Persistent $\alpha_1$-adrenergic receptor function in the nucleus locus coeruleus causes hyperexcitability in AD/HD model rats

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Igata S, Hayashi T, Itoh M, Akasu T, Takano M, Ishimatsu M. Persistent $\alpha_1$-adrenergic receptor function in the nucleus locus coeruleus causes hyperexcitability in AD/HD model rats. J Neurophysiol 111: 777–786, 2014. First published November 27, 2013; doi:10.1152/jn.01103.2012.—Spontaneously hypertensive rats (SHR) are widely used as a model of attention deficit hyperactivity disorder (ADHD) as their ADHD-like behaviors are restored by methylphenidate. However, a postnatal neural development in SHR is unknown. We performed whole cell patch clamp recordings from locus coeruleus (LC) neurons in neonatal (postnatal day (P) 3–5), juvenile (P21–28), and adult (P 49–56) SHR and age-matched Wistar rats to evaluate $\alpha_1$- and $\alpha_2$-adrenergic receptor (ARs) activities at each developmental period. LC neurons in neonatal Wistar rats and SHR showed no difference in resting membrane potential and spontaneous firing rate, while juvenile and adult SHR LC neurons showed depolarized resting membrane potential and faster spontaneous firing rate than in Wistar rats. Blockade of $\alpha_1$-AR activity by prazosin hyperpolarized the membrane and abolished spontaneous firings in all developmental periods in SHR LC neurons, but not in juvenile and adult Wistar rats. $\alpha_1$-AR stimulation by phenylephrine evoked an inward current in juvenile LC neurons in SHR, but not in juvenile Wistar rats. This phenylephrine-induced inward current was abolished by nonselective cation channel blockers. By contrast, $\alpha_2$-AR stimulation-induced outward currents in the presence of an $\alpha_1$-AR antagonist were equivalent in SHR and Wistar LC neurons. These data suggest that Wistar LC neurons lose $\alpha_1$-AR function during development, whereas $\alpha_1$-ARs remain functional in SHR LC neurons. Thus persistent intrinsic activity of $\alpha_1$-ARs may be a neural mechanism contributing to developmental disorders in juvenile SHRs.

The nucleus locus coeruleus (LC) is the major noradrenergic nucleus of the brain and provides the noradrenergic projections to multiple brain areas, including the prefrontal cortex (Aston-Jones et al. 1991; Foote et al. 1980). Resting membrane potential (RMP) and a pattern of spontaneous firing in LC neurons are associated with arousal and vigilance and are potentially affected by $\alpha_1$- and $\alpha_2$-AR activation (Aston-Jones and Bloom 1981a, 1981b; Aston-Jones and Cohen 2005; Foote et al. 1980; Howells et al. 2012). Application of clonidine to rat LC neurons activates a class of G protein-coupled inwardly rectifying potassium (GIRK) channels via the $\alpha_2$-AR, resulting in membrane hyperpolarization and inhibition of the spontaneous firing of the action potentials (Aghajanian and Vander-Maalen 1982; Arima et al. 1998; Williams et al. 1985, 1988). By contrast, Williams and Marshall (1987) reported that $\alpha_1$-AR agonists such as phenylephrine (PE) induced membrane depolarization and accelerated spontaneous firing rates (SFRs) by activating unknown cation channels in neonatal rat LC neurons. While $\alpha_1$-ARs are known to couple with a Gq protein, it remains unclear what cationic channel is involved in the PE-induced membrane depolarization. Moreover, they reported that the effects of $\alpha_1$-AR on membrane potential were only detected in LC neurons from neonatal rats, but not from older rats [older than postnatal day (P) 21]. These studies suggest that the activities of LC neurons under control of both $\alpha_1$- and $\alpha_2$-ARs could be altered during postnatal developmental periods (neonatal, juvenile and adult).

