Activation of PI3K/Akt Prevents Hypoxia/Reoxygenation-Induced GnRH Decline via FOXO3a

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Summary

Recent studies have suggested that the hypothalamus has an important role in aging by regulating nuclear factor-κB (NF-κB)-directed gonadotropin-releasing hormone (GnRH) decline. Moreover, our previous study has shown that ischemia-reperfusion (IR) injury activates NF-κB to reduce hypothalamic GnRH release, thus suggesting that IR injury may facilitate hypothalamic programming of system aging. In this study, we further examined the role of phosphoinositide 3-kinase (PI3K)/Protein kinase B (Akt) pathway, a critical intracellular signal pathway involved in the repair process after IR, in hypoxia-reoxygenation (HR)-associated GnRH decline *in vitro*. We used GT1-7 cells and primarily-cultured mouse GnRH neurons as cell models for investigation. Our data revealed that the activation of the PI3K/Akt/Forkhead box protein O3a (FOXO3a) pathway protects GnRH neurons from HR-induced GnRH decline by preventing HR-induced *gnrh1* gene inhibition and NF-κB activation. Our results further the understanding of the regulatory mechanisms of HR-associated hypothalamic GnRH decline.

Key words

Hypoxia-reoxygenation • Nuclear factor-κB • Gonadotropin-releasing hormone • Forkhead box protein O3a

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Introduction

Cerebral ischemia and reperfusion (IR) injury is the leading cause of mortality worldwide. It causes inflammation, increases oxidative stress, and may even lead to irreversible neurodegeneration by causing synaptic dysfunction and neuronal death [1]. Recent studies have suggested that hypothalamic neurons are more vulnerable to IR than cortical and hippocampal neurons [2,3].

It has also been suggested that the hypothalamus has an important role in aging [1,4]. Hypothalamus-driven programmatic aging has been associated with NF-κB-directed gonadotropin-releasing hormone (GnRH) decline and GnRH decline-induced impairment of neurogenesis [1,4]. NF-κB has been demonstrated to inhibit GnRH secretion from hypothalamus via inhibition of *gnrh* gene [1,4]. Moreover, our previous study has shown that IR injury activates NF-κB to reduce hypothalamic GnRH release, suggesting that hypoxia-reoxygenation (HR) injury may facilitate hypothalamic programming of system aging [2]. The PI3K/Akt pathway is a critical intracellular signal pathway involved in the repair process of various tissues during cerebral ischemic insult [3,5,6]. Phosphorylation of Akt by PI3K promotes cell survival against cerebral ischemic damage by phosphorylation and subsequent inactivation of many proapoptotic proteins, such as glycogen synthase kinase 3β, Bad, and Forkhead transcription factors (FOXO) [3,5,6]. However, whether PI3K/Akt pathway has a role in HR-induced GnRH reduction remains unclear. In this study, we further examined the role of the PI3K/Akt pathway in HR-associated GnRH decline *in vitro*, using GT1-7 cells and primarily-cultured mouse GnRH neurons as cell models for investigation.

Methods

Cell culture

GT1-7 cells (BioVector NTCC Inc) were cultured in Dulbecco's modified eagle medium (DMEM; American Type Culture Collection) supplemented with 10% FBS, Invitrogen; Thermo Fisher Scientific, Inc.), 100 IU/ml penicillin (Invitrogen; Thermo Fisher Scientific, Inc.), and 100 g/ml streptomycin (Invitrogen; Thermo Fisher Scientific, Inc.) in a humidified atmosphere containing 5%CO_2/95% air at 37ºC.

Moreover, primary hypothalamic neurons were isolated from E17 CD1 mice embryos as previously reported [7]. A total of five hypothalami from embryonic mice were isolated. Single-cell suspension was then obtained using trituration. Cells were cultured in DMEM supplemented with 10% FBS, 10% heat-inactivated defined horse serum, 1% penicillin-streptomycin, and 20 mM D-glucose in fresh sterile cell dish coated with 100 µg/ml poly-L-lysine (Sigma-Aldrich; Merck KGaA) at 37˚C with 5% CO_2.

