Modulation of Transient and Persistent Inward Currents by Activation of Protein Kinase C in Spinal Ventral Neurons of the Neonatal Rat

Yue Dai, Larry M. Jordan, and Brent Fedirchuk

Department of Physiology, University of Manitoba, Winnipeg, Manitoba, Canada

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Dai Y, Jordan LM, Fedirchuk B. Modulation of transient and persistent inward currents by activation of protein kinase C in spinal ventral neurons of the neonatal rat. J Neurophysiol 101: 112–128, 2009. First published October 22, 2008; doi:10.1152/jn.01373.2007. Neuronal excitability can be regulated through modulation of voltage threshold (\(V_{th}\)). Previous studies suggested that this modulation could be mediated by modulation of transient sodium currents (\(I_{T}\)) and/or persistent inward current (PIC). Modulation of \(I_{T}\) and PIC through activation of protein kinase C (PKC) has previously been described as a mechanism controlling neuronal excitability. We investigated modulation of \(I_{T}\) and PIC by PKC in neonatal rat spinal ventral neurons. In whole cell voltage clamp, activation of PKC by application of 1-oleoyl-2-acetyl-sn-glycerol (OAG, 10–30 \(\mu M\)) resulted in 1) a reduction of \(I_{T}\) amplitude by 33% accompanied an increase in half-width and a decrease in the maximal rise and decay rates of the \(I_{T}\); 2) a reduction of PIC amplitude by 49%, with a depolarization of PIC onset by 4.5 mV. Activation of PKC caused varied effects on \(V_{th}\), for eliciting \(I_{T}\), with an unchanged \(V_{th}\) or depolarized \(V_{th}\), being the most common effects. In current-clamp recordings, PKC activation produced a small but significant depolarization (2.0 mV) of \(V_{th}\) for action potential generation with an increase in half-width and a decrease in amplitude and the maximal rise and decay rates of action potentials. Inclusion of PKC19–36 (10–30 \(\mu M\)), a PKC inhibitor, in the recording pipette could block the OAG effects on \(I_{T}\) and PIC. The ability of serotonin to hyperpolarize \(V_{th}\) was not altered by PKC activation or inhibition. This study demonstrates that activation of PKC decreases the excitability of spinal ventral neurons and that \(V_{th}\) can be modulated by multiple mechanisms.

INTRODUCTION

The nervous system has the ability to alter motoneuron excitability to adapt for different conditions such as walking. Modulation of the voltage threshold (\(V_{th}\)) for generation of action potentials appears to be one of the fundamental means that the nervous system uses to regulate neuronal excitability. The state-dependent modulation of \(V_{th}\) has been observed in fictive locomotion. In decerebrate cats (Krawitz et al. 2001), the \(V_{th}\) for action potentials in lumbar motoneurons is hyperpolarized during fictive locomotion induced by stimulation of the mesencephalic locomotor region (MLR) and returns to the control level shortly after the termination of stimulation of MLR. A persistent change in \(V_{th}\) is also observed in 16-wk endurance-trained rats, where the \(V_{th}\) is found to be hyperpolarized in hindlimb motoneurons compared with the untrained rats (Beaumont et al. 2003). In contrast, the \(V_{th}\) of lumbar motoneurons is depolarized in rats in which the hindlimbs are unweighted for 2 wk (Cormery et al. 2005) and in cats that are chronically spinalized for 6 wk (Hochman and McCrea 1994). These studies demonstrated that \(V_{th}\) can be modulated for different motor states or behavior and the modulation of \(V_{th}\) could be mediated by different pathways. We have previously shown that application of serotonin (5-hydroxytryptamine [5-HT]) or norepinephrine or activation of descending serotonergic fibers produced a \(V_{th}\) hyperpolarization of ventral horn neurons in the isolated spinal cord of the neonatal rats (Fedirchuk and Dai 2004b; Gilmore and Fedirchuk 2004). However, the mechanisms underlying the depolarization of \(V_{th}\) remain unknown. Our modeling studies suggest that sodium channels play a dominant role in regulation of \(V_{th}\) and can result in either hyperpolarization (Dai et al. 2002; Gardiner et al. 2006) or depolarization of \(V_{th}\) (Cormery et al. 2005). Therefore we expect sodium channel modulation to be a key mechanism regulating \(V_{th}\).

It has become evident that sodium channels themselves are the target of modulation mediated by phosphorylation at specific sites (see Cantrell and Catterall 2001; Catterall 2000). In particular, phosphorylation of the \(\alpha\) subunit by protein kinase C (PKC) decreases the peak \(Na^{+}\) current in reconstituted brain sodium channels (Costa and Catterall 1984; Murphy and Catterall 1992; Numann et al. 1991). \(Na^{+}\) channels can be phosphorylated through activation of different neurotransmitter pathways. Phosphorylation of \(Na^{+}\) channels by cAMP-dependent protein kinase A (PKA) decreases peak \(I_{Na}\) in cultured brain neurons (Li et al. 1992), mammalian cells (Li et al. 1992, 1993), and Xenopus oocytes (Gershman et al. 1992; Smith and Goldin 1996). This phosphorylation has been shown to be voltage dependent and mediated by activation of D1-like dopamine receptors in acute isolated hippocampal neurons (Cantrell et al. 1997, 1999). Phosphorylation of \(Na^{+}\) channels by activation of PKC also reduces sodium currents and this PKC-mediated phosphorylation is shown to be activated by muscarinic acetylcholine receptors in rat hippocampal neurons (Cantrell et al. 1996). In mouse prefrontal cortex neurons, activation of PKC through 5-HT1A receptors decreases the rapid inactivating \(I_{Na}\) by reducing the maximal \(I_{Na}\) and shifting the fast inactivation voltage dependence (Carr et al. 2002). Many studies have explored the PKC pathway activated by 5-HT1A and 5-HT2 receptors (for review see Raymond et al. 2001). All these studies suggest that PKC-mediated modulation of \(I_{Na}\) via neurotransmitters is a fundamental way for the nervous system to regulate the neuronal excitability, although little information is available about this modulation in spinal ventral neurons.

Address for reprint requests and other correspondence: B. Fedirchuk, Department of Physiology, University of Manitoba, 745 Bannatyne Ave., Winnipeg, MB, Canada R3E 0J9 (E-mail: brent@scrc.umanitoba.ca).

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Since PKC activation reduces peak sodium currents, it may be expected that PKC activation decreases neuronal excitability. This has been shown as the suppression of intrinsic bursting (Alroy et al. 1999) and the down-regulation of the persistent Na\(^{+}\) current in rat hippocampal pyramidal cells (Mittmann and Alzheimer 1998) and a reduction of dendritic excitability in mouse neocortical neurons (Carr et al. 2002) when PKC pathways are activated by PKC activators in these neurons. In contrast, however, activation of PKC has also been shown to increase neuronal excitability through a hyperpolarization of voltage threshold for action potentials in mouse neocortical neurons (Astman et al. 1998) or amplification of subthreshold depolarization in rat pyramidal neurons (Franceschetti et al. 2000). The increased neuronal excitability is attributed to the PKC-mediated hyperpolarization of onset voltages of persistent inward current (PIC). These contradictory findings suggest that 1) PKC is involved in regulating neuronal excitability; 2) the modulation of PIC by the PKC pathway could play an important role in this regulation; and 3) activation of PKC may exert a different influence on neuronal excitability in different systems.

Modulation of sodium currents via the PKC pathway has been studied intensively in many neuron types and systems (Cantrell and Catterall 2001; Catterall 2000) and the PKC-mediated modulation of PIC has also been reported in rodent brain neurons (Astman et al., 1998; Franceschetti et al. 2000). However, little is known about the modulation of \(I_{\text{Na}}\) and PIC in the spinal motor system, especially the interactions between the PKC and serotonergic pathways in modulating neuronal excitability. The goals of this study are to test the hypothesis that alterations in activity in the PKC pathway modulate the transient sodium currents and persistent inward currents of spinal ventral neurons of neonatal rats and alter the voltage threshold and neuronal excitability. This study also explores the interaction between the PKC- and 5-HT-mediated modulation of the \(V_{\text{th}}\) and demonstrates multiple pathways existing for modulation of \(V_{\text{th}}\). Preliminary data have been reported in abstract form (Fedirchuk and Dai 2004a).

