Activator of G Protein Signaling 3 Null Mice: I. Unexpected Alterations in Metabolic and Cardiovascular Function


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Activator of G protein signaling (AGS)-3 plays functional roles in cell division, synaptic plasticity, addictive behavior, and neuronal development. As part of a broad effort to define the extent of functional diversity of AGS3-regulated-events in vivo, we generated AGS3 null mice. Surprisingly, AGS3 null adult mice exhibited unexpected alterations in cardiovascular and metabolic functions without any obvious changes in motor skills, basic behavioral traits, and brain morphology.

Receptor-independent activators of G protein signaling (AGS) offer alternative modes of signal processing for the G protein signaling system that have broad mechanistic and functional significance. AGS proteins are mechanistically divided into three groups: guanine nucleotide exchange factors (group I, AGS1), guanine nucleotide dissociation inhibitors (group II, AGS3–6); and group III (AGS2, AGS7–10) that interact with Gβγ (1, 2). Group II AGS proteins each possess one or more G protein regulatory (GPR) motifs that bind GαiGDP and GαtGDP > GαoGDP (1). The GPR motif is also referred to as the GoLoco motif (54).

GPR proteins may promote dissociation of the Gαβγ complex independent of nucleotide exchange or bind GαGDP during the G protein activation-deactivation cycle before it can reassociate with Gβγ (3). Alternatively, GPR proteins may be complexed with GαGDP independent of any initial formation of heterotrimeric Gαβγ. In the latter situation, nonreceptor guanine nucleotide exchange factors such as Ric-8A may activate Gα-GPR in a manner analogous to that by which a G protein-coupled receptor promotes activation of Gαβγ (4). These modes of signaling provide a previously unknown mechanism for signal integration and related studies in various model organisms revealed unexpected functional roles for Gα and GPR proteins in asymmetric cell division, autophagy, membrane protein transport, neuronal development, and/or synaptic plasticity (4–18).

The discovery of such surprising functionality for GPR proteins and the G-switch in model organisms resulted from unbiased functional screens and suggests that there are additional functional roles for this signaling module yet to be identified.

As a first step to address this thought, we generated a mouse line with a conditional AGS3 null allele. Based on previous observations indicating a role for AGS3 in neuronal development and synaptic plasticity, we hypothesized that the loss of AGS3 expression would result in developmental defects in the central nervous system. Surprisingly, elimination of AGS3 did not alter basal behavior or gross brain morphology but rather resulted in altered cardiovascular and metabolic homeostasis.

Materials and Methods

Materials

pFloxFLPNeo was kindly provided by Dr. James Shayman (Department of Internal Medicine, University of Michigan, Ann Arbor, MI) (19). AGS3 monoclonal antibody was purchased from BD PharMingen (San Diego, CA). Gia3-specific antiseraum 976 was generated as described (20). All other reagents and materials were obtained as described elsewhere (21).

Generation of conditional null allele of AGS3

All protocols and procedures were approved by the University Committee on Use and Care of Animals [Louisiana State University Health Sciences Center (LSUHSC), Pennington Biomedical Research Center,
and Medical University of South Carolina. To create AGS3 null mice, we used the targeting vector pFlox-FLP-Neo (19), which contains two loxP sites and a PGK-neo resistance cassette flanked by FLP recombinase target (FRT) sites that are used by Cre/loxP and FLP/FLPe/FRT recombinases, respectively. The targeting construct pFloxFLPNeo/Gspm1 contains 8.8 kb AGS3 genomic sequence divided in two regions of AGS3 homology (3.0 and 5.7 kb in size) and a 410-bp sequence encompassing AGS3 exon 3 cloned within flanking loxP sites. The targeting construct was linearized and electroporated into W4 mouse embryonic stem (ES) cells derived from 129S6/SvEvTac (22). The 480 G418-resistant ES cell clones were screened by PCR using AGS3-specific primers (5'-ATG ATT GAG GGC TGT CTT GTG GGG AAG G-3'; 5'-CTG TGG TGG GCA GTA GAG TAG AGG-3'), and subjected to SDS-PAGE and immunoblotting with AGS3 antisera. Primers for AGS3 exon 3 cloned within flanking loxP sites were designed as follows: for AGS3-fl (AGS3-FL) and AGS3-short (25). A prominent immunoreactive species of Mr approximately 45,000 is also evident in the heart and in untransfected COS7 cells (Fig. 1B, right panel). The Mr approximately 45,000 species is observed in both WT and AGS3 null mice and is variably and inconsistently detected with the different panel of AGS3 antibodies available (PEP22, PEP32, PEP98, AGS3 monoclonal) and as such appears to be unrelated to full-length AGS3.

