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Locus Coeruleus Dysfunction in Transgenic Rats with Low Brain Angiotensinogen

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SUMMARY

Aims: Transgenic TGR(ASrAOGEN)680 (TGR) rats with specific downregulation of glial angiotensinogen (AOGEN) synthesis develop cardiovascular deficits, anxiety, altered response to stress, and depression. Here, we evaluated whether these deficits are associated with alteration of the integrity of the noradrenergic system originating from locus coeruleus (LC) neurons. **Methods:** Adult TGR rats were compared to control Sprague Dawley rats in terms of the following: tissue levels of transcripts encoding noradrenergic markers, tissue tyrosine hydroxylase (TH) protein level, *in vivo* TH activity, density of TH-containing fibers, behavioral response to novelty, locomotor activity, and polysomnography. **Results:** TH expression was increased in the LC of TGR rats compared to controls. In LC terminal fields, there was an increase in density of TH-containing fibers in TGR rats that was associated with an elevation of *in vivo* TH activity. TGR rats also displayed locomotor hyperactivity in response to novelty. Moreover, polysomnographic studies indicated that daily paradoxical sleep duration was increased in TGR rats and that the paradoxical sleep rebound triggered by total sleep deprivation was blunted in these rats. **Conclusions:** Altogether, these results suggest that disruption of astroglial AOGEN synthesis leads to cardiovascular, cognitive, behavioral, and sleep disorders that might be partly due to LC dysfunction.

Introduction

There is growing evidence that the brain renin–angiotensin system (RAS) plays a critical role in hypertension, cardiac hypertrophy, heart failure, Alzheimer's disease, stroke, alcoholism, and depression [1,2]. Thus, the brain RAS could be a versatile therapeutic target. Therefore, it is essential to identify how alterations of specific brain RAS components can affect the function of other brain systems.

Several rodent models have been engineered to discriminate the role of brain-derived angiotensins from that of their circulating counterparts, and to determine how a specific perturbation in brain RAS function results in disease [3–8]. One of the most characterized models, the TGR(ASrAOGEN)680 (TGR) rat, was developed based on the observation that the main source of brain angiotensinogen (AOGEN), the only known precursor of brain-derived angiotensins, is provided by astrocytes [9,10]. In adult TGR rats, brain levels of AOGEN and of two of its bioactive metabolites, angiotensin (Ang)-II and Ang-(1-7), are reduced by ~90% compared to control rats [8,11], without any alteration in plasma AOGEN, Ang-I, Ang-II and Ang-(1-7) levels, due to the expression of an AOGEN antisense RNA (AS RNA) which is under the control of the glial fibrillary acidic protein (GFAP) gene promoter [8]. The AS RNA is highly expressed in the brain of TGR rats and its expression pattern is similar to that of the endogenous GFAP promoter. Adult TGR rats display obvious signs of brain RAS dysfunction, including reduced behavioral response to intracerebroventricular infusions of renin and Ang-II, and decreased plasma level of vasopressin [8,12]. These rats also develop profound cardiovascular [8,13–16] and metabolic [16] dysfunctions, and exhibit behavioral deficits, that is, increased sensitivity to stress and anxiety [17–19]. Although these behavioral deficits could be related to brainstem noradrenergic dysfunction [20–22], the neurophysiological and neurochemical integrity of locus coeruleus (LC) neurons, the main source of noradrenaline (NA) to the brain [23], has not been investigated in TGR rats, so far.

It has long been suggested that the brain RAS exerts its functions by targeting hypothalamic and brainstem noradrenergic neurons [24–28]. Accordingly, our laboratory presented data suggesting that noradrenergic neurons of the nucleus tractus solitarius are targeted by the brain RAS [29]. Indeed, we demonstrated in TGR that chronic glial AOGEN depletion in rats results in alteration of the ontogeny of these neurons. Brainstem LC neurons might also be important mediators of the effects of the brain RAS. Indeed, initial

studies performed 30 years ago demonstrated that intracerebroventricular injection of Ang-II elicits an increase in NA turnover within the LC of adult rats [27,30]. Subsequently, *in vitro* studies revealed that exogenously applied Ang-II depresses glutamate depolarizations and postsynaptic potentials in LC neurons [31,32]. In addition, more recent *in vivo* studies demonstrated that peripheral administration of angiotensin receptor antagonists alters the expression of tyrosine hydroxylase (TH), the rate-limiting enzyme in catecholamine biosynthesis [33], within the LC of normotensive and of spontaneously hypertensive rats [34–36]. However, there is no direct evidence, to date, that the endogenous brain RAS controls the catecholaminergic activity and function of LC neurons.

