Changes in Membrane and Synaptic Properties of Thalamocortical Circuitry Caused by Hydrogen Peroxide

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Frantseva, Marina V., Jose L. Perez Velazquez, and Peter L. Carlen. Changes in membrane and synaptic properties of thalamocortical circuitry caused by hydrogen peroxide. J. Neurophysiol. 80: 1317–1326, 1998. Free radical (FR) production was linked to the generation of epileptiform activity. We performed electrophysiological recordings in rat thalamocortical slices to investigate the effects of FRs on the intrinsic and synaptic properties of thalamic and cortical neurons. Whole cell recordings from identified cortical pyramidal neurons and thalamic neurons of the ventrobasal nucleus revealed that exposure to the FR-forming agent H₂O₂ (2.5 mM) decreased γ-aminobutyric acid-A– and γ-aminobutyric acid-B– mediated inhibition to 35.3 ± 13.4% and 13.7 ± 4.4% (means ± SE) of control in cortical neurons and to 41.8 ± 14.8% and 33.6 ± 11.6% of control in thalamic neurons. H₂O₂ application increased excitatory transmission in thalamic neurons to 162.9 ± 29.6% of control but caused no change in cortical neurons. H₂O₂ altered significantly the characteristic low-pass filter behavior of cortical and thalamic cells as determined by their input impedances. After 35 min of superfusion, the impedance of cortical neurons decreased by 67.0 ± 14.5%, and thalamic decreased by 76.3 ± 2.7% for the frequencies in the range 1–50 Hz while remaining constant for frequencies >200 Hz. Neuronal hyperexcitability was manifested during H₂O₂ exposure by continuous firing and long depolarizing shifts in response to extracellular stimulation in both thalamocortical and cortical neurons only in slices preserving thalamocortical connections. In slices with severed thalamocortical connections, cortical neurons did not show signs of hyperexcitability. These observations indicate that FRs could promote hyperexcitabilty of thalamocortical circuits by altering the balance between excitation and inhibition and by transforming the characteristic low-pass filter behavior into a flat band-pass filter.

INTRODUCTION

Head trauma, hyperoxygenation, and hypoxia, which are all associated with a sharp increase in free radical (FR) production (Hall 1993; Hyslop et al. 1995; Jerrett et al. 1973; Kryzhanovskii et al. 1980; Perez Velazquez et al. 1997), are often complicated by the development of seizures (Behnke 1940; Jennett 1973; Jensen et al. 1991; Katz et al. 1987). Observations from a variety of experimental epilepsy models employing FR generating systems, such as intracerebral application of alumina gel (Ward 1972), pial or subpial application of iron salts (Willmore and Rubin 1981), and hyperbaric oxygenation (Jerrett et al. 1973), are consistent with the hypothesis that FR might play a role in the pathogenesis of epilepsy. Antioxidants were shown to reduce the development of seizures in some models; pretreatment with the antioxidant vitamin E prevented hyperbaric oxygen-induced seizures in rats (Jerrett et al. 1973) as well as iron-induced epileptiform discharges (Willmore and Rubin 1981). Moreover, vitamin E adjunctive therapy was shown to cause improvement in seizure frequency in a group of poorly controlled epileptic children as well as normalization of interictal EEG patterns in one-half of the patients studied (Ogunnkan and Hwang 1989). Production of epileptiform discharges in hyperbaric and iron-induced experimental epilepsy is believed to be mediated by membrane lipid peroxidation (LPO) initiated by FR (Jerrett et al. 1973; Willmore and Rubin 1981).

Attempts to demonstrate the epileptogenic action of reactive oxygen species in vitro were mostly unsuccessful and contradictory. For example, the most common FR-generating compound hydrogen peroxide (H₂O₂) at 3.3 mM concentration hyperpolarized hippocampal CA1 and CA3 pyramidal neurons (Seutin et al. 1995). In addition, H₂O₂ (at 2.94 mM) in the presence of iron salts was reported to markedly decrease synaptic efficacy and impair the ability of CA1 neurons to produce action potentials (Pellmar 1987). These effects can hardly account for a putative epileptiform effect of FR observed in vivo. In contrast, Muller et al. (1993) reported that hydrogen peroxide at 300 μM concentration produced an excitatory effect on CA3 pyramidal cells in rat hippocampal slice cultures by decreasing inhibitory postsynaptic potentials (IPSPs), eventually leading to epileptiform bursting.

