The synergistic inhibitory actions of oxcarbazepine on voltage-gated sodium and potassium currents in differentiated NG108-15 neuronal cells and model neurons

Chin-Wei Huang\textsuperscript{1,2}, Chao-Ching Huang\textsuperscript{2,3}, Ming-Wei Lin\textsuperscript{4}, Jing-Jane Tsai\textsuperscript{1} and Sheng-Nan Wu\textsuperscript{4,5}

\textsuperscript{1} Department of Neurology, \textsuperscript{2} Institute of Clinical Medicine, \textsuperscript{3} Department of Pediatrics, \textsuperscript{4} Institute of Basic Medicine, \textsuperscript{5} Department of Physiology, National Cheng Kung University School of Medicine, Tainan, Taiwan

Abstract

Oxcarbazepine (OXC), one of the newer anti-epileptic drugs, has been demonstrating its efficacy on wide-spectrum neuropsychiatric disorders. However, the ionic mechanism of OXC actions in neurons remains incompletely understood. With the aid of patch-clamp technology, we first investigated the effects of OXC on ion currents in NG108-15 neuronal cells differentiated with cyclic AMP. We found OXC (0.3–30 \textmu M) caused a reversible reduction in the amplitude of voltage-gated Na\textsuperscript{+} current (\(I_{\text{Na}}\)). The IC\textsubscript{50} value required for the inhibition of \(I_{\text{Na}}\) by OXC was 3.1 \textmu M. OXC (3 \textmu M) could shift the steady-state inactivation of \(I_{\text{Na}}\) to a more negative membrane potential by approximately \(-9\text{ mV}\) with no effect on the slope of the inactivation curve, and produce a significant prolongation in the recovery of \(I_{\text{Na}}\) inactivation. Additionally, OXC was effective in suppressing persistent \(I_{\text{Na}}\) (\(I_{\text{Na,p}}\)) elicited by long ramp pulses. The blockade of \(I_{\text{Na}}\) by OXC does not simply reduce current magnitude, but alters current kinetics. Moreover, OXC could suppress the amplitude of delayed rectifier K\textsuperscript{+} current (\(I_{\text{K(DR)}}\)), with no effect on M-type K\textsuperscript{+} current (\(I_{\text{K(M)}}\)). In current-clamp configuration, OXC could reduce the amplitude of action potentials and prolong action-potential duration. Furthermore, the simulations, based on hippocampal pyramidal neurons (Pinsky–Rinzel model) and a network of the Hodgkin–Huxley model, were analysed to investigate the effect of OXC on action potentials. Taken together, our results suggest that the synergistic blocking effects on \(I_{\text{Na}}\) and \(I_{\text{K(DR)}}\) may contribute to the underlying mechanisms through which OXC affects neuronal function in vivo.

Received 2 September 2007; Reviewed 17 October 2007; Revised 6 November 2007; Accepted 15 November 2007; First published online 10 January 2008

Key words: Delayed-rectifier K\textsuperscript{+} current, differentiated NG108-15 cell, oxcarbazepine, voltage-gated Na\textsuperscript{+} current.

Introduction

Oxcarbazepine (OXC; 10,11-dihydro-10-oxo-5H-dibenzo[b,f]-azepine-5-carboxamide), a keto derivative of carbamazepine (CBZ), is an anti-epileptic drug that has been approved as monotherapy and adjunctive therapy for the treatment of partial seizures with or without secondarily generalized seizures, and generalized tonic-clonic seizures (Beydoun et al., 2000; Wellington and Goa, 2001). It has also been reported to be the potential drug of choice in the management of trigeminal neuralgia (Zakrzewska and Patsalos, 2002) and bipolar disorders (Pratoomsri et al., 2006). The anti-epileptic properties of OXC were thought to be mediated through the effects on neuronal ion fluxes by blocking voltage-gated Na\textsuperscript{+} channels (Wamil et al., 1994), similar to CBZ. However, the ion mechanisms through which this drug can either modulate seizure activity or improve mood disorder are incompletely understood.
The NG108-15 cell line has been widely used as a neuron model in electrophysiology and pharmacology research (Meves et al., 1999; Tsai et al., 2006; Wu et al., 2001, 2002). It is a hybrid cell line derived from the fusion of mouse neuroblastoma (N18TG-2, a subclone of mouse C1300 neuroblastoma cells) and rat glioma (C6BV-1, a subclone of rat C6 glioma). This cell line was reported to express Kv3.1 mRNAs and to exhibit the activity of delayed rectifier K\(^+\) (K\(_{\text{DR}}\)) channels. More importantly, this cell line was thought to be a suitable model for investigating the mechanisms of neuronal development and differentiation (Nirenberg et al., 1983; Tojima et al., 2003). Moreover, there was an increase in the expression levels of Na\(_{\text{i,1,7}}\) in NG108-15 cells when neuronal differentiation was induced by pretreatment with a cyclic AMP (cAMP) analogue (Kawaguchi et al., 2007).