![Diagram of the strategy for generation of Gpsm1 null mice](https://academic.oup.com/endo/article-abstract/149/8/3842/2455541/fig1)

**A.** Targeting vector for the conditional null Gpsm1 allele and PCR genotyping strategy to confirm the loss of Gpsm1 exon 3 from mouse tail genomic DNA using primers (a and b) flanking the floxed exon. An expected 565-bp wild-type band is amplified from WT DNA (+/+), whereas a single 310-bp band representing the loss of exon 3 is produced from Gpsm1 null mice (−/−). Heterozygous null (+/−) mice contain both fragments. ASTERISK (*) represents the stop codon introduced by a shift in reading frame due to the loss of exon 3. C. ClaI; A. ApIIb; B. BamHI; H. HincII. Additional information regarding Gpsm1 gene targeting can be found in supplemental information. B, Left panel, Brain lysates (75 µg per lane) prepared from COS7 cells transfected with 10 µg pcDNA3 empty vector (V) or pcDNA3::AGS3-Short (SH) (25) as well as heart tissue lysates (150 µg per lane) from WT and Gpsm1−/− were subjected and subjected to SDS-PAGE and immunoblotting with AGS3, LGN, and Gia2 specific antisera as described in Materials and Methods. Right panel, Lysates (75 µg per lane) prepared from COS7 cells transfected with 10 µg pE23A-Ags3 Short (SH) (25) as well as heart tissue lysates (150 µg per lane) from WT and Gpsm1−/− were subjected to SDS-PAGE and immunoblotting with AGS3 antisera (PEP22), which detects both full-length AGS3 (AGS3-FL) and AGS3-short (25). A prominent immunoreactive species of Mr approximately 45,000 is also evident in the heart and in untransfected COS7 cells (Fig. 1B, right panel). The Mr approximately 45,000 species is observed in both WT and AGS3 null mice and is variably and inconsistently detected with the different panel of AGS3 antibodies available (PEP22, PEP32, PEP98, AGS3 monoclonal) and as such appears to be unrelated to full-length AGS3.
AGS3− /− mice were viable and fertile; however, the ratio of inheritance was slightly different from the Mendelian frequency of 1:2:1 expected from a heterozygous cross. Of 220 offspring, 50 (23%) were AGS3+/+, 134 (61%) were AGS3− /−, and 36 (16%) were AGS3+ /−. A two-tailed χ2 analysis showed that the observed ratios of + /+ to + /− to − /− were significantly different from the expected 1:2:1 ratio (p < 0.001), suggesting a potential role of AGS3 in reproductive efficiency. Male mice backcrossed to C57BL/6J three or more times were used in this study, and all experiments used paired littermates of AGS3 null and wild-type mice.

**Tissue analysis**

Tissues were removed, weighed, frozen in liquid nitrogen, and stored at −70 °C until processed for analysis. Lysates were prepared by homogenizing tissues in 1% Nonidet P-40 buffer and processed for SDS-PAGE, transfer to polyvinyl difluoride membranes, and immunoblotting as described (21).