Lentivirus-mediated shRNA silencing of FOXO3a

Lentivirus-mediated shRNA silencing was performed as follows [8, 9]. The FOXO3a shRNA-expressing plasmid was purchased from Origene Technologies, Inc. A negative “scrambled” control was purchased from Miaoling (www.miaolingbio.com). The FOXO3a shRNA-expressing and control plasmid, the packaging plasmid (pCMV-dr8.2 dvpr; Miaoling), and the envelop plasmid (pCMV-VSV-G, Miaoling) were transfected into HEK293T cells using FuGENE 6 (Promega Corporation). Virus-containing supernatants, collected three days post-infection, were then used to infect GT1-7 cells or primary hypothalamic neurons in the presence of polybrene (8 µg/ml, overnight). To select stable clones expressing the shRNA, cells were incubated for 48 h in complete medium and then subjected to puromycin (2 µg/ml; 3 days) selection.

HR induction

HR induction was conducted as previously described [2]. Briefly, cells in the HR group were treated with an ischemia-mimetic solution and incubated in 95% N_2 and 5% CO_2 for 8 h. Cells were then incubated in DMEM with 4.5 g/l glucose (pH 7.2-7.4) at 37 °C with 95% air and 5% CO_2 for 2 h. Cells of the normoxic group were exposed to DMEM with 4.5 g/l glucose (pH 7.2-7.4) at 37 °C with 95% air and 5% CO_2 for 10 h. To activate the PI3K/Akt pathway, 20 µM PI3K activator 740 Y-P (ApexBio Technology LLC) was added 30 min before HR induction.

GnRH release

GT1-7 cells were plated at 1×10^6/ml while primary hypothalamic neurons were plated at 2×10^6/ml. After drug treatment, the supernatant was collected and GnRH levels were determined using the luteinizing hormone-releasing ELISA kit (MyBioSource, Inc.) following the manufacturer’s instructions [10]. Spectrophotometric data were obtained at 450 nm in a Victor-2 Multilabel counter (Perkin-Elmer Inc.).

Phosphorylation of nuclear factor of Akt, κ light polypeptide gene enhancer in B-cells inhibitor α (IκBα) and FOXO3a by PI3K activator

Phosphorylation of phosphorylated p-Akt, p-IκBα and p-FOXO3a were analyzed using Western blot analysis as previously described [10]. Following HR induction, cells were lysed in lysis buffer containing Protease/Phosphatase Inhibitor Cocktail (Cell Signaling Technology, Inc.). Cell lysates were subjected to SDS–PAGE and electrotransferred to a polyvinylidene fluoride (PVDF) membrane. The membrane was then incubated with primary antibodies against p-IκBα (1:1,000; Ser32/36; Cell Signaling Technology, Inc.), GAPDH (1:1,000; Abcam), p-Akt (1:1,000; Ser473; Cell Signaling Technology, Inc.) or total Akt (1:1,000; Cell Signaling Technology, Inc.), or p-FOXO3a (1:1,000; Thr32; Cell Signaling Technology, Inc.) followed by 1:3,000 Goat anti-mouse or anti-rabbit IgG-HRP (Santa Cruz Biotechnology, Inc.). Protein bands were quantified using BioRad GS800 densitometer equipped with “Quantity One” software package.

Nuclear localization of FOXO3a and NF-κB

The nuclear localization of FOXO3a and NF-κB was analyzed using Western blot. Briefly, cells were lysed in subcellular fractionation buffer (250 mM sucrose;20 mM HEPES;pH 7.4;10 mM KCl; 1.5 mM $MgCl_2$;1 mM EDTA;1 mM EGTA;1 mM PVDF;1 mM dithiothreitol) after drug treatment. Cell lysates were passed through a 25-gauge needle ~10 times and then incubated on ice for 20 min. The nuclear pellet was obtained after centrifugation of cell lysates at 3000 rpm for 5 min. The cytosolic pellet was obtained after centrifugation of the supernatant at 8000 rpm for 10 min. Nuclear and cytosolic fractions were mixed with SDS

