Title

FSH mediated cAMP signalling upregulates the expression of Gα subunits in pubertal rat Sertoli cells

Authors

Bhattacharya, Indrashis

Sharma, Souvik Sen

Sarkar, Hironmoy

et al.

Publication Date

2021-09-01

DOI

10.1016/j.bbrc.2021.06.094

Peer reviewed

Biochemical and Biophysical Research Communications 569 (2021) 100–105

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

FSH mediated cAMP signalling upregulates the expression of Gα subunits in pubertal rat Sertoli cells

Indrashis Bhattacharya [a, b, **, 1], Souvik Sen Sharma [a, c, 1], Hironmoy Sarkar [a, d, 1], Alka Gupta [a, 1], Bhola Shankar Pradhan [a], Subeer S. Majumdar [a, c, *]

[a] Cellular Endocrinology Laboratory, National Institute of Immunology, Aruna Asaf Ali Marg, New Delhi, 110067, India
[b] Dept. of Zoology, HNB Garhwal University, Srinagar, 246174, Uttarakhand, India
[c] National Institute of Animal Biotechnology, Hyderabad, 500 032, Telangana, India
[d] Department of Microbiology, Raiganj University, West Bengal, 733134, India

ARTICLE INFO

Article history:
Received 16 June 2021
Accepted 28 June 2021

Keywords:
Sertoli cell
G-protein
FSH
Adenyly cyclase
cAMP

ABSTRACT

Follicle Stimulating Hormone (FSH) acts via FSH-Receptor (FSH-R) by employing cAMP as the dominant secondary messenger in testicular Sertoli cells (Sc) to support spermatogenesis. Binding of FSH to FSH-R, results the recruitment of the intracellular GTP binding proteins, either stimulatory $G\alpha_s$ or inhibitory $G\alpha_i$ that in turn regulate cAMP production in Sc. The cytosolic concentration of cAMP being generated by FSH-R thereafter critically determines the downstream fate of the FSH signalling. The pleiotropic action of FSH due to differential cAMP output during functional maturation of Sc has been well studied. However, the developmental and cellular regulation of the Gα proteins associated with FSH-R is poorly understood in Sc. In the present study, we report the differential transcriptional modulation of the Gα subunit genes by FSH mediated cAMP signalling in neonatal and pubertal rat Sc. Our data suggested that unlike in neonatal Sc, both the basal and FSH/forskolin induced expression of *Gαs*, *Gαi-1*, *Gαi-2* and *Gαi-3* transcripts was significantly ($p < 0.05$) up-regulated in pubertal Sc. Further investigations involving treatment of Sc with selective $G\alpha_i$ inhibitor pertussis toxin, confirmed the elevated expression of Gi subunits in pubertal Sc. Collectively our results indicated that the high level of Gαi subunits serves as a negative regulator to optimize cAMP production in pubertal Sc.

1. Introduction

Testicular Sertoli cells (Sc) regulate the division and differentiation of male germ cells (Gc) to sperm [1,2]. During the neonatal/infantile period, immature Sc are incapable of supporting the robust differentiation of Gc [3]. However, functional maturation of Sc attained during puberty is concomitant with the spermatogenic onset [4,5]. Follicle Stimulating Hormone (FSH) and testosterone (T) are the major endocrine regulators orchestrating the maturation of Sc and spermatogenesis [6]. Although gene knock-out studies have demonstrated FSH to be dispensable for male fertility [7–9], a mutated FSH Receptor (*Fshr*) has recently been shown to restore complete male fertility in LH deficient mice without any support from T [10]. Similarly, experimental depletion of circulating FSH by hCG in adult men leads to poor sperm counts, which has been shown to be restored upon FSH supplementation alone [11]. Such studies indicate critical role of FSH in male fertility and suggest the urgent need of further research on FSH mediated regulation of spermatogenesis [12,13].

FSH mediates its effects on Sc by binding to its receptor- FSH-R. FSH-R, a G-protein coupled receptor (GPCR), undergoes a conformational change upon ligand (FSH) binding which results in the activation of the intracellular GTP binding proteins (G-proteins) associated with the receptor [12]. G proteins are of many different types and the presence of stimulatory $G\alpha_s$ (coded by *Gnas*) or inhibitory $G\alpha_i$ (coded by *Gnai1, Gnai2 & Gnai3*) in Sc has been reported previously [14]. Dual coupling of $G\alpha_s$ or $G\alpha_i$ to FSH-R differentially modulates the activity of adenyly cyclase (AC) to regulate FSH induced cAMP production within Sc [12,14]. The

* Corresponding author. National Institute of Animal Biotechnology, India.
** Corresponding author. Cellular Endocrinology Laboratory, National Institute of Immunology, Aruna Asaf Ali Marg, New Delhi, 110067, India.
E-mail addresses: indrashis.bhattacharya@gmail.com (I. Bhattacharya), subeer@nii.ac.in, subeer@niab.org.in (S.S. Majumdar).
[1] All have contributed equally.

https://doi.org/10.1016/j.bbrc.2021.06.094
0006-291X/

concentration of cAMP subsequently directs the multiple downstream signalling cascades such as canonical Protein Kinase A (PKA) or other (PKC, PI3K, Akt/PKB and ERK1/ERK2) pathways highlighting the pleiotropic effects of FSH in Sc [15,16]. The robust cAMP response in Sc results in the activation of PKA which in turn phosphorylates (thereby activates) cAMP Response Element Binding protein (CREB) to induce the transcription of genes such as *Kitlg*, *Gdnf*, *Abp*, *Transferrin* et cetera, that play an indispensable role in regulating spermatogenic progression [15]. Interestingly, cAMP has been shown to induce the expression of phosphodiesterases (PDEs) in Sc; PDEs cleave cAMP to yield AMP, attenuating the cAMP dependent PKA pathway. Induction of PDEs by cAMP constitutes an important negative feedback mechanism, regulating cAMP mediated signal transduction within the cell [17,18].

