× 800, Bio-Tec Instruments, USA).

Cell Proliferation

HUVECs were grown to 50-60% confluence; medium was then changed to contain 5% FCS (low FCS) for a further 12 h of incubation. Quiescent cells were then changed to normal medium, and incubated with normal saline or various concentrations of metallothionein for 22 h in the presence of H_2O_2 (10 mM)/Fe^{+2} (50 μM). Subsequently, treated cells in 24-well plates were further incubated with MTT (0.5 mg/ml) for 3 h. Culture medium was removed by aspiration, and cells were solubilized in DMSO (0.5 ml). The extent of reduction of MTT to formazan within cells was quantified by the measurement of OD_{550} with the microplate reader (ZLx 800, Bio-Tec Instruments). Proliferation of HUVECs was measured using a colorimetric assay based on the ability of mitochondria in viable cells to reduce MTT as previously described.[21]

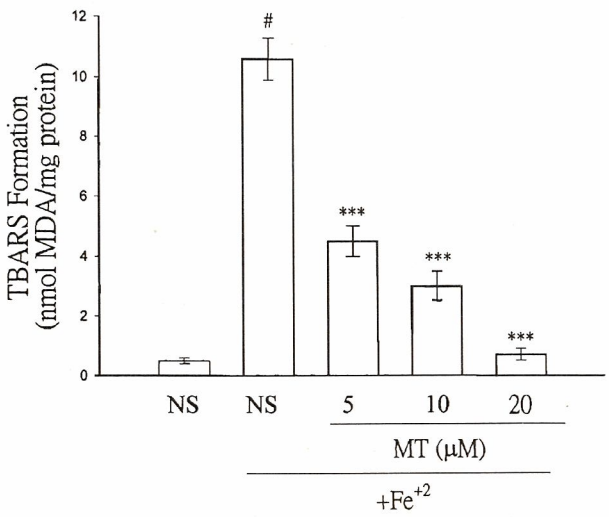

Fig. 1. Antioxidation activities of of metallothionein in the thiobarbituric acid-reactive substance (TBARS) test. Rat brain homogenates were preincubated with normal saline or various concentrations of metallothionein at 37 °C for 10 min; then 0.4 mM NADPH/100 μM Fe^{+3}/4 mM ADP and 0.2 mM Fe^{+2} were added, and incubation continued for another 30 min. Data are presented as the means ± S.E.M. (n = 5). $^{\#}p < 0.001$ as compared with the normal saline (NS) group; $^{***}p < 0.001$ as compared with the normal saline (NS) group challenged by Fe^{+2}.

Statistical Analysis

Data are presented as the means ± S.E.M. with the number of experiments indicated. Statistical analysis was performed using Student unpaired *t*-test unless specifically mentioned, and a *p* value less than 0.05 was considered statistically significant.

RESULTS

Effects of Metallothionein on Lipid Peroxidation

Metallothionein (5-20 μM) exerted a concentration-dependent inhibition of iron-catalyzed lipid peroxidation in rat brain homogenates (Fig. 1). At a higher concentration (50 μM), metallothionein also significantly inhibited spontaneous lipid peroxidation by 90% (data not shown). Metallothionein (50 μM) did not interfere with the absorption at 532 nm when added to rat brain homogenates that were either intact or already oxidatively modified (data not shown).

Stable Free Radical (DPPH)-scavenging Action

Diphenyl-*p*-picrylhydrazyl decolorization was used to evaluate the ability of compounds to exert their activity as free radical scavengers. The scavenging activity of metallothionein was expressed in a concentration-dependent manner (Fig. 2). The biphasic pattern of decolorization induced by metallothionein was similar to that by Trolox or α-tocopherol, but not by butylated hydroxytoluene (data not shown).

Effects of Metallothionein on Superoxide Anion Production in Human Neutrophils

The inhibitory effect of metallothionein on superoxide anion production of human neutrophils elicited by FMLP is shown in Fig. 3. The initial rate of human neutrophil superoxide generation induced by 100 nM FMLP was 2.8 ± 0.2 nmol/min/10^6 cells (n = 5). Metallothionein inhibited the FMLP-induced respiratory burst in a concentration-dependent manner. Metallothionein at a higher concentration (50 μM) markedly inhibited FMLP-induced superoxide anion