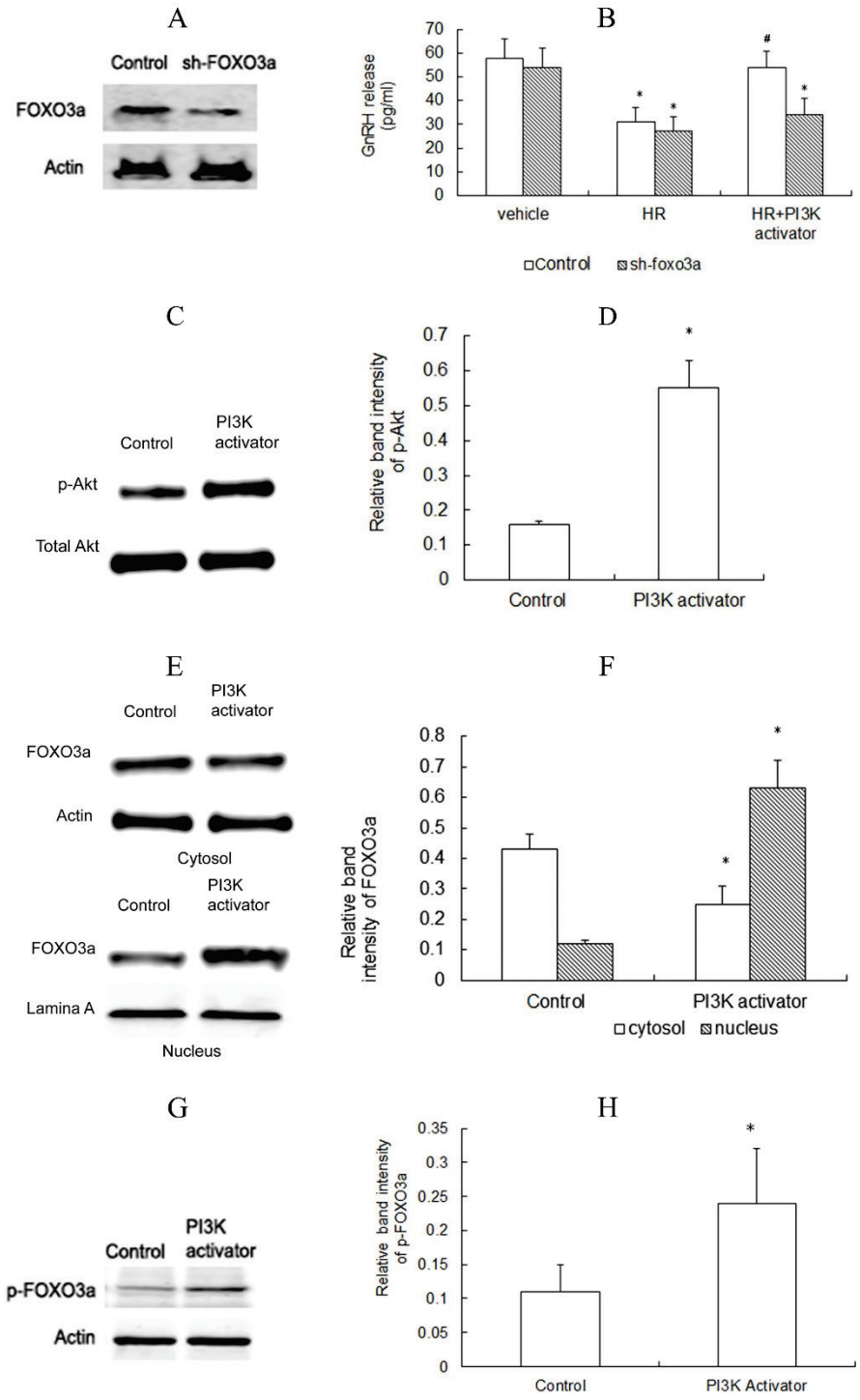

Fig. 1. Activation of PI3K/Akt/FOXO3a pathway mitigates HR-induced decrease of GnRH release. **(A)** Cells were transfected with FOXO3a shRNA or control shRNA. FOXO3a was then analyzed by Western blot. Representative western blot image for FOXO3 was shown. **(B)** Cells transfected with FOXO3a shRNA or control shRNA were treated with a PI3K activator, after which were exposed to HR. Then, GnRH released into the medium was measured by an EIA kit. n=5. **P*<0.01, compared with the control group; # *P*<0.01, compared with HR group. **(1C-1H)** Cells were treated with or without a PI3K activator for 30 min. Levels of p-Akt, FOXO3a and p-FOXO3a were then analyzed by Western blot. n=4. **P*<0.01, compared with the control group. Representative western blot image for p-Akt was shown in C. The relative band intensities of p-Akt were qualified and shown in D. Representative western blot image for FOXO3a was shown in E. The relative band intensities of FOXO3a were qualified and shown in F. Representative western blot image for p-FOXO3a was shown in G. The relative band intensities of p-FOXO3a were qualified and shown in H.