The documented FR-induced epilepsy in vivo mentioned might be a network phenomenon, whereas in vitro experiments are at best limited by the local circuitry. For example, the spike and wave discharges and behavioral manifestations of iron-induced epilepsy in the rat (Singh and Pathak 1990) are suggestive of absence epilepsy originating in thalamocortical circuitry. Absence epilepsy is thought to be caused by enhanced γ-aminobutyric acid (GABA)–mediated hyperpolarizations driven by thalamic reticular neurons, followed by rebound low-threshold calcium spikes in reticular and thalamocortical neurons (Gloor et al. 1990; Steriade and Contreas 1995). We examined the effects of FR on synaptic and membrane properties of thalamic and cortical neurons. We used hydrogen peroxide as a generator of FR, and we examined synaptic (excitatory and inhibitory) and intrinsic membrane properties of thalamic and cortical neurons were investigated by means of whole cell recordings.

METHODS

Brain slices and solutions

Thalamocortical slices were prepared as described by Agmon and Connors, (1991). Briefly, 14–20 day old Wistar rats were anesthetized with halothane (Fluothane, Ayerst Laboratories, Mon-
Electrophysiological recordings

**WHOLE CELL RECORDINGS.** Neuronal recordings were obtained with the use of the whole cell configuration of the patch-clamp technique (Hamill et al. 1981) from cells in layers IV–V of the somatosensory cortex and from the ventralbasal nucleus (VB) of the thalamus. Patch pipettes were pulled from borosilicate capillary tubing (World Precision Instruments, New Haven, CT). Electrodes, filled with internal solution [(in mM) of 150 potassium glutonate, 10 N-[2-hydroxyethyl]piperazine-N'-2-ethanesulfonic acid (HEPES), 2 Mg-ATP, 5 KCl, and 0.1 ethylene glycol-bis(β-aminoethyether) -N,N',N''-tetraacetic acid (EGTA), at pH 7.2 adjusted with KOH and osmolarity 275 ± 5 mOsm] had tip resistances between 4 and 5.5 MΩ. The resistance to ground of the whole cell seal was 2–8 GΩ before breakthrough. Neuronal responses were recorded with the use of an Axoclamp 2-A amplifier in bridge mode. For extracellular stimulation a bipolar stimulating electrode was placed in the VB of thalamus (unless otherwise indicated). Initial low-stimulus intensities were gradually increased to elicit stable postsynaptic responses and were maintained at this level for the duration of the experiment.

**EXTRACELLULAR RECORDINGS.** For extracellular stimulation, current pulses (single current stimuli 150 μs at 0.66 Hz; or trains 100 Hz, 2 s) were delivered through a bipolar stimulating electrode positioned in the VB nucleus of the thalamus. Extracellular recording electrodes filled with ACSF were placed in the IV–V layer of somatosensory cortex and in the VB of the thalamus.

Pclamp software (Axon Instruments) was used for analysis of membrane potential, input resistance (which was measured from the linear part of the current-voltage plot), and amplitudes of postsynaptic responses. The amplitudes of the postsynaptic responses were measured from the peaks to the baseline. To test for statistical significance the paired Student's t-test was used. Significance was considered at P < 0.05; numerical values are expressed as means ± SE.

**White noise stimulation and impedance estimation**

A 3.2-s zero-mean Gaussian white noise current input was injected via patch electrodes into thalamic and cortical neurons. The input was generated by the computer as described previously (Wright et al. 1996). For estimation of the neuronal input impedance (Z_N), the power level of the noise input was chosen such that no action potentials were triggered. The acquisition software was written in a combination of C language and 8088 assembly language.

**INPUT IMPEDANCE ESTIMATION.** Linear time-invariant systems are characterized by the impulse response function or first-order (linear) Wiener kernel (Schetzen 1989). If the input to the system is current and the output response is voltage, then the input impedance is estimated by performing a Fourier transform of the first-order kernel. For the computation of the first-order kernel we used the Laguerre expansion of the kernels technique (Marmarelis 1993) with 100 Laguerre functions as described in detail previously (Wright et al. 1996). The fast Fourier transform (FFT) of the first-order (linear) kernel was implemented with the routine described by Press et al. (1992) to obtain a 4,096-point characterization of the cellular input impedance magnitude over a frequency range of 0–5,000 Hz with a 1.22-Hz frequency resolution. For accurate high-frequency values the calculated impedance was adjusted by a factor of (2πfT/1/2) to account for the effect of the antialiasing filter with cutoff time constant τ and by a factor of (Tπf)/sin(Tπf) to compensate for the finite width T of the sequence of input pulses.

