Redox Sensitive Calcium Stores Underlie Enhanced After Hyperpolarization of Aged Neurons: Role for Ryanodine Receptor Mediated Calcium Signaling

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Bodhinathan K, Kumar A, Foster TC. Redox sensitive calcium stores underlie enhanced afterhyperpolarization of aged neurons: role for ryanodine receptor mediated calcium signaling. J Neurophysiol 104: 2586–2593, 2010. First published September 8, 2010; doi:10.1152/jn.00577.2010. A decrease in the excitability of CA1 pyramidal neurons contributes to the age related decrease in hippocampal function and memory decline. Decreased neuronal excitability in aged neurons can be observed as an increase in the Ca\textsuperscript{2+}-activated K\textsuperscript{-} mediated post burst afterhyperpolarization (AHP). In this study, we demonstrate that the slow component of AHP (sAHP) in aged CA1 neurons (aged-sAHP) is decreased ~50% by application of the reducing agent dithiothreitol (DTT). The DTT-mediated decrease in the sAHP was age specific, such that it was observed in CA1 pyramidal neurons of aged (20–25 mo), but not young (6–9 mo) F344 rats. The effect of DTT on the aged-sAHP was blocked following depletion of intracellular Ca\textsuperscript{2+} stores (ICS) by thapsigargin or blockage of redox sensitive RyRs by ryanodine, suggesting that the age-related increase in the sAHP was due to release of Ca\textsuperscript{2+} from ICS through redox sensitive RyRs. The DTT-mediated increase in the aged-sAHP was not blocked by inhibition of L-type voltage gated Ca\textsuperscript{2+} channels (L-type VGCC), inhibition of Ser/Thr kinases, or inhibition of the large conductance BK potassium channels. The results add support to the idea that a shift in the intracellular redox state contributes to Ca\textsuperscript{2+} dysregulation during aging.

afterhyperpolarization, aging, oxidative stress, redox state, neuronal excitability, ryanodine receptor, hippocampus

INTRODUCTION

An age-related decline in hippocampus-dependent memory is thought to result from dysregulation of Ca\textsuperscript{2+}-dependent processes in CA1 pyramidal neurons including synaptic plasticity and neuronal excitability (Burke and Barnes 2010; Foster 2007, 1999; Kumar et al. 2009; Magnusson et al. 2010; Oh et al. 2010). One of the well characterized markers of aging in CA1 pyramidal neurons is an increase in the slow component of the Ca\textsuperscript{2+} - activated, K\textsuperscript{-} mediated afterhyperpolarization (sAHP) (Disterhoft et al. 1996; Kumar and Foster 2007; Kumar and Foster 2002, 2004; Landfield and Pitler 1984; Matthews et al. 2009; Moyer et al. 1992; Thibault et al. 2007; Tombaugh et al. 2005).

The exact mechanism that underlies the age-related increase in sAHP is unknown. The increase in sAHP may be due to altered Ca\textsuperscript{2+} regulation, including an increase in L-type voltage gated Ca\textsuperscript{2+} channels (L-type VGCC) (Thibault and Landfield 1996; Veng and Browning 2002), increased release of Ca\textsuperscript{2+} from intracellular Ca\textsuperscript{2+} stores (ICS) (Gant et al. 2006; Kumar and Foster 2004), or an increase in the function or density of K\textsuperscript{+} channels that mediate the sAHP (Power et al. 2001; Power et al. 2002). Importantly, aging is associated with increased oxidative stress that could influence the highly redox sensitive ryanodine receptor (RyR), which regulates Ca\textsuperscript{2+} release from the ICS (Bull et al. 2008; Eager and Duldhunty 1998; Hidalgo et al. 2004; Huddleston et al. 2008). Moreover, aged neurons are characterized by a decrease in their redox buffering capacity (Bodhinathan et al. 2010; Parihar et al. 2008) and recent work from our lab demonstrates that the shift in redox state contributes to decreased N-methyl d-aspartate receptor (NMDAR) function involving altered CaMKII activity in CA1 neurons from aged animals (Bodhinathan et al. 2010). Based on these observations, we tested the hypothesis that the redox state of the aged neuron contributes to the increase in sAHP (Foster 2007; Kumar et al. 2009).