FSH shows its pleiotropic effects during Sc maturation without any apparent change in the expression of FSH-R [19–21]. FSH acts as a mitogen in fetal and neonatal/infantile Sc via the ERK-MAPK pathway favoured by low concentrations of cAMP, whereas during the onset of puberty the proliferation of Sc ceases with a dominant cAMP-dependent PKA signalling [22–24]. We have previously reported that a developmental shift in FSH response occurs around 12-days of postnatal age in maturing rat Sc due to an elevated binding ability of FSH to FSH-R with high cAMP production leading to the rapid transition of spermatogonia A to B [19].On the other hand, unlike infancy, the upregulated expression and activity of $G\alpha_s$ has been suggested to be critical for determining the robust cellular cAMP response in pubertal primate Sc [21,25]. Despite such information on FSH mediated signal transduction during Sc maturation, the molecular mechanisms regulating the expression of G proteins associated with FSH-R in Sc are not clearly defined. In the present study, we report the role of FSH mediated cAMP signalling in regulating the expression of $G\alpha_s/G\alpha_i$ subunits in rat Sc.

2. Materials and methods

2.1. Animals and reagents

Wistar rats (*Rattus norvegicus*) were housed and used as per the national guidelines provided by the Committee for the Purpose of Control and Supervision of the Experiments on Animals (CPCSEA). Ovine (o) FSH and anti-cAMP antibody were obtained from National Hormone and Pituitary Program (NHPP), National Institutes of Health (NIH; Torrance, CA). All other reagents, unless stated otherwise, were procured from Sigma Chemical Co. (St. Louis, MO).

2.2. Isolation of Sc

Testes were obtained from 5-day (neonatal) and 19-day old (pubertal) rats. Sc were isolated using a sequential enzymatic digestion as described previously [19]. For freshly isolated Sc (FrSc), isolated cell-clusters were exposed to 20 mM Tris-HCl (pH 7.4) for 3–5 min (min) to remove Gc and saved in Trizol before storing at −80 °C for subsequent RNA extraction.

2.3. Long term culture

Isolated Sc clusters were seeded in 24-well plates and were cultured in DMEM/nutrient mixture Ham F-12 (DMEM/F12 HAM) media containing 1% Fetal Calf Serum (FCS) for 24 h (hr) in a humidified 5% CO_2 incubator at 34 °C. The next day, cells were washed with pre-warmed media and cultured further in serum replacement growth factor media (GF media) containing 5 μg/ml sodium selenite, 10 μg/ml insulin, 5 μg/ml transferrin, and 2.5 ng/ml epidermal growth factor. On 3rd day, residual Gc, if any, were removed by hypotonic shock with 20 mM Tris-HCl (pH 7.4) for 3–5 min at 34 °C. Sc were then washed twice to remove dead Gc and the culture was continued further in GF media. On 4th day, one portion of Sc of each age group was treated with Trizol and stored in −80 °C for RNA extraction (0 h) and the rest were given various treatments [19].

2.4. In vitro treatments for gene expression

On day 4 of culture, Sc were treated with i) GF media alone i.e. vehicle as Control (C) ii) vehicle with 50 ng/ml of o-FSH and iii) vehicle with 10 μM forskolin (FORSK) for 24hr at 34 °C. On day 5 of culture, the treatment was terminated and the cells were saved in Trizol and stored at −80 °C for mRNA analysis [19].

2.5. Reverse transcription and quantitative real time PCR (RT-q-PCR)

Total mRNA was extracted from Trizol samples and cDNAs were prepared by reverse transcription (Promega, USA). Quantitative Real-time PCR (q-PCR) amplifications were performed by Realplex^S (Eppendorf, Germany) using Power SYBR Green Master Mix (Applied Biosystems, USA)]. Primers for each gene (target gene as well as internal control *18S rRNA*) were validated by a standard curve calculated from the C_t values of real time amplification from serial dilutions of the cDNAs. Primers with an efficiency of 1 ± 0.2 were used. The RT-q-PCR reaction involved melting of cDNA at 95 °C for 15 min, followed by 40 amplification cycles (30 s at 95 °C, 45 s at 60 °C and 45 s at 65 °C). Melting curve analyses for each gene were performed to detect the specific amplification peak for each gene. The expression of mRNA for the target genes were evaluated by the efficiency-corrected ΔCt method [quantity = $(\text{Efficiency}+1)^{-Ct}$] as described previously [26]. The mean (±SEMs) of at least 3 individual experiments was evaluated for each treatment group for the target gene. Primers used in the study are detailed in Supplemental Table SI.

2.6. Cyclic AMP assay

On day 4 of culture, Sc were pre-incubated with GF media containing 0.1 mM IBMX and 100 ng/ml of PT for 2 h followed by treatment with i) GF media containing 0.1 mM IBMX (vehicle) considered as Control (C), ii) vehicle containing o-FSH (50 ng/ml) for 24 h at 34 °C. For all the cultures, Sc conditioned media was used to determine cAMP by radio-immuno-assay (RIA) as reported by us previously [21].