The spontaneously hypertensive rat (SHR) is the most widely used animal model of ADHD (Adriani et al. 2003; Russell 2007; Sogvolden 2000; Sogvolden et al. 1993). A juvenile SHR (P21–28) starts to display behaviors characteristic of ADHD with no hypertension, which can be restored by intra-abdominal injection of therapeutic agents such as MPH (Adriani et al. 2003). Although there are several behavioral studies using SHR, electrophysiological studies using brain slice preparations are limited. In particular, SHR LC activities in the different developmental periods are critical to understanding local neural communication in developmental disorders. In the present study, we evaluated the $\alpha_1$- and $\alpha_2$-AR contribution to the membrane properties of LC neurons using neonatal, juvenile, and adult SHR and compared them with age-matched Wistar rats. We also explored the unknown cationic channel activated by $\alpha_1$-AR stimulation.
METHODS

Slice preparation. All experimental procedures were approved by the Institutional Animal Use and Care Committee of Kurume University School of Medicine and were performed in accordance with the guiding principles of the Physiological Society of Japan. All animals were housed in a controlled environment room under a 12:12-h light-dark cycle with free access to food and water. Male Wistar rats and SHRs during P3–5, P21–28 and P49–56 were decapitated under pentobarbital sodium anesthesia (50 mg/kg ip). The brains were quickly removed and immersed for 8–10 s in a cooled sucrose artificial cerebrospinal fluid (ACSF, 4–6°C) that was prebubbled with 95% O₂-5% CO₂. Horizontal brain slices (240 μm thick) were cut with a vibrating microtome (VT1000s; Leica, Wetzlar, Germany) in cooled sucrose ACSF. The sucrose ACSF was composed of 199 mM sucrose, 2.5 mM KCl, 2.4 mM MgCl₂, 23.8 mM NaHCO₃, 12.5 mM NaHPO₄ and 10 mM D-glucose. Brain slices were left to recover for 1 h in oxygenated normal ACSF at 32°C. A hemisected slice was then transferred to a recording chamber and submerged in the normal ACSF at 32–34°C with a perfusion rate of 2.5 ml/min. The composition of the normal ACSF was 126 mM NaCl, 2.5 mM KCl, 2.4 mM CaCl₂, 1.2 mM MgCl₂, 21 mM NaHCO₃, 1.2 mM NaHPO₄ and 11 mM D-glucose (pH 7.4, 279–288 mOsm).

Electrophysiological recordings. Whole cell recordings were made from LC neurons using the slice patch technique. Patch pipettes were prepared from borosilicate glass capillaries with a micropipette puller (PP83; Narishige, Tokyo, Japan). Patch pipettes were filled with an internal solution of 140 mM K-gluconate, 10 mM NaCl, 0.3 mM CaCl₂, 1 mM MgCl₂, 1 mM ethylene glycol-bis(2-aminoethyl ether)-N,N',N''-tetraacetic acid (EGTA), 0.25 mM guanosine 5'-triphosphate sodium salt hydrate (GTP) and 10 mM HEPES (pH 7.3 adjusted by KOH, 280–285 mOsm). The tip resistance of the whole cell patch pipette was 3–5 MΩ. The liquid junction potential of −10.8 mV for K-gluconate was corrected. Whole cell recordings were sampled at 10 kHz using an Axopatch 200B amplifier (Axon Instruments, Sunnyvale, CA).
vate, CA). Spontaneous action potentials and RMP were recorded from LC neurons using the whole cell current-clamp mode. Voltage clamp was analyzed at a holding potential of \(-60\) mV. Membrane potential and current were filtered at 1 kHz for analysis with pClamp software (Axon Instruments). The drugs used in the present study were GTP, EGTA, norepinephrine (noradrenaline (NA)), yohimbine, prazosin, PE, 2-aminoethyl diphenylborinate (2-APB), flufenamic acid (FFA), MPH (Sigma, St. Louis, MO), BaCl2 (Wako, Tokyo, Japan) and tetrodotoxin (Nacalai Tesque, Kyoto, Japan). Yohimbine, prazosin, 2-APB and FFA were dissolved in dimethyl sulfoxide, with a final concentration of 0.02% dimethyl sulfoxide.