The results reveal that the sAHP is decreased by the reducing agent dithiothreitol (DTT) in an age-dependent manner. Application of ryanodine, to block RyRs, prevented the DTT-mediated decrease in sAHP in the aged neurons. Depletion of ICS by the application of thapsigargin also blocked the DTT effect on aged sAHP. The DTT-mediated decrease in the aged-sAHP was independent of the activity of L-type VGCC or Ser/Thr kinase activity. Finally, inhibition of large conductance potassium (BK) channel activity did not influence the DTT-mediated decrease in the aged-sAHP. The results point to an ICS-dependent and RyR-mediated mechanism that links altered redox state during aging with the enhanced sAHP in CA1 pyramidal neurons. Reversal of the redox state of aged hippocampal CA1 pyramidal neurons is a potential target to ameliorate Ca\textsuperscript{2+} dysregulation, decrease sAHP, and restore normal functionality in aged neurons.

METHODS

Animals

Procedures involving animals have been reviewed and approved by the Institutional Animal Care and Use Committee and were in accordance with guidelines established by the U.S. Public Health Service Policy on Human Care and Use of Laboratory Animals. Male Fischer 344 rats, young (3–8 mo) and aged (20–25 mo), were obtained from National Institute on Aging colony at Harlan Sprague Dawley Inc (Indianapolis, IA). All animals were group housed (2 per cage), and maintained on a 12:12 h light schedule, and provided ad lib access to food and water.

Hippocampal slice preparation

The animals were deeply anesthetized using isoflurane (Webster, Sterling, MA) and decapitated with a guillotine (MyNeurolab, St Louis, MO). The brains were rapidly removed and hippocampi were...
The values of the holding current are presented as range and mean ± S.E. The number in parentheses indicates the number of cells from the young and aged animals.

Table 2: DTT effect on absolute sAHP amplitude from young and aged rats

<table>
<thead>
<tr>
<th>Holding current, nA</th>
<th>Young Control (n = 12)</th>
<th>Young DTT (n = 3)</th>
<th>Aged Control (n = 40)</th>
<th>Aged DTT (n = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>sAHP amplitude, mV</td>
<td>4.23 ± 0.17</td>
<td>4.38 ± 0.15</td>
<td>6.44 ± 0.32</td>
<td>3.70 ± 0.78</td>
</tr>
<tr>
<td>Range</td>
<td>-0.8 to +0.4</td>
<td>-0.2 to +0.1</td>
<td>-0.7 to +0.7</td>
<td>-0.2 to +0.2</td>
</tr>
<tr>
<td>Mean ± SE</td>
<td>-0.183 ± 0.114</td>
<td>-0.067 ± 0.088</td>
<td>-0.083 ± 0.051</td>
<td>0.032 ± 0.078</td>
</tr>
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</table>

The values for sAHP amplitude are indicated as mean ± S.E. The number in parentheses indicates the number of cells from the young and aged animals. The values of the holding currents are presented as range and mean ± S.E.

Intracellular electrophysiological recordings

Intracellular recordings were performed on CA1 pyramidal neurons to record the sAHP as previously described (Kumar and Foster 2004).

FIG. 1. Age-dependent reduction in the sAHP by DTT. (A) Time course of the change in the normalized sAHP amplitude in the aged (filled circles) (n = 5) and young (open triangles) animals (n = 3), following application of DTT for 40 min. (B) Representative traces illustrating the change in the AHP of aged (left) and young (right) animals under control conditions and at the end of 40 min application of DTT. The line beneath the traces indicates the onset and offset of the step current used to elicit a train of 5 action potentials. In this and subsequent figures, the faint line within the traces represents the baseline. Calibration bars: 200 ms, 10 mV. Inset: Magnified representation of change in the aged and young AHP under control condition, and 40 min after DTT application.

FIG. 2. Oxidizing agent decreases sAHP in young animals. (A) Time course of the change in the normalized sAHP amplitude in young animals (n = 5), following application of X/XO for 50 min. (B) Representative traces illustrating the change in the AHP for a young animal under control conditions (black trace) and at the end of 50 min application of X/XO (gray trace). Calibration bars: 200 ms, 10 mV. Inset: Magnified representation of change in the young AHP under control condition, and 50 min after X/XO application.
resistance. Depolarizing current pulses (0.1 – 1.0 nA, 100 ms duration) were delivered every 20 s through the microelectrode to elicit sodium spike bursts containing a train of 5 action potentials. The AHPs in the control and in the experimental conditions were elicited at the constant HMP of \(-63\) mV, by manually clamping the membrane potential with DC current injection not exceeding \(-1\) nA. The sAHP amplitude was measured as the difference between the average membrane potential recorded during the 100-ms period immediately preceding the onset of the depolarizing current, and the average membrane potential recorded over a 100 ms window spanning the 400 – 500 ms after the offset of the depolarizing current pulse. The amplitude of the sAHP was compared before and during drug administration in the same neuron.

