Cerebral blood flow is mediated by brain cells expressing glucose transporter 2

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PURPOSE A global elevation of blood flow in all brain regions is one of the immediate responses to hypoglycemia. The normal regulation of such blood flow responses can be impaired in diabetic patients. Glucose transport isoform 2 (glut2) positive brain cells have been shown to be sparsely distributed and play a very important role in control of glucagon plasma levels simulated by hypoglycemic stimuli (1). The aim of the present study was to illustrate a role of glut2 positive brain cells in control of cerebral blood flow (CBF).

METHODS

Animals: All studies were performed with the approval of local animal care and use committee. Glut2 positive brain cells were genetically knocked out (NG2KO) as previously described (1). At age of 16-20th weeks, six of each male NG2KO and their coutertype cohorts were prepared with a catheter in one femoral vein under 2% isoflurane mixed with 100% air and thereafter well maintained under 1-2% isoflurance to maintain breathing rates >100bpm, which had been shown capable of maintaining mice under physiological conditions, such as $PaCO_2$ in the range of 35-45mmHg. Such condition would minimize the effect of CO_2 on CBF. Tail bleeds were sampled and measured for glucose levels (Breeze glucose meter) immediately before and right after MR measurements at euglycemia, which were approximately lasting for one hour. An adjusted insulin (1U/ml) infusion protocol, i.e. a bolus followed by a continuous rate, induced hypoglycemia in all mice. Once tail bleed glucose levels were reached less than 2mM, hypoglycemic condition, the animals were scanned again.

MR measurements: All MR measurements were performed at 9.4T (26cm diameter). CBF was measured using a well-established continuous arterial spin labeling (CASL) technique in combination with a home-built actively-detuned system. Four segmented semi-adiabatic EPI sequence was adopted with a labeling module to implement the CASL sequence (3 consecutive 2mm-thick slices, 23×15mm^2, 128×64 data matrix). Cerebral blood flow (CBF) was assessed at euglycemia and hypoglycemia. **Data Analysis:** CBF maps were calculated from 16 paired labeled and controlled images with a labeling efficiency 0.8 (2). Significant difference was when $p< 0.05$.

RESULTS AND DISCUSSION Blood glucose levels before measurements were 8.5±1.0mM (mean±SEMs), in the wild-type mice, similar to 9.8±2.9mM in the NG2KO mice. The resting euglycemic CBF in was globally increased in NG2KO mice and reached a significant level in both hypothalamus and the entire brain (Figure 1 & Table 1).

Table1 Summary of CASL on regional blood flow (ml/100g/min) of wild type (WT) and NG2KO mice at euglycemia

	WT (n=6)	NG2KO (n=6)	*p-value*
hippocampus	107±6	120±4	0.087
hypothalamus	92±4	110±3*	0.004
cortex	100±3	106±5	0.388
brain	87±2	107±2*	0.001

"*" indicated p<0.01 using unpaired student t-test.

While the amounts of insulin used to induce acute hypoglycemia were similar in the NG2KO mice and their wild-type ones, the very similar hypoglycemia condition was reached, i.e. 1.7±0.1mM in the wild types and 1.6±0.1mM in the NG2KO mice, respectively (Figure 2). At insulin-induced hypoglycemia, CBF changes of the wild-type mice were in the range of 12-25% and highly significantly from those at resting euglycemia condition (paired student t-test $p<0.016$). NG2KO$^-$ mice did not increase (Figure 1 & 3) then instead, decreased 6±3% significantly in hypothalamus ($p<0.03$, paired student t-test).

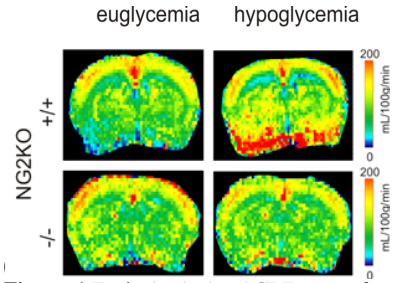

Figure 1 Typical calculated CBF maps of two mouse brains from one of each group at 9.4T.

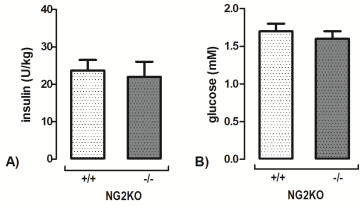

Figure 2 Summary of insulin dosages (A) (mean ± SEMs) and the insulin-induced hypoglycemic glucose levels (B) in NG2KO (-/-) mice and their wild type controls (+/+).

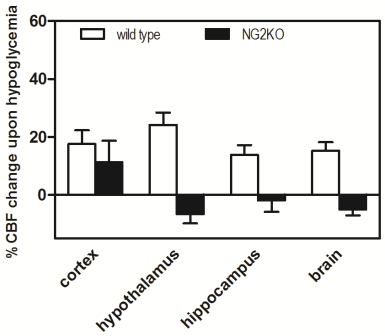

Figure 3. Summary of percent of CBF changes under insulin induced acute hypoglycemia from NG2KO mice and their counter types when compared to those under euglycemia. The percent CBF changes of wild type mice were significantly higher than those of NG2KO mice ($p<0.05$).

The observed CBF increases upon hypoglycemia in the WT mice were consistent, but with a less degree, with previous study in other rodents (3), which might due to the effects of isoflurane (4). The slight elevation of CBF at resting euglycemia condition and the blunted elevation of CBF upon insulin induced hypoglycemia are consistent with glucogan findings (1,5).

CONCLUSION We conclude that brain cells expressing glut2 are implicated in regulating the CBF response to hypoglycemia. We further postulate that glut2 positive brain cells are implicated in activation-induced increase in CBF, which may be a mechanism linking neuronal activation to vascular changes.

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