During ischemia-reperfusion the heart may use energy substrates such as amino acids and lactate. In cardiac surgery, high concentrations of glutamate in cardioplegic solutions enhances myocardial protection. However, neither the metabolic role of glutamate nor the expression of glutamate transporters are well characterized in the heart. We used immunoisolation and Western blotting to identify and quantify cardiac content of glutamate transporters, and identified (in man and rat) subtype 1, 3, and 4, but not 2. We used a novel high affinity blocker, LL-TBOA, to investigate the functional roles of glutamate transporters in ischemia and reperfusion. LL-TBOA blocks all glutamate transporter subtypes, thereby preventing uptake and leakage of glutamate due to reversal of the transporters during ischemia. Rat hearts were isolated and perfused with 1.6 mM LL-TBOA for 5 min before 30 min of induced global ischemia and 60 min of reperfusion (n=7). Hearts perfused with the solvent DMSO (n=5) or no pretreatment (n=7) were used as controls. Infarct size was evaluated by triphenyl tetrazolium chloride staining. LL-TBOA reduced infarct size from 32±15% in controls to 17±5% (mean±SD) in LL-TBOA (p=0.007), but DMSO had no effect (35±6%). In conclusion, the heart expresses three glutamate transporters. Blockage of glutamate transporters has a beneficial effect against ischemia-reperfusion injury, mimicking the effect of high glutamate concentrations.