**Body composition and indirect calorimetry**

Male C57BL/6J wild-type (n = 7) and Gpsm1− /− null (n = 9) were weaned onto Purina Rodent Diet (no. 5001, 4% fat; St. Louis, MO) at 3 wk of age and housed in a controlled environment at 22 °C on a 12-h light, 12-h dark cycle with free access to food and water. Body weights were measured once weekly. Fat mass, lean mass, and fluid mass were determined in triplicate for each animal once weekly by nuclear magnetic resonance (NMR) with a Bruker Mice Minispec NMR analyzer (Bruker Optics, Inc., Billerica, MA). Lean mass was added to liquid mass to produce the variable fat-free mass (FFM) that was used for analysis. At 21 wk of age, energy expenditure was measured by indirect calorimetry (Oxymax system; Columbus Instruments, Columbus, OH). Oxygen consumption and carbon dioxide production were measured at 48-min intervals for 4 d. Mice had free access to food and water. Energy expenditure (EE) was expressed as kilojoules per kilogram FFM per hour.

**Cardiovascular measurements**

Arterial pressure and heart rate were monitored in conscious, freely moving male mice with a battery-operated (PA-C10) telemetry probe (Transoma Medical; St. Paul, MN) as part of the Cardiac and Vascular Function Core in the Department of Pharmacology and Experimental Therapeutics at LSUHSC (New Orleans, LA). Animals were anesthetized with a mixture of ketamine (100 mg/kg, ip) and xylazine (10 mg/kg). A ventral midline skin incision was made from the lower abdomen to the thoracic cavity, and the body of the telemetry probe was inserted and advanced up to the aorta. The body of the telemetry probe was placed in a sc pouch along the animal’s right flank and a second cannula inserted into the thoracic aorta via the carotid artery. Penicillin G (50,000 U/kg) was administered i.m. in the hind limb. Transoma acquisition software was used to monitor heart rate and mean arterial pressure (MAP). Data collection began 7–10 d after surgery after the return of regular diurnal cycles. Heart rate was calculated from the arterial pressure recording. Arterial pressure and heart rate data were collected for 10 sec every 10 min for 24–50 h.

**Baroreceptor reflex sensitivity and heart rate variability**

Baroreceptor reflex sensitivity and heart rate variability were measured using HemoLab software (http://www.intergate.com/harald/ HemoLab/HemoLab.html) and calculated baroreceptor-heart rate reflex sensitivity according to Bertinieri et al. (24). Briefly, sequences of three consecutive increases (or decreases) in arterial pressure were matched to corresponding decreases (or increases) in heart rate and used to calculate baroreceptor reflex gain for each sequence. The average reflex gain was calculated from a minimum of 20 sequences. The same software package was used to calculate heart rate variability. Blood pressure was recorded using radiotelemetry using a sampling rate of 500 Hz and blood pressure waveforms extracted using Dataquest ART software (DSI, St. Paul, MN). The software then calculated the interbeat intervals between successive arterial pulses. Fast Fourier transformation was then used to calculate the spectrum of heart rate variability, from which the low frequency (LF; 0.02–0.2 Hz) and high frequency (HF; 0.2–0.6 Hz) bandwidths were extracted. Area under the curve was calculated for the LF and HF bands as indices of the sympathetic and parasympathetic modulation of heart rate. The ratio of LF to HF was then calculated for each group.

**Results and Discussion**

**Generation of Gpsm1 null mice**

To address the potential functional diversity for the GPR-G-switch signaling module on a broad scale, we generated a mouse line with conditional disruption of the AGS3 gene, Gpsm1 (Fig. 1 and supplemental Fig. 1). Our strategy involved Cre-mediated excision of Gpsm1 exon 3, which shifted the reading frame resulting in a premature stop codon (supplemental Fig. 1A). RNA blots and sequencing of Gpsm1 cDNA from Gpsm1− /− mice confirmed the loss of the second coding exon and the presence of the premature stop codon (supplemental Fig. 1C). The absence of the AGS3 protein in Gpsm1− /− mice was confirmed by immunoblotting of brain and heart, two tissues enriched in AGS3 mRNA (Fig. 1). The gene targeting strategy did not disrupt the expression of AGS3-Short in heart consistent with the postulate that AGS3-Short transcription involves an alternative promoter (Fig. 1) (25). We do not yet know whether individual cells express both full-length AGS3 and AGS3-Short (25) and, if so, whether these proteins function in a coordinated manner to influence G protein signaling. In cells expressing both full-length and AGS3-Short, the loss of full-length AGS3 in the AGS3 null mice may influence this mechanism of coordinated regulation and contribute to the observed phenotype.

