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Restoration of Normal Cerebral Oxygen Consumption with Rapamycin Treatment in a Rat Model of Autism–Tuberous Sclerosis

Oak Z. Chi³, Chang-Chih Wu¹, Xia Liu³, Kang H. Rah³, Estela Jacinto¹, and Harvey R. Weiss²

Harvey R. Weiss: hweiss@rutgers.edu

¹Department of Biochemistry and Molecular Biology, Rutgers Robert Wood Johnson Medical School, Piscataway, NJ 08854, USA

²Department of Neuroscience and Cell Biology, Rutgers Robert Wood Johnson Medical School, 675 Hoes Lane West, Piscataway, NJ 08854, USA

³Department of Anesthesiology, Rutgers Robert Wood Johnson Medical School, Piscataway, NJ 08854, USA

Abstract

Tuberous sclerosis (TSC) is associated with autism spectrum disorders and has been linked to metabolic dysfunction and unrestrained signaling of the mammalian target of rapamycin (mTOR). Inhibition of mTOR by rapamycin can mitigate some of the phenotypic abnormalities associated with TSC and autism, but whether this is due to the mTOR-related function in energy metabolism remains to be elucidated. In young Eker rats, an animal model of TSC and autism, which harbors a germ line heterozygous *Tsc2* mutation, we previously reported that cerebral oxygen consumption was pronouncedly elevated. Young (4 weeks) male control Long–Evans and Eker rats were divided into control and rapamycin-treated (20 mg/kg once daily for 2 days) animals. Cerebral regional blood flow (¹⁴C-iodoantipyrine) and O₂ consumption (cryomicrospectrophotometry) were determined in isoflurane-anesthetized rats. We found significantly increased basal O₂ consumption in the cortex (8.7 ± 1.5 ml O₂/min/100 g Eker vs. 2.7 ± 0.2 control), hippocampus, pons and cerebellum. Regional cerebral blood flow and cerebral O₂ extractions were also elevated in all brain regions. Rapamycin had no significant effect on O₂ consumption in any brain region of the control rats, but significantly reduced consumption in the cortex (4.1 ± 0.3) and all other examined regions of the Eker rats. Phosphorylation of mTOR and S6K1 was similar in the two groups and equally reduced by rapamycin. Thus, a rapamycin-sensitive, mTOR-dependent but S6K1-independent, signal led to enhanced oxidative metabolism in the Eker brain. We found decreased Akt phosphorylation in Eker but not Long–Evans rat brains, suggesting that this may be related to the increased cerebral O₂ consumption in the Eker rat. Our findings suggest that rapamycin targeting of Akt to restore normal cerebral metabolism could have therapeutic potential in tuberous sclerosis and autism.

Correspondence to: Harvey R. Weiss, hweiss@rutgers.edu.

Conflict of interest None.

Keywords

Mammalian target of rapamycin; Cerebral blood flow; Cerebral oxygen consumption; Rapamycin; Autism spectrum disorders

Introduction

Mutations in *Tsc1* or *Tsc2* genes occur in tuberous sclerosis (TSC), a genetic disorder that is characterized by the development of numerous benign tumors affecting multiple organs. TSC is also associated with autism spectrum disorders (ASD). Approximately 50–70 % of individuals with TSC are diagnosed with ASD, while TSC accounts for 1–4 % of all cases of autism (Ehninger and Silva 2011). Thus, understanding the pathogenesis of TSC might reveal clues to some of the molecular aberrations that could occur in ASD. TSC proteins form a complex consisting of TSC1 (hamartin) and TSC2 (tuberin) and act as tumor suppressors. Together, they function to downregulate cell growth by negative regulation of mTOR signaling (Huang and Manning 2008). mTOR is part of two protein complexes, mTORC1 and mTORC2, and is involved in the control of cell growth and metabolism (Laplane and Sabatini 2012). Among the two complexes, mTORC1 is better understood largely due to the inhibitory effects of rapamycin on this complex. The most well-characterized rapamycin-sensitive mTORC1 target is S6K1, a protein kinase that phosphorylates the ribosomal subunit S6 to control translation initiation. Inhibition of mTORC1 by rapamycin downregulates S6K activation and S6 phosphorylation. mTORC2, which is not acutely rapamycin sensitive, responds to growth factors and promotes full activation of Akt via phosphorylation (Oh and Jacinto 2011). Prolonged rapamycin treatment can indirectly inhibit mTORC2 and Akt activation by preventing association of mTOR with mTORC2 components rictor and SIN1 (Sarbasov et al. 2006). Active Akt impinges on mTORC1 signaling via negative regulation of TSC1/2 and consequently relieves suppression of mTORC1 by TSC1/2 (Huang and Manning 2008).