**Immunohistochemistry in LC.** Six rats were anesthetized and transcardially perfused with heparinized saline followed by 300 ml of 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). Each brain was removed and a section containing the LC was cut and postfixed in 4% paraformaldehyde at 4°C for 18 h. The section was cryoprotected in 0.1 M phosphate buffer containing 30% (wt/vol) sucrose for 18 h. The horizontal section was further cut into 70-μm-thick slices on a freezing microtome. The slices were incubated in 5% normal donkey serum for 1 h at room temperature (RT). Free-floating slices were incubated with a polyclonal rabbit anti-α1A-adrenoceptor antibody (1:200; Alomone, Jerusalem, Israel), a polyclonal rabbit anti-α2B-adrenoceptor antibody (1:200; Alomone) or a monoclonal anti-tyrosine-hydroxylase (TH) antibody (1:2000; Sigma) for 24 h at 4°C in phosphate-buffered saline containing 0.5% (vol/vol) Triton X-100, 1% (wt/vol) bovine serum albumin and 0.1% (wt/vol) NaN3. After several rinses in phosphate-buffered saline, the slices were incubated in Alexa Fluor 488 Goat Anti-Rabbit IgG (1:1000; Invitrogen, Carlsbad, CA) and Alexa Fluor 568 Goat Anti-Mouse IgG (1:500; Invitrogen) for 4 h at RT. After washing, the specimens were mounted using PermaFluor aqueous mounting medium (Thermo, Fremont, CA). The preparations were examined on a FluoView 1000 laser scanning confocal microscope system (Olympus, Tokyo, Japan). The controls included omission of the primary antibody.

**Statistical analyses.** Each experimental value was presented as the mean ± SE, and data were analyzed by unpaired Student’s t-test. All statistical comparisons were made using Prism version 4 software (GraphPad, San Diego, CA). P values <0.05 were considered statistically significant.

**RESULTS**

To examine LC neuron membrane properties during postnatal development, we compared the RMP and SFR at three developmental time points, P3–5 (neonatal), P21–28 (juvenile) and P49–56 (adult), using a whole cell patch clamp technique (Fig. 1). Neonatal RMP was \(-54.8 \pm 0.8\) mV and \(-53.5 \pm 1.0\) mV in Wistar rats (n = 10) and SHR (n = 8), respectively. The SFR was \(0.95 \pm 0.23\) Hz and \(1.05 \pm 0.56\) Hz in Wistar rats (n = 10) and SHR (n = 8), respectively (Fig. 1A). In juvenile animals, RMP and SFR were significantly increased from neonatal values. In adult animals, RMP and SFR were significantly decreased from juvenile values. The effects of prazosin (500 nM) on membrane potential and spontaneous firing of LC neurons are shown in Fig. 2. Representative traces were obtained from neonatal (A), juvenile (B), and adult rats (C) (neonatal: Wistar n = 8 from five rats; SHR, n = 8 from four rats; juvenile: Wistar, n = 6 from five rats; SHR n = 7 from four rats; adult: Wistar, n = 7 from four rats, SHR, n = 5 from four rats). Note that all LC neurons except from juvenile and adult Wistar rats were hyperpolarized by prazosin. Juvenile and adult Wistar LC neurons were prazosin resistant.
was $-57.4 \pm 0.7 \text{mV (n = 18)}$ in Wistar rats and $-53.1 \pm 1.2 \text{mV (n = 19)}$ in SHR, and SFR was $0.57 \pm 0.06 \text{Hz (n = 18)}$ in Wistar rats and $1.23 \pm 0.17 \text{Hz (n = 19)}$ in SHR (Fig. 1B). In adult animals, RMP was $-57.2 \pm 0.6 \text{mV (n = 12)}$ in Wistar rats and $-54.3 \pm 0.9 \text{mV (n = 12)}$ in SHR, and SFR was $0.65 \pm 0.16 \text{Hz (n = 12)}$ in Wistar rats and $1.7 \pm 0.13 \text{Hz (n = 12)}$ in SHR (Fig. 1C). In the neonate, RMP and SFR were not statistically different between SHR and Wistar rat LC neurons, while RMP was significantly depolarized ($P < 0.01$) and SFR was significantly faster ($P < 0.01$) in SHR LC neurons in the juvenile and adult periods compared with age-matched Wistar rat. Both $\alpha_1$- and $\alpha_2$-ARs are known to be involved with RMP of LC neurons via a cation channel and an inwardly rectifying potassium channel, respectively. Williams and Marshall (1987) reported that an inward current coupled with $\alpha_1$-ARs is a characteristic membrane property in neonatal LC neurons.

