A Role for Superoxide in Protein Kinase C Activation and Induction of Long-term Potentiation*

(Received for publication, March 5, 1997, and in revised form, November 6, 1997)

Eric Klann‡§, Erik D. Roberson¶, Lauren T. Knapp¶, and J. David Sweatt‡§

From the ‡Department of Neuroscience and the ¶Center for the Neural Basis of Cognition, University of Pittsburgh, Pittsburgh, Pennsylvania 15260 and the †Division of Neuroscience, Baylor College of Medicine, Houston, Texas 77030

The Journal of Biological Chemistry Vol. 273, No. 8, Issue of February 20, pp. 4516–4522, 1998

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Printed in U.S.A.

Ca2+-dependent protein kinases are involved in the induction of LTP (5, 6). The activation of protein kinase C (PKC) is one of the requisite biochemical steps necessary for the induction of LTP, as selective PKC inhibitors can block induction of LTP (5, 7, 8). Furthermore, both the second messenger-independent activity of PKC (autonomous activity; see Ref. 9) and total, cofactor-stimulated PKC activity (cofactor-dependent activity; see Refs. 9 and 10) are increased shortly after LTP-inducing HFS. Mechanisms that have been proposed for the activation of PKC immediately following the induction of LTP (15 s to 2 min after the final HFS) include translocation of PKC to the membrane (11, 12), conformational changes in PKC that result in the unmasking of activator sites (10), and decreases in protein phosphatase activity that might result in increased phosphorylation and activity of PKC (10).

An additional mechanism that might be involved in the activation of PKC during the induction of LTP is an oxidative mechanism involving reactive oxygen species (ROS), particularly the superoxide anion (O₂⁻). A variety of evidence suggest this possibility. First, levels of superoxide may increase during the induction of LTP, as it has recently been demonstrated that NMDA receptor activation in area CA1 of hippocampal slices results in the production of superoxide (13). Second, if superoxide levels increase, PKC is a possible target, given that treatment of several different types of cells and of hippocampal homogenates with superoxide or other ROS has been shown to increase both autonomous and cofactor-dependent PKC activity (14–16). Coupled with recent studies implicating superoxide dismutase (SOD), which catalyzes removal of superoxide, in neuronal function and the neuropathology of Alzheimer’s disease (17) and amyotrophic lateral sclerosis (18), these findings prompted us to determine whether superoxide was involved in the induction of LTP, and if so, to determine whether superoxide was involved in the regulation of PKC activity during LTP.

EXPERIMENTAL PROCEDURES

Preparation of Hippocampal Slices and Induction of LTP—Hippocampi from male Sprague-Dawley rats (100–150 g) were removed and 400-μm slices were prepared with a McIlwain tissue chopper. The slices were perfused for 1 h with a standard saline solution (124 mM NaCl, 4.4 mM KCl, 26 mM NaHCO₃, 10 mM d-glucose, 2 mM CaCl₂, 2 mM MgCl₂, gassed with 95% O₂, 5% CO₂, pH 7.4) in an interface tissue slice chamber at 32 °C. After 1 h, baseline responses to Schaffer collateral stimulation in the CA1 region were monitored for at least 20 min before LTP-inducing HFS to assure a stable baseline. Test stimuli were given at a frequency of 0.1 Hz, which produced 50% of the maximum population EPSP (usually between 30 and 40 μA). Responses were measured every 2.5 min as an average of four individual traces.

In our previous study (9), LTP-inducing HFS consisted of three sets of stimuli, each set was delivered 5 min apart. Each set included two 1-s trains of stimuli (100 Hz), given 20 s apart at the current needed to elicit a maximum population EPSP. Using this stimulus paradigm, cofactor-dependent PKC activity increased 2 min after the final HFS train. However, because this paradigm spanned a period of 10 min, the LTP-associated increase in cofactor-dependent PKC activity was actually measured 12 min after the first HFS train.