All drugs were prepared according to the manufacturer’s specifications and ultimately dissolved in ACSF prior to bath application on the hippocampal slices. Nifedipine (Sigma, St. Louis, MO), ryanodine (20 \(\mu\)M) (Calbiochem, San Diego, CA), \((\pm)\)1-(5-Isouquinolinesulphonyl)-2-methylpiperazine dihydrochloride (H-7, 10 \(\mu\)M) (Tocris Bioscience, Ellisville, MO), and xanthine oxidase (0.25 U/mg xanthine, Roche Diagnostics, Indianapolis, IN) were directly dissolved in ACSF.

**Statistical analysis**

All statistical analyses were performed using Stat View 5.0 (SAS Institute Inc, NC). Student’s \(t\)-test were used to examine for differences between treatments or groups with significance set at \(P < 0.05\). Paired Student’s \(t\)-test were used to analyze the effect of treatment before and after application of drugs and unpaired Student’s \(t\)-test were used to examine the effect of treatments between age groups. Where stated, \(n\) represents the number of slices used in each experiment. All data are reported as group mean \pm standard error of mean (SEM). In general only one or two slices per animal were employed for a given experimental condition; although, several conditions could be examined using slices from the same animal.

**RESULTS**

**Age dependent decrease in the sAHP following DTT application**

To study the effects of altered redox state on the aged-sAHP, the reducing agent DTT was applied to aged and young hippocampal CA1 pyramidal neurons while continuously recording the sAHP. In confirmation of previous reports (Kumar and Foster 2007; Kumar and Foster 2004; Landfield and Pitler 1984; Matthews et al. 2009; Moyer et al. 2000; Moyer et al. 1992; Power et
al. 2002; Tombaugh et al. 2005) the sAHP was significantly ($P < 0.05$) increased in aged ($6.44 \pm 0.32$ mV, $n = 40$) relative to young CA1 pyramidal neurons ($4.23 \pm 0.17$ mV, $n = 12$). The properties of the CA1 pyramidal neurons recorded from the young and aged animals are indicated in Table 1. In a subset of these neurons, after a stable baseline recording for 10 min, DTT was applied for 40 min. Application of DTT significantly ($P < 0.05$) decreased the sAHP amplitude from the baseline levels in the aged ($48 \pm 14\%$ of baseline, $n = 5$), but not in the young animals ($105 \pm 10\%$, $n = 3$) (Fig. 1, A and B).

As such, the sAHP amplitude in aged following DTT application was similar to that observed in young animals (Table 2). The DC holding current required to maintain the membrane potential at $-63$ mV was similar for baseline recording and 45 min after DTT application (Table 2) indicating that the DTT-mediated reduction in sAHP was not associated with altered membrane properties. Finally, application of xanthine/xanthine oxidase (X/XO) to hippocampal slices from young animals increased the AHP in 4 out of 5 cells resulting in an overall significant increase in the sAHP amplitude ($150 \pm 21\%$ of the baseline, $n = 5$) (Fig. 2, A and B). The results point to a link between altered redox state and the increased sAHP amplitude in aged neurons.

**DTT-mediated decrease in aged-sAHP involves intracellular Ca$^{2+}$ stores and ryanodine receptors**

To test the hypothesis that the DTT-mediated decrease in the aged-sAHP was due to decreased Ca$^{2+}$ mobilization from ICS, thapsigargin was applied prior to and during the application of DTT to aged hippocampal slices. Application of thapsigargin for 30 min significantly ($P < 0.05$) the amplitude of aged-sAHP to $58 \pm 8\%$ ($n = 7$) of the baseline levels (Fig. 3A). In a subset of these cells ($n = 4$), a new baseline was established and DTT was applied in the presence of thapsigargin. Application of DTT for 50 min failed to decrease the aged-sAHP amplitude ($104 \pm 23\%$) (Fig. 3, B and C). The results suggest that ICS provide a redox sensitive Ca$^{2+}$ source that contributes to the age-related increase in the sAHP.

