Involvement of Carbon Monoxide in Long-Term Potentiation in the Dentate Gyrus of Anesthetized Rats

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ABSTRACT—To elucidate the role of carbon monoxide (CO) in hippocampal long-term potentiation (LTP), the effect of an inhibitor of heme oxygenase, which produces carbon monoxide, was investigated in the dentate gyrus of anesthetized rats. Administration of zinc protoporphyrin IX (100 nmol, i.c.v.) 30 min before the tetanic stimulation of 30 pulses at 60 Hz attenuated the intensity of LTP. However, this drug did not affect the established LTP. These results suggest that CO participates in the generation of LTP in vivo.

Keywords: Heme oxygenase inhibitor, Zinc protoporphyrin IX, Hippocampus

Much evidence has established a major role for nitric oxide (NO) as a messenger molecule in the brain as well as in white blood cells and blood vessels (1). NO is a short-lived free radical gas that can activate soluble guanylyl cyclase. In 1993, Verma et al. proposed that carbon monoxide (CO) is also a neural messenger that activates guanylyl cyclase. CO can be formed by heme oxygenase through the metabolism of heme in many tissues of the body, including in the brain (2).

Long-term potentiation (LTP) of excitatory synaptic transmission in the hippocampus is a model of synaptic plasticity and is considered to be the cellular basis of learning and memory (3). LTP is triggered by activation of postsynaptic NMDA channels, and its maintenance requires both presynaptic and postsynaptic alterations of quantal character (4), indicating that a retrograde messenger must be sent from the postsynaptic neuron to presynaptic terminals. NO has been a good candidate for such a messenger (1). Recently, carbon monoxide has been nominated as a putative retrograde messenger in the Schaffer collateral-CA1 pyramidal cell synapses (5, 6). However, the role of CO in LTP in the dentate gyrus synapses has not yet been reported. In the present study, we investigated the possible involvement of CO in the generation of LTP in the dentate gyrus in vivo by testing the effects of a selective and potent heme oxygenase inhibitor, zinc protoporphyrin IX (ZnPP; Aldrich Chemical Co., Milwaukee, WI, USA).

Evoked potentials were recorded as described in detail in our previous papers (7, 8). Male Wistar rats (7- to 9-week-old) were anesthetized with a combination of urethane (1 g/kg, i.p.) and α-chloralose (25 mg/kg, i.p.) and then fixed in a stereotaxic frame. The perforant path (8.1 mm posterior to the bregma, 4.4 mm lateral to the midline, approximately 3.0 mm ventral to the dura) was stimulated with a pair of electrodes, and evoked population spikes were recorded extracellularly from the granular cell layer of the ipsilateral dentate gyrus (3.5 mm posterior, 2.0 mm lateral, approximately 3.5 mm ventral to the bregma) (9). A single test stimulus (0.08-msec duration) was given at an interval of 30 sec, and the evoked population spike was recorded extracellularly. The amplitude of the population spike was defined as the mean of the first negative deflection (the difference between the positive peak and the following negative peak) and the positive deflection (the difference between the negative peak and the second positive peak) (10). Stimulus intensity was set to a level that produced a population spike of about 50% of the maximum amplitude. ZnPP was diluted to the desired concentrations with dimethyl sulphoxide (DMSO). After the response became stable, 5 μl of the desired concentration of the drug was injected into the contralateral ventricle (0.8 mm posterior to the bregma, 1.5 mm lateral to the midline, 4.5 mm ventral to the dura) using a microsyringe (9). The responses to test stimuli were observed for 30 min so that the drug would diffuse thoroughly and ensure stability of the baseline responses. Brief tetanic stimulation (30 pulses at 60 Hz) was applied at the same stimulus intensity 30 min after the injection of the drug, and the responses to test stimuli were recorded