* This work was supported by National Institutes of Health Grants NS08950 (to E. K.), NS34007 (to E. K.), and MH46186 (to J. D. S.), National Institutes of Health Training Grant MH18273 (to L. T. K.), a University of Pittsburgh CRDF Award (to E. K.), grants from the McKnight Foundation (to J. D. S.), the Winters Foundation (to E. K.), and the Life and Health Insurance Medical Research Fund (to E. D. R.).

† To whom correspondence should be addressed: Dept. of Neuroscience, University of Pittsburgh, 446 Crawford Hall, Pittsburgh, PA 15260. Tel.: 412-624-4610; Fax: 412-624-9198; E-mail: klann@brain.bhs.pitt.edu.

‡§ This work was supported by National Institutes of Health Grants NS08950 (to E. K.), NS34007 (to E. K.), and MH46186 (to J. D. S.), National Institutes of Health Training Grant MH18273 (to L. T. K.), a University of Pittsburgh CRDF Award (to E. K.), grants from the McKnight Foundation (to J. D. S.), the Winters Foundation (to E. K.), and the Life and Health Insurance Medical Research Fund (to E. D. R.).

¶ The abbreviations used are: LTP, long-term potentiation; HFS, high frequency stimulation; NMDA, N-methyl-d-aspartate; PKC, protein kinase C; ROS, reactive oxygen species; SOD, superoxide dismutase; DMPO, 5,5-dimethylpyrrolinol 1-oxide; EPSP, excitatory postsynaptic potential; NOS, nitric oxide synthase; NO, nitric oxide; NOArg, N-nitro-arginine.
To determine changes in PKC activity that more accurately reflect changes during the induction of LTP, we modified our LTP-inducing HFS in this study to consist of three 1-s trains of stimuli (100 Hz), given 20 s apart at the current needed to elicit a maximal population EPSP (usually between 60 and 80 μA). In experiments in which PKC activity was assayed, slices were frozen 2 min after the final HFS train, dissected, and homogenized. In experiments in which LTP was examined, responses were measured every 2.5 min as an average of four individual traces for 1 h after the final HFS. Post-HFS responses were generated with the same test stimuli used prior to HFS. LTP was defined as an increase of 20% or greater in the initial EPSP slope.

Application of ROS Scavengers, Spin Trap, or NOS Inhibitor to Hippocampal Slices—Slices were prepared as described above. After a 1-h incubation in standard saline solution, hippocampal slices were perfused with standard saline solution containing SOD (Calbiochem), SOD/catalase (Calbiochem), DMPO (OMRF Spin Trap Source), or NOArg (Sigma) for at least 30 min prior to LTP-inducing HFS. In more than 90% of the experiments, slices given HFS in the presence of SOD, SOD/catalase, DMPO, or NOArg were compared with slices from the same animal given HFS in the absence of the agents in an adjacent recording chamber (LTP controls).

Protein Kinase C Assays—Autonomous and cofactor-dependent PKC activity in control and potentiated tissue from hippocampal area CA1 was determined using the selective PKC substrate NG(28–43), a substrate for activated PKC. PKC activity was assayed in the presence of 2 mM EGTA. Cofactor-dependent PKC activity was defined as activity assayed in the presence of 100 μM CaCl₂, 320 μM phosphatidylserine, and 30 μg/ml diocetylphosphatidylcholine, with the autonomous PKC activity subtracted (9). Expressed in terms of absolute specific activities, autonomous NG(28–43) phosphorylation was 0.65 ± 0.10 pmol/min/μg in control slices and 1.10 ± 0.33 pmol/min/μg in slices given LTP-inducing HFS; cofactor-dependent NG(28–43) phosphorylation was 7.07 ± 0.63 pmol/min/μg protein in control slices and 11.27 ± 1.30 pmol/min/μg protein in slices given LTP-inducing HFS.