loading dye, subjected to SDS–PAGE, and electrotransferred to a PVDF membrane. The membrane was probed with antibodies to FOXO3a (1:1,000; Santa Cruz Biotechnology, Inc.) or p65 (1:400; Abcam) followed by 1:5,000 Goat anti-rabbit IgG-HRP (Santa Cruz Biotechnology, Inc.). Actin (Santa Cruz Biotechnology, Inc.) was used as a cytoplasmic marker, and lamina A (Santa Cruz Biotechnology, Inc.) was used as a nuclear marker.

Gnrh1 promoter activity

Gnrh1 promoter activity was determined using the Dual-Luciferase kit (Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol. Briefly, cells were seeded into 96-well plates (10,000 cells/well) for 12 h. Cells were then transfected with pGL3-based *gnrh1* promoter-driven luciferase plasmids (47.5 ng/well; Miaoling) using Lipofectamine for 48 h [11]. Meanwhile, cells were transfected with pRL-SV40 Renilla luciferase

plasmid (47.5 ng/well; Promega Corporation) as an internal control. Cells were lysed after drug treatment, and a Luciferase Dual Assay was performed according to the manufacturer's protocol using the same Victor-2 Multilabel counter. All experiments were repeated in triplicate.

Statistical analysis

Statistical analysis was conducted using SPSS software (SPSS, Inc.) version 15.0. Normal distribution of data was analyzed using the Ryan-Joiner test, and variance homogeneity was analyzed using Levene's test of Equal Variances. A two independent sample t-test was applied to compare the means of two groups. A one-way ANOVA was applied to compare the means of multiple groups.

Results

Activation of the PI3K/Akt pathway mitigates HR-induced GnRH decline via FOXO3a

To investigate whether the PI3K pathway activation affects the HR-related GnRH reduction, GT1-7 cells were exposed to HR in the presence or absence of 20 μM PI3K activator 740 Y-P. The results showed that the release of GnRH was significantly decreased under HR conditions; yet, the PI3K activator could mitigate this process ($P<0.01$ vs. control; Fig. 1B). Moreover, the beneficial effect of the PI3K activator was abrogated by the FOXO3a knockdown (Fig. 1A, B).

Since Akt and FOXO3a have been identified as direct substrates of PI3K [12-14] we further investigated whether Akt and FOXO3a are associated with the protective effect of PI3K activator against HR-induced GnRH reduction. The PI3K activator induced the phosphorylation of Akt and enhanced the phosphorylation and nuclear localization of FOXO3a in GT1-7 cells ($P<0.01$; Fig. 1C-1H). These results revealed that the activation of the PI3K/Akt/FOXO3a pathway could mitigate the decrease of GnRH release under HR.

Activation of the PI3K/Akt pathway prevents HR-induced inhibition of the gnrh1 gene via FOXO3a

Our previous study revealed that HR-related GnRH decrease is associated with reduced hypothalamic *gnrh1* promoter activity [1,4]. The present study further discovered that the *gnrh1* promoter activity was reduced to ~50% under HR. To verify whether the activation of the PI3K/Akt/FOXO3a pathway affects the decrease of *gnrh1* promoter activity under HR, FOXO3a-ablated and scrambled control cells were exposed to HR in the presence or absence of PI3K activator. In control cells, the PI3K activator prevented the inhibition of *gnrh1* promoter activity under HR ($P<0.01$; Fig. 2); yet, this was not observed in FOXO3a-ablated cells (Fig. 2).

