SUPPLEMENTARY INFORMATION

Experimental Protocols

Series 7: The effects of blockade of the entry of extracellular calcium ($CdCl_2$, 100 μM) as well as the mobilization of intracellular calcium stores (2-APB, 75 μM) on GAD67 activity was measured in duplicate in PC12 cells exposed to normoxia and 60 cycles of IH (n = 4 experiments in each group). Inhibitors were added to the cell culture medium 30 min prior to either normoxic or IH exposure and remained in the medium during the entire duration of gas challenges.

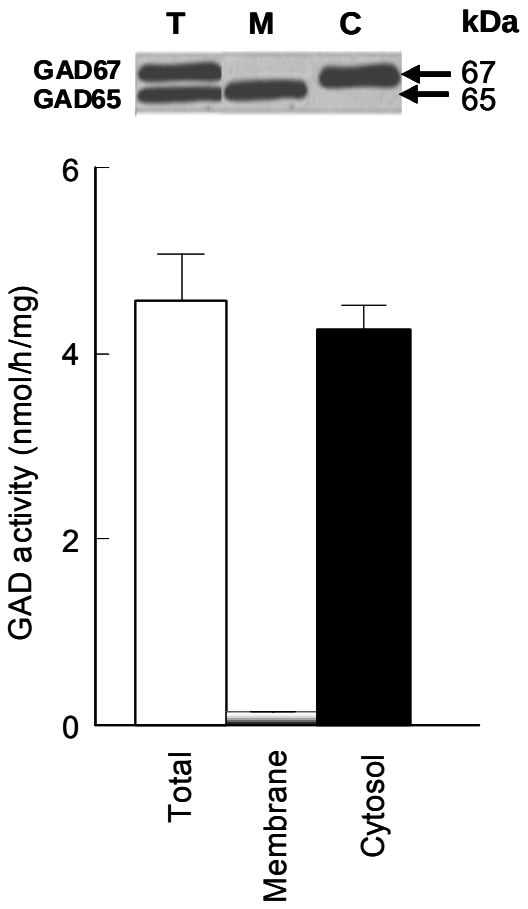
Series 8: GAD67 activity was measured in triplicate in PC12 cells exposed to either normoxia or 60 cycles of IH in the presence of a potent D1R specific antagonist (SKF 83566, 0.5 and 5 nM) or a β-adrenergic receptor antagonist (ICI 118551, 0.1 and 1 μM; n = 4 experiments in each group). The antagonists (Tocris Bioscience, MO) were added to the cell culture medium 30 min prior to either normoxic or IH exposure and remained in the medium during the entire duration of gas challenges.

FIGURE LEGENDS

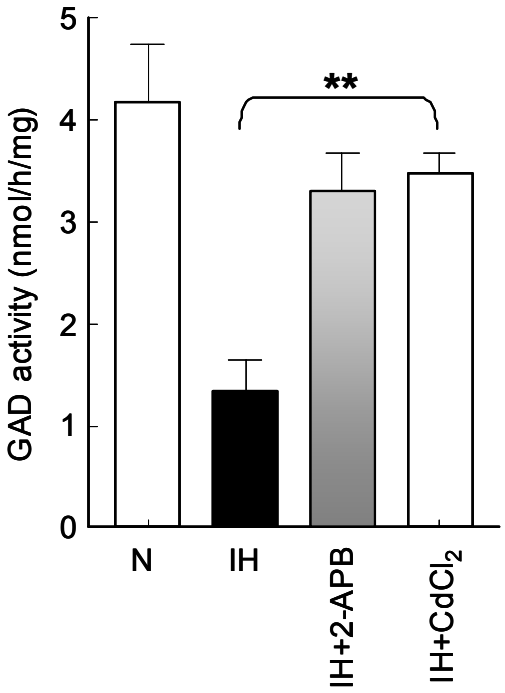
Figure S1: Distribution of GAD activity in PC12 cells. PC12 cells cultures under normoxic conditions were fractionated into cytosolic (C) and membrane enriched (M) fractions as described in "Methods" and GAD activity was measured fluorimetrically (n=4 experiments). (T)= total cell lysate.

