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Biphasic effect of hydrogen peroxide on field potentials in rat hippocampal slices

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Abstract

In the CA1 region of rat hippocampal slices, H_2O_2 (0.294–2.94 mM) caused initial augmentation, and subsequent long-lasting depression, of population spikes and excitatory postsynaptic potentials. The effect of H_2O_2 may not be mediated by its degradation product, hydroxyl radicals, because an iron chelator deferoxamine did not block the effect. A catalase inhibitor 3-amino-1,2,4-triazole only modestly attenuated the initial augmentation, suggesting that the effect of H_2O_2 is not attributable to catalase-dependent O_2 generation, either. An *N*-methyl-D-aspartate receptor antagonist DL-2-amino-5-phosphonovaleric acid had no influence on the effect of H_2O_2, whereas a γ-aminobutyric acid type A receptor channel blocker picrotoxin attenuated long-lasting depression, indicating that γ-aminobutyric acid-mediated inhibition is altered during the depression phase. The initial augmentation but not subsequent depression was attenuated by a phospholipase A_2/C inhibitor 4-bromophenacyl bromide, suggesting the involvement of lipid signaling molecule(s) in the enhancement of excitatory synaptic transmission. These results suggest that H_2O_2 regulates hippocampal synaptic transmission via multiple mechanisms. © 1997 Elsevier Science B.V.

Keywords: Free radical; H_2O_2; Hippocampus; CA1 region; Synaptic plasticity

1. Introduction

Free radicals and reactive oxygen species are continuously generated in the course of normal cellular metabolism but are well controlled by intrinsic detoxifying enzyme systems and antioxidants. Under certain pathological conditions, however, this balance can be disrupted. Brain tissue is particularly vulnerable to oxidative stress because of large amounts of oxygen consumption and high contents of easily oxidized constituents such as poly-unsaturated fatty acids. Oxidative insults are implicated in many pathological processes in the brain, including acute injuries like ischemia and trauma, and more slowly progressing disorders like Parkinson's and Alzheimer's disease (Halliwell, 1992; Coyle and Puttfarcken, 1993).

On the other hand, accumulating evidence suggests that reactive oxygen species such as hydrogen peroxide (H_2O_2) play important roles in physiological cellular events as intermediates of intracellular and/or intercellular signaling (Lander, 1997). Indeed, neuronal functions assessed by electrophysiological measurements are shown to be directly affected by reactive oxygen species (Pellmar, 1987; Pellmar et al., 1989; Muller et al., 1993; Seutin et al., 1995). Furthermore, several species such as nitric oxide and carbon monoxide are implicated in the underlying mechanisms of long-lasting changes in synaptic transmission, candidate processes of information storage during learning (Zhuo et al., 1993). In the present study we describe novel, complex actions of H_2O_2 on evoked excitatory field potentials in the CA1 region of hippocampal slices.

2. Materials and methods

Hippocampal slices were prepared from young adult (4–7 weeks old) male Wistar rats. After decapitation, the brain was rapidly removed, hippocampi were dissected and placed in gassed (95% O_2/5% CO_2) standard extracellular solution containing 124 mM NaCl, 5 mM KCl, 2.4 mM $CaCl_2$, 1.3 mM $MgSO_4$, 1.24 mM KH_2PO_4, 26 mM $NaHCO_3$ and 10 mM D-glucose. Transverse slices (400–450 μm thick) were cut with a microslicer DTK-2000

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(Dosaka EM, Kyoto, Japan). Slices were then maintained in an incubation chamber for at least 1 h at 37°C in the standard solution. At the time of an experiment, individual slices were transferred to a submersion recording chamber where they were constantly perfused with the standard solution (2.5 ml/min) at 37°C.

Extracellular recordings were obtained from the pyramidal cell layer of the CA1 region using 5 to 10 MΩ glass electrodes filled with 0.9% NaCl. A bipolar electrode was placed on the stratum radiatum to stimulate Schaffer collateral/commissural pathway. Stimuli of 50 μs in duration were applied every 30 s. The stimulus intensity was set to evoke 50% of the maximal amplitude of field population spikes. In several experiments, field excitatory postsynaptic potentials (EPSPs) were recorded with a glass electrode placed on the apical dendritic layer of the CA1 region. Recorded responses were amplified, temporarily stored on a storage unit EA-602J (Nihon Kohden, Tokyo, Japan) and written out on a chart recorder. The amplitude of population spikes or EPSPs was measured from the chart paper using a digitizing tablet. Data are presented as mean ± S.E.M.