We then conducted initial phenotype studies for the Gpsm1− /− mouse focusing on brain morphology and behavior as well as cardiovascular and metabolic homeostasis based on AGS3 tissue distribution and tissues in which Gi and/or Goα play important roles in signal processing. The initial studies reported herein focused on the Gpsm1− /− mice, and we have not yet studied the phenotype of the Gpsm1+ /+ mice. The heterozygotes (Gpsm1+ /−) do exhibit an expected 50% reduction in the expression of AGS3 in brain and white adipose tissue providing a tool for examining stochiometric considerations in subsequent studies.

**Brain morphology and behavior profile**

As an initial approach to determine the phenotype of the Gpsm1− /− mice, we evaluated them independently through PhenoFirst (Charles River Labs, www.criver.com/flex_content_area/documents/rm_tg_r_phenofirst_panel.pdf), a modified SHRPA panel (SmithKline Beecham Pharmaceuticals; Harwell, MRC Mouse Genome Centre and Mammalian Genetics Unit; Imperial College School of Medicine at St. Mary’s; Royal London Hospital, St. Bartholomew’s and the Royal London School of Medicine phenotype assessment) (26), which measures primary neurobehavioral observations in three general areas: spinocebellar function (body position, gait, tail elevation, locomotor activity); sensory function (transfer arousal, touch escape, palpebral reflex, corneal re-
flex, pinna reflex); and autonomic function (startle response, skin color, piloerection, urination, defecation). There were no apparent differences between the wild-type (WT) and Gpsm1/H11002/H11002 mice in this phase I neurobehavioral profile. We also analyzed brain sections from WT and Gpsm1/H11002/H11002 mice. Surprisingly, Nissl staining showed no gross differences in brain morphology or cellularity in 3-wk-old, 6-wk-old, or 6-month-old Gpsm1/H11002/H11002 mice and revealed no obvious increase in Nissl-stained cells or cortical thickness (data not shown). Immunohistochemistry of mouse brain sections using anti-NeuN to identify neurons and anti-γ aminobutyric acid also revealed no obvious abnormalities in cellularity (data not shown).

These data contrast with observations in various model systems implicating AGS3 in neuronal development and behavior. Indeed, short hairpin RNA knockdown of AGS3 at embryonic day (E) 12.5 increased the number of cortical neurons at the expense of cortical neuronal progenitors in E14 mouse embryos (10). AGS5/LGN, which is widely expressed in the brain, may provide some compensation for the loss of AGS3 during development although there was no apparent change in AGS5/LGN expression in Gpsm1/H11002/H11002 tissues (Fig. 1B). Alternatively, the increased number of cortical neurons observed at E14 after short hairpin RNA knockdown (10) may not result in a readily observable morphological change in the brain due to the continued remodeling of the neural circuitry that occurs as the animal undergoes further development. Alterations in the circuitry as a result of a loss of AGS3 may not be obvious from analysis of brain morphology and will only be revealed with specific behavioral challenges and interventions that provide a functional readout of synaptic plasticity (5, 27, 28).

**Energy homeostasis**

While profiling expression of AGS3 among tissues in the mouse, we observed selective expression of AGS3 in white but not brown adipose tissue (Fig. 2A). This is of particular note given the central role that Giα and Gsα play in the integration of signals controlling lipid metabolism in the

![Fig. 2. Adipose tissue expression and metabolic profile of Gpsm1/H11002/H11002 mice.](https://academic.oup.com/endo/article-abstract/149/8/3842/2455541)