**Lucifer yellow staining and morphology**

Pyramidal cortical neurons were identified by their morphology and specific patterns of firing (Agmon and Connors 1992; Cauli et al. 1997; Connors et al. 1982). For morphological assessment neurons were dialyzed with 0.05% dipotassium salt (Lucifer yellow, Sigma Chemical, St. Louis, MO) via a patch electrode. Slices were then fixed overnight at 4°C in 4% paraformaldehyde, dehydrated in a graded ethanol series, and cleared in methyl salicylate. Images were taken with the use of a Bio-Rad MRC 600 laser scanning confocal microscope with long-working distance objective with the use of a fluorescein filter block. Micrographs were printed on a Kodak SV6500 video printer.

**RESULTS**

**H2O2 changes intrinsic membrane properties in thalamic and cortical cells**

We sought to investigate specific alterations of cellular properties induced by hydrogen peroxide that might cause cortical hyperexcitability in response to thalamic stimulation. For that we followed H2O2-induced changes in the intrinsic properties of neurons and local inhibitory and excitatory synaptic actions. As shown in Fig. 2, membrane input resistance (R_in) was decreased after 10 min of H2O2 (2.5 mM) application in six of six cortical neurons from 140.6 ± 15.6 to 99.0 ± 11.3 MΩ (P < 0.005) and in nine of nine thalamic neurons from 138.7 ± 9.0 to 100.4 ± 11.3 MΩ (P < 0.0005). Most of the
cortical neurons recorded (23 of 26, layers IV–V) were regular spiking cells (Connors et al. 1982) with morphological characteristics of pyramidal neurons (Fig. 1). All thalamic cells recorded in the VB showed a rebound (low-threshold spike) response to hyperpolarizing current pulses, typical of thalamic neurons (Deschenes et al. 1984; Jansen and Llinas 1984). The resting membrane potential (RMP) was hyperpolarized during H₂O₂ perfusion by 2.1 ± 0.7 mV in 50% of cortical cells and by 1.0 ± 1.0 mV in all thalamic cells (n = 9). RMP did not change in 5 of 16 cortical neurons (33%), and it depolarized in 2 neurons. As shown in Fig. 2, exposure to hydrogen peroxide for 10 min decreased the membrane time constant (τ) in all cortical and thalamic cells to 61.4 ± 8.0% and 54.0 ± 12.3% of control, respectively.

The intrinsic properties of cortical and thalamic neurons were investigated further by measuring membrane input impedance at different frequencies (Zᵢ) with the use of white noise analysis. The first-order linear kernel was obtained by the Laguerre method (Marmarelis 1993) as detailed by Wright et al. (1996). A typical kernel is shown in Fig. 3A. The amplitude of the kernel reflects the high-input resistance of the neuron. Assuming linearity, the first-order kernel can be interpreted as the impulse response, which is represented by the membrane time constant τ. The kernel decays much faster in H₂O₂ (Fig. 3A, right), confirming the shorter τ measured in response to current steps (see Fig. 2).

The Zᵢ was obtained by FFT of the first-order linear kernel as explained in METHODS. The input resistance (the value

![Fig. 1. Morphological and electrophysiological characterization of a cortical neuron of layer IV–V of somatosensory cortex. A: whole cell recording obtained in response to hyperpolarizing and depolarizing current pulses reveals regular spiking characteristics. B: confocal image of the Lucifer yellow–filled cell identifies it as a pyramidal neuron. Note a prominent apical dendrite extending toward the pia and a high density of basal dendritic processes. Scale bar 100 μm.](http://jn.physiology.org/)

