performed according to standard methods.[7]

Northern blot analysis

[“-^{32}P] dCTP-labeled cDNA probes were prepared using the multiprime DNA labeling system (Amersham). The prepared cDNA inserts and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) probes were added directly into the prehybridization solution (Quikhyb®) at a radioactivity of 1×10^6 ct/min per ml. Hybridization was performed at 68 EC for 70 min. The membrane was washed with 2X SSC, 0.1% SDS wash buffer at room temperature twice for 40 min and with the same solution at 40 EC once for 20 min. The wet blot membranes were sealed in plastic foil and exposed to medium-sensitive medical x-ray film (Fuji, Tokyo, Japan) at –70 °C using intensifier screens. Exposition times were 2-3 days for Northern blots. Hybridization intensity of autoradiographic signals was measured using 2-dimensional densitometry. The obtained density (in optical units) was calculated versus the value of the slot blot for GAPDH to quantify mRNA.

Statistics

All values are presented as the mean ± SD from each group. Statistical significance was evaluated using Kruskal-Wallis test when multiple groups were compared; where only 2 groups were compared, Mann-Whitney rank sum test was used. A level of $p < 0.05$ was considered significant.

RESULTS

The baseline control group (n = 8) was administered normal saline (1 ml/kg) and the levels of SOD activity and mRNA were set as 100%. After 3 days of injections into the peritoneal cavity, levels of SOD activity and mRNA revealed no change. Results of the vehicle control (normal saline plus 0.5% ethanol) were similar to those of the baseline control group (Fig. 1).

After 3 days of intraperitoneal injection of vitamin E (200 mg/ml/kg plus 0.5% ethanol), the activity of SOD increased about 50% compared to the control groups (Fig. 2). This increase of activity was more significant in liver ($p < 0.001$). Changes in SOD-mRNA also paralleled the activity, i.e., increased gene expressions in the liver and brain were more prominent (Fig. 2).

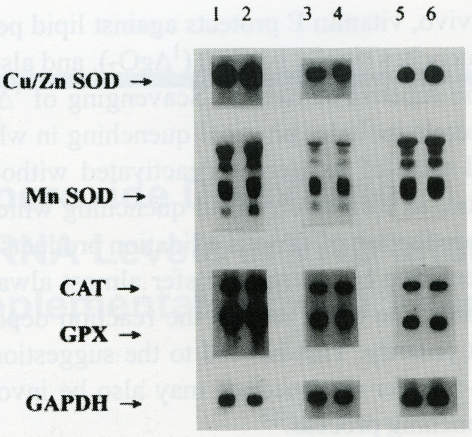

Fig. 1. Northern blots. mRNA levels of antioxidant enzymes in rat organs (n = 8) revealed changes after 3 days of supplementation with vitamin E. 1, 2 = liver; 3, 4 = brain; 5, 6 = kidney. Cu/Zn = copper/zinc; Mn = manganese; CAT = catalase; GPX = glutathione peroxidase. 1, 3, 5 = mRNA before vitamin E administration (saline administration); 2, 4, 6 = mRNA after vitamin E administration.

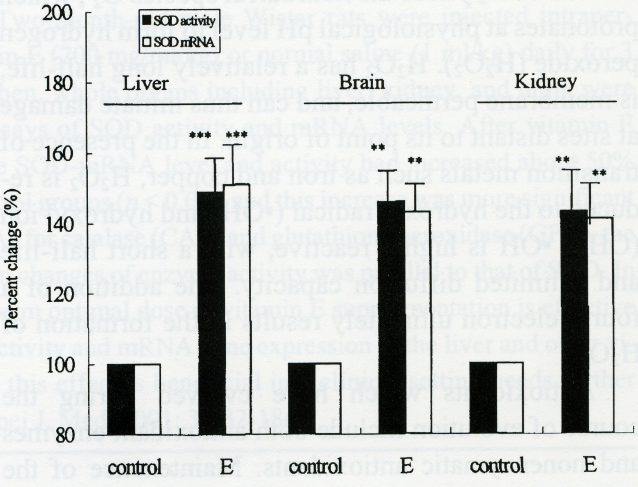

Fig. 2. Percentage of changes of SOD activity and mRNA in rat organs (n = 8) after 3 days of supplementation with vitamin E (200 mg/ml/kg + 0.5% ethanol saline). The control group used 0.5% ethanol in saline. E = vitamin E. $^{**}p < 0.01$, $^{***}p < 0.001$. (vitamin E vs. control).