RyRs mobilize Ca$^{2+}$ from the ICS and are highly redox sensitive. To test whether RyRs were involved in the DTT-mediated decrease in aged-sAHP, RyRs were blocked by ryanodine prior to and during the application of DTT. Application of ryanodine for 40 min, significantly ($P < 0.05$) decreased the aged-sAHP amplitude to $47 \pm 10\%$ ($n = 4$). Application of DTT for 50 min failed to further decrease the aged-sAHP amplitude such that it was $54 \pm 7\%$ ($n = 4$) of the original baseline (Fig. 4, A and B).

Both DTT (Fig. 1) and ryanodine (Fig. 4) decreased the aged-sAHP to $\sim 50\%$ of baseline. The similar magnitude of the response for the two conditions raises the possibility of a “floor effect” of ryanodine, which may have masked DTT influences on the sAHP. To address this issue, the sAHP of aged neurons was enhanced by increasing the extracellular Ca$^{2+}$ concentration from 2 mM to 4 mM. Increasing the extracellular Ca$^{2+}$ to 4 mM increased the sAHP almost two fold, from $6.71 \pm 0.79$...

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**FIG. 5.** RyR blockade inhibits the DTT-mediated decrease in aged-sAHP when the AHP is increased by increasing Ca$^{2+}$ in the recording medium. (A) Representative traces illustrating the AHP from aged neurons recorded under conditions of 2 mM or 4 mM Ca$^{2+}$ in the ACSF. Calibration bars: 200 ms, 10 mV. (B) Quantification of the mean sAHP amplitude in aged neurons recorded under 2 mM Ca$^{2+}$ ($n = 11$, open bar), under 4 mM Ca$^{2+}$ ($n = 6$), under 4 mM Ca$^{2+}$ with ryanodine ($n = 5$), and under 4 mM Ca$^{2+}$ with ryanodine + DTT ($n = 5$); all values under 4 mM Ca$^{2+}$ are represented as filled bars. (C) Time course of the change in the normalized sAHP amplitude recorded in 4 mM ACSF from aged animals and incubated with ryanodine ($n = 5$) prior to and during DTT application. (D) Representative traces illustrating the AHP of aged animals under control condition, and at the end of a 40 min application of ryanodine and at the end of 50 min application of ryanodine + DTT. Calibration bars: 200 ms, 20 mV. Inset: Magnified representation of change in the aged AHP (4 mM Ca$^{2+}$) under control condition, ryanodine, ryanodine + DTT.
mV (n = 11) to 11.06 ± 1.09 mV (n = 6) (Fig. 5, A and B). In five cells, a baseline was recorded in 4 mM Ca\(^{2+}\), followed by application of ryanodine for 40 min, which was then followed by the application of DTT for 50 min (Fig. 5C). Application of ryanodine decreased (P < 0.05) the aged-sAHP amplitude to 53 ± 6% (5.31 ± 0.86 mV) and application of DTT for 50 min failed to further decrease the aged-sAHP amplitude (51 ± 7%, 5.06 ± 1.05 mV) of the original baseline (Fig. 5, B–D). Thus DTT failed to reduce the sAHP amplitude under high Ca\(^{2+}\) and ryanodine application, despite the fact that sAHP amplitude was similar to the baseline under normal 2 mM Ca\(^{2+}\) conditions (Fig. 5B). The results indicate that the ryanodine blockade of the DTT-mediated decrease in the sAHP was not due to a floor effect of the sAHP during ryanodine application. Rather, these data suggest that the DTT effect on sAHP in aged animals is mediated by RyRs.

Finally, application of DTT for 40 min, decreased the aged-sAHP amplitude to 44 ± 7% (n = 4) of the baseline (similar to that observed in Fig. 1). In two of these cells, application of ryanodine for 40 min failed to further decrease the aged-sAHP amplitude such that it was 55 ± 6% (n = 2) of the original baseline. In the other two cells, application of thapsigargin also failed to further decrease the aged-sAHP amplitude such that it was 41 ± 8% (n = 2) of the original baseline. This suggests that application of DTT occluded the effect of ryanodine and thapsigargin on the aged-sAHP amplitude.