A. AGS3 immunoblot of adipose tissue from WT, Gpsm1/H11002/H11002, and Gpsm1/H11002 mice. Lysates were isolated, prepared, and subjected to SDS-PAGE and immunoblotting with AGS3-specific antisera as described in *Experimental Procedures*. BAT, Brown adipose tissue; IWAT, inguinal white adipose tissue; EWAT, epididymal white adipose tissue; RPWAT, retroperitoneal white adipose tissue. B. Body weight for wild-type and Gpsm1/H11002/H11002 mice was measured weekly. C. Food consumption was measured three times per week and is presented as grams per day per gram body weight (*right side of graph, , p = 0.003*) and as absolute food consumption as grams per day per mouse (*left side of graph, , p = 0.04*). D. Repeated measures of body composition were determined in each animal using a small animal NMR as described in *Materials and Methods*, and group means for each ratio were calculated from the respective ratios of individual mice in each group at each time point. The differences in both body weight (B) and body composition (D) between WT and Gpsm1/H11002/H11002 mice were statistically significant as determined by ANOVA (p = 0.0001). E. EE was measured using indirect calorimetry during a 4-d period at 21 wk of age. Oxygen consumption and carbon dioxide production were measured at 48-min intervals. Group means for both WT and Gpsm1/H11002/H11002 were calculated for each interval, and means for each interval were binned in groups of three intervals. EE is expressed as kilojoules per hour per unit of FFM (kilograms). Gray bars indicate night.
adipocyte (29–31). To address the potential significance of this observation, we examined fat vs. protein deposition during postweaning growth. Weaning weight did not differ between WT and Gpsm1<sup>−/−</sup> mice (Fig. 2B). However, Gpsm1<sup>−/−</sup> mice exhibited a decreased body weight first apparent at 10–12 wk. Body composition was similar between the genotypes for the first month after weaning, but by 10 wk of age, the Gpsm1<sup>−/−</sup> mice also exhibited a decrease in fat deposition coinciding with the reduction in body weight, compared with WT. By 5.5 months of age, the Gpsm1<sup>−/−</sup> mice had an approximately 6% reduction in fat mass to body weight ratios compared with WT mice (Fig. 2B). Serum glucose, total cholesterol and triglycerides were unaltered in the Gpsm1<sup>−/−</sup> mice (serum glucose: WT, 179.5 ± 11.8 mg/dl; Gpsm1<sup>−/−</sup>, 198.8 ± 6.7 mg/dl; total cholesterol: WT, 84 ± 6 mg/dl; Gpsm1<sup>−/−</sup>, 85.3 ± 6 mg/dl; triglycerides: WT, 87.3 ± 7.8 mg/dl; Gpsm1<sup>−/−</sup>, 90.6 ± 7.2 mg/dl).

Such differences in fat disposition could reflect decreased food intake or increased EE. The Gpsm1<sup>−/−</sup> mice actually had higher food consumption per unit body weight, compared with WT (Fig. 2C). Subsequent studies of EE by indirect calorimetry indicated that over a 4-d period nocturnal EE was 30% higher in Gpsm1<sup>−/−</sup> vs. WT mice (Fig. 2E), but this difference was absent during the day. There were no apparent differences in locomotor activity (supplemental Fig. 2). These data indicate that the absence of AGS3 perturbs energy balance by increasing energy expenditure and that metabolic fuels are less efficiently used for fat deposition in the absence of AGS3. Generally, brown adipose tissue, which is more prominent in rodents, is involved in generating heat, whereas white adipose tissue is primarily involved in the storage of fat which can be mobilized for energy expenditure when required. Thus, the observed changes in fat deposition and energy expenditure are consistent with the expression of AGS3 in white but not brown adipose tissue.