In this study, we used adult TGR rats to determine whether phenotypic attributes of brainstem noradrenergic LC neurons are altered when astrocytic AOGEN synthesis is specifically disrupted. These rats are a particularly relevant study model to address this question, as astrocytes are the only source of AOGEN in the LC region [37,38].

Materials and Methods

Animals

The animal protocols used in this study were approved by the Claude Bernard University Animal Care and Use Committee (protocol # BH2013-02) and were in accordance with the guidelines of the European Union (directive 2010/63/UE) for the care and use of laboratory animals, in compliance with the French law (decree 2013-118). All adult (12-week-old males) TGR and age-matched Hannover Sprague Dawley rats (SD; parent strain originally used for the generation of TGR rats) were obtained from our breeding colony. Breeders were originally obtained from the Max-Delbrück Center for Molecular Medicine (MDC), Berlin-Buch, Germany. The animals were kept at room temperature (25 ± 1°C) with a 12 h/12 h darkness (19:00–07:00 h)/light (07:00–19:00 h) cycle. They received food and water *ad libitum*.

Experimental Design and Tissue Preparation

In a first set of experiments, nine Sprague Dawley and nine TGR rats were anesthetized with sodium pentobarbital, then their brain was removed and quickly frozen in isopentane. Five hundred-micrometer-thick slices were cut throughout the pontine region using a freezing microtome and LC was micropunched bilaterally, as previously described [29]. For each animal, tissue punches were pooled and homogenized in distilled water containing a cocktail of proteinase inhibitors. These LC samples were used to quantify tissue TH levels using the dot blot technique.

In a second set of experiments, five Sprague Dawley and five TGR rats were used to prepare LC tissue extracts as described above. LC punches were homogenized in RNAse-free water, and the resulting homogenate was used to measure TH, dopamine-β-hydroxylase (DBH) and NA transporter (NET) gene transcript levels using RT-qPCR.

In a third set of experiments, three rats of each experimental group were perfused transcardially with 4% paraformaldehyde. Their brain was dissected, postfixed at 4°C for 2 h, cryoprotected at 4°C in 25% sucrose and frozen in isopentane. Twenty-five-micrometer-thick coronal sections were performed using a cryomicrotome (Leica) and processed free-floating for TH immunohistological detection in the brainstem and in the forebrain.

In a fourth set of experiments, *in vivo* TH activity was assessed in the LC, hippocampus and frontal cortex of five TGR rats and five controls. Rats received a single i.p. injection of the aromatic amino acid decarboxylase inhibitor NSD-1015 (100 mg/kg; Sigma-Aldrich, St. Quentin Fallavier, France) and were decapitated 20 min later. Then, their brain was extracted and frozen in isopentane. Five hundred-micrometer-thick slices were cut throughout the pontine and forebrain regions of each brain using a freezing microtome, and the LC, hippocampus and frontal cortex were subsequently microdissected. Brain tissues were homogenized and processed for determination of tissue L-DOPA concentration using high-pressure liquid chromatography.

In a last set of experiments, we measured anxiety-induced locomotor activity as well as daily locomotor activity in five Sprague Dawley and five TGR rats using an open-field test. Subsequently, rats were prepared for polysomnographic recordings.

In Vitro Protocols

Tissue TH Protein Levels

Tyrosine hydroxylase protein level was determined in LC samples using an immunoautoradiographic technique, as previously described [29,39].

Quantification of Transcript Levels

Total RNA was extracted from LC punches and the messenger RNAs (mRNAs) contained in 1 μg of purified RNA extracts were then reverse transcribed as previously described [40]. smRNA was used to normalize the RT step (Morales and Bezin, patent WO2004.092414). The cDNAs obtained after RT were quantified by real-time PCR using the 1.2 LightCycler System (Roche Diagnostics, Indianapolis, IN, USA) and the QuantiTect SYBR Green PCR Kit (Qiagen, Valencia, CA, USA). Results obtained for the targeted mRNAs were normalized against those obtained for the smRNA. Sequences of the different primer pairs used are listed in Table 1. All primer pairs were designed with the Primer 3 software (Whitehead Institute for Biomedical Research; http://primer3.ut.ee).