**DTT-mediated reduction in the aged-sAHP is independent of L-type VGCCs**

L-type VGCCs are another major source of Ca\(^{2+}\) for the sAHP. To test the hypothesis the DTT-mediated decrease in the aged-sAHP involves the L-type VGCC, nifedipine was applied prior to and during the application of DTT to aged hippocampal slices (Fig. 6A). Application of nifedipine for 20 min decreased the sAHP to 68 ± 4% (n = 5) of the baseline. Subsequent application of DTT for 30 min further decreased the amplitude of aged-sAHP to 34 ± 4% (n = 5) of the original baseline. The results suggest that the effects of nifedipine and DTT may be independent. In fact, using the sAHP responses recorded in nifedipine (bath application for ≥20 min) as the baseline, application of DTT decreased the amplitude of the aged-sAHP to 48 ± 6% (P < 0.05; n = 6) (Fig. 6B), a decrease comparable to that observed following DTT application in the absence of nifedipine (Fig. 6B). The activity of BK channels is sensitive to oxidation (DiChiara and Reinhart 1997). Moreover, an increase in BK channel activity can reduce the sAHP amplitude by decreasing the action potential spike width (Giese et al. 1998; Murphy et al. 2004; Shao et al. 1999). To test the hypothesis that the DTT-mediated decrease in aged-sAHP involves the BK channels, paxilline was first applied to inhibit BK channel activity (Sanchez and McManus 1996). Aged hippocampal slices were incubated in paxilline for ≥60 min prior to recording the sAHP and applying DTT. Paxilline failed to block the DTT-mediated decrease in aged-sAHP, such that DTT application was still able to decrease the amplitude of the aged-sAHP to 28 ± 11% (n = 3) of the baseline (Fig. 7, A and B). Furthermore, the DTT-mediated decrease in the presence of paxilline was not significantly (P > 0.05) different from the decrease observed in the presence of DTT alone.

**Serine/threonine kinases provide another potential mechanism for regulating RyRs and the K\(^{+}\) channels that mediate the sAHP.** Protein kinase A increases the activity of cardiac RyRs (RyR subtype 2) (Danila and Hamilton 2004; Morimoto et al. 2009; Xiao et al. 2007; Yoshida et al. 1992), and kinase activity inhibits the sAHP (Madison and Nicoll 1986; Malenka and Storm 1993). To test whether the changes in kinase activity underlie the decrease in sAHP of aged neurons on application of DTT, the broad spectrum serine/threonine kinases inhibitor H-7 was applied prior to and during the application of DTT. Aged hippocampal slices were incubated with H-7 for ≥60 min before recording the sAHP. In the presence of H-7, application of DTT significantly (P < 0.05) decreased the aged-sAHP to 53 ± 14% (n = 3) of the baseline (Fig. 8A). The
results suggest that DTT is not altering the sAHP through modulation of kinase activity.

Figure 8B summarizes the change in the sAHP amplitude of aged CA1 neurons following DTT application in the presence of various inhibitors. In each case, the response was normalized to the preDTT baseline. Treatments that blocked Ca\(^{2+}\) release from ICS (thapsigargin, ryanodine) blocked the DTT-mediated reduction in the sAHP. However the DTT-mediated reduction in sAHP was not blocked in the presence of nifedipine, paxilline, or H-7. Consistent with previous reports, we observed \(\sim 50\%\) and \(\sim 30\%\) reduction in sAHP amplitude on application of ryanodine (Gant et al. 2006; Kumar and Foster 2004) and nifedipine (Disterhoft et al. 2004; Power et al. 2002) respectively.

**DISCUSSION**

The results demonstrate a link between the age-related increase in the sAHP and redox state, through the release of Ca\(^{2+}\) from ICS. A shift in Ca\(^{2+}\) regulation and altered Ca\(^{2+}\) channel function is a characteristic of aging CA1 neurons (Burke and Barnes 2010; Foster 2007, 1999; Gant et al. 2006; Hemond and Jaffe 2005; Kumar et al. 2009; Landfield and Pitler 1984; Magnusson et al. 2010; Oh et al. 2010; Thibault et al. 2001; Thibault and Landfield 1996). Recently, we demonstrated that DTT could reverse an age-related decrease in NMDA receptor function in region CA1 (Bodhinathan et al. 2010). In the current study, the reducing agent, DTT, decreased the sAHP in aged, but not in young CA1 neurons. The data are consistent with the altered redox buffering in aged animals as a mechanism contributing to Ca\(^{2+}\) dysregulation and electrophysiological changes observed in aged neurons.