One of the primary conduits for the regulation of adipose tissue function is the sympathetic nervous system with increased sympathetically mediated oxidative capacity of adipose tissue. Translocation of sympathetic input into short-term metabolic responses and longer-term transcriptional responses is complex and dependent on other factors including species, sex, age, and the specific adipose depot in question. In general, activation of β-adrenergic receptors (primarily β<sub>2</sub>-adrenergic receptor in rodents), acting through G<sub>α</sub> and adenyl cyclase to increase cAMP levels, ultimately leads to protein kinase A-dependent phosphorylation of perilipin and hormone-sensitive lipase, resulting in lipolysis and the release of free fatty acids from white adipose tissue. Studies in a number of animal models indicate that any perturbation that leads to increased cAMP signaling or β-adrenergic sensitivity in adipose tissue produces a lean, obesity-resistant phenotype (32–34). Interestingly, a decrease in G<sub>α</sub> expression or a change in subcellular distribution of G<sub>α</sub> also results in increased lipolysis and inhibition of receptor-G<sub>α</sub> coupling with pertussis toxin induces lipolysis in rat adipocytes, indicating that G<sub>α</sub> provides and maintains significant inhibitory input into cAMP signaling in adipose tissue (35–37). The decreased adiposity and increase in EE observed in the Gpsm1<sup>−/−</sup> mice, which lack AGS3 as a modulator of G<sub>α</sub> signaling, may result from an altered balance of G<sub>α</sub>-G<sub>i</sub> signaling and subsequent amplification of signals that increase intracellular cAMP concentration in adipocytes. A similar mechanism has been proposed for AGS3 in the modulation of neuronal responses via D<sub>1</sub> and D<sub>2</sub> receptors during sensitization and withdrawal from drugs of abuse (5).

**Cardiovascular dynamics**

Heterotrimeric G proteins clearly play a central role in cardiovascular function and aberrant G protein signaling is associated with cardiovascular dysfunction (38, 39). Changes in the expression of G<sub>α</sub> are associated with hypertension and systolic heart failure (40–44). G<sub>α</sub> expression increases postnatally in spontaneously hypertensive rats and the onset of this increase in expression corresponds to the development of hypertension (45–49). Uncoupling of G protein-coupled receptors and G<sub>α</sub> by treatment with pertussis toxin normalizes the expression of G<sub>α</sub> in spontaneously hypertensive rats and results in a reduction in blood pressure to normotenensive levels (50, 51). Systolic heart failure results in increased expression of G<sub>α</sub>, and this is postulated to account in part for the desensitization to catecholamines (39–44).

Given the role of G<sub>α</sub> in cardiovascular reactivity and the expression of AGS3 in the heart (Fig. 1B) (25), we examined cardiovascular function in Gpsm1<sup>−/−</sup> mice.

AGS3 is enriched in brain and a short form of AGS3 (AGS3-Short) lacking the TPR domain is expressed in heart. AGS3 protein and mRNA is also found in vascular smooth muscle and lymphoid tissues as well (25) (Blumer J. B., Q. Yang, and S. M. Lanier, unpublished observations). Immunoblots of mouse heart tissue indicated the expression of both full-length AGS3 and AGS3-Short in WT mice (Fig. 1B, right panel). Gpsm1<sup>−/−</sup> and WT mice were implanted with telemetry probes. The mean heart rate was nearly identical in the two groups (Fig. 3A, left panel); however, the MAP was significantly (p = 0.006) lower in the Gpsm1<sup>−/−</sup> mice than the WT mice (Fig. 3A, right panel). Gpsm1<sup>−/−</sup> mice also exhibited significantly reduced (p = 0.001) diurnal variations in MAP (Fig. 3A, right panel). Diurnal variations in heart rate were similar in WT and Gpsm1<sup>−/−</sup> mice (Fig. 3A, left panel), suggesting that overall activity, indirectly reflected as heart rate fluctuations, was not different in Gpsm1<sup>−/−</sup> mice as discussed above.

The reduced mean arterial pressure and decreased diurnal variation in arterial pressure in the Gpsm1<sup>−/−</sup> mice, compared with WT mice, may reflect either altered sympathetic drive to the vasculature or altered vascular responsiveness to vasodilator or vasoconstrictor stimuli. As an initial step to address this question, heart rate variability was analyzed to assess the relative contributions of the sympathetic and parasympathetic nervous systems to cardiovascular control. There were no differences in the ratio of the low frequency/high frequency peaks in the interbeat intervals between Gpsm1<sup>−/−</sup> and WT mice (1.67 ± 0.48 vs. 1.53 ± 0.51, respectively), suggesting that the sympathetic and parasympathetic systems contributed equally to cardiovascular control in the two groups of mice.