Next, we examined a possible involvement of $\alpha_1$-ARs in the membrane potential in juvenile and adult rat LC neurons. First, we tested the effect of prazosin on RMP and spontaneous firing. Prazosin (500 nM) induced membrane hyperpolarization and inhibition of spontaneous firing in neonatal rat LC neurons (Wistar: $5.5 \pm 0.7 \text{mV, n = 8}$; SHR: $5.9 \pm 0.6 \text{mV, n = 8}$; $P = 0.58$, Fig. 2A). Juvenile SHR LC neurons also exhibited membrane hyperpolarization ($6.9 \pm 0.7 \text{mV, n = 7}$), which was not observed in age-matched Wistar rats ($n = 6$) (Fig. 2B). We also confirmed that LC neurons obtained from adult SHRs showed hyperpolarization by prazosin ($6.3 \pm 0.8 \text{mV, n = 5}$), which was not observed in adult Wistar rats ($n = 7$) (Fig. 2C). These results show that the membrane properties of juvenile and adult SHR are qualitatively equivalent. It was previously reported that SHR ($P < 0.05$) become hypertensive, which could alter a central and/or a peripheral catecholaminergic system. We performed the following experiments using neonatal and juvenile SHR. In neonatal rats, prazosin (500 nM) in the presence of tetrodotoxin (1 $\mu$M) and yohimbine (1 $\mu$M) induced outward currents in LC neurons (Wistar: $13.8 \pm 1.3 \text{pA, n = 7}$; SHR: $17.2 \pm 2.1 \text{pA, n = 7}$; $P = 0.44$, Fig. 3A). In juvenile rats, while prazosin also evoked an outward current ($18.7 \pm 1.6 \text{pA, n = 7}$) in SHR LC neurons, Wistar LC neurons were not affected by prazosin ($n = 8$) (Fig. 3B). Next, we tested the effect of PE, an $\alpha_2$-AR agonist. Bath application of PE (30 $\mu$M) in the presence of yohimbine (1 $\mu$M), an $\alpha_2$-AR antagonist, caused inward currents accompanied by an increase in the conductance in LC neurons in neonatal rats (Wistar: $-20.2 \pm 0.5 \text{pA, n = 10}$; SHR: $-22.9 \pm 1.4 \text{pA, n = 16}$; $P = 0.117$, Fig. 4A) and in juvenile SHR ($-17.9 \pm 1.4 \text{pA, n = 21}$). However, no juvenile Wistar LC neurons ($n = 15$) showed an inward current by PE (Fig. 4B). A steady inward current with no obvious desensitization.
zation was observed by continuous application of PE (30 μM) up to 15 min in juvenile SHR LC neurons (data not shown). This PE-induced inward current was blocked by the α₁-antagonist, prazosin (500 nM) (Fig. 5A).