Tsc mutant rodents provide a relevant model to study the molecular defects associated with ASD. Although heterozygous mutations of *Tsc* genes do not cause obvious abnormalities in brain structure, rapamycin treatment can reverse the learning and memory impairments in *Tsc2*^{+/-} mice (Ehninger and Silva 2011). Heterozygous *Tsc* mutant mice also display behavioral deficits characteristic of ASD (Waltereit et al. 2011). Rapamycin treatment of *Tsc2*^{+/-} mice reversed the impaired social interaction, a behavior relevant to ASD in humans (Sato et al. 2012). The Eker rat, which harbors a spontaneous germ line mutation in *Tsc2*, has served as an invaluable model in understanding TSC (Habib 2010). Recent studies have revealed that this animal model also displays similar molecular defects found in other mouse models of TSC and ASD. Furthermore, autism-like behavioral abnormalities have been observed in the Eker as well (Waltereit et al. 2011). We have shown that the young Eker rat has significantly elevated cerebral O₂ consumption (Weiss et al. 2007). Our initial studies suggested that the increased cerebral metabolism in the Eker rat was not due to increased activity of the glutamatergic excitatory neurotransmitter system (Weiss et al. 2007, 2009). Despite the emerging link between mTOR signaling and ASD, the pathogenic mechanisms remain poorly defined. Studies from cellular and cancer models have uncovered

that mTOR is a central regulator of metabolism and senses conditions of energy and oxygen levels (Yuan et al. 2013, Laplante and Sabatini 2012). Functions of mTORC1 in protein synthesis require high levels of cellular energy in the form of ATP, and thereby mTORC1 is sensitive to energy depletion (Dennis et al. 2001).

The purpose of this study was to test the hypothesis that blocking the activity of mTOR with rapamycin would reduce the increased regional cerebral O₂ consumption in the Eker rat model of ASD. Regional cerebral blood flow (¹⁴C-iodoantipyrine) and cerebral O₂ consumption (microspectrophotometry) were determined in control and Eker rats, and various components of mTOR signaling were also examined. Rapamycin had no effect on cerebral O₂ consumption in control rats. We found that Eker rats had significantly higher cerebral O₂ consumption than control rats and that rapamycin treatment significantly reduced the cerebral metabolic rate in the Eker rats. This appeared linked to mTOR-related Akt but not S6K1 signaling.

Materials and Methods

This investigation was conducted in accordance with US Public Health Service Guidelines using the Guide for the Care of Laboratory Animals (DHHS Publication No. 85-23, revised 1996) and was approved by our Institutional Animal Care and Use Committee. Fourteen male Long-Evans rats (70–100 g) and 14 male Eker rats (70–100 g) were divided into control ($n = 7$) and rapamycin-treated ($n = 7$) groups for the cerebral oxygen consumption portion of the study. These are young animals approximately 4 weeks old and were used to determine cerebral blood flow and cerebral arterial–venous oxygen saturation differences. An additional five Long-Evans and five Eker rats were used for the molecular biology portion of this study.

The rats were initially anesthetized with 2 % isoflurane in an air and oxygen mixture through a tracheal tube to maintain the arterial PO₂ above 100 mmHg. A femoral artery and vein were cannulated. The venous catheter was used to administer the radioactive tracer. The arterial catheter was connected to Statham P23Db pressure transducer and an iWorx data acquisition system to monitor heart rate and blood pressure. This catheter was also used to obtain arterial blood samples for analysis of hemoglobin, blood gases and pH using a Radiometer blood gas analyzer. Body temperature was maintained at 38 °C with a servo-controlled rectal thermometer probe and a heating lamp. Upon completion of the surgery, the isoflurane concentration was reduced to 1.4 %.