**METHODS**

Experiments were conducted on the slice preparations in accordance with guidelines for the ethical treatment of animals issued by the Canadian Council on Animal Care and with the approval of our institutional protocol review committee.

**Preparation of slices**

The slice experiments were carried out on neonatal (postnatal day 1 [P1] to P5) Sprague–Dawley rats. The rats were anesthetized with an intraperitoneal injection of ketamine (100 mg/kg). After decapitation, the rats were eviscerated and pinned ventral side up in a Sylgard-lined dish filled with 4°C dissecting artificial cerebral spinal fluid (aCSF) bubbled with 95% O\(_2\)-5% CO\(_2\). The spinal cords were then dissected. The lumbar segments (T13–L6) were isolated and introduced into a dish filled with 4°C dissecting artificial cerebral spinal fluid (aCSF) bubbled with 95% O\(_2\)-5% CO\(_2\). The chamber was perfused with recording aCSF at a rate of 0.5–1 ml/min, bubbled with 95% O\(_2\)-5% CO\(_2\). Neurons in ventral areas (lamina VII–X) were visualized using an infrared cube and recorded in whole cell patch clamp using glass pipette electrodes. The pipette electrodes were pulled from borosilicate glass (MTW 150F-4, WPI) using a P-87 puller (Sutter Instruments) and had resistances of 5–8 MΩ when filled with intracellular solution. A MultiClamp 700A patch-clamp amplifier, Digidata 1322 A/D converter, Minidigi 1A, and pClamp 9.0 software (all from Axon Instruments) were used for data acquisition. The whole cell patch recordings were made in both voltage- and current-clamp mode. Bridge balance and capacitance compensation were made before the recordings. Series resistance was monitored (usually <30 MΩ) and compensated. Data were low-pass filtered at 3 kHz and sampled at 10 kHz. The data were analyzed using self-written codes with Igor Pro (4.0) and Axon Clampfit (9.0). Student’s t-test and \(\chi^2\) test were performed with statistical significance defined as \(P < 0.05\). Results are shown as means ± SD. Activation and inactivation curves are presented as fitted means ± SE.

**Measurement of cell membrane properties**

The fast transient sodium current and persistent inward current (PIC) were generally recorded with potassium channel blockers (tetraethylammonium [TEA] and 4-aminopyridine [4-AP]) applied to the recording solution in this study. In some cells, however, these blockers were not used to study the membrane properties of the cells in both voltage- and current-clamp recordings. In this case the transient sodium current would not be isolated. Instead, it became a dominant component of the whole transient inward current \(I_T\). Therefore we use \(I_T\) to represent all the transient inward currents recorded in the present study, which could be either purely mediated or dominated by the fast transient sodium current. The \(I_T\) and PIC were recorded in voltage-clamp protocol shown in Fig. 1. \(I_T\) was recorded through a succession of 2-s voltage steps, with a step of either 2 or 5 mV, and a holding potential of −70 mV (Fig. 1A1). The maximal \(I_T\) elicited by the voltage steps was used to calculate a set of \(I_T\) parameters including the amplitude, half-width, maximal rise rate, and maximal decay rate. The step voltage at which the first \(I_T\) was elicited was defined as the voltage threshold \(V_{\text{th}}\). In some cells the voltage dependence of \(I_T\) was constructed by fitting the Boltzmann equation

\[
I_T = \frac{\text{amplitude}}{1 + \exp(\frac{V_{1/2} - V}{\sigma})}
\]

where the \(V_{1/2}\) and \(\sigma\) were half-maximal activation (or inactivation) of \(I_T\) and \(V_{\text{th}}\) is the slope of activation (or inactivation). To obtain the best-fitting values for the Boltzmann equation, interpolating points were used in some cells. The \(I_T\) was recorded in the aCSF with normal sodium concentration (152 mM) in most of the experiments. In some cells, however, a lower sodium (50 mM) aCSF was used to measure \(I_T\).}

In normal sodium concentration solution the \(I_T\) is usually seen as all-or-none currents in most of the cells due to the limited space clamp of neurons recorded in fresh slice. Poor space clamp could cause a distortion of \(I_T\), which included the repetitive spikes (unclamped spikes) in one voltage step, delayed inward currents (longer time to reach peak), and bumps and notches in the inward currents. Recordings with any of these phenomena were excluded for calculation of \(I_T\) parameters. Poor space clamp could also cause unchanged amplitude of \(I_T\) in subsequent one or more depolarizing steps. Data characterized by this phenomenon were excluded for fitting the Boltzmann equation.

In general, the PIC was mediated by sodium and calcium currents. In this study it was recorded by applying a slow voltage bi-ramp of 10 s, with peak voltage of 20–50 mV and holding potential of −70 mV (Fig. 1B). The leak current was subtracted from the recordings before calculating the parameters of onset voltage and amplitude for PIC. The PIC evoked in the ascending phase of the ramp voltage was measured using an infrared cube and recorded in whole cell patch clamp using glass pipette electrodes. The pipette electrodes were pulled from borosilicate glass (MTW 150F-4, WPI) using a P-87 puller (Sutter Instruments) and had resistances of 5–8 MΩ when filled with intracellular solution. A MultiClamp 700A patch-clamp amplifier, Digidata 1322 A/D converter, Minidigi 1A, and pClamp 9.0 software (all from Axon Instruments) were used for data acquisition. The whole cell patch recordings were made in both voltage- and current-clamp mode. Bridge balance and capacitance compensation were made before the recordings. Series resistance was monitored (usually <30 MΩ) and compensated. Data were low-pass filtered at 3 kHz and sampled at 10 kHz. The data were analyzed using self-written codes with Igor Pro (4.0) and Axon Clampfit (9.0). Student’s t-test and \(\chi^2\) test were performed with statistical significance defined as \(P < 0.05\). Results are shown as means ± SD. Activation and inactivation curves are presented as fitted means ± SE.
used to calculate the parameters, details of which are illustrated in Fig. 1B. After leak subtraction, a straight line was drawn along the rising phase of the current trace (dashed line in Fig. 1B); the last point where the straight line was tangent to the current trace was defined as the onset of PIC ($I_o$) and the corresponding voltage on the ramp was defined as the onset voltage of PIC ($V_o$). The lowest point on the current trough was defined as the peak of PIC ($I_p$) and the corresponding voltage as peak voltage ($V_p$) of PIC. The amplitude of PIC was calculated as the difference between $I_o$ and $I_p$ (i.e., $PIC = I_p - I_o$). In some cells a manual adjustment was required to determine the points for $I_o$ and $I_p$ to avoid the errors generated from the noise, excitatory or inhibitory postsynaptic current (EPSC or IPSC), respectively.

Current-clamp recordings were also made to study the properties of the action potential (AP) in some cells. A 3-s step current with a step of 10–20 pA and a 10-s biramp current with peak of 300–500 pA were used to evoke repetitive firing. The rheobase, amplitude, half-width, maximal rise rate, and maximal decay rate of action potentials were used to evoke repetitive firing. The rheobase, amplitude, half-width, maximal rise rate, and maximal decay rate of $I_T$ (inset). The step voltage at which the first $I_T$ is elicited is defined as the voltage threshold ($V_{th}$) for eliciting the $I_T$. The voltage dependence of $I_T$ is constructed by fitting the Boltzmann equation with the data recorded with protocols shown in A1 (for activation) and A2 (for inactivation). $V_{th}$ is recorded by a slow-voltage bi-ramp of 10 s with peak voltages of 20–50 mV and holding potential of −70 mV. The leak current is subtracted from the recordings before calculating the parameters of onset voltage and amplitude for PIC.

C relation between the voltage thresholds measured in voltage clamp (VC) ($V_{th,VC}$) and current clamp (IC) ($V_{th,IC}$). $V_{th}$ values were measured from 11 cells in both voltage- and current-clamp protocols. The $V_{th,VC}$ is the voltage at which the first $I_T$ was elicited in the step voltages and the $V_{th,IC}$ is the voltage at which the dV/dt ≥ 10 mV/ms in the rising phase of the action potential in the same cell. The $V_{th,VC}$ was calculated as −36.8 ± 7 mV on average and the $V_{th,IC}$ was −33.7 ± 6 mV. The mean difference between the $V_{th,VC}$ and $V_{th,IC}$ was −3.1 ± 3 mV. The solid line was the linear regression of the data and the dashed line is the unity line.