DL-2-Amino-5-phosphonovaleric acid (APV), 3-amino-1,2,4-triazole and deferoxamine were obtained from Sigma (St. Louis, MO, USA). All other reagents and chemicals were obtained from Wako (Osaka, Japan).

3. Results

Fig. 1 shows the effects of H_2O_2 on population spikes recorded from the CA1 pyramidal cell layer, and EPSPs recorded from the CA1 apical dendritic layer. Bath application of H_2O_2 (1.47 mM; 0.005%) for 20 min induced biphasic changes in the amplitude of population spikes. The field responses were initially augmented, which continued in a steady-state level during the presence of H_2O_2 in the perfusing solution. After washout of H_2O_2, the responses were promptly depressed and gained a new steady-state level below the baseline (Fig. 1A and B). Similar results were obtained when the amplitude of EPSP was measured in the same experimental conditions (Fig. 1A and C).

These biphasic effects were evident when H_2O_2 was applied at concentrations of 0.294 mM or higher. The initial augmenting effect was most prominent at 1.47 mM H_2O_2; higher or lower concentrations of H_2O_2 resulted in smaller degrees of augmentation (Fig. 2A). On the other hand, the magnitude of subsequent depression was similar at a range of 0.294–2.94 mM. Depression caused by 0.294 mM H_2O_2 was partially reversible after 30 min of washout, although higher concentrations of H_2O_2 induced stable, long-lasting depression of population spikes (Fig. 2B).

We tested the influences of duration of H_2O_2 application. When H_2O_2 (1.47 mM) was perfused for 10 min, subsequent depression of population spikes was transient,

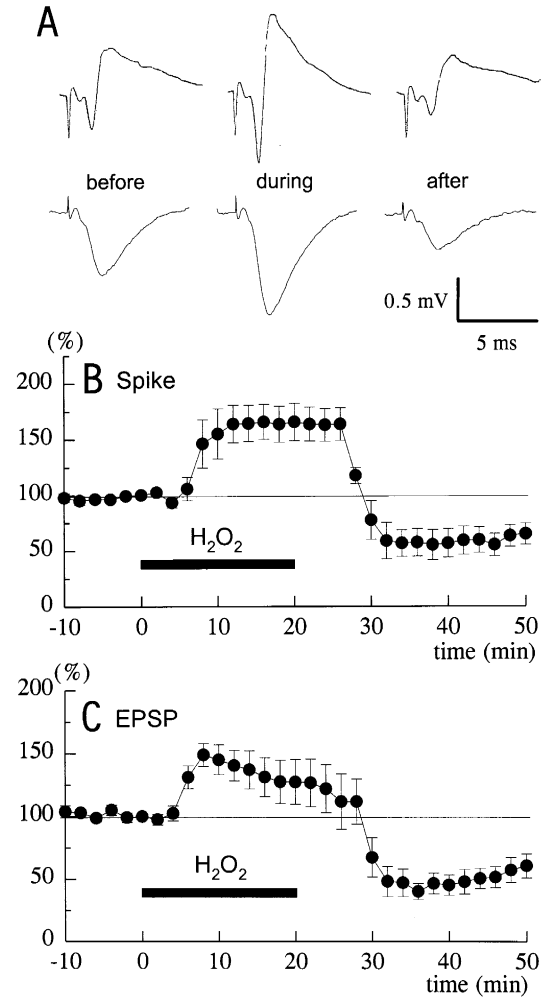

Fig. 1. (A) Representative traces of population spikes (upper) and EPSPs (lower) before, during and 30 min after application of 1.47 mM H_2O_2 for 20 min. (B) and (C) Time-courses of the changes in the amplitudes of population spikes (B, $n = 5$) and EPSPs (C, $n = 8$) induced by bath application of 1.47 mM H_2O_2. H_2O_2 was applied during the time indicated by bars.

and the amplitude showed recovery within 30 min after washout. More prolonged (over 20 min) perfusion resulted in permanent depression of the responses (Fig. 3A). When H_2O_2 was applied for 40 min or longer, depression of the population spike was already evident during the prolonged perfusion of H_2O_2 (Fig. 3B), suggesting that the depression phase is not dependent on the removal of H_2O_2.