RESULTS

Superoxide Modulates the Induction of LTP—The hypothesis that superoxide plays a role in the induction of LTP was investigated in area CA1 of rat hippocampal slices. LTP was induced with HFS consisting of three 1-s trains of 100-Hz stimulation delivered 20 s apart at a stimulus intensity that produced a maximum initial slope of the extracellular field excitatory postsynaptic potentials (EPSPs). This stimulus paradigm resulted in LTP in 96% (25 out of 26) of the control slices for the experiments shown in Fig. 1. The slope of the EPSPs was increased to 155.7 ± 4.0% (n = 26) of pre-HFS control levels (within-slice comparison) 60 min after the final HFS train (Fig. 1, A–C). To determine whether superoxide was involved in LTP induction, we gave slices HFS in the presence of the superoxide scavenger SOD. SOD is highly specific for superoxide and is not known to react enzymatically with other ROS (21). LTP was blocked in eight out of nine slices 60 min after the final HFS train (average slope of EPSPs = 101.0 ± 7.5% of control, n = 9) (Fig. 1A). In contrast, slices from the same animal in an adjacent recording chamber given HFS without SOD exhibited LTP in all nine experiments (average slope of EPSPs = 158.7 ± 7.6% of control, n = 9) (Fig. 1A). These data suggest that superoxide plays a role in the induction of LTP.

An alternative explanation of the results in Fig. 1A is that one of the products of the reaction between SOD and superoxide inhibits the induction of LTP. Specifically, SOD converts superoxide to H₂O₂ and O₂ (22), and H₂O₂ has been shown to inhibit LTP (23). To address this possibility, slices were given HFS in the presence of bath-applied SOD along with catalase, an enzyme which scavenges H₂O₂ and catalyzes its conversion to O₂ and water (22). In these experiments, LTP was inhibited in 10 out of 15 slices (average slope of EPSPs = 113.3 ± 4.7% of control for all 15 slices and 102.8 ± 2.4% of control for the 10 slices that did not meet our criteria for LTP) (Fig. 1B). In control experiments, LTP was produced in 12 out of 13 slices (average slope of EPSPs = 150.4 ± 4.7% of control, n = 13) (Fig. 1B). Interestingly, the five slices incubated with SOD and catalase that did exhibit LTP after HFS were potentiated to a lesser extent when compared with LTP control slices (average slope of EPSPs = 134.6 ± 7.6% of control, n = 5). Slices incubated with catalase alone exhibited LTP (average slope of EPSPs = 168.5 ± 12.5% of control, n = 3) similar to that observed in LTP control slices (average slope of EPSPs = 166.6 ± 7.4% of control, n = 3). Our interpretation of these data is that the effects of SOD on LTP induction are not solely a result of increased levels of H₂O₂. However, our findings do not exclude the possibility that H₂O₂ not removed by catalase may contribute to the observed effects of SOD on LTP.

As mentioned, SOD is a specific enzyme not known to catalyze the removal of any ROS besides superoxide. However, SOD, like most other proteins, contains histidine, tryptophan, and methionine, each of which can react nonenzymatically with other ROS (21). To demonstrate that the effects of SOD are not due to these nonenzymatic reactions with other ROS, we incu-
NMDA receptor-dependent, as they were blocked completely by 50 mM MgCl₂ and 4 mM CaCl₂. The EPSPs recorded in this manner were intensity used for the HFS; data from SOD/catalase slices were then to the amplitude of the EPSP produced by a single pulse at the stimulus measurement (not shown). Measurements in all slices were normalized was made for the first and the second half of the HFS separately; no over the last 50 ms of the HFS). In addition, the integral measurement was made for the same animal which received LTP-inducing HFS in an identical adjacent recording chamber. LTP was significantly attenuated by DMPO (p < 0.005, paired Student’s t test).

Although the concentrations of SOD (121 units/ml) and catalase (260 units/ml) we used have been shown in previous studies to have no effect on baseline electrophysiological responses in area CA1 of guinea pig hippocampal slices (24), we performed a series of experiments to verify that the superoxide scavengers used in our studies did not have nonspecific effects on synaptic transmission. We observed no alteration of electrophysiological responses to test stimuli in area CA1 in rat hippocampal slices exposed to SOD and catalase (Fig. 2A). Moreover, SOD and catalase had no effect on the induction of LTP in control slices (average slope of EPSPs 162.3 ± 11.3% of control, n = 4) (Fig. 1C). Thus, the effects of SOD and catalase on LTP are not attributable to nonenzymatic removal of ROS other than superoxide.