**Difference in $V_{th}$ measured in voltage- and current-clamp recordings**

The term “$V_{th}$” is used in the present study as voltage threshold either for eliciting the fast transient inward current ($I_T$) in voltage-clamp recording or for generating an action potential in current-clamp recording. Although the $I_T$ recorded in voltage clamp should be dominated by the same current underlying the APs recorded in current clamp, the $V_{th}$ measured in voltage clamp is not the same as the $V_{th}$ measured in current clamp. However, the validity of using both voltage- and current-clamp methods to assess $V_{th}$ in this study was based on our experimental observations. Figure 1C shows $V_{th}$ measured from 11 cells recorded in both voltage- and current-clamp protocols. Except for one cell, the $V_{th}$ determined by voltage clamp is equal to or less than the $V_{th}$ determined by current clamp. The difference between the mean $V_{th}$ for the voltage-clamp protocol (−36.8 ± 7 mV) and $V_{th}$ for the current-clamp protocol (−33.7 ± 6 mV) is only 3 ± 3 mV. The $V_{th}$ determined by voltage-clamp protocol is also shown to be well correlated with the $V_{th}$ determined by current-clamp protocol, suggesting that any change in either $V_{th}$ would correspond to a change in the same direction for $V_{th}$ measured with the other protocol, although the amount of change might be different (see Figs. 6 and 7 as examples). Based on these results we concluded that the $V_{th}$ values obtained from either protocol describe the common property of the cells in initiating spikes. Thus for simplicity we use the $V_{th}$ for values obtained from either protocol in the present study.
**Solutions and chemicals**

**EXTRACELLULAR SOLUTIONS.** The dissecting aCSF (for slice preparation only) contained (in mM): NaCl (25), sucrose (188), KCl (1.9), NaH₂PO₄ (1.2), MgSO₄ (10), NaHCO₃ (26), kynurenic acid (1.5), glucose (25), and CaCl₂ (1.0). The recording aCSF contained (in mM): NaCl (125), KCl (2.5), NaHCO₃ (26), NaH₂PO₄ (1.25), d-glucose (25), MgCl₂ (1), and CaCl₂ (2.5). The pH of these solutions was adjusted to 7.3 with KOH. Osmolarity was adjusted to 305 mOsm/L.

**INTRACELLULAR SOLUTIONS.** Solutions contained (in mM): CsCl (135), TEA-Cl (20), MgCl₂ (5), BAPTA (2), HEPES (10), Na₂ATP (5), and Na₃GTP (0.5). In current-clamp recordings the intracellular solutions contained (in mM): K-gluconate (120), NaCl (5), HEPES (10), EGTA (5), MgCl₂ (2), CaCl₂ (1), Mg-ATP (5), and GTP (0.5) (pH was adjusted to 7.3 with CsOH; osmolality was adjusted to 305 mOsm/L).

**BLOCKERS.** TEA (10 mM), 4-AP (4 mM), APV (20 µM), CNQX (10 µM), bicuculline (10 µM), and strychnine (10 µM) were used in most experiments. In some experiments tetrotodotoxin (TTX, 2 µM) or nifedipine (20–30 µM) was used to block the calcium currents. The low sodium concentration aCSF (145 mM) was replaced by BaCl₂; CdCl₂ (0.4), BaCl₂ (2.5), and glucose (20) (pH = 7.3 with CsOH; osmolality 300–305 mOsm/L).

**RESULTS**

All the data presented in this study were collected from neonatal rat spinal ventral neurons. Our experiments started with a hypothesis that the $V_{th}$ can be modulated by activation of the PKC pathway. Two currents—$I_T$ and PIC—are targeted in this study. These two currents have been shown to be modulated by activation of PKC and to contribute to the hyperpolarization of $V_{th}$ in previous studies (Astman et al. 1998; Franceschetti et al. 2000). In this study we first characterized the basic properties of these two currents and then investigated the modulation of these properties by PKC activation. Finally, we studied the interactions between the PKC and 5-HT pathways in the regulation of $V_{th}$.

**Characterization of basic properties of $I_T$ and PIC**

The $I_T$ and PIC are characterized with the recordings from voltage-clamp protocols (see Methods for details). Results from 95 cells show that the $V_{th}$ for eliciting $I_T$ is at $-42.7$ mV, with $I_T$ amplitude of 422 pA, half-width of 10 ms, maximal rise rate of 533 pA/ms, and maximal decay rate of 284 pA/ms. Results from the same group of the cells indicate that the onset voltage for activation of PIC is $-53.4$ mV with PIC amplitude of 64 pA (see “Whole sample” in Table 1 for details). The cells recorded in this study were widely distributed from lamina VII to X. To explore differences in the properties of $I_T$ and PIC for neurons in different lamina, we classified these neurons into laminal groups ranging from VII to X. The results are shown in Table 1. These results indicate that cells in lamina IX, presumably including motoneurons, usually have a lower $V_{th}$ for $I_T$, larger $I_T$ amplitude, faster rise and decay rates of $I_T$, higher onset voltage of PIC, and larger PIC amplitude, compared with the parameters of cells in other laminae. However, the statistically significant differences are observed only for the amplitudes of $I_T$ and PIC among the neurons from lamina VII to X. This might be due to fact that motoneurons are generally larger in size than interneurons and could possess a larger total conductance of $I_T$ and PIC than the interneurons in lamina VII, VIII, and X.

**OAG induced reduction of $I_T$ and PIC and could depolarize $V_{th}$**

Activation of PKC induced varied effects on $V_{th}$, although the OAG-induced reduction of $I_T$ was observed in all cells

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**Table 1. Properties of $I_T$ and PIC classified in lamina distribution**

<table>
<thead>
<tr>
<th>Lamina</th>
<th>$V_{th}$, mV</th>
<th>Amplitude, pA</th>
<th>Rise Rate, pA/ms</th>
<th>Decay Rate, pA/ms</th>
<th>Onset, mV</th>
<th>PIC, pA</th>
</tr>
</thead>
<tbody>
<tr>
<td>VII</td>
<td>−42.8</td>
<td>313*</td>
<td>9</td>
<td>487</td>
<td>−53.0</td>
<td>48.3†</td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>8 (238)</td>
<td>11</td>
<td>372</td>
<td>401</td>
<td>11</td>
<td>41</td>
</tr>
<tr>
<td>VIII</td>
<td>−39.1</td>
<td>482*</td>
<td>6</td>
<td>451</td>
<td>−57.3</td>
<td>43†</td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>6 (360)</td>
<td>8</td>
<td>368</td>
<td>168</td>
<td>8</td>
<td>42</td>
</tr>
<tr>
<td>IX</td>
<td>−46.3</td>
<td>653*</td>
<td>8</td>
<td>629</td>
<td>−51.6</td>
<td>119†</td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>11 (375)</td>
<td>6</td>
<td>704</td>
<td>473</td>
<td>17</td>
<td>110</td>
</tr>
<tr>
<td>X</td>
<td>−40.2</td>
<td>482*</td>
<td>7</td>
<td>634</td>
<td>−53.7</td>
<td>53†</td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>8 (308)</td>
<td>7</td>
<td>665</td>
<td>171</td>
<td>10</td>
<td>44</td>
</tr>
<tr>
<td>Whole</td>
<td>−42.7</td>
<td>422</td>
<td>10</td>
<td>533</td>
<td>−53.4</td>
<td>64</td>
</tr>
<tr>
<td>Sample (SD)</td>
<td>8 (317)</td>
<td>12</td>
<td>501</td>
<td>378</td>
<td>12</td>
<td>69</td>
</tr>
</tbody>
</table>