Voltage-gated Na\(^+\) current (I\(_{\text{Na}}\)) is responsible for action potential (AP) initiation and propagation of APs in electrically excitable cells. Experimental evidence suggests that anti-epileptic drugs, such as phenytoin, CBZ and lamotrigine, exert a voltage-dependent effect on voltage-gated Na\(^+\) channels by preferentially acting on inactivated channels (Kuo and Bean, 1994; Ragsdale et al., 1996; Willow et al., 1985). In addition, the persistent Na\(^+\) current (I\(_{\text{Na(p)}}\)), known to play an important role in regulating the firing characteristics in neurons, is also responsible for the generation of epileptiform activity (Segal and Douglas, 1997). CBZ and topiramate have been found to potentially block I\(_{\text{Na(p)}}\) (Sun et al., 2007).

There are important K\(^+\) currents that can regulate neuronal excitability. Among them, the delayed rectifier K\(^+\) current (I\(_{\text{K(OR)}}\)), present in differentiated NG108-15 cells, has been known to contribute to changes in membrane potential in these cells (Tsai et al., 2006; Wu et al., 2001). Another is the M-type K\(^+\) current (I\(_{\text{K(M)}}\)), which is active at depolarized potentials around the threshold of AP firing, displaying slowly activating and deactivating K\(^+\) currents with distinct electrophysiological and pharmacological properties (Brown and Adams, 1980; Meves et al., 1999). Network modelling has also shown that an interplay between I\(_{\text{Na(p)}}\) and I\(_{\text{K(M)}}\) could account for the generation of synchronized oscillations of neocortex (Golomb et al., 2006).

Therefore, the goals of this study were to examine the properties of I\(_{\text{Na}}\) to study the effect of OXC on I\(_{\text{Na}}\), I\(_{\text{Na(p)}}\), I\(_{\text{K(OR)}}\) and I\(_{\text{K(M)}}\) in differentiated NG108-15 neuronal cells and to address the issue of whether this drug can affect the configuration of APs and neuronal excitability.

Materials and methods

Cell preparation and differentiation

The clonal strain NG108-15 cell line, formed by Sendai virus-induced fusion of the mouse neuroblastoma clone N18TG-2 and the rat glioma clone C6 BV-1, was originally obtained from the European Collection of Cell Cultures (ECACC-88112302; Wiltshire, UK). NG108-15 cells were kept in monolayer cultures at a density of 10\(^6\)/ml in plastic dishes containing Dulbecco's modified Eagle's medium (Life Technologies; Grand Island, NY, USA) supplemented with 100 \(\mu\)M hypoxanthine, 1 \(\mu\)M aminopterin, 16 \(\mu\)M thymidine, and 5% fetal bovine serum as the culture medium, in a humidified incubator equilibrated with 90% air/10% CO\(_2\) at 37 \(^\circ\)C (Wu et al., 2001). The experiments were generally performed after 5 d of subcultivation (60–80%).

To induce neuronal differentiation, culture medium was replaced with medium containing 1 mM dibutyryl cAMP and cells were cultured in the incubator for 1–7 d. NG108-15 cells could proliferate well in the culture medium; however, they could stop proliferating and show the growth of neurites in response to dibutyryl cAMP (Kawaguchi et al., 2007; Tojima et al., 2003). The numbers of neurites and varicosities were found to be significantly increased in NG108-15 cells treated with 1 mM dibutyryl cAMP.

Electrophysiological measurements

Immediately before each experiment, cells were dissociated and an aliquot of cell suspension was transferred to a recording chamber mounted on the stage of an inverted DM-IL microscope (Leica Microsystems, Wetzlar, Germany). The microscope was coupled to a digital video camera (DCR-TRV30; Sony, Tokyo, Japan) with a magnification up to \(\times 1500\) to monitor cell size during the experiments. Cells were bathed at room temperature (20–25 \(^\circ\)C) in normal Tyrode’s solution containing 1.8 mM CaCl\(_2\). Patch pipettes were pulled from Kimax-51 glass capillaries (1.5–1.8 mM o.d., Kimble; Vineland, NJ, USA) using a two-stage electrode puller (PP-830; Narishige, Tokyo, Japan) and the tips fire-polished with a microforge (MF-83; Narishige). The pipette used had a resistance of 3–5 M\(\Omega\) when immersed in normal Tyrode’s solution. Ion currents were measured with glass pipettes in whole-cell configuration of the patch-clamp technique, using an RK-400 (Biologic, Clai, France) or an Axopatch 200B patch-clamp amplifier (Molecular Devices, Sunnyvale, CA, USA) (Huang et al., 2004; Wu et al., 2000). All potentials were corrected for liquid
play to NG108-15 cells through a digital-analogue waveforms of APs used as voltage templates were relations for ion currents. In some experiments, the generally used to measure the current–voltage response data for inhibition of $I_{\text{Na}}$ and $I_{\text{K}}$ with a modified form of the Hill equation:

$$y = \frac{(1-a) \times [\text{OXC}]^{-n}}{[\text{OXC}]^{-n} + I_{\text{Na}}^{\alpha} } + a,$$

where $y$ is the normalized amplitude of $I_{\text{K(DR)}}$ or $I_{\text{Na}}$, $[\text{OXC}]$ represents the concentration of OXC, and $I_{\text{Na}}$ and $n$ are the concentration required for a 50% inhibition and Hill coefficient, respectively. This also allows estimation of maximal inhibition $1-a$.