To demonstrate that a structurally and functionally distinct superoxide scavenger would also inhibit LTP induction, we incubated slices with boiled SOD and catalase and then examined their effect on the induction of LTP. In slices incubated with the heat-inactivated enzymes, LTP was indistinguishable (average slope of EPSPs 154.4 ± 9.4% of control, n = 4) (Fig. 1C). Thus, the effects of SOD and catalase on LTP are not due to nonspecific effects on synaptic transmission. We observed no alteration of electrophysiological responses to test stimuli in area CA1 in rat hippocampal slices exposed to SOD and catalase (Fig. 2A). Moreover, SOD and catalase had no effect on high-frequency synaptic transmission, as measured by either the total depolarization or the steady-state depolarization produced during HFS (Fig. 2C). These results suggest that inhibition of LTP induction by superoxide scavengers is not due to effects on baseline synaptic transmission or NMDA receptor function. Overall, these data suggest that superoxide has a role in modulating the induction of LTP in area CA1.

**Fig. 2.** Effect of SOD and catalase on baseline synaptic transmission and the NMDA receptor-mediated component of the EPSP. A, stable baseline responses were recorded for 20 min before slices were incubated with SOD (121 units/ml) and catalase (260 units/ml) for 60 min. Responses were recorded for additional 20 min after washout of SOD and catalase (n = 4). B, NMDA receptor-mediated EPSPs were isolated in 20 μM 6-cyano-7-nitroquinoxaline-2,3-dione, 0 mM MgCl₂, and 4 mM CaCl₂. The EPSPs recorded in this manner were NMDA receptor-dependent, as they were blocked completely by 50 μM APV (left). Representative traces taken during baseline recording (a) and after APV application (b) are shown at the top (calibration bars: 2 mV and 4 ms) and ensemble averages (n = 7) at the bottom. Addition of SOD (121 units/ml) and catalase (260 units/ml) for 60 min had no effect on NMDA receptor-mediated EPSPs (right). Representative traces during baseline (a) and after SOD/catalase (b) are at the top and ensemble averages (n = 7) at the bottom. C, after recording stable baseline responses for 10 min in standard saline solution, slices were stimulated with LTP-inducing HFS, as described under “Experimental Procedures.” Left, the responses to the first HFS were analyzed by both integrating the entire HFS-response trace (Integral) and measuring the level of steady-state depolarization during HFS (SS Depol; averaged over the last 50 ms of the HFS). In addition, the integral measurement was made for the first and the second half of the HFS separately; no differences between control and SOD/catalase were observed in either measurement (not shown). Measurements in all slices were normalized to the amplitude of the EPSP produced by a single pulse at the stimulus intensity used for the HFS; data from SOD/catalase slices were then expressed as percent of control (n = 3). Right, representative HFS response traces from control and SOD/catalase slices, and the two traces overlaid (calibration bar: 2 mV and 90 ms).

**Fig. 3.** Effect of DMPO on the induction of LTP in area CA1. Stable baseline responses were recorded for 20 min before slices were given HFS. The open squares in each panel are ensemble averages from control LTP experiments (n = 5). The closed squares are ensemble averages from slices given LTP-inducing HFS with 10 mM DMPO present (n = 5). LTP was inhibited in three out of five experiments in which DMPO was present in the bath. Hippocampal slices given LTP-inducing HFS in the presence of DMPO were compared with a control slice from the same animal which received LTP-inducing HFS in an identical adjacent recording chamber. LTP was significantly attenuated by DMPO (p < 0.005, paired Student’s t test).
Superoxide Activates PKC and Modulates Induction of LTP

Figure 4: Effect of SOD and catalase on the maintenance of LTP in area CA1. Stable baseline responses were recorded for 20 min before LTP was induced. Fifteen min after the tetanus, SOD (121 units/ml) and catalase (260 units/ml) were washed into the chamber, and the potentiation monitored for the ensuing hour (n = 3).