*Significant difference with $P < 0.05$; †$P < 0.001$ in the selected properties from lamina VII to lamina X (ANOVA, single factor).
(35/35) and the reduction of PIC amplitude was seen in 94% of the cells (33/35). Figure 2 is an example in which OAG induced a reduction of $I_T$ and PIC and a depolarization of $V_{th}$. The $I_T$ of $-450.3$-pA amplitude was elicited by the voltage step to $-35$ mV in control (Fig. 2A, left). Bath application of $15 \mu M$ OAG produced a reduction of $I_T$ by 18% with 5-mV depolarization of $V_{th}$ (Fig. 2A, right). The half-width of $I_T$ was increased by 15% and the maximal rise and maximal decay rates of $I_T$ were reduced by 19 and 28%, respectively (Fig. 2A, inset, dashed line for control). The current–voltage ($I$–$V$) curve (Fig. 2B, left) constructed by the peak of $I_T$ indicated that OAG reduced the $I_T$ and depolarized the activation of $I_T$. This resulted in a 4.7-mV positive shift in half-maximal activation of $I_T$, with an almost unchanged slope for the activation in Boltzmann equation (Fig. 2B, right panel and table). The OAG-induced reduction of PIC was also observed in the same cell. A 10-s biramp voltage was applied to the cell (Fig. 2C) and the PIC of 89.9 pA was evoked at an onset voltage of $-51$ mV (Fig. 2C, gray trace). A small reduction of PIC was observed in 5 min after bath perfusion of $15 \mu M$ OAG. By 15 min later, OAG reduced the PIC by 35% with a 3.2-mV depolarization of the onset voltage (Fig. 2C, black traces). Of 35 cells recorded with OAG, 7 of the cells showed a depolarization of $V_{th}$ with reduction of $I_T$ in all these cells and reduction of PIC in 5 of the 7. One cell showed a depolarization of $V_{th}$ with an increase in PIC. Although PKC activation reduced $I_T$ and PIC with depolarization of $V_{th}$, the alteration in $V_{th}$ did not necessarily accompany the OAG-induced reduction of $I_T$ and PIC (see the following text).

**OAG induced reduction of $I_T$ and PIC and could leave $V_{th}$ unchanged**

In more than half of the cells recorded with OAG, activation of PKC altered the properties of $I_T$ and PIC but did not change the $V_{th}$ for $I_T$. An example of OAG-induced reduction of $I_T$ and

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**FIG. 2.** OAG (1-oleoyl-2-acetyl-sn-glycerol)-induced reduction of $I_T$ and PIC with a depolarization of $V_{th}$: A: the $I_T$ is elicited at $-35$ mV by the voltage steps in control (single asterisk). Bath application of $15 \mu M$ OAG reduced the $I_T$ by 18% with a 5-mV depolarization of $V_{th}$ (double asterisks). The half-width of $I_T$ was increased by 15% and the maximal rise and maximal decay rates of $I_T$ were reduced by 19 and 28%, respectively. The changes in shape and size of $I_T$ are shown in the inset (dashed line for control). B: the reduction of $I_T$ and depolarized activation of $I_T$ are reflected in the current–voltage ($I$–$V$) curve (left) constructed by the peak of $I_T$. This results in a 4.7-mV positive shift in half-maximal activation of $I_T$, with almost unchanged slope for the activation in the Boltzmann equation (right and table). The dashed line with open circles is for control and the solid line with closed circles for OAG. The activation curves (right) were plotted with means ± SE. C: the OAG-induced reduction of PIC is shown in the same cell. A 10-s biramp voltage is applied to the cell (bottom trace), and the PICs of 89.9 pA are evoked with onset voltage of $-51$ mV (gray trace). The reduction of PIC was observed in 5 min after bath perfusion of 15 $\mu M$ OAG. At 15 min later, the PIC was reduced by 35% with 3.2-mV depolarization of the onset voltage (black traces).
PIC with an unchanged $V_{th}$ is shown in Fig. 3. The recordings were made in low sodium concentration (50 μM) aCSF in this cell. $I_T$ was measured as $-607.6$ pA with a $V_{th}$ of $-40$ mV in control. OAG (10 μM) did not alter the $V_{th}$ in this cell, but reduced the peak amplitude of $I_T$ by 60% to $-240.8$ pA, with a 46% increase in half-width and 60 and 77% decreases in maximal rise and decay rates, respectively. The reduction of peak $I_T$ in the whole range of step voltages is shown in the $I-V$ curves in Fig. 3A (left). The changes in size of the $I_T$ at $V_{th} = -40$ mV is shown in the inset in Fig. 3A (dashed line for control; solid line for OAG). The voltage dependencies of $I_T$ described by using the Boltzmann equation (Fig. 3A, right) show that OAG induced a 3.6-mV right shift in the half-maximal activation of $I_T$ and a 2.3-mV left shift in half-maximal inactivation of $I_T$, with small changes in the slopes (see table in Fig. 3A). PIC was reduced by OAG in the same cell. As shown in Fig. 3B, the PIC was measured as 146.9 pA with an onset voltage of $-58.8$ mV (Fig. 3B, gray trace). A small reduction of PIC was observed in only 3 min after OAG application. Eight minutes later, the PIC was reduced by about half. By 15 min after OAG administration, OAG reduced PIC amplitude by 83% to 25.3 pA and depolarized the PIC onset voltage by 7.3 mV (Fig. 3B, black traces). In a total of 35 cells recorded with OAG (2 of these cells were recorded in low sodium concentration aCSF), 20 of the cells showed no substantial changes in $V_{th}$, whereas the reduction of $I_T$ was observed in all of the cells and a reduction of PIC in 19 of the 20 cells. One of the cells showed an increase in PIC.

**Varied effects of PKC activation on $V_{th}$**

It has been shown in previous studies that activation of PKC could induce variable changes in voltage dependence of fast inward sodium currents. Although no substantial change in voltage dependence of the sodium currents could be observed in many studies (Cantrell et al. 1996; Numann et al. 1991; Sigel and Baur 1988; West et al. 1991), both the depolarization of activation (Dascal and Lotan 1991) and hyperpolarization of activation and inactivation (Franceschetti et al. 2000) of the sodium currents have been reported. In the present study we show that activation of PKC could induce either a depolarization of $V_{th}$ (Fig. 2) or leave $V_{th}$ unchanged (Fig. 3). Activation of PKC could also result in a complete blockade of transient inward currents or hyperpolarization of $V_{th}$. Results from 35 cells recorded with OAG showed (Fig. 4A, white bars) that more than half of the cells (57%, 20/35) showed no change in $V_{th}$ in the presence of OAG, whereas 20% of the cells (7/35) showed a depolarization of $V_{th}$ and 9% (3/35) demonstrated a hyperpolarization of $V_{th}$. For another 14% (5/35) of the cells OAG blocked the $I_T$.

Despite the variable effects on $V_{th}$, the OAG-induced changes in other properties of $I_T$ and PIC are very consistent. Activation of PKC produced a reduction of $I_T$, an increase in $V_{th}$, and a reduction of PIC with an unchanged $V_{th}$ for $I_T$.
The significant changes (denoted by the asterisks) in parameters for PIC include the onset voltage and double asterisks represent significant changes with PKCI. The single asterisks represent the significant changes with PKCI.

The results from 15 cells recorded with PKCI are summarized in Fig. 4 (black bars). As shown in Fig. 4A, the PKCI blocked the OAG effect on $V_{th}$ by significantly reducing the number of cells that display a blockade of $I_T$ (6%, 1/15) or a depolarization of $V_{th}$ (6%, 1/15). This resulted in a significant increase in the number of cells that showed an unchanged $V_{th}$ (73%, 11/15). A small increase in the number of cells that displayed hyperpolarization of $V_{th}$ (13%, 2/15) was also observed. The most striking effect of PKCI is its ability to block OAG-induced changes in $I_T$ and PIC as shown in Fig. 4B (black bars). With PKCI (15–30 μM) included in the recording electrodes, the OAG-induced changes in $I_T$ ($V_{th}$ amplitude, half-width, maximal rise, and decay rates) and PIC (onset voltage, amplitude, and conductance) are greatly reduced.

Differences from control in any of these parameters are <8% and in the presence of PKCI, OAG failed to produce any statistically significant change. These results clearly show that the PKC-mediated changes in $I_T$ and PIC can be efficiently blocked by the PKC inhibitor. The details of the PKCI results are shown in Table 3.