Tissue L-DOPA Concentration

Catecholamines were extracted and L-DOPA concentration measured by high-pressure liquid chromatography followed by electrochemical detection, as previously described [29].

TH Immunohistochemistry

Tyrosine hydroxylase protein was detected by immunohistochemistry using a mouse monoclonal anti-TH antibody (1:1000; Millipore MAB5280) and a biotinylated horse anti-mouse IgG secondary antibody (1:1000; Vector BA 2001). At the end of the staining protocol, sections were digitized and the density of TH-immunolabeled processes was quantified in the LC, dentate gyrus and frontal cortex using the Leica QWin™ software (Leica Micro-

Table 1 Sequences of the primer pairs used for qPCR

Target cDNA	GenBank reference #	Forward primer sequence	Reverse primer sequence	Product size (base pairs)
DBH	NM_013158.2	5′-CGA AAT CTG GAA TCC GCA TCT-3′	5′-TCA TTG CCG AAC CGG TTT ACT-3′	376
NET	NM_031343.1	5′-TTG CTA TGC CTG ATG GTC GTT-3′	5′-AAA TCC AGC TCC CAG GGA AAA-3′	258
TH	NM_012740.3	5′-ACT GTC CGC CCG TGA TTT TC-3′	5′-TCC CCA TTC TGT TTA CAT AGC CC-3′	273

systems, Nanterre, France), as described previously [29]. Measurement boxes with constant size and location were drawn onto digitized pictures. The density of pixels which intensity was above background level was measured inside the boxes. Results were expressed as the percent surface occupied by positive pixels in the measurement boxes.

In Vivo Protocols

Locomotor Activity

An open-field test was used to measure the novelty stress-induced locomotor activity and daily locomotor activity of Sprague Dawley and TGR rats (n = 5 per strain). Locomotor activity was monitored in a polycarbonate cage (W260 × L410 × H200 mm; Imetronic, Pessac, France) equipped with an array of four pairs of infrared beams. The lower beams were positioned 40 mm above the floor of the cage. The activity cage was connected to a computer interface that counted photocell beam breaks. A locomotor event was recorded when two successive horizontal or vertical breaks occurred. A first set of experiments was performed to measure the locomotor activity of rats facing a new environment, which constitutes a stressful condition. For this purpose, rats were recorded for 20 min after their first introduction inside the cage; the number of movements was pooled and collected every minute during this protocol. Two days after the novelty stress test, rats were recorded continuously for 26 h in the same cage to determine whether TGR rats exhibit motor deficits; the number of movements was pooled and collected every 30 min during this protocol.

Polysomnographic Recordings

Rats were implanted for chronic sleep recordings (EMG, EcoG) and cerebellar temperature measurement (Tbr) under deep anesthesia (sodium pentobarbital, 50 mg/kg i.p.), as previously described [41]. After 10 days of recovery and habituation to the recording conditions, baseline sleep–wake activity was recorded for three consecutive days using a 16-channel digital recorder (Somnologica™; Flaga Group hf., Reykjavik, Iceland) and data were stored online on the hard drive of a computer. Animals were then placed in a water tank on top of a small platform and subjected to a 10-h total sleep deprivation (TSD) [41]. At the end of the TSD, rats were put back to their home cages and recorded continuously for the next 24 h. Polygraphic recordings were visually scored by 30-second epochs, according to the classical criteria of the vigilance stages, that is, slow wave sleep (SWS), paradoxical sleep (PS) and wakefulness (Wake). Baseline Wake, SWS and PS durations were calculated from the data collected during the 72 h preceding TSD.

Statistical Analysis

Differences between control and TGR rats for TH protein concentration, the density of TH-immunolabeled processes, mRNA levels, L-DOPA concentration, stress-induced locomotor activity and duration of sleep–wake stages were tested using the ANOVA I followed by a post hoc Newman–Keuls' test. Differences between control and TGR rats for the daily locomotor activity were tested using a two-way repeated-measures ANOVA (RM ANOVA II; time = factor 1 and strain = factor 2).