Figure 5: Effect of SOD and catalase on LTP-associated increases in PKC activity. LTP-inducing HFS was delivered to the CA1 region of hippocampal slices that were assayed for either autonomous (A) or cofactor-dependent (B) PKC activity using NG(28–43) as an exogenous substrate. Experimental slices were compared with control slices from the same hippocampus. Autonomous and cofactor-dependent PKC activity are expressed as percent of control for slices given HFS (n = 12), and slices given HFS in the presence of bath applied SOD (121 units/ml) and catalase (260 units/ml) (n = 8). Error bars are S.E. for the indicated number of determinations. Significance was determined with a paired Student’s t test. * denotes p < 0.05.

(average slope of EPSPs = 160.4 ± 7.8% of control, n = 5). In addition, DMPO had no effect on electrophysiological responses to test stimuli (data not shown). These results are consistent with results observed with SOD and catalase and provide further evidence of a role for superoxide in the induction of LTP. We conclude from this series of experiments that removing superoxide reduces the probability of LTP induction and the magnitude of LTP when potentiation occurs. Because neither SOD, catalase, nor DMPO can cross cell membranes, these data also suggest that superoxide may act as a transcellular messenger in LTP.

The results of the experiments in Figs. 1 and 3 indicate that superoxide modulates the induction of LTP, but they do not address the possibility of a role for superoxide in the maintenance of LTP. To test this possibility, we induced LTP and added SOD and catalase 20 min after the final LTP-inducing HFS. Pre-established LTP was unaffected by addition of SOD and catalase when monitored up to 1 h after the addition of the enzymes (Fig. 4). These results indicate that superoxide modulates the induction, but not the maintenance, of LTP.

Superoxide Contributes to PKC Activation during LTP—We next sought to begin to elucidate the biochemical targets of superoxide in LTP. We began by testing the hypothesis that superoxide regulates PKC during the induction of LTP. We previously have shown that the induction of LTP is associated with increases in both autonomous (9) and cofactor-dependent PKC activity (9). To investigate a role for superoxide in regulating PKC activity during the induction of LTP, we first measured PKC activity after LTP-inducing HFS (see "Experimental Procedures"). Slices were frozen 2 min after the final train of HFS, dissected, and homogenized. Homogenates from control slices and slices given HFS were assayed for autonomous and cofactor-dependent PKC activity using the selective PKC substrate peptide NG(28–43) (9, 26, 27). In comparisons of slices given LTP-inducing HFS and control slices that received only test stimuli, we observed an increase in both autonomous and cofactor-dependent PKC activity 2 min after the HFS (183.4 ± 19.6 and 144.8 ± 11.6% of control, respectively, n = 12, p < 0.05) (Figs. 5, A and B). These results agree with previous work demonstrating that HFS results in an increase in both autonomous (9) and cofactor-dependent PKC activity (9, 10), and are consistent with physiological studies showing that blockade of PKC inhibits induction of LTP (5, 7, 8).

To test the hypothesis that superoxide mediates the increases in autonomous and cofactor-dependent PKC activity associated with the induction of LTP, we assayed PKC activity after incubating slices with SOD/catalase and delivering LTP-inducing HFS to area CA1. We observed an attenuated but significant increase in autonomous PKC activity in slices given HFS in the presence of SOD/catalase (146.8 ± 20.1% of control, n = 8, p < 0.05) (Fig. 5A). In contrast, the increase in cofactor-dependent PKC activity associated with the induction of LTP was blocked completely (91.4 ± 9.6% of control, n = 8) (Fig. 5B). In control experiments, addition of SOD/catalase to both the slices after LTP-inducing HFS and to the homogenization buffer had no significant effect on the LTP-associated increases in either autonomous PKC activity (177.5 ± 23.4% of control, n = 3) or cofactor-dependent PKC activity (160.3 ± 15.7% of control, n = 3). These results suggest that the increase in cofactor-dependent PKC activity associated with the induction of LTP is mediated either directly or indirectly by superoxide. Furthermore, these data suggest that superoxide contributes to the generation of autonomously active PKC, consistent with the hypothesis that multiple mechanisms of PKC activation are associated with the induction of LTP (9, 12, 28).