2.7. Data representation and statistical analysis

One treatment group comprised of 3 wells, within one culture set. At least 3 such sets of cultures for each age group (performed on different calendar dates) were used to analyse and interpret the data. Testes from about 25 to 30 male rats were pooled for 5-days of age and 6–10 male rats from 19-days of age for Sc cultures. Statistical analysis was performed using Graph Pad Prism 5.0 software. Details of the statistical tests are provided in the figure legends.

3. Results and discussion

The present study investigated the molecular mechanisms underlying the differential regulation of Gα subunits associated with FSH-R during post-natal development of rat Sc. The transition of Sc from a proliferative, immature state to a functionally mature state occurs during the onset of puberty and is associated with a remarkable shift in FSH signalling in these cells [3,6]. FSH induced

cAMP signalling in pubertal Sc is critical for the robust initiation of Gc differentiation, leading to the progression of the first spermatogenic wave [27]. In the present study, we demonstrated the differential expression of Gα subunits in neonatal and pubertal rat Sc and have established the role of FSH mediated cAMP signalling in regulating the expression of these genes in Sc.

3.1. Differential expression of G-protein subunits in neonatal and pubertal rat Sc

Our results suggested that the basal level mRNA expressions of *Gαs* (coded by *Gnas*), *Gαi1*, *Gαi2* and *Gαi3* transcripts (coded by *Gnai1*, *Gnai2* and *Gnai3*) were up-regulated in pubertal Sc (both FrSc and cultured cells) as compared to that found in neonatal Sc (Fig. 1 – I&II). These intracellular GTP binding proteins are recruited by FSH-bound FSH-R to modulate the activity of adenylyl cyclase (AC) thereby regulating production of cAMP in Sc [14]. There are multiple sub-types of such GTP binding proteins, like $G\alpha_s$, $G\alpha_i$, $G\alpha_0$, $G\alpha_{q/11}$ and $G\alpha_{12/13}$ which have been reported to be associated with various GPCRs including FSH-R [16,28]. Since testicular Sc do not express either $G\alpha_0$ (known to induce K^+ ion channels) or $G\alpha_{q/11}$ (activates the phospholipase-C β enzyme) [29], FSH-R mainly gets differentially coupled with either cholera toxin (CT) sensitive stimulatory $G\alpha_s$ or pertussis toxin (PT) sensitive inhibitory $G\alpha_i$ proteins [30]. Locking of $G\alpha_s$ with FSH-R induces cAMP generation, whereas coupling with $G\alpha_i$ inhibits cAMP production [14,16]. FSH-R coupled with both $G\alpha_s$ or $G\alpha_i$, has been shown to induce ERK-MAPK mediated Sc proliferation in fetal and neonatal/pre-pubertal testes or to activate the cAMP-dependent canonical PKA pathway leading to the cessation of Sc proliferation during puberty [23]. Although the elevated expression of $G\alpha_s$ mRNA and protein have been reported in maturing rat testis and pubertal primate Sc [21,25,31], the pubertal rise in the expression of $G\alpha_i$ mRNA in mature rat Sc observed in the present study is a novel finding. Interestingly, despite such significant ($p<0.05\%$) developmental differences in the m RNA expression of *Gαs*, *Gαi1*, *Gαi2* and *Gαi3* transcripts observed between neonatal and pubertal Sc (Fig. 1. I and II), we found that the expression of these transcripts remained similar within Sc of a particular age (5-day or 19-day) group (Fig S1 A-B).

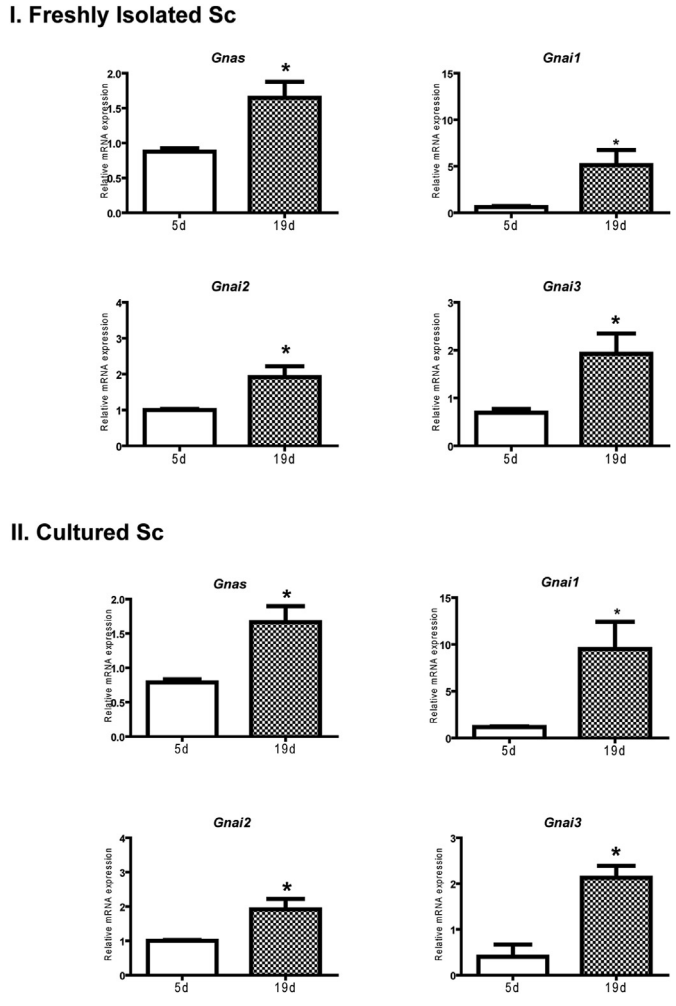

Fig. 1. Relative Expression of *Gα* subunit genes in neonatal (5-day) and pubertal (19-day) rat Sc

RT-q-PCR analyses of *Gnαs, Gnαi1, Gnαi2* or *Gnαi3* expression in neonatal (5-day, 5 d) and pubertal (19-day, 19 d) rat Sc. Freshly isolated (FrSc) Sc from 5-day and 19-day old rat testes **(I)**; 5-day and 19-day old Sc cultured for 4 days *in-vitro* at 0 h **(II)**. The shown data (mean ± SEM) is representative of at least three independent experiments. Unpaired *Student's t-test* was used for assessing statistical significance between the two age groups. $P < 0.05$ was considered to be statistically significant.