Results

TH Expression is Altered in LC Neurons of TGR Rats

Tyrosine hydroxylase protein concentration within the LC of TGR rats was 126 ± 7% of control rats (Figure 1A; $P < 0.05$, ANOVA I). This was associated with a + 62 ± 20% increase in TH-mRNA level in the LC of TGR rats (Figure 1B; $P < 0.05$, ANOVA I). This increase seemed rather specific to TH as we could not detect any alteration of the levels of DBH and NET-mRNAs in TGR rats compared to controls (Figure 1B). Although TH expression was increased in TGR rats, *in situ* distribution of the enzyme within the LC region of these rats was similar to that of controls (Figure 1C–E).

TH Protein Distribution is Altered in the Hippocampus and Cortex of TGR Rats

As we detected abnormally high levels of TH protein and mRNA within the LC of TGR rats, we next compared the distribution of TH protein in the hippocampus and cortex between control and TGR rats using immunohistochemistry. We specifically focused on the subgranular zone of the dentate gyrus (SGZ) and the molecular layer of the frontal cortex (MLFC), which receive dense noradrenergic innervation arising exclusively from LC neurons and are devoid of dopaminergic terminals [42,43]. We detected dense networks of TH-immunopositive processes in the SGZ and MLFC, both in control and TGR rats (Figure 2A–D). However, the density of TH-containing axons in these forebrain regions was dramatically increased in TGR rats compared to controls, by 101 ± 12% in the SGZ and 74 ± 14% in the MLFC (Figure 2E; $P < 0.001$, ANOVA I).

In Vivo TH Activity is Increased in the Hippocampus and Cortex of TGR Rats

We further investigated whether increased TH protein concentration was associated with enhanced activity of the enzyme in the LC, hippocampus and cortex of TGR rats. Although TH activity

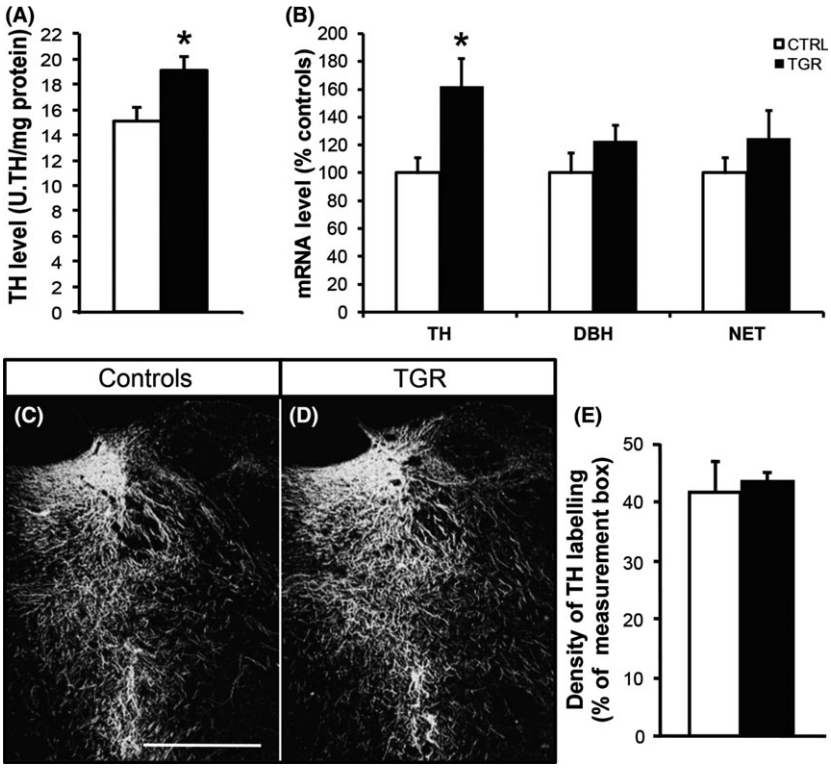

Figure 1 Tyrosine hydroxylase expression is altered in the locus coeruleus of TGR rats. (**A**) TH level is increased in the LC of TGR rats (black bars) compared to controls (white bars). Results are the mean ± SEM (n = 9). *$P < 0.05$, ANOVA I. (**B**) TH-mRNA expression is concomitantly increased in the LC of TGR rats; however, the concentration of the mRNA encoding dopamine beta-hydroxylase (DBH) and the NA transporter (NET) in TGR rats is similar to controls. Results are the mean ± SEM (n = 5). *$P < 0.05$, ANOVA I. (**C** and **D**) TH protein is detected by immunohistochemistry in the LC region in both control (**C**) and TGR rats (**D**). Scale bar: 500 μm. (**E**) Density of TH immunolabeling is similar in the LC of control and TGR rats. Results are the mean ± SEM (n = 3).