Values were provided as means $\pm$ S.E.M. with sample sizes (n) indicating the number of cells from which the data were obtained. The paired or unpaired Student’s t test and one-way analysis of variance with the least-significance-difference method for multiple comparisons were used for the statistical evaluation of differences among means. Statistical significance was determined at a $p$ value of $<0.05$.

**Data recordings and analyses**

The signals were displayed on an analogue/digital oscilloscope (HM-507; Hamag Inc., East Meadow, NY, USA) and on a Dell 2407WFP-HC colour monitor (Round Rock, TX, USA). The data were stored online in a laptop computer (Slimnote VX3; Lemel, Taipei, Taiwan) via a universal serial bus port at 10 kHz through an analogue/digital interface (Digidata 1322A; Molecular Devices). This device was controlled by commercially available software (pCLAMP 9.0; Molecular Devices). Cell-membrane capacitance of 36–51 pF (47.3 $\pm$ 6.3 pF, $n = 21$) was compensated. Series resistance, always in the range of 5–15 M$\Omega$, was electronically compensated. Currents were low-pass filtered at 1 or 3 kHz. Ion currents recorded during whole-cell experiments were digitally stored and filtered at 1 or 3 kHz. Ion currents recorded during whole-cell experiments were digitally stored and analysed subsequently by use of the pCLAMP 9.0 software, the Origin 7.5 software (Microcal Software Inc., Northampton, MA, USA), the SigmaPlot 7.0 software (SPSS Inc., Apex, NC, USA), or custom-made macros in Microsoft Excel (Redmond, WA, USA). The pCLAMP-generated voltage-step protocols were generally used to measure the current–voltage relations for ion currents. In some experiments, the waveforms of APs used as voltage templates were replayed to NG108-15 cells through a digital-analogue converter (Digidata 1322A) (Wu et al., 2002).

To calculate the percentage decrease in $I_{\text{Na}}$, each cell was depolarized from $-80$ mV to $-10$ mV and the peak amplitude elicited by depolarizing pulse was measured. For $I_{\text{K(DR)}}$, the voltage was applied from $-50$ mV to $+50$ mV and current amplitude at the of depolarizing pulse was measured. Concentration–response data for inhibition of $I_{\text{Na}}$ or $I_{\text{K(DR)}}$ were fitted with a modified form of the Hill function:

$$y = \frac{(1-a) \times [\text{OXC}]^{-n}}{[\text{OXC}]^{-n} + I_{\text{Na}}^{\alpha} } + a,$$

where $y$ is the normalized amplitude of $I_{\text{K(DR)}}$ or $I_{\text{Na}}$, $[\text{OXC}]$ represents the concentration of OXC, and $I_{\text{Na}}$ and $n$ are the concentration required for a 50% inhibition and Hill coefficient, respectively. This also allows estimation of maximal inhibition $1-a$.

Values were provided as means $\pm$ S.E.M. with sample sizes (n) indicating the number of cells from which the data were obtained. The paired or unpaired Student’s t test and one-way analysis of variance with the least-significance-difference method for multiple comparisons were used for the statistical evaluation of differences among means. Statistical significance was determined at a $p$ value of $<0.05$.

**Drugs and solutions**

OXC (oxcarbazepine; 10,11-dihydro-10-oxo-5H-dibenz[b,f]-azepine-5-carboxamide; Trileptal) was obtained from Novartis Pharma AG (Basle, Switzerland). CBZ, dibutyryl cAMP, linopirdine, tetraethylammonium chloride, adenosine, adenosine deaminase, and tetrodotoxin were from Sigma Chemicals (St Louis, MO, USA). Dendrotoxin was obtained from Calbiochem (La Jolla, CA, USA) and 8-cyclopentyl-1,3-dipropylxanthine was from Toecris Bioscience (Bristol, UK). Tissue culture media and trypsin/EDTA were obtained from the American Type Culture Collection (Manassas, VA, USA). l-glutamine, penicillin-streptomycin and fungizone were obtained from Life Technologies (Grand Island, NY, USA). All other chemicals were commercially available and of reagent grade.

The composition of normal Tyrode’s solution was 136.5 mM NaCl, 5.4 mM KCl, 1.8 mM CaCl$_2$, 0.53 mM MgCl$_2$, 5.5 mM glucose, and 5.5 mM Hepes-NaOH buffer (pH 7.4). To record $K^+$ currents or membrane potential, the patch pipette was filled with a solution consisting of 140 mM KCl, 1 mM CaCl$_2$, 3 mM Na$_2$ATP, 0.1 mM Na$_2$GTP, 0.1 mM EGTA, and 5 mM Hepes-KOH buffer (pH 7.2). To measure Na$^+$ current, $K^+$ ions inside the pipette solution were replaced with equimolar Cs$^+$ ions, and the pH was adjusted to 7.2 with CsOH.