3.2. Induction of $G\alpha_s/G\alpha_i$ mRNA expression by cAMP in Sc

As the expression of the G protein subunits was found to be elevated in pubertal Sc, we hypothesized a role of FSH mediated cAMP signalling in regulating the expression of these genes in these cells. To this end, neonatal and pubertal Sc were treated with FSH or the adenyly cyclase activator, forskolin (FORSK) and the expression of the Gαs/Gαi transcripts was evaluated. A significant ($p<0.05\%$) increase in the expression of *Gnαs, Gnαi1, Gnαi2* and *Gnαi3* transcripts was observed in response to FSH in pubertal Sc as compared to that in neonatal Sc (Fig. 2A-D). Moreover, the effect of FSH on the expression of these genes was mimicked by treatment of Sc with FORSK, indicating a role of cAMP mediated signalling in regulating G-protein expression in Sc. Importantly, unlike in pubertal Sc, FSH failed to induce the expression of $G\alpha_s/G\alpha_i$ transcripts in neonatal Sc. This can be attributed to the lack of FSH mediated cAMP dependent PKA pathway in neonatal Sc, as has been described previously [19,23]. However in contrast to FSH, treatment of neonatal Sc with FORSK which directly activates AC by bypassing FSH-R, significantly ($p<0.05\%$) augmented the expression of *Gαs Gαi1, Gαi2* and *Gαi3* mRNAs in neonatal Sc, indicating the competence of the downstream signalling proteins [e.g. PKA or CREB (cAMP response element binding protein) etc [32]] to induce a pubertal like transcriptional pattern in immature Sc (Fig. 2A-D). Cyclic-AMP supplementation has been reported to augment the expression of $G\alpha_s$ and $G\alpha_{i\text{-}2}$ mRNAs in cultured astroglial cells, thyroid follicles [33–35] and S49 mouse lymphoma cell lines [36]. Furthermore, treatment of pubertal Sc with a combination of FSH and T has been reported to transiently down-regulate the expression of $G\alpha_{i\text{-}1}$, $G\alpha_{i\text{-}2}$ mRNAs and up-regulate $G\alpha_{i\text{-}3}$ mRNA at 6 h, whereas such expression profiles get recovered to the basal level at 24 h [37]. It is to be noted that the Gα subunit gene expression patterns in neonatal and pubertal rat Sc observed in the present study are different from what has been previously reported by us in primates [21]. For instance, the expression pattern of *Gnai2* remains uniform in infant and pubertal monkey Sc and FSH down regulates *Gnai2* in pubertal monkey Sc in a cAMP independent manner [21]. These observed variations may be due to the species-specific regulation of G proteins in rodent and primate Sc.

The increase in the levels of $G\alpha_i$ transcripts in pubertal Sc upon treatment with FSH or FORSK appeared to be counterintuitive to the established role(s) of elevated cAMP in orchestrating Sc maturation. In order to determine if the increase in the transcript levels of $G\alpha_i$ genes was reflected at the protein level as well, neonatal and pubertal rat Sc were treated with pertussis toxin (PT)