In the rapamycin-treated animals, 20 mg/kg of rapamycin (LC Laboratories, Woburn, MA) dissolved in normal saline and 10 % DMSO was injected ip once a day for 2 days. Experiments were conducted 48 h after the first injection. In the control group, vehicle was injected. Upon completion of the final surgery, arterial blood pressure and heart rate were recorded and an arterial blood sample was withdrawn anaerobically for the analysis of PO₂, PCO₂, pH and hemoglobin concentration. Then 20 µCi of ¹⁴C-iodoantipyrine was infused intravenously for the determination of cerebral blood flow. When the isotope entered the venous circulation, the arterial catheter was cut to a length of about 20 mm. Ten-microliter arterial blood samples were obtained from the arterial catheter approximately every 3 s

during the next 60 s. Immediately after the last sample was collected, the rats were decapitated. The heads were frozen in liquid nitrogen.

The brains were sampled from four regions: cortex, hippocampus, cerebellum and pons. The brain samples were sectioned (20 μ m) on a microtome–cryostat, and the sections were exposed to X-ray film for 4 days to obtain an autoradiogram. The ^{14}C -iodoantipyrine concentrations of the tissues were determined by reference to eight precalibrated standards (40–1069 nCi/g, Amersham) using a computer-based microdensitometer system. At least eight average density measurements were taken from each of the brain regions examined. Blood samples were placed in a tissue solubilizer and 24 h later put into a counting fluid. The isotope counts were quench corrected. Regional blood flow determinations were calculated from the following equation:

$$\text{Ci}(T) = \lambda K \int C_A e^{-K(T-t)} dt;$$

where $\text{Ci}(T)$ equals the tissue concentration of ^{14}C -iodoantipyrine at the time of decapitation, λ equals the tissue/blood partition coefficient, C_A is the arterial concentration of the tracer, t equals the time and K is defined as follows: $K = mF/\lambda W$, where m is a constant, F/W equals the flow per unit mass of tissue and the λ value of 0.80 was used.

Alternate sections from the same frozen sample used for blood flow measurements were utilized for the determination of arterial and venous O_2 saturation (Buchweitz-Milton and Weiss 1987; Zhu and Weiss 1991). The regions were mounted with embedding medium in a microtome–cryostat at -35°C under a N_2 atmosphere. The sections were transferred to precooled glass slides and covered with degassed silicone oil and a coverslip. These slides were placed on a microspectrophotometer fitted with a N_2 -flushed cold stage to obtain readings of optimal density at 568, 560 and 523 nm. This three-wavelength method corrects for the light scattering in the frozen blood. The measuring spot was 8 μ m in size. To ensure that the path of light only traversed the blood, only vessels in transverse section were studied. Readings were obtained to determine O_2 saturation in five arteries, and eight veins found in each region. To determine the O_2 content of blood, the percent of O_2 saturation was multiplied to the hemoglobin concentration times 1.36, the maximal binding capacity of hemoglobin for O_2 per gram. Regional O_2 extraction, the difference between the average arterial and venous O_2 contents, was then obtained. Using the Fick principle, the O_2 consumption for each region was calculated as the product of average regional blood flow and O_2 extraction.

To analyze total extracts, brain tissue was lysed in RIPA buffer (150 mM NaCl, 25 mM Tris pH 7.4, 1 % NP-40, 0.25 % sodium deoxycholate, 1 mM EDTA, 1 mM Na_3VO_4 , 1 mM NaF and protease inhibitor cocktail). The lysates were centrifuged at 16,000g for 30 min at 4°C . Total extracts were resolved by SDS-PAGE followed by immunoblotting. Antibodies to S6, p-S6 (Ser235/236), Akt, p-Akt (Ser473), AMPK, p-AMPK (Thr172), TSC2, p-TSC2 (Thr1462) were from cell signaling (Danvers, MA), IRS-1 was from Millipore (Billerica, MA), and calnexin was from Santa Cruz Biotechnology (Santa Cruz, CA).