An α₁-AR is known to couple with Gq protein, which was reported to activate nonselective cation channels (NSCC) (Clapham 2003; Montell 2005). Thus we examined whether the NSCC blockers 2-APB and FFA could abolish the PE-induced inward current. When the PE-induced inward currents reached the maximum amplitude, addition of prazosin, 2-APB, or FFA to the superfusate solution successfully blocked the inward current in juvenile SHR LC neurons (prazosin: 2.42 ± 3.3 pA, n = 8; 2-APB: 12.4 ± 1.9 pA, n = 9; FFA: 9.1 ± 3.3 pA, n = 8; Fig. 5, A and B). These results suggest an involvement of NSCC via α₁-AR activation on RMP in LC neurons in neonatal Wistar and SHR rats, and in juvenile SHR rats, but not in juvenile Wistar rats.

An inwardly rectifying potassium channel (GIRK) coupled to α₂-ARs is known to contribute to RMP in rat LC neurons. To evaluate the amount of outward current via α₂-AR under physiological conditions, we examined the role of α₂-ARs on the membrane current of LC neurons from juvenile SHR and Wistar rats. High-dose NA (>10 μM) induced a smaller outward current in juvenile SHR LC neurons compared with Wistar rats (Fig. 6C). Low-dose NA (3 μM) induced an inward current in juvenile SHR LC neurons only (~20.2 ± 2.2 pA, n = 6), but not in Wistar rats (n = 10) (Fig. 6, A and B). Dose-dependent effects of NA are illustrated in Fig. 6C. NA (3 μM) evoked an inward current, while the NA (>3 μM) evoked an outward current in juvenile SHR LC neurons. By contrast, all juvenile Wistar neurons showed an outward current by NA (3–300 μM).

Surprisingly, NA (3–100 μM)-induced outward currents obtained in the presence of prazosin (500 nM), an α₁-antagonist, were virtually equivalent between juvenile SHR and Wistar LC neurons (Fig. 6D). A larger outward current was obtained by NA (≥10 μM) in the presence of prazosin from juvenile SHR LC neurons. Additionally, the Ba²⁺, GIRK inhibitor, sensitive NA current was identical in both SHR and Wistar juvenile LC neurons (data not shown). We also examined the effect of MPH (30 μM) on the membrane current in LC neurons using whole cell voltage clamp recording at a holding potential of −60 mV. All LC neurons from juvenile Wistar rats showed outward currents (36 ± 5.01 pA, n = 15). By contrast, bath application of MPH to juvenile SHR caused varied currents, depending on the cells (~35 to +30 pA; mean ± SE = −2.0 ± 5.0 pA; n = 15 from six rats): inward current in five cells, outward current in five cells, and no effect in five cells. Lower concentrations of MPH (1 μM) induced no obvious current in both Wistar and SHR (Fig. 6E).

Finally, we tested the possibility that α₁-ARs of LC neurons were absent in juvenile Wistar rats using immunohistochemistry for α₁A- or α₁B-AR (green), TH (red) and cell nuclei (blue). TH immunoreactivity in the region of the LC neurons is shown in Fig. 7, A and D. Two distinct α₁A- and α₁B-ARs...
were located within a field of dense TH-positive neurons in both SHR and Wistar rats (Fig. 7, C and F).

DISCUSSION

In the present study we examined the membrane properties of LC neurons in SHR and Wistar rats at different postnatal developmental stages. SHR are widely used as an ADHD model animal as they display ADHD-like characteristic behaviors, such as hyperactivity and impulsivity (Adriani et al. 2003; Russell 2007; Sagvolden 2000; Sagvolden et al. 1993) in the juvenile period (P21–28) before development of hypertension (>P35). In previous reports, the Wistar Kyoto rat strain was recently reported to represent a type of depression animal model (Andrus et al. 2012; Hurley et al. 2013; Yamada et al. 2013). Therefore, we used Wistar rats as control animals in the present study.