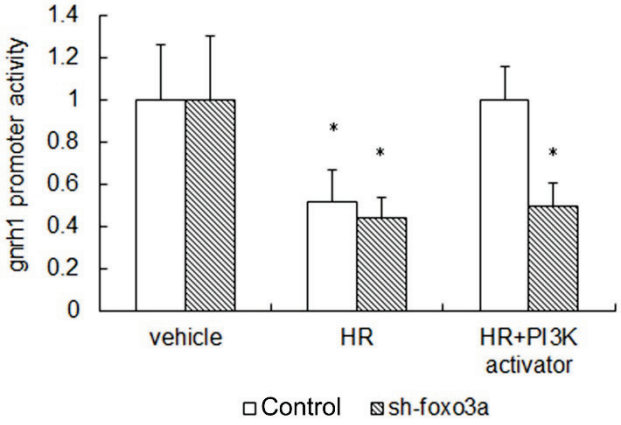

Fig. 2. Activation of PI3K/Akt/FOXO3a pathway prevents HR-induced inhibition of *gnrh1* gene. Cells transfected with a plasmid expressing a *gnrh1*-promoter + luciferase reporter gene and FOXO3a shRNA (or control shRNA) were treated with PI3K activator, after which were exposed to HR. *Gnrh1* promoter activities were then measured using the Dual-Luciferase kit. n=5. *$P<0.01$, compared with the control group.

Activation of the PI3K pathway abrogates HR-induced NF-κB activation via FOXO3a

NF-κB has been reported to be involved in an aging-related decrease in hypothalamic *gnrh1* promoter activity and GnRH release [1,4]. Cytoplasmic inactivated NF-κB binds to IκBα protein, a member of a family of cellular proteins, whose function is to inhibit the NF-κB [11,15]. NF-κB can be activated by different mechanisms, including phosphorylation and subsequent degradation of IκBα [11, 15]. In this study, we found that HR promotes IκBα phosphorylation and enhances the nuclear localization of NF-κB. Yet, treatment of cells with PI3K activator abrogated HR-induced activation of NF-κB ($P<0.01$; Fig. 3). Moreover, the PI3K activator exhibited no effects on FOXO3a-ablated cells (Fig. 3). This result suggested that the PI3K/Akt/FOXO3a pathway abrogates HR-induced NF-κB activation.

Activation of the PI3K/Akt pathway mitigates HR-induced GnRH decline in primary hypothalamic neurons via FOXO3a

To confirm the involvement of the PI3K/Akt/FOXO3a pathway in HR-induced GnRH decline, some experiments were repeated in primary hypothalamic neurons (Fig. 4). As expected, HR significantly decreased the release of GnRH from primary

hypothalamic neurons ($P<0.01$). Contrary, the treatment of cells with PI3K activator prevented HR-induced GnRH decline ($P<0.01$; Fig. 4); this process could be abrogated by the knockdown of FOXO3a (Fig. 4).

Discussion

The mammalian FOXO family consists of FOXO1, 3a, 4, and 6 [16,17]. FOXO factors are predominantly localized to the nucleus, where they regulate genes associated with the cell cycle, cell death, and oxidative stress response [18]. In the presence of growth factors, FOXOs are phosphorylated by Akt, after which they translocate to the cytoplasm [19, 20]. FOXO3a is believed to be the key target of the PI3K/AKT pathway [12-14,21]. A previous study discovered that the activation of the PI3K/Akt/FOXO3a the PI3K/Akt/FOXO3a pathway promotes cell apoptosis