**Mathematical modelling**

Spontaneous discharge of neurons was simulated with the aid of the model for hippocampal CA3 pyramidal neurons (Table 1), which was originally derived from Pinsky and Rinzler (1994). This model consists of a fast and transient Na$^+$ current, a persistent, depolarization-activated Na$^+$ current, a low-threshold Ca$^{2+}$ current, a high-threshold Ca$^{2+}$ current, a Ca$^{2+}$-activated K$^+$ current, a transient outward K$^+$ current, a slowly inactivating K$^+$ current (i.e. delayed rectifier K$^+$ current), and a hyperpolarization-activated cation current. The source file for this model is readily available at http://senselab.med.yale.edu/modeldb. The solutions to the different sets of ordinary differential equations in the simulations were generally approximated using the X-Win32 version of XPPAUT on a Hewlett Packard (HP xw9300) workstation (Palo Alto, CA, USA) (Ermentrout, 2002; Huang et al., 2007).

A network of spiking neurons with Hodgkin–Huxley type (Brette et al., 2007) was simulated to
ethylammonium chloride is effective in suppressing pipette solution and differentiated NG108-15 cells.

In an initial set of electrophysiological experiments, NG108-15 neuronal cells depolarized from significantly reduced. For example, when cells were exposed to a CsCl-containing pipette solution free Tyrode’s solution containing 0.5 mM CdCl₂ and 10 mM tetraethylammonium chloride. CdCl₂ can block Ca²⁺ currents, while tetraethylammonium chloride is effective in suppressing K⁺ currents. As shown in Figure 1a, the cell was held at −80 mV, and I_Na was elicited in response to a series of depolarizing voltage pulses. When cells were exposed to OXC, the peak amplitude of I_Na was significantly reduced. For example, when cells were depolarized from −80 mV to −20 mV, the addition of 3 µM OXC decreased the amplitude of I_Na from 2061 ± 245 pA to 792 ± 169 pA (n = 6). After washout of OXC, the amplitude of I_Na was returned to 1866 ± 207 pA (n = 4). Similarly, 1 µM tetrodotoxin could suppress the amplitude of I_Na by about 95% in these cells. Figure 1b illustrates the averaged current density vs. membrane potential relationships for I_Na obtained with or without application of 3 µM OXC. The results showed that this drug was able to block tetrodotoxin-sensitive I_Na with no change in overall current density-voltage configuration of this current in differentiated NG108-15 cells.

Results

Effect of OXC on the inactivation curve of I_Na

To characterize the inhibitory effects of OXC on I_Na, we further examined the voltage dependence of the effect of OXC on I_Na in differentiated NG108-15 cells. Figure 2 shows the steady-state inactivation curve of I_Na obtained in the absence and presence of 3 µM OXC. In these experiments, the interval between two sets of voltage pulses was 30 ms to avoid incomplete recovery of I_Na. As shown in Figure 2b, the relationships between conditioning potentials and the normalized amplitudes of I_Na in the absence and presence of 3 µM OXC were fitted to a Boltzmann function using a nonlinear least-squares regression analysis: I = I_max f [1 + exp((V - Vₐ)/k)], where I_max is the maximal activated I_Na, V is the membrane potential in mV, Vₐ is the membrane potential for a half-maximal inactivation, and k is the slope factor of the inactivation curve. In the absence of OXC, Vₐ = −48.0 ± 1.6 mV and

Table 1. Default parameters used for the modelling of hippocampal CA3 pyramidal neurons

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
<th>Value</th>
</tr>
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<tbody>
<tr>
<td>C_m</td>
<td>Membrane capacitance</td>
<td>3 µF</td>
</tr>
<tr>
<td>SNa</td>
<td>Na⁺ current conductance</td>
<td>18 nS</td>
</tr>
<tr>
<td>SK(DR)</td>
<td>Delayed rectifier K⁺ conductance</td>
<td>15 nS</td>
</tr>
<tr>
<td>SK(Ca)</td>
<td>Ca²⁺-activated K⁺ current conductance</td>
<td>20 nS</td>
</tr>
<tr>
<td>SK(ATP)</td>
<td>ATP-sensitive K⁺ current conductance</td>
<td>0.3 nS</td>
</tr>
<tr>
<td>VCa</td>
<td>Ca²⁺ reversal potential</td>
<td>+80 mV</td>
</tr>
<tr>
<td>VNa</td>
<td>Na⁺ reversal potential</td>
<td>+60 mV</td>
</tr>
<tr>
<td>VK</td>
<td>K⁺ reversal potential</td>
<td>−75 mV</td>
</tr>
</tbody>
</table>

mimic the conditions in which OXC was applied. In this model, 200 excitatory and 50 inhibitory neurons with random connectivity were incorporated with Hodgkin–Huxley kinetics. As current injections were randomly applied to the neurons, persistent and stochastic activity of neuronal spiking can be readily simulated. This model consists of two populations of excitatory (100 cells) and inhibitory (50 cells) neurons. Applied current or ion conductances can be randomly applied. Theoretically, neural networks in normal or epileptic brain can be applied and compared.
$k = 9.4 \pm 0.5 \text{ mV} \ (n = 6)$, whereas in the presence of $3 \mu M$ OXC, $V_1 = -57.1 \pm 1.7 \text{ mV}$ and $k = 9.2 \pm 0.5 \text{ mV} \ (n = 5)$. Therefore, OXC did not simply reduce the maximal conductance of $I_{Na}$, it also shifted the inactivation curve to a hyperpolarized potential ($9.1 \pm 1.3 \text{ mV}$, $n = 5$). However, we found no significant change in the slope (i.e. $k$) of the curve in the presence of this drug. Taken together, the results suggest that OXC could shift the inactivation curve of $I_{Na}$ in differentiated NG108-15 cells.