The cerebral cortex from Long–Evans and Eker rat brains was homogenized in buffer (10 mM Tris, pH 7.4, 0.5 mM EDTA, 25 mM sucrose, supplemented with a mixture of protease and phosphatase inhibitors) by passing through a 27G1/2 needle (Becton–Dickinson & Co, Franklin Lakes, NJ) 15 times. The homogenate was centrifuged at 1000g for 10 min at 4 °C to separate LSP, and the supernatant was recentrifuged at 200,000g for 1 h at 4 °C to obtain cytosol and HSP fractions. The LSP and HSP were resuspended with RIPA and IP buffer (20 mM Tris, pH 7.5, 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1 % Triton X-100), respectively.

A one-way analysis of variance was applied to the various measurements of hemodynamics, O₂ supply and consumption parameters that performed to determine the difference between treatment groups in the Eker and Long–Evans rats. A repeated-measures design was used to study the multiple brain regions within groups. Post hoc multiple comparisons were made using Tukey's procedure. All values are expressed as mean ± SEM. A value of $p < 0.05$ was considered statistically significant.

Results

Hemodynamic and blood gas parameters in the young Long–Evans (referred herein as control) and Eker, untreated and rapamycin-treated groups, were within the normal ranges for anesthetized rats (Table 1). Mean arterial pressures were similar in all groups. Heart rates were also similar between the control and Eker rats. Rapamycin treatment did not significantly affect arterial blood pressure or heart rate in either group of animals. Arterial blood gases were controlled and also were not significantly different between the various groups.

Regional cerebral blood flows were significantly higher in the untreated Eker rats compared to that found in the control rats in all examined regions under basal conditions (Table 2). The cerebral blood flow averaged approximately 2.2 times higher in the brains of the untreated Eker rats compared to the control rats. Treatment with rapamycin significantly lowered cerebral blood flow in all brain regions of the Eker rats. However, there were no significant changes from baseline in cerebral blood flow after rapamycin administration in any examined brain regions of the control rats. Regional cerebral oxygen (O₂) extractions were significantly lower in the control rats compared to the Eker untreated rats under baseline conditions (Table 2). This difference was found in all regions. Rapamycin treatment had no significant effect on cerebral O₂ extraction in either the control or the Eker rats.

Cerebral O₂ consumption was significantly elevated in all examined regions at baseline in the young Eker rats compared to the young control rats (Fig. 1). The cerebral O₂ consumption averaged approximately 3.1 times greater in the untreated Eker rats. These differences were significant in all examined brain regions (Fig. 1). In the Eker rats, the administration of rapamycin significantly lowered regional cerebral O₂ consumption. These differences were significant in the cortex (–53 %), hippocampus (–51 %) cerebellum (–54 %) and pons (–50 %) (Fig. 1). In the control rats, administration of rapamycin had no significant effect on cerebral O₂ consumption in any brain region. After rapamycin, the Eker and control rats had similar cerebral O₂ consumption in all examined brain regions. These

results revealed that inhibition of mTOR by rapamycin could restore the elevated cerebral O₂ consumption in the Eker rat, while having no significant effect on basal O₂ consumption of the control rats. The cerebral O₂ supply/consumption ratios were similar in all brain regions of both the Eker and control rats, Table 2. Rapamycin did not significantly affect the cerebral regional O₂ supply/consumption ratio in either group of rats.

To investigate the molecular mechanism involved in the increased cerebral O₂ consumption of the Eker rat and how rapamycin restores this abnormality, we used tissues from the brain cortex. We first examined phosphorylation of mTOR at Ser2448, whose phosphorylation is increased in some cancer cells (Holz and Blenis 2005, Sekulic et al. 2000). Phosphorylation at Ser2448 was comparable in control and Eker rats and was abolished after rapamycin treatment (Fig. 2). Next, we analyzed the autophosphorylation site at Ser2481, which was reported to be detected in both mTORC1 and mTORC2 immunoprecipitates (Foster andingar 2010) but is abolished upon mTORC2 disruption (Oh et al. 2010). Phosphorylation at this site was comparable in control and Eker rats and appeared to be more significantly decreased in the Eker upon rapamycin treatment. We then examined mTORC1 and mTORC2 effectors, S6 and Akt, respectively. We first examined S6 phosphorylation at the S235 and found no significant difference between control and Eker rats (Fig. 2). Rapamycin treatment abrogated S6 phosphorylation in both control and Eker rats. Since S6 phosphorylation was comparable and rapamycin could abolish S6 phosphorylation whereas it did not have any effect on cerebral O₂ consumption in the control rats, these results suggested that the mTOR-dependent elevation of O₂ consumption in the Eker was unlikely due to defective mTORC1/S6 signals.