Figure S2: Effect of blockade of dopamine release on IH-induced GAD67 inhibition. PC12 cells were exposed to normoxia (N) or IH in the presence or absence of 2-APB (75 μM) and $CdCl_2$ (100 μM). GAD67 activity in the cytosolic fraction was measured. Both 2-APB and $CdCl_2$ inhibited hypoxia-evoked DA release in IH treated PC12 cells (Kim et al., 2004). Data from 4 independent experiments are expressed as mean $\pm$ SEM. ** denote $p < 0.01$.

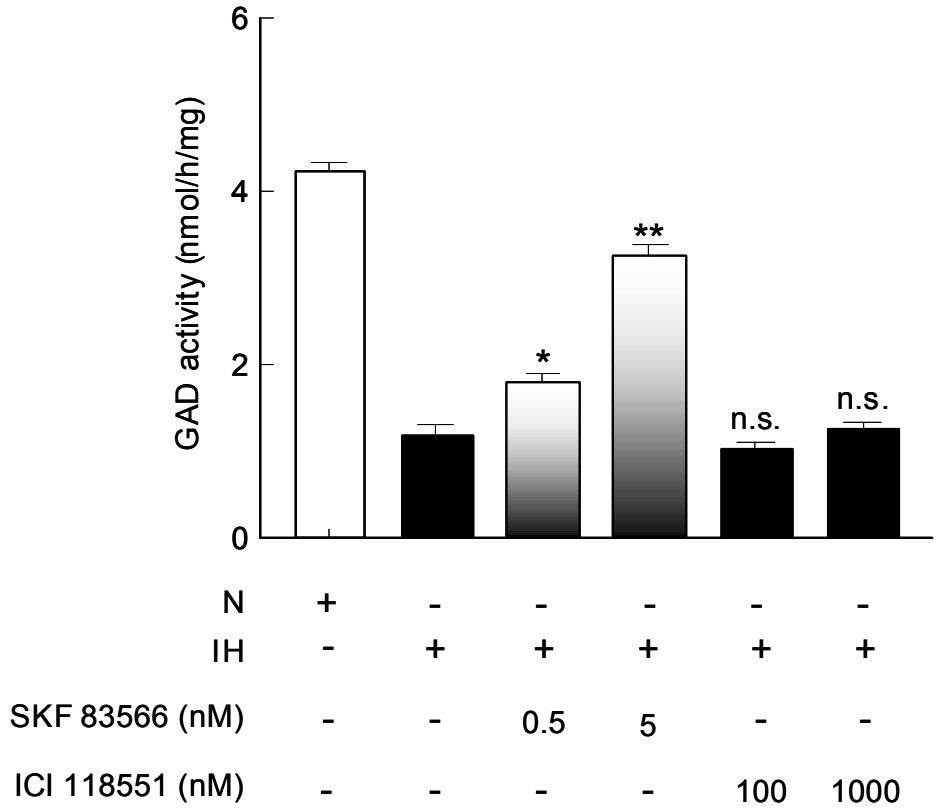
Figure S3: D1R but not β-adrenergic receptor antagonist reverses IH-induced GAD67 inhibition. GAD67 activity was measured in PC12 cells exposed to either normoxia or 60 cycles of IH in the presence and absence of either D1R specific antagonist (SKF 83566, 0.5 and 5 nM) or β-adrenergic receptor antagonist (ICI 118551, 0.1 and 1 μM). Data from 4 independent experiments are expressed as mean $\pm$ SEM. * & ** denote $p< 0.05$ and $p < 0.01$ respectively, n.s. – not significant and N – normoxia.



Supplemental Fig. S1



Supplemental Fig. S2



Supplemental Fig. S3