In the next set of experiments, we examined whether the effects of H_2O_2 are mediated by its degradation products. Hydroxyl radicals can be generated through the Fenton reaction; H_2O_2 reacts with Fe^{2+} to produce this extremely reactive species responsible for covalent modification and damages to macromolecules (Coyle and Puttfarcken, 1993). If hydroxyl radicals are involved, chelation of endogenous Fe^{2+} by a potent iron chelator deferoxamine should alter the effect of H_2O_2. However, pretreatment of slices with 20 μM deferoxamine failed to block the effect

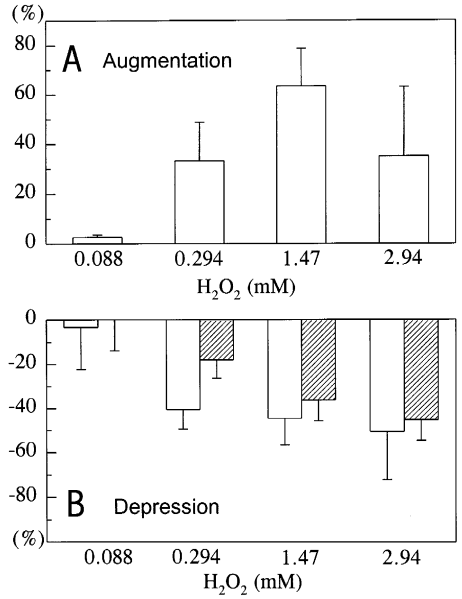

Fig. 2. Concentration dependence of the effects of H_2O_2 on the population spike amplitude. H_2O_2 was applied for 20 min at indicated concentrations. Values shown are percent increases observed at the end of H_2O_2 application (A) and percent decreases observed at 10 min (open columns) and 30 min (hatched columns) after washout (B). $n = 4–5$ for each condition.

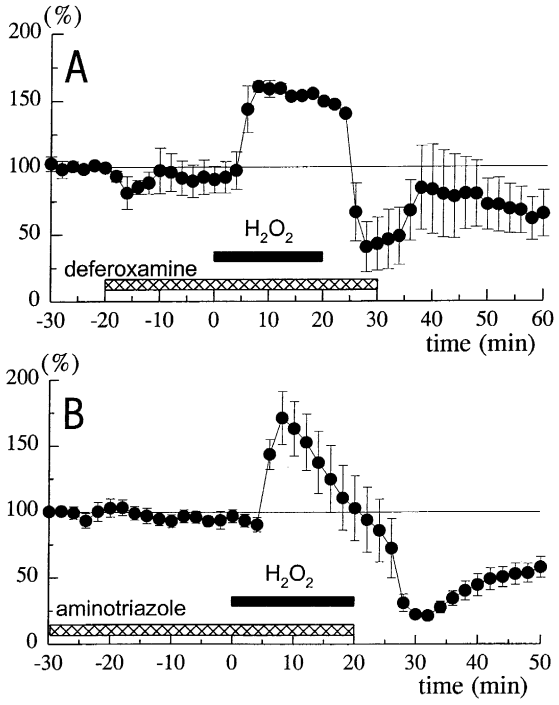

Fig. 4. Effects of deferoxamine (A, $n = 4$) and 3-amino-1,2,4-triazole (B, $n = 7$) on H_2O_2 (1.47 mM)-induced changes in population spike amplitude. Deferoxamine (20 μM) and 3-amino-1,2,4-triazole (10 mM) were applied during the time indicated by a hatched bar.