(tissue L-DOPA accumulation after inhibition of the aromatic amino acid decarboxylase) within the LC tissue was up by 23 ± 12% in TGR rats compared to controls (controls vs. TGR: 1.65 ± 0.15 vs. 2.03 ± 0.20 ng L-DOPA/mg tissue), which is in the range of the increase in TH protein concentration measured in this brain region (see Figure 1A), our data could not reach statistical significance (Figure 3A). In contrast, TH activity was significantly increased ($P < 0.05$, ANOVA I) by 55% in both the hippocampus and the cortex of TGR rats compared to controls: hippocampus (Figure 3B), 0.049 ± 0.005 (controls) versus 0.077 ± 0.006 (TGR) ng L-DOPA/mg tissue; cortex (Figure 3C), 0.074 ± 0.005 (controls) versus 0.115 ± 0.009 (TGR) ng L-DOPA/mg tissue.

TGR Rats Exhibit Hyperactivity in Response to Novelty

Abnormal LC neurotransmission may lead to behavioral deficits related to stress adaptation [44]. Indeed, release of NA within the cortex is a strong modulator of the response to stressful events. It is particularly important for adaptation to novelty [45–47]. Thus, we exposed rats to a novel environment and measured their locomotor activity in response to novelty. TGR rats exhibited hyperactivity during the first 5 min of a 20-min open-field test, compared to controls (Figure 4A). Within this period of time, we measured abnormally high numbers of horizontal movements (locomotion) and vertical movements (rearing) in TGR rats. This was not associated with an alteration of the daily locomotor activity of TGR rats, as compared to controls (Figure 4B,C).

Basal Paradoxical Sleep Duration and Sleep Rebound Following Total Sleep Deprivation are Altered in TGR Rats

Gonzalez et al. [48,49] showed that the duration of the PS phase is increased in rats during the day following a 10-h TSD. This PS

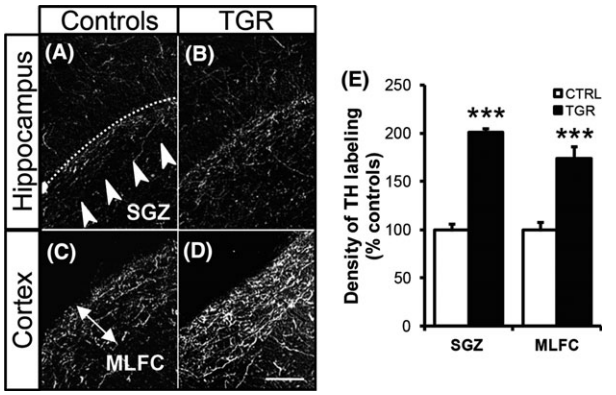

Figure 2 Tyrosine hydroxylase concentration and distribution are altered in the terminal fields of locus coeruleus neurons of TGR rats. TH protein is detected by immunohistochemistry within the subgranular layer of the dentate gyrus (SGZ; **A,B**) and the molecular layer of the frontal cortex (MLFC; **C,D**) in both control (**A** and **C**) and TGR rats (**B** and **D**). Scale bar: 100 μm. (**E**) Density of TH immunolabeling is increased in the SGZ and MLFC of TGR rats, as compared to controls. Results are the mean ± SEM (n = 3). ***$P < 0.001$, ANOVA I.