Nitric Oxide Also Contributes to PKC Activation during LTP—The results described above indicate that superoxide can modulate induction of LTP, possibly through interactions with PKC. In many systems, superoxide exerts its effects in conjunction with nitric oxide (NO) (see Ref. 22). Furthermore, it is known that inhibitors of nitric oxide synthase (NOS) modulate
the induction of LTP induced by many stimulus paradigms (29–34). In this study, slices given HFS in the presence of the NO synthase inhibitor N-nitro-arginine (NOArg, 100 μM) exhibited LTP (average slope of EPSPs = 135 ± 5% of control, n = 4). However, the slices were potentiated to a lesser extent (p < 0.05, paired Student’s t test) when compared with LTP control slices from the same animal in an adjacent recording chamber (average slope of EPSPs = 166 ± 10% of control, n = 4). Therefore, we next sought to determine whether NO might be involved in the regulation of PKC activity during the induction of LTP. To examine interactions between NO and PKC during the induction of LTP, hippocampal slices were incubated with NOArg (100 μM), given LTP-inducing HFS, and assayed for changes in PKC activity. As with superoxide scavengers, in the presence of NOArg we observed an attenuated but significant increase in autonomous PKC activity (141.4 ± 20.5% of control, n = 10, p < 0.05) (Fig. 6A), and complete inhibition of the increase in cofactor-dependent PKC activity (98.7 ± 14.6% of control, n = 10) (Fig. 6B) associated with the induction of LTP. These results suggest that activation of NOS and production of NO play an important role in the activation of PKC during the induction of LTP.

There is an alternative interpretation of these results, as NOS is known to produce superoxide as a by-product (35). If NOS were in fact the source of superoxide production during LTP induction, the requirement for NOS activity could reflect the need for superoxide, rather than a direct role for NO in activating PKC. To distinguish between these two possibilities, we tested whether hemoglobin, which binds NO avidly (36), but does not block effects of superoxide alone on PKC activity could block the changes in PKC activity associated with the induction of LTP. We delivered LTP-inducing HFS to hippocampal slices incubated with 20 μM hemoglobin and then assayed PKC activity. The increase in autonomous PKC activity associated with the induction of LTP was attenuated (146.7 ± 10.6% of control, n = 8, p < 0.05), as was the increase in cofactor-dependent PKC activity (120.4 ± 19.6% of control, n = 8, p > 0.05) in slices incubated with hemoglobin. We interpret these results to suggest that NO, like superoxide, plays an important role in PKC activation during the induction of LTP. However, it should be noted that hemoglobin can also bind other ROS, including superoxide, albeit with a lower affinity than NO. Therefore, we cannot completely rule out the possibility that hemoglobin is blocking effects of superoxide or another ROS on PKC activity. It is interesting to note that hemoglobin, like superoxide, blocks effects of superoxide or another ROS on PKC activity in this study. This suggests that NO, like superoxide, plays an important role in PKC activation and NO during LTP.

The results in this report provide evidence for a role for superoxide in the induction of LTP. Our experiments with superoxide scavengers indicate that superoxide increases the probability of LTP induction and the magnitude of LTP when it occurs. Moreover, the demonstration of a superoxide-dependent change in PKC activity during the induction of LTP provides evidence that superoxide levels increase during the induction of LTP. Thus, we have shown that superoxide is likely to be produced during the induction of LTP, and that it has both biochemical and physiologic effects.