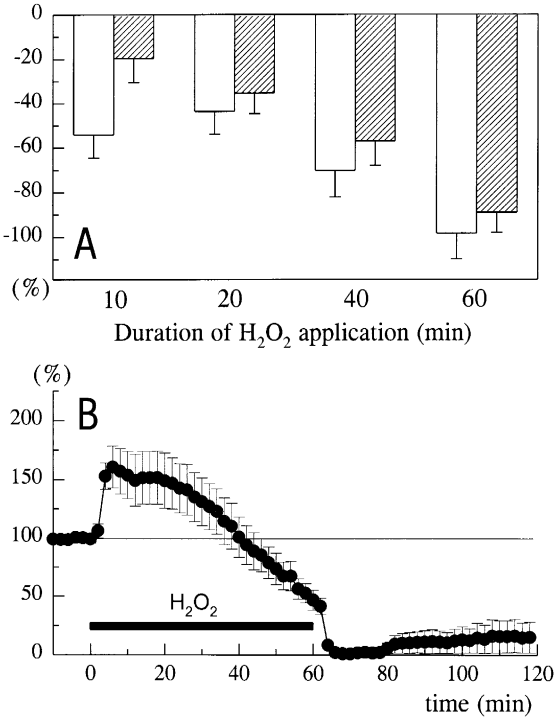

Fig. 3. Prolonged application of H_2O_2 results in large magnitudes of depression. (A) H_2O_2 (1.47 mM) was applied for indicated durations. Values are percent decreases in population spike amplitude observed at 10 min (open columns) and 30 min (hatched columns) after washout of H_2O_2. $n = 4–5$ for each condition. (B) Time-course of the changes in population spike amplitude induced by 60 min application of 1.47 mM H_2O_2, $n = 5$.

of H_2O_2. Both augmenting and depressing actions of H_2O_2 on population spikes were similarly observed in the presence of deferoxamine (Fig. 4A).

Another possibility is that H_2O_2 may support synaptic transmission by serving as a source of molecular oxygen O_2 (Llinas and Sugimori, 1980). This is due to the property of H_2O_2, depending on catalase activity intrinsic to brain tissues (Walton and Fulton, 1983), to be degraded into O_2 and H_2O. Therefore, we used a catalase inhibitor 3-amino-1,2,4-triazole to investigate whether the actions of H_2O_2 on population spikes were mediated by catalase-dependent O_2 generation from H_2O_2. As shown in Fig. 4B, the only difference observed in slices treated with 3-amino-1,2,4-triazole was that augmentation during H_2O_2 application was not persistent and the amplitude of population spikes gradually declined to the level at or below the baseline. However, the magnitude of initial augmentation at the beginning of H_2O_2 application and that of enduring depression after the washout of H_2O_2 were not significantly different from those observed in control slices (Fig. 1B). These results indicate that the effects of H_2O_2 are not attributable to catalase-dependent O_2 generation, although endogenous catalase does modulate the actions of H_2O_2.

The modulation of evoked field potentials could result from alteration of transmission efficacy either at excitatory synapses or at inhibitory synapses. To clarify these alternatives, H_2O_2 application was performed in the presence of picrotoxin (50 μM), a blocker of $GABA_A$ receptor chan-

nels. As shown in Fig. 5A, picrotoxin had no effect on the augmentation of population spikes during application of H_2O_2. In contrast, depression observed after washout of H_2O_2 was affected by picrotoxin. Although the transient component of depression shortly after H_2O_2 washout was accelerated, the sustained component long after washout was attenuated. The amplitude of population spikes gradually returned to the level near the baseline. Thus, H_2O_2-induced augmentation may be the consequences of the enhancement of excitatory synaptic transmission, whereas long-lasting depression by H_2O_2 may involve alteration of transmission efficacy at inhibitory synapses.

The augmentation by H_2O_2 seems to be different from long-term potentiation of synaptic transmission, a well-known form of synaptic plasticity at hippocampal excitatory synapses. First, increases in field responses were observed only during application of H_2O_2 and disappeared promptly after washout, which is not consistent with the characteristic persistence of electrically-induced long-term potentiation. Second, although the activation of NMDA receptors is critical for the induction of long-term potentiation by electrical stimulation in CA1 synapses (Bliss and Collingridge, 1993), a selective NMDA receptor antagonist APV (50 μM) showed no effect on the magnitude of augmentation induced by H_2O_2 (Fig. 5B). In addition, the depression of population spikes observed after washout of H_2O_2 was not altered by APV, either.

