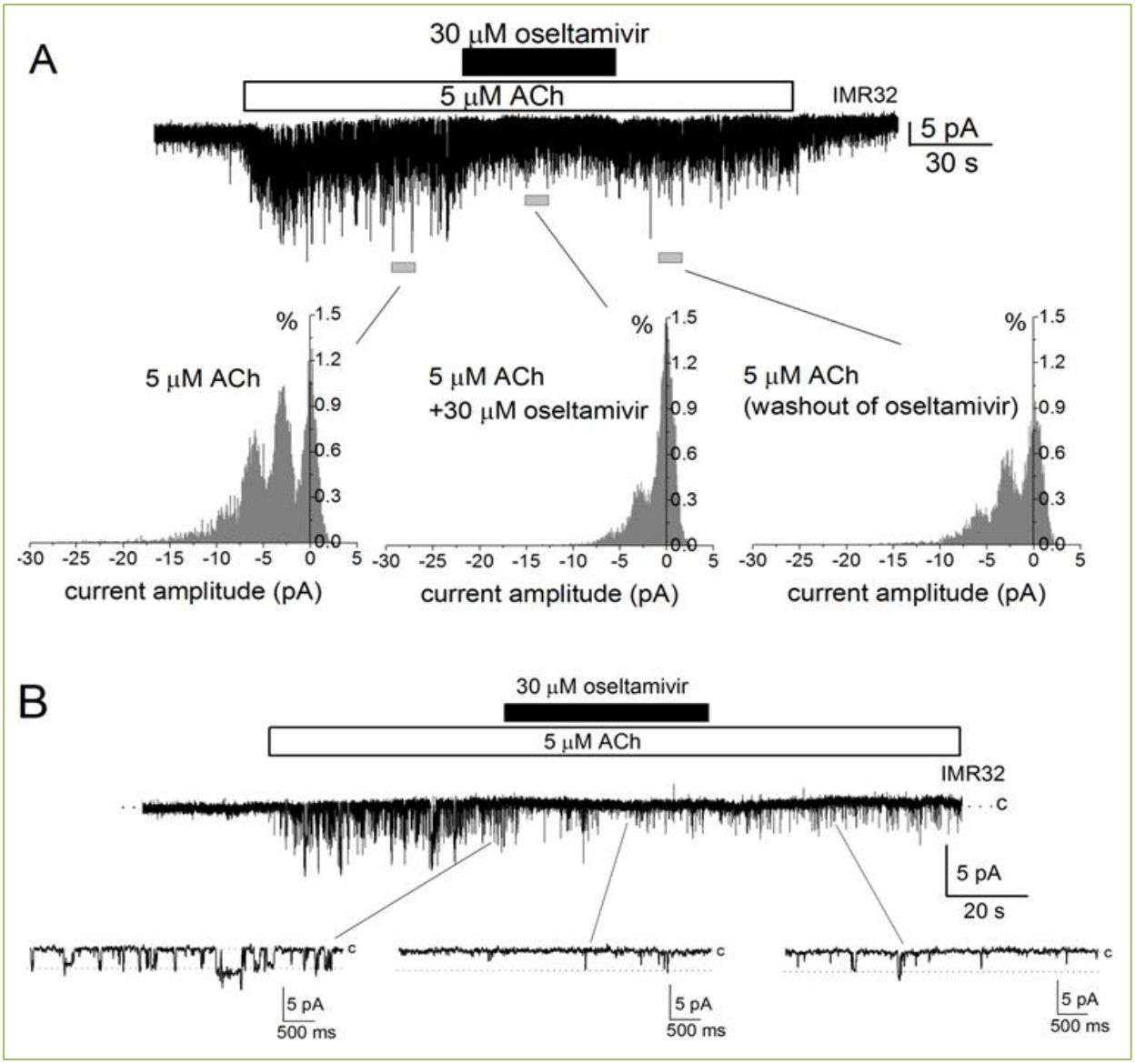

Figure 1. Channel events of nAChRs recorded from IMR32 cells in whole-cell mode. (A) A representative current trace in an IMR32 cell which included at least 4 channels is shown. After 5 μM ACh was applied to the cell at a holding potential of −90 mV, oseltamivir (30 μM) was added. Inset figures show the histogram of current amplitude for 10 s before, during, and after the washout of oseltamivir in the presence of ACh. (B) A representative current trace in an IMR32 cell in which two nAChR channels were active is shown. After 5 μM ACh was applied at a holding potential of −90 mV, oseltamivir (30 μM) was added. Inset figures show the expanded current traces before, during, and after the washout of oseltamivir in the presence of ACh.

sympathetic nerve activity [1]. In contrast, orally administered oseltamivir had no hypothermic action in rats [3]. Therefore, in our recent study [15] entitled "oseltamivir blocks human neuronal nicotinic acetylcholine receptor-mediated currents", we tested effects of *in vitro* oseltamivir and OC on nAChRs in human peripheral neuroblastoma IMR32 cells and on human-type α3β4 nAChRs heterologously co-expressed in human embryo kidney (HEK) cells (HEK-α3β4 nAChRs).