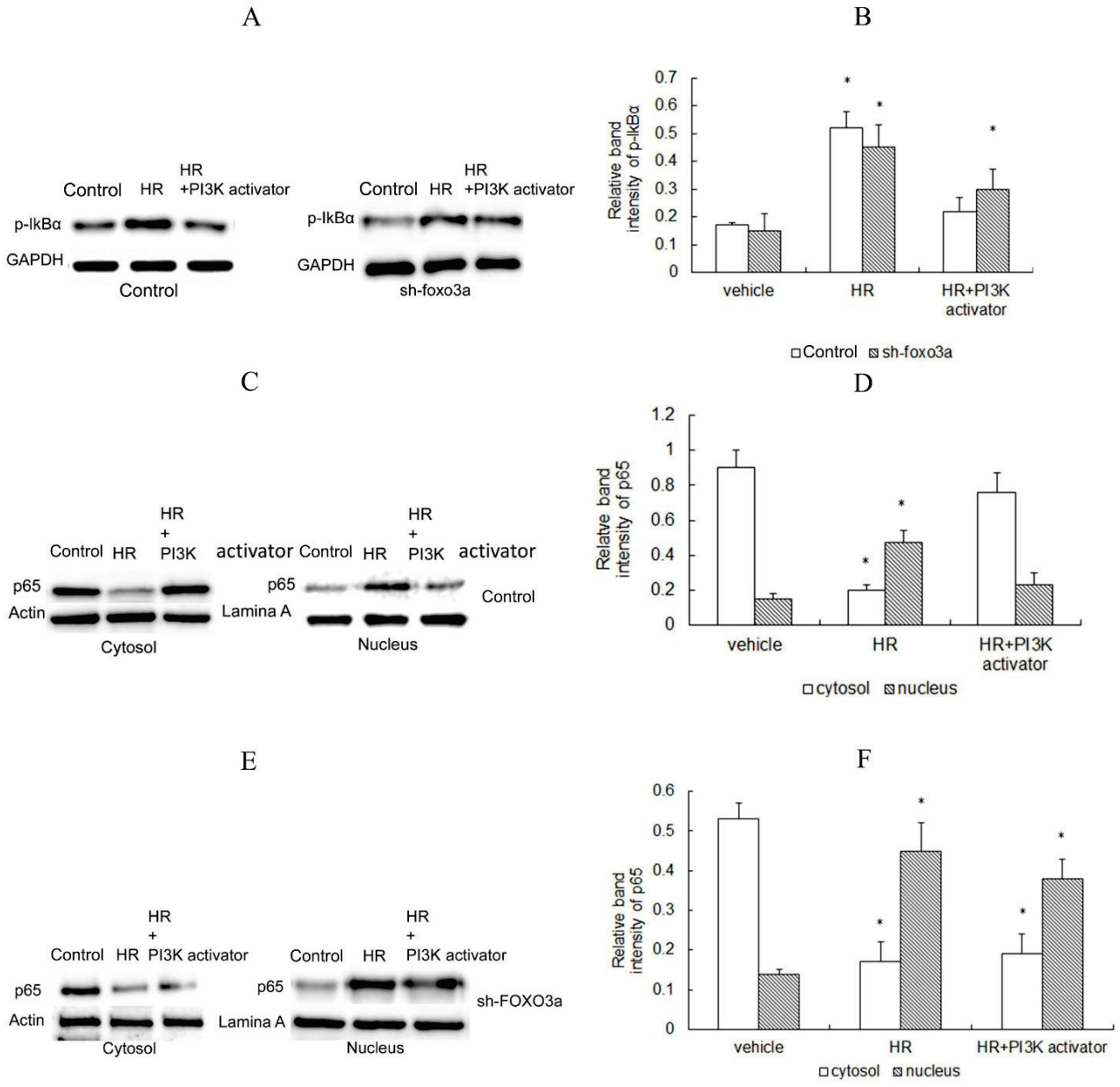

Fig. 3. Activation of PI3K/Akt/FOXO3a pathway abrogates HR-induced NF-κB activation. Cells transfected with FOXO3a shRNA or control shRNA were exposed to a PI3K activator, after which were exposed to HR. p-IκBα and NF-κB p65 were then analyzed by Western blot. n=4. *P<0.01, compared with the control group. p-IκBα and NF-κB p65 were then analyzed by Western blot. n=4. *P<0.01, compared with the control group. Representative western blot image for p-IκBα was shown in **A**. The relative band intensities of p-IκBα were qualified and shown in **B**. Representative western blot image for NF-κB p65 of WT cells was shown in **C**. The relative band intensities of NF-κB p65 of WT cells were qualified and shown in **D**. Representative western blot image for NF-κB p65 of cells transfected with FOXO3a shRNA was shown in **E**. The relative band intensities of NF-κB p65 of cells transfected with FOXO3a shRNA were qualified and shown in **F**.

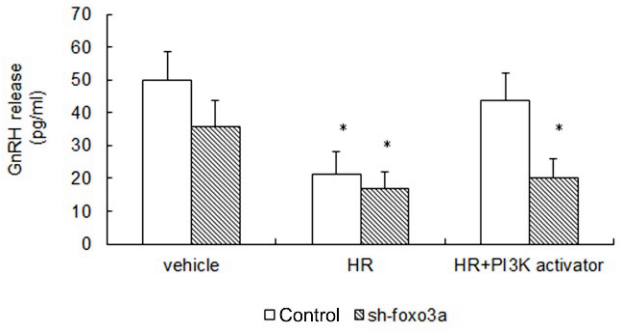

Fig. 4. Activation of PI3K/Akt/FOXO3a pathway prevents HR-induced decrease of GnRH release in primary hypothalamic neurons. Neurons were treated with a PI3K activator, after which were exposed to HR. Then, GnRH released into the medium was measured by an EIA kit. n=5. **P*<0.01, compared with the control group.

pathway stimulates spinal cord regeneration following injury in adult rats [21]. In contrast, the inactivation of [12-14]. In this study, we hypothesized that the activation of the PI3K/Akt pathway might positively affect HR-associated GnRH decline. To the best of our knowledge, this is the first study that demonstrated that PI3K/Akt pathway prevented GnRH reduction under HR *via* FOXO3a.