![Fig. 2. Comparison of intrinsic membrane properties in thalamic (A) and cortical (B) neurons before and 10 min after the beginning of exposure to 2.5 mM H₂O₂. Input resistance is significantly decreased because of the action of hydrogen peroxide in both thalamic (n = 7) and cortical (n = 9) neurons. Membrane time constant (τ) is also significantly decreased because of H₂O₂ application. * Statistically significant difference (P < 0.05). Inset: whole cell recordings of a thalamic neuron in response to hyperpolarizing and depolarizing current pulses before and after exposure to H₂O₂. Rₛ, membrane input resistance.](http://jn.physiology.org/)
of the input impedance at 0 Hz) decreased because of exposure to H$_2$O$_2$ in six of six cortical and seven of seven thalamic cells (Figs. 3 and 4). As shown in Table 1, after 35 min of H$_2$O$_2$ application the average input resistance in cortical neurons was 32.3 ± 17.2% of the initial value, and in thalamic neurons it was 28.0 ± 24.2%. Also H$_2$O$_2$ caused a reduction in the membrane impedance ($Z_N$) at low frequencies (ranging from 1 to 50 Hz). As indicated in Table 1, 35 min of exposure to H$_2$O$_2$ reduced membrane impedance at 40 Hz ($Z_{40}$) to 67.0 ± 14.5% of the initial value in cortical neurons and to 76.9 ± 4.1% in thalamic neurons. Overall changes in $Z_N$ during peroxide perfusion show a flat band-pass response, with $Z_N$ values remaining constant or increasing for frequencies >200 Hz (Figs. 3B and 4A). To rule out the possibility that these changes of membrane properties were induced by long-term impaling of the cellular membrane with the patching electrode, measurements were performed for an equivalent time in the absence of hydrogen peroxide (Fig. 4B). The average value of input resistance after 35 min of patching was 96.7 ± 1.3% of the initial value ($n = 3$), and the input impedance at 40 Hz was 110.7 ± 15.3% of the initial value (Table 1). Exposure of cortical and thalamic cells ($n = 3$) to hydrogen peroxide for 45–60 min before patching further confirmed our findings; the initial part of the impedance curve was flattened, confirming that changes in the neuronal response function determined by $Z_N$ were in fact caused by the action of H$_2$O$_2$. These alterations in the input impedance indicate a loss of low-pass filter behavior characteristic for thalamic and cortical neurons (Puil et al. 1994).

**H$_2$O$_2$ reduces IPSP magnitude in thalamic and cortical cells**

To investigate the effects of H$_2$O$_2$ on inhibitory neurotransmission in thalamocortical circuits, intracellular postsynaptic responses were measured in cortical and thalamic cells before, during, and after H$_2$O$_2$ exposure in the presence of the glutamate receptor blockers CNQX and d-AP5 [to block excitatory postsynaptic potentials (EPSPs)]. IPSPs in thalamic neurons were evoked by local extracellular stimulation in the VB nucleus, whereas IPSPs measured in somatosensory cortex (layer IV–V) were evoked by stimulation of the local white matter. Stimulation evoked GABA$_A$– and GABA$_B$–mediated IPSPs that were blocked by bicuculline and phaclofen and had reversal potentials of –71.6 ± 2.25 mV and –78.6 ± 2.11 mV, respectively ($n = 5$ thalamic neurons). H$_2$O$_2$ progressively decreased both GABA$_A$ and...
GABA<sub>B</sub> components of inhibitory postsynaptic responses in thalamic (7/7; Fig. 5) and in cortical (9/9) neurons. After 4 min of H<sub>2</sub>O<sub>2</sub> application the average GABA<sub>A</sub>-mediated IPSP amplitude was decreased to 41.8 ± 14.8% of control in thalamic and to 35.3 ± 13.4% in cortical cells. Similarly, the mean amplitude of the GABA<sub>B</sub>-mediated IPSP was 33.6 ± 11.6% of control in thalamic and 13.7 ± 4.4% in cortical neurons (Table 2). IPSPs were abolished by 6.0 ± 1.4 min in six of nine cortical neurons and by 6.2 ± 1.4 min in four of seven thalamic neurons. Superfusion with normal ACSF for 5 min partially restored IPSPs in two of two thalamic neurons and in five of seven cortical neurons.

To test whether the effects of hydrogen peroxide were in fact caused by the action of FRs, H<sub>2</sub>O<sub>2</sub>-induced changes of IPSPs were determined in the presence of FR scavengers. Slices were superfused for ≈10 min before hydrogen peroxide exposure with 100 μM vitamin E and 1 mM glutathione, which prevented the rapid decrease of IPSPs. The averages of GABA<sub>A</sub>- and GABA<sub>B</sub>-mediated amplitudes after 10 min of H<sub>2</sub>O<sub>2</sub> exposure were 118.8 ± 37.8% and 88 ± 31.5%, respectively (n = 5).