**Effect of OXC on recovery of $I_{Na}$ from inactivation**

The effect of OXC on the recovery of $I_{Na}$ from inactivation was studied with the use of a double-pulse protocol. In this protocol, a 40-ms conditioning step to $-20 \text{ mV}$ inactivated most of the $I_{Na}$, and the recovery of $I_{Na}$ from inactivation at the holding potential of $-80 \text{ mV}$ was examined at different times with a test step ($-20 \text{ mV}$, 40 ms), as shown in Figure 3. In the control condition, the peak amplitude of $I_{Na}$ almost completely recovered from inactivation when the recovery time was 120 ms. The time-course of recovery from inactivation was well fitted to a single exponential function with a time constant of $25.8 \pm 1.8 \text{ ms} \ (n = 5)$. However, during cell exposure to $3 \mu M$ OXC, recovery from inactivation was significantly prolonged, with a time constant of $38.9 \pm 2.1 \text{ ms} \ (n = 6)$. In other words, after a 120-ms interval, the peak amplitude of $I_{Na}$ was found to recover completely.

Figure 1. Inhibition of OXC on voltage-gated Na$^+$ current ($I_{Na}$) in NG108-15 cells differentiated with 1 mM dibutyryl cAMP. In these experiments, cells were bathed in Ca$^{2+}$-free Tyrode’s solution containing 0.5 mM CdCl$_2$ and 10 mM tetraethylammonium chloride. The pipette was filled with a Cs$^+$-containing solution. (a) Original current traces obtained in the absence (upper) and presence (lower) of $3 \mu M$ OXC. (b) Current density vs. membrane potential relationships of $I_{Na}$ under control and during the exposure to $3 \mu M$ OXC. ● Control; ○, OXC. (c) Comparison of $I_{Na}$ density in the presence of various compounds. Each cell was depolarized from $-80 \text{ mV}$ to $-20 \text{ mV}$ and the peak amplitude of $I_{Na}$ was measured. Bar (i) undifferentiated NG108-15 cells; (ii) differentiated NG108-15 cells (i.e. control cells); (iii) $3 \mu M$ OXC; (iv) $3 \mu M$ CBZ; (v) $1 \mu M$ tetrodotoxin. * Significantly different from undifferentiated NG108-15 cells [bar (i)]. ** Significantly different from controls [bar (ii)]. (d) Concentration–response relationship for OXC-induced inhibition of $I_{Na}$. Each point represents the mean ± S.E.M. ($n = 5–8$). The grey smooth line represents the best fit to the Hill equation as described in the Materials and methods section. The IC$_{50}$ value, maximally inhibited percentage of $I_{Na}$, and Hill coefficient were $3.1 \mu M$, 99% (i.e. $a = 0.01$), and 1.0, respectively.
from inactivation in control conditions; however, in the presence of OXC, a substantial blockade of $I_{\text{Na}}$ was still observed. It is thus clear that OXC produces a significant prolongation of the recovery from $I_{\text{Na}}$ inactivation in differentiated NG108-15 cells. 

**Inhibitory effect of OXC $I_{\text{Na}P}$ in differentiated NG108-15 cells**

The $I_{\text{Na}P}$ can be responsible for initiation of APs and some forms of epilepsy (Beurrier et al., 2000;
Stafstrom, 2007). It was found to be present in differentiated NG108-15 cells. We further examined whether OXC could have any effects on the amplitude of $I_{Na(P)}$. In these experiments, the cell was held at $-80\,\text{mV}$ and a 1.5-s long ramp pulse from $-120\,\text{mV}$ to $+20\,\text{mV}$ was applied as described previously (Beurrier et al., 2000). Our experimental results showed that OXC at a concentration of 10 $\mu\text{M}$ could inhibit the peak amplitude of $I_{Na(P)}$ by about 40% (Figure 4). For example, 10 $\mu\text{M}$ OXC decreased $I_{Na(P)}$ from $356 \pm 58$ to $205 \pm 34\,\text{pA}$ ($n=5$). Similarly, 1 $\mu\text{M}$ tetrodotoxin almost completely blocked $I_{Na(P)}$ in these cells. Therefore, $I_{Na(P)}$ present in differentiated NG108-15 cells can also be sensitive to blockade by OXC.