Previous studies have reported that the HR-related decrease of hypothalamic GnRH is associated with an inflammation-induced decrease of hypothalamic *gnrh1* promoter activity [1, 4]. In support of this finding, the present study demonstrated that gnrh1 promoter activity was suppressed under HR. The PI3K/Akt/FOXO3a pathway prevented HR-induced inhibition of *gnrh1* promoter activity, suggesting that the PI3K/Akt/FOXO3a pathway prevents HR-induced GnRH reduction via the regulation of the *gnrh1* gene.

Hypothalamic GnRH release is mediated by NF-κB signaling [1,4]. NF-κB in an inactive state is located in the cytoplasm and sequestered with IκBα [11,15]. Pleiotropic extracellular factors can initiate phosphorylation cascades that lead to the degradation of IκBα by proteasomes, allowing translocation of active NF-κB into the nucleus [11,15]. NF-κB activity is regulated by a number of different mechanisms, including the degradation of IκB [11,15]. In the present study, HR enhances IκB phosphorylation and NF-κB activation in GT1-7 cells. The PI3K/Akt/FOXO3a pathway can prevent HR-induced NF-κB signaling, thus suggesting that the PI3K/Akt/FOXO3a pathway could inhibit NF-κB activation via IκB. NF-κB has been demonstrated to be involved in an aging-related decrease in hypothalamic *gnrh1* promoter activity and GnRH release [1,4]. In the current study, the PI3K/Akt/FOXO3a pathway prevented GnRH decline under HR, possibly through the direct inhibition of *gnrh1* gene and/or indirect inhibition of *gnrh1* promoter activity through the promotion of NF-κB activation.

In summary, the current study indicated that activation of the PI3K/Akt/FOXO3a pathway protects GnRH neurons from HR-induced GnRH decline by preventing HR-induced *gnrh1* gene inhibition and NF-κB activation. These results further the understanding of the regulatory mechanisms of HR-associated hypothalamic GnRH decline.

Conflict of Interest

There is no conflict of interest.

Acknowledgements

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Authors' contributions

Han Guo and Shuchuan Xuanyuan are involved in performing experiments and statistical analysis. Chun Shi and Bensi Zhang are involved in manuscript writing. All authors read and approved the final manuscript.

References

1. Zhang G, Li J, Purkayastha S, Tang Y, Zhang H, Yin Y, Li B, Liu G, Cai D. Hypothalamic programming of systemic ageing involving IKK-β, NF-κB and GnRH. Nature 2013;497:211-216. https://doi.org/10.1038/nature12143