H<sub>2</sub>O<sub>2</sub> enhances excitatory transmission in thalamic neurons

Long-lasting EPSPs were whole cell recorded in thalamic neurons of the VB nucleus (by local stimulation) in the presence of phaclofen and bicuculline to eliminate IPSPs. In thalamic neurons, hydrogen peroxide caused biphasic changes in excitatory neurotransmission (Fig. 6). As shown in Table 3, the first maximum EPSP amplitude was recorded after 3.4 ± 0.1 min and was 145.4 ± 18.6% compared with control (5/6 neurons). Within the next several minutes the EPSP height was stable, reaching a second maximum (162.9 ± 29.6% of control in 5/6 neurons) after 7.4 ± 0.3 min of H<sub>2</sub>O<sub>2</sub> application. EPSP amplitude decreased after 9.9 ± 0.1 min by 19.2% (reaching 143.7 ± 29.0% compared with control, 5/6 cells). This effect was reversible in five of five thalamic cells; EPSP amplitude recovered to 92.3 ± 8.5% of control after superfusion with normal ACSF for 4 ± 0.5 min. In three of seven of these thalamic neurons, repeated stimulation in the presence of 2.5 mM H<sub>2</sub>O<sub>2</sub> evoked large depolarizing shifts, accompanied by high-frequency

### TABLE 1. Comparison of R<sub>n</sub> and Z<sub>40</sub> in cortical and thalamic cells before and after H<sub>2</sub>O<sub>2</sub> application

<table>
<thead>
<tr>
<th>Time after H&lt;sub&gt;2&lt;/sub&gt;O&lt;sub&gt;2&lt;/sub&gt; exposure (min)</th>
<th>Cortex, n = 6</th>
<th>Thalamus, n = 7</th>
<th>Control, n = 3</th>
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<tbody>
<tr>
<td>10</td>
<td>20</td>
<td>35</td>
<td>35</td>
</tr>
<tr>
<td>R&lt;sub&gt;n&lt;/sub&gt; (%)</td>
<td>65.5 ± 16.1</td>
<td>49.7 ± 14.9</td>
<td>32.3 ± 17.2</td>
</tr>
<tr>
<td>Z&lt;sub&gt;40&lt;/sub&gt; (%)</td>
<td>94.0 ± 1.9</td>
<td>83.7 ± 6.9</td>
<td>67.0 ± 14.5</td>
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</table>

Values are means ± SE and represent averaged percentages of control values (before H<sub>2</sub>O<sub>2</sub> application) of the R<sub>n</sub> and Z<sub>40</sub>. Membrane input resistance (R<sub>n</sub>) and impedance at 40 Hz (Z<sub>40</sub>) were significantly decreased after H<sub>2</sub>O<sub>2</sub> exposure. Control column represents average percentage changes of R<sub>n</sub> and Z<sub>40</sub> superfused by artificial cerebrospinal fluid in the absence of H<sub>2</sub>O<sub>2</sub>.

**FIG. 4.** Input impedance in cortical neurons in control and during peroxide application. A: changes in the magnitude of the impedance of a cortical cell during bath application of hydrogen peroxide (2.5 mM). As seen for thalamic neurons (Fig. 3), Z<sub>n</sub> decreases for frequencies 1–200 Hz, creating a flat band-pass characteristic as opposed to the low-pass in control. B: another cortical neuron was patched for an equivalent time (45 min), and its Z<sub>n</sub> was determined to control for possible changes caused by the patching procedure: seal characteristics and effects of the internal electrode solution on cell properties. In this case we observed a slight decrease of input resistance (Z<sub>n</sub> at 0 Hz) and an increase of impedance at 40 Hz (Table 1).

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**FIG. 5.** Comparison of R<sub>n</sub> and Z<sub>40</sub> in cortical and thalamic cells before and after H<sub>2</sub>O<sub>2</sub> application.
**FIG. 5.** Reduction of inhibitory postsynaptic potentials (IPSPs) in thalamic neurons after H$_2$O$_2$ application. A: IPSP in a thalamic neuron evoked by near-site stimulation and recorded intracellularly under control conditions decreases on superfusion with hydrogen peroxide in the continuous presence of d-2-amino-5-phosphopentanoic acid and 6-cyano-7-nitroquinoxaline-2,3-dione ($V_m = -65$ mV). Superfusion with H$_2$O$_2$-free artificial cerebrospinal fluid (ACSF) for 10 min partially restored the IPSP. B and C: time course of changes in amplitude of $\gamma$-aminobutyric acid-A (GABA$_A$) - (B) and $\gamma$-aminobutyric acid-B (GABA$_B$) - mediated (C) IPSPs caused by application and subsequent wash of 2.5 mM H$_2$O$_2$. Amplitudes of both GABA$_A$ and GABA$_B$ components of the IPSP decreased dramatically because of the action of hydrogen peroxide. IPSP amplitude was partially restored by superfusion with normal ACSF. The amplitude was measured from the peak of the IPSP to the baseline levels. $V_m$ was kept at $-63$ mV.

spiking, which eventually led to lasting sustained depolarization and intense firing (60–100 Hz, see Fig. 7A). These changes were reversed after 5–6 min of return to control ACSF in the continuous presence of GABA receptor blockers (Fig. 7A). EPSP magnitude tended to decrease after >20 min of exposure to H$_2$O$_2$.