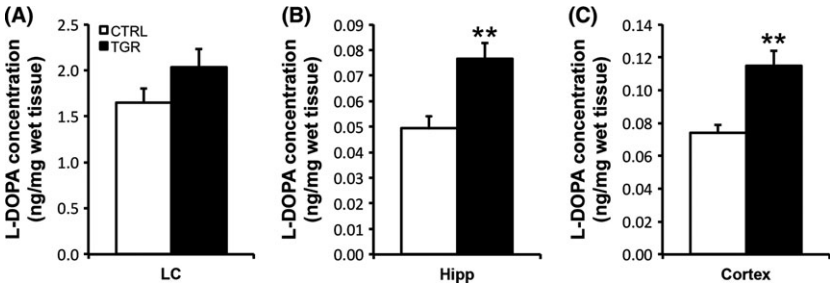

Figure 3 *In vivo* TH activity is increased in the hippocampus and cortex of TGR rats. *In vivo* TH activity was estimated by measuring the regional accumulation of L-DOPA in LC (**A**), hippocampal (**B**) and cortical (**C**) tissues extracted from control (white bars) and TGR rats (black bars) 20 min after the injection of the DOPA decarboxylase blocking agent NSD-1015. Although TH activity tends to be increased in the LC region of TGR rats compared to controls, there is no significant difference between the two groups of rats. In contrast, TH activity is significantly increased in the hippocampus and frontal cortex of TGR rats compared to controls. Results are the mean concentration of L-DOPA ± SEM (n = 5). $^{**}P < 0.01$, ANOVA I.

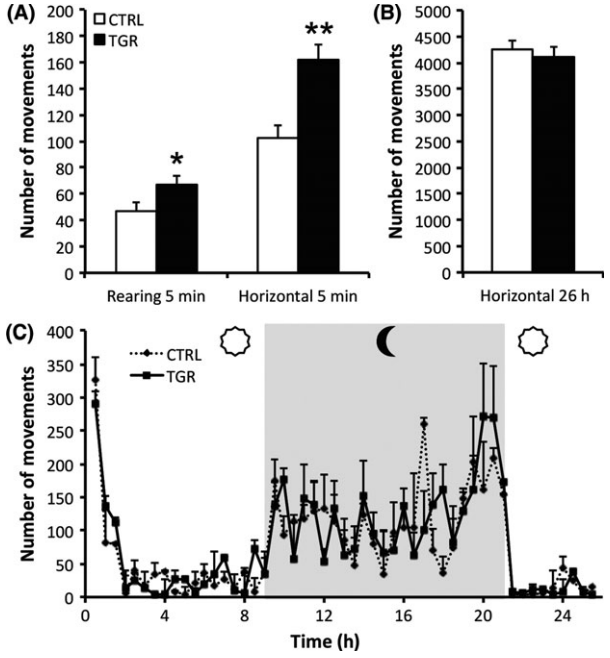

Figure 4 TGR rats exhibit locomotor hyperactivity in response to novelty. (**A**) The number of horizontal and vertical movements counted during the first 5 min of the 20-min open-field test is greater in TGR rats (black bars) than in controls (white bars). (**B,C**) The daily locomotor activity profile is not different between TGR and control rats, suggesting that the hyperactivity displayed by TGR rats in response to novelty is not the consequence of locomotor dysfunction. Results are the mean ± SEM (n = 5). $^{*}P < 0.05$, $^{**}P < 0.01$, ANOVA I.

rebound is, at least in part, modulated by the LC noradrenergic system. Thus, we measured the duration of wakefulness, SWS and PS during 24 h in control and TGR rats in basal conditions and one day after TSD. We found that duration of the PS phase was increased in TGR rats compared to controls in basal conditions (Figure 5A; $P < 0.01$, ANOVA I). TSD increased the duration of the PS phase in both control and TGR rats (Figure 5B); however, the amplitude of the post-TSD PS rebound was blunted in TGR rats (Figure 5C; $P < 0.05$, ANOVA I).

Discussion

The data presented here reinforce the hypothesis that LC noradrenergic neurons are a main target for the brain RAS *in vivo*. In addition, our results suggest that chronic brain RAS disturbances might lead to long-term LC dysfunction and associated behavioral and cognitive alterations.

In this study, we used the TGR(ASrAOGEN) rat line 680 to explore the functional relationship between the brain RAS and the NA neurotransmitter system. Originally, two transgenic lines expressing the AS RNA transgene were analyzed in detail, the TGR680 and TGR1 [8]. Heterozygous animals of these lines were characterized with regard to the expression pattern of the AS RNA and pathophysiological alterations. In TGR1 rats, the transgene is transmitted exclusively to male offspring, indicating that it is most likely integrated into the Y chromosome. By contrast, transgene-positive rats of both genders were obtained in the 680 line, proving the existence of alternative integration sites. The pathophysiological alterations observed in male TGR1 and TGR680 are similar, therefore, it is likely that they are caused by the expression of AS RNA rather than by insertional mutations.