We then examined Akt levels and phosphorylation. Akt levels were similar between control and Eker rats. Akt phosphorylation was also similar in the untreated Eker and control rat brains. Prolonged rapamycin treatment of control rats slightly reduced the level of Akt but did not reduce Akt phosphorylation significantly. Rapamycin treatment of Eker rats reduced both Akt levels and phosphorylation in the brain. These results parallel the effect of rapamycin on cerebral oxygen consumption in control versus Eker rat. Thus, downregulating Akt signals could restore normal cerebral oxygen consumption in the Eker rat.

Discussion

The Eker rat serves as a model of tuberous sclerosis–autism spectrum disorders. It has a defect in Tsc2 that is associated with a significantly elevated cerebral O₂ consumption (Weiss et al. 2007, 2012). The major finding of the current study was that inhibition of the TSC–mTOR pathway with rapamycin restored regional cerebral O₂ consumption to normal in the Eker rat, while rapamycin had no significant effect on cerebral metabolism in control animals. The effect of inhibition of mTOR signaling with rapamycin appeared not to be related to mTORC1/S6 signaling, but rather to Akt signaling in the Eker rat.

We found significant increases in basal cerebral blood flow, O₂ extraction and oxygen consumption in the Eker rats compared to young Long–Evans controls in the current study. Thus, the increased consumption was associated with an increase in both flow and O₂ extraction. This is similar to our previous reports using this Eker rat model (Weiss et al.

2007, 2012). The increased basal cerebral O₂ consumption in the Eker rats may have important implications for neurological issues found in tuberous sclerosis–ASD. This increased cerebral metabolism does not appear to be related to increased activity in excitatory glutamate receptors (Weiss et al. 2007, 2009, 2012). However, some of the increased cerebral oxygen consumption may be related to reduced activity of gamma-aminobutyric acid (GABA) inhibitory receptors (Weiss et al. 2008).

It is not clear whether the enhanced cerebral O₂ consumption seen in the Eker rat is also found in children with ASD. However, metabolic deregulation is known to occur in ASD. A study on children with TSC who underwent MRI as well as PET scans has shown correlation between glucose hypermetabolism in cerebellar regions and stereotypical behaviors and impaired social interaction (Asano et al. 2001). Widespread reductions in specific metabolites (Baruth et al. 2013) and mitochondrial dysfunction (Rossignol and Frye 2012) have also been associated with ASD. Elevated cerebral glucose utilization in autism has been reported previously (Rumsey et al. 1985), and more recent reports suggested that ASD is associated with increased regional brain activity and function (Allen et al. 2004; Mizuno et al. 2006). However, some studies suggest that there is reduced cerebral function, metabolism and blood flow in several brain regions in ASD (Haznedar et al. 2006).

Problems with Tsc1 or Tsc2 lead to changes in mTOR signaling and energy metabolism (Astrinidis and Henske 2005). Defective mTOR signaling is strongly linked to the molecular defects found in TSC and ASD. Many studies have used rapamycin to ameliorate learning, behavioral and other phenotypic abnormalities associated with ASD using both humans and animal models (de Vries 2010; Ehninger et al. 2008) (Zhou et al. 2009). Some studies have suggested that impaired regulation of translation by mTORC1 contributes to neuronal and synaptic defects associated with ASD (Gkogkas et al. 2013; Kelleher and Bear 2008). Given the abnormal energy metabolism that occurs in neurological conditions including ASD and the well-established function of mTORC1 in the control of cellular metabolism, we examined whether the defect in cerebral oxygen consumption in the Eker could be due to deregulated mTOR signaling. Our findings revealed that with haploinsufficient Tsc2 in the brain of Eker rats, there was relatively normal baseline mTOR signaling but a pronounced defect in metabolism.