We explored the local neural mechanisms in LC underlying the developmental disorder in SHR. It was reported that the firing patterns of LC neurons are critical for attention and vigilance. Two firing patterns of LC neurons have been reported: phasic and tonic. Animals showed good performance in selective attention tasks during phasic firing, while the performance became poor when LC neurons showed tonic firing (Aston-Jones and Bloom 1981a, 1981b; Aston-Jones and Cohen 2005; Foote et al. 1980; Howells et al. 2012; Rajkowski et al. 1994). Although we did not analyze the firing patterns of LC neurons, the RMP will change the firing frequency and possibly alter the firing patterns. A depolarized membrane and faster firing frequency in juvenile SHR LC neurons would affect cognitive function in juvenile SHR. RMP and SFR in neonatal LC neurons were not statistically different between SHR and Wistar rats, whereas juvenile and adult SHR showed a significantly depolarized membrane potential and an increase in SFR compared with age-matched Wistar rats.

The LC is the major noradrenergic nucleus in the central nervous system. We examined the involvement of $\alpha_1$- and $\alpha_2$-ARs in the depolarized membrane potential observed in juvenile SHR LC neurons. $\alpha_1$-ARs are Gq-coupled and have been reported to cause membrane depolarization by both augmentation of a nonselective cationic conductance and inhibition of a potassium conductance (Montell 2005; Wu et al. 2010). Activation of $\alpha_1$-ARs in neurons leads to the production of inositol 1,4,5-trisphosphate and diacylglycerol, which mobilizes $Ca^{2+}$ from internal $Ca^{2+}$ stores and $Ca^{2+}$ influx from outside the cell through a NSCC. Our results showed that PE induced an inward current in juvenile and neonatal SHR, as well as in neonatal Wistar LC neurons, but not in juvenile Wistar LC neurons. The PE-induced inward current of juvenile SHR LC neurons was inhibited by prazosin, as well as by the NSCC channel blockers 2-APB and FFA. 2-APB and FFA are also used as TRP channel blockers. TRP channels can be activated by $\alpha_1$-ARs through the Gq protein. TRP channels are a family of NSCC that can form receptor-operated channels and store-operated channels (Clapham 2003; Clapham et al. 2003; Montell 2005; Venkataraman and Montell 2007; Wu 2010). PE was reported to activate an unknown cation channel in neonatal rat LC neurons (Williams and Marshall 1987). It is possible that the PE-induced inward current in LC neurons may be due to activation of NSCC. In this study, NSCC blockers revealed a small outward current following inhibition of the PE-induced inward current. Moreover, prazosin, an $\alpha_1$-AR antagonist, in the presence of the $\alpha_2$-AR antagonist, induced an outward current in juvenile SHR LC neurons, suggesting that $\alpha_1$-AR activity may involve RMP in juvenile SHR LC neurons. When this intrinsic $\alpha_1$-AR activity is inhibited by NSCC blockers or prazosin, the outward current is revealed. This persistent intrinsic $\alpha_1$-AR activity in juvenile SHR could produce membrane depolarization in LC neurons compared with juvenile Wistar rats. Moreover, the depolarized membrane potential through $\alpha_1$-AR activation may have
induced the increase of spontaneous firing in juvenile SHR LC neurons.

The $\alpha_2$-AR channel is coupled with a GIRK channel. $\alpha_2$-AR-activated GIRK channels may also contribute to RMP in LC neurons. $\alpha_2$-AR activation hyperpolarizes the membrane and leads to a reduction in the firing rate. $\alpha_2$-AR stimulation by NA is known to activate GIRK channels and produce outward currents at membrane potentials positive to K$^+$ equilibrium potential (Aghajanian and VanderMaelen 1982; Arima et al. 1998; Williams et al. 1985, 1988). A low concentration of NA (3 $\mu$M) induced inward currents in juvenile SHR LC neurons, but not in juvenile Wistar LC neurons. NA (3–100 $\mu$M) induced outward currents in the presence of prazosin in both juvenile SHR and Wistar rat LC neurons that were virtually identical. These results indicate that the $\alpha_2$-AR coupled with the GIRK channel was equivalent in SHR and Wistar rats in juvenile LC neurons. While MPH (30 $\mu$M)-induced currents varied from the inward to outward currents in juvenile SHR, all Wistar LC neurons exhibited the outward current. These results suggest that the total current evoked by NA or MPH consists of the sum of $\alpha_1$- and $\alpha_2$-AR-activated currents in juvenile SHR.