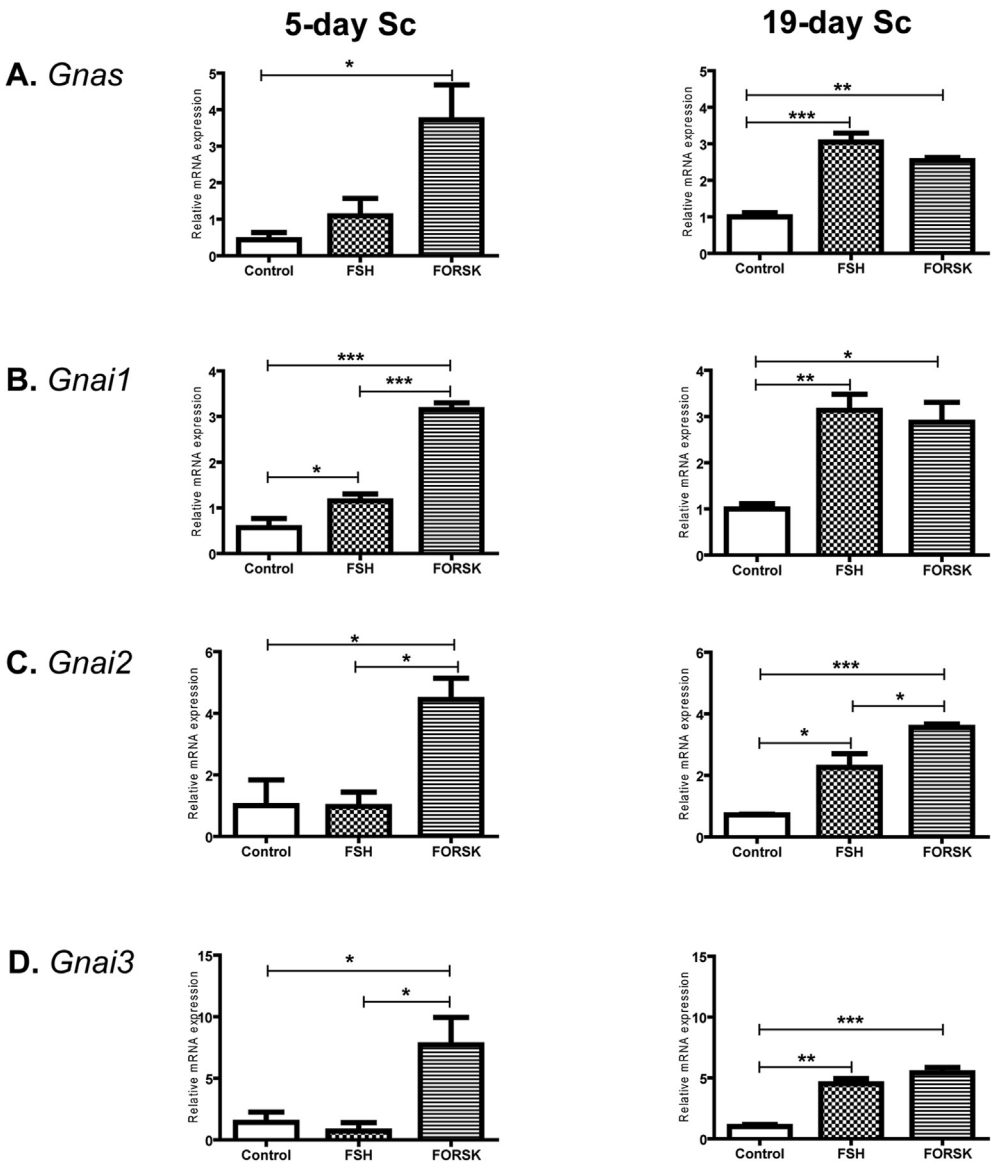

Fig. 2. Effect of FSH and forskolin (FORSK) on the expression of *Gnαs, Gnαi1, Gnαi2* and *Gnαi3* genes in neonatal (5-day) and pubertal (19-day) rat Sc.
RT-q-PCR analyses of *Gnαs, Gnαi1, Gnαi2* or *Gnαi3* gene expression in neonatal and pubertal rat Sc treated with ovine FSH (50 ng/ml) or adenylyl cyclase activator forskolin (10 μM), for 24 h **(A–D)**. Data (mean ± SEM) is representative of at least three independent experiments. One-way ANOVA followed by *Tukey's multiple comparison test* was used for assessing statistical significance. $P < 0.05$ was considered to be statistically significant.

to inactivate $G\alpha_i$ protein in Sc, followed by assessment of FSH mediated cAMP induction in the cells. It was observed that the selective inactivation of Gαi subunits by PT (100 ng/ml) supplementation resulted in a significant ($p<0.05\%$) rise in FSH induced cAMP production in neonatal and pubertal Sc (Fig. 3A-B). However, the fold rise in FSH induced cAMP production after PT pre-incubation was much higher in pubertal Sc as compared to neonatal Sc (Fig. 3A-B). This observation was concordant with the gene expression data and indirectly indicated the presence of elevated levels of $G\alpha_i$ sub-units in pubertal Sc as compared to neonatal Sc.

Our data further revealed an auto-regulatory mechanism of FSH mediated cAMP signalling, by which cAMP concentration is optimized in pubertal Sc via two parallel feedback loops, positive feedback by augmenting expression of stimulatory Gαs and negative feedback via inducing expression of inhibitory Gαi1, Gαi2 and Gαi3 subunits (Fig. 4A-B). FSH/FORSK mediated increase in the expressions of $G\alpha_i$ mRNAs in pubertal Sc is indicative of such negative regulation (Fig. 4B). Such negative regulatory mechanisms for cAMP signalling have been reported in Sc previously. For instance, cAMP directly induces the transcription of cAMP degrading enzyme PDE (isoform-PDE-4D, in particular for Sc and testis) to down-regulate its own concentration [17,18] or at stage II-VI of adult rat testes, FSH induced PKC selectively inhibits FSH-R-$G\alpha_s$ mediated cAMP response [38,39].

To conclude, the present study has demonstrated for the first time, the regulation of Gα subunits by FSH mediated cAMP signalling in rat Sc. Our results further indicated the existence of a possible negative feedback loop involving the induction of $G\alpha_i$ subunits by FSH dependent cAMP signalling, which may potentially maintain the optimal cAMP concentration in pubertal Sc, ensuring proper spermatogenic progression. However, future studies are