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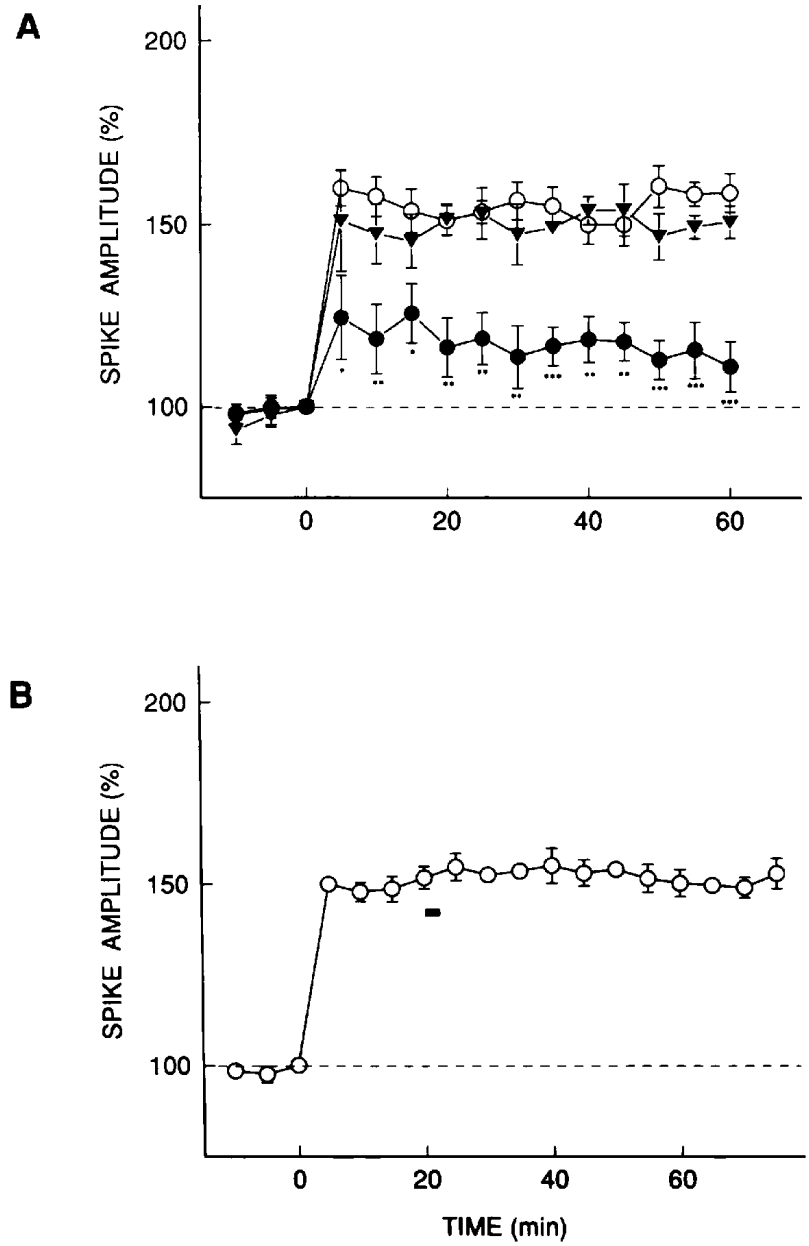

Fig. 1. Effects of zinc protoporphyrin IX (ZnPP) on long-term potentiation (LTP) in the dentate gyrus of anesthetized rats. A: tetanus-induced potentiation of the population spike in the dentate gyrus of rats injected i.c.v. with vehicle (○, n=6), 40 nmol ZnPP (▼, n=4) or 100 nmol ZnPP (●, n=5). The vehicle or the drug solution was injected 30 min before the tetanus. The abscissa indicates the time in min after tetanic stimulation (30 pulses at 60 Hz). The ordinate indicates the spike amplitude expressed as a percentage of baseline values at 0 min (immediately before tetanic stimulation). Significance of differences was determined by Student's *t*-test following Bartlett's test. *$P<0.05$, **$P<0.01$, ***$P<0.001$ vs. the vehicle-injected group. B: effect of 100 nmol ZnPP on established LTP (n=4). Drug was injected 20 min after tetanus-application (indicated by a black bar). All data represent the means±S.E.M.

for 60 min.

When tetanic stimulation was applied in the vehicle-injected group, the amplitude of the population spike was greatly potentiated, and LTP was generated in all of the six animals tested (Fig. 1A). The shape of the population spike and the magnitude of LTP were hardly different between the intact group and the vehicle-injected group (data not shown). Injection of 100 nmol ZnPP did not influence the amplitude of the evoked population spike before the tetanic stimulation, but significantly attenuated the generation of LTP after the tetanus (Fig. 1A). ZnPP (100 nmol) did not affect the basal spike amplitude nor

shape (n=2, data not shown). We also investigated the effect of ZnPP after application of tetanus. LTP was induced by application of tetanic stimulation and 100 nmol ZnPP was administered 20 min after the tetanus. As shown in Fig. 1B, ZnPP did not affect the established LTP.

We have shown for the first time that a heme oxygenase inhibitor blocks the generation of LTP in the dentate gyrus and that this heme oxygenase inhibitor is capable of affecting LTP in vivo. The data support the previous finding that CO is involved in LTP in the CA1 region in vitro by two groups (5, 6). CO is probably produced in the early state of LTP, e.g., during the tetanus, and triggers the generation of LTP. This consideration is supported by the finding that heme oxygenase is present in the granule cells of the dentate gyrus (2). Although the present data suggest the role of CO as a retrograde messenger, the postsynaptic effect of CO cannot be ruled out. Involvement of various intracellular messengers, e.g., calcium, calmodulin dependent protein kinase II, protein kinase C, in the generation of LTP has been suggested. However, the correlation of CO with these factors are not clear. Further studies are necessary to clarify the mechanism of how CO modulates the LTP.

Stevens et al. (1993) reported that LTP is reversible by application of ZnPP in the CA1 region (5). However, ZnPP had no influence on the established LTP in the dentate gyrus (Fig. 1B), suggesting that CO is involved in the introduction of LTP but is not essential to maintain LTP in the dentate gyrus. There may be regional differences in the role of CO for maintenance of LTP. Mizutani et al. (1993) suggested the role of NO in LTP in the dentate gyrus in vivo (8). The present results suggest that CO also contributes to the induction of LTP, probably as an intercellular messenger in the dentate gyrus.

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