**H$_2$O$_2$ does not change excitatory transmission in cortical neurons but causes hyperexcitability in cortical neurons when thalamocortical connectivity is preserved**

Application of hydrogen peroxide to cortical slices without thalamocortical connectivity did not cause significant changes of excitatory postsynaptic responses in cortical neurons evoked by stimulation of local white matter. After 4 min of H$_2$O$_2$ exposure, EPSP amplitude changed by +3.8 ± 20.76% and by $-6.1 \pm 19.8\%$ after 10 min (6/6 neurons). Prolonged (up to 20 min) application of H$_2$O$_2$ gradually decreased EPSP amplitude in three of six cortical neurons.

To test whether hydrogen peroxide causes seizure-like activity in thalamocortical circuits, brain slices preserving thalamocortical connections were exposed to 2.5 mM H$_2$O$_2$. A slice was considered to have intact thalamocortical connectivity if a focal electrical stimulus applied to the VB elicited an intracellular or extracellular response in the somatosensory cortex, layers IV–V. The majority (70%) of synaptic cortical responses (layers IV–V) resulting from thalamic stimulation (see Fig. 7B) appeared to be polysynaptic. All cells exhibiting polysynaptic connections were identified as regular spiking pyramidal neurons (Connors et al. 1982) by their firing pattern and staining with Lucifer yellow (n = 7; see Fig. 1). Dual extracellular recordings from the somatosensory cortex and the VB nucleus in the presence of H$_2$O$_2$ did not reveal spontaneous synchronized field potential activity in thalamocortical slices (n = 6). Furthermore, stimulation of the VB nucleus with single current pulse or pulse trains (100 or 40 Hz for 2 s) did not result in the onset of spontaneous synchronized field potential activity (n = 6). However, whole cell recordings from cortical pyramidal and thalamic neurons revealed hyperexcitable events, occurring on thalamic stimulation after 5–9 min in H$_2$O$_2$ (6/7 cortical neurons and 4/6 thalamic; see Fig. 7). As shown in Fig. 7, A and B, hyperexcitable events induced by exposure to H$_2$O$_2$ in response to thalamic stimulation were of the following two types: 1) EPSPs of increasing complexity and duration or 2) a complex polysynaptic response, comprising recurrent large depolarizations with superimposed action potentials. These effects were reversible on superfusion with control ACSF in two of two cortical neurons and in four of four thalamic neurons. As illustrated in Fig. 7C, no indication of
TABLE 2. Hydrogen peroxide-induced changes in IPSP amplitude in thalamic and cortical cells

<table>
<thead>
<tr>
<th>Time</th>
<th>Cortex</th>
<th>Thalamus</th>
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<tbody>
<tr>
<td></td>
<td>4 min</td>
<td>5 min wash</td>
</tr>
<tr>
<td>IPSPn (%)</td>
<td>35.3 ± 13.4</td>
<td>47.5 ± 6.2</td>
</tr>
<tr>
<td>IPSPm (%)</td>
<td>13.7 ± 4.4</td>
<td>27.7 ± 10.1</td>
</tr>
<tr>
<td>n</td>
<td>9/9</td>
<td>4/7</td>
</tr>
</tbody>
</table>

Values are means ± SE and represent GABA,-mediated inhibitory post-synaptic potential (IPSPn) and GABA₂-mediated IPSP (IPSPm). Values correspond to the averaged percentages of control IPSPn and IPSPm amplitudes (before exposure to hydrogen peroxide). IPSP amplitudes were estimated by measuring the peaks of IPSP. Four-minute wash partially reversed the effect (columns 2 and 4).

hyperexcitability was found in cortical neurons from brain slices where the thalamocortical connections were severed (6/6 neurons), suggesting that cortical hyperexcitability induced by FR requires some degree of thalamocortical connectivity.