Previous studies have confirmed the existence of $\alpha_1$-ARs in brain slice preparations from the adult rat using PCR (Osborne et al. 2002; Pullen et al. 1985). Immunohistochemical results also illustrated the presence of both $\alpha_1$- and $\alpha_2$-ARs in juvenile Wistar and SHR LC neurons, suggesting that the attenuation of functional $\alpha_1$-ARs in developed Wistar LC neurons might relate to loss of second messenger. However, the function of $\alpha_1$-ARs in the adult rat LC neurons has not been previously reported. By examining the membrane properties of LC neu-

Fig. 6. Effect of noradrenaline (NA) on the membrane current of LC neurons. Current recordings are from juvenile Wistar LC neurons (A) and juvenile SHR LC neurons (B). C: concentration response curve of NA-induced outward currents. The amplitudes of outward currents were 29.9 ± 0.6, 48.7 ± 2.6, 94.4 ± 6.5, 107.8 ± 6.3 and 108.7 ± 10.1 pA in juvenile Wistar rat LC neurons and 13.5 ± 0.4, 44.7 ± 2.6, 73.2 ± 10.4, 77.8 ± 5.9 and 83.5 ± 2.7 pA in juvenile SHR LC neurons following application of 3, 10, 30, 100 and 300 $\mu$M of NA, respectively. D: concentration-response curve of NA-induced outward current in the presence of prazosin (500 nM). The amplitudes of outward currents were 21.6 ± 3.3, 61.6 ± 2.9, 100.5 ± 15.2 and 125.7 ± 11.9 pA in juvenile Wistar rat LC neurons, and 21.6 ± 1.7, 60.2 ± 2.3, 102.7 ± 10.8 and 118.8 ± 8.7 pA in juvenile SHR LC neurons following application of 3, 10, 30 and 100 $\mu$M of NA, respectively. Each point represents the mean ± SE obtained from 4–8 LC neurons. Differences between Wistar (○) and SHR (○) rats at specific concentrations were analyzed by Student’s unpaired t-test. *P < 0.05, **P < 0.01. E: summary of the effect of methylphenidate (30 $\mu$M) on the membrane current of juvenile LC neurons. (Wistar, n = 15 from five rats; SHR, n = 15 from six rats). **P < 0.01.
In SHR and Wistar rats in postnatal developmental stages, we found a persistent $\alpha_1$-AR activity in SHR, which disappeared in Wistar rats. It is possible that the delayed maturation may have influenced the persistent $\alpha_1$-AR activity in juvenile SHR. In fact, Shaw et al. (2007) reported that human ADHD patients showed delayed maturation of the cortex and striatum. In voltage clamp experiments, we avoided using older SHR (P35) as they develop hypertension, which might alter the adrenergic system. RMP and SFR were not statistically different between juvenile and adult SHR LC neurons. Nevertheless, we confirmed that LC neurons obtained from older SHRs (P49–56) could also induce hyperpolarization by prazosin as well as for juvenile SHRs. Thus these data suggest that $\alpha_1$-ARs remain in the older SHR LC neurons.