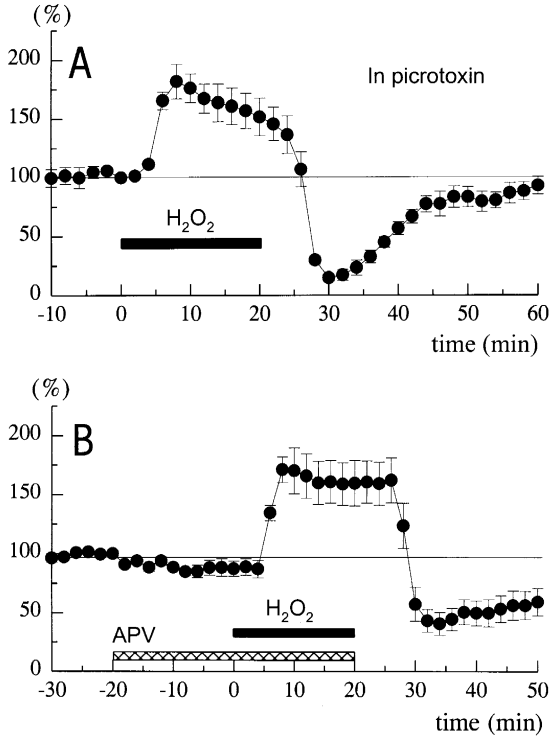

Fig. 5. Effects of picrotoxin (A, $n = 5$) and DL-2-amino-5-phosphonovaleric acid (APV) (B, $n = 5$) on H_2O_2 (1.47 mM)-induced changes in population spike amplitude. Picrotoxin (50 μM) was present during the entire course of experiments, whereas APV (50 μM) was applied during the time indicated by a hatched bar.

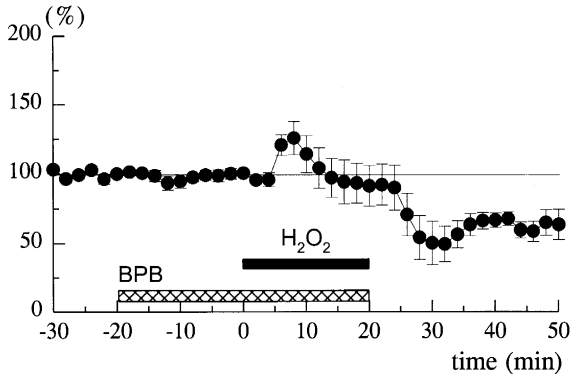

Fig. 6. Effect of 4-bromophenacyl bromide (20 μM) on H_2O_2 (1.47 mM)-induced changes in population spike amplitude ($n = 5$). 4-Bromophenacyl bromide was applied during the time indicated by a hatched bar.

It is becoming increasingly evident that various kinds of cellular biochemical processes are influenced by reactive oxygen species. Among them is phospholipase A_2, which is reported to be activated by reactive oxygen species (Goldman et al., 1992). Considering the evidence that several products of phospholipases, such as arachidonic acid and its metabolites, directly affect synaptic transmission at central synapses (Drapeau et al., 1990), we speculated that phospholipase-mediated processes may be involved in the effects of H_2O_2. Accordingly, we examined the effect of a phospholipase C/A_2 inhibitor 4-bromophenacyl bromide on H_2O_2-induced changes in excitatory field responses. Slices treated with 4-bromophenacyl bromide (20 μM) prior to and during the application of H_2O_2 (1.47 mM) showed only small, transient augmentation of population spikes during the presence of H_2O_2 (Fig. 6). In contrast, depression after washout of H_2O_2 was not affected by treatment with 4-bromophenacyl bromide.

4. Discussion

The present results demonstrate that H_2O_2 affects evoked field responses in the hippocampal CA1 region. The effect apparently consisted of two phases: initial augmentation and subsequent long-lasting depression.