DISCUSSION

In the current study we report that 2.5 mM H₂O₂ causes reduction of inhibitory neurotransmission, temporary augmentation of excitatory neurotransmission in thalamic neurons, and changes in the intrinsic properties of neuronal membranes. We also document that H₂O₂ application induces hyperexcitable events in neurons of the somatosensory cortex and thalamus on thalamic stimulation. Prevention of H₂O₂-mediated decrease of IPSP by a mixture of antioxidants suggests that these effects are caused by FR-mediated reactions.

There are several lines of evidence suggesting that FR might have an epileptiform effect in brain tissue. These include 1) temporal correlation between FR overproduction and development of seizures in some pathological conditions in vivo (e.g., head trauma (Jennett 1973), ischemia (Jensen et al. 1991), and hyperoxygenation (Behnke 1940; Jerrett et al. 1973)); 2) use of FR-generating systems for induction of seizures in a variety of in vivo experimental epilepsy models (e.g., intracerebral application of alumina gel (Ward 1972), pial or subpial application of iron salts (Willmore and Rubin 1981), and hyperbaric oxygenation (Jerrett et al. 1973)); and 3) the efficacy of oxygen radical scavengers in the reduction of seizures in clinical trials (Kovalenko et al. 1984; Ogunmekan and Hwang 1989) and in in vivo studies (Jerrett et al. 1973; Rubin and Willmore 1980). However, little is known about the electrophysiological mechanisms underlying the seizure-like action of FR. The attempts to reproduce FR-induced seizures in in vitro systems were mostly unsuccessful. Various FR-generating compounds were reported to hyperpolarize RMP in different cell types (Kripeit-Drews et al. 1994; Muller et al. 1993; Seutin et al. 1995). H₂O₂ at 0.01% (2.94 mM) concentration in the presence of iron salts was shown to decrease synaptic efficacy and impair the ability of hippocampal neurons to produce action potentials (Pellmar 1987). The lack of in vitro evidence for FR involvement in development of seizures observed in vivo could be caused by the fact that the effects caused by short-lived and highly reactive FR on physiological systems would vary with concentration and time of exposure. For example, Muller et al. (1993) showed that 300 μM H₂O₂ produced an excitatory effect on CA3 pyramidal neurons in rat hippocampal slice cultures (because of a decrease in evoked IPSP amplitude). However, neither 2.94 mM nor 3.3 mM of H₂O₂ application resulted in hyperexcitability of hippocampal neurons (Pellmar 1987; Seutin et al. 1995). Also, chronic exposure of cultured mouse dorsal root ganglia neurons to low concentrations of H₂O₂ (0.47 mM) was found to increase the amplitude and duration of action potentials (Scott and Lew 1988).

The epileptogenic action of FR might be based on the specific neuronal circuitry. Epilepsy induced by FeCl₃ injection into the rat brain and attributed to LPO (Singh and Pathak 1990) resembled absence epilepsy. Therefore we hypothesized that one of the possible mechanisms of FR-in-
duced seizures might be the H$_2$O$_2$-induced hyperpolarization of thalamic neurons (reported to be one causative factor in absence epilepsy) (see Steriade and Contreras 1995). The results of our experiments did not confirm this hypothesis; we never observed spontaneous synchronized activity when recording cortical and thalamic field potentials in thalamocortical slices. Also, the membrane potential of thalamic cells was not hyperpolarized significantly to evoke sustained rebound responses mediated by low-threshold calcium currents (Jansen and Llinas 1984).

However, thalamic (VB) stimulation, after 5–9 min of H$_2$O$_2$ application, resulted in hyperexcitability at the cellular level observed with whole cell recordings from pyramidal cortical (layers IV–V) and VB thalamic neurons. These hyperexcitable events consisted either of slowly developing depolarizations with superimposed action potentials or EPSPs of increasing complexity and duration. Interestingly, no hyperexcitability was seen when recording from cortical neurons in brain slices deprived of direct thalamic input. This suggests that H$_2$O$_2$-mediated augmentation of cortical postsynaptic responses requires intact thalamocortical circuitry. This result might in part explain the elusive character of FR excitatory action in in vitro studies, where neuronal circuitry is not intact.