**Effect of OXC on $I_{K(DR)}$ in differentiated NG108-15 cells**

The effect of OXC on $I_{K(DR)}$ in these cells was also examined. To record $K^+$ outward currents, cells were bathed in Ca$^{2+}$-free Tyrode’s solution containing 1 $\mu\text{M}$ tetrodotoxin and 0.5 mM CdCl$_2$. As shown in Figure 5, when the cell was held at $-50\,\text{mV}$ and the depolarizing pulses from $-50\,\text{mV}$ to $+50\,\text{mV}$ in 10-mV increments were applied, a family of $K^+$ outward currents with little inactivation was elicited. This type of outward currents has been previously identified as $I_{K(DR)}$ (Wu et al., 2001). Within 1 min of exposing the cells to 10 $\mu\text{M}$ OXC, the amplitude of $I_{K(DR)}$ measured at the end of 300-ms depolarizing pulses was reduced at the potentials ranging from $+20\,\text{mV}$ to $+100\,\text{mV}$. For example, when the depolarizing pulses from $-50\,\text{mV}$ to $+50\,\text{mV}$ were applied, the presence of 10 $\mu\text{M}$ OXC significantly decreased current density at the end of the voltage pulses from $41 \pm 4\,\text{pA/pF}$ to $22 \pm 2\,\text{pA/pF}$ ($n=8$). This inhibitory effect was readily reversed on washout of OXC.

Effects of OXC, CBZ and dendrotoxin on $I_{K(DR)}$ in differentiated NG108-15 cells were also examined and compared. As shown in Figure 5b, 1 $\mu\text{M}$ dendrotoxin, a known inhibitor of $I_{K(DR)}$, was effective in suppressing the density of $I_{K(DR)}$. CBZ (3 $\mu\text{M}$) exerted an inhibitory effect on $I_{K(DR)}$ density more potently than OXC at the same concentration. Averaged current density vs. membrane potential relationships for $I_{K(DR)}$ in the absence and presence of 10 $\mu\text{M}$ OXC are shown in Figure 5c.

The relationship between the OXC concentration and the percentage inhibition of $I_{K(DR)}$ was also constructed. As shown in Figure 5d, 0.3–30 $\mu\text{M}$ OXC suppressed the amplitude of $I_{K(DR)}$ in a concentration-dependent manner. The IC$_{50}$ value for OXC-induced inhibition of $I_{Na}$ was 7.2 $\mu\text{M}$. The results indicate that in addition to inhibition of $I_{Na}$, OXC can be effective in suppressing $I_{K(DR)}$ present in differentiated NG108-15 cells.

**No association of OXC effects on ion currents with its binding to adenosine receptors**

Previous reports showed that OXC-induced antinociceptive effects in a rat model of inflammatory hyperalgesia could be mediated via its binding to peripheral

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**Figure 4.** Inhibitory effect of OXC on persistent $I_{Na}$ ($I_{Na(P)}$) in differentiated NG108-15 cells. In these experiments, each cell was held at $-80\,\text{mV}$ and a 1.5-s long ramp pulse from $-120\,\text{mV}$ to $+20\,\text{mV}$ was applied. (a) Original current traces in response to ramp pulse obtained in the absence (i) and presence (ii) of 10 $\mu\text{M}$ OXC. The inset shows the voltage protocol used. (b) Summary of data showing the effect of OXC and tetrodotoxin on the peak amplitude of $I_{Na(P)}$ in differentiated NG108-15 cells. Each point represents the mean ± s.e.m. ($n=6–9$). OXC, 10 $\mu\text{M}$ oxcarbazepine; TTX, 1 $\mu\text{M}$ tetrodotoxin. * Significantly different from control.
adenosine A<sub>1</sub> receptors (Tomicˇ et al., 2006). However, in our study, subsequent application of either adenosine deaminase (1 U/ml) or 8-cyclopentyl-1,3-dipropylxanthine (DPCPX; 1 μM) was found to have no effects on OXC-induced blockade of I<sub>Na</sub> or I<sub>K(DR)</sub> present in differentiated NG108-15 cells (data not shown). DPCPX was thought to be a potent antagonist of adenosine A<sub>1</sub> receptors (Deckert et al., 1993; Tomic ´ et al., 2006; Wu et al., 1998). In addition, neither I<sub>Na</sub> nor I<sub>K(DR)</sub> was affected in the presence of 30 μM adenosine.

Therefore, the inhibitory effects of OXC on ion currents presented here are not associated with its binding to adenosine A<sub>1</sub> receptors.

No effect of OXC on I<sub>K(M)</sub> in differentiated NG108-15 cells

Previous studies have demonstrated that, undergoing differentiation with cAMP, NG108-15 cells could also express the activity of M-type K<sup>+</sup> channels (Meves et al., 1999). We further studied whether OXC could have any influence on I<sub>K(M)</sub>. The results demonstrated the inability of this drug to affect the amplitude of I<sub>K(M)</sub> in these cells. When cells were hyperpolarized from −40 mV to −60 mV, I<sub>K(M)</sub> measured at the end of the hyperpolarizing pulse in the control did not significantly differ from that obtained during the
exposure to 10 μM OXC (Figure 6). However, at the same voltage protocol, 10 μM linopirdine, a specific inhibitor of I_{K(M)}, could be effective in suppressing I_{K(M)}.

**Effect of OXC on APs in differentiated NG108-15 cells**

In another series of experiments, we examined the effect of OXC on changes in membrane potential. In these experiments, cells were bathed in normal Tyrode’s solution containing 1.8 mM CaCl_2. Current-clamp configuration was made in a K^+–containing pipette solution. The resting membrane potential and AP duration in differentiated NG108-15 cells were −69 ± 8 mV and 123 ± 19 ms, respectively (n = 18). The APs recorded from these cells were sensitive to tetrodotoxin (1 μM). The typical effect of OXC on APs in differentiated NG108-15 cells is illustrated in Figure 7. OXC at a concentration of 3 μM significantly decreased the amplitude of APs from 71 ± 4 mV to 59 ± 4 mV and prolonged the duration of APs from 121 ± 16 ms to 158 ± 24 ms (n = 6).