2. Shi C, Li G, Guo H, Liu X. Forkhead transcription factor FOXO1 is involved in hypoxia/reoxygenation-induced gonadotropin-releasing hormone decline. Neuroreport 2020;31:1296-1301. https://doi.org/10.1097/wnr.0000000000001548
3. Liu F, Huang X, Luo Z, He J, Haider F, Song C, Peng L, Chen T, Wu B. Hypoxia-Activated PI3K/Akt inhibits oxidative stress via the regulation of reactive oxygen species in human dental pulp cells. Oxid Med Cell Longev 2019;2019:6595189. https://doi.org/10.1155/2019/6595189
4. Kim K, Choe HK. Role of hypothalamus in aging and its underlying cellular mechanisms. Mech Ageing Dev 2019;177:74-79. https://doi.org/10.1016/j.mad.2018.04.008
5. Kwon DS, Kwon CH, Kim JH, Woo JS, Jung JS, Kim YK. Signal transduction of MEK/ERK and PI3K/Akt activation by hypoxia/reoxygenation in renal epithelial cells. Eur J Cell Biol 2006;85:1189-1199. https://doi.org/10.1016/j.ejcb.2006.06.001
6. Song HP, Chu ZG, Zhang DX, Dang YM, Zhang Q. PI3K-AKT pathway protects cardiomyocytes against hypoxia-induced apoptosis by MitoKATP-mediated mitochondrial translocation of pAKT. Cell Physiol Biochem 2018;49:717-727. https://doi.org/10.1159/000493037
7. Belsham DD, Cai F, Cui H, Smukler SR, Salapatek AM, Shkreta L. Generation of a phenotypic array of hypothalamic neuronal cell models to study complex neuroendocrine disorders. Endocrinology 2004;145: 393-400. https://doi.org/10.1210/en.2003-0946
8. Essaghir A, Dif N, Marbehant CY, Coffer PJ, Demoulin JB. The transcription of FOXO genes is stimulated by FOXO3 and repressed by growth factors. J Biol Chem 2009;284:10334-10342. https://doi.org/10.1074/jbc.M808848200
9. Shi C, Viccaro K, Lee HG, Shah K. Cdk5-Foxo3 axis: initially neuroprotective, eventually neurodegenerative in Alzheimer's disease models. J Cell Sci 2016;129:1815-1830. https://doi.org/10.1242/jcs.185009
10. Shi C, Luo X, Wang J, Long D. Incorporation of β-sitosterol into the membrane prevents tumor necrosis factor-α-induced nuclear factor-κB activation and gonadotropin-releasing hormone decline. Steroids 2015;96:1-6. https://doi.org/10.1016/j.steroids.2014.12.014
11. Kalaitzidis D, Gilmore TD. Transcription factor cross-talk: the estrogen receptor and NF-kappaB. Trends Endocrinol Metab 2005;16:46-52. https://doi.org/10.1016/j.tem.2005.01.004
12. Santo EE, Stroeken P, Sluis PV, Koster J, Versteeg R, Westerhout EM. FOXO3a is a major target of inactivation by PI3K/AKT signaling in aggressive neuroblastoma. Cancer Res 2013;73:2189-2198. https://doi.org/10.1158/0008-5472.Can-12-3767
13. Fujiki K, Inamura H, Matsuoka M. Phosphorylation of FOXO3a by PI3K/Akt pathway in HK-2 renal proximal tubular epithelial cells exposed to cadmium. Arch Toxicol 2013;87:2119-2127. https://doi.org/10.1007/s00204-013-1077-6
14. Snoeks L, Weber CR, Wasland K, Turner JR, Vainder C, Qi W, Savkovic SD. Tumor suppressor FOXO3 participates in the regulation of intestinal inflammation. Lab Invest 2009;89:1053-1062. https://doi.org/10.1038/labinvest.2009.66
15. Giridharan S, Srinivasan M. Mechanisms of NF-κB p65 and strategies for therapeutic manipulation. J Inflamm Res 2018;11:407-419. https://doi.org/10.2147/jir.S140188
16. Van Der Heide LP, Hoekman MF,Smidt MP. The ins and outs of FoxO shuttling: mechanisms of FoxO translocation and transcriptional regulation. Biochem J 2004;380:297-309. https://doi.org/10.1042/bj20040167
17. Wang X, Hu S, Liu L. Phosphorylation and acetylation modifications of FOXO3a: Independently or synergistically? Oncol Lett 2017;13:2867-2872. https://doi.org/10.3892/ol.2017.5851
18. Accili D, Arden KC. FoxOs at the crossroads of cellular metabolism, differentiation, and transformation. Cell 2004;117:421-426. https://doi.org/10.1016/s0092-8674(04)00452-0
19. Brunet A, Bonni A, Zigmond MJ, Lin MZ, Juo P, Hu LS, Anderson MJ, Arden KC, Blenis J, Greenberg ME. Akt promotes cell survival by phosphorylating and inhibiting a Forkhead transcription factor. Cell 1999;96:857-868. https://doi.org/10.1016/s0092-8674(00)80595-4
20. Zhang M, Zhang X. The role of PI3K/AKT/FOXO signaling in psoriasis. Arch Dermatol Res 2019;311:83-91. https://doi.org/10.1007/s00403-018-1879-8

21. Lu H, Zhang LH, Yang L, Tang PF. The PI3K/Akt/FOXO3a pathway regulates regeneration following spinal cord injury in adult rats through TNF-α and p27kip1 expression. Int J Mol Med 2018;41:2832-2838. https://doi.org/10.3892/ijmm.2018.3459