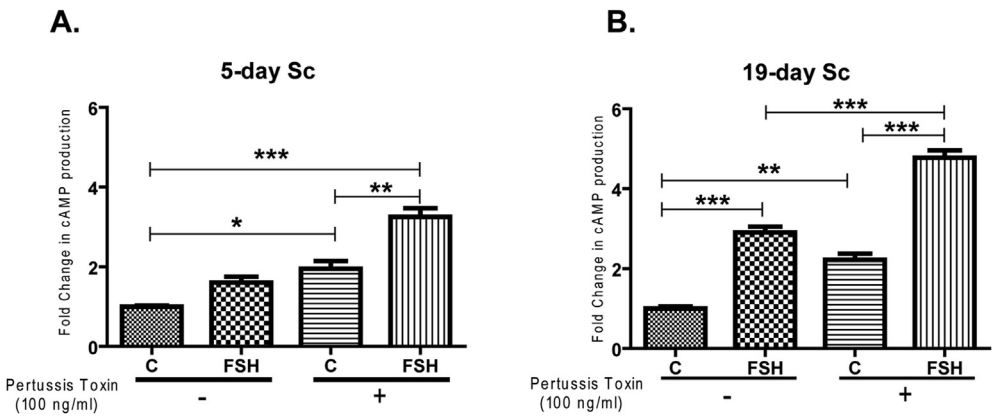

Fig. 3. Effect of Gαi inhibition on FSH mediated cAMP production by neonatal (5-day) and Pubertal (19-day) rat Sc.
Pre-Incubation (2hr) with Gαi inhibitor pertussis toxin (100 ng/ml PT) increased both the basal and FSH induced cAMP production in neonatal (**A**) and Pubertal (**B**) Sc at 24 h, (−) = without PT, (+) = with PT. One-way ANOVA followed by *Tukey's multiple comparison test* was used for assessing statistical significance between the treatment groups.

Figure 4

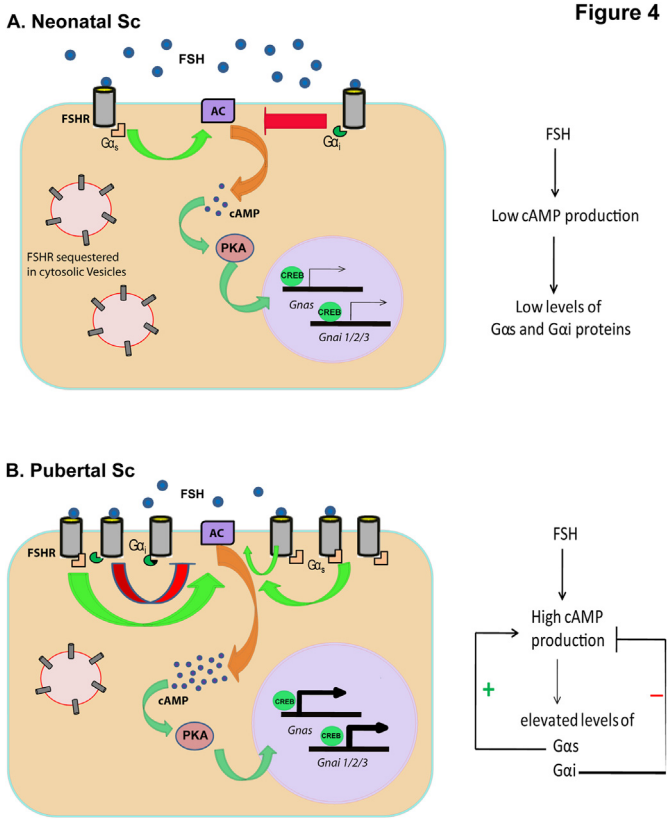

Fig. 4. Cartoon showing a diagrammatic representation of the differential regulation of G-proteins by cAMP signalling in neonatal (5-day) and pubertal (19-day) rat Sc.
Neonatal Sc express few FSH-R on the membrane-surface, thereby limiting the generation of cAMP inside the cell. Weak cAMP stimulation results in a moderate expression of *Gαs* and *Gαi* mRNAs and proteins in these cells **(A).** Pubertal Sc express higher number of FSH-Receptors on the membrane-surface, leading to a robust cAMP output with higher expression of *Gαs* and *Gαi* mRNAs and proteins in these cells. As per this model depicted above, the increase in the levels of Gαi-subunits in response to cAMP establishes a negative feedback loop that keeps a check on FSH mediated cAMP in these cells **(B)**. Pointed arrows with green positive (+) signs and closed line with red negative (−) sign represent the augmenting and antagonizing feedback loops respectively, regulating FSH-R signalling in pubertal Sc. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

required to investigate the cross-talk between FSH and T signalling and their role(s) in regulating G-protein expression and activity in pubertal Sc.

Author contributions

IB, SSS and SSM conceived the concept. IB designed all experiments. IB, SSS, HS, AG and BSP have done all experiments and generated the data. IB, SSS and SSM analysed the data. IB wrote the first draft of this manuscript, Tables, and Figures. SSS and HS prepared the final figures. IB, SSS, HS, AG and SSM edited the manuscript to produce the final draft.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement and Funding

We are thankful to all the staff of the Small Animal Facility. We are grateful to the past and present Directors, NII for valuable support. This study was funded by Tata Innovation Fellowship from the Department of Biotechnology, (BT/HRD/35/01/012010), J.C. Bose Fellowship from the Department of Science and Technology (SERB-JCB/2017/000027) to SSM. IB is supported by Department of Science and Technology (ECR/2018/000868).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbrc.2021.06.094.

References

[1] S.S. Majumdar, I. Bhattacharya, Genomic and post-genomic leads toward regulation of spermatogenesis, Prog. Biophys. Mol. Biol. 113 (2013) 409–422, https://doi.org/10.1016/j.pbiomolbio.2013.01.002.
[2] M.D. Griswold, 50 Years of Spermatogenesis : Sertoli Cells and Their Interactions with Germ Cells, 99, 2018, pp. 87–100, https://doi.org/10.1093/biolre/ioy027.
[3] R.M. Sharpe, C. McKinnell, C. Kivlin, J.S. Fisher, Proliferation and functional maturation of Sertoli cells, and their relevance to disorders of testis function in adulthood, Reproduction 125 (2003) 769–784.