Effect of PKC activation on action potential properties

To test the effect of PKC activation on $V_{th}$ for generation of action potentials, we did some experiments with current-clamp recordings (see METHODS for details). In five cells, we made both voltage- and current-clamp recordings. Figure 6 shows an example of one of the cells recorded in both protocols. A 3-s
current pulse (Fig. 6A1) and 10-s current ramp (Fig. 6A2) were delivered to the cell to evoke repetitive firing in both control and OAG conditions. Traces recorded from both conditions were overlapped (dashed for control and solid for OAG) and $V_{th}$ was measured from each spike with circles marked on the traces (open for control and closed for OAG). OAG (20 μM) did not change the rheobase in this cell but depolarized the $V_{th}$ of the first spike by 1.8 mV (3.1 mV on average for all spikes) with pulse current injection and by 3.9 mV (2.0 mV on average) with ramp current injection. The firing frequency was reduced by 1 Hz from 18 to 17 Hz in the pulse current injection. There was no change in rheobase (40 pA) but a small increase in AP width by 0.5 ms from 4.7 ms. Small reductions were observed in AP amplitude by −3.4 mV from 37.6 mV, maximal rise rate by −1 mV/ms from 15 mV/ms, and maximal decay rate by −0.2 mV/ms from 7 mV/ms. (Note: the AP amplitude was measured from $V_{th}$.) Voltage-clamp recordings made in the same cell showed that OAG depolarized the $V_{th}$ for $I_T$ by 5 mV with a 17% (~120 pA) reduction of the amplitude of $I_T$ (Fig. 6B). This cell demonstrated that activation of PKC could decrease the neuronal excitability by depolarization of $V_{th}$ for action potentials and a reduction of repetitive firing frequency.

In 12 cells recorded with the current-clamp protocol, OAG depolarized the $V_{th}$ for action potentials by 2.4 ± 0.8 mV in 8 of the cells and hyperpolarized the $V_{th}$ by 1.8 ± 0.4 mV in 3 of them. One of the cells showed no change in $V_{th}$. Of the 12 cells, OAG produced either an increase ($n = 4$) or no change ($n = 8$) in rheobase. Results from the 12 cells are summarized in Fig. 7A. The averaged values measured in control include the rheobase (64 ± 43 pA), $V_{th}$ (−39 ± 6 mV), AP amplitude (43 ± 13 mV measured from $V_{th}$), width (6 ± 2 ms), maximal rise rate (18 ± 7 mV/ms), and maximal decay rate (9 ± 4 mV/ms). Results in Fig. 7A indicate that activation of PKC could produce a small but significant depolarization of $V_{th}$ (2 ± 1 mV, $P < 0.05$) with a significant increase in AP width (0.6 ± 0.2 ms, $P < 0.05$) and decrease in AP amplitude (8 ± 3 mV, $P < 0.05$), maximal rise rate (5 ± 2 mV/ms, $P < 0.05$), and maximal decay rate (2 ± 1 mV/ms, $P < 0.05$). The rheobase was increased by 14 ± 17 pA but not significantly ($P > 0.08$). Five of the 12 cells were recorded in both current and voltage protocols. The averaged activation of $I_T$ was fitted by Boltzmann equation and is shown in Fig. 7B. In current-clamp recording OAG depolarized the $V_{th}$ for action potential by 1.8 ± 0.5 mV in these cells, whereas in voltage-clamp recording OAG induced a positive shift in the half-maximal activation of $I_T$ by 2.3 ± 1.7 mV, with little change in activation slope.

**A different pathway is used by PKC and 5-HT for modulation of $I_T$**

Activation of PKC with OAG can induce a depolarization of $V_{th}$, which is opposite to the hyperpolarization of $V_{th}$ induced by bath application of 5-HT or noradrenaline (Fedirchuk and Dai 2004b) or activation of descending serotonergic fibers (Gilmore and Fedirchuk 2004). In prefrontal cortex neurons the PKC pathway mediates 5-HT–induced inhibition of sodium currents (Carr et al. 2002). In spinal ventral neurons, however, it is unclear whether the PKC and serotonergic pathways could play a common role in modulation of $V_{th}$. In this study we tested the possible interaction between these two systems. We assessed the ability of 5-HT to induce $V_{th}$ hyperpolarization with or without PKCI when OAG was applied. Figure 8 shows recordings from two cells recorded without PKCI. In control, the $I_T$ of −1.324 pA was elicited at $V_{th}$ of −35 mV (single asterisks, black traces in Fig. 8A1). Bath application of 20 μM 5-HT hyperpolarized $V_{th}$ by 5 mV to −40 mV (double asterisks, red traces in Fig. 8A1). Bath application of 20 μM OAG with 5-HT completely blocked the $I_T$ (Fig. 8A1, blue traces). Note that $I_T$ could not be elicited even when the voltage step was raised to −25 mV, 10 mV higher than that in control. However, the $I_T$ could partially recover after a 25-min washout with fresh aCSF (Fig. 8A1, green traces). This cell showed a 5-HT–induced hyperpolarization of $V_{th}$ “antagonized” by OAG (see inset in Fig. 8A1 for the changes in shape and size of $I_T$).
PKCI blocked the OAG effects on $I_T$ and PIC

**A**

![Graph showing the effect of PKCI on $I_T$ and PIC.](image)

**B**

![Graph showing the effect of PKCI on $I_T$ and PIC.](image)

FIG. 5. Blockade of OAG effect on $I_T$ and PIC by PKCI. A: the $I_T$ is recorded with 20 µM PKCI in the recording electrode. The amplitude of $I_T$ is measured as $-467$ pA with $V_{th}$ of $-35$ mV in control. By 10 min after bath application of 20 µM OAG the $I_T$ is measured as $-487$ pA and the $V_{th}$ is unchanged. Changes in shape and size of $I_T$ are shown in the inset. The PKCI blocks the OAG-induced reduction of $I_T$ and alteration of $V_{th}$ in this cell. B: the PKCI also blocks the OAG-induced changes in PIC in the same cell. The PIC has amplitude of $-181$ pA and onset voltage of $-60.3$ mV in control (gray trace). By 7 min after application of OAG (black trace), the PIC was measured as $-180$ pA with almost unchanged onset voltage (see inset for details).

The OAG- and 5-HT–induced changes in PIC were also recorded in the same cell. As shown in Fig. 8A2, the PIC of $-60$ pA was evoked by a voltage bi-ramp at an onset voltage of $-69.4$ mV (Fig. 8A2, black trace). 5-HT induced some EPSC in this cell but did not change the properties (amplitude and onset voltage) of the PIC (Fig. 8A2, red trace). However, OAG completely removed the PIC (Fig. 8A2, blue trace), which was then partially recovered from washout (Fig. 8A2, green traces). Note that the reduction of peak of the passive ramp current with OAG could be caused by an inhibition of potassium conductance by PKC activation (Murbartian et al. 2005), which could be partially washed out with fresh aCSF.

To further test the interaction between the pathways of 5-HT and PKC, we switched the sequence of application of 5-HT and OAG in Fig. 8A. An example is shown in Fig. 8B. In this cell the $I_T$ was reduced (not blocked) by OAG. The $I_T$ was measured as $-780$ pA with $V_{th}$ of $-45$ mV in control (single asterisks, black traces in Fig. 8B). Bath application of 20 µM OAG did not alter the $V_{th}$ but reduced the $I_T$ by $-270$ pA to $-510$ pA (single asterisks, blue traces in Fig. 8B). Following bath application of 15 µM 5-HT with OAG, the $V_{th}$ was hyperpolarized by 5 mV to $-50$ mV and the $I_T$ was further reduced by $-115$ pA to $-395$ pA (double asterisks, red traces). Both OAG and 5-HT induced a reduction of $I_T$ in this cell (Fig. 8B, inset). This cell demonstrated that OAG did not block the ability of 5-HT to induce a hyperpolarization of $V_{th}$.