**Inhibitory effects of OXC on ion currents in response to AP waveforms in differentiated NG108-15 cells**

The size and time-course of ion currents (e.g. I_{Na} and I_{K(DR)}) in response to change in AP waveforms have been described to be distinguishable from those elicited by rectangular voltage-clamp pulses. The effects of OXC on ion currents during APs generated from NG108-15 cells were thus further investigated. In these experiments, the waveforms of APs were digitally generated as voltage templates and replayed to evoke ion currents (Wu et al., 2002). As shown in Figure 8, the amplitudes of I_{Na} and I_{K(DR)} in response to AP waveforms were decreased in the presence of OXC. For example, after application of 3 μM OXC, the peak amplitude of I_{Na} elicited by the waveforms of APs was decreased by about 40%. Moreover, I_{K(DR)} in response to AP waveforms was suppressed by about...
30%. Thus, the results suggest that $I_{\text{Na}}$ and $I_{\text{K(DR)}}$ evoked by AP waveforms can be more sensitive to blockade by this drug than that during rectangular pulses.

Effects of OXC on repetitive firing of APs in modelled neurons

In order to determine how OXC alters the discharge pattern of hippocampal neurons and to address questions that are difficult to be experimentally studied, a simulation model, originally derived from Pinsky and Rinzel (1994), was implemented (Huang et al., 2007). In this modelled neuron, when the $I_{\text{Na}}$ conductance was decreased from 18 nS to 16.5 nS, the peak amplitude of $I_{\text{Na}}$ was readily reduced, along with termination of APs [Figure 9a(i)]. When the conductance of $I_{\text{K(DR)}}$ was decreased from 15 nS to 14 nS, membrane potential was depolarized and the firing of APs was slowed compared with the control condition [Figure 9a(ii)]. As a result, the reduced $I_{\text{Na}}$ and $I_{\text{K(DR)}}$, which mimics the condition in which OXC is applied, can act in concert to affect the firing of neuronal APs. The simulation results can be allowed to predict that changes in the activity of KDR channels could contribute to electrical behaviour of hippocampal neurons.

Drugs known to affect voltage-gated Na$^+$ or K$^+$ currents have been recently demonstrated to have significant effects on the ~10 Hz oscillations of excitatory motor cortex networks (Castro-Alamancos et al., 2007). A simulated network of spiking neurons with Hodgkin–Huxley kinetics was thus evaluated to study the possible action of OXC. As shown in Figure 9b(i–iii), as the $I_{\text{Na}}$ conductance was arbitrarily decreased from 120 $\mu$S to 90 $\mu$S at 100 ms, the firing of APs was abolished in simulated excitatory neurons. Moreover, after a further reduction of $I_{\text{K(DR)}}$ conductance from 36 $\mu$S to 34 $\mu$S, compared to the control condition, the firing of APs in the modelled cells were reduced along with random firing in a stochastic manner. Therefore, it is tempting to speculate that the combination of decreased conductance of $I_{\text{Na}}$ and $I_{\text{K(DR)}}$ which is used to mimic the OXC action, can readily modulate neuronal firing in a network in vivo.

Discussion

The major findings of this study are as follows. First, in differentiated NG108-15 neuronal cells, OXC inhibited the amplitude of $I_{\text{Na}}$ in a concentration-dependent manner. Second, OXC could produce a negative shift in the steady-state inactivation curve of $I_{\text{Na}}$. Third, OXC prolonged the recovery of $I_{\text{Na}}$ inactivation. Fourth, OXC suppressed the amplitude of $I_{\text{K(DR)}}$ in a concentration-dependent manner. Fifth, the
The morphological and functional changes in NG108-15 cells treated with dibutyryl cAMP in our study were consistent to a previous report (Kawaguchi et al., 2007; Tojima et al., 2003). The density of \( I_{\text{Na}} \) in differentiated NG108-15 cells was significantly enhanced. The IC\( _{50} \) value of OXC required for the inhibition of \( I_{\text{Na}} \) in these cells was 3.1 \( \mu \text{M} \). The \( I_{\text{Na}} \) in response to AP waveforms was more sensitive to
blockade by OXC than that elicited by rectangular steps. In addition, we found that it could block the amplitude of \( I_{K(DR)} \) with an IC\(_{50} \) value of 7.4 \( \mu \)M. Therefore, it is possible that when APs in neurons in vivo fire more frequently, the inhibitory action of OXC on \( I_{Na} \) and \( I_{K(DR)} \) would be enhanced. The ion mechanism could account for a previous study showing that OXC-induced inhibition of AP firing depended on firing frequency (Wamil et al., 1994). Our experimental data are in agreement with previous studies showing that OXC suppressed the sustained high-frequency firing of sodium-dependent APs in mouse neurons in cell culture (Schmutz et al., 1994).