[4] W.H. Walker, Molecular mechanisms controlling Sertoli cell proliferation and differentiation, Endocrinology 144 (2003) 3719–3721, https://doi.org/10.1210/en.2003-0765.
[5] L.R. França, R.A. Hess, J.M. Dufour, M.C. Hofmann, M.D. Griswold, The Sertoli cell: one hundred fifty years of beauty and plasticity, Andrology 4 (2016) 189–212, https://doi.org/10.1111/andr.12165.
[6] I. Bhattacharya, S. Sen Sharma, S.S. Majumdar, Pubertal orchestration of hormones and testis in primates, Mol. Reprod. Dev. 86 (2019) 1505–1530, https://doi.org/10.1002/mrd.23246.
[7] M.H. Abel, A.N. Wootton, V. Wilkins, I. Huhtaniemi, P.G. Knight, H.M. Charlton, The effect of a null mutation in the follicle-stimulating hormone receptor gene on mouse reproduction [1], Endocrinology 141 (2000) 1795–1803, https://doi.org/10.1210/endo.141.5.7456.
[8] A. Dierich, M.R. Sairam, L. Monaco, G.M. Fimia, A. Gansmuller, M. Lemeur, P. Sassone-Corsi, Impairing follicle-stimulating hormone (FSH) signaling in vivo: targeted disruption of the FSH receptor leads to aberrant gametogenesis and hormonal imbalance, Proc. Natl. Acad. Sci. U. S. A. 95 (1998) 13612–13617, https://doi.org/10.1073/pnas.95.23.13612.
[9] T.R. Kumar, Y. Wang, N. Lu, M.M. Matzuk, Follicle stimulating hormone is required for ovarian follicle maturation but not male fertility, Nat. Genet. 15 (1997) 201–204, https://doi.org/10.1038/ng0297-201.
[10] O.O. Oduwole, H. Peltoketo, A. Poliandri, L. Vengadabady, M. Chrusciel, M. Doroszko, L. Samanta, L. Owen, B. Keevil, N.A. Rahman, I.T. Huhtaniemi, Constitutively active follicle-stimulating hormone receptor enables androgen-independent spermatogenesis, J. Clin. Invest. 128 (2018) 1787–1792, https://doi.org/10.1172/JCI96794.
[11] A.M. Matsumoto, A.E. Karpas, W.J. Bremner, Chronic human chorionic gonadotropin administration in normal men: evidence that follicle-stimulating hormone is necessary for the maintenance of quantitatively normal spermatogenesis in man, J. Clin. Endocrinol. Metab. 62 (1986) 1184–1192, https://doi.org/10.1097/00006254-198702000-00020.
[12] L. Casarini, P. Crépieux, Molecular mechanisms of action of FSH, Front. Endocrinol. 10 (2019) 1–10, https://doi.org/10.3389/fendo.2019.00305.
[13] I. Huhtaniemi, A short evolutionary history of FSH-stimulated spermatogenesis, Hormones (Basel) 14 (2015) 468–478, https://doi.org/10.14310/horm.2002.1632.
[14] M. Simoni, J. Gromoll, E. Nieschlag, The follicle-stimulating hormone receptor: biochemistry, molecular biology, physiology, and pathophysiology, Endocr. Rev. 18 (1997) 739–773, https://doi.org/10.1210/er.18.6.739.
[15] W.H. Walker, J. Cheng, FSH and testosterone signaling in Sertoli cells, Reproduction 130 (2005) 15–28, https://doi.org/10.1530/rep.1.00358.
[16] A. Ulloa-Aguirre, E. Reiter, P. Crepieux, FSH receptor signaling: complexity of interactions and signal diversity, Endocrinology 159 (2018) 3020–3035, https://doi.org/10.1210/en.2018-00452.
[17] C. Mehats, C.B. Andersen, M. Filopanti, S.L.C. Jin, M. Conti, Cyclic nucleotide phosphodiesterases and their role in endocrine cell signaling, Trends Endocrinol. Metab. 13 (2002) 29–35, https://doi.org/10.1016/S1043-2760(01)00523-9.
[18] G. Levallet, J. Levallet, H. Bouraïma-Lelong, P.J. Bonnamy, Expression of the cAMP-phosphodiesterase PDE4D isoforms and age-related changes in follicle-stimulating hormone-stimulated PDE4 activities in immature rat sertoli cells, Biol. Reprod. 76 (2007) 794–803, https://doi.org/10.1095/biolreprod.106.055343.
[19] I. Bhattacharya, B.S. Pradhan, K. Sarda, M. Gautam, S. Basu, S.S. Majumdar, A switch in Sertoli cell responsiveness to FSH may be responsible for robust onset of germ cell differentiation during prepubartal testicular maturation in rats, Am. J. Physiol. Endocrinol. Metab. 303 (2012) E886–E898, https://doi.org/10.1152/ajpendo.00293.2012.
[20] S.S. Majumdar, K. Sarda, I. Bhattacharya, T.M. Plant, Insufficient androgen and FSH signaling may be responsible for the azoospermia of the infantile primate testes despite exposure to an adult-like hormonal milieu, Hum. Reprod. 27 (2012) 2515–2525, https://doi.org/10.1093/humrep/des184.
[21] I. Bhattacharya, S. Basu, K. Sarda, M. Gautam, P. Nagarajan, B.S. Pradhan, H. Sarkar, Y.S. Devi, S.S. Majumdar, Low levels of Gαs and Ric8b in testicular sertoli cells may underlie restricted FSH action during infancy in primates, Endocrinology 156 (2015) 1143–1155, https://doi.org/10.1210/en.2014-1746.