In the study [15], oseltamivir reversibly blocked nAChRs-mediated membrane currents in neuroblastoma IMR32 cells and in HEK-α3β4 nAChRs cells in a concentration-dependent manner (0.3-100 μM) when these nAChRs were stimulated with nicotine and ACh. However, these nAChRs-mediated membrane current responses were not affected by 30 μM OC, an active metabolite of oseltamivir. Moreover, the inhibition of nAChRs by oseltamivir was voltage-dependent and the nicotine-induced desensitization of nAChRs was accelerated in the presence of oseltamivir, suggesting that oseltamivir binds to a site in the channel pore for the blockade.

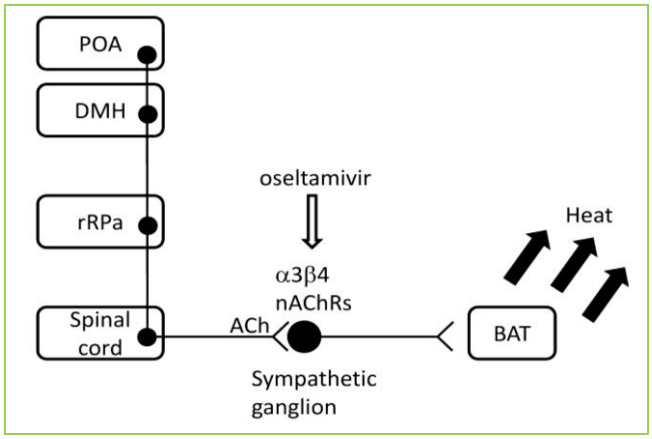

Figure 2. Scheme of effects of oseltamivir on BAT-related thermoregulation. POA: preoptic area of hypothalamus, DMH: dorsomedial hypothalamus, rRPa: rostral raphe pallidus nucleus, BAT: brown adipose tissue.

To further confirm the mechanism of oseltamivir action on nAChRs at a molecular level, we analyzed the single channel events of ACh-evoked membrane currents in the whole-cell configuration in IMR32 cells. Addition of 30 μM oseltamivir effectively reduced the number of channels opened by 5 μM ACh (Fig.1A, a representative cell) while the unitary channel events evoked by ACh alone were not significantly different from those by ACh plus oseltamivir (slope conductance: 5 μM ACh: 36.6 ±3.7 pS, n=7; 5 μM ACh plus 30 μM oseltamivir: 38.3 ± 6.2 pS, n=6; P>0.05). In contrast, when we analyzed the channel open time in the presence of 5 μM ACh, an addition of 30 μM oseltamivir effectively reduced the open time, and the withdrawal of oseltamivir reversed the reduction (Fig. 1B, a representative result out of five cells). Therefore, oseltamivir, which binds to a site in the channel pore of human nAChRs, directly blocks the channel.

These results strongly indicate that oseltamivir affects nAChRs as a channel blocker. Consistently, pre-administered nicotinic antagonists (mecamylamine and hexamethonium) potentiated oseltamivir-induced hypothermia in mice, and the hypothermia caused by intracerebroventricularly (i.c.v.)-administrated nicotine was effectively inhibited by pretreatment with i.c.v.-administrated oseltamivir [1]. On the other hand, oseltamivir at 30 μM inhibited the binding of blockers to Na^+ and Ca^{2+} channels, and ionotropic NMDA receptors although each inhibition was less than 50% [16]. Therefore, oseltamivir possibly changes the function of several ion channels including other subtypes of human nAChRs which were not used in our study. Further functional assays are required for understanding adverse effects of oseltamivir and OC on ion channels.

Since oseltamivir affects the function of α3β4 nAChRs in the peripheral ganglia, it is reasonable that the blockade causes to lower body temperature via reduction in sympathetic neuron activity in mice [1, 2]. Indeed, dysfunction of nAChRs in sympathetic nerve causes the hypothermia in mice whose acetylcholinesterase is genetically disrupted [17]. Moreover, it has been shown that the sympathetic nerve which controls function of brown adipose tissue (BAT) can regulate human body temperature [18-20], hence suggesting that oseltamivir with an anti-nicotinic action may cause hypothermia in human (Fig.2, [21-23]). Forty four cases of hypothermia after ingestion of oseltamivir had been collected in MHLW (the Japanese Ministry of Health, Labor and Welfare) between April, 1, 2004 and March, 20, 2007 [24]. The Food and Drug Administration (FDA) also updated The Adverse Reaction section of the labeling for oseltamivir to include hypothermia in November 2010 [25].

In our recent study [15] and this report, we show that oseltamivir but not OC inhibits the function of human nAChRs in a concentration dependent manner, due to reducing the channel open time of nAChRs without affecting the channel conductance. These results add to the evidence suggesting that besides the virus neuraminidase, nAChRs are a site for pharmacological actions of oseltamivir in mammals.

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