Activation of $\alpha_2$-ARs improves working memory performance in rats, and $\alpha_2$-AR agonists can improve attention and

Fig. 7. Confocal micrographs from horizontal LC sections. Sections were triple stained for tyrosine-hydroxylase (TH; A and D; red), $\alpha_1$A- and $\alpha_2$B-AR (B and E; green) and nuclei (blue). Location of $\alpha_1$A- and $\alpha_2$B-AR are within a field of dense TH neurons in both rats. C and F: merged image. Scale bar, 100 $\mu$M.
behavioral inhibition. High levels of noradrenaline release can impair cognitive function through α1-AR activation (Arnsten and Pliszka 2011; Arnsten et al. 2007; Birnbaum et al. 1999). By contrast, α2-AR activation is necessary for MPH-induced improvement in sustained attention (Berridge et al. 2012). Low-dose MPH increases NA in the prefrontal cortex and improves cognitive function (Berridge et al. 2006; Devilbiss and Berridge 2006). In support of these reports, we demonstrated that stimulation of α1- and α2-ARs has counteracting effects, and that MPH induced an inward current in juvenile SHR LC neurons. LC neurons also project to the prefrontal cortex. Thus α1-AR activity in LC neurons may affect the release of NA in the prefrontal cortex. Moreover, the α1-AR mRNA levels decrease during postnatal development in SD rats (Nakamura et al. 1988; Osborne et al. 2002; Williams and Marshall 1987). On the other hand, there are some reports that MPH can improve cognitive function in healthy adults (Agay et al. 2010; Camp-Bruno and Herting 1994; Linssen et al. 2011; Smith and Farah 2011; Tomasi et al. 2010). We demonstrated that functional α1-ARs in the LC are attenuated during the developmental period in Wistar rats, while α1-ARs remain functional in other central nervous system areas, including the medial prefrontal cortex (mPFC) in adult animals. The mPFC is a critical area of the cerebral cortex involved in cognitive function (Berridge et al. 2006; Devilbiss and Berridge 2006). Noradrenergic activity at α1-ARs in the mPFC facilitates cognitive performance (Lapiz et al. 2006). Devoto et al. (2005) also demonstrated that electrical stimulation of the LC increased NA and dopamine (DA) in the mPFC using adult SD rats. Therefore, the effects of MPH on cognitive function in healthy adulthood could result from the action of MPH on α1-NA and/or DA receptors in the mPFC. Cognitive function consists of a variety of higher brain functions, including screening targets, sustained attention, working memory, and executive function. NA and DA receptors are involved in these cognitive functions. Of these various functions, some may be improved by α1-AR, α2-AR and DA receptors, while some may not. The role of α1- and α2-ARs and DA receptors in complex cognitive function requires further examination.

In the present study, we demonstrated that functional α1-ARs persisted in juvenile and adult SHR LC neurons, which may cause membrane depolarization and increase spontaneous firing. Although the noradrenergic receptor has three subtypes (α1, α2 and β), in the present study we only focused on the α1- and α2-ARs in LC neurons. The noradrenergic α1-ARs exist primarily at postsynaptic sites, whereas α2-ARs exist at both pre- and postsynaptic sites (Berridge and Waterhouse 2003). Noradrenergic signals are thought to be inhibited by an auto-receptor via an axon-recurrent collateral. Persistent functional α1-ARs in LC neurons and a projecting axon terminal that counteracts α2-ARs in the LC soma could interfere with the autoreceptor. Weakened α2 noradrenergic signaling by persistent α1-AR function could interrupt the action of LC neurons on cognitive function and sustained attention in juvenile SHR.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: S.I. and M. Ishimatsu conception and design of research; S.I., T.H., and M. Ishimatsu performed experiments; S.I. and M. Ishimatsu analyzed data; S.I. and M. Ishimatsu interpreted results of experiments; S.I. and T.H. and M. Ishimatsu prepared figures; S.I. and M. Ishimatsu drafted manuscript; S.I. and M. Ishimatsu edited and revised manuscript; S.I., T.H., M. Itoh, T.A., M.T., and M. Ishimatsu approved final version of manuscript.

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