In six cells recorded with 5-HT (15–20 µM) and OAG (10–20 µM), 5-HT hyperpolarized $V_{th}$ for $I_T$ in all of the cells by $-7$ ± 5 mV. Bath application of OAG induced a 5 ± 2-mV depolarization of $V_{th}$ in two of the cells and a 5-mV hyperpolarization of $V_{th}$ in one cell. One cell displayed a blockade of $I_T$ and two cells showed no change in $V_{th}$. The above-cited results indicate that activation of serotonergic receptors in spinal ventral neurons does not stop the PKC effects on $I_T$, especially on $V_{th}$. To further test whether the modulation of the $V_{th}$ by these two agents is mediated by different pathways, we did additional experiments with PKCI (20–30 µM) in the recording pipette. Figure 9 shows an example of the results. The recordings were made with 20 µM PKCI in the micro-electrode. The $I_T$ of $-1,140$ pA was elicited at $V_{th}$ of $-20$ mV in control (marked by single asterisks). Bath application of 20 µM OAG did not change the $V_{th}$ or the shape and size of $I_T$ (Fig. 9, inset in OAG condition, dashed trace for control). However, bath application of 15 µM 5-HT with OAG hyperpolarized the $V_{th}$ by 5 mV to $-25$ mV (double asterisks) with a small (~17%) reduction in $I_T$ amplitude (see inset in 5-HT condition, dashed trace for control). PKCI blocked the effect of OAG but not that of 5-HT on $I_T$ and $V_{th}$.

In a total of five cells recorded with PKCI, bath application of OAG did not alter the $V_{th}$ or make any significant change in the amplitude of $I_T$ in all of the cells. However, application of 5-HT hyperpolarized $V_{th}$ by $-6$ ± 5 mV in four of the cells.

**TABLE 3.** Properties of $I_T$ and PIC and changes induced by OAG with PKCI

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<tr>
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<td>$-5.0$</td>
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All values in this table were obtained with PKCI in the recording pipette.

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Activation of PKC modulated both calcium and sodium components of PIC

The persistent inward current is composed of two currents: 1) the L-type calcium current (Ca–PIC), which is dihydropyridine sensitive, and 2) the persistent sodium current (Na–PIC), which is TTX sensitive. Since no blockers of these components were used in the preceding experiments, the PIC present in the above-cited study is presumably mediated by both calcium and sodium currents. To investigate the effect of PKC activation on either component of PIC, we did additional experiments with either TTX (2 μM) or nifedipine (20–30 μM) administrated in the recording solution. Examples are shown in Fig. 10. Our results showed that activation of PKC significantly reduced both calcium and sodium components of PIC. Of four cells recorded with TTX, bath application of OAG (20–30 μM) reduced Ca–PIC by about 25% with 1.3-mV depolarization of PIC onset (see Fig. 10A), whereas the similar amount of OAG reduced Na–PIC (n = 4) by about 70% with 8.7-mV depolarization of PIC onset (Fig. 10B). Although the Ca– and Na–PIC onset was not significantly depolarized by OAG due to the small number of samples, the significant reduction of the PIC amplitude clearly indicated that Ca–PIC and Na–PIC were modulated by activation of PKC pathway. Furthermore, the effect of PKC activation might be stronger on Na–PIC than that on Ca–PIC in terms of reduction of PIC and depolarization of PIC onset. See Table 4 for the details.

Correlation analysis

To study the mechanisms underlying the changes in $I_T$ and PIC by PKC activation we did correlation analysis among some parameters measured from $I_T$ and PIC. It is known that the ascending phase of $I_T$ is dominated by activation of sodium currents, which could be described by the amplitude and rise rate of $I_T$, whereas the descending phase of $I_T$ is determined by inactivation of $I_T$ and activation of some potassium conductances such as the delayed rectifier, which could be described by decay rate and width of the $I_T$. To explore some putative mechanisms underlying the OAG-induced changes in $I_T$ and PIC, we analyzed the correlation between the maximal rise and decay rates (Fig. 11A) and the correlations between the amplitude of $I_T$ and the maximal rise (Fig. 11B) and decay rates (Fig. 11C). For example, alteration of both the activation and inactivation properties of sodium channels might be expected to alter both the rise rate and decay rate of $I_T$. We also examined the correlations between the OAG effect on PIC and the effect on the amplitude of $I_T$ (Fig. 11D). Results from 15 cells are shown in Fig. 11, where the plots in the left column show the relations between two selected parameters measured from the recordings in control and OAG conditions (dashed line with open circles is for control and the solid line with closed circles is for OAG) and the plots in the right column show the relations of the changes induced by OAG between the two parameters. These results are discussed in the following section.

Mechanisms mediating the OAG-induced changes in $I_T$

Phosphorylation of sodium channels through the PKC pathway was previously reported to reduce the peak of the transient

One cell did not respond to 5-HT. These results indicate that the ability of 5-HT to induce a $V_{th}$ hyperpolarization was not blocked by PKCI and suggest that the modulation of the $V_{th}$ by 5-HT and PKC is mediated by different pathways.

FIG. 6. OAG-induced depolarization of $V_{th}$ in current-and voltage-clamp protocols. A: current-clamp recordings. A 3-s current pulse (A1) and 10-s current ramp (A2) are delivered to the cell to evoke repetitive firing in both control and OAG conditions. The traces from both conditions are overlapped (dashed for control; solid for OAG) and $V_{th}$ is measured from each spike with circles marked on the traces (open for control; closed for OAG). OAG (20 μM) did not change the rheobase in this cell but depolarized the $V_{th}$ of the first spike by 1.8 mV (3.1 mV on average for all spikes) in pulse current injection (A1) and by 3.9 mV (2.0 mV on average) in ramp current injection (A2). B: voltage-clamp recordings. The recordings made from the same cell showed that OAG depolarizes the $V_{th}$ for eliciting the $I_T$ by 5 mV with 17% reduction of the peak amplitude of $I_T$. This cell did not show PIC.
OAG conditions. These correlations are maintained between control and voltage protocols. The averaged activation of values instead of a percentage. Bars represent SD. Note that the changes in each property are expressed as real action potential by 1.6 mV in these cells, whereas in voltage-clamp recording OAG induced a 2.3 ± 1.7-mV positive shift in the half-maximal activation of I_T with an almost unchanged slope of activation (see text for the details). The activation curves were plotted with means ± SE.

Mechanisms mediating the OAG-induced changes in PIC

In this study we show that activation of PKC generally results in a depolarization of PIC onset voltage and reduction of PIC amplitude (and conductance, as well) in neonatal rat spinal ventral neurons. The mechanisms underlying the OAG-induced decrease in PIC are unknown. However, from the correlation analysis we expect that the reduction of PIC could be related to the reduction of I_T. As shown in Fig. 11D1, there is no correlation between the amplitudes of PIC and I_T in both control and OAG conditions. However, the reduction of PIC amplitude is correlated to the reduction of I_T amplitude after OAG administration (Fig. 11D2), indicating that the OAG-induced reduction of PICs might result in part from the reduction of I_T. Supporting evidence can be seen in Figs. 3 and 8. In Fig. 3, the OAG-induced shifts in voltage dependence of I_T resulted in a reduction of window current (Fig. 3A, right), which could partially account for the reduction of the PIC (Fig. 3B). In Fig. 8A, the PIC was completely removed with the blockade of I_T by OAG (Fig. 8A, blue traces), which partially recovered with a partial recovery of I_T after washout (Fig. 8A, green traces). Interestingly, the 5-HT–induced reduction of I_T did not change the PIC in this cell (Fig. 8A, red traces). The hyperpolarization of V_th by 5-HT, which could enhance the window current, might play a role in maintaining the PIC in this cell. Ptak et al. (2005) showed that in the pre-Bötzing complex region the transient sodium current can be reduced by a low concentration (3 μM) of riluzole via a higher affinity for the inactivation state. This could lead to a reduction of Na–PIC. In hippocampal neurons PKC-induced phosphorylation of transient sodium current via muscarinic acetylcholine receptors
results in a reduction of the sodium current as well as Na–PIC (Cantrell et al. 1996). Based on these findings we expect that the PKC-dependent reduction of PIC observed in neonatal rat spinal ventral neurons could partially result from the modulation of $I_T$ inactivation (Cantrell et al. 1996; Chen et al. 2006) and partially from the modulation of Ca–PIC.

**DISCUSSION**

The present study demonstrates that activation of protein kinase C reduces the amplitude of the fast transient inward current and persistent inward current in the neonatal rat spinal ventral neurons. Activation of PKC also depolarizes the onset voltage of PIC and produces varied effects on voltage threshold for inducing the fast transient inward currents or eliciting action potentials. $V_{th}$ can be modulated by 5-HT and OAG, but the modulation of $V_{th}$ by the serotonergic pathway is not dependent on the activation of PKC pathway, which in general decreases the excitability of the neonatal rat spinal ventral neurons.