Redox modulation has been observed for several ion channels including K\(^{+}\) and Ca\(^{2+}\) channels (Chiamvimonvat et al. 1995; DiChiara and Reinhart 1997; Hidalgo et al. 2004; Ruppersberg et al. 1991; Stephens et al. 1996), which could contribute to the sAHP. The identity of the K\(^{+}\) channel that underlies the sAHP is unknown (Furuichi et al. 1994; Sah and Faber 2002); however, the amplitude to the sAHP is reduced by activation of Ser/Thr kinases, including PKA (Madison and Nicoll 1986; Pedarzani and Storm 1993), CaMKII (Muller et al. 1992), and PKC (Malenka et al. 1986). In the current study, the broad spectrum Ser/Thr kinase inhibitor H-7 had no influence on the DTT-mediated decrease in aged-sAHP, indicating that the reduction was not mediated through kinase activity.

In the case of K\(^{+}\) channels, previous reports indicate that cysteine specific oxidation decreases BK channel activity (Tang et al. 2001; Tang et al. 2004), and that the reducing agent...
DTT increases BK channel activity (DiChiara and Reinhart 1997). The BK channels are involved in repolarization of action potential, and an increase in BK channel activity will reduce the width of the action potential (Shao et al. 1999). Moreover, a decrease in the spike width can decrease the sAHP, by limiting the duration of depolarization-induced Ca²⁺ entry through L-type VGCCs (Giese et al. 1998; Murphy et al. 2004). Thus DTT could be acting on the BK channels to decrease L-type VGCC activity and the sAHP amplitude. However, several pieces of evidence suggest that this might not be the case. First, blockade of BK channels with paxilline did not block the DTT-mediated decrease in aged-sAHP. Second, blockade of VGCC’s with nifedipine did not influence the DTT-mediated decrease in aged-sAHP. Finally, the fast AHP, which is mediated by the BK channel, is not altered with age (Matthews et al. 2009).

The amplitude of the sAHP is dependent on the level of cytosolic Ca²⁺. L-type VGCCs play a role in determining the amplitude of the sAHP (Landfield and Pitler 1984; Moyer et al. 1992; Norris et al. 1998) and contribute to the increase in the sAHP during aging (Thibault and Landfield 1996; Veng and Browning 2002). However, it does not appear that the DTT-mediated reduction in the aged-sAHP is acting through L-channels. The DTT-mediated reduction in the sAHP was larger than that observed for L-channel blockade, and was specific to aged animals. Previous research indicates that the decrease in the sAHP following blockade of L-channels is quantitatively similar across ages, suggesting other mechanisms contribute to the age-related increase in the sAHP amplitude in aged animals (Disterhoft et al. 2004; Power et al. 2002). In the present study, blockade of the L-channel reduced the aged-sAHP ~30%, consistent with previous reports (Norris et al. 1998; Power et al. 2002). Regardless of L-channel function, DTT reduced the aged-sAHP by ~50% and the effect of DTT was specific to aged animals. The results suggest that DTT is acting on mechanisms other than the L-channel, which may mediate the age-related increase in the sAHP.

**RyR and redox state dependent effects on sAHP**

Release of Ca²⁺ from ICS through RyR activation, plays a role in determining the sAHP amplitude (Borde et al. 2000; Davies et al. 1996; Sah and McLachlan 1991; Usachev et al. 1993; van de Vrede et al. 2007) and Ca²⁺ from ICS contributes to altered physiology during aging (Gant et al. 2006; Kumar and Foster 2004, 2005). In addition, the RyRs are highly redox sensitive (Bull et al. 2008), such that oxidation of the cysteine residues increases the Ca²⁺ sensitivity and activity of RyR (Eager and Duhunty 1998; Hidalgo et al. 2004; Huddleston et al. 2008). In the present study, the DTT-mediated decrease in the sAHP was blocked on depletion of ICS by thapsigargin or blockade of RyRs by ryanodine, indicating the involvement of ICS and RyRs in the decreased Ca²⁺ mobilization by DTT application. Together, the results suggest that the increase in the sAHP in aged neurons is related to reduced sensitivity of RyR mobilization from the ICS through the RyRs.

Treatments that modify intracellular redox state may provide a novel therapeutic strategy to restore Ca²⁺ homeostasis in the aged neurons. Interestingly, a learning mediated decrease in the sAHP is observed in aged memory-unimpaired rats (Matthews et al. 2009; Moyer et al. 2000; Murphy et al. 2006; Tombaugh et al. 2005). Future research should include an examination of a learning induced shift in redox state (Shvets-Teneta-Gurii et al. 2007) as a possible mechanism mediating the decrease in the sAHP of memory unimpaired animals.

**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the author(s).

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