TGR rats display behavioral deficits, that is, increased sensitivity to stress and anxiety, as well as indices of depression [17–19,50]. Furthermore, we report, here, that TGR rats exhibit sleep disorders associated with increased daily PS duration and blunted adaptation to sleep deprivation. So far, behavioral deficits detected in TGR rats have been attributed to dysfunction of the serotonin neurotransmitter system [19,50]. However, these behavioral deficits could be related to noradrenergic dysfunction as well. Indeed, the central NA neurotransmitter system is involved in stress adaptation and participates to the alternation of sleep–wake stages [20–22,48,49]. It is also required for adequate regulation of the cardiovascular function (blood pressure and baroreflex) [51], which is disturbed in TGR rats [8,13]. Unfortunately, this particular neurotransmitter system has been largely underinvestigated in TGR rats [29]. Our data show, for the first time, that TH expression is increased in the LC of TGR rats compared to controls. In addition, we detected abnormally high level of TH and increased tissue TH activity in the cortex and hippocampus of these rats, while *in situ* distribution and activity of the enzyme within the LC region was unaffected. Thus, our data suggest that, in TGR rats, an excess

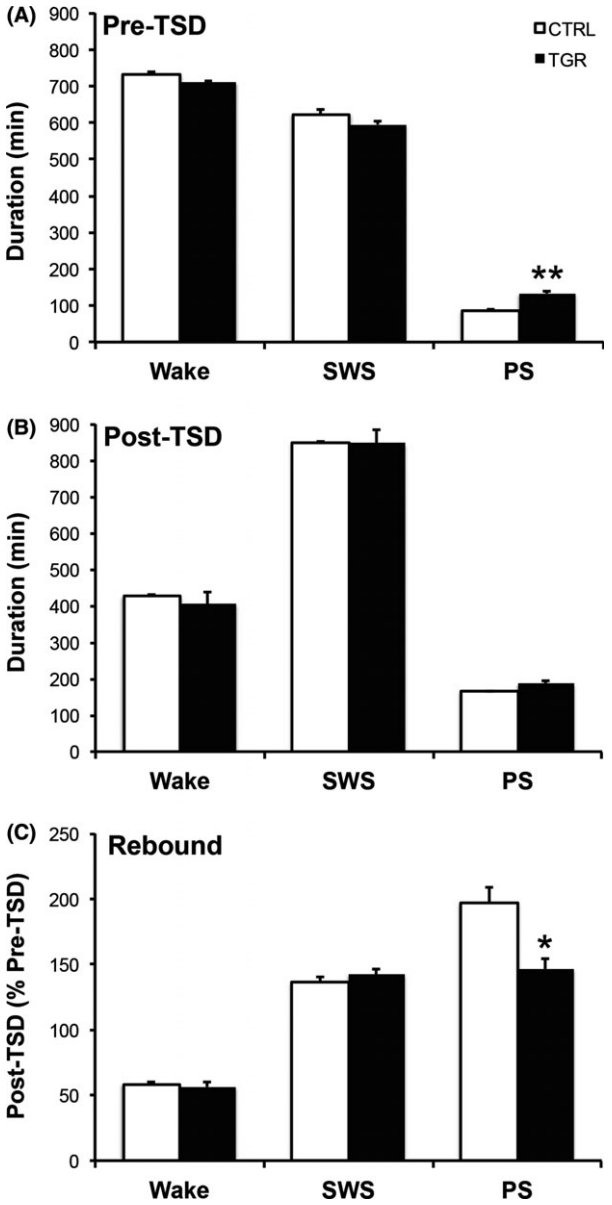

Figure 5 Daily paradoxical sleep duration and sleep rebound following total sleep deprivation (TSD) are altered in TGR rats. (**A**) Daily duration of wakefulness (Wake), slow wave sleep (SWS) and paradoxical sleep (PS) was recorded in basal conditions (baseline) in control (white bars) and TGR (black bars) rats. Baseline PS duration was significantly increased in TGR rats compared to controls. (**B**) There was no significant difference in the duration of Wake, SWS and PS between TGR rats and their controls during the 24 h following TSD. (**C**) Consequently, the ratio between PS duration after and before TSD, called PS rebound, is reduced in TGR rats compared to controls. Results are the mean ± SEM (n = 5). $*P < 0.05$, $**P < 0.01$, ANOVA I.

amount of TH is produced by LC neurons and preferentially targeted toward their axons, likely to support greater NA neurotransmission in remote brain areas. Although further *in vivo* studies are needed to support this hypothesis, enhanced release of NA in the forebrain regions innervated by LC neurons is likely to contribute to cardiovascular, behavioral and cognitive deficits exhibited by adult TGR rats (see below).