**Alteration of neuronal excitability by activation of PKC**

Modulation of transient sodium current by activation of protein kinase alters neuronal excitability (for review see

**FIG. 8.** Modulation of $I_T$ and PIC by serotonin (5-HT) and PKC. A: the $I_T$ with an amplitude of −1,324 pA was elicited at $V_{th}$ of −35 mV in control (single asterisks and black trace, A1). Bath application of 20 µM 5-HT hyperpolarized $V_{th}$ by 5 mV to −40 mV (double asterisks and red trace, A1). The $I_T$ was completely blocked by bath application of 20 µM OAG with 5-HT (blue trace, A1). Note that no $I_T$ could be elicited even though the voltage step was raised to −25 mV, 10 mV higher than that in control. However, the $I_T$ partially recovered after a 25-min washout with normal ACSF (double asterisks and green traces, A1). The $I_T$ recorded in each condition is overlapped in the A1 inset to show the changes in the shape and size of $I_T$. The 5-HT and OAG induced changes in PIC in the same cell (A2). The PIC was measured to be −59.7 pA with an onset voltage of −69.4 mV (black trace, A2). 5-HT induced some excitatory postsynaptic current in the cell without altering the properties of the PIC (red trace, A2). However, OAG completely removed the PIC (blue trace, A2), which partially recovered after a 25-min washout (green traces, A2). B: these recordings are made in a different cell. The $I_T$ was measured as −780 pA with $V_{th}$ of −45 mV in control (single asterisks, black traces). Bath application of 20 µM OAG did not alter the $V_{th}$ but reduced the $I_T$ to −510 pA (single asterisks, blue traces). Following bath application of 15 µM 5-HT with OAG, the $V_{th}$ was hyperpolarized by 5 mV and the $I_T$ was further reduced to −395 pA (double asterisks, red traces). The changes in shape and size of $I_T$ are shown in the inset.
Catterall 2000). It has been shown in previous studies that the neuronal excitability could be reduced via the PKA pathway being activated by dopaminergic receptors (Calabresi et al. 1987; Cantrell et al. 1997, 1999). A similar conclusion about the effect of PKC activation on neuronal excitability might be drawn since the peak of transient sodium current is reduced by phosphorylation of sodium channels via PKC. However, previous studies have shown that the PKC activation increases the neuronal excitability by hyperpolarization of voltage threshold in mouse neocortical neurons (Astman et al. 1998) or by amplification of subthreshold depolarization in rat pyramidal neurons (Franceschetti et al. 2000). The increased excitability was attributed to the hyperpolarization of onset voltage of PIC. Contradictory observations have also been reported. For example, activation of PKC via muscarinic receptors suppresses the intrinsic bursting in hippocampal CA1 pyramidal cells (Alroy et al. 1999). This inhibitory effect is mediated by a down-regulation of the persistent Na\(^+\) current (Mittmann and Alzheimer 1998). Activation of PKC is also found to reduce dendritic excitability in prefrontal cortex pyramidal neurons (Carr et al. 2002). In the present study we show that the PKC activation reduces the excitability of neonatal rat spinal ventral neurons. This decreased neuronal excitability could result from a mixed effect of a depolarization of \(V_{th}\) for \(I_T\) (or AP) and onset voltage for PIC and a reduction of amplitude of \(I_T\) (or AP) and PIC. In some cells the \(V_{th}\) depolarization was also accompanied by an elevation of rheobase currents. On the other hand, PICs have been shown to play a role in initiation of spikes in repetitive firing not only in spinal interneurons (Theiss et al. 2007; Zhong et al. 2007) but also in cultured cells (Kuo et al. 2006). Therefore our findings suggest that neuronal excitability might be regulated by a balance of multiple effects of the PKC pathway and that the different effects of PKC activation could be manifest in different neuron populations.

The effect of PKC activation on the neuronal input–output relation was not tested in this study. Using a five-compartment model built with cat lumbar motoneuron properties (Dai et al. 2002) we showed that a 2- to 3-mV depolarization of \(I_{Na}\) activation and inactivation in the initial segment did not produce a substantial change in the primary range of the frequency–current (\(f-I\)) relation but could induce a fair amount of change in the secondary range of the \(f-I\) relation (shifting the \(f-I\) curve and altering the \(f-I\) slope; unpublished data). Therefore it is unlikely that OAG-induced changes in \(V_{th}\) alone could significantly alter the input–output relation of the cells in normal conditions (primary range). The effect of a small depolarization of \(V_{th}\) on motoneuron recruitment remains unknown. The recruitment of motoneurons was determined by several mechanisms including the current and voltage threshold of the motoneurons and strength of synaptic inputs to the motoneuron pools (Heckman and Binder 1990, 1993; Pinter 1990). In a previous study with a large-scale simulation we showed that alteration of \(V_{th}\) (7 mV) produced only a small change (<3 Hz) in firing frequency of the motoneurons, although it could alter the number (10–45%) of recruited motoneurons in the pools, depending on the cell type and excitatory synaptic strength (Dai et al. 1999). Therefore a 2-mV depolarization of \(V_{th}\) by activation of PKC might not be expected to alter the firing frequency of the neurons, but it might reduce the number of recruited neurons, depending on the functional role, synaptic inhibitions as well as other compensatory mechanisms (primary range).

![Figure 9](http://jn.physiology.org/lookup/doi/10.1152/jn.00647.2009)

**FIG. 9.** Modulation of \(I_T\) by 5-HT with inhibition of PKC. The \(I_T\) was measured as 1.140 pA at a \(V_{th}\) of 20 mV in control with 20 \(\mu\)M PKCI in the electrode (single asterisks). Bath application of 20 \(\mu\)M OAG did not make any substantial change in \(V_{th}\) and the shape and size of \(I_T\) (single asterisks). However, the \(V_{th}\) was hyperpolarized by 5 mV with about 17% reduction of \(I_T\) amplitude after bath application of 15 \(\mu\)M 5-HT with OAG (double asterisks). The changes in \(I_T\) size and shape are shown in the insets in each condition (dashed trace for control). Therefore PKCl blocked the effect of OAG but not that of 5-HT on \(I_T\) and \(V_{th}\).

![Figure 10](http://jn.physiology.org/lookup/doi/10.1152/jn.00647.2009)

**FIG. 10.** Activation of PKC reduced calcium- and sodium-mediated PIC. A: the recording was made with 2 \(\mu\)M tetrodotoxin (TTX) in the solution. The PIC was measured as 26.3 pA with onset of –26.2 mV. Bath application of 25 \(\mu\)M OAG reduced PIC by 13.4 pA (51%), with unchanged onset voltage in this cell. The PIC was partially recovered after a 50-min washout (gray trace). B: the recording was made with 30 \(\mu\)M nifedipine in the solution. The PIC was evoked at –51.7 mV with amplitude of 82.1 pA. More than 50% of the PIC was reduced 7 min after bath administration of 30 \(\mu\)M OAG. By 15 min after OAG, the PIC reduced by 57.8 pA (70%), with no substantial change in PIC onset voltage in this cell.
PKC activation modulated \( I_T \) and PIC conductances

Although PKC activation induced large reductions of \( I_T \) and PIC amplitudes, it did not induce a large depolarization of \( V_{th} \). This apparent paradox might be explained in several ways. First, any change in \( V_{th} \) is mainly dominated by a modulation of \( I_T \) activation property. Reduction of \( I_T \), however, could be induced by a reduction of \( I_T \) conductance (availability of the channels) and/or alteration of voltage dependence of \( I_T \) (a depolarization of activation and/or hyperpolarization of inactivation). Our data presented in this study suggest that modulation of \( I_T \) conductance instead of its activation property might be the major effect of PKC activation on neonatal rat spinal ventral neurons. Second, our data also show that a large reduction of PIC (~50%) by OAG is not accompanied by a large depolarization of PIC onset either (only 4.4 mV; see Table 2). This result suggests that OAG does not induce a substantial change in the subthreshold depolarizing current that might be mediated by PIC and play a role in regulating the \( V_{th} \) or PIC onset. Thus similar to the case of \( I_T \), modulation of PIC by PKC activation might mainly target the PIC conductance rather than its voltage dependence. Third, we have demon-

| TABLE 4. Ca– and Na–PIC modulated by OAG |
|-----------------|-----------------|
|                 | Onset, mV        | PIC, pA |
| Ca–PIC (n = 4)  |                 |        |
| Control         | \(-35.0 \pm 13\) | \(75.0 \pm 43\) |
| OAG             | \(-33.6 \pm 13\) | \(56.2 \pm 33\) |
| Changes         | \(1.3 \pm 1.4\)  | \(-18.9 \pm 12^*\) |
| Na–PIC (n = 4)  |                 |        |
| Control         | \(-55.3 \pm 3\)  | \(62.3 \pm 40\) |
| OAG             | \(-46.6 \pm 7\)  | \(19.1 \pm 10\) |
| Changes         | \(8.7 \pm 8.8\)  | \(-43.2 \pm 36^*\) |

*Significant changes with \( P < 0.05 \) (paired \( t \)-test).

connectivity, and membrane properties of the neurons. Future studies are required to test this prediction.