The administration of rapamycin led to a profound decrease in cerebral blood flow and oxygen consumption in all examined regions of the Eker rat brain. There was no significant effect of rapamycin on these parameters in the control rat brains. This differential effect of rapamycin may have important implications for the treatment of tuberous sclerosis and ASD in man (de Vries 2010).

Rapamycin reduced mTORC1 signaling equally in both Eker and control rat brains as assessed by conventional mTORC1 readouts including phosphorylation of mTOR at Ser2448 and S6. The finding that the mTORC1/S6K1/S6 signaling branch appeared normal in the Eker rat was not surprising given previous reports that mTORC1 signaling is not significantly elevated in non-tumor tissues obtained from the Eker rats (Kenerson et al. 2002). Hyperactivation of mTORC1 has been well documented in immortalized or transformed cell lines, such as in TSC^{-/-} murine embryonic fibroblasts (Kwiatkowski et al.

2002). In these cells, rapamycin could reduce mitochondrial membrane potential, oxygen consumption and alter mitochondrial gene expression and phosphoproteome (Cunningham et al. 2007; Schieke et al. 2006). In our in vivo Eker rat model, rapamycin abolished S6 phosphorylation and had a dramatic effect on cerebral oxygen consumption. However, the effect of rapamycin on cerebral oxygen consumption does not parallel S6 phosphorylation since rapamycin also abrogated its phosphorylation in control rats that have normal oxygen consumption. In agreement with our findings, S6K1 also did not affect mitochondrial respiration and expression of mitochondrial genes (Morita et al. 2013; Cunningham et al. 2007). Thus, although we cannot exclude that the mTORC1/S6K pathway does play a role in control of oxygen consumption, other rapamycin-sensitive mTOR targets or effectors are likely more directly involved in this metabolic process.

Our results suggest that the mTOR-dependent control of cerebral oxygen consumption could be mediated via mTORC2/Akt. Similar to the lack of effect of rapamycin on oxygen consumption, there was a minimal effect on Akt phosphorylation in the control rats. Strikingly, Akt levels and phosphorylation were also depressed by rapamycin in these animals. We have previously reported that in addition to allosterically activating Akt by phosphorylation, mTORC2 also stabilizes Akt by phosphorylation during translation (Oh et al. 2010). Although mTORC2 is not directly sensitive to rapamycin, the prolonged rapamycin incubation used in our studies could indirectly inhibit mTORC2 activity (Sarbasov et al. 2006), thus strongly downregulating Akt. Why increased cerebral oxygen consumption in the untreated Eker did not lead to elevated Akt phosphorylation is puzzling, but Akt activation is also regulated by other mechanisms such as compartmental localization. Nevertheless, the decreased Akt phosphorylation and protein levels after rapamycin in the Eker brain correlated with the restoration of cerebral metabolism to basal amounts in these animals. This links Akt and cerebral O₂ consumption, which would be consistent with previous studies that demonstrated a crucial role for Akt in glucose metabolism and mitochondrial function (Robey and Hay 2009). The link between the effects of rapamycin on oxygen consumption and Akt phosphorylation may explain our findings, but the mechanism remains to be elucidated. Akt has been shown to regulate interaction of hexokinase and the voltage-dependent anion channel (VDAC) at the outer mitochondrial membrane and that could increase coupling of glucose metabolism to oxidative phosphorylation (Gottlob et al. 2001). Whether this role of Akt is controlled by mTOR is also not clear although mTORC2 has been linked to Akt-mediated regulation of mitochondrial functions (Betz et al. 2013). Further work is necessary to explore the role of mTORC2 and Akt in mitochondrial metabolism.

In conclusion, our findings reveal that TSC2 haploinsufficiency leads to excess energy metabolism in the brain of Eker rats. This defect is exclusively corrected by rapamycin in the Eker rat, without metabolic effects in control rat brains. This does not appear to be related to changes in mTORC1/S6 signals but is related to aberrant Akt, possibly mTORC2, signaling in the Eker rat. This may be linked to the underlying metabolic perturbations that lead to seizures, cognitive and social impairments, associated with TSC-related ASD. A more detailed examination of the aberrant metabolic targets of the mTOR pathway might reveal insights on the pathology of TSC and ASD.

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Fig. 1.