The initial augmenting effect was not influenced by blockade of $GABA_A$ receptor channels, indicating that it results from the enhancement of synaptic transmission at excitatory synapses. Earlier studies have shown that H_2O_2 can support synaptic transmission in brain slice preparations, even in the absence of added O_2 (Llinas and Sugimori, 1980; Walton and Fulton, 1983). This effect is exerted by generation of O_2 from H_2O_2, and is entirely

dependent on catalase activity intrinsic to the tissues (Walton and Fulton, 1983). In this study, we observed that the inhibition of endogenous catalase by 3-amino-1,2,4-triazole prevented the persistence of augmentation during H_2O_2 application, which suggests that endogenous catalase does play some role in controlling the modulatory effects of H_2O_2 on synaptic transmission. However, pretreatment with 3-amino-1,2,4-triazole failed to block initial augmentation at the beginning of H_2O_2 application. This result indicates that O_2 supply by catalase-dependent degradation is unlikely to be a primary mechanism of the augmenting effect of H_2O_2. Moreover, deferoxamine did not affect H_2O_2-induced augmentation, demonstrating that the hydroxyl radical is not involved in this effect. Taken together, these results suggest that H_2O_2 itself, rather than its degradation products, is responsible for the augmentation of excitatory synaptic transmission.

On the other hand, phospholipase A_2/C inhibitor 4-bromophenacyl bromide largely attenuated the augmenting effect of H_2O_2. In several preparations, reactive oxygen species including H_2O_2 have been demonstrated to activate phospholipase A_2 (Goldman et al., 1992; Rao et al., 1993). Lipid signaling molecules such as arachidonic acid and platelet-activating factor are generated by phospholipase A_2 activity, and can regulate the efficacy of hippocampal synaptic transmission (Drapeau et al., 1990; Clark et al., 1993). Therefore, it is likely that these lipid mediators are involved in the augmentation of synaptic responses induced by H_2O_2. In this context, increase in spontaneous miniature excitatory postsynaptic currents induced by a brief period of anoxia is blocked by 4-bromophenacyl bromide (Katchman and Hershkowitz, 1994), suggesting that phospholipase products, possibly arachidonic acid and/or its metabolites, can augment excitatory synaptic transmission.

Depression observed after washout of H_2O_2 was long-lasting, when sufficient concentrations of H_2O_2 and durations of application were employed. This long-lasting depression was not attenuated either by an iron chelator or by a catalase inhibitor, leaving the possibility that it is not due to non-specific deterioration of tissue viability caused by oxidative injury. It is interesting to note that H_2O_2 induces a long-lasting inhibition of Ca^{2+}-dependent glutamate release from cerebrocortical synaptosomes even in the presence of deferoxamine, therefore, apparently without damaging the synaptosomes (Zoccarato et al., 1995). In addition, picrotoxin blocked the persistence of depression, indicating that alterations in GABA-mediated inhibitory control are at least in part involved in the long-lasting depression of synaptic responses observed after H_2O_2 treatment. The exact mechanisms still remain unclear and require further clarification: although arachidonic acid or its metabolites are reported to participate in long-lasting depression of synaptic transmission under certain conditions (Bolshakov and Siegelbaum, 1995; Normandin et al., 1996), this may not be the case with the present observations because 4-bromophenacyl bromide had no effect on H_2O_2-induced depression.

Our present results are not consistent with those reported by Pellmar and colleagues, who examined the effects of H_2O_2 on field potentials in the CA1 region of guinea pig hippocampal slices (Pellmar, 1987; Pellmar et al., 1989). First, they have not described an augmenting action of H_2O_2. Second, although they found that H_2O_2 decreased synaptic responses, in their studies deferoxamine blocked the effect of H_2O_2, leading them to the conclusion that hydroxyl radicals are responsible for this effect. Reasons for these discrepancies are not clear: besides the difference in species of animals, several subtle differences in experimental conditions such as temperature (30°C vs. 37°C in the present study) may be taken into consideration. For example, the involvement of nitric oxide in the induction of hippocampal synaptic plasticity is reported to be strictly dependent on temperature (Williams et al., 1993).

In conclusion, we have demonstrated that synaptic transmission at hippocampal CA1 synapses is significantly modulated by H_2O_2. This phenomenon may be relevant to pathological processes where reactive oxygen species are massively produced, and physiological processes such as information storage where enduring changes in transmission efficacy are involved.

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