The recovery from \( I_{Na} \) inactivation in the presence of OXC observed in this study was slow, as 200 ms in duration was required for the channel to recover completely. As a result, OXC-induced blockade of \( I_{Na} \) will become even more significant when a train of APs occur, because of the fact that under these conditions, the availability of \( K(DR) \) channels is decreased as a function of firing frequency. The frequency-encoded neurons are likely to be more susceptible to OXC.

OXC could produce a distinct shift in the steady-state inactivation curve of \( I_{Na} \) in these cells. It is thus likely that OXC preferentially binds to the inactivated state of the voltage-gated \( Na^+ \) channel. More interestingly, the ability of OXC to produce a negative shift in the inactivation curve suggests that OXC-induced blockade of \( I_{Na} \) is voltage dependent. Although the kinetic profiles of OXC-induced blockade of \( I_{Na} \) remain to be further elucidated, it is possible that this drug can block the voltage-gated \( Na^+ \) channel in a state-dependent manner.

As persistent neuronal sodium currents (\( I_{Na(p)} \)) may be a common underlying mechanism in some forms of epilepsy (Lossin et al., 2002; Segal, 2002), the inhibitory effect on \( I_{Na(p)} \) of OXC, as shown in our study, potentially supports the role of OXC in treating a broad spectrum of epileptic syndromes. OXC-induced blockade of ion currents may also be responsible for its inhibitory effects on the release of glutamate and other neurotransmitters (Waldmeier et al., 1995). However, its blockade of these ion channels in neurons is not associated with a mechanism linked to the binding to adenosine receptors, although OXC was reported to have antinociceptive actions through its binding to peripheral adenosine A\(_1 \) receptors (Tomić et al., 2006).

The inhibition by OXC of \( I_{Na} \) could be indirectly altered by its blockade of \( I_{K(DR)} \). The reason is due to the possibility that inhibition of \( Na^+ \) channels is further exacerbated by the blockade of \( I_{K(DR)} \). Inhibition of \( I_{K(DR)} \) may delay and weaken the repolarization of neurons following an AP. Therefore, OXC may influence the availability of voltage-gated \( Na^+ \) channels as its blockade of \( I_{K(DR)} \) is responsible for maintenance of high electrical excitability in a network of neurons. Inhibition of \( I_{Na} \) can retard the influx of \( Na^+ \) ions into the cells, while blockade of \( I_{K(DR)} \) renders the cells relatively excitable. It thus remains to be determined whether OXC-induced blockade of ion currents contributes to its important side-effect (e.g., hypotension) (Kothare et al., 2006).

Oscillatory activities (~10 Hz) are known to be associated with several forms of epilepsy, such as frontal lobe epilepsy partialis continua (Chauvel et al., 1992), cortical myoclonus in humans (Castro-Almancos, 2006) and abnormal twitching in rats (Semb and Komisaruk, 1984). It has been recently found that changes in the amplitude of \( I_{K(DR)} \) are able to sustain these oscillations (Castro-Almancos et al., 2007). It is thus postulated that the inhibitory effects of OXC might have therapeutic potential in these specific disorders.

The differences between OXC and CBZ in treating epileptic patients are still under investigation. It has been demonstrated that OXC should be preferred over CBZ, in terms of efficacy on seizure control and side-effect profile (Schmidt and Elgar, 2004). From our study, although OXC shares similar efficacy with CBZ in \( I_{Na} \) antagonism, CBZ exerts more potent \( I_{K(DR)} \) antagonism. It has been clearly noted that there was relatively higher paradoxical seizure worsening potential in CBZ in idiopathic generalized epilepsy (Thomas et al., 2006), compared to other anti-epileptic drugs including OXC. CBZ can act as a blocker of the voltage-dependent \( Na^+ \) channel and enhance membrane stabilization. However, it has another property that may indirectly increase hypersynchronization of neuronal discharges in a thalamocortical loop in which intensified oscillatory activity in absence epilepsy was shown (McLean and Macdonald, 1986). Whether this difference in anti-epileptic efficacy relates to an \( I_{K(DR)} \) antagonism needs further evaluations.

In our experiments, a relatively large reduction of \( Na^+ \) current conductance (\( g_{Na} \)) by OXC blockade does not abolish APs. The model simulation does not appear to completely come up with experimental results. In simulation, a small reduction of \( g_{Na} \) from 18 nS to 16.5 nS would be sufficient to abolish the AP firing. Thus, there would be some quantitative differences between the experimental results and model simulation. One of the reasons might be due to OXC-induced blockade of \( I_{K(DR)} \). In addition, different types of neurons are also required to be further considered.
In conclusion, this study suggests the role of OXC as an effective anti-epileptic drug and OXC-mediated action could be in part associated with the direct inhibition of these two types of ion currents in neurons.

Acknowledgements

This work was partly aided by grants from the National Science Council (NSC-94-2320B-006-019, NSC-95-2314-B-006-046, NSC-95-2745-B006-002-MY2 and NSC-96-2314-B-006-059) and the Programme for Promoting Academic Excellence & Developing World Class Research Centers, Ministry of Education, Taiwan.

Statement of Interest

Taiwan.

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This provides a list of references for the document.


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