The hyperexcitable events observed during peroxide application could be attributed to the progressive decrease of inhibitory synaptic transmission, starting almost immediately after H$_2$O$_2$ exposure. H$_2$O$_2$ application for 6 min abolished inhibitory postsynaptic responses in 67% of thalamic and in 70% of cortical neurons. Also, H$_2$O$_2$ caused a transient enhancement of excitatory neurotransmission in thalamic neurons. The amplitude of EPSPs peaked twice, at 3.4 ± 0.1 min and 7.4 ± 0.3 min after H$_2$O$_2$ exposure. This enhancement can be explained in light of the experimental observations that FRs cause inhibition of glutamate uptake in synaptosomal preparations (Berman and Hastings 1997) as well as the increase (after 4 ± 1 min of exposure to t-butyl hydroperoxide) and then decrease (15 ± 1 min later) of Ca$^{2+}$ currents observed in the rabbit sinoatrial node (Sato et al. 1989). In general our observations suggest that the excitatory action of H$_2$O$_2$ was much more pronounced in thalamic than in cortical neurons. For example, EPSPs evoked by local stimulation were significantly enhanced in thalamic neurons but not in cortical cells. Also, three of seven thalamic neurons developed bursting after application of H$_2$O$_2$.

In other studies, inhibitory responses were also found to be particularly susceptible to a variety of insults known to be accompanied by massive production of FR. For example, studies in juvenile monkeys subjected to hypoxia (Sloper et al. 1980) and in monkeys with alumina cream epileptic foci (Ribak et al. 1979, 1982) suggested that inhibitory interneurons might be especially vulnerable to these types of insult. A similar sensitivity was shown by glycinergic inhibitory

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<tr>
<th>Time After H$_2$O$_2$ Exposure, min</th>
<th>EPSP (%)</th>
<th>n</th>
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<tbody>
<tr>
<td>3.4 ± 0.1</td>
<td>145.4 ± 18.6</td>
<td>6/6</td>
</tr>
<tr>
<td>7.4 ± 0.3</td>
<td>162.9 ± 29.6</td>
<td>5/6</td>
</tr>
<tr>
<td>9.9 ± 0.1</td>
<td>143.7 ± 29.0</td>
<td>5/6</td>
</tr>
<tr>
<td>Wash</td>
<td>92.3 ± 8.5</td>
<td>5/5</td>
</tr>
<tr>
<td>4 ± 0.5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE and represent excitatory postsynaptic potential (EPSP) amplitudes, measured from the peaks to the baseline, expressed as the averaged percentages of the EPSP amplitudes before H$_2$O$_2$ application. Four-minute wash partially reversed the effect (column 5).
interneurons in spinal cord induced by ischemia (Davidoff et al. 1967). It is tempting to speculate that decrease of inhibitory neurotransmission reported in these studies was induced by FR-mediated reactions. The reversibility of H2O2-mediated effects (see Pellmar 1986, 1987) is difficult to explain. Complete depletion of the antioxidant glutathione in hippocampal slices prevented any recovery from the hydrogen peroxide damage, while not enhancing its effects (Pellmar et al. 1992). It is conceivable that antioxidative defense mechanisms might be capable of fast repair of the oxidative damage, reported to be reversible (e.g., accumulation of membrane lipid peroxides leading to increase and then decrease of membrane fluidity) (see Meerson 1991). These observations suggest that reversible effects of hydrogen peroxide (e.g., alterations of neurotransmission) are mediated by modified lipid-protein interactions rather than irreversible protein oxidation.

The passive membrane properties in thalamic and cortical neurons were also altered by H2O2; input resistance and membrane time constant in thalamic and cortical neurons were decreased. Time course of input resistance changes as well as opposite effects of H2O2 on IPSP (decrease) and EPSP (increase) magnitude do not suggest that changes in neurotransmission were caused by changes in membrane resistance (significant changes of membrane input resistance were observed after 10 min of H2O2 superfusion, whereas alterations of synaptic responses were registered between 3 and 4 min after the beginning of H2O2 application). Input impedance changes during peroxide perfusion revealed the elimination of the characteristic low-pass filter behavior in both types of neurons. This suggests that, as FR-mediated reactions continue, neurons could become equally responsive to high and low frequencies. Loss of inhibitory input, amplified by temporary enhancement of excitatory neurotransmission in thalamic neurons, might predispose an otherwise normal thalamocortical circuitry to seizure activity.

In summary, these results suggest that FR formation might contribute to the development of seizures in thalamocortical circuitry by attenuation of inhibitory neurotransmission, transient enhancement of excitatory neurotransmission, and changes in the intrinsic properties of neuronal membranes, eliminating the low-pass filter characteristics.

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