[22] J.M. Orth, The role of follicle-stimulating hormone in controlling sertoli cell proliferation in testes of fetal rats, Endocrinology 115 (1984) 1248–1255, https://doi.org/10.1210/endo-115-4-1248.
[23] P. Crépieux, S. Marion, N. Martinat, V. Fafeur, Y. Le Vern, D. Kerboeuf, F. Guillou, E. Reiter, The ERK-dependent signalling is stage-specifically modulated by FSH, during primary Sertoli cell maturation, Oncogene 20 (2001) 4696–4709, https://doi.org/10.1038/sj.onc.1204632.
[24] S.J. Meachem, S.M. Ruwanpura, J. Ziolkowski, J.M. Ague, M.K. Skinner, K.L. Loveland, Developmentally distinct in vivo effects of FSH on proliferation and apoptosis during testis maturation, J. Endocrinol. 186 (2005) 429–446, https://doi.org/10.1677/joe.1.06121.
[25] I. Bhattacharya, S. Basu, B.S. Pradhan, H. Sarkar, P. Nagarajan, S.S. Majumdar, Testosterone augments FSH signaling by upregulating the expression and activity of FSH-Receptor in Pubertal Primate Sertoli cells, Mol. Cell. Endocrinol. 482 (2019) 70–80, https://doi.org/10.1016/j.mce.2018.12.012.
[26] I. Bhattacharya, M. Gautam, H. Sarkar, M. Shukla, S.S. Majumdar, Advantages of pulsatile hormone treatment for assessing hormone-induced gene expression by cultured rat Sertoli cells, Cell Tissue Res. 368 (2017) 389–396, https://doi.org/10.1007/s00441-016-2410-1.
[27] M.A. Wood, P. Mukherjee, C.A. Toocheck, W.H. Walker, Upstream stimulatory factor induces Nr5a1 and Shbg gene expression during the onset of rat sertoli cell differentiation, Biol. Reprod. 85 (2011) 965–976, https://doi.org/10.1095/biolreprod.111.093013.
[28] E. Ghosh, P. Kumari, D. Jaiman, A.K. Shukla, Methodological advances: the unsung heroes of the GPCR structural revolution, Nat. Rev. Mol. Cell Biol. 16 (2015) 69–81, https://doi.org/10.1038/nrm3933.
[29] T.B. Haugen, R.H. Paulssen, V. Hansson, Cell-specific expression of Gq/11 protein and mRNA in rat seminiferous tubules, FEBS Lett. 329 (1993) 96–98, https://doi.org/10.1016/0014-5793(93)80201-5.
[30] R.H. Paulssen, E.J. Paulssen, J.O. Gordeladze, V. Hansson, T.B. Haugen, Cell-specific expression of guanine nucleotide-binding proteins in rat testicular cells, Biol. Reprod. 45 (1991) 566–571, https://doi.org/10.1095/biolreprod45.4.566.
[31] S. Lamsam-Casalotti, M. Onoda, V. Papadopoulos, M. Dym, Developmental expression of GTP-binding proteins in rat testes, J. Reprod. Fertil. 99 (1993) 487–495, https://doi.org/10.1530/jrf.0.0990487.
[32] W.H. Walker, L. Fucci, J. Habener, Expression of the gene encoding transcription factor cyclic adenosine 3'5'- Monophosphate (cAMP) Response Element-Binding Protein (CREB): regulation by Follicle-Stimulating Hormone-Induced cAMP signaling in primary rat Sertoli cells, Endocrinology 136 (1995) 3534–3545.
[33] K. Dib, A. el Jamali, C. Jacquemin, C. Corrèze, Cyclic AMP regulation of messenger RNA level of the stimulatory GTP-binding protein Gsα: isoproterenol, forskolin and 8-bromoadenosine 3′:5′-cyclic monophosphate increase the level of Gsα mRNA in cultured astroglial cells, Eur. J. Biochem. 219 (1994) 529–537, https://doi.org/10.1111/j.1432-1033.1994.tb19968.x.
[34] A. El Jamali, N. Rachdaoui, K. Dib, C. Corrèze, Cyclic AMP regulation of G(iα2) and G(iα3) mRNAs and proteins in astroglial cells, J. Neurochem. 71 (1998) 2271–2277, https://doi.org/10.1046/j.1471-4159.1998.71062271.x.
[35] B. Saunier, K. Dib, B. Delemer, C. Jacquemin, C. Correze, Cyclic MP regulation of gs protein, J. Biochem. 265 (1990) 19942–19947.
[36] J.R. Hadcock, M. Ros, D.C. Watkins, C.C. Malbon, Cross-regulation between G-protein-mediated pathways. Stimulation of adenylyl cyclase increases expression of the inhibitory G-protein, G(iα2), J. Biol. Chem. 265 (1990) 14784–14790, https://doi.org/10.1016/s0021-9258(18)77181-0.
[37] F. Loganzo, P.W. Fletcher, Follicle-stimulating hormone increases guanine nucleotide-binding regulatory protein subunit αi-3 mrna but decreases αi-1 and αi-2 mrna in sertoli cells, Mol. Endocrinol. 6 (1992) 1259–1267, https://doi.org/10.1210/mend.6.8.1328874.
[38] L. Monaco, M. Conti, Inhibition by phorbol esters and other tumor promoters of the response of the Sertoli cell to FSH: evidence for dual site of action, Mol. Cell. Endocrinol. 49 (1987) 227–236, https://doi.org/10.1016/0303-7207(87)90217-6.
[39] H. Nikula, K. Vihko, I. Huhtaniemi, Protein kinase C and Gi-protein mediated modulation of cAMP production in different stages of the rat seminiferous epithelium, Mol. Cell. Endocrinol. 70 (1990) 247–253, https://doi.org/10.1016/0303-7207(90)90215-T.