As a next step to address the mechanism for the difference in diurnal variation and blood pressure, we analyzed baroreceptor reflex sensitivity and responses to the vasodilating...
agent sodium nitroprusside (SNP). Baroreceptor reflex sensitivity was assessed by examining heart rate responses elicited by spontaneous changes in arterial pressure (24). In Gpsm1/H11002/H11002/H11002 mice, the gain of the baroreceptor reflex was significantly enhanced compared with wild-type mice (Fig. 3B). The Gpsm1/H11002/H11002/H11002 mice also exhibited a markedly increased sensitivity to the vasodilator SNP. Both WT and Gpsm1/H11002/H11002/H11002 mice responded to SNP (87.5 g/kg) with an expected drop in arterial pressure. Whereas the WT readily compensated for the vasodilation with a full return of arterial pressure to pre-SNP levels, the Gpsm1/H11002/H11002/H11002 mice did not (Fig. 3C), suggesting altered vascular control mechanisms involving heterotrimeric G protein signaling mechanisms. Similar prolonged recovery of MAP after SNP administration occurred in mice lacking smooth muscle actin (52), which also had a lower resting MAP than WT mice, and in a mouse model of Hutchinson-Gilford progeria syndrome caused by a lamin A G608G mutation (53).

The modulation of G protein signaling by AGS3 may involve a typical cell surface G protein-coupled receptor or another intracellular checkpoint under the control of the G-switch (2). The development of thoughts related to accessory proteins and G protein signaling systems has led to the realization that Gα and Gβγ are also processing intracellular signals distinct from their role as transducers for cell surface G protein-coupled receptors and that they are engaged in previously unrecognized functional roles for the G-switch. The exact pathway modulated by AGS3 and related GPR proteins as well as the mechanism involved is likely signal and cell-specific and developmentally regulated with vary-

![Fig. 3. Cardiovascular profile of Gpsm1/H11002/H11002/H11002 mice. A, Top panel, Heart rate (HR) and MAP were averaged from all animals (WT, n = 4; Gpsm1/H11002/H11002/H11002, n = 5) over a 24-h period. WT HR = 561 ± 9 beats per minute (bpm); Gpsm1/H11002/H11002/H11002 HR = 562 ± 6 bpm; WT MAP = 117 ± 1 mm Hg; Gpsm1/H11002/H11002/H11002 MAP = 107 ± 2 mmHg. *, p = 0.0001. Also shown is the difference in HR and MAP between the peak nocturnal increase and the day time nadir. WT ΔHR = 170 ± 23 bpm; Gpsm1/H11002/H11002/H11002 ΔHR = 107 ± 16 bpm; p = 0.055. WT ΔMAP = 37 ± 4 mm Hg; Gpsm1/H11002/H11002/H11002 ΔMAP = 13 ± 2 mm Hg. *, p < 0.05. Bottom panel, Telemetry recordings of arterial pressure and heart rate from conscious, unrestrained WT (n = 4) and Gpsm1/H11002/H11002/H11002 (n = 5) mice over a 50-h period. MAP and HR recordings were taken for 10 sec every 10 min, and tracings plotted over time are presented. Black and white bars underneath the tracings represent night and day, respectively. B, Spontaneous baroreflex gain (SBG) was measured as described in Materials and Methods. WT (n = 4) SBG mean = 1.28 ± 0.07 msec/mm Hg; Gpsm1/H11002/H11002/H11002 (n = 5) SBG mean = 1.88 ± 0.21 msec/mm Hg. *, p < 0.05. C, MAP and HR telemetry recordings from WT and Gpsm1/H11002/H11002/H11002 mice at 14 wk of age after administration of 87.5 μg/kg SNP. Recordings were taken continuously and the time of drug delivery is marked by the vertical line in the tracing. Data are representative of three WT and five Gpsm1/H11002/H11002/H11002 mice.](https://academic.oup.com/endo/article-abstract/149/8/3842/2455541)
Acknowledgments

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