A number of studies have explored the effects of lesions or stimulation of LC neurons on stress, sleep and the cardiovascular function in rodents. 6-OH-DA-induced lesions of the LC and subsequent brain NA depletion are associated with reduced exploratory behavior in novel environment paradigms [47] as well as exaggerated sleep rebound following total sleep deprivation [48,49]. Furthermore, microinjection of glutamate or homocysteic acid into the LC decreases both blood pressure and heart rate [51]. Thus, it is plausible that increased brain NA neurotransmission may be associated with lower sleep rebound following sleep deprivation, diminished blood pressure and baroreflex activity as well as increased exploratory activity in novel environment tests, all of which are displayed by adult TGR rats. To further support a role for NA neurotransmission in behavioral and cardiovascular deficits developed by TGR rats, future experiments will aim at measuring if alleviation of these deficits can be achieved by treating TGR rats with NA depleting agents, downregulating TH synthesis within the LC using local siRNA injections, or by optogenetic inhibition of the activity of LC neurons.

Locus coeruleus neurons express relatively high levels of AT2 receptors [52,53] *in vivo* while only limited AT1 receptor mRNA level and AT4 receptor binding sites have been detected [54,55]. However, little is known about how AT2 receptors influence the activity of LC neurons *in vivo*. Recent work has demonstrated that intracerebroventricular injection of the AT2 receptor agonist CGP42112 decreases the hypothalamic NA release triggered by electrical stimulation of the LC [56], but the underlying mechanisms have not been investigated. A series of *in vitro* studies have established that hypothalamic and brainstem noradrenergic neurons isolated from newborn rats coexpress AT1 and AT2 angiotensin receptor subtypes *in vitro*, both of which are activated following treatment with Ang-II [57,58]. These studies revealed that chronic treatment with Ang-II increases the expression of TH protein through AT1 receptor stimulation and subsequent activation of the MAP kinase intracellular pathway; on the other hand, activation of AT2 receptors by Ang-II in these neurons has an inhibitory effect on the MAP kinase pathway. Thus, AT2 stimulation could potentially decrease TH expression in noradrenergic neurons, especially in LC neurons. The results presented here are in line with this hypothesis. Indeed, we found that drastic downregulation of brain AOGEN synthesis, associated with low levels of brain angiotensins [8,11], results in increased TH expression within LC neurons and enhanced density of TH-containing axons within the cortex and hippocampus of TGR rats. To determine if hypostimulation of AT2 receptors is directly responsible for such alterations, future studies might investigate whether (1) expression of AT2 receptors in the LC is maintained or downregulated in LC neurons of TGR rats and, if maintained, (2) systemic or local (intracerebral injection) treatment with agonists that are highly specific to AT2 receptors, and do not bind to AT1 receptors, can restore TH expression in adult TGR rats down to control level.

The authors acknowledge that the work presented here has few methodological and technical limitations. Indeed, as the transgenic line used has constitutive expression of the antisense transgene, it is difficult to determine whether the effects of brain

AOGEN depletion on TH expression and distribution are set during development or are ongoing. Nevertheless, prior studies from our laboratory have demonstrated that TH expression and distribution are altered in the medulla oblongata of 4-week-old TGR rats [29]. Thus, it is reasonable to assume that the phenotypic alterations of LC noradrenergic neurons detected in adult TGR rats may be due to both developmental and ongoing effects of brain AOGEN depletion.

Conclusion

Altogether, we provide strong evidence indicating that glia-derived angiotensin peptides play a critical role in the regulation of TH gene expression in LC neurons. In addition, our results suggest that noradrenergic dysfunction associated with disruption of the brain RAS may be responsible for cognitive and stress response deficits developed by TGR rats.

Acknowledgment

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Conflict of Interest

The authors declare no conflict of interest.

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