Cerebral regional O₂ consumption in control and rapamycin-treated Long-Evans and Eker rats. These rats are vehicle treated (*unhatched bars*) or treated with rapamycin (*hatched bars*). The measured regions are cortex (CORT), hippocampus (HIPPO), cerebellum (CEREB) and pons. In Eker rats, rapamycin lowered oxygen consumption in all examined brain regions. In the Long-Evans rats, rapamycin had no significant effect on cerebral O₂ consumption. Control Eker rats had significantly higher cerebral O₂ consumption than all other groups. Values are mean $\pm$ SEM. *Asterisk* indicates a value different from all other groups

Fig. 2.

Protein and signaling for mTOR, S6 and Akt are shown for control Long-Evans and Eker rat brains. Protein expression in control Long-Evans and Eker rat cortices untreated (Con) or treated with rapamycin (Rapa) is shown for two representative animals for each treatment group. After rapamycin, mTOR Ser2448 and S6 phosphorylation decreased dramatically in both the Long-Evans and Eker cortices. The effect of rapamycin on Akt phosphorylation is minimal in the control rat brain and more pronounced in the Eker rat brain. The differential effect on Akt may help explain the reduction in cerebral O_2 consumption after rapamycin in

the Eker rat brain. Results are quantitated and plotted. For p-mTOR Ser2448 and Ser2481 $n = 2$; p-S6 and p-Akt $n = 3$. *Error bars* SEM

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Table 1

Hemodynamic and blood gas parameters in the Long-Evans and Eker rats

	<u>Long Evans</u>		<u>Eker</u>	
	Control	Rapamycin	Control	Rapamycin
Mean blood pressure (mmHg)	71 ± 4	86 ± 2	81 ± 8	94 ± 14
Heart rate (beats/min)	411 ± 19	436 ± 11	413 ± 28	440 ± 12
Arterial PO ₂ (mmHg)	109 ± 3	114 ± 7	112 ± 3	119 ± 7
Arterial PCO ₂ (mmHg)	33 ± 4	35 ± 2	38 ± 3	39 ± 5
Arterial pH	7.30 ± .04	7.35 ± .03	7.33 ± .06	7.34 ± .05

Values are mean ± SEM (*N* = 7 per group)

Table 2

Regional cerebral blood flow (ml/min/100 g) and cerebral O₂ extraction (ml O₂/100 ml) in Long–Evans and Eker control and rapamycin-treated rats

Blood flow	Long Evans		Eker	
	Control	Rapamycin	Control	Rapamycin
Cortex	75 ± 6	104 ± 13	180 ± 28 [†]	89 ± 9
Hippocampus	84 ± 6	106 ± 15	188 ± 36 [†]	101 ± 8
Cerebellum	95 ± 7	115 ± 16	217 ± 28 [†]	107 ± 8
Pons	101 ± 5	133 ± 19	197 ± 29 [†]	102 ± 8
O ₂ extraction				
Cortex	3.7 ± 0.2	3.9 ± 0.2	4.9 ± 0.3 [*]	4.6 ± 0.2 [*]
Hippocampus	3.6 ± 0.2	3.8 ± 0.2	4.8 ± 0.3 [*]	4.5 ± 0.3 [*]
Cerebellum	3.6 ± 0.2	3.8 ± 0.2	4.7 ± 0.3 [*]	4.5 ± 0.2 [*]
Pons	3.6 ± 0.2	3.8 ± 0.2	4.7 ± 0.3 [*]	4.5 ± 0.2 [*]
O ₂ supply/consumption				
Cortex	2.72 ± 0.04	2.83 ± 0.06	2.78 ± 0.05	2.71 ± 0.05
Hippocampus	2.72 ± 0.10	2.82 ± 0.05	2.80 ± 0.05	2.79 ± 0.06
Cerebellum	2.76 ± 0.04	2.86 ± 0.03	2.85 ± 0.02	2.80 ± 0.03
Pons	2.71 ± 0.05	2.84 ± 0.03	2.87 ± 0.05	2.77 ± 0.03

Values are presented as mean ± SEM (*N* = 7 per group)

^{*} Significantly different from comparable Long–Evans group

[†] Significantly different from all other groups