**FIG. 11.** Correlation analysis of the properties of \( I_T \) and PIC. \( A \): the relations between the maximal rise and decay rates of the \( I_T \) are plotted in both control (dashed line with open circles) and OAG (solid line with closed circles) conditions \( (A1) \). A linear regression is applied to the relations to show the strength of the correlations between the 2 selected parameters. The same relation is plotted between the amount of OAG-induced reduction of the maximal rise and decay rates with a linear regression applied to the relation \( (A2) \). The similar relations as shown in \( A1 \) and \( A2 \) are plotted between the following paired parameters: the maximal rise rate and the amplitude of \( I_T \) \( (B) \); the maximal decay rate and the amplitude of \( I_T \) \( (C) \); the amplitude of PIC and the amplitude of \( I_T \) \( (D) \).
strated that PIC was mediated by sodium and calcium currents (Fig. 10). Compared with \( I_T \) the mixed currents of PIC have a larger time constant (over tens of milliseconds) for activation and might not make a significant contribution to the rising phase of the \( I_T \), which is activated within 1–2 ms and therefore determined the \( V_{th} \).

**Variable effects of PKC activation on \( V_{th} \)**

As shown in Fig. 4, activation of PKC could induce various changes in \( V_{th} \), whereas the reduction of the \( I_T \) was consistent and observed in all cells recorded. Reduction of transient sodium current by PKC activation has been reported uniformly in many cell types (for review see Cantrell and Catterall 2001; Catterall 2000). However, the alterations of voltage dependence of the transient sodium current by PKC activation are different for different cell types, experimental conditions, and concentration of PKC agents. Activation of PKC could induce varied changes in voltage dependence of sodium channels, which are shown as the depolarization of activation (Dascal and Lotan 1991), hyperpolarization of activation and inactivation (Franceschetti et al. 2000), or an unchanged voltage dependence (Cantrell et al. 1996; Numann et al. 1991; Sigel and Baur 1988; West et al. 1991). It is unclear whether the variable effects of PKC activation on \( V_{th} \) observed in this study could be attributable to the different populations of cells in spinal cord. The cells recorded in our experiments were from a heterogeneous population distributed in lamina VII–X from T13–L6 (see Table 1). Significant differences were observed in amplitudes (conductances) of \( I_T \) and PIC among the cells from lamina VII–X (Tables 1 and 2). Therefore the variable effects of PKC activation on \( V_{th} \) (and PIC) could be related to the different functions of the cells having different intrinsic membrane properties and laminar distributions. It may therefore reflect a complex modulation of the cell membrane properties through the PKC pathway. The multiple mechanisms also provide the nervous system with more flexibility to regulate the neuronal excitability to be appropriate for different motor tasks.

**Modulation of \( I_T \) and PIC versus animal ages**

There is little information about PKC mediated modulation of \( I_T \) and PIC with developmental age. Reduction of sodium currents through phosphorylation of sodium channels has been reported in different ages of animals and types of neurons. For example, PKC-mediated reduction of sodium currents was observed in brain neurons from P20 embryos of rats (Numann et al. 1991), in hippocampal neurons of adult (>P25) rats (Cantrell et al. 1996, 1997, 1999), in prefrontal cortex (PFC) neurons of 3- to 5-wk-old C57BL6 mice (Carr et al. 2002), and in pyramidal neurons of Sprague–Dawley rats aged 10–25 days (Franceschetti et al. 2000). In this study we demonstrate that activation of PKC reduces the transient and persistent inward current in the spinal ventral neurons of P1–P5 neonatal rats. It appears to be a uniform phenomenon that phosphorylation of sodium channels via PKC decreases peak sodium current. It is unclear, however, if variation of voltage dependence of sodium channels with PKC activation is related to animal ages. More studies are required to address this issue in the future.

**Multiple pathways for modulation of \( V_{th} \) by 5-HT and PKC**

The present study demonstrates multiple pathways for modulation of \( V_{th} \) by 5-HT and PKC in neonatal rat spinal ventral neurons. Activation of 5-HT receptors hyperpolarizes the \( V_{th} \) in neonatal rat spinal neurons (Fedirchuk and Dai 2004b) and also facilitates the sodium (Harvey et al. 2006a,b) and calcium PIC (Li et al. 2006) in rat spinal motoneurons. These 5-HT–mediated effects on the \( V_{th} \) and PIC are opposite to the effects of PKC activation observed in this study. Furthermore, we have shown in this study that the ability of 5-HT to induce \( V_{th} \) hyperpolarization is not altered by PKC activation or inactivation, suggesting that these two pathways are independent of each other. The functional role of these two pathways in modulation of \( I_T \) and PIC during locomotion remains unknown. However, activation of the serotonergic pathway generally enhances the neuronal excitability, whereas activation of the PKC pathway reduces the excitability. The balance of these two pathways may provide the motor system with more precise control of the configuration of the motor system for different motor tasks.

**Space-clamp issues**

Incomplete space clamp is a problem for almost all studies using whole cell patch-clamp techniques (Armstrong and Gilly 1992; Hodgkin and Huxley 1952; Rall and Segev 1985; Taylor et al. 1985), especially to those studies using fresh slices or whole tissues in the recording. Any voltage-dependent current could be contaminated by the unclamped currents (Bar-Yehuda and Korngreen 2008). Incidents of distortion of the inward currents by poor space clamp were observed in the present study (see Methods for details). The unclamped currents could cause an increase in the inward currents that could not be estimated in the present study. In theory, these distorted inward currents could lead to an overestimation of the inward current conductance. However, this error in our study is limited and small. First we excluded data with overt space-clamp issue from further analysis (see Methods). For the remaining cells the portion of the unclamped currents in the recorded whole cell currents should be small. Second, our study focused on the OAG-induced changes in \( I_T \) and PIC properties that were given in both absolute and relative values in our results (see Fig. 4 and Table 2). The absolute values of \( I_T \) and PIC amplitude alone, which might theoretically be distorted by unclamped currents, are not essential to the overall findings of this study. The fact that activation of the PKC pathway could induce about 30% reduction of \( I_T \), which was comparable to the reduction of \( I_{Na} \) in other type of neurons (Cantrell and Catterall 2001), suggests that the unclamped inward currents did not confound our results.

Poor space clamp could also cause an error in calculation of the kinetics of \( I_T \). In this study, a steep slope (small \( V_c \)) in the Boltzmann function was observed. However, the half-maximal activation and inactivation of \( I_T \) usually fell within the normal range in most of the cells. Only cells that showed a normal range of voltage dependence and flat slope in the Boltzmann function were selected for kinetics study. Further, the conclusions of our study again rely more on the relative changes in the kinetics rather than on the absolute values.

Poor space clamp had a relatively small effect on the parameters of \( I_T \) (width, maximal rise and decay rates) since they are
dependent more on the gating properties of the channels rather than the total conductance. Calculation of these parameters from the maximum \( I_T \) (see METHODS) could also reduce the errors from the poor space clamp since these parameters are very stable as soon as a spike was completely elicited. On the other hand, however, we could not absolutely rule out possible error from poor space clamp determination of \( V_{th} \). This might account for some small variation in \( V_{th} \). However, since the OAG-induced changes in \( V_{th} \) were small (\( \sim 2 \text{ mV} \) on average) and the PKC could significantly block these changes (Fig. 4), the variable \( V_{th} \) observed in this study should be due to the modulation of intrinsic membrane properties by PKC activation and not to error introduced by poor space clamp.

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