The idea of a role for superoxide in synaptic plasticity is consistent with a number of findings from previous studies. First, NMDA receptor activation, a key step in most forms of LTP, results in the production of superoxide in area CA1 of hippocampal slices (13) and in other types of neurons (25). Second, activation of other glutamate receptors can produce superoxide through a number of biochemical pathways (37). In addition, superoxide increases the release of glutamate in hippocampal slices (38), which suggests a means by which it might modulate synaptic efficacy. Finally, it is interesting to note that although the intracellular form of Cu/Zn-SOD, SOD1, is abundantly expressed in the dentate gyrus and area CA3 of the hippocampus, it is expressed to a much lesser degree in area CA1, the hippocampal subregion studied herein (39); thus, area CA1 is likely to have less capacity for removal of superoxide, consistent with a physiologic role for superoxide in this region.

There are a variety of possible sources for superoxide production during LTP induction. First, NOS, which is known to be activated during the induction of LTP (33), is a source of superoxide (35). The fact that NOS inhibitors and superoxide scavengers have similar effects is consistent with a role for NOS in producing superoxide, although it is difficult to dissect this role of NOS from its required role in producing NO. Another possible source of superoxide in LTP is arachidonic acid metabolism. Superoxide is produced by lipoxygenase, which initiates leukotriene synthesis from arachidonic acid, and prostaglandin endoperoxide synthase, which initiates prostaglandin synthesis from arachidonic acid (40). Interestingly,
PKC activity suggests that these species may serve as trans-
these compounds to block the effects of superoxide and NO on
ing, as all are confined to the extracellular space. The ability of
changes in PKC activity is another interesting question. LTP is
active research.
Furthermore, peroxynitrite decomposes to form hydroxyl radi-
oxidant that mediates oxidation of protein sulfhydryls (46).

To form the highly reactive peroxynitrite intermediate, a potent
are known to react rapidly
form the highly reactive peroxynitrite intermediate, a potent
oxidant that mediates oxidation of protein sulfhydryls (46).
Furthermore, peroxynitrite decomposes to form hydroxyl rad-
cellular membranes, although it has been proposed that superoxide can
traverse cell membranes through anion channels. Consistent
with this notion, it has been reported that NMDA receptor
activation in cerebellar granule neurons results in the genera-
tion of superoxide that can be trapped extracellularly (25). An
alternative explanation for our results is that the scavenging
compounds could be acting as extracellular sinks to pull ROS
out of the postsynaptic neurons, preventing activation of PKC
postsynaptically.

Although ROS traditionally have been thought of as toxic
agents involved in cell injury, they recently have been specu-
lated to act as cellular messengers. This is evidenced by reports
showing that ROS increase: 1) the phosphorylation of the ribo-
somal S6 protein (51); 2) PKC activity in mouse epidermal JB6
cells (15); 3) tyrosine phosphorylation in human neutrophils
(52); and 4) p21<sup>ras</sup> GTPase activity in PC12 cells (53).
In addition, it has been shown that generation of hydrogen peroxide
is necessary for platelet-derived growth factor signal transduc-
(54) and may be involved in lysophosphatic acid signal transduc-
(55). To our knowledge, the results in this report are the first to demonstrate ROS regulation of protein kinase
activity in the brain and are consistent with the idea that ROS
act as normal cellular messengers under certain physiological
conditions.

Finally, our data are consistent with the hypothesis that ROS modulate the early expression of LTP via interactions with PKC. However, ROS also have been shown to inactivate protein phosphatases, including calcineurin (56). Calcineurin is a calcium/calmodulin-dependent protein phosphatase that has been shown to be necessary for NMDA receptor-dependent hippocampal long-term depression (57), a use-dependent decrease in synaptic strength. Thus, the production of ROS after induction of LTP, in addition to increasing PKC activity, might also serve to inactivate protein phosphatases such as cal-
cineurin, thereby preventing long-term depression and favoring
the expression of LTP.

In closing, the data in this report indicate that superoxide
modulates the induction of LTP, and that superoxide, in con-
junction with NO, mediates increases in PKC activity associ-
ated with the induction of LTP. These findings present an
interesting physiologic and biochemical context for the effects
of ROS in the nervous system.

Acknowledgments—We thank C. Wyatt and Dr. C. M. Powell for help
with electrophysiologic experiments and Drs. E. Aizenman and E.
Thiels for critically reading previous versions of this manuscript.

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