Glycemic control by the brain renin-angiotensin system: Role for peripheral AT2 receptors

Benjamin J. Weidemann, Nicole K. Littlejohn, Curt D. Sigmund, and Justin L. Grobe

ABSTRACT:

The effect of hyperactivity of the brain renin-angiotensin system (RAS) on metabolism was explored using double-transgenic "sRA" mice, which express human renin via the synapsin promoter and human angiotensinogen via its own promoter. sRA mice exhibit hypertension, polydipsia, and elevated resting metabolic rate (RMR). Compared to control littersmates (n=9), body mass was lower in sRA (n=9) mice (25.1±1.26 vs 18.9±0.87 g, P=0.05), and i.p. glucose tolerance (ipGTT, 2 g/kg total body) was improved (genotype x time P=0.03). Lean mass by NMR was reduced in sRA (16.5±0.79 vs 12.6±0.83, P=0.05), but proportional lean mass was normal (66.1±5.23 vs 66.8±3.18 % total). Lean lipolysis (2g/kg lean body) was normal, and tolerance to a bolus dose of glucose was reduced (20 mg/mg/min, P=0.01), sRA mice were more sensitive to insulin, as i.p. insulin (0.5 U/kg total body) caused dramatic drops in blood glucose (control nadir 72±5 vs sRA nadir 21±5 mg/dl, and occasionally death, genotype x time P<0.02). As the RMR increase in sRA is mediated through suppression of adipose AT2 receptor activation, we tested the effects of chronic infusion of CGP-42112a (50 μg/kg/min) on ipGTT. Chronic CGP-42112a significantly reduced ipGTT (2 g/kg total body, AUC +23%, P=0.008) in both control and sRA mice. Together, these data suggest that the brain RAS may improve glycemic control through tonic suppression of peripheral AT2 activation.

HYPOTHESES:

• Elevated brain RAS activity in the sRA model results in improved glucose tolerance, through increased tissue sensitivity to insulin.
• Altered glycemic control in sRA mice is mediated through suppressed circulating angiotensin, and more specifically, through loss of tonic angiotensin AT2 receptor activation.

BACKGROUND:

• The renin-angiotensin system (RAS) is well known for its roles in blood pressure, volume and composition control. Drugs that interrupt the activity of the RAS constitute a major fraction of the clinically-available therapies for hypertension, cardiac and renal diseases, and complications of diabetes.
• Currently there are only 3 FDA-approved drugs for treating obesity, and all of them work through reducing caloric intake. Understanding the mechanisms of cross-talk between the circulating and brain RAS, and the mechanisms of metabolic rate control by these systems, will hopefully lead to the identification of a completely novel class of anti-obesity, specifically, drugs to increase resting metabolism and thereby energy expenditure.
• We and others have implicated the local tissue-level brain RAS in the regulation of resting metabolic rate, and determined that this is mediated through both stimulation of the sympathetic nervous system and suppression of the circulating RAS. Ongoing work supports a primary role for AT2, as opposed to AT1, receptors in these effects, as chronic subcutaneous infusion of the selective AT2 agonist CGP-42112a normalizes most of the metabolic phenotypes of mice with elevated brain RAS activity.
• Here we examine whether alterations in brain RAS activity, and subsequent changes in circulating RAS activity, result in altered glycemic control.

Figure 1: The sRA model of elevated brain renin-angiotensin system activity. (A) The renin-angiotensin system. (From Grobe et al., Pharmacology, 2008.) Renin cleavage of angiotensinogen is the rate-limiting step in the production of all angiotensin peptides, and this reaction is very species-specific. (B) "sRA" mice are the product of crossing mice that express human renin via the neuron-specific synapsin promoter with mice that express human angiotensinogen via its own promoter (C57 background). Because of the species-specificity of the reaction, RAS hyperactivity only occurs in sites of overlapping transgene expression, and thus sRA mice exhibit brain-specific RAS hyperactivity. (C) sRA mice are small and (D) lean. (E) Oxygen consumption is elevated in sRA mice. Core temperature is elevated, but physical activity is normal, underscoring an activity-independent thermogenesis. From Grobe et al., Cell Metabolism 2010. *P<0.05 vs control littermates.

Figure 2: Intraperitoneal glucose tolerance test in control and sRA mice, with 2 g dextrose / kg total body mass. (A) Body masses. (B) Blood glucose versus time, with injection of 20% dextrose solution at time zero. * P<0.05 vs control.

Figure 3: Nuclear Magnetic Resonance (NMR)-based body composition analyses in control and sRA mice, divided by sex. (A) Total body masses. (B) Lean mass. (C) Lean mass, as a fraction of total body mass. (D) Fat mass. (E) Fat mass, as a fraction of total body mass. * P<0.05 vs control by 2-way ANOVA / Bonferroni post-hoc.

Figure 4: Intraperitoneal glucose tolerance test in control and sRA mice, with 2 g dextrose / kg lean body mass. (A) Body masses. (B) Blood glucose versus time, with injection of 20% dextrose solution at time zero. * P<0.05 vs control.

Figure 5: Intraperitoneal insulin tolerance test in control and sRA mice, with 0.5 insulin Units / kg total body mass. (A) Body masses. (B) Blood glucose versus time, with injection of insulin at time zero. * P<0.05 vs control.

Figure 6: Effect of chronic AT2 receptor agonist infusion on glycemic control in sRA and control littermate mice. (A) 5-hour fasted blood glucose, CGP-42112a is a peptide agonist that is selective for the angiotensin II type 2 (AT2) receptor. Control n=8 vehicle, 7 CGP-42112a, and sRA n=8 vehicle, 7 CGP-42112a. * P<0.05 vs control, and + P<0.05 vs vehicle. (B) Intraperitoneal glucose tolerance test performed based on total body mass. (C) Area under the curve analysis of data in panel B. (D) Area under the curve analysis of data in panel B, with "time zero" values subtracted.

SUMMARY:

Previously:
• sRA mice are small (-20%), hypertensive (+20 mmHg), and exhibit robust polydipsia (10-fold), and a large elevation in resting metabolic rate (+18%). Food intake is almost normal (-7% grams / day, or +25% grams / body mass / day).
In the present study:
• sRA mice exhibit a reduced 5-hour fasted blood glucose concentration. sRA mice are smaller than control littermate mice, but exhibit a normal lean-to-total body mass ratio.
• Although glucose tolerance appears to be improved in sRA mice based on tests performed using total body mass, performing the test using mass alone demonstrates that the glucose clearance rate is similar across genotypes.
• sRA mice are substantially more responsive to acute insulin, and repeating the test with insulin injection based on lean mass alone would likely uncover an even greater group difference.
• Chronic infusion of CGP-42112a resulted in increased fasted blood glucose levels in all mice, and resulted in significantly reduced glucose tolerance in all mice.

CONCLUSIONS & IMPLICATIONS:

• These data support a major role for the brain RAS, likely through suppression of the circulating RAS (and more specifically, AT2 receptor activation), in glycemic control.
• We hypothesize that this effect is mediated through modulation of insulin sensitivity in thermogenic organs such as skeletal muscle and brown/beige adipose tissues.

ONGOING / FUTURE STUDIES:

• Direct measures of insulin at baseline and at various points during the glucose tolerance test
• Assessment of insulin tolerance following chronic AT2 activation
• Determination of site of increased insulin sensitivity (liver, skeletal muscle, various fat pads) by phosphorylated AKT protein (Western blot) analyses

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